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DNA REPAIR AND MUTAGENESIS IN THE UV-SENSITIVE MUTANT *UVSI* OF *ASPERGILLUS NIDULANS*

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Short Title

DNA repair and mutagenesis in UV-sensitive mutant *uvsI* of *Aspergillus*

Suhn-Kee Chae

Ph.D. Thesis

Department of Biology

To Eugene, Erica, and Lee

ABSTRACT

The effects of a newly mapped DNA repair-defective mutant, *uvsI*, on mutagen sensitivities and mutation were investigated. Results showed that *uvsI* differs for most of the investigated properties from other *uvs* mutants of *A. nidulans* which are known to belong to three different epistatic groups, "UvsF", "UvsC", and "UvsB". Most of these mutants are sterile and many of them alter mitotic recombination frequencies, while *uvsI* exhibits normal levels of meiotic and mitotic recombination. In addition, *uvsI* strains are not more sensitive than wild type to MMS (methyl methanesulfonate) to which all other *uvs* strains are sensitive. However, the *uvsI* mutant was found to be very sensitive to the killing effects of UV light and the chemical mutagen, 4-NQO (4-nitro-quinoline-*N*-oxide). In line with the distinct phenotype of *uvsI*, no epistatic interactions were found for this mutant with any members of the established three epistatic groups. The effects of *uvsI* on mutagenesis are highly specific and dependent on the mutational test systems. In the *uvsI* mutant, two types of forward mutation were not affected, but spontaneous and UV-induced reversion frequencies of *choA1* and *pabaA1* were significantly reduced. Specific effects were further demonstrated in reversion tests of various *sC* alleles originally isolated as selenate resistant mutants by treatment with EMS (ethyl methanesulfonate), which leads mainly to G:C to A:T transitions. After EMS treatment *uvsI* mutants showed highly *reduced* reversion frequencies for all these *sC* alleles (except one) compared to uvs^+ strains. These results suggest that the *uvsI* mutation may be defective in AT to GC transition mutagenesis, while increasing transversion(s) from A:T base pairs. In contrast, *uvsI* affected the frequencies of spontaneous and UV-induced reversions for these *sC* alleles in a variety of ways. Thus, *uvsI* may well represent a fourth functional and epistatic group of DNA repair and possibly be involved in a minor mutagenic DNA repair pathway in *Aspergillus nidulans*.

RÉSUMÉ

La mutation uvsI, récemment cartographiée, est responsable d'une déficience de la réparation de l'ADN. Ses effets sur la sensibilité aux agents mutagènes et sur les taux de mutation ont été étudiés. Les résultats ont montré que les mutants uvsI diffèrent, quant aux propriétés étudiées, des autres mutants uvs chez Aspergillus nidulans, lesquels peuvent être rassemblés en trois groupes épistatiques différents, appelés "UvsF", "UvsC" et "UvsB". La plupart de ces mutants sont stériles et beaucoup ont une fréquence de recombinaison mitotique modifiée par rapport au type sauvage, alors que le mutant uvsI a présenté des taux de recombinaison méiotique et mitotique normaux. De plus, les souches uvsI n'étaient pas plus sensibles que les souches sauvages au sulfonate de méthyl-méthane (SMM), alors que tous les autres mutants y sont sensibles. Par contre, le mutant uvsI s'est montré très sensible à l'effet létal de la lumière UV, ainsi qu'à l'agent mutagène chimique N-oxyde de 4-nitro-quinoline (ONQ). Corroborant le phénotype distinct d'uvsI, aucune interaction épistatique n'a été trouvée entre ce mutant et n'importe quel autre membre des trois groupes établis.

La mutation uvsI a aussi des effets sur la mutagénèse. Ces effets sont différents de ceux provoqués par les autres mutations uvs. Ils sont extrêmement spécifiques et dépendent du système de test mutationnel. Chez le mutant uvsI, les deux sortes de mutation avancée étudiées n'étaient pas affectées. Par contre, pour les allèles choA1 et pabaA1, les fréquences de réversion spontanée et induite par les rayons UV étaient réduites de façon significative. De plus, des tests de réversion de divers allèles sC ont révélé des effets spécifiques. Les mutants sC ont été originalement isolés

comme étant résistants au sélénate suite à un traitement au sulfonate d'éthyl-méthane (SEM), agent mutagène qui produit principalement des transitions G:C→A:T. Pour tous les allèles sC (sauf un), la fréquence de réversion après traitement au SEM était extrêmement réduite chez les mutants uvsI, comparés aux souches uvs^+. Par contraste, les fréquences de réversion spontanée et induite aux UV pour ces mêmes allèles étaient affectées par la mutation uvsI de diverses façons. Ces résultats suggèrent que le mutant uvsI pourrait être défectueux dans la mutagénèse de transition A:T→G:C, tout en augmentant les transversions des paires de bases A:T. Donc uvsI pourrait bien représenter un quatrième groupe fonctionnel et épistatique de réparation de l'ADN et pourrait être impliqué dans une voie mineure de réparation mutagénique de l'ADN chez A. nidulans.

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PREFACE

As required by the faculty of graduate Studies and Research of McGill University the following statement is quoted:

> "The candidate has the option, subject to the approval of the Department, of including as part of the thesis the text, or duplicated published text, of an original paper, or papers. In this case the thesis must still conform to all other requirements explained in the Guidelines Concerning Thesis Preparations. Additional material (procedural and design data as well as descriptions of equipment) must be provided in sufficient details (e.g., in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported. The thesis should be more than a mere collection of manuscripts published or to be published. It must included a general abstract, a full introduction and literature review and a final overall conclusion. Connecting texts which provide logical bridges between different manuscripts are usually desirable in the interest of cohesion.
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This thesis consists of an Abstract, Résumé, Introduction, Literature Review (chapter 1), three investigations presented in manuscript forms, as Summary, Introduction, Materials and Methods, Results and Discussion (chapter 2, 3, and 4) , Concluding remarks (chapter 5), Appendix, and Literature Cited.

Chapter 2 has been accepted (in press) for publication in **Current Genetics** as: Chae S.-K. and Kafer E. (1993) *uvsI* mutants defective in UV mutagenesis define a fourth epistatic group of *uvs* genes in *Aspergillus*.

Chapter 3 has been accepted (pending revision) in **Current Genetics** as: Kafer E. and Chae S.-K. (1993) Phenotypic and epistatic grouping of hypo- and hyperrec *mus* mutants in *Aspergillus*.

Chapter 4 has not been submitted yet, but a short version will be ready to submit for publication . The complete reference is,
Chae S.-K. and Kafer E. (1993) Differential and base pair-specific effects of *uvsI* on forward *versus* reverse mutagenesis and *uvsI* interaction with *uvsC*, a mutator strain of *Aspergillus nidulans*.

The literature cited in this thesis has been combined and placed at the end of the dissertation.

All the results cited in **chapter 2** and **4** are solely the work of the candidate.

In **chapter 3**, all results for *musN* and *musN;uvs* double mutants (i.e., Fig. 3.2.A-C; Fig. 3.4), mapping of *uvsI* (Table 3.1), and section 3.7 (Fig. 3.6 - 3.12) are solely the work of the candidate.

ABBREVIATIONS

6-4	pyrimidine-pyrimidone [6–4] lesion
AP	apurinic or apyrimidinic site
CHO	Chinese Hamster Ovary
EDTA	ethylenediaminetetraacetic acid
EMS	ethyl methanesulfonate
ENU	*N*-ethyl-*N*-nitrosourea
ERCC	excision repair cross complementing
MMS	methyl methanesulfonate
MNNG	*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine
MNU	*N*-methyl-*N*-nitrosourea
4-NQO	4-nitroquinoline 1-oxide
SDS	sodium dodecyl sulfate
Tris	tris (hydroxymethyl) aminomethane
ts	temperature sensitive
UV	ultraviolet light
XP	xeroderma pigmentosum

Gene symbols for *A. nidulans* : see Appendix II (p 194)

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INTRODUCTION

In living cells, the dynamic properties of DNA lead to constant changes which often cause mispairing with wrong bases during DNA replication or blocks of DNA replication. Deamination and loss of bases occur at varying frequencies dependent on pH and temperature. Numerous physical and chemical agents also generate DNA damage. Pyrimidine dimers and 6-4 photoproducts are induced by UV irradiation. Ionizing radiation cause DNA strand breaks and base ring opening. On the other hand, chemical agents modify DNA in a variety of ways. In consequence, cells may be destined for death or yield mutations unless such errors are rapidly corrected. Considering all these sources of DNA damage, the extremely high stability of DNA is very astounding. Such a stability is mainly achieved by various DNA repair processes which restore DNA damage by means of normal DNA sequence in complementary strand of double-helical DNA.

The remarkable feats of DNA repair processes have first been identified in prokaryotes, especially in *E. coli* and its phages. In *E. coli*, various different repair mechanisms have been characterized and these are of four basic types (reviewed by Sancar and Sancar 1988). By now over 50 genes and many enzymes are known in detail which contribute to the removal of damaged or inappropriate bases. Two of these occur in non-dividing cells, namely, 1) simple damage reversal, and 2) excision repair. Two others are postreplication repair mechanisms, either, 3) recombinational repair, or 4) error-prone repair, leading to increased mutation. Each of these includes multiple alternate processes involving only a few pathway-specific genes.

In eukaryotes, some evidence for the DNA repair processes corresponding to those of *E. coli* have been obtained, but none have yet been fully elucidated. However, in many cases, highly conserved genes with similar fundamental functions show

recognizable amino acid homologies among widely differing species, from *E. coli* to *Homo sapiens*. In addition, functional equivalence can be verified for gene products which exhibit no sequence homology but do complement defects of foreign cells. Such cross-complementation tests identify corresponding functions that have evolved in different ways, often in parallel with more complex organization and differentiation.

In the yeast, *Saccharomyces cerevisiae*, three large and mostly non-overlapping epistatic groups were identified for radiation repair, each encompassing many genes (reviewed by Haynes and Kunz 1981; Friedberg 1988). This grouping is primarily based on the comparison of the sensitivity to UV or X-ray in double mutants to that in component single mutant strains. Furthermore, biochemical and molecular biological evidence have supported that these three epistatic groups also represent three functional groups of different DNA repair pathways. From the studies of DNA repair in yeast, it has become evident that many more specific genes are likely to be involved in equivalent processes, and that the modes and organization of DNA repair pathways are expected to be largely different from those of prokaryotes. Such hypotheses are supported by the qualitative differences especially in the complexity of genomic organization and replication. Eukaryotes not only differ in having multiple replication origins, and therefore many simultaneous replication forks, but also chromatin of a complex structure and more elaborate gene organization.

In *Neurospora* and *Aspergillus*, organization of radiation repair probably differs from that found in *E. coli* or *Saccharomyces cerevisiae*, judged by results from attempts at epistatic grouping of repair-deficient mutants. In these filamentous fungi, three epistatic groups were found originally, based on sensitivities of single and double mutants to UV or to MMS to which practically all highly UV-sensitive types were cross-sensitive (Käfer 1983; Käfer and Mayor 1986). However, none of the obtained groups corresponded well to those of *E. coli* or yeast. For example, one group in each species (i.e., "UVS-2" in *N. crassa* and "UvsF" in *A. nidulans*) included mutants which generally showed increased

UV-induced mutation, as is found typically for excision-defective types of *E. coli* and yeast. However, other properties of some members of these groups do not fit; e.g., X-ray sensitivity and meiotic defects. Such properties are not found in excision-defective mutants of *E. coli* (i.e., *uvrA*, *B*, and *C*) nor those of yeast (i.e., mutants of the RAD3 group). More significantly, earlier findings in *Neurospora crassa*, suggesting defective excision of UV dimers in members of the "Uvs-2" group, were not confirmed in more reliable recent tests (Baker et al. 1990).

In *Aspergillus nidulans* the three epistatic groups of DNA repair include *uvs* mutations with similar properties: namely, 1) the "UvsF" group of *uvs* mutations which increase UV mutagenesis and spontaneous mitotic recombination as found typically for excision repair types in *E. coli* and yeast; 2) the "UvsC" group of mutants which are sterile and abolish spontaneous mitotic recombination, but in addition increase spontaneous and practically lack UV-induced mutation (Jansen 1972; Käfer and Mayor 1986); and 3) the "UvsB" group of mutations which greatly increase chromosomal aberrations and deletions, probably as a result of unrepaired chromosome breaks, and therefore show low ascospore viability and increased mitotic recombination of a non-reciprocal type.

The *uvsI* mutant which was the main subject of this study has been isolated and shown to be defective in UV-mutagenesis. Therefore, *uvsI* is a likely candidate for a gene involved in UV-induced mutagenic repair (Han et al. 1983). This type of mutation was not previously represented among the various types included in the three epistatic groups of *Aspergillus nidulans*.

The main aim of this study was to examine the interrelationship between *uvsI* and all formerly characterized *uvs* mutants. To this end interaction of these mutations and their epistatic grouping was investigated. In addition, phenotypes were characterized and compared as an aid to functional grouping, especially, detailed analysis of *uvsI* effects on spontaneous and mutagen-induced mutagenesis.

CHAPTER 1

LITERATURE REVIEW

1.1. DNA REPAIR

DNA repair can be defined in a strict sense as two major cellular responses; namely, i) direct reversal of DNA damage through a single enzymatic reaction, and ii) excision of DNA damage and resynthesis using undamaged complementary strand as template. On the other hand, DNA damage can be tolerated by either i) replicative bypass with a temporary gap left at the damaged site and gap filling by homologous recombination, or ii) translesion synthesis without creating a gap, which is an error-prone process (Friedberg 1985).

Different from simple DNA reversals, other DNA repair events are usually mediated by genes involving multistep reactions or complexes. Many DNA repair genes have been sorted out and placed into specific pathways. Genetically, epistatic analysis has been accomplished by tests for the phenotypic effects on survival of combinations of mutant loci (largely described for radiation sensitive *rad* mutants of yeast by Game and Cox 1973; Haynes and Kunz 1981). **Epistatic interactions** can be seen if two genes control different steps in a single pathway. In this case, the double mutants should have the phenotype of one the single mutants which acts at the earlier of the two steps affected, i.e., the double mutants should be no more sensitive than the most sensitive of the two component single mutant strains. Similar interaction also would be expected when both gene products are physically associated and required for the activity of the resulting enzyme complex. On the other hand, if two mutations affect proteins involved in entirely independent and alternate pathways, **synergism** will be observed, i.e., the survival fraction of the double mutants will be equal to or more than the multiple of the survival fractions of the two component single mutant strains. Such a interaction would be expected when a common substrate is used competitively in an important step of the two alternate pathways. On the other hand, **additivity** would be expected if two genes act independently on different substrates. In this case, the effect of double mutation on

survival should be additive compared to that of each component single mutation. Interpretation of such an interaction, however, is only reliable if null mutations are used, because a leaky mutant allele (i.e., missense mutations producing partially active proteins) can give unexpected deviations. Such leaky alleles of the same epistatic group are therefore expected and found to exhibit apparently additive interactions (Game and Cox 1972, 1973; Brendel and Haynes 1973).

Epistatic grouping by genetical analysis largely has been successful in assigning more than 40 genes into the three RAD groups for radiation repair in yeast (summarised by Friedberg 1991). Such an epistatic grouping has been further supported by the results obtained biochemically and molecularly.

Biochemical studies often have provided direct evidence for excision-defective enzymes. In addition, molecular cloning of DNA repair genes has made possible gene disruption, over-expression, and comparison of sequences, which somtimes revealed the primary functions of these genes.

1.1.1. Damage reversal systems

The best-known example of simple direct reversal of DNA damage is enzymatic **photoreactivation** (PR) of UV-induced cyclobutane dimers. Photoreactivation is mediated by a DNA photolyase which catalyzes monomerization of dimers dependent on a light source (>300 nm; reviewed by Sancar 1990). Recently the *phr* gene of *E. coli* which codes for this photolyase enzyme has been cloned and molecular analysis of reaction mechanisms has become possible (Kim et al. 1992). Photolyase appears to increase the efficiency of nucleotide excision repair by enhancing both the recognition of dimers by *uvrABC* excinuclease and the turnover rate (Yamamoto et al. 1984).

Photoreactivating enzyme activities also have been found in various species of

eukaryotes (e.g., PHR1 of yeast; Resnick and Setlow 1972). Furthermore, physiological properties of several purified photolyases from prokaryotes and eukaryotes have indicated that remarkable similarities exist. Comparison of amino acid sequences of *phr* genes in various organisms revealed a very high degree of sequence identity, especially within the carboxy-terminal domains (reviewed by Sancar 1990). Exchange of the *E. coli phr* gene with those of other species showed that the corresponding enzymes are indeed functionally equivalent (Kobayashi et al. 1990).

Another case of DNA damage reversal is the direct removal of methyl groups by "suicidal" **methyltransferases** (MTase) which become inactivated in the process. The principal lesion among methylated bases in DNA is O^6-methylguanine which is responsible for the mutagenicity of methylating agents. In *E. coli*, two types of O^6-methylguanine-DNA methyltransferases, coded by *ada* and *ogt* genes, have been identified (Potter et al. 1987; Rebeck et al. 1988). The Ada MTase is induced as part of the adaptive response to alkylating agents, while Ogt MTase (DNA MTase II) is constitutively expressed (reviewed by Lindahl et al. 1988).

In eukaryotes, O^6-methylguanine DNA methyltransferases genes have been cloned by complementing *E. coli ada⁻ ogt⁻* double mutant cells with random genomic fragments, or cDNAs, from yeast (Xiao et al. 1991), CHO and human cells (Katakoa et al. 1986; Tano et al. 1991).

1.1.2. Excision repair in prokaryotes

Excision repair removes damaged or inappropriate nucleotides by three different mechanisms involving many specific enzymes. On the other hand, subsequent replacement occurs by general DNA replicating enzymes which use the normal opposite strand as a template for DNA resynthesis.

a) Base excision repair

Base excision involves removal of bases by damage-specific glycosylases. The resulting abasic (AP) sites are further processed by AP endonucleases, or in some cases by enzymes which have both activities.

DNA **glycosylases** catalyze excision of free bases from DNA by hydrolysis of the *N*-glycosylic bond (reviewed by Sakumi and Sekiguchi 1990). Most of the glycosylases are highly specific for a particular form of base damage in DNA. In *E. coli*, several DNA glycosylases have been identified, e.g., uracil DNA glycosylase (coded by the *ung* gene), 3-methyladenine DNA glycosylases I (*tag*), and 3-methyladenine DNA glycosylase II, the product of *alkA* gene.

The **AP endonucleases** cleave the DNA strand by two different mechanisms, either 5' to an AP site leaving a 3'-OH (called class II AP endonucleases, e.g.,endonuclease IV and exonuclease III in *E. coli*), or alternatively, 3' to an AP site leaving a 3'-α,β-unsaturated aldehyde (class I AP endonuclease = AP lyase; reviewed by Doetsch and Cunningham 1990). The major AP endonuclease of *E. coli* is exonuclease III which is multifunctional, having AP endonuclease, 3' to 5' dsDNA specific exonuclease, and RNAase H activities. This enzyme accounts for more than 85% of AP endonuclease activity in *E. coli*. However, exonuclease III mutants *(xth⁻)* show surprisingly low mutagen sensitivities (except to H_2O_2). This is due to an alternate pathway of repair with overlapping specificities as discovered in tests of double mutants of *xth* and mutation in the gene for endonuclease IV (*nfo*). Such *nfo* mutations greatly enhanced the very slight sensitivities of *xth* mutants to MMS and g-rays in double mutant strains, i.e., synergistic interactions were seen in the double mutants (Cunningham et al. 1986).

The well-known UV endonuclease is an example which has both activities of AP endonuclease and glycosylase, namely an **AP endonuclease associated with**

specific glycosylase activity. This enzyme is specific for pyrimidine dimers and represents an important type of the major UV damage (Haseltine at al. 1980). Surprisingly, this enzyme was found as being a major activity only in two species, namely *Micrococcus luteus* and the T4 phage of *E. coli*. In other species, pyrimidine dimers are eliminated mainly by nucleotide excision, even though *N*-glycosylase/AP lyase enzyme similar to that from *Micrococcus* and T4 phage was discovered in yeast (Hamilton et al. 1992). Such an alternate mode of repair has been found also in *Micrococcus*, accounting for the wild type level of UV-sensitivity in mutants lacking UV-endonuclease (Tao et al. 1987). Moreover, Nakayama et al. (1992) showed that double mutants, in which both UV endonuclease and nucleotide excision repair are deficient, exhibited higher UV sensitivity than either single mutant strain. This demonstrates that UV-endonuclease indeed contributes to cell survival after UV treatment. On the other hand, T4 "endo V", a product of the *denV* gene, has an important role in DNA repair, since *denV* mutants exhibited high sensitivity to UV light (Lindahl 1982). The partially purified endonuclease V of T4 has been used years ago to demonstrate defects in DNA repair for UV-sensitive human cells obtained from patients with Xeroderma pigmentosum (XP; Tanaka et al. 1975). In addition, the cloned *denV* gene has been also employed to identify lack of dimer incision and to substitute for it in UV-sensitive cells by transferring into other organisms, e.g., *rad1* and *rad2* of yeast (Valerie et al. 1986), human XP cells (Valerie et al. 1987), or *mei-9* and *mus-201* of *Drosophila* (Banga et al. 1989). These findings, however, do not indicate that all these mutants have defects in genes coding for UV-endo-like enzymes. Rather, such findings reflect that the *denV* gene product can provide a function, i.e., incision at UV damage, which is lacking in the mutants.

b) Nucleotide excision repair

Nucleotide excision results in the replacement of damaged nucleotides by short tracts of newly synthesized DNA. In *E. coli*, the step of the damage-specific incision in nucleotide excision repair (NER) is carried out by 3 genes, *uvrA, B*, and *C*, which code for the UvrABC nuclease (reviewed by Van Houten 1990). In contrast to the *denV* protein, this enzyme complex has very broad specificity, presumably recognizing distortions of various types in the DNA double helix. Therefore, mutants defective in any one of the three genes are sensitive not only to UV, but also to various other agents, e.g., the UV-mimetic chemical 4-NQO and cross-linking compounds. However, such mutants are not usually hypersensitive to ionizing radiation.

The UvrA protein is a DNA-independent ATPase and DNA binding protein (Seeberg and Steinum 1982). In its amino acid sequence, two sets of zinc finger DNA-binding domains were recognized (Doolittle et al. 1986). The *uvrA* gene product is active as a dimer in the presence of ATP (Oh et al. 1989). The UvrB polypeptide has a ATP binding site, but ATPase activity is only found after cleavage near the carboxy terminus of UvrB (i.e., UvrB*; Caron and Grossman 1988). Such cleavage is presumably mediated by the Ada protease which proteolyzes Ada protein, because conserved amino acid sequences between UvrB and Ada proteins were detected. The UvrC protein has a DNA-binding property (Sancar et al. 1981). The UvrC shares amino acid homology with the UvrB protein in two domains (Backendorf et al. 1986) and also with the ERCC1 protein (Van Duin et al. 1988).

The reaction of the UvrABC nuclease proceeds in two major steps. The first step is a UvrA- and UvrB-dependent recognition of the damaged site (Seeberg and Steinum 1982). The UvrA dimer associates with UvrB polypeptide to form the $UvrA_2B$ complex in the ATP dependent manner as evidenced by DNAse I footprint analysis (Van Houtten et al. 1987). When this complex encounters a DNA damage site, stable UvrB-DNA pre-

incision complex is formed by losing the $UvrA_2$ with the hydrolysis of ATP. The second step is the strand cleavage reaction . UvrC polypeptides bind to the pre-incision complex at the site of the lesion. The UvrC then promotes an incision in the DNA on both sides of the damaged site. As a result, these reactions release a DNA fragment, 12-13 nucleotides long from the substrate molecule (i.e., scission occurs at the eighth phosphodiester bond 5' and the fourth or the fifth phosphodiester bond 3' to the lesion; Sancar and Rupp 1983).

The *uvrA*, *uvrB*, and possibly *uvrC* are under the control of a major regulatory process, dependent on a LexA repressor and the *recA* gene (for *uvrA*, Kenyon and Walker 1981; for *uvrB*, van den Berg et al. 1981; for *uvrC*, van Sluis et al. 1983). In *uvrB*, tandem promoters of P1 and P2 are responsible for its regulation (van den Berg et al. 1981; Sancar et al. 1982). Binding of a LexA repressor appeared to inhibit transcription from P2 of the cloned *UvrB* gene, but not from P1 *in vitro*.

c) Mismatch repair

Correction of mismatched pairs of normal bases is carried out by several special repair systems in *E. coli*. Some of these have recently been identified and described in detail (reviewed by Modrich 1991). These systems correct mismatches of spontaneous origin, resulting either i) from errors in DNA replication or, ii) from heteroduplex formation in recombination.

i) DNA replication errors are corrected by at least 3 systems which recognize different mismatches. These are methyl-directed long-patch mismatch correction, VSP (very short-patch) mismatch repair, and MutY-dependent short-patch G:A to C:G correction. The first of these is methylation directed; namely, the "old" methylated strand which is identified by adenine methylations at d(GATC) sequences is used as a template,

and the mismatched base in the "new", i.e., newly replicated and not yet methylated, strand is replaced (Lu et al. 1983; Pukkila et al. 1983).

In the methyl-directed ***long-patch mismatch repair***, three gene products (*mutH, mutL, and mutS*) are required for the mismatch recognition and strand discrimination. The *mutS* gene codes for a specific enzyme which recognizes all eight possible mismatched base pairs. The function of the MutL protein which binds to MutS-heteroduplex complexes is not well understood, but no simple enzymatic activity nor affinity for heteroduplex was demonstrated (Grilley et al. 1989). The *mutH* gene product has been shown to be involved at the strand discrimination step and contains a d(GATC) endonuclease activity (Welsh et al. 1987). MutHLS-dependent incision appears to occur between either 3' or 5' to the heteroduplex site and the nearest d(GATC) site (Su et al. 1989). Then, excision of the DNA strand containing a mismatched base occurs in either direction of 3' to 5' or 5' to 3' dependent on the orientation of the unmethylated d(GATC) sequence relative to the mismatched base. Exonuclease I presumably participates in 3' to 5' excision (Lahue et al. 1989), while a candidate enzyme for 5' to 3' excision is either exonuclease VII or RecJ exonuclease (Lovett and Kolodner 1989).

A second repair system, ***VSP mismatch repair***, generates a very short excision-repair tract (≤ ten nucleotides; Lieb 1983). In the VSP repair pathway, only G-T mismatches are corrected exclusively to normal G:C base pairs (Lieb 1985). MutL, MutS, DNA polymerase I and Vsr protein appear to be required for this process (Dzidic and Radman 1989). The *vsr* gene codes for a mismatch-specific DNA endonuclease (Hennecke et al. 1991).

A third repair type, ***MutY-dependent mismatch repair***, is not methylation directed. It depends on *mutY*, and is highly specific for G-A mispairs. The product of this gene is an adenine glycosylase with homology to endonuclease III (Au et al. 1989), but the mechanism of recognition and replacement is still unknown.

ii) **During recombination**, random mismatch correction may occur, as has been shown many years ago in bacteria, by transformation with λ phage DNA containing artificial heteroduplexes. Of these mismatch correction processes one depends on two recombination genes (*recF* and *recJ*), but not on *mutHLS* (Smith 1989). Evidence also comes from analysis of meiotic products in ascomycetes. If single strand exchanges between genetic variants produce heteroduplex DNA, correction will eliminate subsequent segregation of alleles and disguise events of recombination. Defects in mismatch correction therefore can increase recombination frequencies and lead to "hyperrec" phenotypes (shown in yeast; Bort s et al. 1990).

1.1.3. Postreplication repair in prokaryotes

Two major modes of post replicational repair, **recombinational** and **mutagenic repair**, occur in cells which are replicating DNA sequences containing unrepaired DNA damage. DNA damage blocks DNA replication. Thus, either translesion synthesis, or a restart of replication with gap formation should be necessary (reviewed by Echols and Goodman 1991). In *E. coli*, both types of repair depend on RecA.

a) Recombinational repair

In recombinational repair, the RecA protein is essential for a strand transfer reaction which involves homologous sequences from undamaged sister duplexes. The transferred single-stranded normal DNA is used as a template for repair-synthesis to fill in gaps, or it may replace damaged ssDNA strands. Such repair restores normal, but recombinant, dsDNA and is usually error-free (i.e., nonmutagenic). Several different recombination pathways have been identified in *E. coli* which work in different situations and with different substrates (reviewed by Cox and Lehman 1987; Clark 1991).

b) Mutagenic repair

Mutagenic processes have been considered as a repair system since cell survival after DNA damage can be enhanced when mutations are generated and lethal damages are eliminated in the process. Translesion DNA synthesis, i.e., replication past DNA damage in the template strand, requires at least four gene products of *E. coli*, RecA, UmuD, UmuC, and polC. The major type of mutagenic repair in *E. coli* is described in detail in section 1.2.2.

1.1.4. Excision repair in eukaryotes

a) Base excision repair

The yeast gene coding for 3-methyladenine DNA glycosylase, which recently has been cloned and sequenced, appears to have homology with *alkA* of *E. coli* and is induced in response to alkylating DNA damage (Chen et al. 1990). 3-methyladenine DNA glycosylases, uracil-DNA glycosylases, AP endonucleases which have functions corresponding to those of *E. coli* have also been identified from various eukaryotic cells (Doetsch and Cunningham 1990).

b) *Mismatch repair*

In eukaryotes, somatic and meiotic mismatch correction have been demonstrated (reviewed by Grilley et al. 1990). In yeast, diploids homozygous for mutations in the *PMS1*, *PMS2*, or *PMS3* gene displayed elevated post-meiotic segregation frequencies (Williamson et al. 1985). In these mutant strains, mismatch correction is dramatically reduced when heteroduplex plasmid substrates are provided (Bishop et al. 1987, 1989).

Homology of *PSM1* with *mutL* of *E. coli* supports the involvement of *PMS1-3* in mismatch repair in yeast (Kramer et al. 1989). Mismatch correction activities have been found in higher eukaryotes using transfection assays or cell-free systems. Moreover, strand targeting was found to occur by methylation of CpG sites (Hare and Taylor 1988). Specific repair of G-T mismatches has also been described in HeLa cell nuclear extracts (Wiebauer and Jiricny 1989).

c) Excision repair of radiation damage in yeast; the RAD3 epistatic group

In yeast, three major types of processes for radiation repair are thought to occur, corresponding to the three epistatic groups of *RAD* genes (called, RAD3, RAD52, and RAD6, after the most prominent member gene of each group; reviewed by Haynes and Kunz 1981; Friedberg 1988; Friedberg et al. 1991). In two of these groups, phenotypes are fairly uniform, and much direct and indirect evidences support the proposal that genes of the larger one, the RAD3 group, functions in nucleotide excision repair. Similarly, genes of the second, RAD52, group are most likely involved in recombinational repair and recombination. In contrast, the third, RAD6, group is rather heterogeneous, but includes especially genes which are required for or are needed modify radiation-induced mutation.

The RAD3 group of genes mediates nucleotide excision repair of bulky lesions (as reviewed in detail, Friedberg 1988). Most mutants in this group are highly sensitive to UV light, but not to ionizing radiation. They were shown to be defective in the incision of cyclobutane dimer-containing DNA by monitoring DNA sedimentation patterns in alkaline sucrose gradients before and after UV irradiation using double mutants of *rad* with *cdc9* (DNA ligase mutation). Mutant strains of the *rad1-rad4*, and *rad10* do not carry out any

damage-specific incision of UV-irradiated DNA, while *rad7*, *rad14*, *rad16*, *rad23*, and *mms19* mutants are partially defective (Wilcox and Prakash 1981). However, a recent study has shown that *rad14*Δ mutants lack incision ability (Bankmann et al. 1992). In addition, a new gene, *RAD25*, was assigned into the RAD3 epistatic group (see below).

The *RAD1* and the *RAD10* genes also function in the mitotic intrachromosomal recombination pathway, and this process of recombination differs from that controlled by genes of the RAD52 epistatic group (Schiestl and Prakash 1990). Recently, specific interaction between the *RAD1* and *RAD10* gene products, Rad1p and Rad10p, has been demonstrated *in vitro* (Bardwell et al. 1992; Bailly et al. 1992).

The *RAD3* gene was cloned (Naumovski and Friedberg 1982) and shown to be an essential gene, i.e., *rad3Δ* mutants are lethal (Higgins et al. 1983). Certain mutant alleles of *RAD3*, *rem* mutants, were isolated as mutations which conferred mutator and hyper-rec phenotypes and these are distinct from the phenotype of other *rad3* alleles (Malone and Hoekstra 1984). In addition, these *rem1* mutants exhibited very slight UV sensitivity, which differs considerably from the *rad3-2* mutant. Interestingly, double mutants of *rem1* with *rad52* are inviable, while *rad3;rad52* double mutants are viable (Montelone et al. 1988). Such a phenotypic difference between *rad3* and *rem1* suggests that *RAD3* may encode a multimeric protein. The *RAD3* gene product, Rad3p, could be purified and was shown to possess both DNA-dependent ATPase and DNA helicase activities (Sung et al. 1987). However, a single amino acid change by *in vitro* mutagenesis abolished both activities of Rad3p (Sung et al. 1988).

A new gene, *RAD25*, was isolated recently using DNA from the cloned human *ERCC3/XPBC* repair gene as a hybridization probe (Park et al. 1992; see section 1.1.7 for *ERCC3* gene). An amber nonsense mutation in *RAD25*, $rad25_{799am}$, confers hypersensitivity to UV light. Double mutants of $rad25_{799am}$ with *rad1*Δ revealed epistatic interaction, while synergisms were obvious in double mutants of $rad25_{799am}$ with *rad6*Δ and *rad52*Δ.

1.1.5. Postreplicational repair in yeast

a) Recombinational repair; the RAD52 epistatic group

Mutants of the RAD52 group (*rad50-rad57*) are sensitive primarily to ionizing radiation (Game and Mortimer 1974) and most have defects in double strand break (DSB) repair (Ho 1975; Resnick and Martin 1976) as well as meiotic and mitotic recombination (reviewed by Haynes and Kunz 1981; Petes et al. 1991). They show quite a variety of effects. Some are acting mainly in meiosis; e.g., *RAD50* is required early for meiotic pairing and possibly formation of synaptonemal complex (Alani et al. 1990). Others are defective also in mitotic recombination in vegetative diploid cells. However, in most cases the function of the primary gene product is unknown. An exception is *RAD51* which has recently been shown to have not only an amino acid sequence homology to RecA of *E. coli* but also to resemble RecA protein in its DNA binding properties (Shinohara et al. 1992). The functional *RAD52* gene appeared to regulate the endoexonuclease which is immunoprecipitable with antibody raised against endoexonuclease of *Neurospora crassa* (Chow and Fraser 1983; Chow and Resnick 1988).

b) RAD6 epistatic group

The RAD6 group contains genes which are functionally heterogeneous. However, this group includes most of the mutants defective in damage-induced mutagenesis. Such mutant strains generally show sensitivity to both ionizing and UV radiation. Mitotic recombination and meiosis are normal in members of the RAD6 group, except for the *rad6* mutations which cause defects in sporulation, i.e., meiosis. None of

the mutants of this group are defective in damage-specific incision of UV-irradiated DNA (Prakash 1977; reviewed by Haynes and Kunz 1981).

Based on such phenotypic differences, the RAD6 group genes can be classified into four subtypes: i) *RAD6*, ii) *RAD18*, iii) *RAD9*, and iv) *REV* loci (McKee and Lawrence 1979). Two of these, *RAD6* and *REV* loci are mainly involved in mutagenic repair (see section 1.2.3, for *RAD6* and *REV* genes).

i) *RAD18*: It is now generally believed that *RAD18* is not involved in DNA damage-induced mutagenesis. However, it certainly is involved in DNA repair because the *rad18* mutants show very high sensitivity to UV, γ-ray, and certain chemicals (reviewed by Haynes and Kunz, 1981; Lawrence 1982). Since postreplication repair appeared to be defective in *rad18* (Prakash 1981) and epistatic interactions between *rad18*Δ and *rad6*Δ deletion mutants were clearly shown after UV-irradiation, the primary function of *RAD18* might be in a *RAD6*-dependent postreplicational error-free type of DNA repair (Johnson et al. 1992). However, with regard to X-ray sensitivity, epistatic interactions to *rad6* were not observed in *rad18* strains (Game and Mortimer 1974). The sequence of the cloned *RAD18* gene showed that the Rad18p has cysteine-rich zinc-fingers suggesting the presence of DNA binding domains and putative nucleotide-binding domains (Chanet et al. 1988; Jones et al. 1988).

ii) *RAD5* (=*REV2*): *RAD5* (Game and Cox 1971) appeared to be an allele of *REV2* (Lemontt 1971) which was isolated as a mutation with reduced reversion of an ocher allele after UV irradiation. However, *rev2* had no effect on the reversion of amber, missense, or frameshift mutations (Lawrence and Christensen 1978b). In addition, forward mutagenesis in *rad5*Δ strains was not significantly reduced, while reversion of ocher alleles was affected as expected (Johnson et al. 1992). Studies of double mutants of *rad5*Δ with *rad18*Δ or with *rad6*Δ confirmed that *RAD5* is epistatic to RAD6 group genes, even though *rad5*Δ mutants showed synergistic interactions with certain members of this group, especially *rev1*Δ and *rev3*Δ. It was suggested that *RAD5* is involved in an

error-free postreplication repair pathway as has been proposed for *RAD18*. The cloned *RAD5* gene revealed DNA-binding motifs (Zinc-fingers) similar to those found for *RAD18* (Ahne et al. 1992). In addition, Rad5p contained all the domains typically associated with DNA helicase (Johnson et al. 1992).

iii) *RAD9*: The *rad9* deletion mutants exhibited wild type level recombination and mutation for both spontaneous and after UV treatment (Schiestl et al. 1989). Since the *rad9* mutants were found to be defective in "G2 arrest" after X-ray treatment (Weinert and Hartwell 1988), mutagen sensitivity of *rad9* may be due to the failure of cells to enter an arrest phase in the G2 cell stage which is required for DNA repair before entering mitosis.

1.1.6. DNA repair genes in filamentous fungi

a) Epistatic groups in Neurospora crassa

Many UV- or MMS-sensitive mutants (e.g., *uvs* and *mus*) have been characterized in *Neurospora crassa* (reviewed by Schroeder 1988; Kafer and Luk 1989; listed by Perkins 1992). Three epistatic groups, i.e., UVS-2, UVS-6, and UVS-3, have originally been postulated based on MMS sensitivity of double mutant strains (Kafer and Perlmutter 1980; Kafer 1983). In addition, *uvs-2;uvs-6* double mutants showed synergistic interaction after UV and X-ray treatment, while double mutants of *uvs-3* with *uvs6* are inviable (Inoue et al. 1981). Lethal interaction was also found for the*uvs-6;mus-9* pair, and often double mutants between members of different epistatic group showed poor viability (e.g., double mutants of *uvs-3* with *uvs-2* and *mus-8*, and *uvs-6;mus-7* double mutant; Kafer 1983) So far, eleven mutagen-sensitive mutants which are mapped have been assigned into three groups: i) UVS-2 group: *uvs-2*, *upr-1*, *mus-8*, *mus-26*, and

uvs-4, ii) UVS-6 group: *uvs-6*, *mus-7*, and *mus-10*, iii) UVS-3 group: *uvs-3*, *mus-9* and *mus-11* (see Table 1 of Baker et al. 1990).

The **UVS-2 group** was believed to contain mutants which are defective in excision repair, since members in this group shared some phenotypes to excision defective mutants of *E. coli* and yeast (See chapter 2, "Discussion" for phenotypes of mutants included in this group). Moreover, the mutants, *uvs-2* and *upr-1*, appeared not to carry out excision repair when tested for loss of tritium labelled cytosine from cyclobutane-dimers separated by chromatography (Worthy and Epler 1973). However, when both the pyrimidine dimer specific endonuclease assay for monitoring single strand breaks in alkaline sucrose gradient, and radioimmunoassay using specific antibody for thymine dimer or the (6-4) photoproduct were performed, defects of excision repair in members of this group were not confirmed (Macleod and Stadler 1986; Baker et al. 1990, 1991). In addition, when two other UV- but not X-ray-sensitive mutants in this group, *upr-1* and *mus-26*, were reexamined using more extensively back-crossed strains, results showed more than additive effects in tests for epistasis with *uvs-2*, contradicting earlier results (Ishii and Inoue 1989), while synergism with members of the other two epistatic groups was confirmed. The *uvs-2* mutants had shown increases of UV-induced *ad-3* mutations in a nonselective forward mutation assay (see section 1.2.5.c). However, after treatment with a base analogue, considerable decrease of point mutations were observed in *uvs-2* strains, while the frequencies of multi-locus deletions were increased compared to wild type (de Serres and Brockman 1991). These later results are also not expected for excision repair defective type and argue for quite a different major function of the *uvs-2* gene .

A second, **UVS-6 group**, contains mutants sensitive to X-ray, MMS and some also to UV. Members of this group showed wild type levels of mutation frequencies both spontaneously and after treatments with UV and 4-NQO (de Serres 1980; Inoue et al. 1981). This group may correspond to the RAD52 group in yeast involving

recombinational repair. Such mutants are all defective for meiosis and sterile in homozygous crosses, but no good evidence for defects in mitotic recombination is available, since no stable vegetative diploids can be isolated in *Neurospora crassa.*

The **UVS-3 group** possibly represents a group of genes involved in DNA damage induced mutagenic repair pathway. Members of this group are sensitive to both UV and X-rays. Mutants in most genes, as found for *uvs-3* (de Serres et al. 1980), *mus-9*, and *mus-11* (Kafer 1981) confer mutator phenotype. Moreover, in *uvs-3* mutants, UV- or γ-ray-induced forward mutations (i.e., *ad-3* mutation) were either completely abolished (de Serres 1980) or considerably reduced (Schüpbach and de Serres 1981). The *mus-9* and the *mus-11* mutant strains also showed reduction of UV-induced recessive lethals (Kafer 1981; see section 1.2.5.c, for assay of recessive lethals). In contrast, all *uvs-3* heterokaryons tested produced increases for UV-induced recessive lethals which were similar to those of wild type (Kafer 1984).

Induced repair of DNA damage was demonstrated by Stadler and Moyer (1981) using the "heterokaryon rescue experiment" in *Neurospora crassa.* In addition, inducible as well as constitutive excision repair processes were identified tentatively (Baker 1983). Several polypeptide changes were found after UV irradiation by two dimensional polyacrylamide gel electrophoresis in which different *uvs-3* alleles showed identical differences to wild type (Howard and Baker 1986).

b) Epistatic groups in Aspergillus nidulans

Several mutagen-sensitive mutants also have been isolated in *Aspergillus nidulans.* Among them, mutants for ten *uvs* and eight *mus* genes are available from the Fungal Genetics Stock Center. Origin of these mutants strains were described in Kafer and Mayor (1986). The properties of *uvs* mutants are summarized in Table 1.1. Based on UV sensitivity of double mutants, three epistatic groups had been identified initially:

Table 1.1. Properties of uvs mutants: Mutagen sensitivities, effects on frequencies of mutation and recombination.

-, - -, - - -, slightly to highly reduced compared to uvs+; NT, not tested; 0, like wild type; +, ++, +++, slightly to highly increased

Tested properties	*uvsF*	*uvsH*		*uvsJ*	*uvsB* *uvsD*	*uvsC* [a] *uvsE*	*uvsA*	*uvsI*
		77	*304*					
Radiation survival								
UV, quiescent (Q)	- - -	- - -	- -	- -	- -	0	0	- - -
pregerminated (P)	-	- - -	NT	- -	- -	-	-[i]	-[j]
Ionizing radiation[b] (Q)	0[c]	- - -	- -	-	(Q) 0[d] (P) 0[d]	(Q) 0 (P) - -[e] (*uvsE* only)	NT	NT
Survival after treatment with chemical mutagens								
4-NQO	- -	- - -	NT	- - -	- -	- -	±[m]	- -[k, m]
MMS (growth on CM+MMS)	- -[b]	- - -	- -	-	- - -	- -	±[m]	0[m]
EMS (Q)	- - (at 37°)[d] ± (at 20°)[d]	- - (37°)[d] - (20°)[d]	NT	- - (30°)[m]	0 (37°)[d] 0 (30°)[m]	- - (30°)[m]	NT	0 (30°)[m]
MNNG (Q)	-[b]	NT	NT	-[b]	NT	NT	NT	0[k]
NA (nitrous acid)	-	NT	-[e]	- -[f]	-[e]	NT	NT	NT
Effects on mutation (selenate resistant forward mutation)								
Spontaneous	+	0	0	+[m]	+	+ + +	0[m]	0[m]
UV-induced	+ +	+ +	+	+ +[m]	+	-	0[m]	0[m]
Effects on meiosis (homozygous crosses)								
Fertility	Normal	Almost sterile	Reduced	Sterile[f]	Almost sterile	Sterile[g]	Normal	Normal[l]
Recombination	Normal[h]	Normal	Normal	Not testable	Abnormal	Not testable	NT	Normal[l]
Spontaneous mitotic recombination								
Allelic	Increased[a]	NT	NT	NT	Increased[a, g]	Reduced[g]	NT	Normal[m]
Intergenic[b]	+ +	+ +	+	+	+	-	NT	Normal[m]

[a] Test of Fortuin (1971b, 1971c), and Jansen (1970a, 1970b, 1972); [b] Kafer and Mayor (1986); [c] Johnston (1969)

[d] Babudri and Polli (1989); [e] Wright and Pateman (1978); [f] Lanker et al. (1968); Chang et al. (1968)

[g] Parag (1977); [h] Sheaffield (1969); [i] Jansen (1967); [j] Han and Kang (1985)

[k] Han et al (1983); [l] Chae (1986); [m] this thesis, Chae (1993); [n] unpublished observation

[After Kafer and Mayor (1986)]

i) UvsF group; *uvsF* and *uvsH*, ii) UvsC group; *uvsC* and *uvsE*, and iii) UvsB group; *uvsB* and *uvsD* (Kafer and Mayor 1986). Phenotypes and possible DNA repair processes are summarized for each group in chapter 2.

The *uvsI* mutantswere isolated after MNNG treatment in a strain from the stock center (A168; Han et al. 1983). Normal photoreactivation and increased UV-survival after liquid holding were demonstrated for this *uvsI* mutant by Han et al. (1983). The *uvsI* mutation was found to confer defect in mutation to acriflavine resistance when treated with UV or 4-NQO, but not after MNNG and EMS treatments. In addition, UV-reversion of *choA1* appeared to be completely absent in this mutant strain (Han and Kang 1985).

Several alkylation-specific DNA repair defective mutants (*sag*) which are hyper sensitive to the alkylating agent, MNNG, but not to UV, have been isolated by Swirski et al. (1988). Of these *sag* mutants, none were defective in 3-methyladenine DNA glycosylase and DNA methyltransferase activities (see chapter 3 "Discussion", for additional types of mutations in *Aspergillus*).

1.1.7. DNA repair genes in Human and mammalian cells

Human cells derived from patients of Xeroderma pigmentosum (XP) are totally or partially defective in UV excision repair (Cleaver 1968; reviewed by Cleaver 1990). Analogous to the multiple genes involved in nucleotide excision repair of yeast, multiple complementation groups, XP-A to H and XP "variant" have been identified by heterokaryon tests between XP cells. Only a few of these genes have been cloned so far, but other human genes for DNA repair have been cloned by transferring human genomic DNA into various UV- or X-ray sensitive mutants of Chinese Hamster Ovary (CHO) cell. These are designated *E*xcision *R*epair *C*ross *C*omplementing (*ERCC*) or *X*-ray *R*epair

*C*ross *C*omplementing (*XRCC*) human genes, e.g., *ERCC1-ERCC3*, *ERCC5*, and *ERCC6*, and *XRCC1* (reviewed by Thompson 1989, 1991). These genes showed specific and efficient correction of CHO mutants. Two of the *ERCC* genes, *ERCC2* and *ERCC3* appeared to complement XP-D and XP-B cells, respectively (for *ERCC2*, Flejter et al. 1992; for *ERCC3*, Weeda et al. 1990). Moreover, amino acid sequence homology between the *ERCC2* and the *RAD3* of yeast was recognized. The *ERCC1* gene also shares amino acid sequence homology with *RAD10* of yeast as well as *UvrA* and *UvrC* of *E. coli* (van Duin et al. 1988). Reversely, a gene from yeast, *yERCC3* (or *RAD25*), was cloned using *ERCC3/XPBC* cDNA as a hybridization probe (Park et al. 1992). Human *XPAC* and *XPCC* genes which complement XP-A and XP-C cells, respectively, have been cloned (for *XPAC*, Tanaka et al. 1989; for *XPCC*, Legerski and Peterson 1992). Homology of amino acid sequence also was detected between *XPAC* and *RAD14* of yeast (Bankmann et al. 1992) and between *XPCC* and *RAD4* of yeast (Legerski and Peterson 1992).

There are a number of other human diseases which alter cellular responses to DNA damage, even though the evidence for a primary defect in the repair of DNA lesions is less well documented. Cells from individuals with Ataxia telangiectasia (AT), Bloom's Syndrome (BS), Cockayne Syndrome (CS), and Fanconi's Anemia (FA) showed increased sensitivity to certain DNA damaging agents (Reviewed by Heim et al. 1992; for Bloom's Syndrome, Nicotera 1991).

1.2. MUTATION, MUTAGENIC DNA REPAIR, AND MUTATIONAL SYSTEMS

1.2.1. Spontaneous mutation

DNA damage arises endogenously in a variety of ways when the physiological condition changes. Moreover, spontaneous DNA damage occurs during normal DNA metabolism as a result of errors produced during DNA replication and repair itself. Considering all sources of spontaneous DNA damage, the extremely high fidelity of DNA replication and stability of DNA is very astounding. Recent research has identified various repair systems which are involved in restoring the intact genomic contents with high levels of accuracy (reviewed by Radman and Wagner 1988; Echols and Goodman 1991).

The fidelity of DNA replication apparently is achieved by the cumulative effects of a series of error avoidance mechanisms; i) the combined effects of a complementary base selection by DNA polymerase, with the immediate verification by exonuclease 3' to 5' proof-reading function, ii) the role of other accessory proteins (ensuring less than 10^{-7} /nucleotide error frequency in *E. coli*), and iii) post-replicative mismatch correction (reducing error frequency to 10^{-10} /nucleotide: reviewed by Loeb and Kunkel 1982; Radman and Wagner 1986). Therefore, disturbance of any one of these steps leads to increases of spontaneous mutations. To elucidate the mechanisms of spontaneous mutations, *mutator* strains, i.e., genetic variants and mutant strains which exhibit increases of spontaneous mutations, have been used. In addition, more direct conclusions resulted from studies of *antimutator* strains which show decreases of spontaneous mutations. Antimutators have lower error frequencies than those of wild type evolved in nature.

a) Mutators

DNA polymerase mutants: Mutator effects can be the results of mutations in components of DNA polymerases. For example, Speyer (1965) observed a high increase of spontaneous mutation frequencies in bacteriophage T4 which was caused by either of two temperature sensitive mutants. These mutations had been isolated as temperature sensitive lethals, and were mapped to gene 43 which codes for the T4 DNA polymerase. Later, it was shown that strong mutator phenotypes were attributed to increases of mainly G:C to A:T base transitions, but to a smaller extent also to transversions from A:T site, when reversions of certain *rII* ocher mutants were performed. This reversion system can distinguish transitions (i.e., base pair changes of either a purine to a purine base or a pyrimidine to a pyrimidine base) from transversions (i.e., a purine to a pyrimidine and vice versa) phenotypically (Ripley and Drake 1972). DNA polymerase mutants which cause mutator phenotypes also have been characterized in *E. coli*. In contrast to the T4 phage enzyme, DNA polymerase III holoenzyme of *E. coli* comprises several subunits. The α subunit which is specified by the gene *dnaE* is responsible for the enzymatic polymerase activity including selection of matching bases. However, the associated exonucleolytic 3' to 5' proofreading activity resides in a separate protein, the ε subunit encoded by the *dnaQ* (=*mutD*) gene. Several alleles of *dnaE* have been reported to increase spontaneous mutations (Hall and Brammar 1973; Sevastopoulos and Glaser 1977). In addition, extraordinarily strong mutator phenotype (up to 10^5-fold higher level of mutation than that of *mutD*$^+$) was associated with *mutD* mutations (Fowler et al. 1979; Scheuermann et al. 1983). Thus, the proofreading function of the *mutD* gene product is very important for the maintenance of genomic fidelity during DNA replication.

In the case of eukaryotic DNA polymerases, Liu et al. (1983) reported a mutator mutant resistant to aphidicolin which is a specific inhibitor of DNA polymerase α in

Chinese hamster V79 cells. A DNA polymerase α variant from this mutant was shown to have a decrease in the *Km* for dCTP *in vitro*.

Mismatch repair defective mutants: Mutants defective in the genes involved in mismatch repair systems exhibit mutator phenotypes, as perhaps expected. Reduced genomic fidelity is presumably the result of reduction in the correction of mismatched bases by mismatch repair enzymes. In fact, many well-known mutator mutants (*mutS*, *mutH*, *mutL*, and *mutU*) were recently shown to be required for mismatch repair (see section 1.1.2.C). Mutations in the *PMS1-PMS3* gene which was shown to be required for mismatch repair in yeast also resulted in an increase of spontaneous mutation (Kramer et al. 1989; Williamson et al. 1985).

DNA repair defective mutants: More broadly DNA-repair defective mutants in *E. coli* and yeast also frequently exhibit mutator phenotypes. For example, mutations in *uvrA* and *uvrB* which are involved in excision repair cause increased spontaneous mutation (Sargentini and Smith 1981). Similarly, in *Saccharomyces cerevisiae*, many radiation-sensitive mutants also exhibited mutator phenotypes (see "Table 1" in Haynes and Kunz 1981). However, the mutator effects of these *rad* mutants often were dependent on the types of target mutations used (reviewed by Lawrence 1982). Recently, molecular studies of spontaneous mutation in several mutator strains have been carried out using the ochre suppressor tyrosine tRNA (*sup4-o*) and mutator strains made isogenic to wild type by gene disruption (see section 1.2.5.b, for the *sup4-o* mutational system). In these studies, sequencing of the mutated tRNA revealed that single base pair substitutions were enhanced in *rad1*, *rad6*, *rad18*, and *rad52* mutator strains (Kunz et al. 1989, 1990, 1991; Kang et al. 1992). Especially, the *rad6* mutator phenotype was associated with considerable increases in the frequencies of base pair transitions and G:C to T:A transversion, but not other types of transversions. On the other hand, while the *rad18*Δ mutants exhibited increases of the same type transversions as *rad6*, this effect was very specific and not accompanied with increases in transitions (Kunz et al. 1991).

Mutator strains also have been found among mutagen-sensitive mutant strains of *Neurospora* and *Aspergillus*. In *Neurospora crassa*, two alleles of *uvs-3* and two MMS-sensitive mutants (*mus-9*, and *mus-11*) exhibited mutator phenotypes when tested for forward mutation or for recessive lethals (de Serres et al. 1980; Kafer 1981, 1984). In *Aspergillus nidulans*, UV-sensitive mutants of *uvsC* and *uvsE* were reported as mutators in several forward mutational test systems (Jansen 1972; Kafer and Mayor 1986; for effect of these mutant strains on UV-induced mutation, see Table 1.1; for mutational system in *Aspergillus*, see section 1.2.5.c).

b) Antimutators

As known for mutator phenotypes, antimutator effects have been observed for a few mutations in DNA polymerase genes. In the case of certain alleles of T4 DNA polymerase (*ts*CB87 and *ts*CB120), antimutator effects were practically limited to the A:T to G:C transition mutation pathway, i.e., reduced reversion frequencies were found only for certain target alleles for which reversions could be increased by base analogues but not by treatment with the cytosine-specific mutagen, hydroxylamine, in a wild type background (Drake and Allen 1968). Interestingly, these alleles of T4 DNA polymerase also showed mutator effects for transversions (Ripley 1975). Biochemical studies of the T4 DNA polymerase enzyme in these antimutator strains suggested that the accuracy of 3' to 5' exonuclease proofreading function of DNA polymerase was enhanced, i.e., the ratio of exonuclease to polymerase activity was shown to be higher and an enhanced rate of nucleotide turnover could be demonstrated (Muzyczka et al. 1972).

One of DNA polymerase mutants of herpes simplex virus type 1 (HSV-1) also produced lower numbers of spontaneous mutants (Hall et al. 1985). In an *in vitro* assay, purified HSV-1 DNA polymerase from the antimutator strain generated fewer replication errors than the wild type enzyme. In addition, elevated *Km* values for normal nucleoside

triphosphates were obtained. In contrast to the suggested mechanism for the T4 antimutators, namely, increased proofreading by an associated exonuclease, these authors proposed that the antimutator phenotype in the HSV-1 mutant resulted from reduced affinity of the polymerase for nucleoside triphosphates, which facilitated release of mismatched bases in the mutant and therefore was faster than in wild type strains.

Recently, Schaaper and Cornacchio (1992) identified an antimutator allele in the *dnaE* gene of *E. coli* DNA polymerase III (*dnaE910*) for which a third mechanism was discovered. This mutation interacted with mutations in the proofreading (*mutD*) or mismatch repair (*mutL* and *mutT*) enzymes. This interaction reduced, i.e., partially suppressed, the mutator effects of the *mutD*, *mutL*, and *mutT* mutations to some extent when they were combined with the *dnaE910* mutation into double mutant strains.

In *Saccharomyces cerevisiae*, the *rev3* mutation (=*ant2*, *pso1*) considerably decreased intragenic reversions of two ocher alleles (i.e., *lys1-1*, 5- to 12-fold and *arg4-17*, 4-fold). It also increased reversion of one specific missense allele (*his1-7*, 4-fold), but had little effect on the frequency of suppressor tRNA mutations (Quah et al. 1980; Lemontt 1971; Cassier et al. 1980). Recently, the *REV3* gene has been cloned and its amino acid sequence revealed substantial similarity to the other nuclear yeast DNA polymerases (Morrison et al. 1989; see also section 1.2.3.b).

The availability of different nucleoside triphosphates for DNA synthesis is known to affect spontaneous mutations indirectly. Such effects are found in mutant strains which show an aberrant ratio for the four dNTPs. For example, the *mud* (*mu*tation *d*efective) mutant which is an allele of the *purB* gene (*pur*ine auxotrophy) in *E. coli* showed an antimutator phenotype (Geiger and Speyer 1977; Lyons et al. 1985). In *mud*$^-$ cells, the relative pool size of DNA precursor dNTPs was altered, namely, an elevation of dCTP and a reduction of all other dNTPs. Effects of such dNTP imbalance in yeast on mutagenesis were extensively studied, as reviewed by Kunz and Kohalmi (1991).

In conclusion, genetical analysis of mutator and antimutator mutant strains demonstrates that replication errors contribute significantly to spontaneous mutagenesis. In addition, it is evident that the efficiency of DNA repair processes also governs the spontaneous mutation frequencies.

Even though the nature of spontaneous mutagenesis is not fully understood, data from direct sequencing of DNA from spontaneous mutants provides a step towards the understanding of the mechanisms (e.g., sequencing of *lacI* mutants in *E. coli* or *sup4-o* in yeast; see section 1.2.5 for *lacI and sup4-o* systems). Especially, the hypothesis that frameshift mutations are mediated by misalignments which was originally proposed by Streisinger et al. (1966) has been extended (reviewed by Ripley 1990). Other source of spontaneous mutations [e.g., directed or substrate-induced mutation in *E. coli* (Cairns et al. 1988; Drake 1991), and RIP in *Neurospora* (*R*epeat *I*nduced *P*oint mutations which occur in premeiotic cells to eliminate or silence unwanted duplications; Cambareri et al. 1989, Selker 1990)] have interesting aspects, but are not relevant and not discussed here.

1.2.2. Induced mutation and error-prone DNA repair in E. coli

Mutation is considered to be induced when DNA damage arises from exogenous action by various agents. Experimentally, levels of mutation resulting from mutagenic background effects (e.g., cosmic radiation, mutagenicity of food, etc.) are considered as "control" levels, i.e., "spontaneous" mutation. Induced DNA lesions can be classified into two basic categories: those which i) inhibit DNA replication (replication blocking) and in *E. coli*, consequently induce the SOS response (described below), and ii) DNA changes which allow further DNA replication of a special type with reduced fidelity, i.e., is "error-promoting" (reviewed by Echols and Goodman 1991). UV-induced pyrimidine

dimers which generate substantial DNA distortion are typical replication-blocking lesions. On the other hand, alkylating agent-induced modified bases, O^6-alkylguanine or O^4-alkylthymine, are error-promoting lesions which increase mispairing between the altered and normal bases during DNA replication. In addition, base analogues (e.g., thymine analogues, 5-bromouracil and 5-fluorouracil; adenine analogue, 2-aminopurine) are error-promoting mutagens, because during DNA replication such unusual bases incorporated in DNA often promote mispairing with wrong bases.

a) UV mutagenesis in E. coli

In *E. coli*, after exposure to DNA-damaging agents, a new regulatory cascade operates to increase cell survival, which is called "SOS response". This includes a serial induction of a number of genes which act on various cellular processes [e.g., excision repair (*UvrA*, *UvrB*, and *UvrC*), recombination (*RecA*, *RecN*, *RecQ*, and *Ruv*), mutagenesis (*UmuC* and *UmuD*), and inhibit cell division (*SulA* and *SulB*); Walker 1984]. In normal conditions, these genes are repressed by the *LexA* gene product. As a result of DNA damage, an activated RecA protease (i.e., RecA*) cleaves the LexA repressor and, in consequence, turns on SOS genes. Autodigestion of RecA to RecA* slows down when cells have recovered from DNA damage, then LexA repressors accumulate which results in normal levels of expression of SOS genes.

Mutagenesis by UV irradiation of *E. coli* is dependent on the induction of SOS functions (reviewed by Echols and Goodman 1990). The main SOS genes involved in UV mutagenesis are the *recA*, *umuD* and *umuC* genes (Kato and Shinoura 1977; Bagg and Kenyon 1981).

I) Multiple roles of RecA

The RecA protein has at least three essential roles in SOS mutagenesis in addition to its major function in recombination. 1) ***Proteolytic cleavage of LexA*** repressor molecules including the *umuDC* operon and the *recA* itself (as described above). 2) ***Proteolytic cleavage of the UmuD protein by RecA****: In *lexA*(Def) and *recA*$^+$ background, amplification of UmuDC was not sufficient for SOS mutagenesis (i.e., no or very weak mutator activity) until *recA441* or *recA730* mutation were introduced (Witkin and Kogoma 1984; Ennis et al. 1985). These mutations in the *RecA* gene resulted in production of truncated RecA protein, i.e., RecA*. Later, it was shown that RecA* protein cleaves UmuD protein to generate activated form of polypeptide UmuD, i.e., UmuD', which is essential for UV mutagenesis (Burckhardt et al. 1988). Further evidence for a RecA role on proteolytic cleavage of UmuD was obtained using the cloned UmuD' gene. In the *recA430* strain, which fails to cleave UmuD and thus lack SOS mutagenesis, engineered synthesis of UmuD' *in vivo* was sufficient to generate mutation (Nohmi et al. 1988). 3) ***Direct role on translesion DNA replication***: In the *recA*Δ strain or the *recA1730* mutant which can form UmuD', UV mutagenesis was not detectable (Bailone et al. 1991). This was the case even when these strains have *lexA*(Def) background and a plasmid containing a properly engineered *UmuDC* operon encoding activated UmuD' and UmuC (Dutriex et al. 1989). Further evidence for the third role of RecA was obtained by Sweasy et al. (1990). They monitored spontaneous mutability in several *recA* alleles and found that SOS mutator activities varied among different *recA* strains which lacked LexA repressor [i.e., *lexA*(Def)] and contained a plasmid encoding UmuD'C. Such differences of spontaneous mutation frequencies in different *recA* alleles indicated that RecA must play an additional role in SOS mutagenesis.

To explain the direct role(s) of RecA* on SOS mutagenesis, the following hypotheses have been proposed: 1) positioning of the incoming polymerase at the site of

lesion (Echols 1982), 2) inhibition of the proofreading activity of ε subunit (Fersht and Knill-Jones 1983; Lu et al. 1986), 3) stretching of the single stranded template DNA, which facilitated base insertion at the lesion site (Lu and Echols 1987), and 4) positioning the UmuD'C complex at the site of lesion (Bailone et al. 1991). However, no one of these hypothesis is clearly preferred.

ii) *UmuDC* operon

The *UmuD* and the *UmuC* genes which constitute an operon have been cloned and sequenced (Kitagawa et al. 1985; Perry et al. 1985). An SOS box i.e., LexA repressor binding site (CTGTatata-a-aCAG) exists in the 5' flanking region of the 5' located *UmuD* gene, as expected, since both genes are SOS inducible. As shown by Woodgate et al. (1989), purified UmuD' (the larger COOH-terminal fragment) and UmuC were associated tightly *in vivo* and *in vitro*. The UmuD'-UmuC complex, therefore, is likely to be responsible for a major aspect of SOS mutagenesis, i.e., facilitates translesion DNA synthesis opposite DNA lesions.

iii) DNA polymerases in SOS mutagenesis of E. coli

All three DNA polymerase, I, II, and III, can be active when UV mutagenesis occurs. DNA polymerase III is the main required enzyme, presumably needed for translesion replication (Lu et al. 1986; Hagensee et al. 1987). On the other hand, DNA polymerase II also may have a role, since Pol II is amplified more significantly than Pol III during SOS induction (Bonner et al. 1988). Involvement of Pol I in the SOS response was suggested by the finding of an altered form of Pol I (Pol I*) which is present only in SOS-induced cells, and also by the lowered fidelity of Pol I* demonstrated *in vitro* by

Lackey et al. (1982). However, apparently Pol I is not essential since in *polA*$^-$, including polA deletion mutants, UV-induced mutation appears to be normal (Bates et al. 1989).

IV) A two-step model for UV-mutagenesis

Bridges and Woodgate (1984) have proposed a model for UV mutagenesis postulating two consecutive steps, namely first "*misincorporation*", i.e., insertion of a wrong base opposite the photoproduct, and then "*bypass replication*", i.e., continuation of DNA replication beyond the misinserted base. Their model was based on the finding that in *umuDC* and *lexA*(Ind$^-$) strains which are normally nonmutable by UV irradiation, mutants could be obtained with the aid of photoreactivation after UV irradiation, i.e., delayed photoreversal (DPR). Such a phenomenon of DPR mutagenesis in *umuD,C* mutant strains implies that UmuDC proteins are required for replication-bypass. Initially, RecA protein was thought to be absolutely required, mainly for the first, the misincorporation, step (Bridges and Woodgate 1985). However, Bridges (1988) showed subsequently that DPR mutagenesis could also occur in *recA* deletion mutants. Thus, it was suggested that the third role of the RecA protein in UV mutagenesis (see above) is most likely in the second step, i.e., in bypass replication of DNA lesions (Bates and Bridges 1991).

Overall, translesion DNA synthesis which consequently produces mutation may be mediated by a protein complex, i.e., "mutasome", a complex which includes at least RecA, UmuD'-UmuC, and Pol III (Woodgate et al. 1989). However, further understanding of the mechanism, for each step as well as the specific role of each protein, requires more biochemical analysis.

V) Deamination model for UV-mutagenesis of Phage S13

Preirradiation of the bacterial host increases survival of UV-irradiated phage λ was demonstrated first by Weigle (1953). This "Weigle reaction" and UV mutagenesis of single-stranded DNA phage S13 were found to be dependent on RecA and UmuDC functions of host genes, but delayed photoreversal (DPR) mutagenesis of single stranded DNA phage S13 was observed in strains with *umuC* or *recA* deletions (Tessman 1985). Initially, such results were interpreted as fitting a two step model similar to that proposed by Bridges and Woodgate for *E. coli* UV-mutagenesis (as described above). Later, however, Tessman and Kennedy (1991) reassessed the applicability of the two-step model. In phage S13, DPR mutagenesis was abolished when the irradiated phage was not stored before photoreactivation. More specifically, phage S13 was held for 2h at 4°, or 30 min at 37°, to obtain maximum mutation frequencies. It is well known that in *E. coli* half of the cytosine in the cyclobutane dimers are deaminated during incubation for 2h at 37° (Setlow et al. 1965). Furthermore, mutation frequencies in a phage which contains only thymine-thymine dimers was found to be lower (7.2x) than in a phage which also has cytosine-containing dimers (Tessman and Kennedy 1991). Thus, deamination of cytosine in UV-induced pyrimidine dimers is the likely source of delayed photoreversal mutagenesis in phage S13.

Tessman et al. (1992) extended this concept of deamination in DPR mutagenesis to SOS mutagenesis in phage S13 by measuring the kinetics of growth and mutagenesis for UV-irradiated phage. These authors observed that DNA replication continued promptly across T-T dimers by inserting the adenine nucleotide noninstructionally opposite DNA legions, confirming the "A-rule" for noninstructive repair in *E. coli*, as originally suggested by Tessman (1976; reviewed by Strauss 1991). In contrast, bypass of cytosine containing dimers could only occur after deamination of cytosine. Hence, deamination of cytosine,which generates uracil,leads to error-free rather than error-prone

processing by incorporation of dATP opposite uracil. Such an explanation agrees well with the observed specific increases of C to T transitions.

The "deamination" model explains persuasively UV- and delayed photoreversal-mutagenesis in phage S13, but not the major scheme for mutagenesis in *E. coli* (discussed by Bridges 1992).

b) Chemical mutagenesis in E. coli

Alkylating agents, especially ethylating compounds, form O^6-alkyl-guanine and, to a lesser extent, O^4-alkyl-thymine in DNA which are widely believed to be the major sites of alkylation mutagenesis. The best-known of these are ethyl methanesulfonate (**EMS**), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (**MNNG**), *N*-methyl-*N*-nitrosourea (**MNU**), and *N*-ethyl-*N*-nitrosourea (**ENU**) which are not only highly mutagenic but also carcinogenic (reviewed by Singer and Kusmierek 1982). These alkylating agents are classified as "direct-acting" mutagens since they produce lesions which promote mispairing or misreplication (Radman et al. 1977). In *E. coli*, mutagenesis by these chemicals is largely SOS-repair independent, i.e., independent of *RecA*, and *UmuD,C* gene products (Schendel and Defais 1980; Shinoura et al. 1983). Treatments with these alkylating agents predominantly induce G:C to A:T transitions, consistent with the miscoding ability of the O^6-alkyl-guanine which can pair with thymine (reviewed by Horsfall et al. 1990).

Even though mutations induced by these alkylating agents are thought to occur primarily by direct mispairing at alkylated guanine, evidence for alternative mechanisms which are SOS-dependent has been obtained (e.g., by Foster and Eisenstadt 1985 for MNNG; and by Couto et al. 1989 for MNU). In *RecA-UmuDC* dependent MNU and MNNG mutagenesis in *E. coli*, the mutational spectrum revealed that the majority of mutagenic events were changes of A:T base pairs. This suggested that premutagenic

lesions other than O^6-methylguanine, i.e., O^4-methylthymine, were responsible for mutagenic repair by SOS mutagenesis.

Similar to the action of MNNG and MNU, it has been shown that **EMS** produces mainly G:C to A:T transitions in various organisms (for the references and more about EMS mutagenesis, see chapter 4, "Discussion"). In addition to direct mispairing of thymine opposite O^6-ethylguanine, another pathway for the production of G:C to A:T transitions by EMS as well as by other alkylating agents has been suggested; namely, deamination of cytosine opposite O^6-alkylguanine, i.e., a "cross-strand deamination" model (Sowers et al. 1987). Direct insertion of adenine opposite such a resulting uracil would then be expected, if uracil is unrepaired. Alternatively, an apyrimidinic (AP) site would likely be generated quite frequently after the removal of uracil by uracil-DNA glycosylase. This AP site could act as premutagenic lesion when *umuD,C* gene products are present (Schaaper et al. 1983). Indeed, levels of uracil-DNA glycosylase appeared to modify the mutagenicity of EMS (Fix et al. 1990).

The mode of mutagenesis by **methyl methanesulfonate** (MMS), another alkylating agent, differs from other alkylating chemicals. MMS shows high killing effect, but induces relatively low mutations compared to other alkylating agents. In addition, in *E. coli*, the mutagenic activity of MMS depends to a large extent on the presence of UmuDC proteins (Todd et al. 1981; Shinoura et al. 1983). In contrast, in *mutS* strains of *E. coli* which are defective in mismatch repair, mutations induced by MMS were not dependent on RecA nor UmuDC proteins, and occurred at different sites from the *UmuDC*-dependent ones (Sledziewska-Gojska and Janion 1989).

The aromatic mutagen, **4-nitroquinoline 1-oxide** (4-NQO), is widely accepted as being an UV-mimetic agent. Recent *in vitro* studies showed that the 4-NQO-modified base, mainly guanine, is excised with a cleavage pattern similar to that found for UV photoproducts (Thomas et al. 1991) Mutagenicity of 4-NQO was shown to be dependent of UmuDC proteins (Shinoura et al. 1983).

1.2.3. Mutagenic DNA repair in yeast

Among radiation sensitive (*rad*) mutants of yeast, proteins which are required for induced mutagenesis are mostly coded by genes of the RAD6 epistatic group (reviewed by Haynes and Kunz 1981; Friedberg 1991). The RAD6 group contains genes which are responsible for mutagenic repair or error-free DNA repair under the *RAD6* control. The latter, especially *RAD18* and *RAD5* were reviewed before (see section 1.1.5.b). Here, genes in the RAD6 group which are required for mutagenesis are specifically reviewed in more detail.

a) RAD6 *gene*

The most interesting and best analyzed gene is *RAD6*. *rad6* mutants show a pleotropic phenotype, especially very high sensitivity to a variety of DNA damaging agents, such as UV, γ-ray, EMS, MMS, and cross-linking agents (Cox and Parry 1968; Zimmermann 1968). In addition, *rad6* strains are defective in induced mutagenesis by most mutagens, irrespective of the type of mutations selected. For spontaneous mutation, however, increases are found, i.e., *rad6* shows mutator phenotype (Hastings et al. 1976). The *RAD6* gene has been cloned (Prakash et al. 1983; Kupiec and Simchen 1984). Disruption of *RAD6* is not lethal and expression of the *RAD6* gene is regulated transcriptionally during the cell cycle (Kupiec and Simchen 1986b). Sequencing of the *RAD6* gene revealed several interesting aspects; the main open reading frame of *RAD6* contains 172 amino acids. The carboxy terminal part is highly acidic with 13 consecutive aspartic acid residues (Reynolds et al. 1985). Surprisingly, the Rad6 protein is a ubiquitin-conjugating enzyme which catalyzes the ubiquitination of histones H2A and H2B, and possibly other targets (Jentsch et al. 1987). The polyacidic tail of Rad6p is essential for this ubiquitination reaction. In addition, and so far not well understood,

mutants which have the polyacidic tail deleted are defective in sporulation as shown in strains which have different *rad6* mutations. However, in such strains, UV sensitivity and mutability remain at wild type levels (Morrison et al. 1988). Rad6p has been purified and its specific antibody recognized presumably homologous proteins from *Drosophila* and human cells (Prakash and Morrison 1988). At this time, the role of *RAD6* in DNA repair and mutagenesis at the molecular level is uncertain.

b) REV *and* UMR *genes*

Several *rev* (defective induced *rev*ersion mutation) mutants have been isolated using different screening test systems; ***rev1*** and ***rev3*** were isolated on the basis of reduced UV-reversion of ocher nonsense alleles (especially *arg4-17*; Lemontt 1971). Mutations of three other genes, ***rev4-rev6***, were isolated using a frameshift allele reversion system (e.g., *his4-38*; by Lawrence et al. 1985a). On the other hand, ***rev7*** was obtained by prescreening for MMS sensitivity and testing for reversion levels of *lys2* (Lawrence et al. 1985b). Mutation deficiencies also have been observed in ***umr*** (*u*ltraviolet *m*utation *r*esistant) mutants isolated on the basis of reduced UV mutability for forward mutation (i.e., $can1^r$; Lemontt 1973, 1977).

These *rev* and *umr* mutations generally confer only slight sensitivity to UV. In addition, their mutation deficiencies after UV irradiation depend on the marker alleles tested, i.e., for different alleles in the same gene (e.g., many *cyc1* alleles) or different type of alleles (e.g., missense or nonsense mutation); UV-induced reversion frequencies in *rev* mutant strains was quite varied and often similar to those in wild type (Lemontt 1971; Lawrence and Christensen 1976, 1978a,b, 1979). However, EMS-induced forward and reverse mutations were not affected in several of these mutants (e.g., *rev1*, *rev3*, and *rev7*; Lemontt 1973; Lawrence et al. 1985c). Such an allele- and mutagen-specific

reversion seems to be common in *rev* and *umr* strains, and the molecular basis in terms of mutational specificity has not yet been documented.

The *REV1* and the *REV3* genes have been cloned and sequenced. Interestingly, the predicted Rev1p shares homology with UmuC of *E. coli* (Larimer et al. 1989), while *REV3* shows homologies with several DNA polymerase and presumably encodes a nonessential DNA polymerase, since disruption of the *REV3* gene is not lethal (Morrison et al. 1989).

c) PSO *genes*

Several *pso* mutants have been isolated on the basis of their sensitivity to the killing effect of bifunctional mutagens, e.g., psoralen plus UVA (364nm UV; reviewed by Henriques and Brendel 1990). ***pso1*** mutations which confer antimutator effects appeared to be allelic to *REV3* gene (see above; Cassier-Chauvat and Moustacchi 1988). ***pso2*** mutants are defective in mutagenesis induced by a cross-linking agent, but exhibited wild type levels of mutation following treatment with UV light (Henriques and Moustacchi 1980; Cassier and Moustacchi 1981).

In ***pso3*** mutants strains, a specific sensitivity to the killing effect of mono- and bi-functional furocoumarins but not to UV, γ-ray, and alkylating agent was observed (as previously quoted). In addition, some reduction of mutation frequencies after treatment with various mutagens at high doses has been demonstrated in *pso3* strains (Cassier et al. 1980). On the other hand, the *pso3* mutation also caused decreases of intragenic recombination, while crossing over was increased in these mutant strains (Andrade et al. 1989b). Therefore, involvement of the *pso3* gene in mismatch repair has been suggested.

pso4 (=*xs9*) mutants which were originally isolated by their X-ray sensitivity (Benathen and Beam 1977) also exhibited extreme sensitivities to inactivation by 8-

methoxypsoralen (8-MOP) photoaddition, but only slightly to UV at the G2 cell stage and not to MMS (Henriques et al. 1989). Of particular interest is that the *PSO4* gene appears to be involved in recombination and mutagenesis pathways, comparable to the SOS repair pathway of *E. coli* (Henriques et al. 1989; Andrade et al. 1989a). In this mutant strain, induced *lys* (new allele) reversion mutagenesis by all agents tested was strongly blocked. In contrast, canavanine resistant forward mutation depended on the mutagen used for treatment, e.g., it was abolished if treated with 8-MOP+UVA or γ-ray, partially inhibited when exposed to UV, MMS, and HN1 (mono-functional nitrogen mustard), but not affected when treated with HN2 (bi-functional nitrogen mustard; Henriques et al. 1989).

In summary, in contrast to *E. coli* in which the *RecA* gene plays a dual role in both mutagenesis and recombination, mutagenesis in *Saccharomyces cerevisiae* so far has not been found to be interdependent with recombinational repair. In addition, inducibility of mutagenesis (Siede and Eckardt 1984; Friedberg 1991) in yeast is still equivocal, in contrast to SOS mutagenesis in *E. coli* which is DNA damage inducible (see section 1.2.2). However, other types of inducible repair or stress responses have been well-documented. The long search for a RecA-like gene in yeast has not been fruitful until recently. Surprisingly, it has recently been shown that the RAD51 has amino acid sequence homology to RecA of *E. coli* (Shinohara et al. 1992). However, so far none of *RAD51* mutant alleles or disruption have caused defects in a mutagenic repair pathway. On the other hand, possible RecA-like roles have been suggested for *PSO4*, since mutations in this gene conferred defects in both recombination and mutation.

It has been proposed that a specialized replicative complex which includes Rev1p and Rev3p may mediate translesion DNA synthesis in yeast in a similar manner to that postulated in *E. coli*, since Rev1p appears to have amino acid homology with UmuC (Larimer et al. 1989) while Rev3p was identified as a nonessential DNA polymerase (Morrison et al. 1989). However, mechanisms of mutagenic DNA repair in yeast are

largely unknown and much more needsto be learned about the primary gene functions related to mutagenesis.

1.2.4. Mutagenesis in Neurospora *and* Aspergillus

Understanding of the mechanisms of mutagenesis in these two fungi is much more limited than in *E. coli* and yeast, and is mostly based on the genetic analysis of DNA repair-defective mutants. In *Neurospora crassa*, mutants of the UVS-3 epistatic group are defective in DNA damage-induced mutation, and genes of this group may well function in a mutagenic DNA repair pathway (see section 1.1.6.a). On the other hand, in *Aspergillus nidulans*, no similar genes are known. Members of the UvsC group (i.e., *uvsC* and *uvsE*) are required for both DNA damage-induced mutagenesis and spontaneous mitotic recombination, similar to *RecA* or *PSO4*. In addition, mutations in *uvsC* or *uvsE* cause homozygotic sterility. On the other hand, *uvsI* appeared to be a gene which is involved in a minor mutagenic DNA repair pathway, while recombination is not affected by this mutation. It shows similarities with *rev* genes of yeast, but *uvsI* mutants are much more sensitive to UV light and 4-NQO than found for such *rev* mutants (see section 1.1.6.b; also chapter 4).

1.2.5. Mutational systems

To identify the types of mutations produced spontaneously or after treatment with various mutagens, sophisticated systems have been developed. These are for the study of the premutagenic lesions, as well as of the role of cellular DNA repair or replication in the processing of such lesions. Moreover, such systems facilitated a more rapid determination of mutational specificity, either by genetic analysis or, more recently, by DNA sequencing.

a) Mutational systems in prokaryotes

A well-known early approach was the study of mutation in the **bacteriophage T4** which led to the discovery of non-randomness of mutation within genes. This was most clearly demonstrated by mutational "hot spots" in the *rII* region of Benzer (1961), later identified as sites of methylation of cytosine. In contrast, frameshift hotspots in the lysozyme gene correctly were postulated to be the result of fortuitous base pair repeats (Streisinger et al. 1966). In addition, based on the knowledge of changes in amino acid sequences and the use of well defined alleles, reversion systems provided information about mutagen specificity. For example, the ***trpA* reversion system** in *E. coli* permitted assessment of the mutational specificity of the first known mutator "gene", *mutT* (Yanofsky et al. 1966; Cox 1973). On the other hand, the the well-known tester strains of the ***hisD* reversion system** in *Salmonella typhimurium*, i.e., the "Ames test", made it possible to identify allele-specific and varied mutagenic effects for a large number of man-made and natural compounds in the environment (Ames et al. 1973; McCann et al. 1975).

The ***lacI* system in *E. coli*** provided a more detailed analysis of mutagenic specificity by monitoring forward mutation to nonsense codons identifiable by genetic

techniques (Miller 1978). *lac*⁻ mutants resulted from mutations on an F*lac,proB* episome which contained the *lac* operon. They were selected on phenyl-ß-D-galactopyranoside (P-gal) plates. Nonsense codons were identified as amber (UAG) or ochre (UAA) mutations after transfer into a set of suppressor strains, on the basis of blue rather than white color in the presence of 5-bromo-4-chloro-3-indolyl-ß-D-galactoside (X-gal) when suppressed. In addition, the site of each nonsense mutation within the *lacI* gene was identified by deletion mapping (Schmeissner et al. 1977). When aligned with the nucleotide sequence of the *lacI* gene (Farabaugh 1978), precise determination of base substitutions in these nonsense mutations could be monitored (Coulondre and Miller 1977; Miller 1983). However, in spite of the many advantages of this approach, it had the following limitations for the study of mutagenesis: i) only base substitutions could be analyzed; ii) A:T to G:C transitions could not detected, because such transitions did not generate nonsense mutations; and iii) since nonsense mutations were generated in specific amino acid codons and within a specific sequence context, the obtained distribution of mutations was not random.

To avoid the above disadvantages, sequencing of *lacI*⁻ mutations was carried out after the recovery of mutations onto a single stranded M13 phage vector by recombination (Schaaper et al. 1985). However, in the *lacI* gene, one frameshift hot spot dominates the mutational spectrum (Schaaper et al. 1986). To circumvent this problem, a special *LacI* allele (*lacI204*) was made by site-directed mutagenesis which abolished the hot spot by introducing silent mutations (Schaaper and Dunn 1991). Furthermore, sequencing of *lacI* mutants generated in this strain concentrated on the N-terminal part of the I^{-d}, i.e. $^{a}_{1}$ dominant repressor strain, because this region did not include any hot spots but frequently contained the newly mutated sites.

Recently, another useful mutational assay system was developed in *E. coli* by Miller's group, namely the ***lacZ* reversion system.** This test enables the investigator to monitor all base substitutions and frameshift mutations without further analysis, by

selecting *lac*$^+$ revertants using a carefully chosen set of *lacZ* mutant strains (Cupples and Miller 1989; Cupples et al. 1990). These *lacZ* alleles were constructed by site-directed mutagenesis of the Glu-461 codon which represents an amino acid which is essential for proper function of the ß-galactosidase enzyme (Cupples and Miller 1988). Therefore, only specific base changes in these alleles can restore wild type phenotype. Another complementary set of *lacZ* mutations also was made to detect frameshift mutations. Target sequences were altered by addition or deletion of 1G, 1A, or 2(-C-G-) in the non-coding strand. This *lacZ* system has proved highly successful for the isolation of mutator strains which specifically increase certain types of mutations; e.g., *mutY* specific for G:C to T:A (Nghiem et al. 1988), *mutM* specific for G:C to T:A and *mutA* and *mutC* which stimulate transversions (Michaels et al. 1990).

b) Mutational systems in yeast

In eukaryotes, equivalent work to the early genetical approaches in prokaryotes was carried out by the use of *CYC1* gene in *Saccharomyces cerevisiae*. Mutations in the *CYC1* gene could be selected and base pair changes were predicted based on early knowledge of the cytochrome C1 amino acid sequence. This system was used not only to evaluate mutagen specificity but also to assess specific effects of radiation sensitive mutants on mutagenesis (Prakash and Sherman 1973; Lawrence and Christensen 1978a,b). However, this and other simpler reversion systems based on selection of revertants (von Borstel et al. 1973) frequently had the disadvantage that quantitative estimates and evaluation of the mutational spectrum could not be determined with certainty; e.g., for *cyc1* mutants, when replacement of the mutant codon with a functionally equivalent amino acid produced revertants that were indistinguishable from backmutations, or when phenotypic reversions of some nutritional mutants could result from intragenic or tRNA suppressor mutations.

In yeast, similar to *lacZ* reversion systems in *E. coli*, a simple and more informative system was devised based on a new type of the ***CYC1* reversion system.** A set of *cyc1* mutant strains was constructed which permits the detection of all possible types of base pair substitutions (Hampsey 1991). Like the set of *lacZ* tester strains, these alleles contain point mutations at important amino acid codon (Cys-22) in the *CYC1* gene, and only unique base pair substitutions can restore wild type phenotype.

In addition, **forward mutational systems** also have been developed which facilitate retrieval of altered DNA. One of them is the ***SUP4-o* system** (Pierce et al. 1987), which is based on inactivation of the *SUP4-o*, an ochre suppressor tyrosine tRNA gene. This gene is inserted into a yeast-*E.coli* shuttle vector (YCpMP2) containing a centromere sequence, and a putative yeast and M13 replication origin. Three types of mutants can be selected; either canavanine resistant strains, or mutants with red pigmentation, or lysine auxotrophs, since the host strain of YCpMP2 carries the ochre suppressible mutant alleles, *can1-100*, *ade2-1* and *lys2-1*. Using this system, mutational specificity of mutator effects of several *rad* mutants has been demonstrated by Kunz and coworkers (see section 1.2.1, for specific references).

c) Genetic mutational systems in Neurospora *and* Aspergillus

In these filamentous fungi, mainly genetic methods have been used for the analysis of mutation, either to investigate the effects of DNA repair-deficiencies on mutation frequencies, or for mutagenicity tests of specific chemicals or various environmental mutagens. In both species, visual systems were used extensively to obtain random mutations of all types in several genes. The main system used in ***Neurospora crassa*** is the ***ad-3* system** originally developed to analyze the effects of various mutagens (reviewed by de Serres 1992b) and later used also to investigate mutagenesis in DNA repair-defective strains (de Serres 1980; de Serres and Brockman 1991).

Spontaneous and induced adenine-requiring mutants in two adjacent genes (*ad-3A* and *ad-3B*) were isolated visually as pigmented red colonies grown from single conidia after limited growth in liquid, while ad^+ types produced normal whitish mycelia. Mutant genotypes were identified by mapping or complementation tests as either *ad-3A* or *ad-3B*, and further classified by tests for intragenic complementation against specific *ad-3B* mutations (see below for the types of *ad-3* mutants; reviewed by de Serres 1992a).

Similarly, in the **2-thioxanthine system** of ***Aspergillus nidulans***, mutations in several genes of the nitrogen biosynthetic pathways could be detected (Scott et al. 1982; Scott and Kafer 1982). Such mutants produce green conidia as distinct from yellow wild type colonies on medium containing the purine analogue 2-thioxanthine. Mutant green colonies are easily detected and usually result from mutations in genes for either the enzyme xanthine dehydrogenase (*XDH*) or for components of the uptake system of 2-thioxanthine (*2-TxR*). *XDH* mutants could be classified further as *hx*, *cnx* or *uaY* mutations, on the basis of their inability to grow on three different nitrogen sources (i.e., hypoxanthine, nitrate or uric acid). Furthermore, mutants in two of these classes (*hx* and *cnx*) could be assigned to one of several genes using complementation tests.

Two more convenient **selective forward mutation** systems were similarly used in both *N. crassa* and *A. nidulans* for the analysis of rare mutational events, namely selection for resistance to toxic analogues, especially to *p*-fluorophenylalanine (FPA), or to selenate. In both these systems mutants are resistant because they are defective for uptake or metabolism of the analogues of normally metabolized compounds. Some of these mutants are therefore auxotrophs, and revertants can be selected on minimal medium. In general, tests for resistance to FPA produce less uniform results and are more difficult to standardize. Residual "background" growth occurs and secondary mutations continue to appear to various extents under different conditions and in different strains.

In Neurospora mutants obtained on the bases of *p*-fluorophenylalanine resistance (or resistance to 5-methyl-tryptophan) generally are mutations in the ***mtr*** **gene** (Stadler 1983). This gene codes for the neutral amino acid permease. When stable *mtr* mutants are selected in a *trp-2* background on media containing anthranilic acid, such mutants cannot grow on MM. Thus, revertants of *mtr* can be selected on MM (Stadler et al. 1991). In *Aspergillus*, most of the mutants resistant to FPA occur in a gene of the tyrosine biosynthetic pathway, and these mutants practically remain prototrophs. Therefore, revertants cannot be selected on MM and the system has been used less extensively.

The reverse situation is found for the two species when mutants are selected for selenate resistance. In *Neurospora*, such mutants are easily selected in wild type strains, but mutations in some of the DNA repair-defective strains produce lower levels of resistance to the toxic effects of this analogue than in wild type strains. Therefore, comparison of mutation frequencies under identical conditions become impossible for different *uvs* and *mus* strains of *N. crassa* (Kafer 1981, and unpublished results).

In ***Aspergillus nidulans***, no such problem has been encountered and the selenate resistance system has been used successfully for the analysis of mutagenesis in all DNA repair defective, *uvs* and *mus*, mutant strains (Jansen 1972; Kafer and Mayor 1986; and results of chapter 4). Selenate resistance in *Aspergillus* is the result of mutations which cause a sulfite requirement due to the loss of function in one of several genes. However, at relatively high concentrations of selenate (0.1 mM in the presence of 0.02 mM D-methionine) mutations are found practically only in two genes, namely *sB*, which codes for sulfate permease, and *sC*, the gene for ATP sulfurylase (Arst 1968; Gravel et al. 1970). Mutants in these two genes can be distinguished by simple tests for growth on chromate to which *sB* but not *sC* mutants are resistant (Arst 1968). Alternatively, if such mutants are inoculated adjacent to standard *sB* and *sC* mutant strains on MM, residual sparse mycelial growth and fusion of hyphal tips occurs. Heterokaryon

formation leads to vigorous growth at the line of contact between colonies only when complementation occurs (as described in detail by Niklewicz 1970). Revertants of such *sB* or *sC* mutations are difficult to isolate on standard MM, but can be obtained easily if the normal agar in MM is replaced by agarose (see Fig. 4.10).

Systems which test **recessive lethal and aberration** frequencies in these two fungi have been also developed. In ***Neurospora crassa***, well-designed 2-component heterokaryons heterozygous for *ad-3* mutants led to the analysis of chromosomal aberrations and mutations in *ad-3* linked essential genes, both spontaneously and after treatments with various mutagens (de Serres 1992). Two classes of mutants were separated by dikaryon tests: i) "repairable" mutants (*ad-3*^{R}) which grew on MM plus adenine as homokaryons (presumed point mutations); and ii) "irrepairable" mutants (*ad-3*^{IR}) that did not grow on MM plus adenine or even CM (possibly multilocus deletions). Mutants of both types were further analyzed in trikaryons with the use of suitable tester strains. *ad-3B* mutants could be located into intragenic complementation groups by allelic complementation tests, while irrepairable mutations were identified as extended deletions or coincident recessive lethals in adjacent essential genes.

Two-component heterokaryons also have been used for the detection of recessive lethal frequencies over the entire genome (de Serres and Malling 1971; Stadler and Crane 1979; Kafer 1984). Recessive lethals could be identified in platings of multinucleate conidia from such heterokaryons. Since each component haploid carried a recessive resistant marker [e.g., either resistance to *p*-fluorophenylalanine or methyl-tryptophan (for *mtr* marker), or to cycloheximide (*cyh* marker)], conidial platings on MM plus inhibitor produced homokaryons of either selectable parental type only if no recessive lethal was present.

In ***Aspergillus nidulans***, the frequencies of induced recessive lethals can be measured for the total genome of diploids which are heterozygous for genetic markers in each mitotic linkage group, i.e., on each chromosome (Tector and Kafer 1962; Kafer and

Chen 1964). When such diploids are haploidized, heterozygous markers segregate 1:1 in mitotic segregants. However, if lethal mutations are present, the allele in coupling on the same chromosome cannot be recovered while markers on all other chromosomes segregate normally. This method is rather tedious because it requires testing for many markers. A simpler variant of this method has, however, been devised by Morpurgo et al. (1978) in which recessive lethals linked to colour markers on three chromosomes can be detected visually when diploids are haploidized on CM containing benlate, i.e., a spindle poison.

Molecular level studies are also being carried out at the *his-3* and *mtr* loci in which mutations were monitored by Southern analysis (Dubans et al. 1989; Stadler et al. 1991). Especially, the *mtr* system has much potential since forward and backward selection may help to distinguish base substitutions in certain regions without the need for sequencing of the total gene.

CHAPTER 2

uvsI mutants defective in UV mutagenesis define a fourth epistatic group of *uvs* genes in *Aspergillus*

2.1. SUMMARY

Three UV-sensitive mutations of *A. nidulans*, *uvsI*, *uvsJ* and *uvsA*, were tested for epistatic relationships with members of the previously established groups, here called "UvsF", "UvsC", and "UvsB" groups. *uvsI* mutants are defective for spontaneous and induced reversion of certain point mutations and differ also for other properties from previously analyzed *uvs* types. They are very sensitive to the killing effects of UV light and 4-NQO (4-nitro-quinoline-*N*-oxide) but not to MMS (methyl-methane sulfonate). When double and single mutant *uvs* strains were compared for sensitivity to these three agents, synergistic or additive effects were found for *uvsI* with all members of the three groups. The *uvsI* gene may therefore represent a fourth epistatic group, possibly involved in mutagenic repair. On the other hand, *uvsJ* clearly was epistatic with members of the UvsF group and fitted well into this group also by phenotype. The *uvsA* gene was tentatively assigned to the UvsC group. *uvsA* showed epistatic interactions with *uvsC* in all tests, and like UvsC group mutants is UV sensitive mainly in dividing cells. However, the *uvsA* mutation does not cause the defects in recombination and UV mutagenesis typical for this group.

Key Words:

DNA repair; epistatic grouping; *uvs* mutants; *Aspergillus nidulans*.

2.2. INTRODUCTION

Defects in DNA repair frequently lead to increased sensitivity to physical or chemical DNA damaging agents. Analysis of such effects in mutant cells has revealed a variety of repair processes which have been classified into three basic repair mechanisms; namely, excision repair in nondividing cells, and two types of postreplication repair, recombinational and mutagenic repair (reviewed by Sancar and Sancar 1988). For each of these basic types of repair, alternate processes have been identified in *E. coli* which usually are characterized by a few pathway-specific genes.

DNA damage caused by UV irradiation is repaired by processes of all three types in bacteria, but most efficiently by excision. The most UV-sensitive *E. coli* mutants are defective in nucleotide excision repair (*uvrA* and *uvrB*; Sancar and Rupp 1983), while in *Micrococcus luteus* UV-induced cyclobutane dimers are mainly removed by base excision repair dependent on a pyrimidine dimer-specific DNA glycosylase (Haseltine et al. 1980). In addition, UV-induced DNA damage is repaired by postreplication processes, and mutants in various genes are UV sensitive; not only mutants defective in UV mutagenesis (e.g., *umuC*; Kato and Shinoura 1977) but also rec$^-$ types (e.g., *recA* and *recB*, but also more specialized types like *ruvC*, defective in a resolvase enzyme: Dunderdale et al. 1991). Evidence for equivalent basic processes in eukaryotes has recently been obtained from cloned genes, especially in yeast and mammalian cells (summarized by Friedberg 1991). Highly conserved enzymes contain protein domains which show homology of amino acid sequences even between unrelated species.

In contrast, quite basic differences are found when the organization of genes and pathways are compared between well-analyzed species. For example, the major pathways and epistatic groups of the yeast *S. cerevisiae* differ considerably from those of *E. coli* and similar differences are also expected between yeast and multicellular organisms. In yeast a large number of radiation repair genes have been organized into three epistatic groups,

based on studies of the relative sensitivities of single and double mutants to killing by UV and ionizing radiation (Game and Cox 1973; Haynes and Kunz 1981). More than ten RAD3 group genes function in nucleotide excision repair (reviewed by Friedberg 1988). At least six of them are absolutely required and mutants are highly sensitive to UV light (Bankmann et al. 1992). On the other hand, RAD52 group genes are involved in repair of double strand breaks and in recombination. Mutants in such genes are sensitive mainly to ionizing radiation and MMS but not to UV (Kunz and Haynes 1981). The RAD6 group is less homogeneous, but mutants generally are UV and X-ray sensitive. Some genes are required for induced mutation but others appear to function mainly in an error-free post-replication repair.

Three epistatic groups of *uvs* genes have also been identified on the basis of UV sensitivity in *Aspergillus nidulans* (Kafer and Mayor 1986). Mutants within each group have fairly uniform phenotypes, and especially their effects on recombination and mutation are very similar. However, for none of the three groups is there a clear correspondence to the typical phenotypes of UV repair types in *E. coli* or of the three epistatic groups in yeast. The function of each group in *Aspergillus* is still unknown, but the three groups presumably are involved in major alternate processes of repair, because pairs of mutations from any two of these groups are lethal or grow very poorly in *uvs* double mutant strains. Mutants of the first epistatic, or UvsF, group (which includes *uvsH*) show increases of spontaneous mitotic recombination and UV-induced mutation, as typically found for excision-defective mutants. However, some *uvsH* mutations in addition cause sensitivity to X-rays and defects in meiosis, and *uvsH* therefore is unlikely to function in nucleotide excision repair. Genes of the second, UvsC, group (*uvsC* and *uvsE*) have multiple functions. Mutants are sterile in homozygous crosses and show practically no spontaneous mitotic recombination, but spontaneous mutation is considerably increased (Jansen 1970b,1972; Fortuin 1971c). Such mutator effects, as well as reduced UV-induced mutation, were found consistently in several forward and reverse mutation tests

(Kafer and Mayor 1986; S.-K. Chae, unpublished results). Mutations of the third type which fall into the UvsB group (*uvsB* and *uvsD*) do not affect mutagenesis, but cause defects in meiosis and few normal ascospores are found. Diploids homozygous for *uvsB* or *D* exhibit increases of spontaneous mitotic recombination, mainly of a nonreciprocal type. In addition, partially aneuploid, abnormally-growing, conidia are extremely frequent. Possibly, these last two genes are required for chromosome break repair. All these *uvs* mutants of *A. nidulans* are sensitive to MMS as well as UV, but only mutants of two genes, *uvsH* and *uvsE*, are sensitive to ionizing radiation.

Recently a mutation has become available in a new gene, *uvsI*, which may be involved in mutagenic repair. *uvsI* mutants have been found to reduce UV-induced mutation of two different types (Han and Kang 1985). A major aim of the present study therefore was to identify the epistatic relationships of *uvsI* with *uvs* mutations of the three identified groups. Double mutant *uvs* strains were constructed for *uvsI* with mutations from all previously analyzed genes and sensitivities to several mutagens were compared. In addition, two other unassigned mutants were similarly tested, *uvsJ1* (Chang et al. 1968) and *uvsA101* (Jansen 1967).

2.3. MATERIALS AND METHODS

2.3.1. Media and methods for genetic analysis

Complete and minimal media (CM, MM) and the techniques used for genetic analysis were those developed by Pontecorvo et al. (1953) as modified by Scott and Kafer (1982). Sodium deoxycholate (0.01%) was added to CM to reduce colony size in conidial platings (Mackintosh and Pritchard 1963). The methods of Kafer (1958, 1977) were used

for gene mapping and chromosome replacement by mitotic segregation. Diploids were haploidized by benomyl (Hastie 1970).

2.3.2. Strains

All strains of *A. nidulans* are derived from a single haploid nucleus and therefore are isogenic except for differences induced by mutagenic treatments (Pontecorvo et al. 1953). "Standard" strains with a few useful markers have been extensively backcrossed to the original wild type (for 5-9 generations). The origin of most *uvs* mutations has been described (Kafer and Mayor 1986). The following mutants were chosen for analysis, using in all cases the most UV-sensitive of the available *uvs* alleles; namely, *uvsF201*, *uvsH77*, *uvsC114*, *uvsE182*, *uvsB110*, and *uvsD153*. The *uvsI501* mutation was isolated by Han et al. (1983) and is available as strain A825 from the Fungal Genetics Stock Center (University of Kansas Medical Center, Kansas City, KS 66103, USA).

Control strains with color and nutritional markers were derived from standard strains. Congenic *uvs* strains were constructed by replacing 6-7 of the 8 chromosomes with those of the uvs^+ control. A set of such strains which are isogenic for most chromosomes was used for tests of mutagen sensitivities (FGSC A829-A839). All these strains carried essentially the same markers, namely *chaA1* (=chartreuse) for conidial color, two or three vitamin requirements (*pabaA1*, *choA1*, *riboB2*) and often *AcrA1* and/or *ActA1* (resistance to acriflavin and/or cycloheximide).

2.3.3. Isolation of double mutants

Double mutant *uvs* strains were isolated as mitotic haploid segregants from diploids heterozygous for two *uvs* alleles located on different chromosomes. An exception were *uvsA;uvsF* strains which were obtained as meiotic recombinants from

intercrosses because both mutations map to chromosome I. When haploid segregants were tested for segregating markers, complete linkage of markers to *uvs* mutations usually identified double mutant strains. *uvs* genotypes always were checked by tests of mutagen sensitivities. Different levels of MMS sensitivity were recognizable on test plates containing various concentrations of MMS (usually 0.015 and 0.03% v/v, and also 0.005 and 0.01% for unusually sensitive types). In doubtful cases, double mutants were confirmed or identified by complementation tests.

2.3.4. Determination of mutagen sensitivities

Conidiospores were harvested in Na-K-phosphate buffer (0.05M, pH 7.0) with 0.001% Tween 80. They were stored overnight at 4° before treatments. Survival was based on colony counts of treated and untreated samples and was determined after 3 days of incubation at 37°.

Treatment with 4-NQO: A stock solution of 4-NQO (Aldrich Chemical Co.) was prepared (1% in acetone) and diluted 20-fold into buffer. Various amounts were added to suspensions of conidia in buffer (10^6/ml) to obtain increasing concentrations of 4-NQO (up to 0.5 mg/ml). Conidia remained quiescent during treatments (for 2 h at 30° with shaking at 200 rpm) and were diluted into 10% $Na_4S_2O_3$ to terminate exposure.

UV survival curves: Quiescent conidia were spread on CM at appropriate concentrations and irradiated on the plates before incubation (Jansen 1970a). The UV dose rate was 1.6 J/m^2/sec (using a GE germicidal 30W lamp, #G30T8, and a Ultraviolet meter™ from Ultra-Violet Product, San Gabriel, CA). Plates were rotated (at 33 1/2 rpm) for even exposure.

Tests for MMS sensitivity: Various amounts of MMS (Aldrich Chemical Co.) were added to melted CM (at 48°). Plates were poured and used promptly or stored at 4°.

Conidia were plated onto MMS media at two densities and the resulting colonies were counted or recorded photographically.

2.4. RESULTS

2.4.1. *Recovery of double mutants from heterozygous* uvs *diploids.*

When diploids are haploidized on benomyl plates, poorly growing segregants, e.g., aneuploid intermediates, are difficult to recover and only the most competitive haploid segregants form frequent conspicuous sectors. Therefore, many sectors (80-250) of all types and sizes were tested from doubly heterozygous *uvs* diploids to recover poorly viable types, or to identify lethal interactions. Because some single mutants also showed less than the expected 25% recovery, the "viability" of double mutants was assessed relative to the less viable of the two respective single mutant types (Table 2.1). Several new cases of lethal interactions were identified (indicated as 0% in the table). Some of these were double mutants combining *uvsE* (of the UvsC group) or *uvsD* (UvsB group) with members of different epistatic groups, as predicted (Kafer and Mayor 1986) but not demonstrated previously. Of the new mutations *uvsJ* caused lethal interaction with *uvsC* and showed poor growth and abnormal conidiation in double mutants with *uvsB*. In contrast, the other two mutations, *uvsI* and *uvsA*, were obtained in viable double mutants with all other *uvs*.

Table 2.1. Percent recovery of double mutant strains, compared to the less viable single *uvs* mutant (No. indicated) among mitotic haploids from diploids heterozygous for two unlinked *uvs* mutations.

Epista-tic group	Second *uvs* mutation	Newly tested *uvs* mutation involved in recovered double mutant strains									
		uvsI		*uvsA*		*uvsJ*[a]		*uvsE*		*uvsD*[a]	
		%	(No.)	%	(No.)	%	(No.)	%	(No.)	%	(No.)
"UvsF"	*uvsF201*	>100	(36)	[NA[f]]		>100	(3)[a]	0	(61)	0	(40)
	uvsH77	46	(28)	47	(18)	[ND[g]]		0	(51)	[0	(8)][d]
"UvsC"	*uvsC114*	74	(80)	>100	(13)	0	(65)[b]	[>100	(46)][d]	1[c]	(68)
	uvsE182	100	(43)	[ND[g]]		[NA[f]]		[NA[c]]		[NA[f]]	
"UvsI"	*uvsI501*	[NA[c]]		89	(18)	>100	(2)	100	(43)	52	(23)
"UvsB"	*uvsB110* [a]	44	(39)	>100	(13)	40[c]	(13)	0	(23)	[>100	(6)][d]
	uvsD153 [a]	52	(23)	[ND[g]]		[NA[f]]		[NA[f]]		[NA[c]]	

a Recovery was often reduced for *uvsB* and *D* single mutant strains and always very poor for *uvsJ*

b Nonrandom sample; *uvsC* selected by linkage to color marker (*chaA*)

c Double mutants *uvsJ;uvsB* and *uvsD;uvsC* showed poor growth

d Previously tested double mutants (Käfer and Mayor 1986)

e, f NA = Not applicable; [e] "homozygous" combination; [f] mutants located on same chromosome

g ND = Not determined

2.4.2. *4-NQO and MMS sensitivities of known epistatic* uvs *pairs*

All viable double mutants of the six *uvs* genes grouped previously on the basis of UV survival were tested for 4-NQO and MMS sensitivity. Results of these tests, which included previously untested pairs of *uvs* mutations, confirmed the epistatic pairs or groups postulated; namely 1) UvsF group: *uvsF* and *H*; 2) UvsC group: *uvsC* and *E*; and 3) UvsB group: *uvsB* and *D* (data not shown).

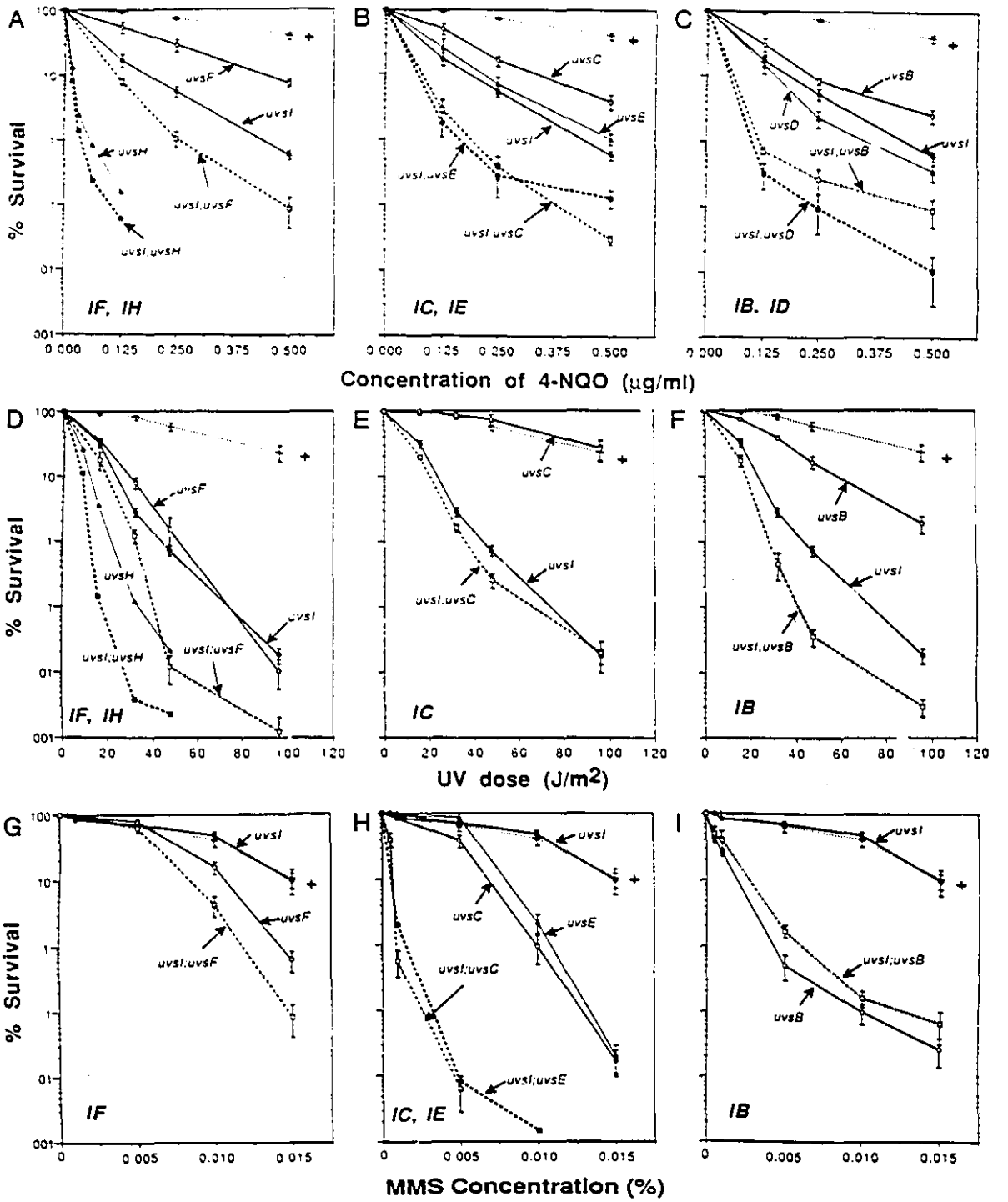
2.4.3. *Quantitative comparison of* uvsI *double mutants with respective single mutant strains for sensitivity to 4-NQO, UV, and MMS, and epistatic grouping of* uvsI

All possible double mutant strains of *uvsI* with mutations of the six classified genes were compared to single mutants for survival after treatment with 4-NQO (Figs. 2.1 A - C). These same strains (except *uvsI; uvsD*) also were irradiated with UV light. In practically all cases double mutants were significantly more sensitive than component single mutant strains (Figs. 2.1 D - F). The exceptions were double mutant strains of *uvsI* with UvsC group mutations when tested for UV survival. The latter tests are uninformative because quiescent conidia of *uvsC* or *E* are not UV sensitive (Fig. 2.1 E; results of a single test of *uvsI;uvsE* not shown). However, when growing cells were treated with UV, synergistic interaction was found for both pairs of mutations (S.-K. Chae; unpublished results).

Tests for MMS sensitivity were also carried out even though *uvsI* strains are not more sensitive than wild type (Figs. 2.1 G - I), because increased sensitivity of various double mutants was evident in MMS tests of conidia when double mutant strains were isolated. Interaction of *uvsI* with mutations from the UvsF group caused moderate increases, as demonstrated in survival curves for *uvsI;uvsF* (Fig. 2.1 G). More striking

Fig. 2.1. Survival of single *uvs* strains, compared to double mutants combining the *uvsI* mutation with members of the three epistatic groups, "UvsF" (including *uvsH*), "UvsC" (and *E*), and "UvsB" (and *D*). Percent survival is plotted vs. dose in semilog format. Values and their standard errors (indicated by error bars) are averages based on at least three experiments (or represent averages of two experiments if no error bars shown).

A - C, Treatment of quiescent conidia with 4-NQO; **D - F**, UV-irradiation of quiescent conidia on CM plates; **G - I**, conidia plated to MMS media and exposed during germination and growth of colonies.


A
B
C
D
E
F
G
H
I
% Survival
uvsF
uvsI
uvsH
uvsI;uvsF
uvsI;uvsH
IF, IH
uvsC
uvsE
uvsI;uvsE
uvsI;uvsC
IC, IE
uvsB
uvsD
uvsI;uvsB
uvsI;uvsD
IB, ID
IC
IB
IF
Concentration of 4-NQO (µg/ml)
UV dose (J/m2)
MMS Concentration (%)

was the highly increased MMS sensitivity found for double mutants of *uvsI* with UvsC group mutations (Fig. 2.1 H). Only for UvsB group mutants was no interaction evident (shown for *uvsI;uvsB* in Fig. 2.1 I).

In conclusion, in tests with all three mutagenic agents no consistent case of epistasis was found for *uvsI* with previously tested *uvs* mutations, and in all cases when double mutants were no more sensitive than single mutant strains, one of the latter showed wild type levels of survival. It therefore appeared unlikely that the *uvsI* gene is a member of one of the three known epistatic groups.

2.4.4. *Epistatic grouping of* uvsJ

For tests of *uvsJ*, double mutants were constructed in which *uvsJ* was combined with one *uvs* mutation from each of the three established epistatic groups or with *uvsI*. Such double mutant strains and controls were tested for sensitivity to 4-NQO, UV, and MMS (Figs. 2.2 and 2.3 A). Survival curves demonstrated epistasis for *uvsJ* with *uvsF* mutations when double and single mutants were treated with 4-NQO or with UV light (Figs. 2.2, A and D). In contrast, *uvsJ* and *uvsC* are unlikely to be epistatic, because *uvsJ;uvsC* double mutants are lethal. Presumably this indicates that these two mutants interrupt alternate processes of repair or of other DNA transactions, and that for survival at least one of these processes must be functional. Furthermore, at least additive interactions were found for *uvsJ* with *uvsB*, and for *uvsJ* with *uvsI* (Figs. 2.2 B,C and E,F). Tests with MMS confirmed epistasis of *uvsJ* with *uvsF* (results not shown) and synergistic interactions of *uvsJ* with *uvsB* (Fig. 2.3 A).

2.4.5. *Epistatic grouping of* uvsA

The *uvsA* mutant is barely sensitive to 4-NQO and UV and grows like wild type on MMS medium (Figs 2.4 and 2.3 B). *uvsA* showed very slight interaction with *uvsF* and *uvsB* in 4-NQO survival curves; however, epistasis with *uvsC* was clear (results not shown). In contrast, highly reduced 4-NQO survival was found for *uvsA* double mutants with *uvsI* (Fig. 2.4 A) and similar synergistic interaction was evident in MMS tests (Fig. 2.3 B; note the higher MMS concentrations compared to Fig. 2.3 A). When double mutants of *uvsA* with members of the UvsF group were tested for UV survival, at least additive interactions between *uvsA* and *uvsF* or *H* could be seen (Fig. 2.4 B). On the other hand, only careful repeated tests and simultaneous controls could demonstrate additive effects for *uvsA* and *uvsB* (Fig. 2.4 C).

Such results clearly ruled out epistasis for *uvsA* with *uvsI*, and also for *uvsA* with members of the *uvsF* group. Furthermore, additive interaction of *uvsA* with *uvsB* is likely but is less well documented. On the other hand, all data are compatible with an epistatic relationship between *uvsA* and *uvsC*. Thus *uvsA* was provisionally assigned to the UvsC group.

2.5. DISCUSSION

The results obtained in this investigation demonstrate that two of three *uvs* mutations tested, *uvsJ* and *uvsA*, could be assigned to previously established epistatic groups. In contrast, the third mutation, *uvsI*, showed at least additive interactions with all tested *uvs* mutations of the three groups. The latter findings are based on evidence of two types. i) Many segregants from each of the possible doubly heterozygous *uvs* diploids

Fig. 2.2. Survival curves of double mutant strains of *uvsJ* with *uvsF*, *uvsB*, and *uvsI*, and their component single mutant strains.

A - C, Treatments with 4-NQO and **D - F**, with UV-light (as in Fig. 2.1).

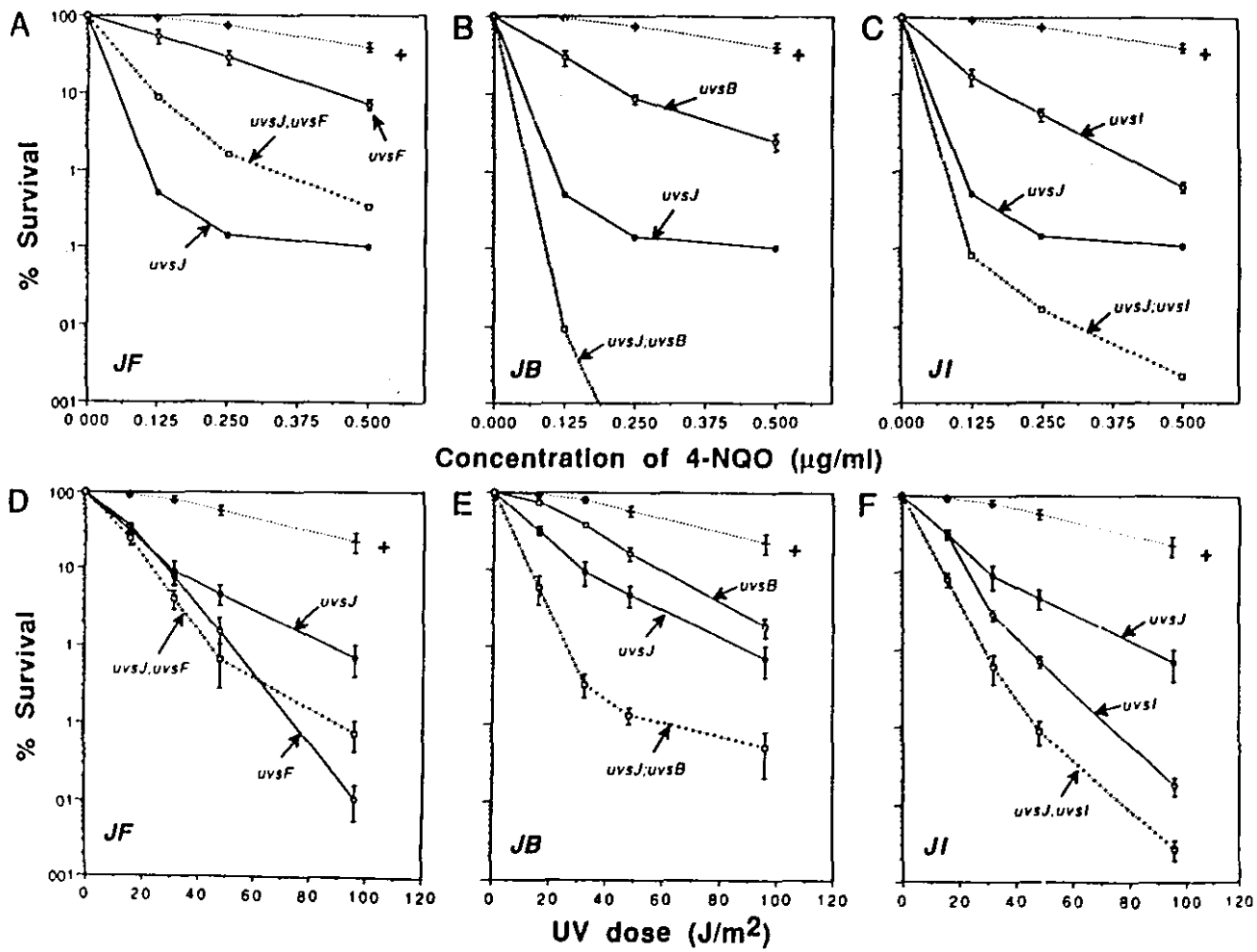

A
B
C
D
E
F
% Survival
100
10
1
01
001
+
uvsJ;uvsF
uvsF
uvsJ
uvsB
uvsJ;uvsB
uvsI
uvsJ;uvsI
JF
JB
JI
0.000
0.125
0.250
0.375
0.500
Concentration of 4-NQO (μg/ml)
0
20
40
60
80
100
120
UV dose (J/m2)

Fig. 2.3. Colony formation on MMS media of double mutants compared to single mutant strains. A, low density plating, incubated 3 days; B, high density plating, incubated 2 days.

A, *uvsJ;uvsB*, respective single mutants, and wild type. In sequence from the top: wild type, *uvsJ* single, *uvsJ;uvsB* double, and *uvsB* single mutant.

B, *uvsA;uvsI* and controls. In sequence from the top: wild type, *uvsA* single, *uvsA;uvsI* double and *uvsI* single mutant.

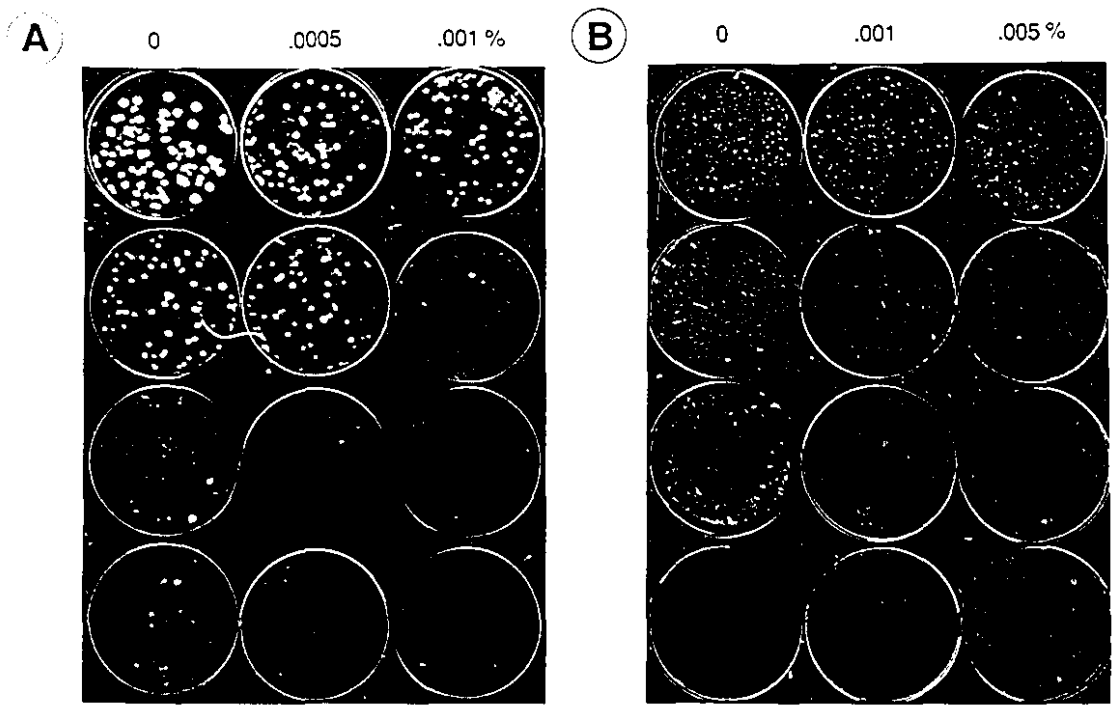
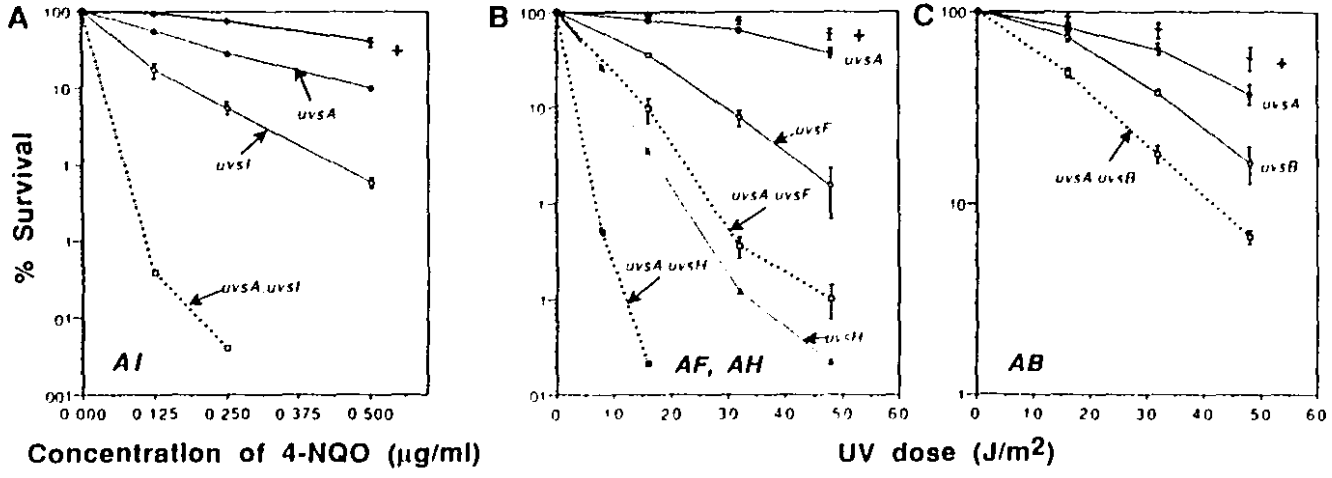

A
0
.0005
.001 %
B
0
.001
.005 %

Fig. 2.4. Survival curves of various *uvsA* double mutant strains and of respective single *uvs* mutants (as in Fig. 2.1); **A**, *uvsA;uvsI* treated with 4-NQO; **B**, UV survival of *uvsA* combined with UvsF group mutants; **C**, *uvsA;uvsB*, and simultaneous controls treated with UV-light.


A
B
C
% Survival
100
10
1
0.1
0.01
+
uvsA
uvsI
uvsA uvsI
AI
0.000
0.125
0.250
0.375
0.500
Concentration of 4-NQO (μg/ml)
uvsF
uvsA uvsF
uvsA uvsH
AF, AH
0
10
20
30
40
50
60
uvsB
uvsA uvsB
AB
UV dose (J/m2)

were tested for MMS sensitivity and *uvsI* double mutants showed uniform and frequently increased sensitivities in all segregants of the same genotype. ii) A few representative double and single mutant strains, isogenic for most chromosomes, were analyzed extensively for survival after treatments with UV, 4-NQO, and MMS. Tests with 4-NQO provided the most useful results, because all *uvs* strains showed some sensitivity to 4-NQO and two of the three *uvs* mutations analyzed here, *uvsI* and *uvsJ*, were very sensitive to 4-NQO, as well as to UV.

Results for the three mutagens were consistent and no case of epistasis was found for *uvsI*. In addition, the obtained results more firmly established the three epistatic groups of *uvs* genes previously identified on the basis of UV survival. Such concordant results are perhaps expected for 4-NQO which is considered UV-mimetic and elicits repair very similar to that of UV light (Thomas et al. 1991). On the other hand, the consistent results obtained with MMS are somewhat surprising, especially considering the complex patterns found in tests with alkylating agents for *rad* mutants of yeast (Cooper and Waters 1987). A possible explanation may perhaps be the uniform sensitivity to MMS of all *uvs* mutants of the three established groups in *Aspergillus*.

The hypothesis that *uvsI* represents a new epistatic group is supported by the finding that the phenotype of *uvsI* mutants considerably differs from that of all other *uvs*. In contrast to all previously analyzed *uvs* mutants, *uvsI* showed wild type levels of growth on MMS media, but produced highly synergistic interactions for MMS sensitivity when combined with UvsC group mutations. Such synergism was especially unexpected the case of *uvsI;uvsA* double mutants when neither single mutant strain was more than slightly MMS sensitive. These results indicate competition for substrates and alternate functions of genes, as has been observed in a few other cases for mutants of different epistatic groups (e.g., for *rad* mutations in yeast, by Dowling et al. 1985). It is therefore very unlikely that *uvsI* is a member of the UvsC group, even though the latter are the only mutations which also cause defects of UV mutagenesis as known for *uvsI*. The effects on

mutation were however entirely different in the two types of mutant strains. *uvsI* mutants reduced spontaneous as well as UV-induced reversion of certain point mutations, but did not noticeably affect forward mutation (e.g., when selenate resistant mutants were selected; S.-K. Chae, unpublished results). In contrast, *uvsC* and *uvsE* of *A. nidulans* were mutators and practically lacked UV mutagenesis in all cases when tested in several forward and reverse mutation systems. In addition, and quite different from *uvsI*, these UvsC group mutations cause defects in recombination, resembling *pso4* of yeast (Andrade et al. 1989a). These phenotypic differences therefore are in line with the synergism found between these two types of mutations and suggest that alternated pathways are affected. The *uvsI* gene may therefore be involved mainly in a minor pathway of spontaneous and induced mutagenesis, while *uvsC* and *uvsE* genes are required for an important type of mutagenic and recombinogenic repair of UV damage. Alternatively, *uvsI* may not represent a completely separate epistatic group but rather a subgroup (as found e.g., within the RAD6 group of yeast for *rev1* and *rev3* which showed synergistic interactions with *rev2=rad5* in recent tests even though all these mutations are epistatic with *rad6*; Johnson et al. 1992).

The phenotype of *uvsI* also differs greatly from that of mutants in the other two epistatic groups, especially UvsF group mutants which show increases of UV-induced mutation. In addition, UvsF and also UvsB group mutations cause increased mitotic recombination and many are defective in meiosis, while *uvsI* strains have normal levels of recombination and fertility (our unpublished results). These phenotypic differences strengthen the evidence from the tests for epistatic interactions which for these cases showed additivity or apparent epistasis in a few cases when one *uvs* mutation was not hypersensitive. We therefore consider it unlikely that such additive effects are the result of leakiness of *uvsI* even though the molecular basis of this mutation is unknown. However, such a possibility cannot completely be ruled out until *uvsI* deletions or gene disruptions become available (the latter will be easy to construct if the *uvsI* gene is located

on the available cosmid which contains two genes, *sA* and *sC*, closely linked to *uvsI*; A. M. Bailey, personal communication).

On the other hand, *uvsA* may well be a leaky mutation. *uvsA* is epistatic with *uvsC* and shares some phenotypic features with members of the UvsC group; e.g., increased UV sensitivity in dividing cells and extreme synergism with *uvsI*. However, *uvsA* mutants have much more normal fertility than other UvsC group mutants and sensitivities of *uvsA* to 4-NQO, UV, and MMS are much less pronounced. As a result, ambiguous findings were obtained in some tests of single vs. double *uvsA* mutants (as described for the leaky *rad14-2* allele by Bankmann et al. 1992). Clearly, further analysis is needed to assess the function of this gene, since little is known about *uvsA* beyond the contradictory evidence for and against involvement in excision repair (Kappas and Bridges 1981; Majerfeld and Roper 1978).

The third mutant, *uvsJ*, shows phenotypic features very similar to mutants of the UvsF epistatic group to which it has been assigned. *uvsJ*, like *uvsF* and *uvsH*, is known to produce increases of UV-induced mutation for *methG* suppressors (Chang et al. 1968) and increased mitotic recombination in homozygous diploids (Kafer and Mayor 1986). Based on such findings, nucleotide excision repair had been considered a possible function of UvsF group genes. However, the increased X-ray sensitivity and meiotic defects of *uvsJ* and *uvsH* are poorly compatible with simple defects in excision repair of UV-damage. In addition, *uvsJ* and *uvsF* strains demonstrated liquid holding recovery after UV treatment (Wohlrab and Tuveson 1969; S.-K. Chae, unpublished results) and such recovery usually is lacking in excision-defective mutants.

While epistatic grouping in *Aspergillus* appears to differ greatly from that found in budding yeast, results show similarity to those obtained in *Neurospora crassa*. In this species three epistatic groups were also identified some time ago (Tuveson 1972; Inoue et al. 1981; Kafer 1983). Genes in one of these, the "UVS-2" epistatic group, had been proposed to function in excision repair, based on tests for UV-dimer removal (Worthy and

Epler 1973). These mutants showed high UV-sensitivity and increased UV-induced mutation (de Serres 1980). However, some of them also caused defects in meiosis (e.g., *mus-8*) and *uvs-2* not only is X-ray sensitive but also showed liquid holding recovery (Schroeder 1974). More recently, *uvs-2* strains and other mutants from this group were found to excise cyclobutane pyrimidine dimers as well as (6-4) photoproducts from UV-treated DNA at wild type rates (Macleod and Stadler 1986; Baker et al. 1990, 1991). Meanwhile, the *uvs-2* gene has been cloned and amino acid comparisons have revealed homology of this gene with *RAD18* of *S. cerevisiae* (Tomita et al. 1993). All these findings reinforce the hypothesis that UVS-2 group members are not involved in excision repair, but they do not shed much light on the possible alternate type of repair involved. On the other hand, a barely UV-sensitive mutant of a putative fourth epistatic group appears to be defective in the incision step of UV dimer excision (Ishii et al. 1991). These results indicate a more complex situation and suggest that alternate repair processes may exist in *Neurospora* even for excision of UV damage, as recently demonstrated for *Micrococcus* (Nakayama et al. 1992).

Overall, it is clear that genetic effects are not very useful indicators for primary gene function of *uvs* genes in *Aspergillus* or *Neurospora*, possibly because repair pathways differ from the models based on evidence from *E. coli* or yeast. Even though many UV-sensitive mutants have been obtained, practically none show features expected for mutants defective in nucleotide excision repair. In *Aspergillus* the only possible candidate is *uvsF* for which direct biochemical tests for excision capacity have not yet been carried out. The alternate approach, namely cloning of *uvsF* (Oza and Kafer 1990) and sequencing of its cDNA has not produced much useful information. Apparently, *uvsF* is not homologous to any known DNA repair gene (E. Kafer, unpublished results) and it will be interesting to find out whether an equivalent gene can be identified in mammals or even yeast. However, of more interest to our ongoing analysis of recombination in *Aspergillus* are mutants of the UvsC and UvsB groups, which have

complex and "unexpected" phenotypes. These genes also appear to be very important for the organism, and a surprising number of mutations have been found in some of them (e.g., >10 alleles of *uvsB* by seven investigators using different criteria for isolation). Their further analysis may provide some interesting information, e.g., about the relationship of single- and double-strand breaks and their repair to mitotic recombination in *Aspergillus*.

We wish to thank Guy L'Heureux for excellent preparation of the photographic illustrations. The main results presented here were obtained by S.-K Chae in partial fulfillment of the requirements for the Ph.D. degree. This project was supported by operating grant A2564 of the Natural Science and Engineering Research Council of Canada.

2.6. ADDITIONAL RESULTS NOT SHOWN IN THE PUBLICATION

2.6.1. 4-NQO sensitivities of known epistatic uvs *pairs*

To check whether tests for epistasis could be carried out equally well with 4-NQO as previously done with UV light, all viable pairs of *uvs* mutations of the three established groups were tested for 4-NQO survival and compared to that of single mutant strains. Each of these pairs, i.e., *uvsF;uvsH*, *uvsC;uvsE*, and *uvsB;uvsD* double mutant strains, showed practically identical 4-NQO survival as the most sensitive component single mutant of each pair (Figs. 2.5.A-C; reported as "data not shown" in the publication). This confirmed epistatic pairs which were previously grouped based on UV-sensitivity (Kafer and Mayor 1986).

2.6.2. Synergism between uvsA *and UvsF group genes for MMS-sensitivity*

Even though *uvsA* and *uvsI* mutants showed wild type levels of sensitivity to MMS, unexpectedly extreme synergism for MMS sensitivity has been observed in *uvsA;uvsI* double mutants (see Fig. 2.3.B). Moreover, double mutants of *uvsI* with members of the UvsF group exhibited higher sensitivity than the more sensitive single mutants (i.e., *uvsF* or *uvsH* mutant strains, in this case). Therefore, it was of interest whether for *uvsA* this also might be the case. Double mutants of *uvsA* with members of the UvsF group were tested for MMS survival. The results showed that double mutants of *uvsA* with two UvsF group genes, *uvsF* and *uvsH*, exhibited extreme synergism for the killing effect of MMS (Fig. 2.6.A,B). Increased sensitivity was also evident for all

Fig. 2.5. 4-NQO survival of double mutants combining pairs of *uvs* mutations previously assigned to the same epistatic groups on the basis of UV sensitivity.

A. Double mutants of *uvsF* with *uvsH*;

B, *uvsC;uvsE* double mutants;

C, *uvsB* with *uvsD*.

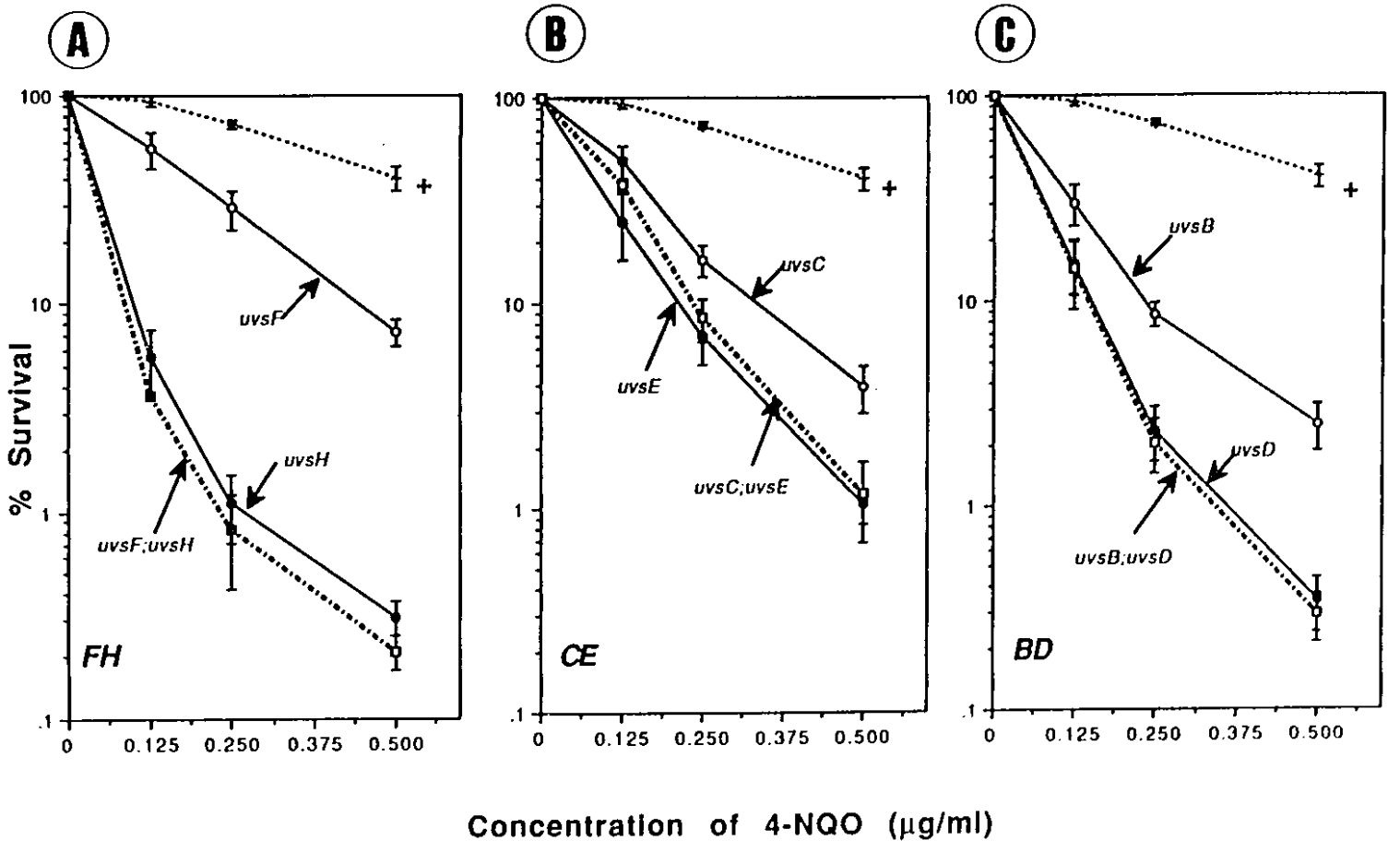
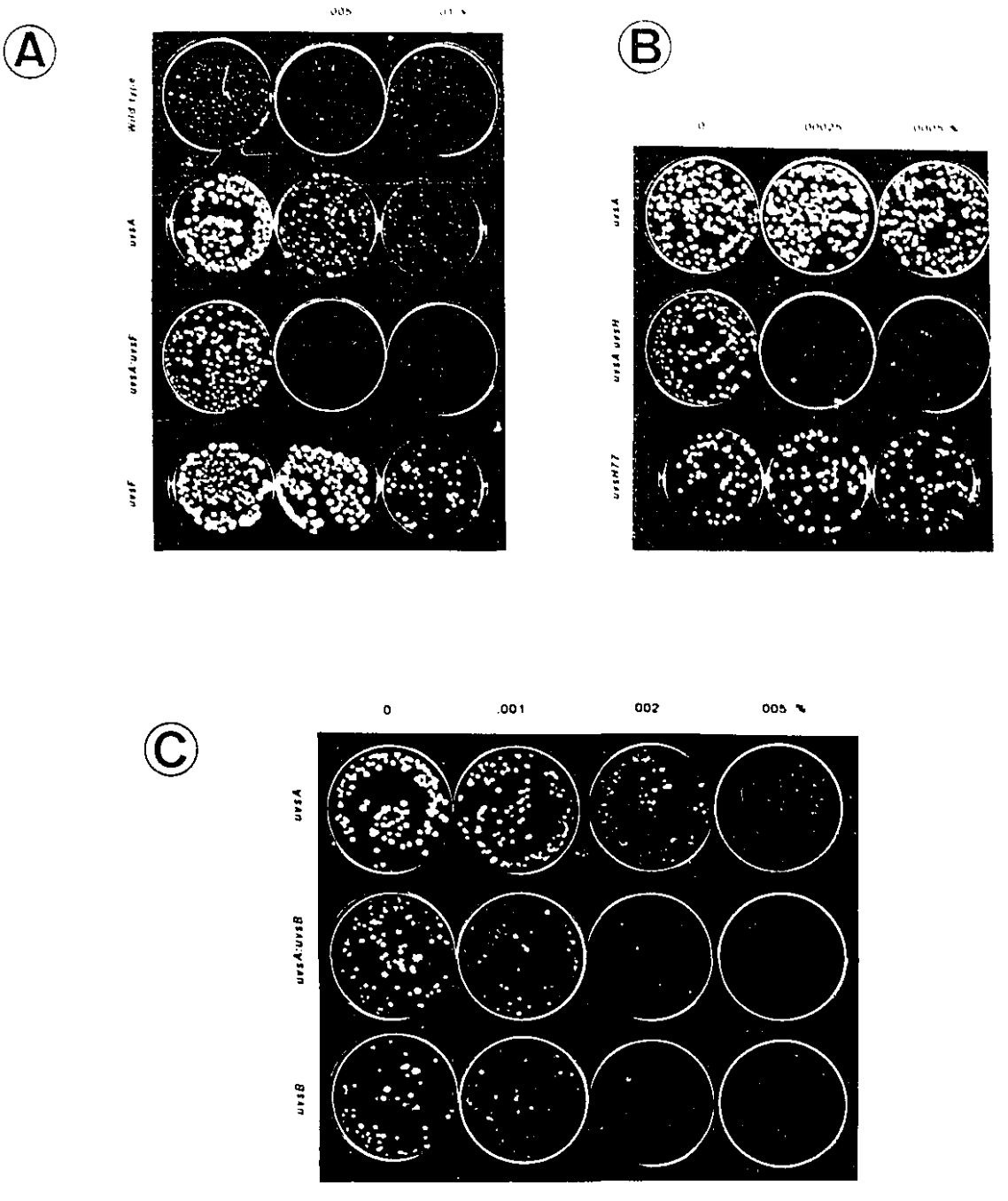

A
B
C
100
10
1
.1
% Survival
+
uvsF
uvsH
uvsF;uvsH
FH
uvsC
uvsE
uvsC;uvsE
CE
uvsB
uvsD
uvsB;uvsD
BD
0
0.125
0.250
0.375
0.500
Concentration of 4-NQO (µg/ml)

Fig. 2.6. Colony formation on MMS media (concentration as indicated) of *uvsA* in double mutant strains with *uvs* of 3 epistatic groups compared to wild type and single mutant strains.

A, with *uvsF* and **B**, with *uvsH*, both of "*uvsF*" epistatic group;

C, *uvsA;uvsB* double mutant strains.

Genotypes as indicated in the figures.


A
B
C
0
.001
002
005 %
uvsA
uvsA:uvsB
uvsB

segregants which were identified as double mutants by linked markers, or in complementation tests from heterozygous crosses (i.e., for double mutants of *uvsA* with *uvsF*) or diploids (i.e., heterozygous for *uvsA* and *uvsH*).

On the other hand, double mutants of *uvsA* with *uvsB* and *uvsC* exhibited similar survival to that of *uvsB* or uvsC single mutant strains, respectively (shown for *uvsA;uvsB* in Fig. 2.6.C). These results are expected since *uvsA* mutants alone are barely sensitive to MMS at the concentrations applied here.

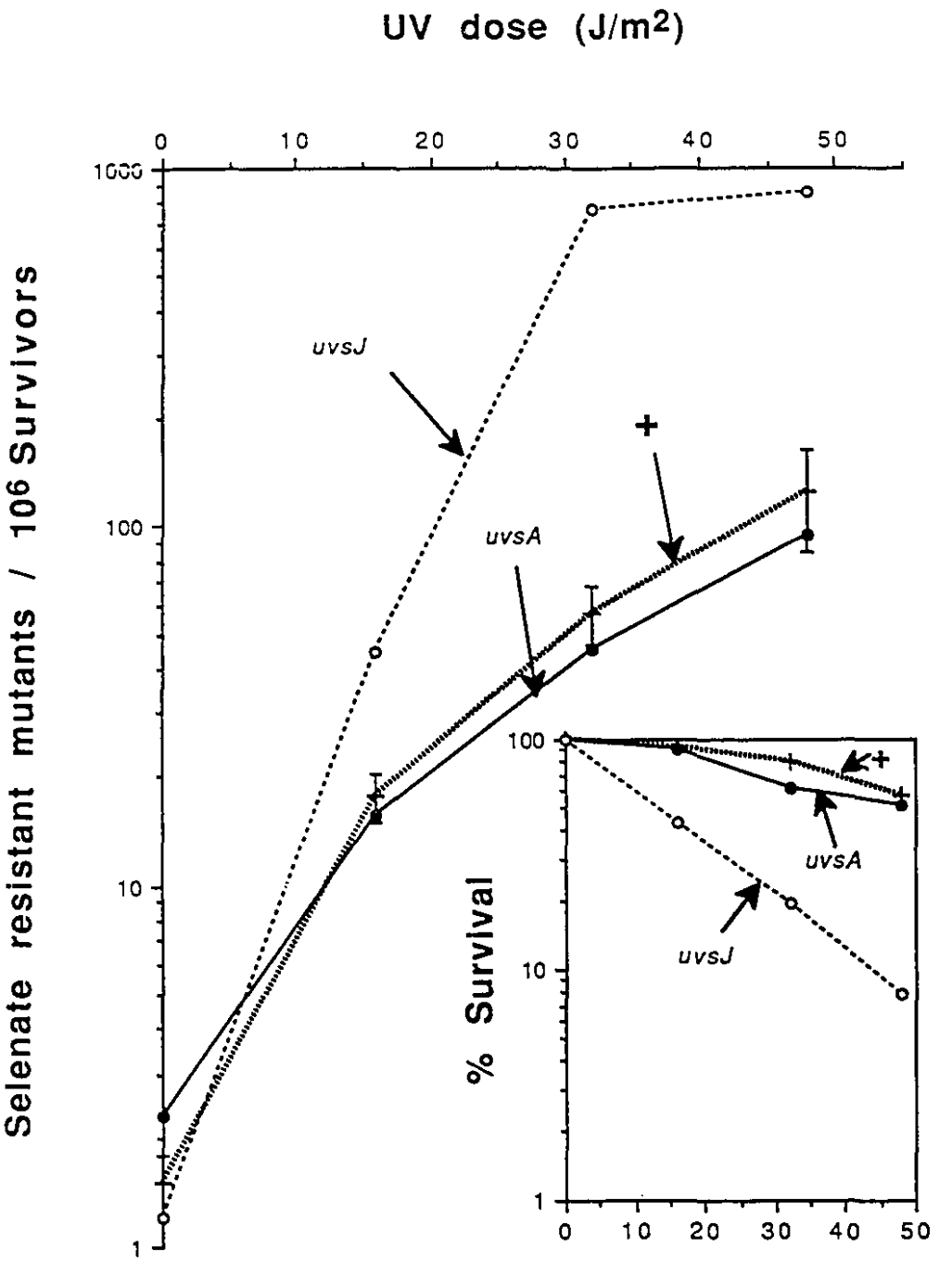
Overall, the *uvsA* gene did not belong to either of the UvsF and the UvsI groups based on MMS sensitivity. This result agreed well with earlier results found with UV and 4-NQO treatment. However, possibility of the *uvsA* gene as a member of the UvsB group could not be ruled out in this test.

2.6.3. Forward mutation to selenate resistance in uvsJ *and* uvsA *mutants*

To compare the effects of *uvsJ* and of *uvsA* on mutation to those of other *uvs* mutations using the same mutational system, selenate resistant mutation frequencies were measured (for selenate resistant mutations in all other *uvs* mutations except *uvsI*, see Kafer and Mayor 1986; for *uvsI* and detailed methods for selecting selenate resistant mutation, see chapter 4).

In *uvsJ* strains, spontaneous mutation frequencies did not deviate from those of uvs^+ strains, while higher increases of selenate resistant mutation frequencies than those in uvs^+ strains were evident after UV treatment (Fig. 2.7). This result supports the assignment of *uvsJ* to the UvsF group. All *uvs* of this group showed increases of UV-induced selenate resistant mutations (i.e., *uvsF* and *uvsH*; Kafer and Mayor 1986). This finding led to the proposal that UvsF group genes may be involved in excision repair,

Fig. 2.7. Tests of *uvsJ* and *uvsA* mutations for effects on UV mutagenesis: Frequencies of UV-induced selenate-resistant mutations in *uvs*$^+$, *uvsJ*, and *uvsA* strains and corresponding survivals (see insert).


UV dose (J/m2)
0
10
20
30
40
50
1000
100
10
1
Selenate resistant mutants / 10^6 Survivors
uvsJ
uvsA
+
100
10
1
% Survival
uvsA
uvsJ
+
0
10
20
30
40
50

since enhanced mutation frequencies after UV irradiation are one of the typical characters for excision defective mutants in *E. coli* and yeast.

In contrast, *uvsA* mutant strains showed wild type levels of mutation both spontaneously and after UV treatment (Fig. 2.7). The *uvsA* gene was tentatively assigned to the UvsC group based on UV sensitivity. However, the effect of *uvsA* on mutation appeared to differ from that of *uvsC* and of *uvsE*, members of the UvsC group. *uvsC* and *uvsE* mutants showed mutator effects (Jansen 1972) and practically lack of UV-induced mutations (Kafer and Mayor 1986; also see chapter 4). However, such properties may not be common for all members of the UvsC group. Recently, *musN* mutation was shown to belong to the UvsC group based on sensitivities to 4-NQO and MMS (see chapter 3). Moreover, *musL*, *musK*, and *musP* mutations also likely are members of the UvsC group when tested for MMS-survival. Similar to *uvsA* mutants, these *mus* strains exhibited wild type levels of mutation for selecting selenate resistance (see chapter 3, Figs. 3.10 and 3.11). Thus, the UvsC group presumably contains members heterogeneous for effects on mutation. Considering properties of *uvsA* which were similar to those of *uvsC* and of *uvsE* strains (see section 2.5, "Discussion"), the *uvsA* gene might belong to the UvsC group. However, for further functional grouping of *uvsA*, tests for recombination are required, since all members of the UvsC group were shown to affect mitotic recombination (for *uvsC* and *uvsE*, Kafer and Mayor 1986; for *musN and musL*, Zhao and Kafer 1992).

2.6.4. *Effects of* uvsI *on mitotic recombination and fertility*

Effects of the *uvsI* mutation on the frequencies of intra- and intergenic mitotic recombination were examined in a diploid strain homozygous for *uvsI* (D3119; for genotype see Table 2.2). This diploid has the same genetic markers crucial for recombination assessment as present in the control *uvs*$^+$ diploid (D2752b; Zhao 1991)

Table 2.2. Effects of *uvsI* mutation on spontaneous intra- and intergenic mitotic recombination

Diploid homozygous for (Diploid No.)	Intragenic $ad^+ \times 10^{-6}$	Intergenic No./100 colonies
uvs$^+$ (D2752b)[b]	4.3 ± 0.7 [a]	4.8 [a]
uvsI (D3119)[c]	3.9 ± 0.4	3.5 ± 0.5

[a] From Zhao (1991)

[b] Genotype: *pabaA6 yA2 adE8; riboB2 chaA1* (M2890) /
SulA1 anA1 adE20 biA1; wA2 cnxE16; ActA1; nicA2; sbA3; fwA2 (M3629)

[c] Genotype: *pabaA6 yA2 adE8; uvsI501; pyroA4; sbA3; chaA1* (M3587) /
SulA1 anA1 adE20 biA1; wA2 cnxE16; uvsI501; nicA2; sB3; choA1; fwA2 (M3740)

which are heterozygous for color markers, *w* (white), *fw* (fawn), *y* (yellow), and *cha* (chartreuse). Intragenic recombinants were selected as adenine prototrophs from these *adE8/adE20* diploids on minimal medium, while color sectors from the same diploids were counted for the frequencies of intergenic recombination (Zhao and Kafer 1992; see Zhao 1991 for details of the methods).

The intragenic recombination frequencies, i.e., frequencies of adenine prototrophs, of *uvsI* strains were not significantly different from those of *uvs*$^+$ strain ($P > 0.1$: Table 2.2). In addition, the frequencies of intergenic recombination, i.e., color sectors, in *uvsI* mutants exhibited wild type levels (Table 2.2). In conclusion, the *uvsI* mutation did not affect the frequency of spontaneous intragenic and intergenic mitotic recombination (reported as "unpublished results" in the publication).

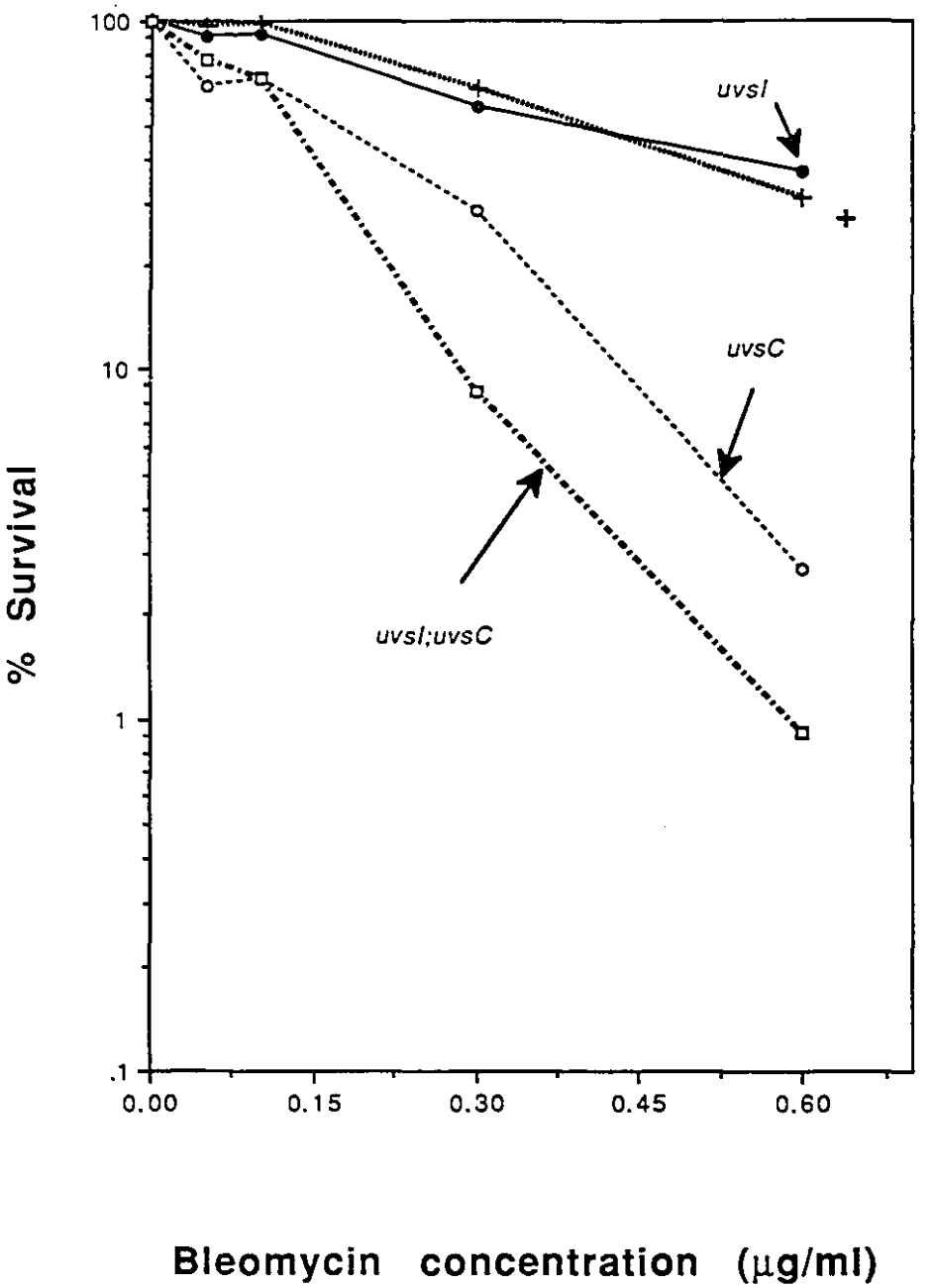
uvsI mutants exhibited normal fertility in crosses either homozygous or heterozygous for *uvsI* mutation. Effect of *uvsI* on meiotic recombination was shown to be normal in crosses homozygous for *uvsI* mutants, when map distances of several markers at the left arm of chromosome I (i.e., *fpaB*, *galD riboA*, and *anA*) were calculated and compared to standard map unit (Chae 1986).

2.6.5. Synergism between uvsI and uvsC for sensitivity to bleomycin and EMS

During the course of mutational studies for *uvsI* and *uvsC* strains, further synergistic interactions of *uvsI* with *uvsC* were observed for sensitivity to bleomycin (Fig. 2.8) and EMS (see chapter 4, Fig. 4.6), even though *uvsI* mutants were not more sensitive than *uvs*$^+$ to both mutagens. However, in the case of bleomycin treatment, synergism was seen only when growing cells were treated (i.e., plated on bleomycin containing CM), but not when quiescent conidia were exposed in buffer (see Fig. 4.14).

Details of the methods for treatment with bleomycin and EMS are described in chapter 4, section on "Materials and methods".

Fig. 2.8. Bleomycin sensitivity of double mutants of *uvsI* with *uvsC*, compared to component single mutant strains and *uvs*$^+$ control. Conidia were plated onto CM containing various concentrations of bleomycin (as indicated).


100
10
1
.1
% Survival
0.00
0.15
0.30
0.45
0.60
uvsI
uvsC
uvsI;uvsC
Bleomycin concentration (μg/ml)

2.6.6. Influence of growth stage on the killing effect of UV light, and interaction between uvs *mutations when irradiated at the G2 cell stage*

A) UV-survivals of uvs$^+$ *and* uvs *strains at hourly time intervals during germination*

It is well known that the cell cycle stage influences the killing effect of radiation. Survival to UV light and ionizing radiation is generally lowest when cells are at the G1 or early S cell stage and increased to a maximum at the G2 stage for wild type strains of a variety of organisms, e.g., *Saccharomyces cerevisiae* (Brunborg and Williamson 1987; Davies et al. 1978), *Schizosaccharomyces pombe* (Fabre 1973; Barale et al. 1982), and Chinese hamster cells (Gillespie et al. 1975). It has been suggested that the greater resistance of G2 cells to radiation is due to duplicated copies of DNA and genes. In addition, there exists a recombinational repair process which is efficient only when a cell contains duplicated DNA (Burnborg and Williamson 1978; Gentner 1981).

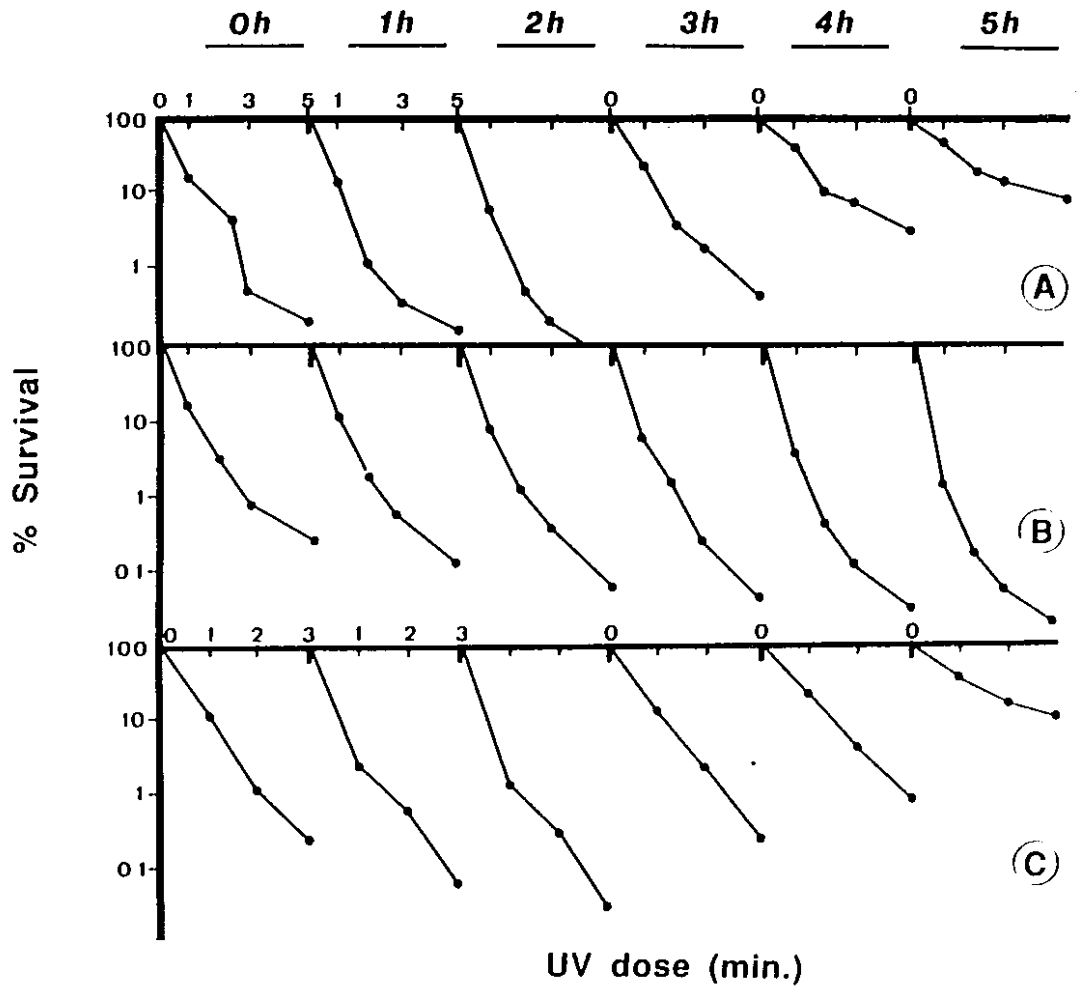
Asexual spores of *A. nidulans* are arrested at G0 cell stage and contain a single nucleus per conidium. When conidia are placed on an appropriate medium (CM), almost synchronous germination and growth are achieved. In addition, when spores are inoculated into liquid CM, S phase is reached about 2-2.5 hours later at 37° and the first mitosis can be observed after 4 - 5 hours incubation (Bainbridge 1971; Wood and Kafer 1969).

Survival of *uvs*$^+$, *uvsI*, *uvsF*, and *uvsC* strains after UV irradiation was measured at hourly time intervals up to and beyond the first mitosis (up to 5h) during incubation at 37° in liquid CM (see Kafer and Mayor 1986, for details of the methods for UV irradiation).

uvs$^+$ strains showed the lowest sensitivity to UV light at the G1 cell stage and maximum resistance at the G2 phase (Fig. 2.9.A). Thus, this result agreed well with

Fig. 2.9. UV-survival curves of *uvs*$^+$, *uvsF*, and *uvsC* measured at hourly time intervals up to 5 hours during incubation at 37° in liquid CM.

A, *uvs*$^+$; B, *uvsC*; C, *uvsF* mutant strains


0h
1h
2h
3h
4h
5h
% Survival
100
10
1
0 1
A
B
C
UV dose (min.)

those obtained for other organisms. Such a pattern was also observed in *uvsI* and *uvsF* mutant strains (*uvsF* in Fig. 2.9.C; data for *uvsI* not shown). The results for *uvsI* confirmed earlier findings which demonstrated "G2 resistance" to UV light at later times during germination on CM plate (Han and Kang 1985). In contrast, *uvsC* mutants showed continuous increases of UV-sensitivity on the duration of incubation, but quiescent conidia of *uvsC* were not more sensitive than wild type strains (Jansen 1970; also see Fig. 2.9.B). However, only slight increases of sensitivity to UV were apparent during incubation in contrast to earlier results for *uvsC* which exhibited drastic reduction (~100x) of UV-survival after 4 - 5 hours incubation in liquid MM at 37° (Fortuin 1971a).

B) Interaction of uvsI *with genes from other epistatic groups for sensitivity to UV in dividing cells*

Germinating single and double mutant spores were irradiated (preincubated for 4h at 37° on CM plate). During that time, cells are likely at the G2 cell stage. Survival of *uvsI;uvsF* double mutants was nearly the same as that of *uvsF* strains, i.e., the most sensitive single mutant (Fig. 2.10.A). This result suggests epistatic interaction between these genes. This was rather unexpected, because when quiescent conidia were irradiated by UV light, additive interactions were obtained. This perhaps implies that, for DNA repair at the G2 stage, *uvsI* and *uvsF* genes may be involved in the same repair process, while they act differently at the G1 cell stage. However, such a hypothesis might not be applicable for the repair of MMS-induced lesions, since survival for MMS sensitivity was measured in growing cells (i.e., on CM containg MMS) and double mutants of *uvsI* with *uvsF* showed additivity under such a condition. Another explanation might be that apparently similar survival of *uvsI;uvsF* to that of *uvsF* may be due to imperfect synchronization among the cell population. Under such a condition, additivity of survival between *uvsI* and *uvsF* strains may not be easily seen, because not only both *uvsI* and *uvsF* strains exhibited highly increased UV-survivals at this cell stage compared to those

found at the G1 phase but also both survival curves were gradually increased during incubation (see Fig. 2.9.B). Alternatively, different progression of cell stages, i.e., differences in the length of certain cell stage, between these mutants may cause "pseudo-epistasis". In fact, different time-length in certain cell stage was often seen among *rad* mutants of yeast (Fingerhut et al. 1984). However, when UV-survivals of *uvsI* and *uvsF* strains were measured at hourly intervals, they showed a very similar pattern of UV-survival curves during growth (see Fig. 2.9.B). This perhaps imply that in both strains progression of cell stages might be similar to each other. For more precise information on cell growth of mutant strains as well as the question of synchronization, time for doubling of nucleus can and will have to be monitored cytologically with the aid of "DAPI staining" (DAPI=4,6-diamidino-2-phenylindole; for the method, see May 1989).

In the case of the double mutant of *uvsI* with *uvsH*, the second UvsF group gene, increased sensitivity to UV was obtained compared to the most sensitive single, i.e., *uvsH*, mutant strains (Fig. 2.10.B). Such increases were more pronounced in double mutants of *uvsI* with *uvsJ*, the third member of the UvsF group (Fig. 2.11.B). For *uvsH* and *uvsJ* single mutant strains, UV-survivals at this G2 cell stage were barely increased or even slightly decreased compared to that measured at the G1 cell stage. Such a lack of "G2 resistance" in these strains was very distinct from the result of *uvsF* strains in which UV-survival at this cell stage was highly increased (~100x) compared to that at the G1 cell stage (see Fig. 2.9.B). This possibly suggests that *uvsH* and *uvsJ* genes may be involved in a recombinational repair process which presumably differs from that mediated by *uvsC* and *uvsE*, rec$^-$ strains of the UvsC group, while *uvsF* gene might not be. The *RAD1* and the *RAD10* genes of yeast were shown to function in both excision repair and mitotic intrachromosomal recombination pathway which is independent of the *RAD52* gene (Schiestl and Prakash 1990). Mutations in both of these genes in yeast confer X-ray sensitivity as well as UV-sensitivity, which is non-typical among members of the RAD3

Fig. 2.10. Tests for epistatic interactions of *uvsI* with UV treatment of dividing cells: UV-survival of 4h preincubated germinating conidia of single *uvs* strains, compared to double mutants combining the *uvsI* mutation with members of the three epistatic groups

A, *uvsI* with *uvsF*; **B**, *uvsI;uvsH* double mutants; **C**, *uvsI;uvsC* double mutants; **D**, *uvsI* with *uvsB*.

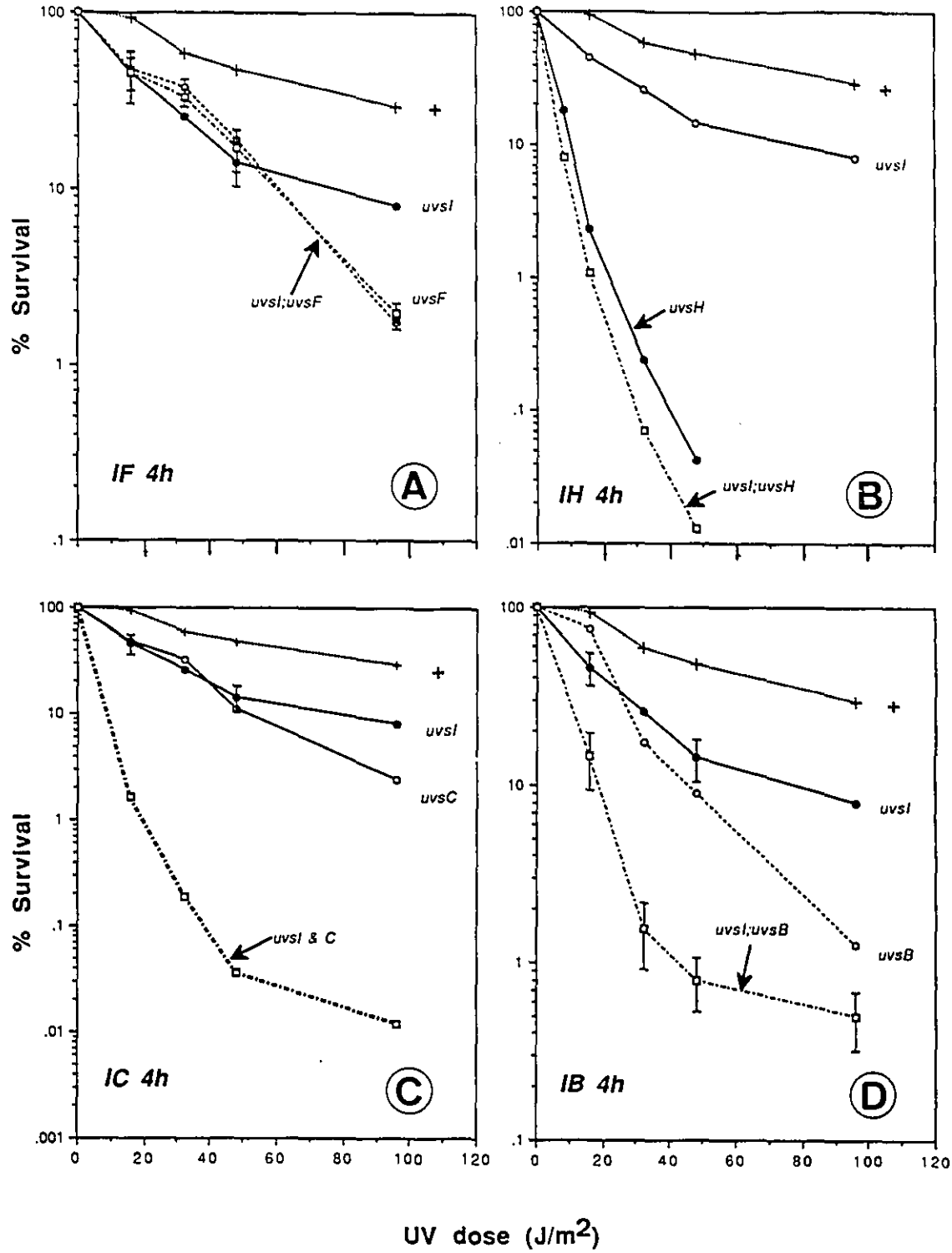
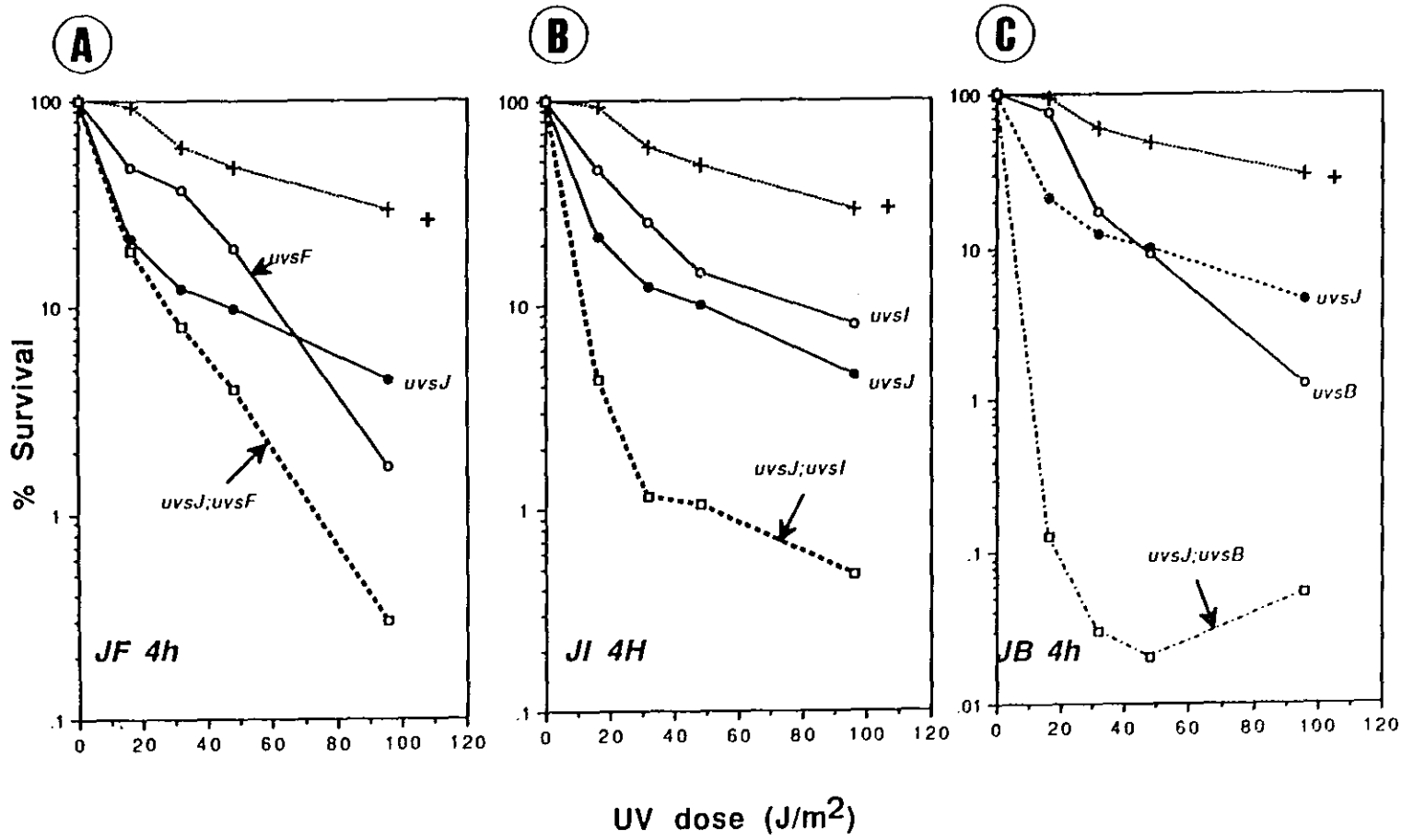

% Survival
100
10
1
.1
.01
.001
+
uvsI
uvsI;uvsF
uvsF
IF 4h
A
uvsH
uvsI;uvsH
IH 4h
B
uvsC
uvsI & C
IC 4h
C
uvsI;uvsB
uvsB
IB 4h
D
0
20
40
60
80
100
120
UV dose (J/m2)

Fig. 2.11. Test of *uvsJ* for epistatic interactions with *uvs* mutations of three groups (interaction with *uvsC* is lethal): UV-survival of 4h germinated conidia (as Fig. 9).

A, *uvsJ* with *uvsF*;

B, *uvsJ;uvsI* double mutants;

C, *uvsJ;uvsB* double mutants.


A
B
C
% Survival
100
10
1
.1
.01
0
20
40
60
80
100
120
uvsF
uvsJ
uvsJ;uvsF
JF 4h
uvsI
uvsJ
uvsJ;uvsI
JI 4H
uvsJ
uvsB
uvsJ;uvsB
JB 4h
UV dose (J/m2)

group (summarized in Haynes and Kunz 1981). Similarly, *uvsH* and *uvsJ* mutants in *Aspergillus* also exhibited sensitivity to γ-rays and UV light (Kafer and Mayor 1986). In contrast to *rad1* and *rad10* of yeast, they showed sterility in homozygous crosses. This indicates that these genes presumably are also required for normal meiosis. These properties of *uvsH* and *uvsJ* strains mentioned above were not found for *uvsF* strains, even though all three genes were assigned to the same epistatic group of the UvsF. In addition, tests for UV-survival in *uvsJ;uvsF* double mutant strains at the G2 cell stage revealed non-epistatic interactions between *uvsF* and *uvsJ* strains (Fig. 2.11.A). This result suggests that these genes may well participate in different repair processes at the G2 cell stage. Unfortunately, UV-survival at the G2 stage of *uvsF;uvsH* and *uvsH;uvsJ* double mutant strains have not yet been carried out.

In the case of the *uvsI;uvsC* and *uvsI;uvsB* double mutant strains, synergistic interactions were observed (Fig. 2.10 C,D; the result of *uvsI;uvsC* strains was reported as "unpublished results" in the publication). Similarly, extreme synergism was seen for *uvsJ;uvsB* double mutant strains (Fig. 2.11.C; *uvsJ;uvsC* pair cannot be treated because of lethal interactions between these genes). These results indicate that *uvsI*, *uvsC*, *uvsB*, and *uvsJ* are non-epistatic to each other.

2.6.7. Changes of survival after liquid holding of UV-irradiated quiescent conidia; "Liquid holding recovery"

Enhanced cell survival after holding of cells in liquid (liquid holding recovery = LHR) in the dark following X-ray or UV irradiation was observed in *E. coli* and also described in yeast (Patrick et al. 1964; reviewed by Haynes and Kunz 1981). Based on findings that many excision defective mutants failed to show LHR, it has been postulated

that LHR is mainly dependent on dimer excision after UV irradiation (Ferguson and Cox 1980). For example, excision defective *rad* mutants in yeast (e.g., *rad1*, *rad2*, and *rad3*) did not exhibit LHR (Parry and Parry 1969; see also "Table 1" in Haynes and Kunz 1981). In addition, *uvr*$^-$ strains of *E. coli* also failed to show LHR, while *rec*$^-$ strains clearly demonstrated LHR (Ganesan and Smith 1969). On the other hand, no changes or decreases of survival (i.e., negative LHR) have been reported in wild type cells of *E. coli*, *Neurospora crassa* (no change) or *Schizosaccharomyces pombe* (negative LHR) after liquid holding (for *E. coli*, Ganesan and Smith 1969; for *N. crassa*, Schroeder 1974; for *S. pombe*, Harm and Haefner 1969). It has been suggested that extensive repair after plating of treated cells even without allowing time for recovery (i.e., liquid holding) might be the reason for negative LHR or for no changes of survival (Haynes and Kunz 1981).

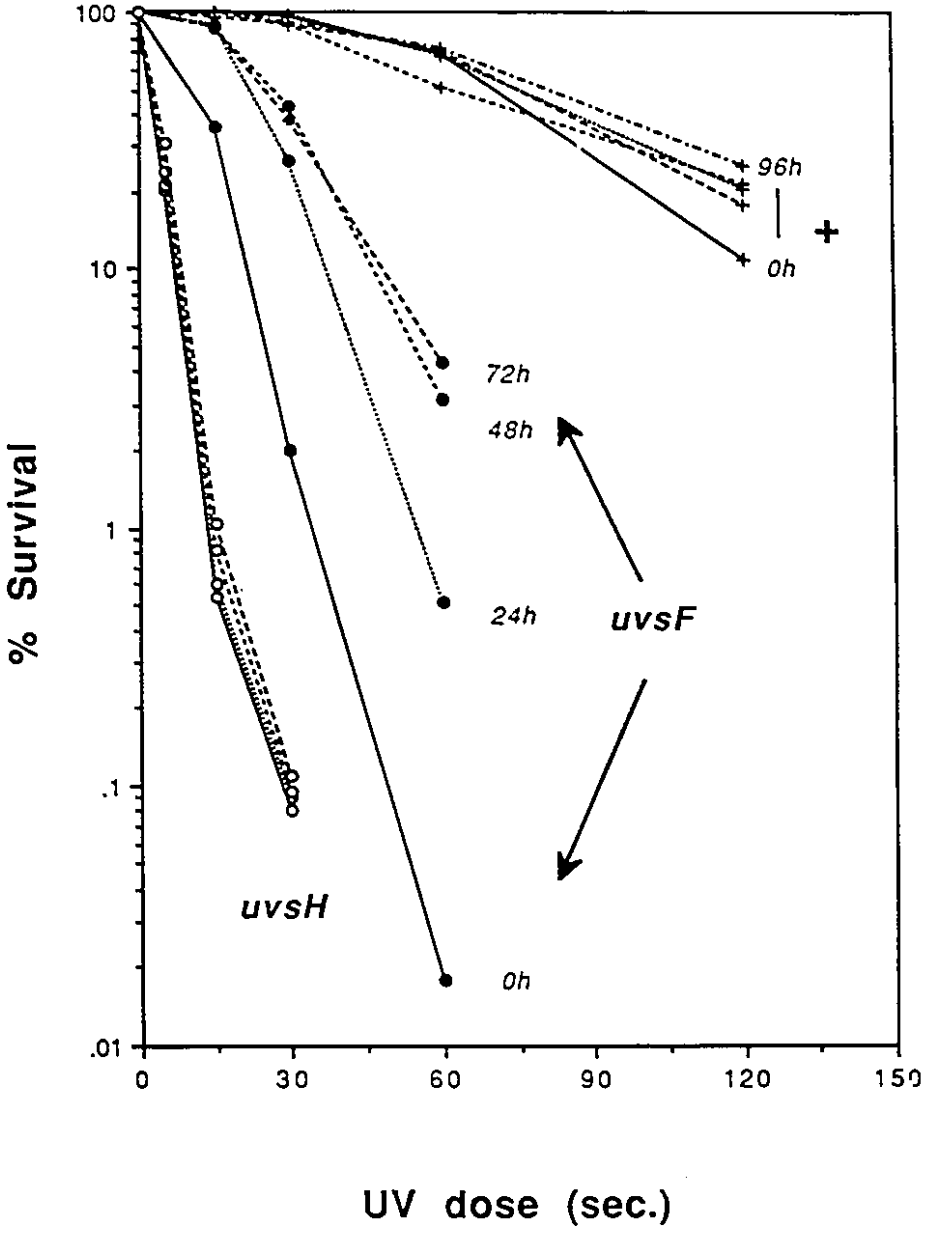
In Aspergillus, no increase of UV-survival of wild type after liquid holding was reported (Wohlrab and Tuveson 1969; confirmed here Fig. 2.12). Some time ago, it had been proposed that UvsF group genes might be involved in excision repair (Kafer and Mayor 1986). This assumption was based on high sensitivity to UV and increased UV-induced mutation in mutant strains of this group compared to *uvs*$^+$ control strains. To obtain further evidence for or against such a hypothesis, *uvsF*, *uvsH*, and *uvsJ* mutants, members of the UvsF group, were examined for their ability to show LHR. UV-irradiated quiescent conidia were held in Na-K-phosphate buffer (0.05 M, pH 7.0) at 18° in the dark, and plated immediately, or at one day intervals up to 3 days (methods for UV irradiation see Kafer and Mayor 1986).

The results of *uvsJ* (not shown) confirmed earlier findings which demonstrated high increases of UV-survival after liquid holding of up to 4 days (Wohlrab and Tuveson 1969). Similarly, enhanced survival (~50x) was obtained for *uvsF* mutants (Fig. 2.12; reported as "unpublished results" in the publication). Ability to show LHR in *uvsF* and *uvsJ* strains suggests that these genes may function in other than an excision repair pathway. However, this result does not completely rule out the possible involvement of

Fig. 2.12. Changes of survival after liquid holding of UV-treated "wild type" *uvs*$^+$ strains (+), *uvsF* (closed circles), and *uvsH* (open circles) mutants.

UV-treated conidia were plated directly (= 0 hour) or held at 18° for 24h, 48h, or 72h (as indicated in Fig.) in Na-K-phosphate buffer (0.05M, pH 7.0).

UV dose rate = 1.6 J/m^2/sec.


100
10
1
.1
.01
% Survival
0
30
60
90
120
150
UV dose (sec.)
96h
0h
+
72h
48h
24h
uvsF
uvsH
0h

these genes in excision repair processes. Some *rad* genes in the RAD3 group of yeast responsible for incision of DNA damage were shown to increase their UV-survival after liquid holding (e.g., *rad4*, *rad10*, and *rad14*; Parry and Parry 1969). Such findings imply that during liquid holding of treated cells more complex situations may occur. Furthermore, LHR may not be entirely dependent on a nucleotide excision repair pathway. Possibly, it is imagined that a base excision repair mediated by UV-endonuclease or other inducible repair processes may play a role in LHR.

In contrast to *uvsF* and *uvsJ* strains, *uvsH* mutants did not exhibit LHR (Fig. 2.12). No changes of survival during liquid holding reflect that the *uvsH* gene may be required for an excision repair pathway. The lack of LHR in *uvsH* strains was rather unexpected, considering the positive result of LHR in *uvsJ* strains. Both strains shared similar characters as mentioned above, i.e., X-ray sensitivity and homozygotic sterility, in addition to common properties among members of the UvsF group. Such characters are non-typical for excision defective mutants of *E. coli* and yeast, and also different from those found for *uvsF* strains. However, *uvsJ* was more close to *uvsF* in terms of abilty to show LHR.

Again as discussed before, such phenotypic differences among *uvsF*, *uvsH*, and *uvsJ*, members of the UvsF group, raise the questions whether UvsF group genes are involved in an excision repair pathway and whether *uvsF* and *uvsH* genes act on the same DNA repair pathway. To obtain more direct answers, first of all, biochemical tests for excision capacity in members of the UvsF group will be required.

CHAPTER 3

Phenotypic and epistatic grouping of hypo- and hyperrec *mus* mutants in *Aspergillus*

3.1. SUMMARY

The radiation-induced mutations, *musK-musS*, which cause increased sensitivity to methyl-methanesulfonate (MMS), were mapped by meiotic recombination, three of them completely linked to translocations breaks. Over half of these mutants were known to be meiotic defective and to affect spontaneous mitotic recombination. As an approach to functional grouping, further phenotypic effects and epistatic interaction with *uvs* mutations were investigated. All *mus* mutants were cross-sensitive to 4-nitro-quinoline-*N*-oxide (4-NQO) but, unexpectedly, none to γ-rays and few to UV light, when quiescent conidia were treated. Double mutants which combined each *mus* with *uvs* mutations of the four epistatic groups of *A. nidulans* ("UvsF", "UvsC", "UvsI" and "UvsB") were compared to single mutant strains for MMS and 4-NQO survival. Of the five *mus* mutations which affect recombination three, *musN, L* and *K*, were found to be epistatic with *uvsC* which is recombination defective. The other two showed synergism with most *uvs* mutations and did not convincingly fit into any group. Three further *mus* mutations showed epistasis or, possibly, no interaction with members of more than one Uvs group, including UvsI in all cases. One of these, *musR*, caused reduced UV-induced reversion for which *uvsI* is defective.

Key words:

DNA repair; MMS-sensitivity; epistatic groups; *Aspergillus nidulans*

3.2. INTRODUCTION

Genetic recombination is one of the most interesting processes, but mechanisms are largely unknown, especially in eukaryotes. To identify genes required for recombination, mutations originally were obtained as strains which showed reduced recombination frequencies in test crosses (e.g., *rec*⁻ in *E. coli*, Clark 1974; or *mei* mutants in *Drosophila*, Baker et al. 1976). In *E. coli*, such mutations frequently caused increased sensitivities to UV or X-rays, and the corresponding genes were shown to be active in recombinational repair pathways. In contrast, uniquely UV-sensitive types were found to be defective in nucleotide excision repair. Mutants within each group were generally epistatic, but inter-group pairs showed synergistic interaction (Howard-Flanders 1968). To obtain recombination-defective mutants, many mutations which caused radiation-sensitivity were therefore isolated in various eukaryotic microorganisms. In yeast, genetic analysis of large numbers of *rad* mutants confirmed the correlation of phenotypic and epistatic grouping and suggested that three groups of genes function in different pathways or processes of radiation repair (Friedberg 1991). Of these, X-ray- but not UV-sensitive types generally are defective in recombination (Cox and Game 1974; Kunz and Haynes 1981).

Tests for sensitivities to chemical mutagens showed that X-ray-sensitive mutants often were also sensitive to MMS (Brendel and Haynes 1973). When MMS-sensitive mutants were isolated, many of them were indeed X-ray-sensitive types and included mutants defective for recombination. For example, over half of the MMS-sensitive strains isolated in *Drosophila* showed reduced survival after treatment with X-rays (Boyd et al. 1981; Oliveri et al. 1990), and similar results were obtained in yeast and *Neurospora* (Prakash and Prakash 1977b; Kafer 1981).

In *Aspergillus nidulans*, many *uvs* mutants were isolated in several laboratories (Babudri and Morpurgo 1988; earlier data summarized by Kafer and Mayor 1986).

Among these UV-sensitive mutants only a few were sensitive to ionizing radiation. UV sensitivity was therefore used to analyze epistatic interactions and three groups of *uvs* genes were identified. Recently this grouping was confirmed in tests using MMS and 4-NQO, to which practically all *uvs* mutants are cross-sensitive. The three groups were called "UvsF", "UvsC" and "UvsB" groups after prominent members. A fourth group was postulated, when *uvsI*, a new type of *uvs* mutant, was not epistatic with any of the three groups (Chae and Kafer 1993). One of these groups, the UvsC group, includes genes required for recombinational repair, but *uvsC* mutants also show increased spontaneous and decreased UV-induced mutation. The *uvsI* mutation, which also is deficient in UV mutagenesis, interacted synergistically with *uvsC*, and is probably involved in a minor specialized process of mutagenic repair. The function of the other two groups is even more uncertain and for none of the groups is there a clear correspondence to any of the epistatic groups in yeast.

To obtain null-mutants with effects on recombination, suitable for epistatic grouping and gene cloning, MMS-sensitive mutants had been induced by γ-rays (Kafer and Mayor 1986). Over half of these *mus* mutants were sterile in homozygous crosses. When analyzed for spontaneous mitotic recombination, five of them were found to decrease or increase inter- and/or intragenic recombination (Zhao and Kafer 1992).

A major objective of the project reported here was therefore to investigate further the phenotypic and epistatic grouping of the *mus* mutants. All *mus* mutants were tested for cross-sensitivity to radiation and to 4-NQO, and epistatic relationships with members of the four Uvs groups were determined. Meiotic mapping was completed and the *mus* mutants were checked for effects on spontaneous and UV-mutagenesis.

3.3. MATERIALS AND METHODS

3.3.1. Strains

Information on the origin of *uvs* mutations and on the isolation of the radiation-induced *mus* mutations has been summarized by Kafer and Mayor (1986). Useful *mus* strains with various markers have been deposited at the Fungal Genetics Stock Center (FGSC Nos. A840-A847; University of Kansas Medical Center, Kansas City, KS 66103, USA). Genotypes of the *uvs* strains, which are isogenic for 6-7 of the eight chromosomes, were described recently (Chae and Kafer 1993). Similar *mus* strains were constructed for tests of UV-induced mutation (Chae 1993).

3.3.2. Media and genetic mapping

Standard media and general genetic techniques were used, as modified from Pontecorvo et al. (1953) and described by Kafer (1977, "Appendix", available on request). For genetic mapping of *mus* mutations and associated translocations, mitotic analysis of hetero- and homozygous diploids and aneuploids was combined with meiotic analysis. Various features specific for Aspergillus have been described recently (Zhao and Kafer 1992). To identify translocations associated with *mus* phenotypes, and to eliminate all others, *mus* segregants from the original mapping diploids were crossed for several generations to translocation-free strains with potentially linked markers. Overall, this involved 6-8 generations for *musK* and *musN*, and 10-14 for other *mus* mutations. After each cross, progeny were checked for translocations, either by monitoring aneuploid frequencies in backcrosses, or interchromosomal linkages in mitotic haploids from vegetative diploids.

3.3.3. Isolation of double mutant strains

To obtain *mus;uvs* strains, diploids were constructed between *mus* mutants and unlinked members of the four epistatic Uvs groups. Among haploid mitotic segregants, 25% of double mutants are expected. When linked color markers, in coupling or repulsion, identified *mus* or *uvs* segregants, these were preferentially isolated and expected frequencies were increased to 50%. To recover poorly viable types or to establish lethal interactions, at least two diploids were usually analyzed.

When *mus* and *uvs* mutations were located on the same chromosome, double mutants were obtained from intercrosses, often recognized as recombinants with unusual levels of MMS or UV sensitivity. When meiotic linkage was close, crossover types were obtained by selection against linked markers used in repulsion. In all cases, putative double mutants with ambiguous phenotypes were outcrossed or checked in complementation tests.

3.3.4. Methods for determination of mutagen sensitivities

Most procedures were as described by Chae and Kafer (1993), especially survival curves after treatment with UV, 4-NQO and MMS. For example, for UV mutagenesis and UV survival, quiescent conidia were plated and irradiated on the plates with low doses of UV (up to 100 J/m^2). However, for tests of radiation sensitivities of the original *mus* and control strains, quiescent conidia were treated in buffer with UV or with γ-rays, and plated after appropriate dilution. The UV dose rate was 1.6 J/m^2/sec (total dose up to 350 J/m^2) and that of γ-rays, from a ^{60}Co source, about 30 krad/h (Kafer and Luk 1989).

To test the UV sensitivity of many segregants from diploids heterozygous for *uvsI*, conidial suspensions were prepared using one very small loop of conidia in 1 ml (of 0.5% saline with 0.001% Tween 80). For each segregant 0.1 ml was spread on a CM

plate used as untreated control, while a second plate was spread with 0.2 ml and irradiated with UV for 1 minute. The dose administered caused almost no kill of *uvs*$^+$ segregants, but practically no colonies of *uvsI* strains survived.

For 4-NQO survival, two sets of tests (1-3 platings per strain in each set) were carried out at different times. Unfortunately, the resulting data could be compared only within each set, because the newly purchased 4-NQO used for the second set had noticeably higher activity.

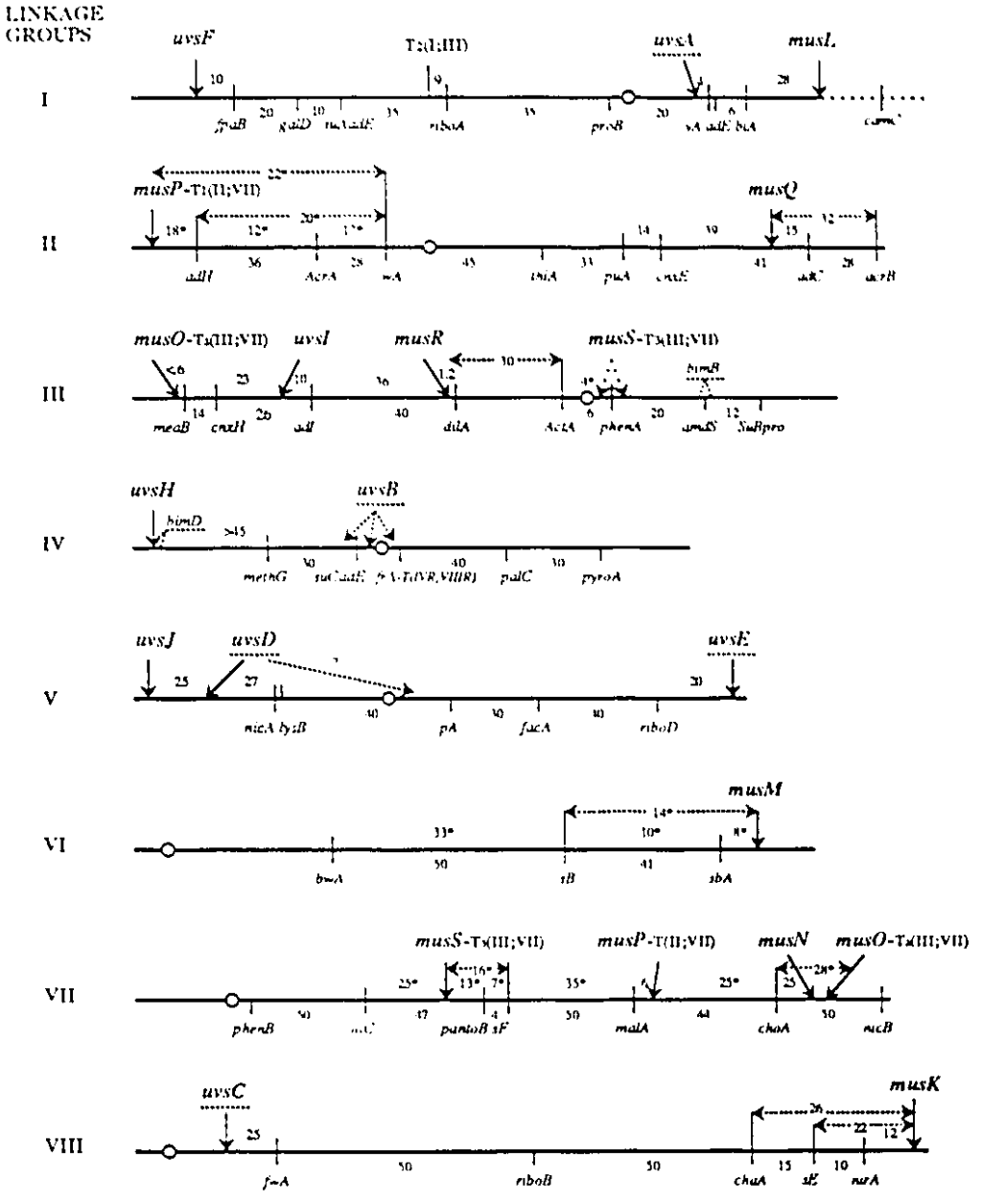
For comparative tests of MMS sensitivity in single and double mutant strains, simultaneous platings of many strains were made on a wide range of MMS concentrations. Conidia from each strain were suspended in saline at approximately the same concentration (one loop in 10 ml) and diluted 10- and 100-fold. From each dilution 100 µl were spread on duplicate MMS plates with 0.01% Na-deoxycholate. The range of MMS in complete medium was either 0.002-0.015%, or 0.00025-0.005% for highly sensitive strains. After 2-3 days of incubation at 37°, approximate size and counts of colonies were recorded, and informative sets of plates were photographed (usually as contact prints, except when negatives were required for documentation).

3.4. RESULTS

3.4.1. Genetic map locations and effects of mus *mutations on fertility*

Extensive mitotic and meiotic analysis located of the genes *musK* - *musS* on the linkage map of *A. nidulans* as shown in Fig.3.1 (summarized in Table 3.2). As expected, induction by γ-rays resulted in induced aberrations. For *musO*, *P* and *S*, translocations

Fig. 3.1. Meiotic linkages of DNA repair genes of *Aspergillus*, especially newly mapped *uvsI*, and *musK* - *musS* genes with associated translocations (for gene symbols see Clutterbuck 1990). Linkage values represent recombination frequencies (in %) between adjacent markers; reduced values from heterozygous aberration crosses are marked with asterisks.


LINKAGE GROUPS
I
uvsF
T3(1;III)
uvsA
musL
II
musP-T1(II;VII)
musQ
III
musO-T3(III;VII)
uvsI
musR
musS-T3(III;VII)
IV
uvsH
uvsB
V
uvsJ
uvsD
uvsE
VI
musM
VII
musS-T3(III;VII)
musP-T(II;VII)
musN
musO-T3(III;VII)
VIII
uvsC
musK

remained associated in many recombinants from several crosses and these *mus* mutations showed meiotic linkage to markers on two chromosomes. In the case of *musP*, unstable duplication progeny from heterozygous crosses identified a practically unidirectional translocation T_1(VII->II). On the other hand, the spontaneous T_2(I;III) translocation was separated from all *mus* mutations and traced to the strain of origin (M2499 = FGSC A605), as confirmed by pulsed field gel electrophoresis (Brody et al. 1991).

Several genes could be mapped accurately by close linkage to known genes (e.g , in group III, *meaB*, *dilA*, and *ActA* close to *musO*, *R*, and *S* respectively, and in VII, *malA* to *musP*; Fig. 3.1). In most other cases, consistent recombination frequencies to adjacent markers identified the map location unambiguously (as shown in Fig.3.1). As is usual for our closely related "Glasgow" *Aspergillus* strains, recombination values were very uniform (e.g., for *musK* distal on VIII R all linkage values shown in Fig.3.1 had standard errors of 0.1%). Only *musL* was difficult to map, because allele ratios were very poor and samples were nonrandom.

In addition, the recently analyzed *uvsI* mutation was mapped in detail on III R (Table 3.1; Fig.3.1). The recombination frequencies obtained (6% with *sC* and 10% with *adI*) placed *uvsI* distal to *sC* in linkage group III, and agreed with previous results (*sC* distal by 4% to *adI*; Kafer 1977).

Of the eight *mus* mutations of *Aspergillus* analyzed here, over half were sterile and produced neither viable ascospores in homozygous crosses nor selfed cleistothecia, even though *A. nidulans* is homothallic (Table 3.2). When analyzed for effects on spontaneous mitotic recombination (Zhao and Kafer 1992), two of the *mus* mutations caused decreases (*musL* and *musK*), and three others increases (*musN*, *Q* and *O*). In Tables 3.2 and 3.3, the eight *mus* mutants are grouped accordingly, and their phenotypes are compared to those of the *uvs* mutants used here (Table 3.2).

Table 3.1. Meiotic mapping of *uvsI:* Linkage to *sC* and *adI* on the left arm of chromosome III

Random samples of ascospores from two crosses

Cross No.	Parental haploid strains: Stock No	Genetic markers and crossover intervals: *cnxII*	I	*uvsI*	II	*sC*	III	*adI*	IV	*methII*	No. and frequencies of progeny (pairs of types): Parentals $P_1 + P_2$	Single crossovers: distal	Single crossovers: proximal	Double CO	Total
3019	2158	$cnxII_3$		+		sC_{12}		+		+	64 + 48	14 + 18	2 + 3	2 + 2	153
	3582	+		$uvsI_{501}$		+		+		+	73%	21%	4%	3%	
3020	2187 +	+		+		adI_{50}				$methII_2$	47 + 61	5 + 6	36 + 37	5 + 5	202
	A 838	+		$uvsI_{501}$		+		+		+	53%	5.5%	36%	5%	

	Intervals:	I	II	III	IV		I	II	II + III	IV
						Recombinants	36	9	21	83
						Total	153	153	202	202
Genetic distances (cM):		23.5	6	4	41	Frequencies :	22.5%	6%	10%	41%

TABLE 3.2. Map location, fertility and mutagen sensitivity of *mus* and *uvs* mutations

+ = like wild type; ±, -, --, --- ---- = increasingly sensitive

Genes and alleles	Map location Chromosome arm or translocation[a]	Fertility in homozygous crosses	Mutagen sensitivity (x = dose response factor) Quiescent conidia treated UV[b]	4-NQO[c]	Growing cells MMS[c]
Hypo-rec mus[d] and uvs[b]					
musL222	I R	none	±[b]	-- (3x)	--- (5-6x)
musK228	VIII R	normal	+	-- (3x)	- (<2x)[c]
uvsC114 or *E182*	VIII R	none	+[b]	-- (4x)	--- (6x)
Hyper-rec types[d]					
musN227	VII R	none	+	- (< 2x)	-- (2-3x)[c]
musQ230	II R	none	+	-- (3x)	-- (2-3x)
musO226	T2(III;VII)	none	- (1.3x)	-- (5x)	-- (2-3x)
uvsF201	I L	normal	--- (6x)	-- (2x)	-- (3-4x)
uvsH77	IV L	none	---- (12x)	---- (15x)	---- (10x)
uvsB221 or *D153*	IV C	poor	-- (3-4x)	--- (7x)	--- (7x)
Mutants with normal recombination					
musP234	T1(VII->II)	none	±[b]	-- (4x)	- (<2x)[c]
musR223	III L	normal	+	-- (3x)	-- (2x)
musS224	T3(III;VII)	normal	+	-- (3x)	-- (3-4x)
uvsI501	III L	normal	--- (8x)	--- (9x)	+

[a] Map locations and meiotic linkages in Fig. 3.1.

[b] See Fig. 3.2; published results included, especially for *uvs* mutations (Kafer and Mayor 1986; Chae and Kafer 1993); *uvsC* is not UV sensitive in tests of quiescent conidia (Jansen 1970); at low dose of UV *musL* and *musP* are not more sensitive than wild type.

[c] Based on results of Figs. 3.3-3.6; at higher levels of MMS, *musK* and *P* are relatively more and and *musN* less sensitive.

[d] Results from tests of *mus* effects on spontaneous mitotic recombination (Zhao and Kafer 1992).

3.4.2. UV-mutagenesis and radiation sensitivity of mus mutants

Eight *mus* mutations were tested for effects on spontaneous and UV-induced mutation. All *mus* strains showed wild type levels for forward mutation to selenate resistance. Similar tests for reversion of *choA1*, for which *uvsI* is defective, identified slightly but consistently lower levels of UV-induced reversion for *musR* (Chae 1993).

Cross-sensitivity to ionizing radiation and UV light was assessed from survival curves as summarized in Table 3.2. Quiescent conidia were treated and the obtained results indicate that none of the *mus* mutants were sensitive to γ-rays (for data, see Zhao 1992). Furthermore, after UV-treatment, practically all *mus* mutants showed survival curves with shoulders and wild-type levels of survival at low UV-doses. Exceptions were two MMS-sensitive mutants which are allelic with *uvsB* (*uvsB221* and *B233*). In addition, at high UV- dose levels, differences from wild type were seen for three mutants, *musL*, *P*, and *O*. Two of them, *musL* and *musP*, showed considerable and very similar UV sensitivity at the highest dose (4 min. exposure, or about 380 J/m^2; for data, see Zhao 1992). For both these mutants normal UV survival at low dose (up to 100 J/m^2) was confirmed in tests with different strains and experimental procedures (Chae and Kafer 1993). Such tests also confirmed slight but consistent UV sensitivity for *musO* even at fairly low UV dose (down to about 50 J/m^2; Chae 1993).

3.4.3. Viability interactions and recovery of double mutant strains

Isolation of some *mus;uvs* double mutant strains turned out to be difficult or impossible, as previously found for *uvs* double mutants. "Lethal" interactions were assumed when practically no double mutants but a considerable number of both single mutant parents were recovered; e.g., in Table 3.3, for *musN;uvsF*, recovery was **0** /

62 *uvsF* + 97 *musN* /219 total tested. In the case of *uvsI*, small numbers of double mutants often were the result of meiotic linkage in intercrosses.

As shown in Tables 3.3 and 3.4, more than half of the *mus* mutants (5/8) were found to be lethal with *uvsF*. Two of these *mus* mutations also were semilethal when combined with *uvsH*, the second member of the UvsF group (indicated as "±lethal" in Table 3.4). In such cases, either only a few of the expected numbers were obtained (<5%) or poor growth was correlated with reduced recovery (usually less than half of the expected frequency) as frequently found for *uvsB* double mutants.

Interestingly, the opposite type of interaction of some *mus* with UvsB group mutations was also observed. Namely, "rescue" from the low growth rate and poor viability caused by UvsB group mutations was observed in *mus;uvsB* (or *D*) strains (Table 3.4). In such cases, double mutants were considerably (3-8x) more frequent than single mutant *uvsB* (or *D*) strains (e. g., for *musR;uvsB*, the recovery was **8** double mutants / 1 *uvsB* + 34 *musR* / 82 total haploids, i.e., >>100% in Table 3.3). The same type of effect was observed also in triple mutant strains, for example, growth rate and conidiation improved when *musN* was incorporated into double *uvs* strains containing a UvsB group mutation. Among haploids from such triply heterozygous diploids, *musN* triple and *N*-double mutant segregants were 2-4 times as frequent as expected (e.g., 18 *musN;uvsC;D* + 66 *musN;uvsD* / 1 *uvsC;D* + 19 *uvsD* / 327 total).

3.4.4. Quantitative assessment of sensitivities to 4-NQO and MMS, and of interactions between mus *and* uvs *mutations in double mutant strains*

As found for *uvs* strains (Chae and Kafer 1993), all *mus* mutants were cross-sensitive to 4-NQO, but none of the *mus* strains were highly sensitive (Table 3.2; Fig.3.2). Furthermore, most *mus* strains showed moderate sensitivity to MMS and only

TABLE 3.3. Recovery of double mutants, as percent of the less viable single mutant strains, among haploids from diploids heterozygous for unlinked *uvs* and/or *mus* mutations (or from intercrosses)[b]

[Number of the less viable, often selected[c], single mutant (No.) and total tested (.T) shown in brackets]

mus gene and linkage	Uvs epistatic groups and *uvs* gene members, and chromosome location									
	UvsF				UvsC		UvsI		UvsB	
	uvsF I		*uvsH*[a] IV		*uvsC* or *E*[a] VIII or V		*uvsI* III		*uvsB* or *D*[a] IV or V	
	%	[No T]	%	[No T]	%	[No T]	%	[No T]	%	[No T]
musL I	0	[11[b] 150]	54	[56[c] 225]	35	[52[c] 133]	>100	[0 130]	12	[157[c] 302]
musK VIII	0	[50[c] 50]	1	[100[c] 110]	33	[15[ac] 91]	23	[43[c] 60]	28[d]	[69[c] 105]
musN VII	0	[62 219]	1	[26 108]	47	[158[c] 158]	75	[87 231]	>100[f]	[38 330]
					>100	[61 437][a]			>>100[f]	[19 271][a]
musQ II	0	[71[c] 112]		----	22	[9 45]	43	[12[c] 50]	3[d]	[90 380]
musO III;VII	47	[15 123]	27	[58[c] 113]	0	[14 80]	(10)[e]	[10[b] 114]	29[d]	[14 132]
musP II;VII	0	[92 333]	12[d]	[26 187]	46	[185[c] 294]	78	[54[c] 183]	>100[f]	[24 280]
musR III	44	[43[c] 120]	75	[12 42]	50	[4[c] 8]	(100)[e]	[10[b] 150]	>>100[f]	[1 82]
musS III;VII	90	[20 104]	100	[4 16]	39	[75[c] 80]	(12)[e]	[16[b] 157]	37[d]	[8 57]

[a] Generally, for UvsF mutations from both, but for other groups only from one gene were used, except for *musN* which was combined with all *uvs* mutations listed.

[b] Recombinants from intercrosses because *mus* and *uvs* map to the same chromosome

[c] Selected for *mus* or *uvs* by phenotype or linked marker

[d] Reduced growth and/or conidiation

[e] Unusual frequency because of meiotic linkage

[f] Rescue from *uvsB* poor phenotype.

one mutant, *musL*, was highly sensitive (Table 3.2; and Fig.3.3.**B**, which illustrates MMS effects on growth rate as well as survival).

In Table 3.4 interactions are classified either as epistatic, when double mutants are no more sensitive than the respective single mutant strains, or as synergistic/additive when double mutants are clearly more sensitive. These classifications are based on survival after 4-NQO treatment (Fig.3.2) and on results from two types of MMS tests (Figs. 3.3-3.5).

Most results obtained for relative sensitivities to 4-NQO are shown as survival curves in Fig.3.2 and summarized in Table 3.4. Only a few cases listed are not shown; occasionally simultaneous controls did not match the bulk of the data; or levels of survival were out of line, because much of the relevant control data had been obtained later, when a more active sample of 4-NQO was used (e.g., for *musP;uvsC* and *musK;uvsE* cases which are questionable are so indicated in Table 3.4). One complete set of early results is shown in Fig. 3.2.**B**.

For each of the *mus;uvs* double mutant strains, MMS sensitivity was judged in two types of tests. i) When single and double mutant strains were isolated, all segregants were tested for MMS using mass transfers of conidia. In such cases, double mutants identified by linked markers always showed uniform MMS sensitivities which frequently were higher than those of single mutant segregants (Fig. 3.5) or in a few cases were unexpectedly lower. ii) Subsequently, the best matched sets of strains were plated onto MMS media for quantitative assessment. In the case of *musN*, survival curves were produced (Fig.3.4) using sets of strains which were isogenic for 6-7 of the eight chromosomes (Chae and Kafer 1993). Alternatively, for the other seven *mus* mutations, sets of well-matched strains were simultaneously plated on a wide range of MMS concentrations (recorded photographically; Fig.3.3). Because *uvsI* strains are not sensitive to MMS, only positive results are listed in Table 3.4 (shown for *N-uvsI* in Fig.3.4).

Fig. 3.2, A - I: Survival curves after treatment with 4-NQO of *mus;uvs* double mutants and respective single *mus* and *uvs* strains. Average values based on 2-3 experiments, more where standard errors are indicated

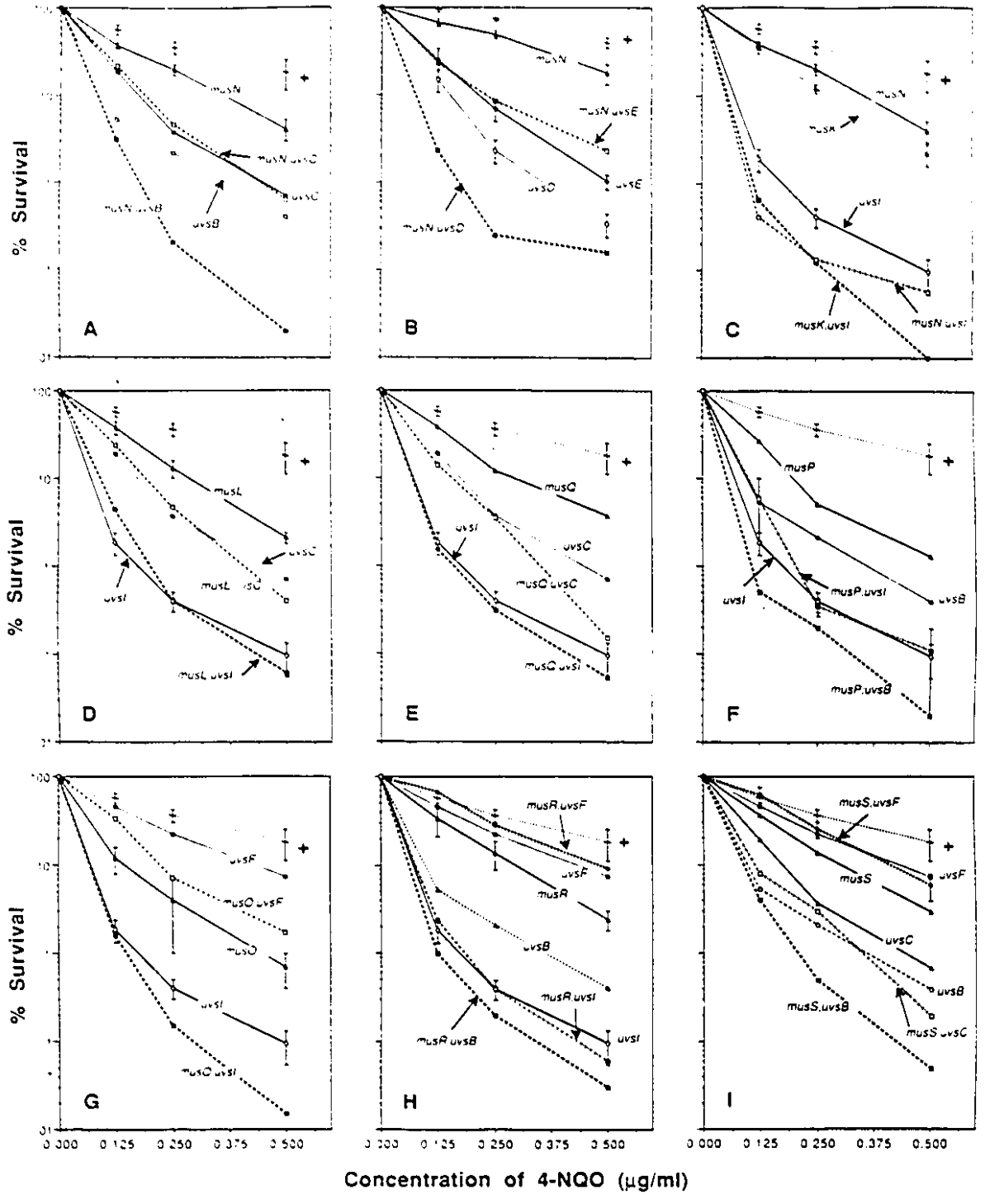

% Survival
A
B
C
D
E
F
G
H
I
musN
musN uvsC
musN uvsB
uvsB
musN uvsE
uvsE
uvsC
musN uvsC
musR
uvsI
musK,uvsI
musL
musL uvsC
musL uvsI
musQ
musQ uvsC
musQ uvsI
musP
musP,uvsI
musP,uvsB
uvsF
musO uvsF
musO
musO uvsI
musR,uvsF
musR uvsB
musR uvsI
musS,uvsF
musS
musS,uvsB
musS uvsC
0.000
0.125
0.250
0.375
0.500
Concentration of 4-NQO (µg/ml)

Fig. 3.3, A - I. Survival and colony size of double compared to single mutant strains grown on MMS plates. Genotypes are indicated on the left, e.g.: + = wildtype, ***L***, ***LH*** and ***H*** = *musL*, *musL;uvsH*, and *uvsH* strains; MMS concentrations are shown in percent; identical numbers of conidia were spread on the three plates shown for each strain.

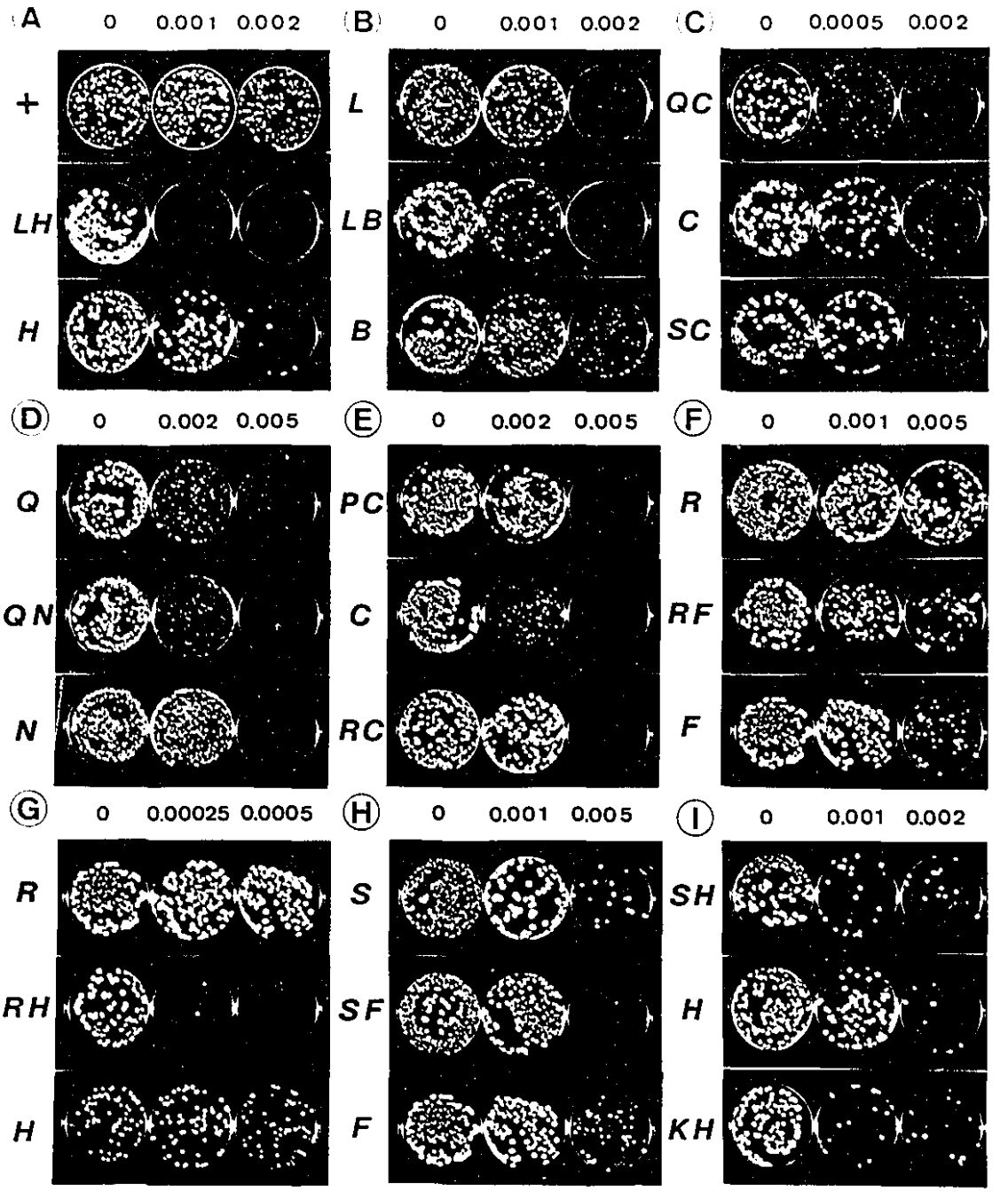
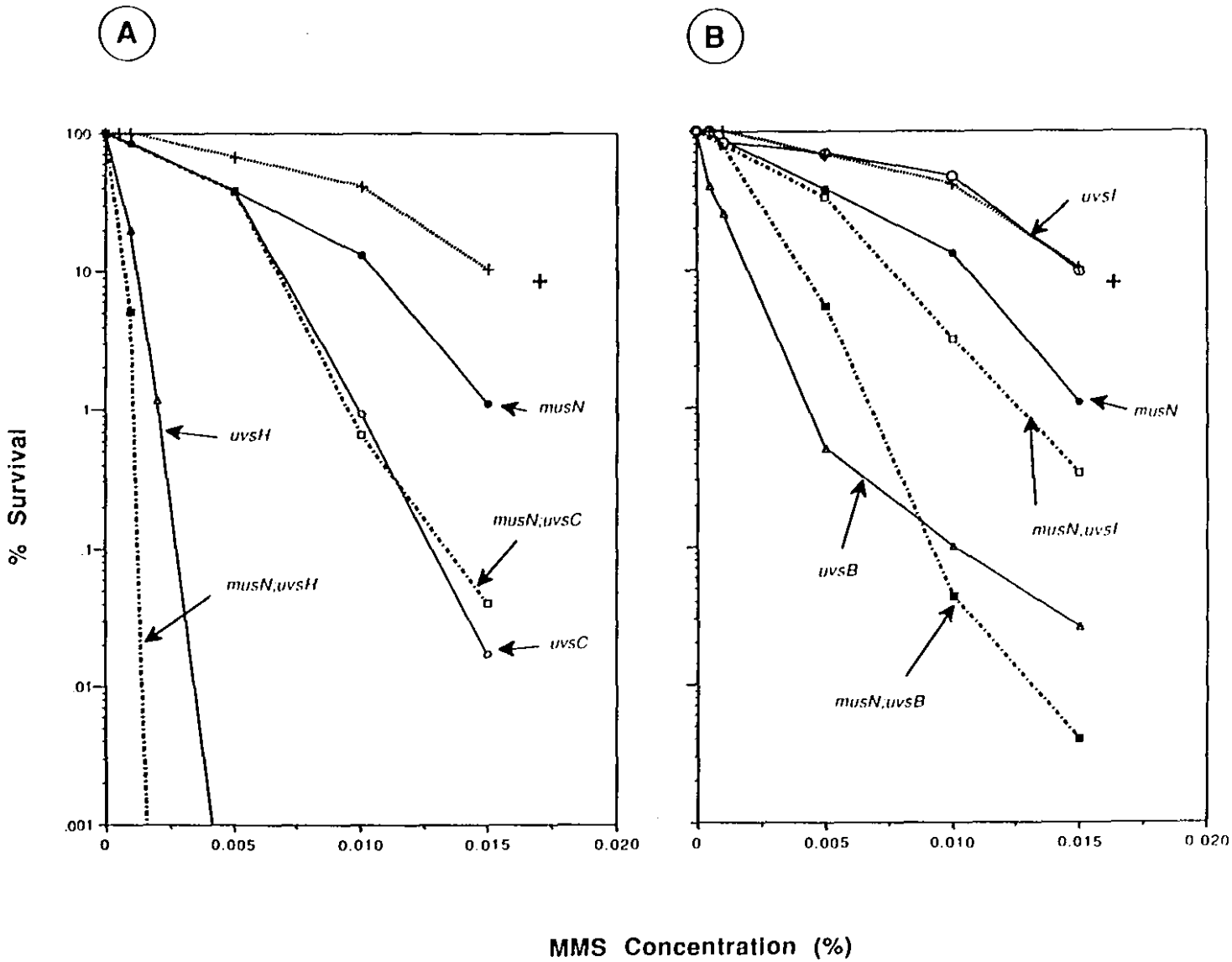

A
0
0.001
0.002
+
LH
H
B
0
0.001
0.002
L
LB
B
C
0
0.0005
0.002
QC
C
SC
D
0
0.002
0.005
Q
QN
N
E
0
0.002
0.005
PC
C
RC
F
0
0.001
0.005
R
RF
F
G
0
0.00025
0.0005
R
RH
H
H
0
0.001
0.005
S
SF
F
I
0
0.001
0.002
SH
H
KH

Fig. 3.4, A - B. MMS survival of *musN* single and *musN;uvs* double mutant strains.

A. Double mutant of *musN* with *uvsH* and *uvsC* and component single mutant strains.

B. *musN;uvsI*, and *musN;uvsB* double and single mutant strains


A
B
100
10
1
.1
01
.001
% Survival
0
0.005
0.010
0.015
0.020
uvsH
musN
musN;uvsC
musN;uvsH
uvsC
uvsI
musN
musN;uvsI
uvsB
musN;uvsB
MMS Concentration (%)

Interactions of *mus* mutations with members of the UvsB group were in no case clearly epistatic, i.e., mutagen sensitivities of double mutants were generally at least additive compared to component single mutant strains. Exceptions were cases of rescue (listed in Table 3.4), but even for these mutations (*musN*, *P*, and *R*) additivity or synergism with *uvsB* was found, if only at high levels of MMS. At lower dose, sensitivity of double mutants was less than that of single UvsB group mutants, in line with increased viability when untreated (e.g., for *N;uvsB*, Fig.3.4.**B**). In most cases, *uvsB* double mutants produced very faint colonies (too faint to show up in Fig. 3.3; e.g., *R;uvsB*, or other semilethal cases).

Based on the results of all tests listed in Table 3.4, the first three mutations were assigned to the UvsC epistatic group, namely *musN* which increases, and *musL* and *K* which reduce mitotic recombination. For *musN*, this assignment is well-supported by results from unusually extensive tests (Figs. 3.2.**A, B** and 3.4). Because typical UvsC group mutants show rec$^-$ phenotypes, such an assignment for the rec$^-$, *musL* mutation, also seems reasonable, in spite of the contradictory finding for 4-NQO survival which suggests epistasis with *uvsI* (Fig. 3.2.**D**).

For all other *mus* genes, no definite assignments were possible. Two of them, *musQ* and *musP* which are lethal with *uvsF*, appear to be epistatic with *uvsI* (Table 3.4), even though there is no overlap of phenotypes. In both cases epistasis with *uvsC* is not completely ruled out (Figs. 3.2.**E** and 3.4.**E**). However, tests for MMS sensitivity of the first *mus* double mutant isolated, *musQ;musN*, clearly showed synergistic interactions (Fig. 3.3.**D**). This finding does not support possible assignment of *musQ* to the UvsC group, since *musN* clearly is a member of this group.

The third hyperrec mutation, *musO*, differs in being lethal with *uvsC* rather than *uvsF*. This mutation showed mainly synergistic or additive interactions, and only 4-NQO survival tests of *musO* with *uvsF* indicated possible epistasis. However, assignment to the UvsF group is doubtful, because MMS sensitivity clearly was much higher for the

Fig. 3.5. Hypersensitivity of *musO;uvsF* double mutants compared to controls. Conidia were transferred onto control medium and 2 MMS plates (0.015 and 0.03%), using 2-4 transfers per strain: top left = wild type strain (2 colonies); middle: 3 colonies each of single mutants, top = *uvsF*, bottom = *musO*; at all other positions *musO;uvsF* double mutant segregants from diploids and crosses were tested (2 colonies per strain)..

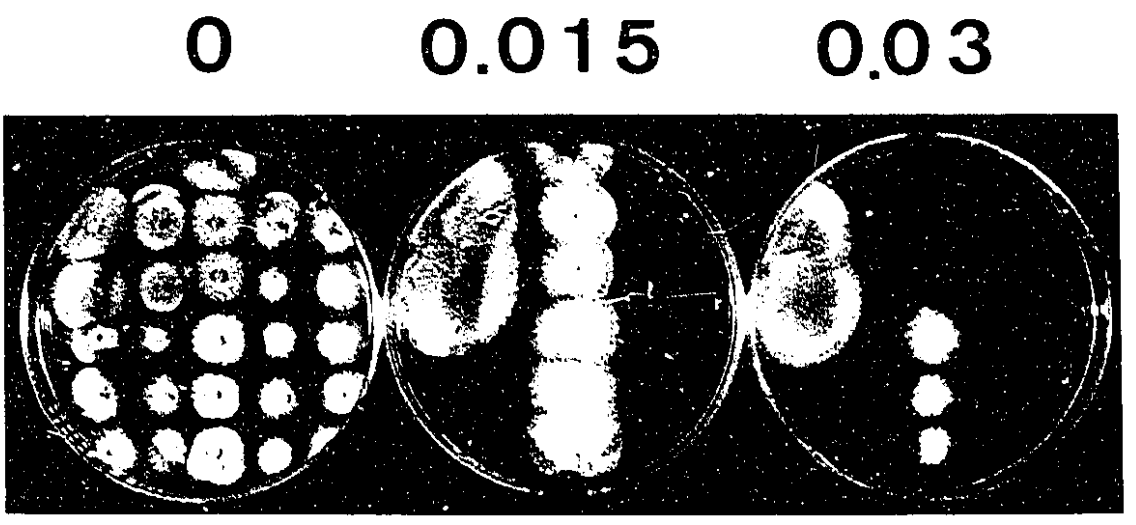

0
0.015
0.03

TABLE 3.4. Types of interaction between *mus* mutations and members of the four epistatic Uvs groups, UvsF, UvsC, UvsI and UvsB (see Table 3.3)

	Interaction for viability			Interaction for mutagen sensitivity[a]				Possible
mus genes	Double mutant lethal	Double mutant ±lethal	Rescue of *uvsB* or *D*	Additive / synergistic 4-NQO	Additive / synergistic MMS	Epistatic 4-NQO	Epistatic MMS	epistatic group
musN	*N-uvsF*	*N-uvsH*	*N-uvsB* > *uvsB*		*N-uvsH*^{4A}	*N-uvsC*^{3A}	*N-uvsC*^{5A}	*uvsC*-
				N-uvsI^{2C}	*N-uvsI*^{4B}	*N-uvsE*^{3B}		group
			N-uvsD > *uvsD*	*N-uvsB*^{2A}	*N*^{c}*-uvsB*^{4B}			
				N-uvsD^{2B}				
musL	*L-uvsF*				*L-uvsH*^{3A}	*L-uvsC*^{2D}	*L-uvsC*^{b}	*uvsC*-
		L-uvsB			*L-uvsI*^{b}	*L-uvsI*^{2D}		group
					L-uvsB^{3B}			likely
musK	*K-uvsF*	*K-uvsH*			*K-uvsH*^{3I}	*K-uvsE*^{d}	*K-uvsE*^{d}	*uvsC*-
		K-uvsB		*K-uvsI*^{2C}	*K-uvsB*^{b}			group?
musQ	*Q-uvsF*			*Q*^{c}*-uvsC*^{2E}	*Q-uvsC*^{3C}			*uvsI*
		Q-musN			*Q-musN*^{3D}			group?
		Q-uvsB			*Q-uvsB*^{b}	*Q-uvsI*^{2E}		
musO	*O-uvsC*	*O-uvsF*			*O-uvsF*^{5}	*O-uvsF*^{2G}		*uvsF* ?
		O-uvsB		*O-uvsI*^{2G}	*O-uvsB*^{b}			or none
musP	*P-uvsF*		*P-uvsB* > *uvsB*	*P-uvsC*?^{d}	*P-uvsH*?^{c}		*P-uvsC*^{3E}	*uvsC* ?
				P-uvsB^{2F}	*P-uvsB*^{c}	*P-uvsI*^{2F}		or *uvsI*
musR					*R-uvsH*^{3G}	*R-uvsF*^{2H}	*R-uvsF*^{3F}	*uvsF*,
			R-uvsB > *uvsB*	*R-uvsB*^{2H}	*R-uvsB*^{b}		*R-uvsC*^{3E}	*uvsC*,
						R-uvsI^{2H}		or *uvsI*?
musS				*S-uvsB*^{2I}	*S-uvsF*^{3H}	*S-uvsF*^{2I}	*S-uvsB*?^{b}	any
					S-uvsH^{3I}	*S-uvsH*?^{d}		group?
				S-uvsC^{2I}		*S-uvsI*^{d}	*S-uvsC*^{3C}	

[a] Superscript numbers refer to Figs. 3.2 - 3.5,
? = result needs confirmation

[b] Poorly growing strains, colonies on MMS media faint and/ or irregular (shown only for *L-uvsB*, Fig. 3.3 B)

[c] Survival curve overlapping that of single mutant strain at low dose levels, but double mutant considerably more sensitive at high dose.

[d] See text

many *musO;uvsF* double mutant recombinants tested from doubly heterozygous diploids and crosses than for either parent single mutant strain (Fig. 3.5).

In the last two cases, *musR* and *musS*, epistatic interaction appears to have occurred with *uvs* mutations of most groups (Table 3.4). For *musR* results from 4-NQO and MMS tests were at least consistent and showed epistasis for UvsF and UvsC group mutants, as well as *uvsI*, suggesting either multiple functions or absence of interaction. In contrast, data for *musS* are entirely contradictory for these two mutagens and no assignment can seriously be considered.

3.5. DISCUSSION

When the MMS-sensitive mutations analyzed here were induced by γ-rays and UV light some years ago, the aim had been to obtain null mutations in radiation repair genes, especially mutants useful for further genetic and molecular analysis of mitotic recombination. In addition, we expected to obtain amino acid requiring mutants which are MMS-sensitive in *Aspergillus* and indeed found about one third to be of this type (Kafer 1987). Among the prototroph mutagen-sensitive isolates, over half showed altered frequencies of spontaneous mitotic recombination and most of these were defective in meiosis (Zhao and Kafer 1992). However, as reported here, none of these *mus* mutants were sensitive to γ-rays and very few to UV light. This finding was very surprising in the light of the high frequency of MMS sensitivity among *uvs* mutants in *Aspergillus*. It also does not agree with the correlation found between MMS and X-ray sensitivity in yeast or *Neurospora*. In both of these species many MMS-sensitive mutants were isolated and the majority were found to be hypersensitive to ionizing radiation and/or to UV light. However all *mus*, as well as all *uvs*, mutants of *Aspergillus* showed

increased sensitivity to 4-NQO which has UV-mimetic activity (Thomas et al. 1991). The two chemical mutagens, 4-NQO and MMS, were therefore used for analysis of epistatic relationships between *mus* genes and members of the four Uvs groups of *Aspergillus*. Satisfactory results were expected, since the excellent agreement found between UV and these chemicals for recent tests of *uvs* mutations (Chae and Kafer 1993). We found, however, that the results from tests of *mus;uvs* double mutants showed considerably less agreement. In various cases inconsistent findings were obtained.

Consistent results were obtained, however, for *musN* for which interactions with all well-analyzed *uvs* mutations were tested. Epistasis was found in all cases only with members of the UvsC group. By phenotype *musN* fits moderately well onto this group. It shares with *uvsC* relatively normal growth of untreated conidia and sterility in homozyous crosses, but differs in not being UV sensitive, not even when growing cells are treated (Chae 1993). In addition, *musN* has hyperrec effects, while *uvsC* is rec$^-$ (Jansen 1970). However, hyperrec as well as rec$^-$ alleles have by now been found for several recombination genes, in *E. coli* as well as in yeast (e.g., for *RAD52* and *RAD50* ; Malone et al. 1988, 1990).

In addition to *musN*, two other mutations appear to be epistatic with *uvsC*, namely *musL* which is rec$^-$, and *musK* which has hyporec effects. Of these, especially *musL* closely resembles *uvsC* (as discussed recently; Zhao and Kafer 1992). All three of these mutants showed normal mutation, while *uvsC* has spontaneous mutator effects and is defective for UV-induced mutation of all types tested (Chae 1993). Possibly these *mus* strains, which are not UV sensitive, would more likely show effects on 4-NQO- or MMS-induced mutation, as found for *mus* mutations in *Drosophila* (Smith and Dusenberg 1989). Alternatively they could interact with *uvsC* only for recombinational repair and not be involved in mutation at all. Recent isolation of genes homologous to *recA*, *RAD51* in yeast and *mei-3* in Neurospora, revealed that related genes may well only retain one of two functions originally present in the ancestral polypeptide (Shinohara et al. 1992;

Cheng et al. 1993). In these cases only the domain responsible for recombination function seems to be present in the fungal genes. This could also be the case for "nontypical" genes of the UvsC group in *Aspergillus*. In addition, *musN, L* and *K* mutations share with *uvsC* lethal interactions with *uvsF*, i.e., mutations which resemble excision repair-defective types. Similar lethal interactions have also been found in yeast for rec⁻ mutation, e.g., *rad52*, when combined with certain excision-defective types, e.g., unusual *RAD3* mutant alleles (Montelone et al. 1988).

In all other cases, no unambiguous assignment of *mus* genes was correlated with similar phenotypic properties, even though for two other mutations which affect recombination, *musQ* and *musO*, results suggest epistasis with mutations of a single group. In the case of *musQ* which appeared to be epistatic with *uvsI*, the difference in phenotype casts doubt on this assignment. Mutations in *uvsI* result in defective UV-induced reversion but recombination is not affected, while the reverse is the case for *musQ*, and the only shared feature is sensitivity to 4-NQO. In the case of the *musO* mutation, a tentative assignment to the UvsF group is based on epistasis in 4-NQO but not MMS tests, and phenotypic differences are considerable. When quiescent conidia are treated, *uvsF* is very sensitive to UV in contrast to *musO*, and *uvsF* is normally fertile, while *musO* is sterile in homozygous crosses. In addition, even though both mutants are lethal in combination with *uvsC* and showed increases in spontaneous recombination in vegetative cells, the latter effects of *musO* are most pronounced for genetic conversion, while those of *uvsF* mainly affect reciprocal intergenic crossing over.

For the three mutations which do not affect recombination, no assignments to epistatic groups of *uvs* mutants could be made. Possibly this is expected, since all these three *mus* show very little overlap in phenotype with *uvs* mutations. Of these three, the *musP* mutation showed the least unusual results, i.e., apparent epistasis with "only" two groups, UvsC and UvsI. Such a finding could mean that the *musP* gene participates in two related, possibly mutagenic, repair processes (as postulated for *cdc40* of yeast;

Kupiec and Simchen 1986a). In contrast, *musR*, apparently showed consistent epistatic interaction with three groups (all except UvsB). This may perhaps signal absence of any significant interactions and suggest minor indirect effects on DNA repair. Or *musR* may truly be epistatic with one of the groups involved, possibly *uvsI*, since both these mutations show similar specific defects for certain types of UV-induced reversion.

In the case of *musS*, results from tests with the mutagens 4-NQO and MMS are quite contradictory and no reasonable explanation comes to mind. Since *musS* (and also *musP*) are mutations associated with translocation breaks, it seems unlikely that these could be leaky alleles. Such partially active mutants might well show the observed low levels of mutagen sensitivities and could cause problems in tests for epistasis. However, leaky mutations could show additivity when mutations are actually epistatic, rather than the reverse as found here, namely apparent epistasis of *mus* mutations with members of two or more Uvs groups.

Two explanations seem worth considering for the surprising lack of radiation sensitivity of the MMS-sensitive mutants in *Aspergillus*. One is the possibility that some of the *mus* mutants which show normal recombination are involved in alkylation repair. For such cases radiation sensitivity and unambiguous assignment to epistatic Uvs groups would not be expected. Such mutations were predicted to be included among *mms* mutants isolated in yeast especially which were not radiation sensitive (Prakash and Prakash 1977a). One of these has recently been cloned and was identified as an alkylation repair-specific gene. The corresponding mutation, *mms5*, was shown to lack an inducible 3-methyl-adenine (m^3A) DNA glycosylase (Chen et al. 1990).

So far none of the expected enzyme defects were found in *Aspergillus* nor in yeast, when mutants specifically hypersensitive to the alkylating agents MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) and MMS, were checked (Swirski et al. 1988; Nisson and Lawrence 1986). One of the enzymes tested in *Aspergillus* had been O^6-methylguanine DNA methyltransferase (MTase), since this species was shown to become

adapted to subsequent challenging doses when pre-exposed to low levels of these alkylating agents (Hooley et al. 1988). While in yeast no evidence for MTase had at that time been obtained, recently yeast clones containing sequences with high homology to *E.coli* and mammalian MTase genes were isolated and gene disruption was shown to cause high sensitivity of yeast cells to MNNG and MMS, but not to 4-NQO (Xiao et al. 1991).

Alternatively, by isolating mutants sensitive to MMS in growth media, we may have selected for mutants which are specifically sensitive during growth. Therefore our tests using quiescent conidia to assess sensitivity to UV, γ-rays and 4-NQO might reveal only a small fraction or none of the sensitivity found in growing cells. This type of growth-dependent UV sensitivity was first for mutations of the UvsC group, including *uvsA* which shares few other phenotypic features with members of that group (Chae and Kafer 1993). In addition, among the alkylation- but not UV-sensitive *sag* mutants of *A. nidulans*, one such case was obtained which showed considerable UV sensitivity only when growing cells were treated (Swirski et al. 1988). In addition, the cell cycle defective mutant *bimD* of Aspergillus, is MMS sensitive and shows UV sensitivity only when treated during growth (Dennison et al. 1993). For most of our *mus* mutants, radiation sensitivity restricted to dividing cells has not yet been ruled out. This is especially a possibility for the three *mus* mutants which show epistasis with members of the UvsC group. Some mutants of this group show not only cell cycle-dependent UV sensitivity, but also X-ray-sensitivity (e.g., *uvsE*; Fortuin 1971). However, when dividing cells were tested for the hyperrec *musN* mutants, no increase in UV sensitivity was found (Chae 1993). These results discouraged further analysis with dividing cells, but do not rule out UV or X-ray sensitivity of germinating conidia for other *mus* mutations, nor the possibility that more consistent results could be obtained if 4-NQO treatment of growing cells were used, as was the case for MMS.

We wish to thank Dorothy Luk for excellent technical assistance in the genetic analysis of the mutagen sensitive strains and Guy L'Heureux for preparing the photographic illustrations. This project was supported by Research Grant OGPO131161 of the Natural Science and Engineering Research Council of Canada.

3.6. APPENDIX I:

While the treatment with γ-rays induced at least ten chromosomal aberrations in ten *mus* strains analyzed, apparently such aberrations resulted in null mutations which caused DNA defects only in four cases. Ironically, of the three *mus* mutations associated with translocation breaks, only one affects recombination and one was actually induced by UV light (*musP234*). The fourth case, *musM225* associated with an intra-chromosomal aberration which reduces meiotic recombination in heterozygous crosses (Fig. 3.1) shows only very slight sensitivity to MMS and has not yet been analyzed. In the light of our experience with radiation-induced translocations in X-ray- and UV-induced mutations in *Aspergillus* these results are not really unexpected, and we resorted to radiation treatment only when attempts to use more suitable agents which induce null-mutations (e.g., 2-epoxyoctane) were unsuccessful. The final meiotic mapping results, however, show up one advantage of the lengthy succession of crosses required for elimination of all unwanted aberrations. Crossing to our highly backcrossed standard and other aberration-free marker strains has increased the isogenicity of the *mus* strains to a point where "standard" meiotic recombination values were obtained. Such effects are expected in the homothallic species of *Aspergillus nidulans* when strains are used which all are derived from the same haploid nucleus. Meiotic recombination values therefore are amazingly homogeneous in Aspergillus and are shown in genetic maps constructed from published data in many different laboratories (Clutterbuck 1990) in contrast to those of other fungi with mating type, e.g., Neurospora (Perkins 1982). It seems very likely that this isogenicity also is the cause of the high recombination frequencies and absence of interference found in *A. nidulans*. Evidence in support of this hypothesis comes from mapping data with highly backcrossed strains of *Neurospora* which show the same pattern of high recombination without interference (Kafer 1982).

While such results show very uniform and reliable recombination values they also produce problems when, e.g., in a three point cross, one of the markers used is closely

adjacent and the other at a considerable distance. In such cases, single and double crossovers are expected and found to be almost equally frequent (Table 1; Kafer 1977). Ordering of markers from a single three point cross is therefore unreliable (and published results have been encountered which erroneously placed a mutation proximal to a known marker, because one more double than single crossovers were observed; e.g., *galD*, which was later shown to be distal to *suAadE* and a closely linked translocation break; Ma and Kafer, 1974).

3.7. DETAILS OF RESULTS NOT SHOWN IN THE PUBLICATION VERSION

3.7.1. MMS test supporting the assignment of musN *to the "UvsC" group:*

Survival curves of conidia plated on MMS media were obtained for double mutants strains combining *musN* with *uvsJ*, a member of the UvsF group. Synergistic interactions were observed in this case (Fig. 3.6). The result for *uvsJ;musN* diploids gives positive evidence for non-epistatic interaction of *musN* with members of the UvsF group. They support the more indirect evidence for the other two members of the UvsF group, which indicated lethality of *musN* double mutants with *uvsF*, and semi-lethality of *musN;uvsH* strains (Table 3.4). In addition, when conidia were plated onto complete medium (CM) containing 0.005% MMS, triple mutants, *musN;uvsH;uvsI*, were shown to be clearly more sensitive than any of the three double mutant strains, namely *musN;uvsH*, or *musN;uvsI*, or *uvsH;uvsI*, (data not shown). This findings further demonstrates that these three mutants all belong to different epistatic groups.

3.7.2 Interaction and rescue of uvsD *by* musN

An interesting type of interaction, i.e., improved recovery and reduced sensitivity to MMS, was discovered for UvsB group mutants with several *mus* mutations, especially *musN*, and also *musP* ("rescue" in Table 3.4; see also section 3.4.3 and Fig. 3.5.B). In the triple mutant strain, *musN;uvsC;uvsD*, conidiation and growth rate are much better than in double mutants *uvsC;uvsD* which have extremely poor growth and conidiation (Fig. 3.7; see also section 3.4.3, for recovery from diploids heterozygous for these mutations). These findings are analogous to those found in certain *recA* mutants of

Fig. 3.6. Survival and colony size on MMS plates, of *musN;uvsJ* double mutant strains.

Top; *uvsJ*, the most sensitive component single mutant strains,

Bottom; *musN;uvsJ* double mutant strains

MMS concentrations shown in percent.

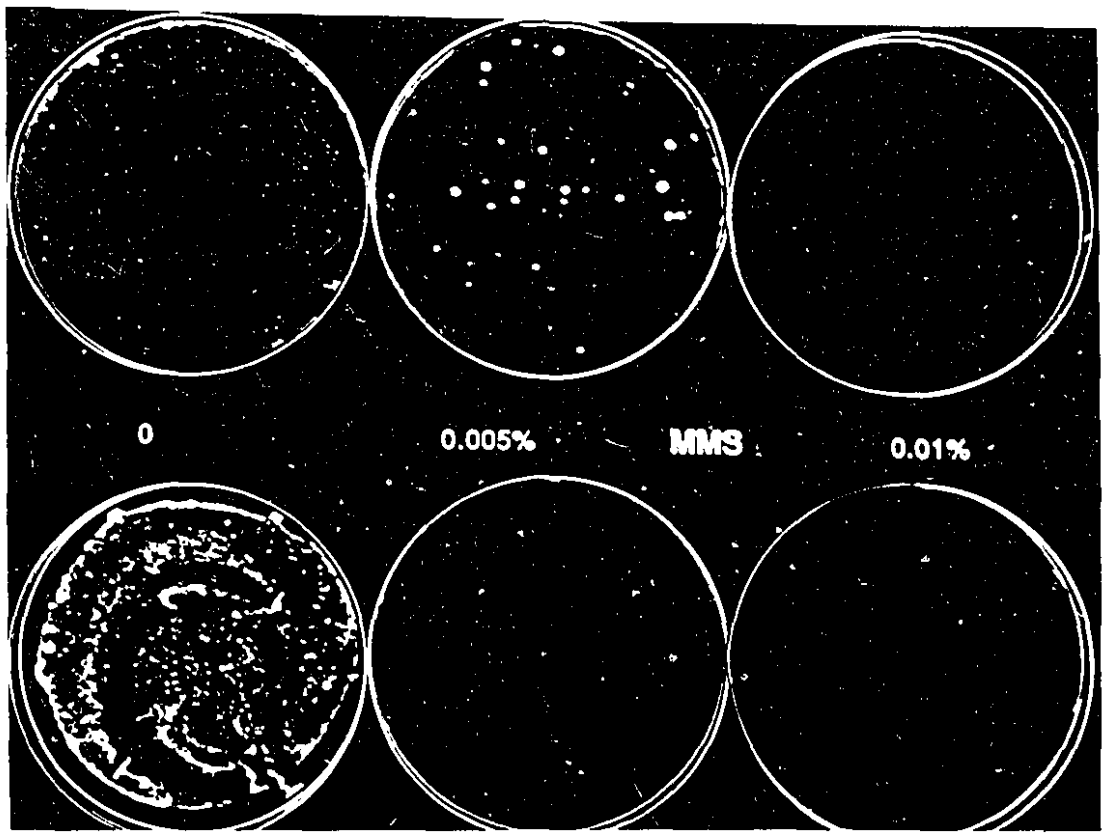
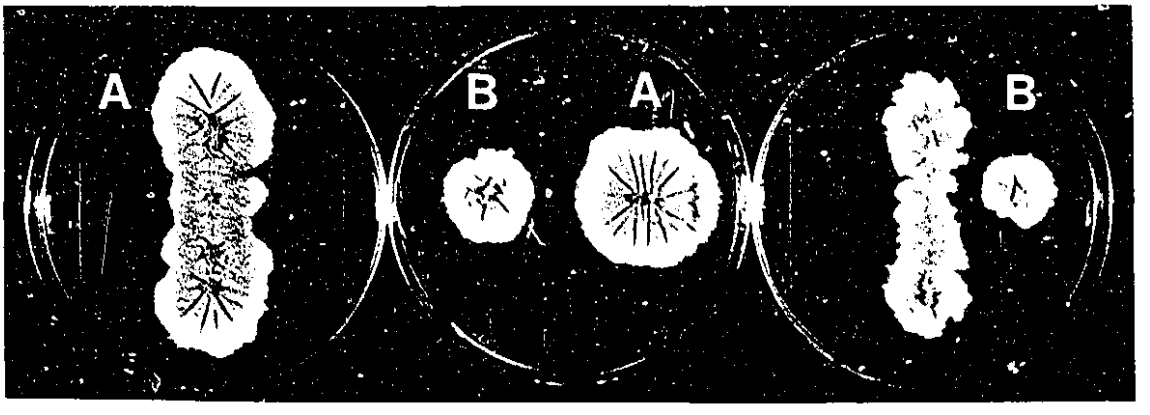

0
0.005%
MMS
0.01%

Fig. 3.7. Viability interaction in *uvsC;uvsD* double mutant *vs* triple mutant of *musN* with *uvsC;uvsD*.

A, *musN;uvsC;uvsD*, triple mutants

B, *uvsC;uvsD*, double mutants

Conidia were either streaked (left and right) or needle-inoculated (middle) on complete medium. All three plates were incubated for 6 days at 37° under same condition.


A
B
A
B

E. coli in which the RecBCD enzyme recklessly degrades DNA, while *recA;recB* double mutants do not show such effects.

3.7.3. UV-survival of musN mutants in dividing cells

The *musN* gene appeared to belong to the UvsC group. Typically, UvsC group genes, *uvsC*, *uvsE*, and *uvsA*, showed increases of sensitivity to UV light during growth compared to that measured with quiescent conidia (for *uvsC* and *uvsE*, Fortuin 1971, also see Fig. 2.9.C; for *uvsA*, Jansen 1967). Thus, survival of *musN* strains to UV light was measured in growing cells (after incubation for 4h at 37°; for UV irradiation on plates, see section 2.3). Different from other members of the UvsC group, *musN* mutant showed wild type levels of UV-sensitivity in growing cells (Fig. 3.8). In addition, no apparent interaction of *musN* with members of the three groups, UvsC, UvsI, and UvsB, was observed as expected, when UV-sensitivities of these double mutants were measured in dividing cells and compared to component single mutant strains (*musN;uvsF* double mutants are lethal; Fig. 3.8.A-C).

3.7.4. Spontaneous and UV-induced mutation in mus strains

To compare the effects of *musN* on mutation with those of *uvsC*, selenate resistant mutants were selected (for details of the procedure, see chapter 4, "Materials and Methods"). Frequencies were compared for *musN* and *uvsC* and their double mutant strain *musN;uvsC*. It was known that both *uvsC* and *uvsE*, members of the UvsC epistatic group, show increased spontaneous mutation frequencies in this system, i.e., they have mutator effects (Jansen 1972), while UV-induced mutations in these strains are much reduced compared to wild type (Käfer and Mayor, 1986).

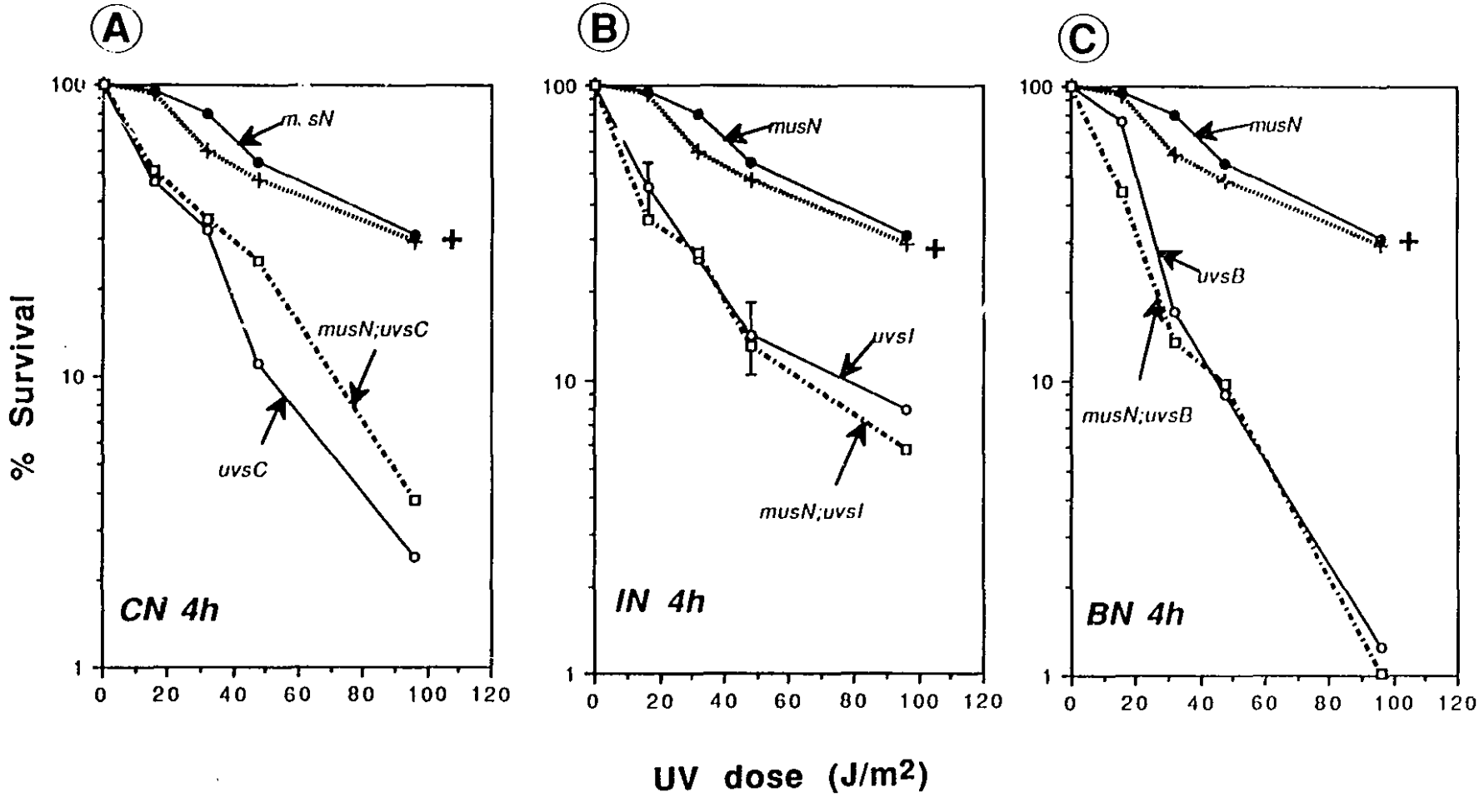
Fig. 3.8. Tests of *musN* for epistatic interactions with *uvs* of three groups using UV treatment of dividing cells

(UV-survival of 4h preincubated, germinating conidia).

A, *musN* with *uvsC*;

B, *musN;uvsI* double mutants;

C, *musN;uvsB* double mutants


A
B
C
100
10
1
% Survival
musN
musN;uvsC
uvsC
CN 4h
musN
uvsI
musN;uvsI
IN 4h
musN
uvsB
musN;uvsB
BN 4h
0
20
40
60
80
100
120
UV dose (J/m2)

It was found that *musN* by itself does not alter the frequencies of spontaneous, nor of UV-induced, selenate resistant mutations (Figs. 3.9 and 3.10). In addition, *musN* is epistatic for spontaneous mutation in the double mutants with *uvsC*, i.e., *musN;uvsC* strains are mutators like *uvsC* alone (Fig. 3.9). However, UV-induced mutation frequencies of the double mutant differ from the reduced levels typical for *uvsC* by showing more induced levels (Fig. 3.10). This result is of interest and unexpected, since the defect of *uvsC* in UV-induced mutagenesis was restored to a small extent by introducing the *musN* mutation. Possibly, a small portion of premutagenic lesions produced by UV light in *uvsC;musN* genetic background may be channeled through other mutagenic repair pathway(s), which results in increases of mutation frequencies compared to those found in *uvsC* strains. On the other hand, premutagenic lesions generated after UV irradiation in *uvsC* strains may not be a good substrate for such pathway(s). Thus, the *uvsC* gene presumably is not responsible for all types of mutations.

Both spontaneous and UV-induced selenate resistance mutations were also analyzed in all other *mus* mutant strains (for genotypes of strains, see Table 3.5). As found for *musN*, all these mutants, namely *musL*, *O*, *K*, *P*, *Q*, R_{Δ} and *musS*, showed wild type levels of spontaneous and UV-induced mutations (Fig. 3.11, A-B). In addition, tests for reversion of *choA1* were carried out, for which *uvsI* is defective (see chapter 4). Results showed that only one of the *mus* mutants, *musR*, exhibited reduced *choA1* reversion frequencies after UV irradiation and none of the other tested *mus* strains significantly deviated from wild type (*musL*, *O*, *P* and *R*; Fig. 3.12).

Table 3.5. Genotypes of *mus* strains for selection of forward mutation to selenate resistance, and for *choA1* reverse mutation

Strain numbers (original Nos.)	*mus*	Genotype
M2892 (2600b.1.15)	+	*pabaA1*; *AcrA1*; *ActA1*; *choA1*; *riboB2 chaA1*
M3752 (2923.4.16)	*musL*	*SulA1 musL222*; *AcrA1*(±); *choA1*; *riboB2*
M3550 (3114.2.7)	*musN*[a]	*pabaA1*; *AcrA1*; *ActA1*;*musN227*; *riboB2 chaA1*
M3765 (2252.7.5)	*musO*	*choA1 musO226* T_2(III;VII); *riboB2 chaA1*
M3046 (2271.2.17)	*musP*	*AcrA1; ActA1; musP234* T_1(VII->II) *choA1; chaA1*
M3874 (2957a.2.14)	*musQ*[a]	*pabaA1 biA1; musQ230; choA1; chaA1*
M3858 (2948b.3.7)	*musR*	*musR223 ActA1; choA1; riboB2 chaA1*
M3245 (2242.3.7)	*musS*	*SulA1 biA1; musS224* T (I;III;VII) *choA1; riboB2 chaA1*

[a] Only used for selection of selenate resistant mutation

For gene symbols, see "Appendix II".

Fig. 3.9. Spontaneous mutation to selenate-resistance in *musN* and *musN;uvsC* double mutant strains.

Individual frequencies of 3-5 independent experiments plotted (closed circles) and their averages with standard error bars indicated (open circles).

For individual frequencies in *uvs*$^+$ and *uvsC* controls, see Fig. 4.1.

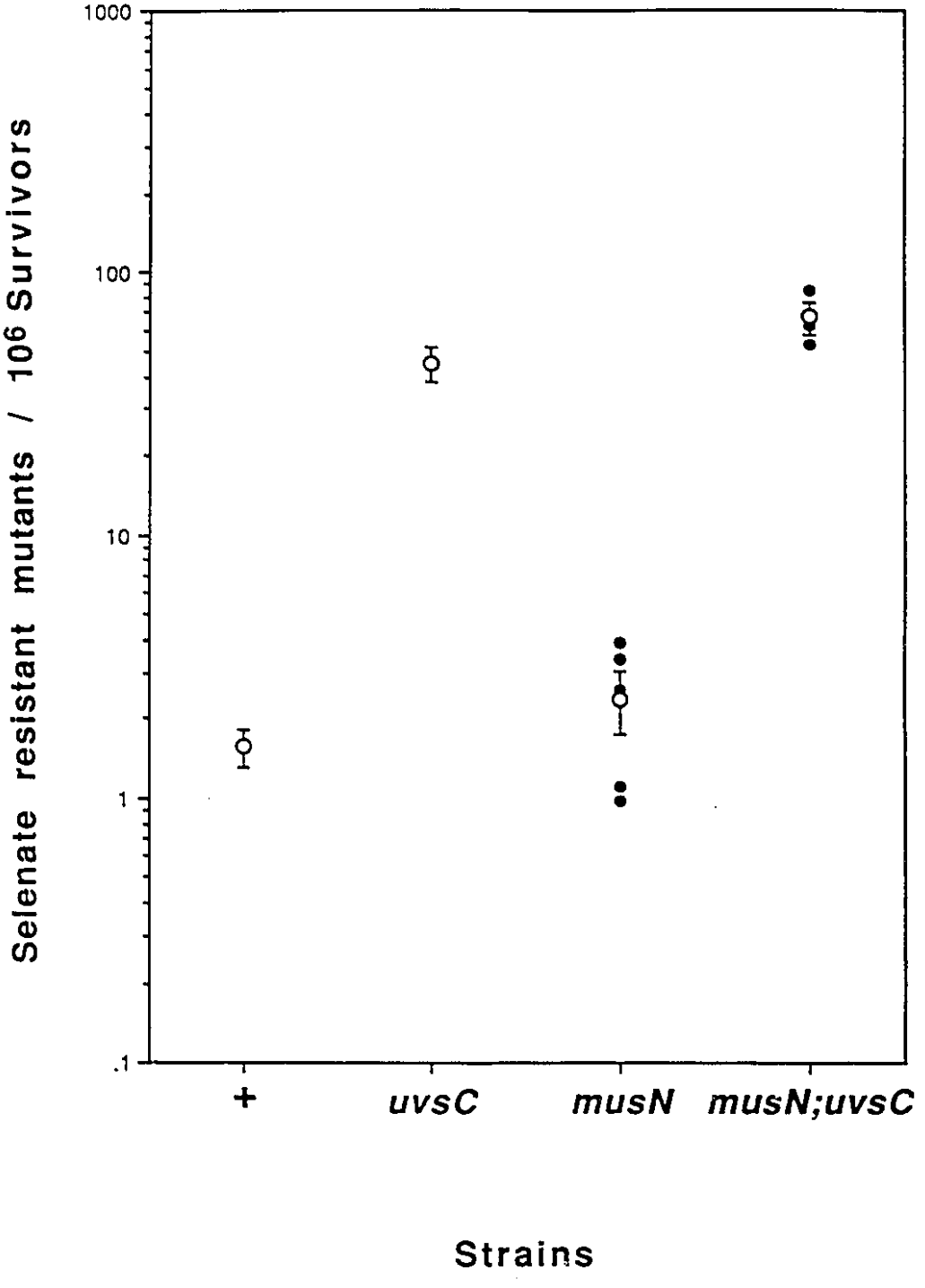

1000
100
10
1
.1
Selenate resistant mutants / 10⁶ Survivors
+
uvsC
musN
musN;uvsC
Strains

Fig. 3.10. UV-induced selenate-resistance mutation in *musN* and *musN;uvsC* double mutant strains.

Frequencies are shown (average of 3 independent experiments for *musN*, *musN;uvsC*).

For UV-survival curves and mutation frequencies (with standard error) of *uvs*$^+$ and *uvsC* strains, see Fig. 4.4.

musN and *musN;uvsC* double mutant strains are no more sensitive than wild type.

UV dose rate = 1.6 J/m^2/sec.

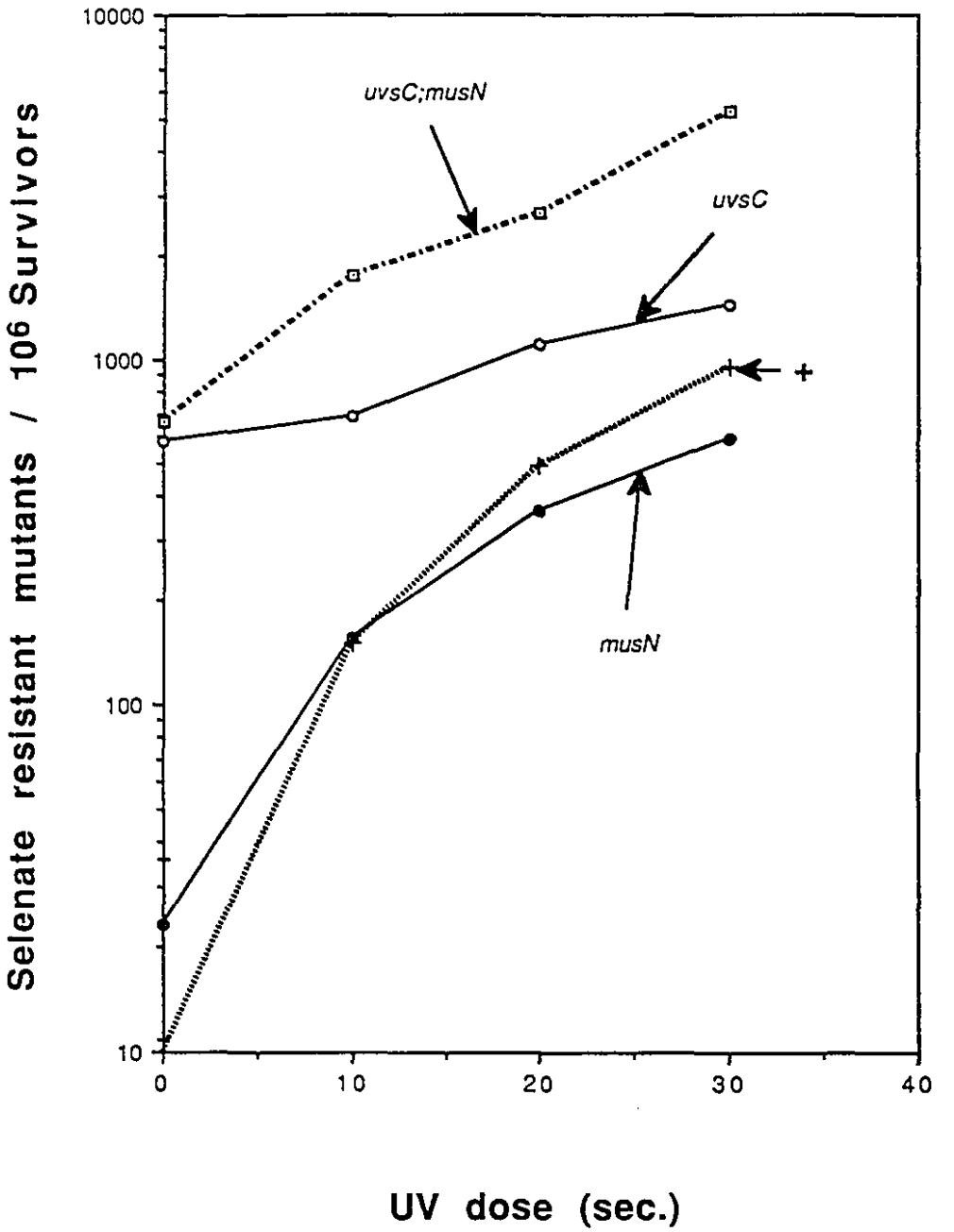

10000
1000
100
10
0
10
20
30
40
uvsC;musN
uvsC
+
musN
Selenate resistant mutants / 10⁶ Survivors
UV dose (sec.)

Fig. 3.11. UV-induced selenate-resistance mutation in *mus*$^+$ control (+), and *mus* strains.

A, *musK*, *musL*, *musN* , and *musO*:

B, *musP*, *musQ*, *musR*, and *musS*.

Symbols as indicated in Figs.

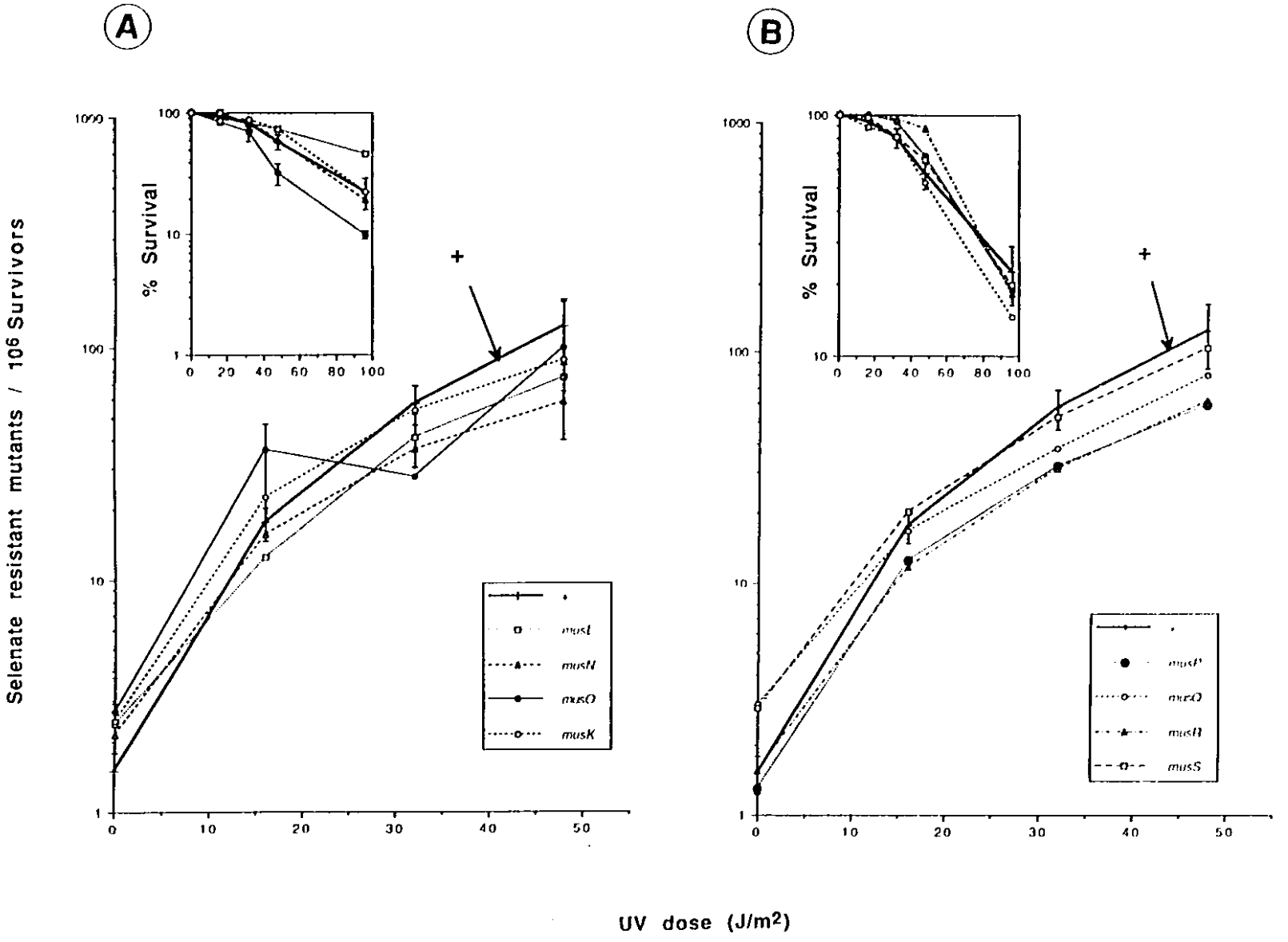
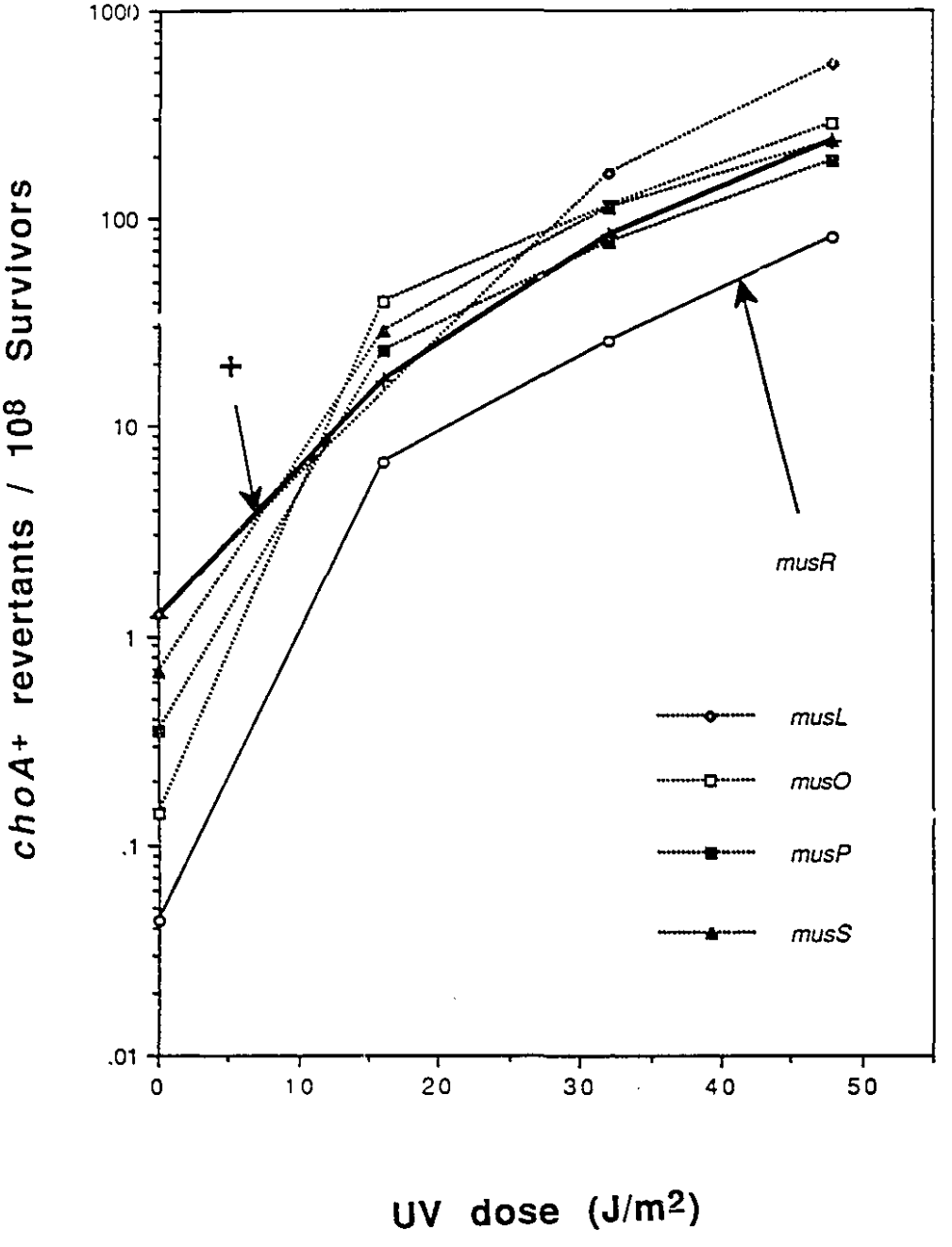

A
B
Selenate resistant mutants / 10⁶ Survivors
% Survival
UV dose (J/m²)
+
musL
musN
musO
musK
musP
musQ
musR
musS

Fig. 3.12. UV-induced *choA1* reversion in *mus*$^+$ control (+), and *mus* strains (*musL*, *musO*, *musP*, *musR*, and *musS*).

Symbols as indicated in Figs.

Frequencies are average of two independent experimetal results.

musR mutants did not produce any *cho*$^+$ revertants in the third experiment, and this result is not included


1000
100
10
1
.1
.01
0
10
20
30
40
50
choA+ revertants / 10^8 Survivors
UV dose (J/m2)
+
musR
musL
musO
musP
musS

CHAPTER 4

Differential and base pair-specific effects of *uvsI* on forward *versus* reverse mutagenesis and *uvsI* interaction with *uvsC*, a mutator strain of *Aspergillus nidulans*

4.1. SUMMARY

Highly specific effects of the *uvsI* mutation on mutagenesis were discovered. These were distinct from the effects of *uvsC* which confers mutator phenotype and defects in UV mutagenesis. Namely, *uvsI* had no detectable (or at most a very minor) effect on spontaneous nor on UV-induced mutation frequencies in two different *forward* mutation systems, but caused greatly reduced *reverse* mutation of *choA1* and *pabaA1*. On the other hand, the *uvsC* mutation showed consistent effects in all systems, namely increased spontaneous forward and reverse mutation and considerably reduced UV-mutagenesis for all types of mutation. Interestingly, *uvsI;uvsC* double mutant strains differed from both single mutant strains. For forward mutations they showed the *uvsC* pattern, i.e., spontaneous mutator effects and reduced UV-induction, but in reversion tests they resembled *uvsI*; i.e., they showed drastic reductions of spontaneous and virtually no UV-induced reversion for *choA1* and *pabaA1*. When the same tests were made using EMS for induction, which mainly leads to GC-> AT base pair changes, one additional specific effect was found. EMS produced results similar to UV, except for induced reversion of point mutations in *uvsI* mutants; namely, in *uvsI* strains reversion of *choA1* was abolished, as found for UV, but reversion of *pabaA1* was not reduced. To follow up this indication of base pair-specific effects of the *uvsI* mutation, reversion tests of many *sC* alleles, isolated initially as selenate resistant mutants after EMS treatment, were carried out. When reversion frequencies in *uvsI* vs. uvs^+ strains were compared for ten such mutations, further evidence for specificity was obtained. Namely in *uvsI* strains, all except one case showed highly reduced *sC* reversion frequencies after EMS treatment compared to the frequencies found for the same *sC* alleles in uvs^+ strains, while effects of *uvsI* on frequencies of UV-induced reversion for these *sC* alleles varied. Similarly, spontaneous mutation was both increased and decreased by *uvsI*, namely mutator effects were demonstrated in reversion tests of several *sC* alleles, in contrast to the antimutator effects

on reversion of *choA1* and *pabaA1* as well as certain *sC* alleles. These results suggest that the *uvsI* mutation may be defective in AT->GC transition mutagenesis, while increasing transversion(s) from A:T base pairs.

Key Words:

Antimutator; mutator; mutagenesis; *Aspergillus nidulans*

4.2. INTRODUCTION

Mutations arise as a consequence of the processing of DNA damage or errors which occurred either endogenously, i.e., are of spontaneous origin, or exogenously, i.e., after treatment with various physical and chemical agents. In spontaneous mutagenesis, DNA replication errors contribute significantly, even though very high genomic fidelity is achieved by the cumulative effects of a series of error avoidance steps (reviewed by Echols and Goodman 1991). Disturbance of any of these steps leads to increases of spontaneous mutation frequencies. This has been demonstrated by analysis of a variety of mutator strains (reviewed by Sargentini and Smith 1985). On the other hand, studies of antimutator strains which exhibit a decrease of spontaneous mutation have led to more direct conclusions about mechanisms of spontaneous mutation.

UV-mutagenesis in *E. coli* is dependent on induction of SOS genes, such as *recA*, *umuD*, and *umuC* (Kato and Shinoura 1977; reviewed by Walker 1984). These gene products are absolutely required for translesion DNA synthesis, i.e., DNA replication past a DNA lesion on the template strand. On the other hand, mutagenesis induced by simple alkylating agents, e.g., ethyl methanesulfonate (EMS) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), which produce "error promoting" DNA lesions, are mostly independent of SOS gene functions (Schendel and Defais 1980; Shinoura et al. 1983). Such DNA lesions allow DNA replication to continue, but with reduced fidelity, which increases mispairing of bases and therefore leads to mutation.

In eukaryotes, the understanding of mechanisms of mutagenesis is more limited than in *E. coli*. However, some genes have been identified which are essential for UV-induced mutation. In the yeast, *Saccharomyces cerevisiae*, such genes, e.g., *RAD6* and *REV* genes, are mostly included in the RAD6 epistatic group, while mutator strains are also frequently found in two other epistatic groups, namely, the RAD3 group in which members mediate nucleotide excision repair or the RAD52 group which contains genes

required for recombinational repair (Reviewed by Haynes and Kunz 1981; Friedberg 1991).

Molecular analysis of *REV* genes revealed that the *REV1* gene product (= Rev1p) clearly had amino acid homology with the UmuC protein of *E. coli* (Larimer et al. 1989). In addition, Rev3p was identified as a nonessential DNA polymerase (Morrison et al. 1989). Thus, it has been proposed that in yeast translesion DNA synthesis may be carried out by a specific complex which includes Rev1p and Rev3p in a manner similar to that postulated for *E. coli* .

In *Aspergillus nidulans*, the *uvsC* mutation is known to alter the frequencies of spontaneous and UV-induced mutations. Mutator effects and highly reduced UV-induced mutation in *uvsC* strains have been demonstrated (Jansen 1972; Kafer and Mayor 1986). In addition, *uvsC* mutants are homozygous sterile and defective in both intragenic (Jansen 1970) and intergenic spontaneous mitotic recombination (Kafer and Mayor 1986; confirmed by Zhao and Kafer 1992). On the other hand, the *uvsI* gene appears to be involved in mutagenic DNA repair (Han et al. 1983), but is not required for either meiotic or mitotic recombination (see chapter 2, "Additional result"). Tests of mutagen sensitivity of *uvsI*, *uvsC*, and of the *uvsI;uvsC* double mutants to various mutagenic agents showed that these two mutations are not epistatic to each other (Chae and Kafer 1993). Moreover, *uvsI* was not epistatic with any members of the previously established three epistatic groups, UvsF, UvsC, and UvsB. However, the possibility of *uvsI* being a member of a subgroup within the UvsC rather than a fourth group was not completely ruled out, even though phenotypes of *uvsI* and *uvsC* differ considerably in most respects except for UV-induced mutation which was known to be reduced in both mutant strains. It was therefore of considerable interest to compare their effects on mutation in various test systems selecting forward and reverse mutations.

In this study, we showed that the effects of *uvsI* on mutation were distinct from those of *uvsC*. In addition, this analysis led to the proposal that *uvsI* may confer base pair-specific effects on mutation.

4.3. MATERIALS AND METHODS

4.3.1 Strains, media and genetic techniques

Strains: Partially isogenic strains of *uvsI*, *uvsC*, *uvsI;uvsC* double mutants, and *uvs*$^+$ control were used for analysis of *uvs* effects on mutagenesis. Mutant *uvs* strains were obtained from appropriately marked heterozygous diploids using benomyl-induced mitotic haploidization. Such *uvs* segregants generally contained six or seven of the eight chromosomes of the control strain. In addition, the one or two exceptional chromosomes which carried the *uvs* mutations in question shared considerable genetic background, since all *A. nidulans* strains used here are derived from a single haploid nucleus (Pontecorvo et al. 1953). The same set of strains was compared for mutation frequencies of three types; namely forward mutation to selenate resistance, reversion of *choA1*, and reversion of *pabaA1*. However, closely related *adE20* strains had to be constructed for the selection of *adE20* suppressors (Table 4.1).

Standard minimal and complete *media* (MM and CM) and *genetic procedures* were those of Pontecorvo et al. (1953), Kafer (1977), and Scott and Kafer (1982).

Table 4.1. Genotypes of *uvs* strains for selection of mutations

A. Strains for selection of forward mutation to selenate resistance, and for *choA1* and *pabaA1* reverse mutation

Strain numbers (original Nos.)	*uvs*	Genotype
M2892 (2600b.1.15)	+	*pabaA1*; *AcrA1*; *ActA1*; *choA1*; *riboB2 chaA1*
M3547 (3113.4.13)	*uvsI*	*pabaA1*; *AcrA1*; *uvsI501*; *choA1*; *riboB2 chaA1*
M3571 (2606.2.3)	*uvsC*	*pabaA1*; *choA1*; *uvsC114 chaA1*
M3630 (3104.11.11)	*uvsI;uvsC*	*pabaA1*; *uvsI501*; *choA1*; *uvsC114 chaA1*

B. Strains for selection of *adE20* suppressor mutations

Strain numbers (original Nos.)	*uvs*	Genotype
M4042 (3122.2.5)	+	*SulA1 adE20*; *AcrA1*; *ActA1*; *pyroA4 ssbA1*; *choA1*; *riboB2 chaA1*
M4043 (3105.8.17)	*uvsI*	*SulA1 adE20*; *AcrA1*; *uvsI501*; *pyroA4 ssbA1*; *sB3*; *choA1*; *chaA1*
M2690 (2408.7.11)	*uvsC*	*adE20 biA1*; *fwA2 uvsC114*
M4044 (3104.3.6)	*uvsI;uvsC*	*SulA1 adE20*; *uvsI501*; *pyroA4 ssbA1*; *sB3*; *choA1*; *uvsC114 chaA1*

For gene symbols, see "Appendix II".

4.3.2 Selection and Induction of mutants

Mutants resistant to selenate were selected on nitrate-free MM containing selenate (0.1 mM Na_2SeO_3; Sigma Chemical Co.), D-methionine (0.02 mM) and urea (5 mM; Arst 1968). Such mutants are unable to grow on sulfate media and generally are alleles of *sB* (coding for sulfate permease) or *sC* (ATP-sulfurylase). These two types could be distinguished by tests for growth on chromate to which *sB* but not *sC* mutants are resistant (0.5 mM Na_2CrO_4 in nitrate-free MM containing 10 mM L-methionine; Arst 1968). In many cases allelism was confirmed by complementation tests using known *sB* and *sC* strains (for additional details, see section 1.5.3).

Revertants of selenate resistant *sC* mutations were selected on minimal medium. To reduce background growth of s^- types, agar of MM was replaced by agarose which has a lower sulfur content (< 0.35%; Sigma Chemical Co.). Suppressors of *adE20* and revertants of *choA1 or pabaA1* were selected on agar MM.

For *UV mutagenesis*, conidia were plated onto appropriate media and irradiated immediately, using a GE germicidal 30W lamp (#G308T8) at a dose rate of 1.6 $J/m^2/sec$. Plates were rotated at 33 1/2 rpm for even exposure.

For *EMS treatment* of quiescent conidia, 10-40 µl/ml of EMS (ethyl methansulfonate, Aldrich Chemical Co.) was added directly into 5 ml conidial suspensions (in Na-K-phosphate buffer, 0.05 M, pH 7.0). Treatment was for 2 h at 30° with shaking at 200 rpm. To terminate exposure, conidia were centrifuged (5 min. at 1,000 rpm) and washed twice in the same buffer.

To obtain *bleomycin-induced sC mutations*, a stock solution in water was prepared (2 mg/ml of bleomycin; Bristol Laboratories, Syracus, N.Y.). Appropriate amounts were added to suspensions of quiescent conidia for final concentrations of 20-120 µg/ml, as indicated. General procedures were the same as for EMS.

Treated conidia were diluted and plated onto CM for estimates of *survival*, while higher concentrations were plated onto selective media for measurements of *mutation frequencies*. Generally, about 10^6 cells/plate were used for selection of selenate resistant mutants or *adE20* suppressors, and 10^8/plate for reversion tests. Plates were incubated at 37° and colonies counted after 4-6 days.

4.3.3 Isolation and Southern analysis of genomic DNA

To isolate genomic DNA of *sC* mutants, strains were cultured for 12 h at 30° (with shaking at 200 rpm). Mycelium was harvested by filtration with miracloth™ and squeeze dried with paper towel. Samples (0.1 g in an eppendorf tube) were vortexed for 5 min. with glass beads (0.4 g) in 0.2 ml extraction buffer (0.5 M NaCl, 0.2 M Tris-Hcl pH 7.5, 0.01 M EDTA, and 1% SDS). For subsequent steps, modified protocols for DNA "mini preparation" were used, following either the method for yeast of Elder et al. (1983), or using the Elu-Quik™ kit (Schleicher & Schuell). For the former method, 300 µl of phenol was added to the mycelial mixture before vortexing. Supernatants were extracted twice with phenol plus chloroform (1:1) and DNA was precipitated with ethanol.

Alternatively, supernatants were mixed with "Elu-Quik" binding buffer and glass beads at a ratio of 100:200:12.5 (for supernatant : binding buffer : glass beads). Subsequent steps were carried out according to the manufacturer's instructions.

Genomic DNAs from induced *sC* mutants were digested with *Hin*dIII restriction enzyme (BRL) which normally generated three nonoverlapping fragments when probed with sC^+ DNA (see Fig. 4.12). Digests were run in agarose gels and transferred onto nylon membrane (Nytran™, S & S) by vacuum transfer for 1.5-2 h (apparatus from Bio-RAD; detailed procedures followed the methods recommended by Maniatis et al. 1982). For Southern blots, i.e., labelling of the probe, hybridization, and colorimetric immunological detections, a kit for nonradioactive DNA labelling and detection (the

Genius™ kit from Boehringer Mannheim) was used and the supplier's instructions were followed. For immunochemiluminescent detection (as in Fig. 4.15), the Lumi-Phos™ 530 substrate/enhancer solution (Lumigen, INC., purchased from Boehringer Mannheim) was used and signals were recorded on X-ray film. Stripping and reprobing of membranes were carried out according to the instruction recommended by the supplier. Briefly, the probe was removed by incubating the membrane in a stripping solution (0.2 N NaOH, 0.1% SDS) for 30 min. at 37°, then washed twice with 2x SSC (1x SSC; contains 0.15 M NaCl, 0.015 M Na-citrate at pH 7.0).

The cloned *sC*$^+$ DNA in plasmid pFB95 (Buxton et al. 1989) was kindly provided by Dr. D. I. Gwynne (Allelix, Toronto). Restriction fragments of pFB95 were isolated from agarose gels using the Elu-Quik™ kit. Various subclones served as probes after labelling with digoxigenin-dUTP.

Stringent hybridization at 68° (for about 12 h) was carried out either in a water bath using leak-proof bags, or in glass roller tubes using a hybridization oven (Bellco Glass, INC.).

4.4. RESULTS

To investigate the effects of *uvsI* on mutagenesis, the levels of spontaneous and induced mutation in *uvsI* strains were compared to control, *uvs*$^+$, strains and to *uvsC*, a mutation known to increase spontaneous and decrease UV-induced mutation. In addition, possible interactive effects of *uvsI* with *uvsC*, were analyzed in double mutant *uvsI;uvsC* strains (called "the double mutants" below). Whenever possible, the same set of strains was compared. Effects on four different types of mutations were measured, two of them forward mutation tests selecting for selenate resistance and suppressors of *adE20*, and two selecting for reversion of the point mutations *choA1* and of *pabaA1*.

4.4.1. Spontaneous mutation frequencies in uvsI *and* uvsI;uvsC *double mutants compared to* uvsC *and* uvs+ *controls*

A) Forward mutation systems

In *uvsI* strains the frequency of spontaneous mutation to **selenate resistance** was found to be very similar to that of wild type (Fig. 4.1). In contrast, in *uvsC* strains a 50-fold increase was found, demonstrating the well-documented mutator effects of *uvsC*. The *uvsI;uvsC* double mutant resembled *uvsC*, but increases were slightly lower, about 20-fold (Fig. 4.1). This difference is statistically significant ($P<0.005$) and presumably represents a slightly moderating effect of *uvsI* on mutations produced in the *uvsC* mutator mutants.

Tests selecting prototrophic revertants of ***adE20*** (adenine requiring) strains produced very similar results. The frequencies in *uvsI* strains were again close to those of wild type (Fig. 4.2), while both *uvsC* and the double mutant strain had mutator phenotypes. In this system, *uvsC* showed 30-fold increases and the double mutant slightly but not significantly lower ones.

Such *adE20* revertants have previously been shown to result from frequent (10^{-6}) recessive **suppressor mutations** in several genes. Three of these suppressor genes (*suA* to *suC*) have been mapped on different chromosomes (Pontecorvo and Kafer 1958; Pritchard 1955; Kafer 1977).

B) Reversion tests

In *uvsI* strains, spontaneous **reversion** of ***choA1*** was significantly reduced compared to wild type ($P<0.025$; Fig. 4.3). *uvsC* again had a strong mutator effect,

Fig. 4.1. Spontaneous mutation to selenate-resistance in *uvs*$^+$ control (+), *uvsI*, *uvsC*, and *uvsI;uvsC* double mutant strains.

Individual frequencies of 9-16 independent experiments plotted (closed circle) and their average with standard error bars indicated (open circle).

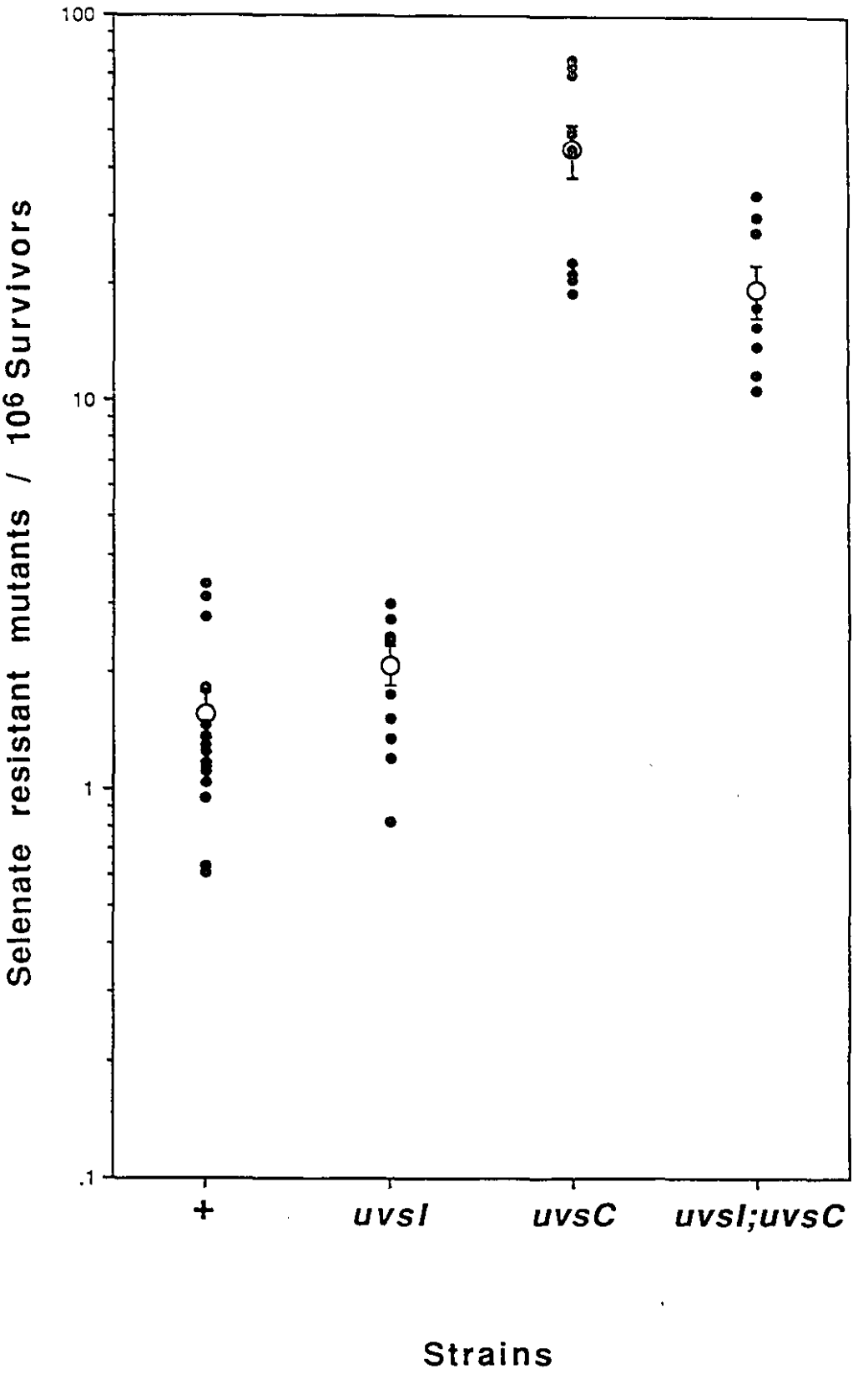

100
10
1
.1
Selenate resistant mutants / 10^6 Survivors
+
uvsI
uvsC
uvsI;uvsC
Strains

Fig. 4.2. Spontaneous mutations of *adE20* suppressors in *uvs*$^+$ and *uvs* strains (as in Fig. 4.1).

Individual frequencies of 4-6 independent experiments (closed triangle) and their average with standard error bars (open triangle).

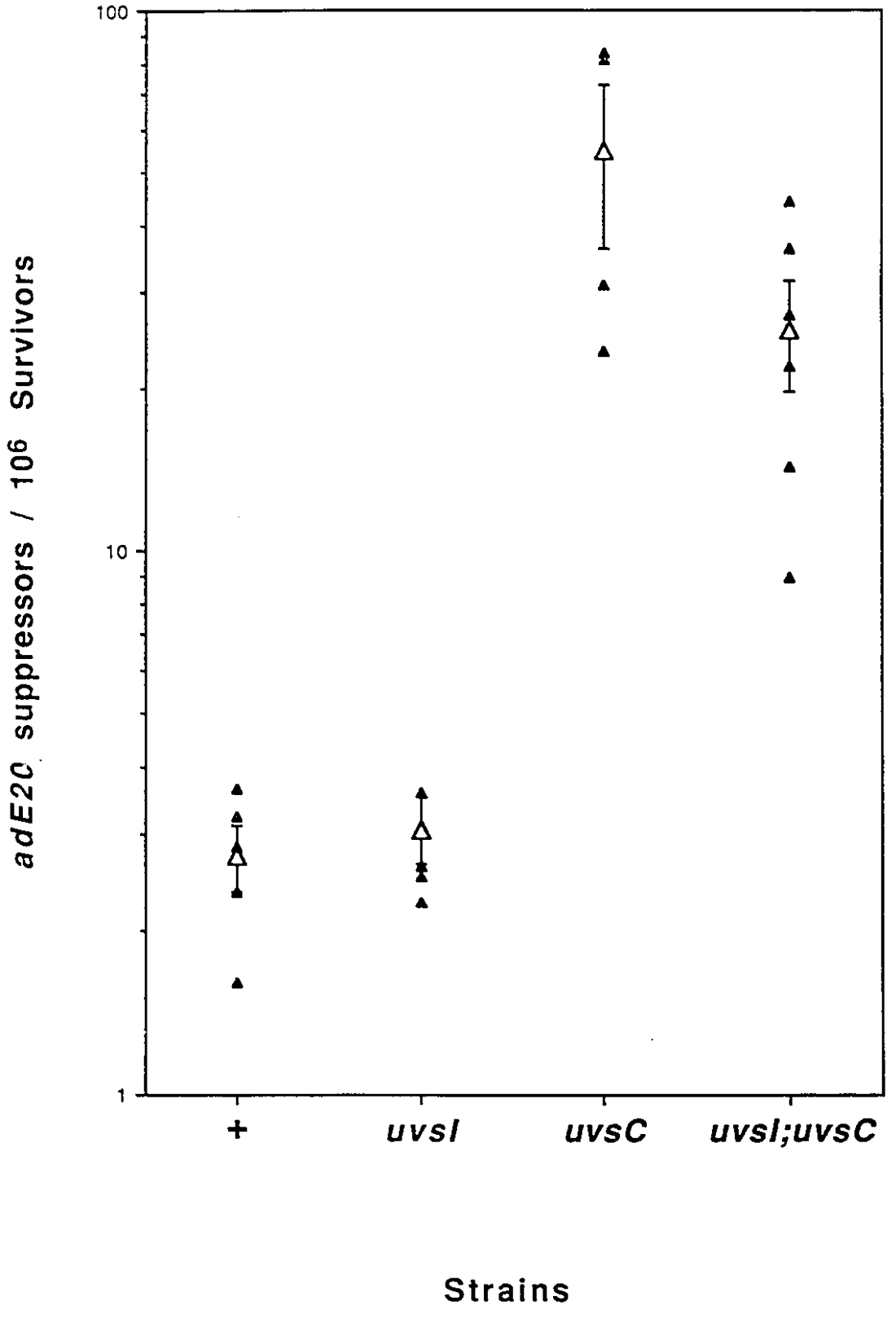
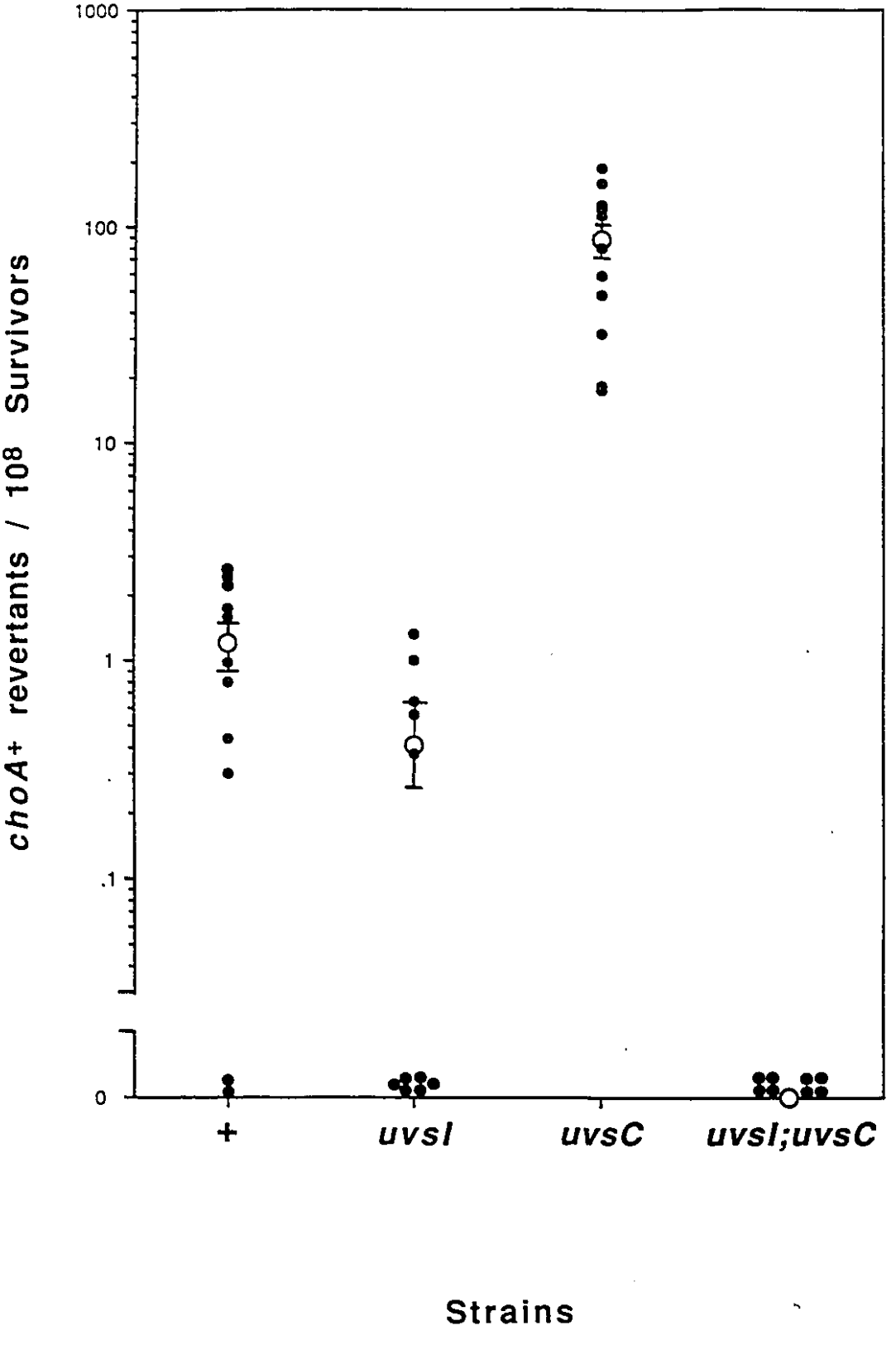

100
10
1
adE2C suppressors / 10^6 Survivors
+
uvsI
uvsC
uvsI;uvsC
Strains

Fig. 4.3. Spontaneous reversion frequencies of *choA1* mutant allele in *uvs*$^+$ and *uvs* strains (as in Fig. 4.1).
Individual frequencies of 8-12 independent experiments (closed circles) and their average with standard error bars (open circle).


1000
100
10
1
.1
0
choA+ revertants / 10^8 Survivors
+
uvsI
uvsC
uvsI;uvsC
Strains

producing 100-fold higher levels of revertants than *uvs*$^+$ control. In this case, however, spontaneous reversion in the *uvsI;uvsC* double mutants was completely abolished.

When spontaneous ***pabaA1* revertants** were selected, results were similar, if slightly more extreme. No revertants could be obtained in *uvsI* nor in double mutant strains, even when large samples were plated. However, the mutator phenotype of *uvsC* was again clearly evident (100-fold increases; Table 4.2).

In summary, *uvsI* had no effects on spontaneous mutation frequencies in forward mutation tests, but caused considerable reduction of reversion frequencies for *choA1* and *pabaA1*. On the other hand, *uvsC* exhibited mutator phenotype for all types of mutation. Surprisingly, interaction of these two *uvs* mutations in the double mutant strains led to opposite effects in forward vs. reverse mutation systems.

4.4.2. UV- and EMS-induced forward and reverse mutation in uvsI, uvsC, *and* uvsI;uvsC *double mutant strains*

A) Forward mutation systems

For **UV-induced mutation frequencies** at the same UV doses, *uvsI* showed either a slight increase (in the case of selenate resistance; Fig. 4.4) or wild type levels (for *adE20* suppressors; Fig. 4.5). On the other hand, practically no UV induction was seen in *uvsC* strains for both types of forward mutation (as found previously for selenate resistance in *uvsC* strains; Kafer and Mayor 1986). In the *uvsI;uvsC* double mutants, UV-induced increases beyond the high spontaneous levels were very slight and resembled those seen for *uvsC* (Fig. 4.4 and 4.5).

After treatment with **EMS, selenate resistant mutations** in *uvsI* strains demonstrated wild type levels of induction, i.e., high increases (100-fold at 40 µl/ml of

Fig. 4.4. UV-induced selenate-resistance mutation in *uvs*$^+$ control (+), *uvsI*, *uvsC*, and *uvsI;uvsC* double mutant strains.

Frequencies of mutants and corresponding survivals shown (see insert).

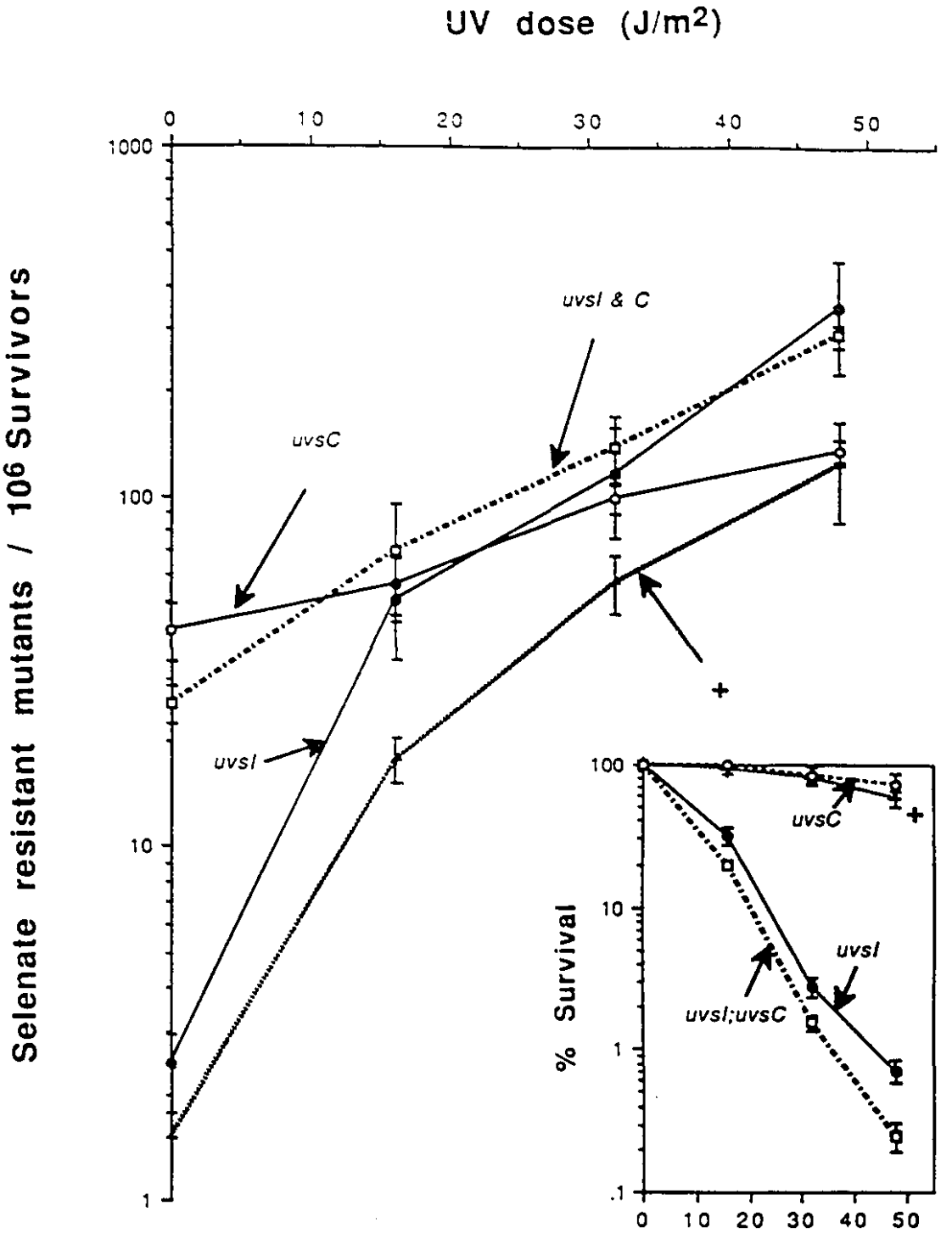
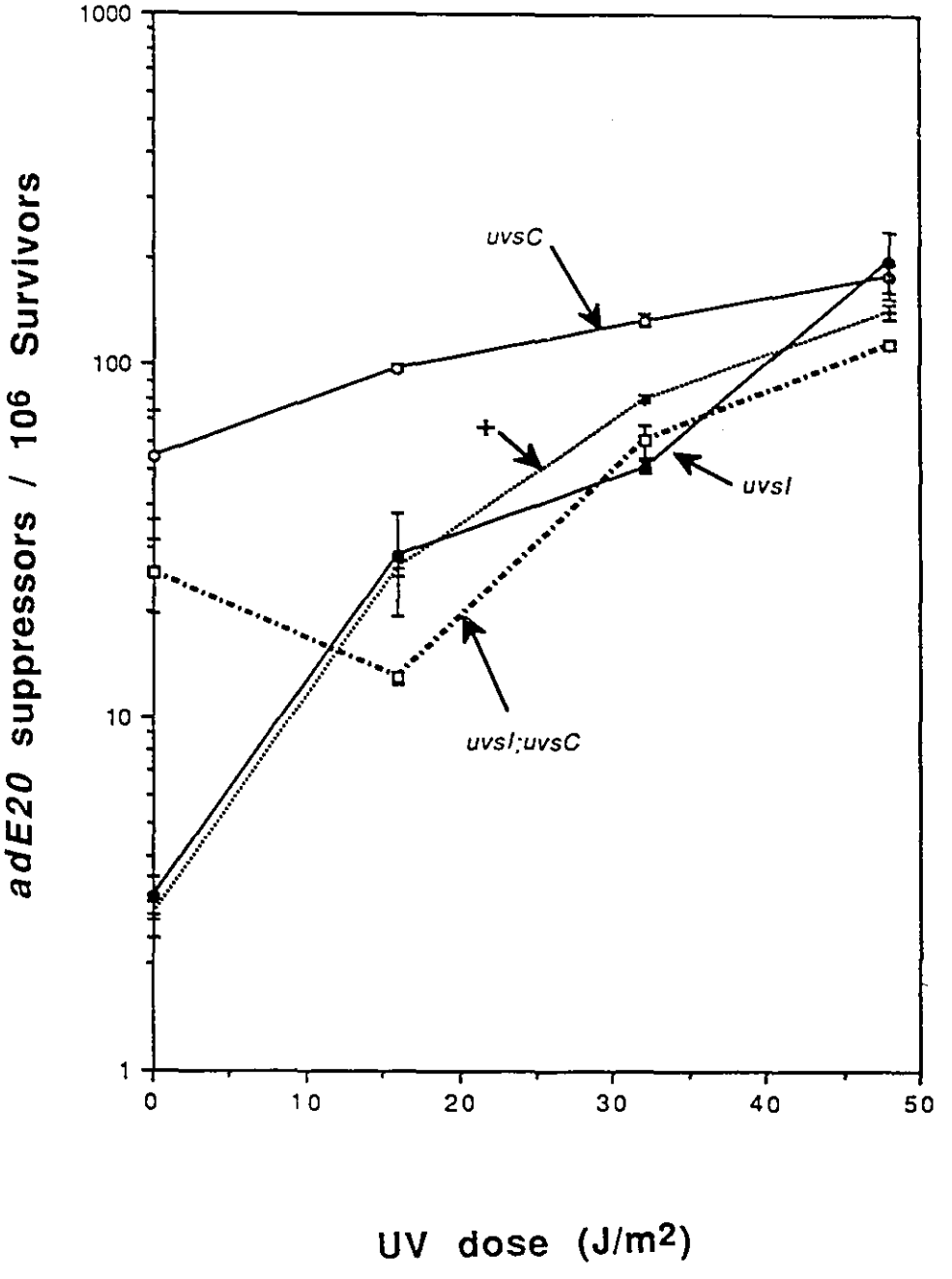

UV dose (J/m2)
0
10
20
30
40
50
1000
100
10
1
Selenate resistant mutants / 10^6 Survivors
uvsC
uvsI & C
uvsI
+
100
10
1
.1
% Survival
uvsC
uvsI
uvsI;uvsC
+

Fig. 4.5. UV-induced *adE20* suppressor mutations in *uvs*$^+$ and *uvs* strains (as in Fig. 4.4).


1000
100
10
1
0
10
20
30
40
50
adE20 suppressors / 10^6 Survivors
UV dose (J/m2)
uvsC
+
uvsI
uvsI;uvsC

EMS; Fig. 4.6). Considerable induction by EMS (40-fold at 40 µl/ml of EMS) was demonstrated also in *uvsC* strains, in contrast to the slight effects of UV. In addition, the double mutants again resembled *uvsC* (55-fold induction), while synergistic interactions between *uvsI* and *uvsC* were apparent for survival after EMS treatment, i.e., EMS sensitivity of the double mutant was extremely high (see insert of Fig. 4.6).

B) UV- or EMS-induced reversion of *choA1* or *pabaA1*

In *uvsI* strains, ***choA1* reversion** was less induced by UV irradiation than in wild type, especially at low doses of UV light (Fig.4.7) and such differences were even larger and highly significant after EMS treatment (Fig. 4.8). In addition, there was no induction of *choA1* revertants by UV or EMS in *uvsC* strains. In double mutant strains, the effects of the two *uvs* mutations apparently were additive, since after treatment with either mutagen no revertants were ever obtained, even when large samples were plated.

After UV-treatment, *uvsI* strains failed to show any ***pabaA1* reversion**, while normal levels of EMS-induced *pabaA1* revertants were produced (Table 4.2). In *uvsC* strains, UV-induced *pabaA1* reversion also was practically absent, and after EMS treatment only slight increases were seen (about 5-fold at 40 µl/ml of EMS; tests of double mutant strains did not produce reliable results since even very large samples yielded insufficient surviving conidia to assess mutation frequencies).

Among selected *pabaA1* revertants, three phenotypes could be distinguished, namely, (i) non-conidiating colonies, (ii) small well-conidiating types, (iii) well-conidiating colonies with normal growth rate (Fig. 4.9). The first type was most frequent in wild type strains, while the third predominated among UV-induced revertants found for *uvsC* strains. No evidence for suppressor mutation was obtained when three well-conidiating revertants were crossed to *paba*$^+$ control strains (0 *paba*$^-$/>200 *paba*$^+$ progeny from each cross). Corresponding crosses of five small aconidial revertants were unsuccessful because no ascospores could be obtained. Presumably such revertants

Fig. 4.6. EMS-induced mutation to selenate resistance and survival curves in *uvs*$^+$ and *uvs* strains (as in Fig. 4.4).

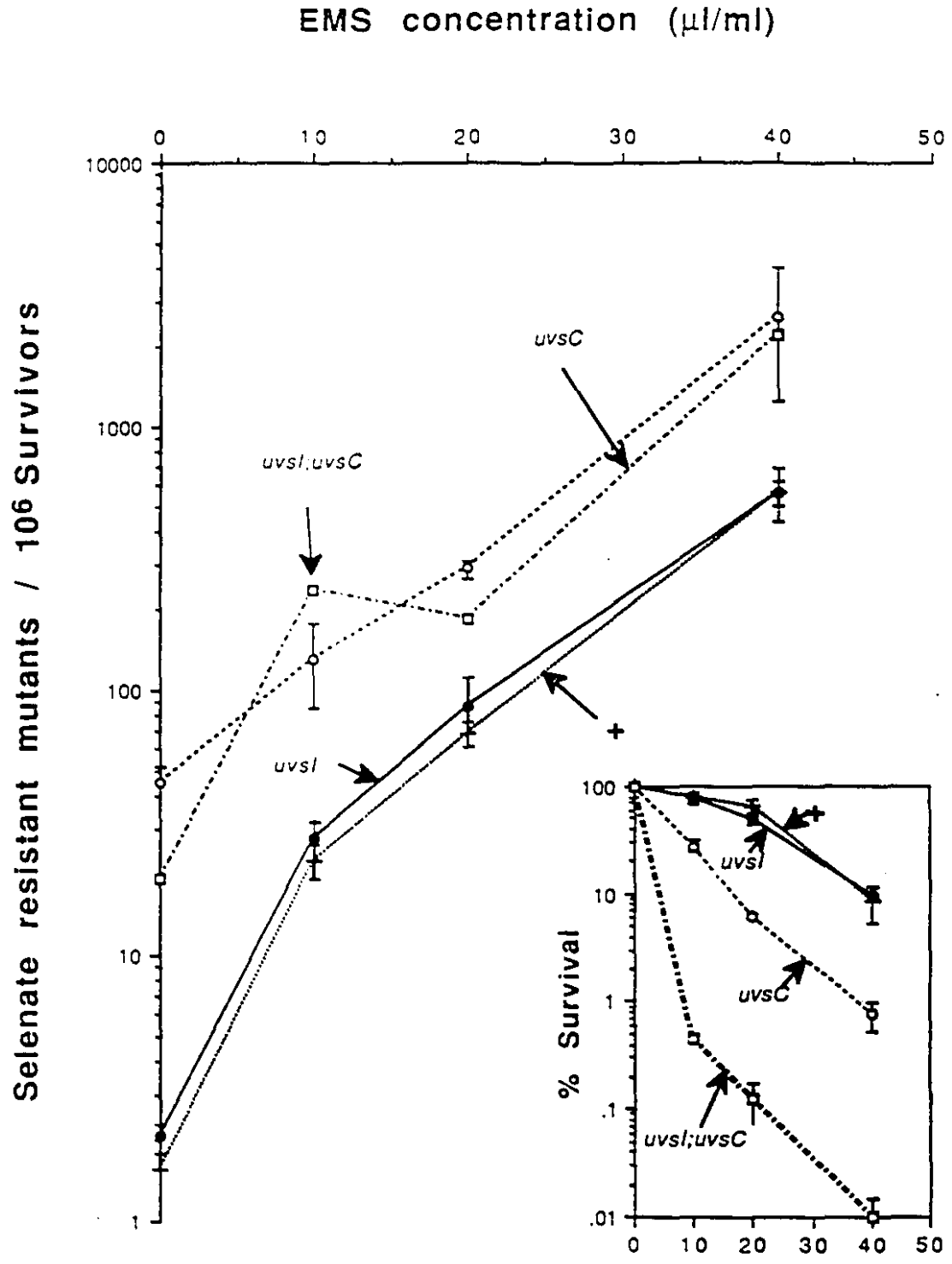

EMS concentration (µl/ml)
Selenate resistant mutants / 10^6 Survivors
0
10
20
30
40
50
10000
1000
100
10
1
uvsC
uvsI;uvsC
uvsI
+
% Survival
100
10
1
.1
.01
uvsI
uvsC
uvsI;uvsC
+

Fig. 4.7. UV-induced *choA1* reversion frequencies in *uvs*$^+$ and *uvs* strains (as in Fig. 4.4).

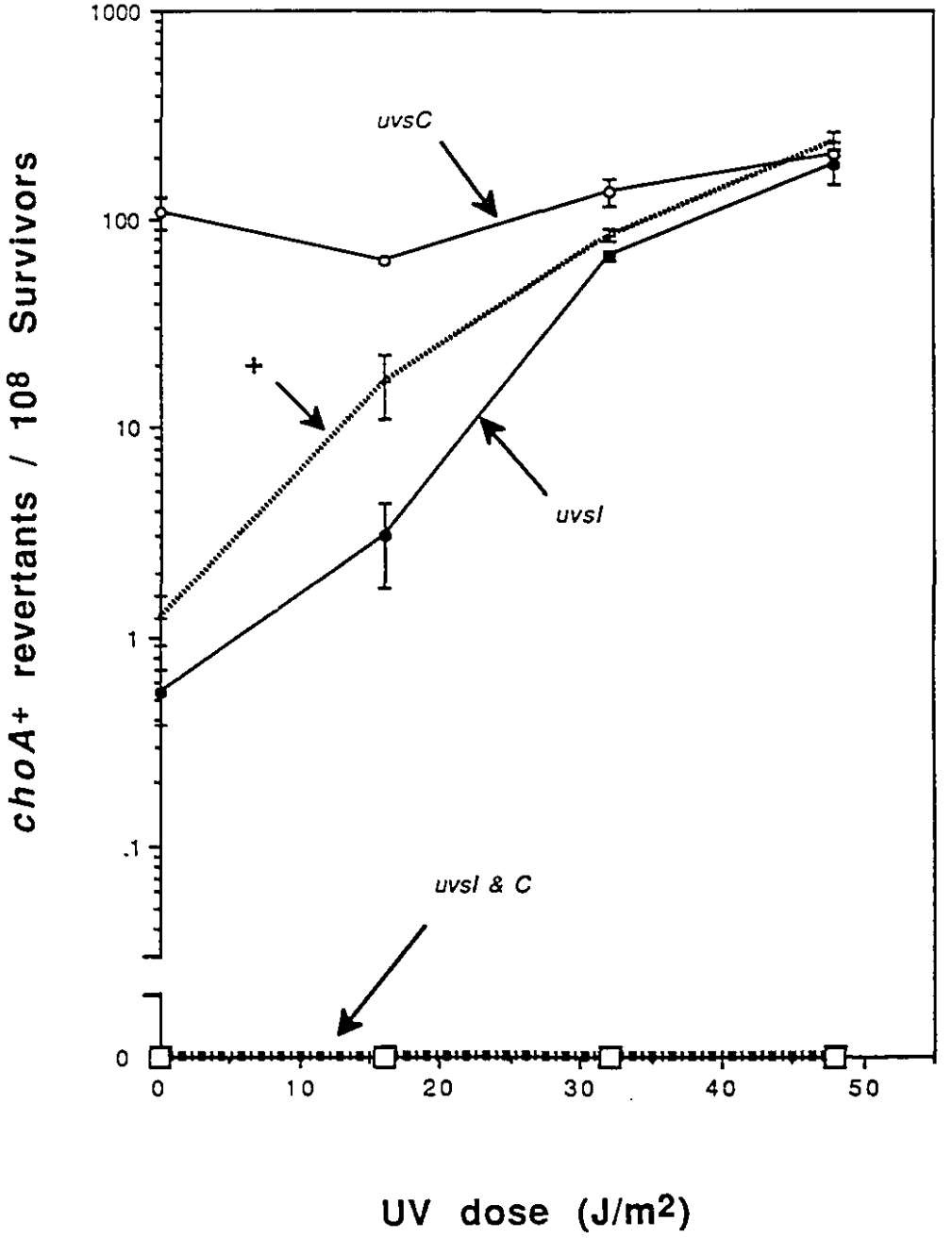

1000
100
10
1
.1
0
0
10
20
30
40
50
uvsC
+
uvsI
uvsI & C
choA+ revertants / 10⁸ Survivors
UV dose (J/m²)

Fig. 4.8. EMS-induced reversion of *choA1* in *uvs*$^+$ and *uvs* strains (as in Fig. 4.4)

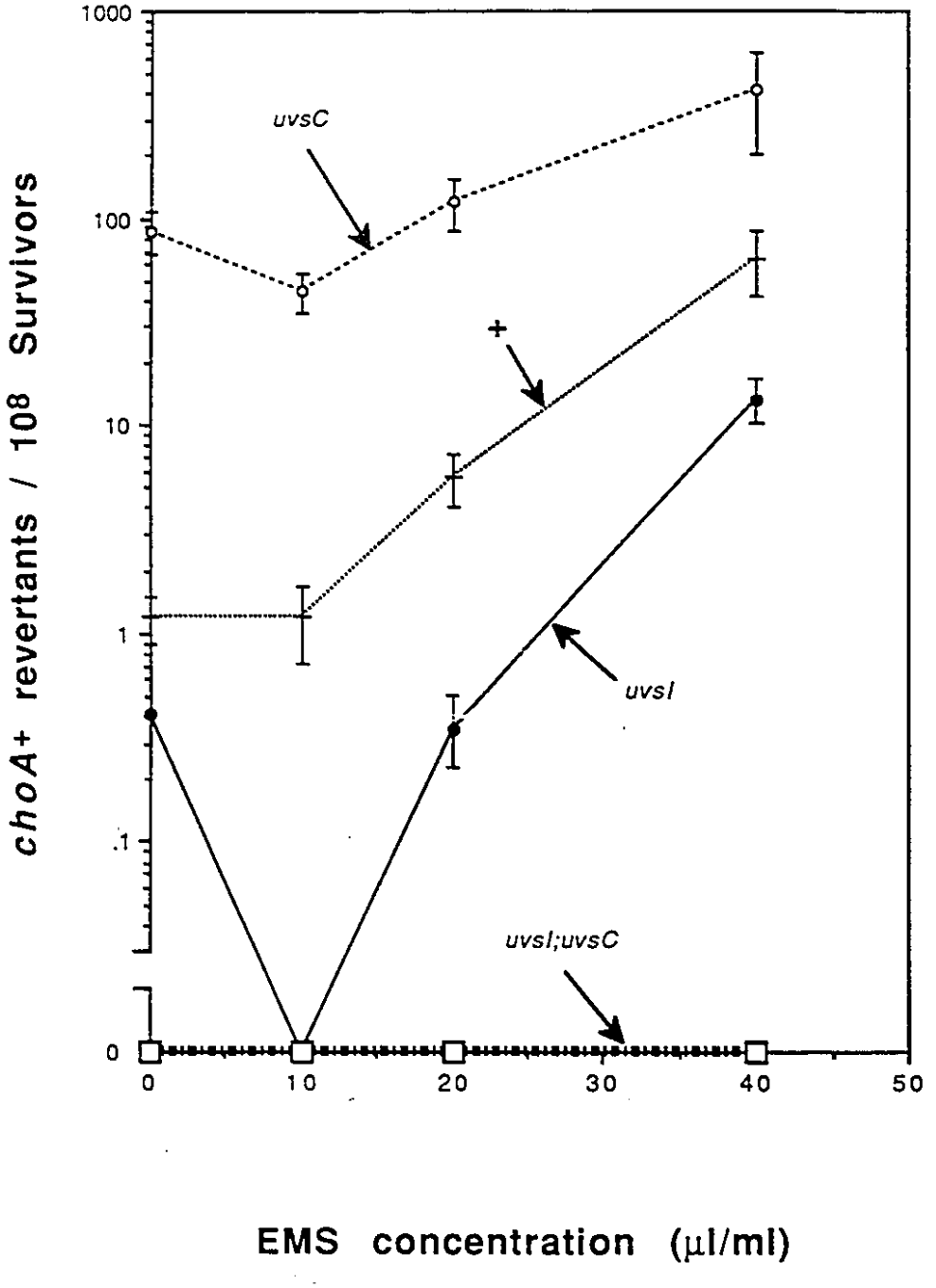

1000
100
10
1
.1
0
0
10
20
30
40
50
uvsC
+
uvsI
uvsI;uvsC
choA+ revertants / 10⁸ Survivors
EMS concentration (µl/ml)

Table 4.2. Frequencies of spontaneous, UV-, and EMS-induced *pabaA1* reversion

Dose and Treatments	Wild Type		*uvsI*		*uvsC*		*uvsI;uvsC*	
	Frequency of *paba*$^+$ ($\times 10^{-8}$)	Survival (%)	Frequency of *paba*$^+$ ($\times 10^{-8}$)	Survival (%)	Frequency of *paba*$^+$ ($\times 10^{-8}$)	Survival (%)	Frequency of *paba*$^+$ ($\times 10^{-8}$)	Survival (%)
0 (= *spontaneous mutation*)	0.2	(100)	0	(100)	37.6	(100)	0	(100)
	0		0		51.6		0	
	0.78		0.32		88		0	
	1.6		0				0	
	0.24		0				0	
Mean ± S.E.	0.56±0.32		0.06±0.07		59.1±18.4		0	
UV-induced mutation [a]								
16 (J/m^2)	0.82	(90.3)	0	(20.7)	41.7	(94.5)	0	(12.7)
32	1.36	(87.9)	0	(3.2)	40	(77.4)	0	(1.3)
48	3.11	(63.7)	0	(0.9)	33	(53.2)	0	(0.7)
EMS-induced mutation [b]								
10 (µl/ml)	6.77	(77.3)	5.53	(74.1)	184	(43.7)	-	-[c]
20	32.8	(61.8)	27.9	(49.6)	336	(12.3)	-	-[c]
40	709	(4.8)	413	(3.2)	324	(1.1)	-	-[c]

[a] Average of three independent measurements
[b] Average of two independent experiments
[c] See Fig.4.6 for survival

Fig. 4.9. Three types of *pabaA1* revertants selected on minimal medium.

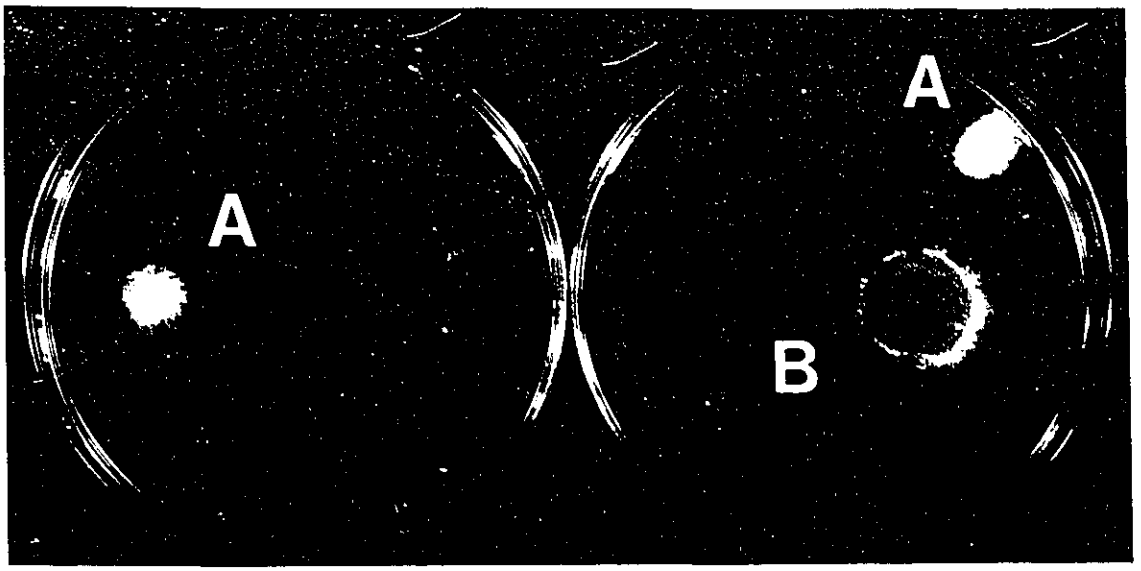
A. Non-conidiating colonies, (frequent in *uvs*$^+$ strains)

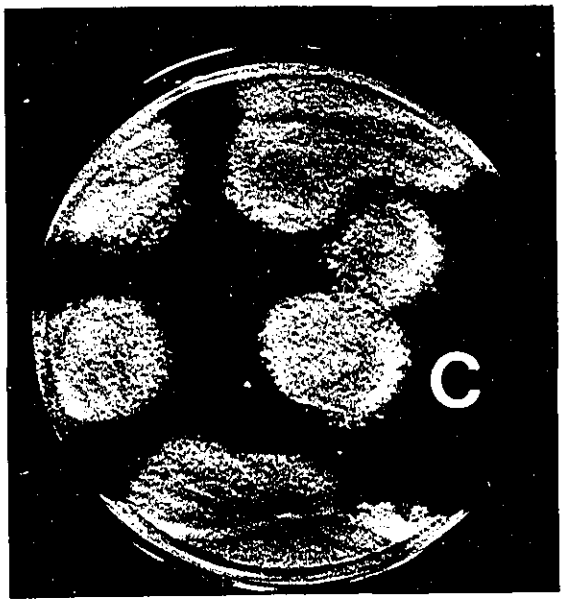
B. Small well-conidiating type, (obtained in *uvs*$^+$ strains)

C. Well-conidiating colonies with normal growth rate, (majority type in *uvsC* strains)

Top; the two plates were incubated for 7 days at 37°,

Bottom; 4 days incubation at 37°.


A
A
B


C

caused sterility even when heterozygous. The genetic basis and significance of the different types of *pabaA1* revertants are not clear at this time.

In summary, in ***uvsI* strains**, wild type levels of UV-induced forward mutations were found, while UV-induced reversion frequencies for *choA1* and *pabaA1* were significantly reduced. Similarly, EMS-treatment of *uvsI* strains resulted in normal induction of forward mutation. However, for effects of *uvsI* on EMS-induced reversion, differences between *choA1* and *pabaA1* were uncovered; namely, EMS-induced reversion was drastically reduced for *choA1*, while *pabaA1* revertants were induced almost at wild type levels.

In ***uvsC* strains**, on the other hand, virtually no induction occurred in all tests after UV irradiation. In contrast, *uvsC* effects on EMS mutagenesis varied; namely, EMS induction was considerable for selenate resistant mutants, slight for *pabaA1* reversion, and lacking for *choA1* reversion.

In the case of ***uvsI;uvsC* double mutant strains**, relative increases of forward mutations after UV and EMS treatments were similar to those in *uvsC*, in line with the finding that *uvsI* does not affect these frequencies. In contrast, drastic reduction was found in reversion tests; i.e, no UV- nor EMS-induced revertants were recovered for *choA1* and *pabaA1*. The latter result indicates at least additive effects of the *uvsI* with the *uvsC* on such type of reverse mutation.

4.4.3. Mutational specificity of uvsI, *demonstrated by spontaneous and induced reversion patterns of EMS-induced* sC *alleles, originating in either* uvs$^+$ *or* uvsI *and transferred into* uvsI *or* uvs$^+$ *strains*

The preceding results suggested that *uvsI* may have specific effects on mutation, i.e., the *uvsI* gene product may be required during the formation of some but not other mutational alterations. To explore such specificity further, reversion tests of many *sC* alleles were carried out (as described below). So far no system is available for *Aspergillus nidulans* which can detect specific mutational changes at the molecular level. Therefore, in an attempt to approximate such systems, tests revealing some mutational specificity were devised, using a chemical which has high mutational specificity. EMS was the chemical of choice, because it efficiently induces point mutations and these mainly result from G:C to A:T transitions in wild type strains (of *E. coli*, Coulondre and Miller 1977, Burns et al. 1986; of yeast, Kohalmi and Kunz 1989; of *Drosophila*, Pastink et al. 1991; of human cells, Lebkowski et al. 1986).

The *sC* mutations to be used in reversion tests were initially isolated as selenate resistant mutants after EMS treatment in either *uvs*$^+$ or *uvsI* strains, and later shown by Southern analysis to contain no detectable aberrations in the *sC* gene region (see below).

A total of 13 *sC* alleles from *uvs*$^+$ and nine from *uvsI* strains were collected after treatment with EMS. Because *uvsI* and *sC* are closely linked, only ten of these 22 *sC* mutations were transferred by crossing, six from *uvs*$^+$ into *uvsI*, and four from *uvsI* into *uvs*$^+$, and thus were available for reversion tests in both *uvs*$^+$ and *uvsI* strains.

The results from tests of **spontaneous reversions** of these ten *sC* alleles in *uvs*$^+$ and *uvsI* strains are given in Table 4.3.A, and B. They clearly show that *uvsI* has large effects on spontaneous reversions; namely, *uvsI* caused reduction of reversion frequencies for *sC* alleles induced originally in the *uvsI* background (i.e., *sC105, 223,*

Table 4.3.A. Frequencies of spontaneous, UV-, and EMS-induced *sC* alleles reversion in *uvs*$^+$ strains.

sC alleles in *uvs*$^+$ origin / No		Spontaneous Frequencies of sC^+ ($\times 10^{-9}$ ± S.E.)	UV-induced[a] Frequencies of sC^+ ($\times 10^{-9}$)	(Survival) (%)	EMS-induced[b] Frequencies of sC^+ ($\times 10^{-9}$)	(Survival) (%)
+	*107*	0	86.4	(88.3)	89.2	(50.4)
	211	0.14 ± 0.18	14	(99.5)	165	(68.4)
	311	1.33 ± 1.26	38.4	(91.9)	1910	(58.7)
	315	1.27 ± 1.17	29.8	(90.8)	775	(70)
	619	3.79 ± 0.93	43.4	(97.6)	61.8	(73.3)
	719	0.82 ± 0.72	10.2	(96.7)	9.6	(53.6)
uvsI	*105*	0.96 ± 1.18	0.3	(100)	56.7	(67.3)
	223	1.1 ± 0.59	12.8	(89.3)	3.9	(62.6)
	309	1.88 ± 0.77	18.2	(96.5)	25.4	(64)
	321	4.2 ± 1.78	30.9	(88.5)	26.7	(55.2)

[a] UV dose; 16 J/m^2
[b] EMS concentration; 20 µl/ml

Table 4.3.B. Frequencies of spontaneous, UV-, and EMS-induced *sC* alleles reversion in *uvsI* strains

sC alleles in *uvsI* origin / No		Spontaneous Frequencies of sC^+ ($\times 10^{-9}$ ± S.E.)	UV-induced[a] Frequencies of sC^+ ($\times 10^{-9}$)	(Survival) (%)	EMS-induced[b] Frequencies of sC^+ ($\times 10^{-9}$)	(Survival) (%)
+	*107*	2.22 ± 0.84	11.9	(35.5)	11.7	(47.8)
	211	3.24 ± 1.99	35.6	(38.1)	20.3	(50.1)
	311	4.78 ± 0.98	40.8	(37.9)	364	(43.2)
	315	15.8 ± 4.91	72.6	(36.4)	528	(47.5)
	619	0.45 ± 0.28	20.9	(27)	30	(61.9)
	719	2.63 ± 1.63	10.9	(26.6)	4.1	(46.8)
uvsI	*105*	0.63 ± 0.77	10.7	(23.3)	2.5	(52.3)
	223	0.62 ± 0.42	1.7	(36.9)	2.7	(55.6)
	309	0.37 ± 0.23	15.8	(27)	34.4	(62.2)
	321	0.22 ± 0.27	35.6	(34.4)	16.3	(50.1)

[a] UV dose; 16 J/m^2

[b] EMS concentration; 20 µl/ml

Table 4.3.C. Comparison of frequencies of spontaneous, UV- and EMS-induced *sC* reversion in *uvsI* strains, to those in *uvs*$^+$.

sC alleles origin / No		*Spontaneous* fold differences (= *uvsI*/*uvs*$^+$)	*UV-induced* Absolute frequencies[a] (x10^{-9}) *uvsI*	*UV-induced* Absolute frequencies[a] (x10^{-9}) +	*UV-induced* (fold differences = *uvsI*/*uvs*$^+$)	*EMS-induced* Absolute frequencies[a] (x10^{-9}) *uvsI*	*EMS-induced* Absolute frequencies[a] (x10^{-9}) +	*EMS-induced* (fold differences = *uvsI*/*uvs*$^+$)
+	*107*	>1[b]*	9.7	86.4	(0.11)	9.5	89.2	(0.11)
	211	23.1	32.4	13.9	(2.33)	17.1	164.9	(0.1)
	311	3.6*	36.0	37.1	(0.97)	359.2	1908.7	(0.19)
	315	12.4*	56.8	28.5	(1.99)	512.2	773.7	(0.66)
	619	0.1*	20.5	39.6	(0.52)	29.6	58.0	(0.51)
	719	3.2	8.3	9.4	(0.88)	1.5	8.8	(0.17)
uvsI	*105*	0.7	10.07	<1	(>10)	1.9	55.7	(0.03)
	223	0.6	1.08	11.7	(0.09)	2.1	2.8	(0.74)
	309	0.2	15.43	16.3	(0.95)	34.0	23.5	(1.45)
	321	0.1*	35.38	26.7	(1.33)	16.1	22.5	(0.71)

[a] Absolute frequencies = UV- or EMS-induced mutation frequency - spontaneous reversion frequency.

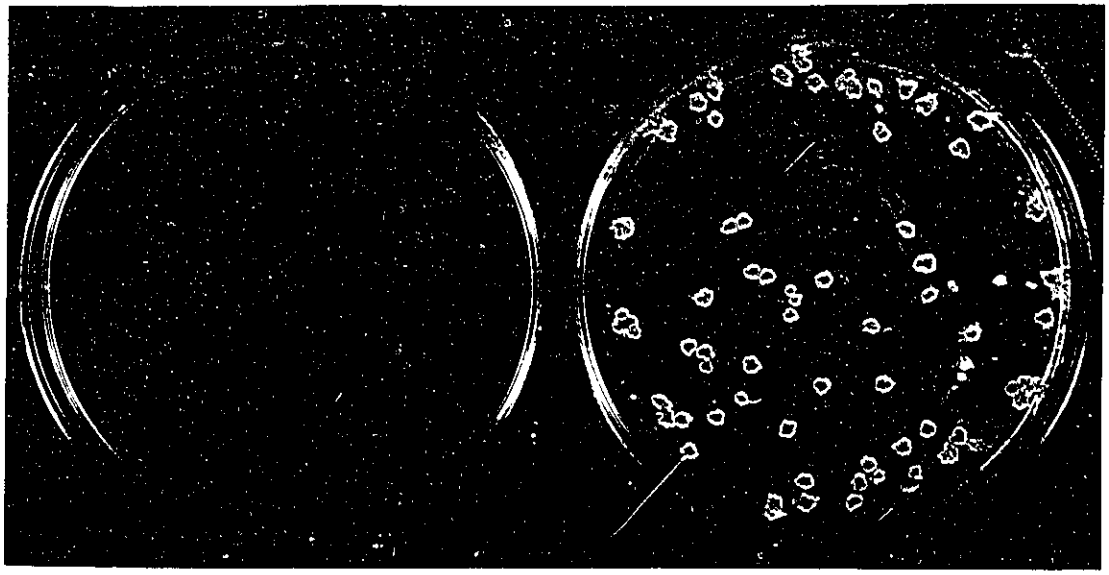
[b] Spontaneous revertants were not detected in *uvs*$^+$.

* Increases or decreases of spontaneous *sC* reversion frequencies in *uvsI* compared to those found for the same alleles in *uvs*$^+$ are statistically significant ($P < 0.05$).

Fig. 4.10. Colonies of UV-induced *sC107* revertants in *uvs*$^+$ strains on agarose minimal medium

Left: Unirradiated control plate (~10^9 cells/plate),

Right: UV-irradiated plate (dose, 16 J/m^2; survival, ~100%)



309, and *321*), while considerable increases were found in *uvsI* strains for *sC* alleles initially generated in *uvs*$^+$ strains (i.e., *sC107*, *211*, *322*, *315*, and *719*; Table 4.3.C). Of the latter alleles, one case, *sC619*, appears to be an exception by showing low reversion frequencies.

After **EMS treatments**, all cases of reversions of such *sC* alleles except one allele (*sC309*) were significantly influenced by *uvsI* mutation; namely, EMS-induced reversion frequencies of most *sC* alleles in *uvsI* mutant strains were far below those obtained for the same *sC* alleles in *uvs*$^+$ control (Table 4.3.C).

In the case of **UV-induced reversions** (see Fig. 4.10, for UV-induced *sC* revertants on agarose MM), effects of *uvsI* varied and were dependent on the alleles tested; i.e., *relative decreases* in the case of some alleles originating from *uvs*$^+$ (*sC107*, *sC619*, and *sC719*) and one allele from *uvsI* (*sC223*), *increases* (*sC211* and *sC315*, originated from *uvs*$^+$; *sC105* and *sC321*, originated from *uvsI*), or *wild type levels* (*sC311* from *uvs*$^+$; *sC309* from *uvsI*) of UV-induced reversion frequencies were demonstrated (Table 4.3.C).

These results clearly demonstrated that *uvsI* had specific effects on mutagenesis, dependent on the nature of the allele tested, i.e., dependent on the molecular type of mutation.

4.4.4. Southern analysis of EMS-induced sC *mutants; evidence for absence of aberrations in* sC *alleles used for reversion test*

To check for detectable chromosomal aberrations in the *sC* gene region of selenate resistant mutants collected after treatment with EMS, Southern analysis of DNA from 22 *sC* mutant strains were performed. Since the *sC*$^+$ plasmid (pFB95) also contains a genomic fragment unlinked to the *sC*$^+$ gene (Buxton et al 1989), genomic DNA digested with *Hind*III was probed with a subcloned 5.5 kb *Sma*I fragment from this plasmid which

contains the *sC*$^+$ gene but not the extra segment (Buxton et al 1989). Thus, when the whole pFB95 plasmid was used as a probe for *Hind*III digested genomic DNA, one extra hybridization band (~4.6 kb) appeared compared to Southern blot using the 5.5 kb *Sma*I fragment (Fig. 4.11 vs. 4.12).

In most cases (i.e., 20 *sC* alleles including all ten of those used for reversion tests out of 22 *sC* alleles induced by EMS), the normal banding pattern was seen (i.e., three bands, of 6.2 kb, 2.9 kb, and 2.0 kb; Fig. 4.12). This finding suggested that point mutations had been induced as expected for the predominant type of mutation after EMS treatment. One of the twelve *sC* mutations which were not included in reversion tests (*sC318*) demonstrated very high spontaneous reversion frequencies in *uvs*$^+$ strains (i.e., 7.9×10^{-5}), much higher than the other ten *sC* alleles tested (see table 4.3.A). Such high reversion frequencies suggest that a very small duplication in the *sC* gene region may cause sulfite requirement of this mutant.

Putative small duplications were detected in two EMS-induced *sC* mutations (i.e., *sC108* and *sC712* ; Figs. 4.12.C-lane 2, A-lane 10). Spontaneous reversion of one of these (*sC108*) in *uvs*$^+$ background occurred with very high frequencies (5.3×10^{-6}, see Table 4.3 for comparison). In contrast, no revertants of the other (*sC712*) were ever obtained, neither spontaneously nor after treatments with UV and EMS in *uvs*$^+$ strains. Possibly, some extra point mutation(s) or small deletion(s) not detectable in Southern analysis, may be involved in this case.

4.4.5. Chromosomal aberrations in the sC *regions of mutants induced by bleomycin*

Bleomycin is a glycopeptide antitumor antibiotic which is considered a radiomimetic agent, because the spectrum of genotoxic effects is similar to that induced by ionizing radiation. Bleomycin induces chromosome breaks and the most frequent

Fig. 4.11. Genomic Southern analysis of the *sC* gene.

Wild type (sC^+) genomic DNA digested with various restriction enzymes was electrophoresed on 0.9% agarose gel, blotted and probed with a DIG-UTP labelled, *Pst*I linearized pFB95 plasmid DNA. Approximate positions of DNA molecular weight markers are indicated (kb).

In Southern blots of genomic DNA an extra band resulted from hybridization with the unlinked genomic DNA fragment included in the pFB95 plasmid (Buxton et al. 1989), namely the second band from the top in lanes of *Hind*III-digested DNA, and the top band after *Bgl*II digestion. Other Blots may contain such bands, but these were not identified specifically.

B, *Bgl*II; **D**, *Dra*I; **E**, *Eco*RI; **EV**, *Eco*RV; **H**, *Hind*III

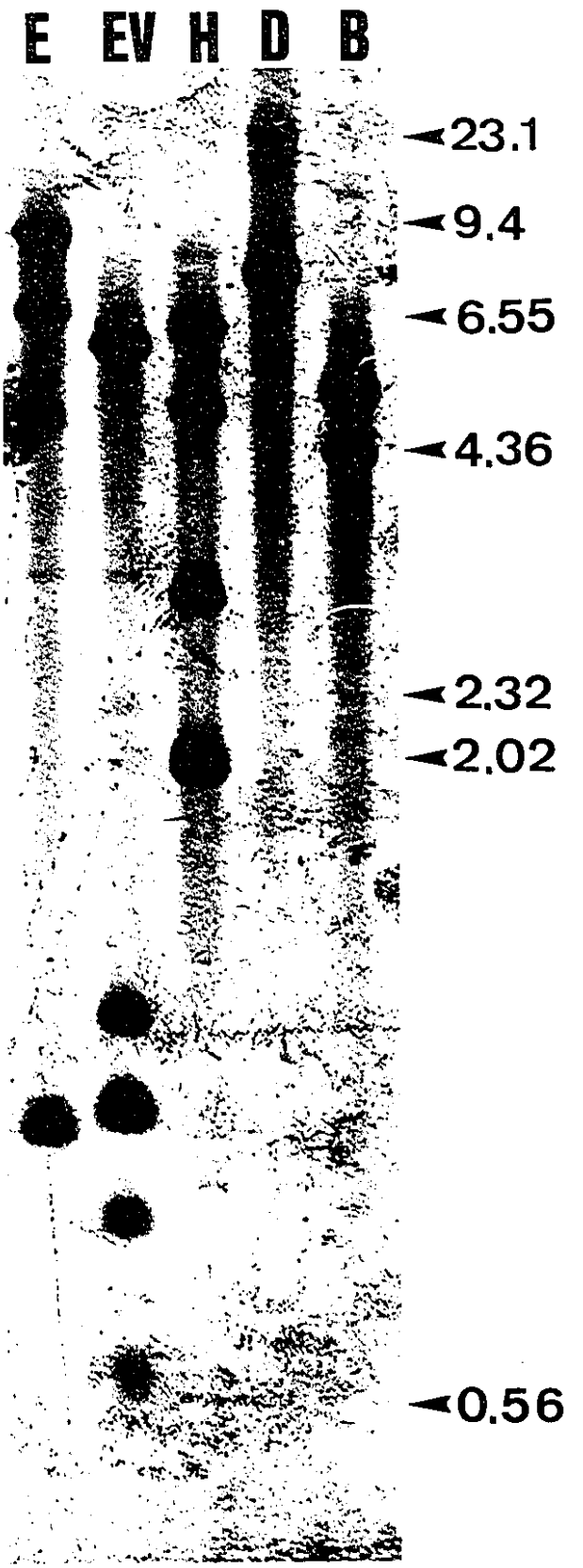

E
EV
H
D
B
23.1
9.4
6.55
4.36
2.32
2.02
0.56

Fig. 4.12. Genomic Southern analysis of EMS-induced *sC* mutants in *uvs*$^+$ and *uvsI* strains.

As Fig. 4.11, but probed with the *sC*$^+$ specific, 5.5 kb *Sma*I DNA fragment from pFB95. The positions of the DNA molecular weight markers are indicated (kb).

A, Genomic Southern blot of *sC* mutants induced by EMS in *uvs*$^+$ strains.

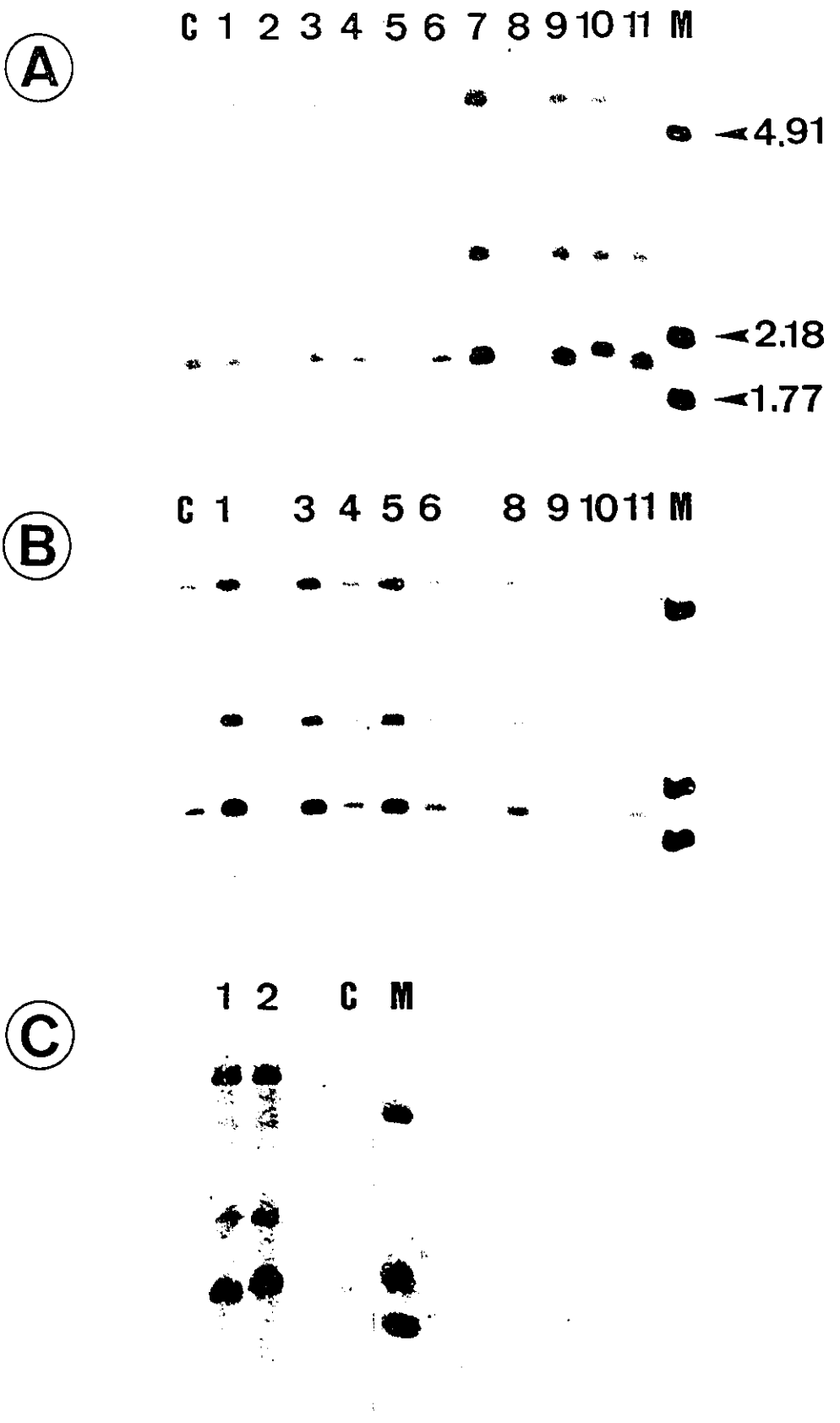
Lanes: **C**, *sC*$^+$ control (M2892; *uvs*$^+$); **1**, *sC107*; **2**, *sC111*; **3**, *sC211*; **4**, *sC308*; **5**, *sC311*; **6**, *sC315*; **7**, *sC 318*; **8**, *sC518*; **9**, *sC619*,; **10**, *sC712*; **11**, *sC719*; **M**, DNA size marker

B, Genomic Southern blot of *sC* mutants induced by EMS in *uvsI* strains.

Lanes: **C**, *sC*$^+$ control (M3547; *uvsI*); **1**, *sC105*; **3**, *sC110*; **4**, *sC115*; **5**, *sC118*; **6**, *sC202*; **8**, *sC210*; **9**, *sC223*; **10**, *sC309*; **11**, *sC321*; **M**, DNA size marker

C, as in A

Lanes: **1**, *sC707*; **2**, *sC108*; **C**, *sC*$^+$ control (M2892; *uvs*$^+$); **M**, DNA size marker


A
C 1 2 3 4 5 6 7 8 9 10 11 M
4.91
2.18
1.77
B
C 1 3 4 5 6 8 9 10 11 M
C
1 2 C M

mutations produced in mammalian cells are chromosomal aberrations, especially multilocus deletions, but also duplications, translocations, and other rearrangements (reviewed by Povirk and Austin 1991; see also Kafer 1990 for bleomycin effects on *Aspergillus nidulans*).

Tests for bleomycin-induced selenate resistant mutations were performed in *uvs*$^+$, *uvsI*, and *uvsC* genetic backgrounds to determine whether these two *uvs* mutations are able to produce normal levels of chromosomal-type mutations. As shown in Fig. 4.13, survival of *uvsI*, *uvsC*, and the double mutants after bleomycin treatment did not deviate from that of *uvs*$^+$ strains. In other words, the two *uvs* gene products apparently are not required for repair of DNA damage produced by bleomycin. This finding, however, was based on tests of nongrowing conidia (for increased sensitivity of *uvsC* to lethal effects of bleomycin during growth and for synergistic interactions of *uvsC* with *uvsI*, see chapter 2, Fig. 2.8).

The frequencies of selenate resistant mutations induced by bleomycin in *uvsI* strains under nongrowing condition were similar to those of wild type (Fig. 4.13). On the other hand, bleomycin-induced increases of mutations were small in *uvsC* strains, especially considering the high levels of spontaneous mutation. In *uvsI;uvsC* double mutant strains, the levels of induction by bleomycin were clearly reduced, not only compared to wild type or *uvsI*, but also compared to the low levels found in *uvsC* single mutant strains.

Southern analysis of bleomycin-induced *sC* mutant strains demonstrated a high proportion of detectable chromosomal mutations in *uvs*$^+$, *uvsI*, and *uvsC* strains (Fig.4.14.A,B,C). Namely, among 14 *sC* mutants tested in *uvsI* strains, seven probably resulted from point mutations, while all others (seven) were caused by aberrations (three cases most likely were deletions, two additions, and two some other type of rearrangements). Similarly, among seven *sC* mutants from *uvsC* strains, one deletion and two rearrangements were detected. Furthermore, even though only three *sC* mutants were

Fig. 4.13. Bleomycin induced selenate-resistance in *uvs*$^+$, *uvsI*, *uvsC*, and *uvsI;uvsC* double mutant strains.

Mutation frequencies (bottom) and survival curves (top) of quiescent conidia treated with bleomycin in buffer. Values are averages of two independent experiments.

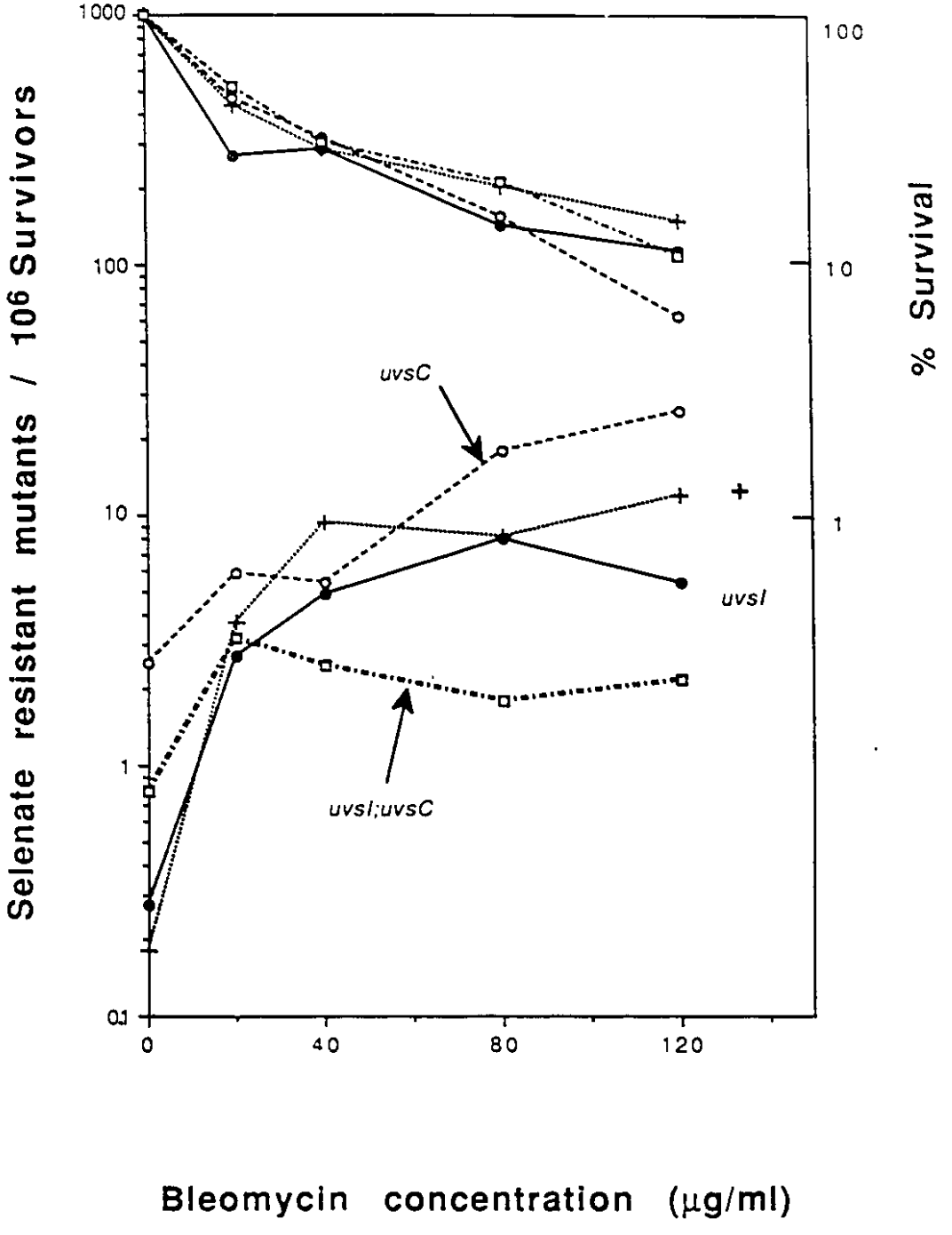

Selenate resistant mutants / 10⁶ Survivors
% Survival
Bleomycin concentration (µg/ml)
1000
100
10
1
0.1
100
10
1
0
40
80
120
uvsC
uvsI
uvsI;uvsC

Fig. 4.14. Genomic Southern analysis of *sC* mutants induced by bleomycin in *uvs*$^+$, *uvsI*, and *uvsC* strains.[as Fig. 4.11 (for A) and Fig. 4.12 (for B and C)].

The positions of the DNA molecular weight markers are indicated (kb). Original genetic background (i.e., *uvs*$^+$ or *uvs*) of bleomycin induced *sC* mutations are represented either as "W" (= wild type, i.e., induced in *uvs*$^+$ strains, for example, *sCW11-24*), or as "I" (= *uvsI* mutants, i.e., induced in *uvsI* strains. e.g., *sCI4-9*), or as "C" (= *uvsC* , e.g., *sCC2-18*).

A, Genomic Southern blots probed with a DIG-UTP labeled, *Pst*I linearized pFB95 plasmid DNA.

Lanes: **1**, *Hind*III digested genomic DNA of *Neurospora crassa*;

2, *Aspergillus sCI4-9*; **3**, *sCI4-5*; **4**, *sCC2-18*; **5**, *sCC2-16*;

6, *sCW11-24*; **7**, *sCW9-22*; **8**, *sCW9-21*;

C, *sC*$^+$ control (M804); M, DNA size marker

Thick arrow indicates hybridization with unlinked genomic DNA fragment (as in Fig. 4.11).

Ⓐ

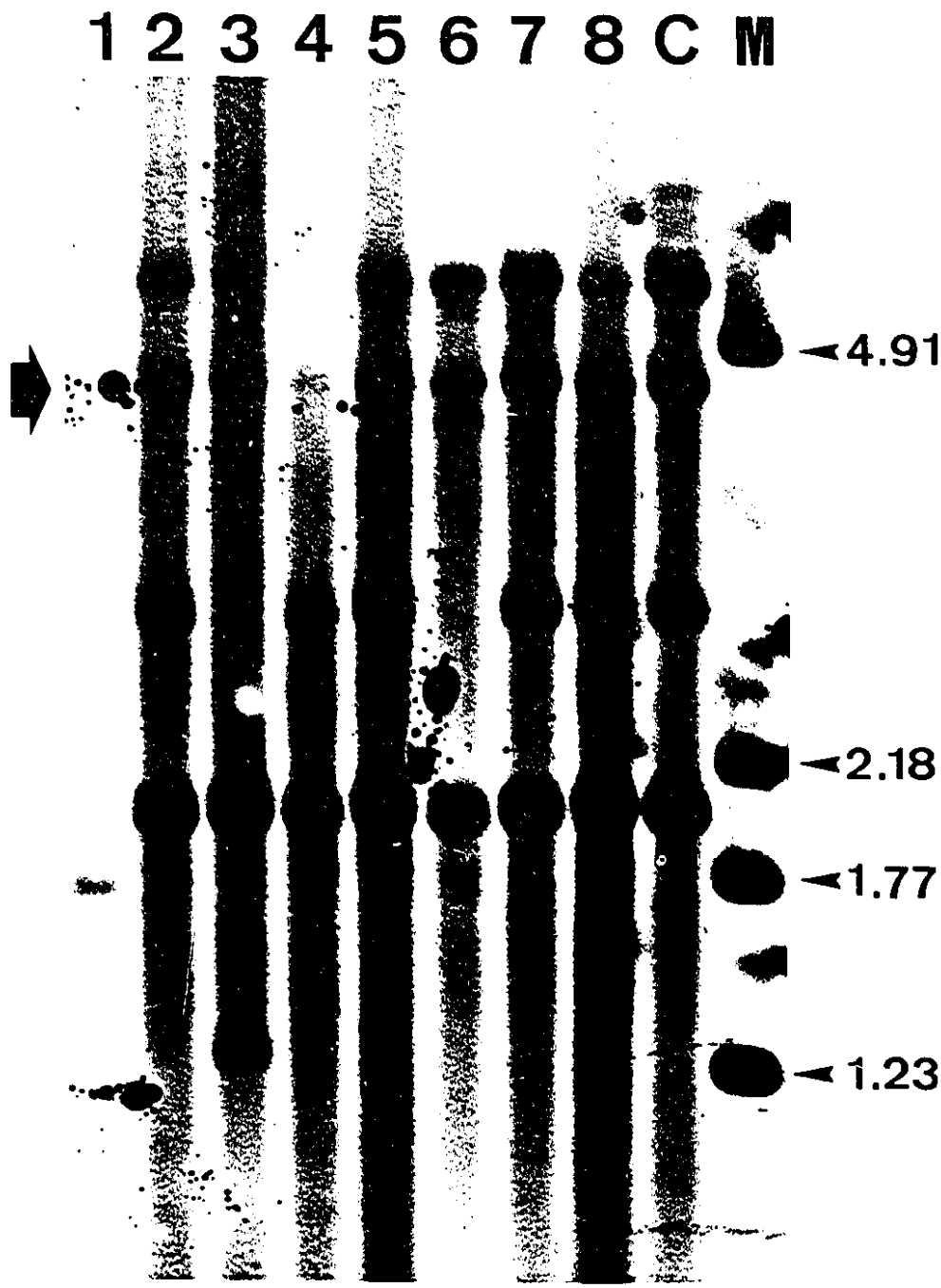

1 2 3 4 5 6 7 8 C M
4.91
2.18
1.77
1.23

Fig. 4.14 (continued)

B, Genomic Southern blots probed with the DIG-UTP labeled, 5.5 kb *Sma*I DNA fragment from the pFB95 plasmid.

Lanes: **1**, *sCC4-21*; **2**, *sCC4-13*; **3**, *sCC4-10*; **4**, *sCC4-8*; **5**, *sCC4-6*; **6**, *sCI6-20*; **7**, *sCI6-18*; **8**, *sCI6-14*; **9**, *sCI6-8*; **10**, *sCI6-7*; **11**, *sCI6-2*; **12**, *sCI5-21*; **13**, *sCI5-20*;

C, sC^+ control (M804); **M**, DNA size marker

The faint hybridization bands (indicated by thick arrow) resulted from hybridization with a contaminating 4.5 kb *Sma*I fragment, rather than with the 5.5 kb *Sma*I fragment DNA.

Ⓑ

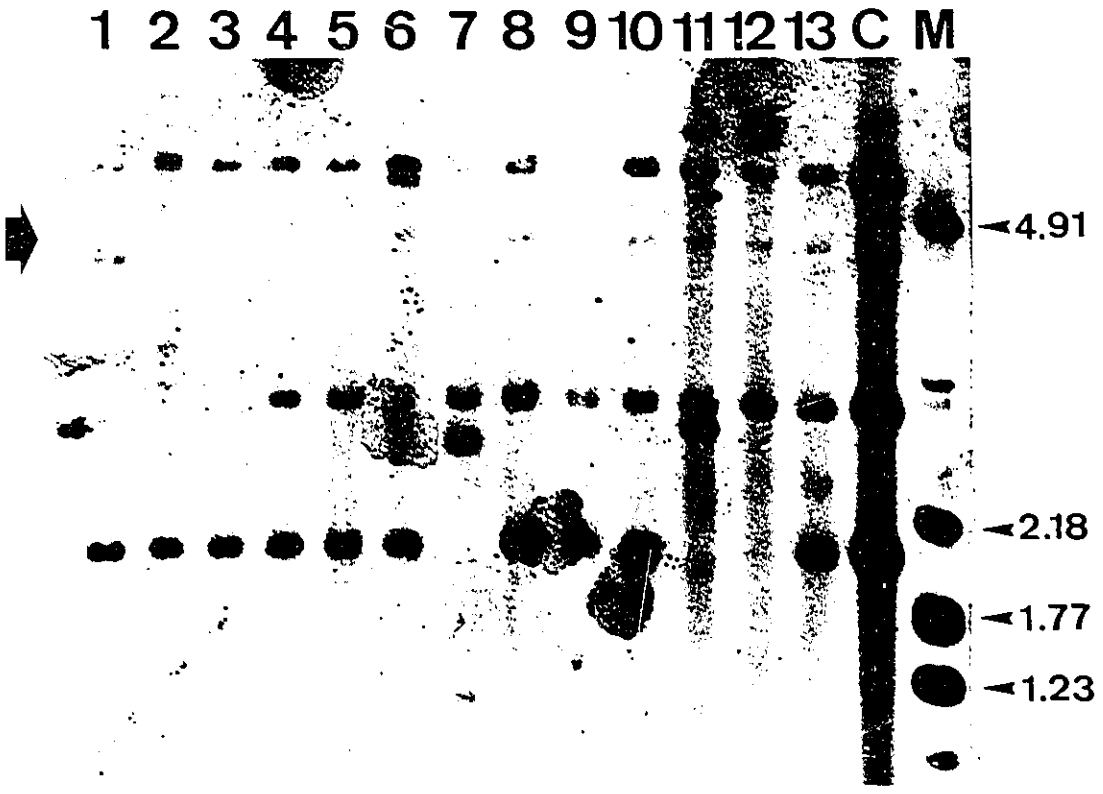

1 2 3 4 5 6 7 8 9 10 11 12 13 C M
4.91
2.18
1.77
1.23

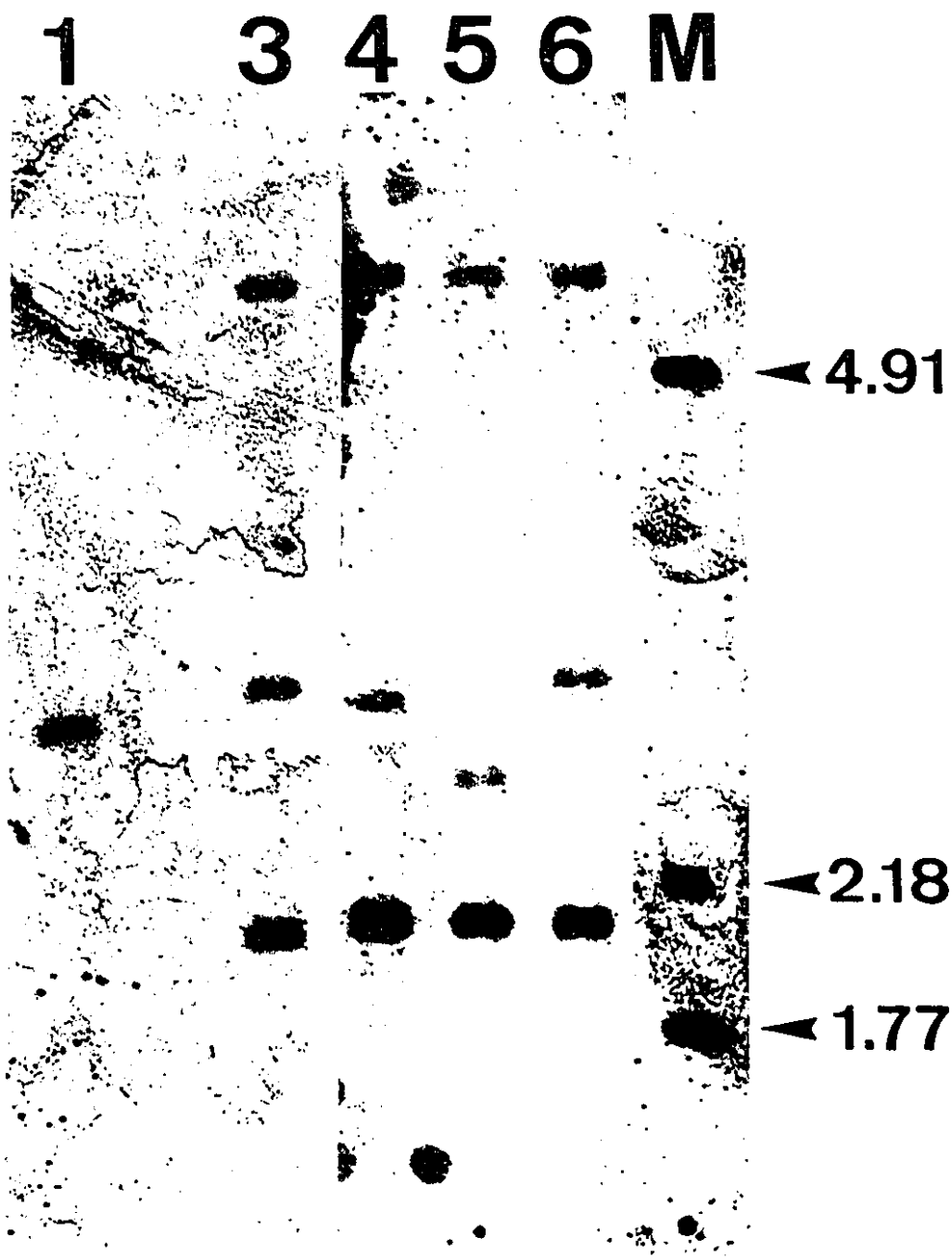
Fig. 4.14 (continued)

C, as B

Lanes: **1**, *Hind*III digested genomic DNA of *Neurospora crassa*;

3, *Aspergillus sCI5-12*; **4**, *sCI5-17*; **5**, *sCI4-21*;

6, *sCI4-18*; **M**, DNA size marker


1
3
4
5
6
M
4.91
2.18
1.77

analyzed in *uvs*$^+$ strains, one case was found which resulted from DNA insertion in the *sC* region.

Further analysis of mutant strains which had DNA insertions or rearrangements identified tandem duplications when a different probe was used [Fig. 4.15.B, *sCI6-18* (lane 6), *sCI6-2* (lane 7); Fig. 4.15.C, *sCC4-13* (lane 3)]. None of these chromosomal aberrations were found to be multilocus deletions including the closely linked *sA* gene , as determined by complementation tests using *sA* control mutant strains (data not shown). This is surprising, because *sA* is located very close to the *sC* gene (the two *s* genes are about 3 kb apart; A. M. Bailey, personal communication).

In conclusion, the results with bleomycin indicate that the frequencies and types of *sC* mutations resulting from chromosomal aberrations were not significantly affected either by *uvsI* or by *uvsC* mutations, when non-growing conidia were treated.

4.5. DISCUSSION

The results presented here showed that the effects of *uvsI* on mutagenesis differ greatly in many aspects from those of *uvsC*. These two mutations were shown to be non-epistatic when determined by relative survival after treatments with various DNA damaging agents (Chae and Kafer 1993). The *uvsI* mutation affected spontaneous mutagenesis differentially, dependent on the test systems used. Namely, different results were obtained when different types of mutations were selected. Antimutator effects of *uvsI* were seen in reversion tests of *choA1*, *pabaA1*, and certain *sC* alleles. In contrast, wild type levels of frequencies were obtained when forward mutations were selected as

Fig. 4.15. Identification of possible tandem duplications in *sC* mutations resulting from insertions and rearrangements after bleomycin treatment (as shown in Fig. 4.14). As Fig. 4.11, but probed with three different DIG-UTP labeled DNA fragments from the pFB95 plasmid DNA.

A, Southern blots of genomic DNA from *sC* mutants caused by chromosomal-type mutation probed with the 5.5 kb *Sma*I DNA fragment (indicated by *probe A* in **D**).

Lanes: **M**, DNA size marker; **1**, *sCW11-24*; **2**, *sCC4-21*; **3**, *sCC4-13*; **4**, *sCC4-10*; **5**, *sCI6-20*; **6**, *sCI6-18*; **7**, *sCI6-2*; **8**, *sCI5-21*; **9**, *sCI5-17*; **10**, *sCI4-21*; **11**, *sCI4-5*; **C**, *sC*$^+$ control (M3547)

B, same as A, but reprobed with a DIG-UTP labeled 2 kb *Hind*III fragment (*probe B*, indicated in **D**) after stripping the membrane (see "Materials and methods").

Lanes; same as A. Tandem duplications are detected in lane 6 (*sCI6-18*) and lane 7 (*sCI6-2*).

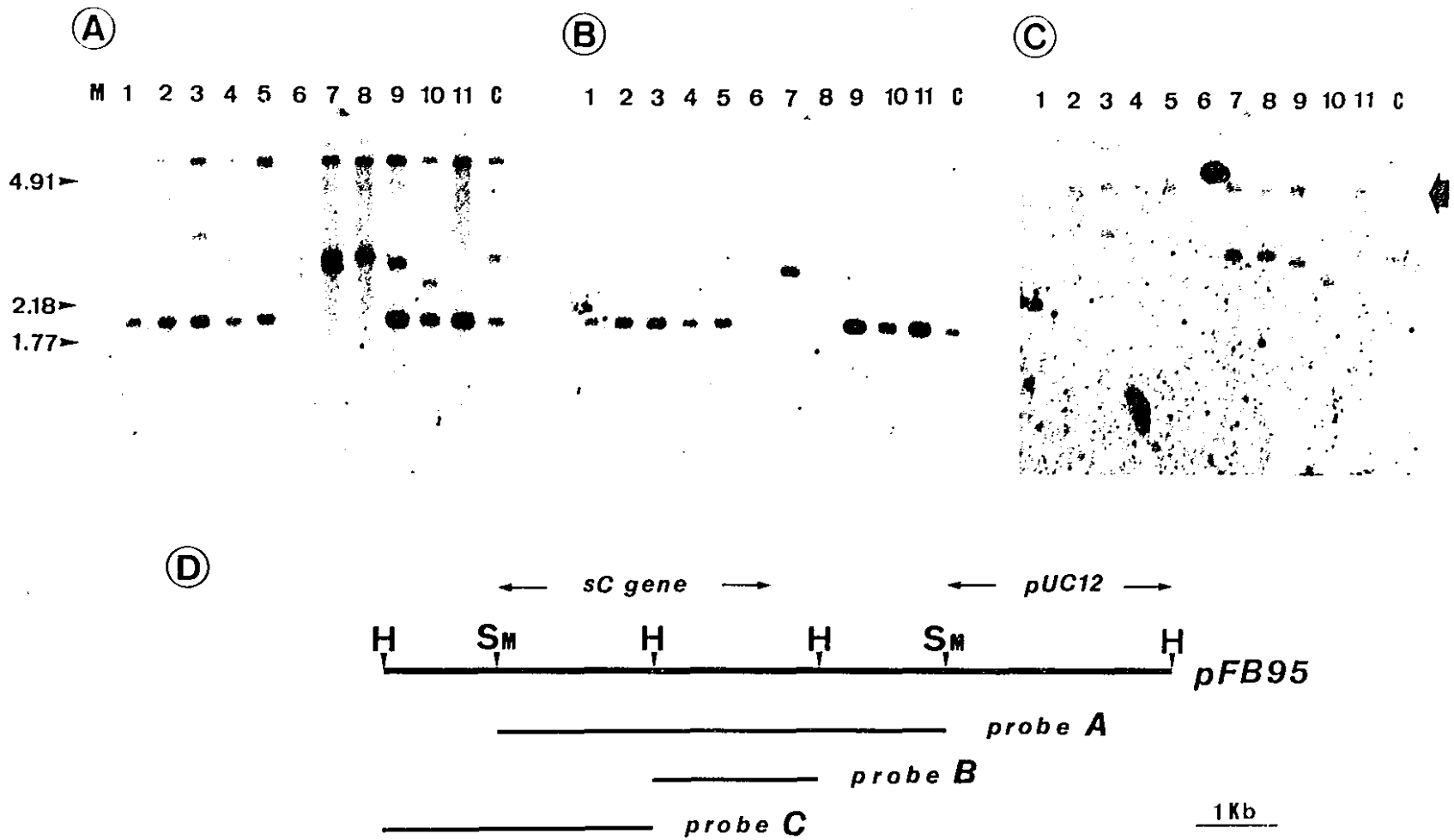
C. same as A, but probed again with a DIG-UTP labeled 3.3 kb *Hind*III fragment DNA (*probe C*, indicated in **D**) after stripping.

Lanes; same as A. Thick arrow; as in Fig. 4.11.

Tandem duplications are detected in lane 3 (*sCC4-13*).

D. Three different probes for Southern blots. The largest size of the *sC* gene and restriction enzyme sites are indicated (Buxton et al. 1989).

H, *Hind*III; **Sm**, *Sma*I


A
M 1 2 3 4 5 6 7 8 9 10 11 C
4.91
2.18
1.77
B
1 2 3 4 5 6 7 8 9 10 11 C
C
1 2 3 4 5 6 7 8 9 10 11 C
D
sC gene
pUC12
H
SM
H
H
SM
H
pFB95
probe A
probe B
probe C
1Kb

selenate resistant mutants or *adE20* suppressors. In addition, mutator phenotype of *uvsI* was demonstrated for spontaneous reversion of several *sC* mutations (Table 4.3.C). In the case of *uvsC*, mutator phenotype was consistently demonstrated whatever test systems were applied, which confirms again earlier results using selenate and *p*-fluorophenylalanine resistance (Jansen 1972; Kafer and Mayor 1986). Different from the effects of *uvsI* on recombination, *uvsC* resembles *recA* of *E. coli* and *pso4* of yeast (Henriques et al. 1989; Andrade et al. 1989) in being defective both for recombination and for DNA damage-induced mutation. However, in contrast to both of these mutations, *uvsC* exhibits pronounced mutator phenotype. Mutator phenotype is typically shown in mutants defective in error avoidance systems or mismatch repair. However, *uvsC* is unlikely to be involved in mismatch repair, since a hyper-rec phenotype is often associated with mutator effects shown by mismatch repair-defective mutants. On the other hand, altered replication fidelity could be one possible cause for the mutator effect. Alternatively, spontaneous premutagenic lesions in *uvsC* mutants may be channeled through other mutagenic DNA repair pathway(s). Such a "channeling hypothesis" has been proposed for explanation of radiation-sensitive, *rad*, mutants which also have mutator effects (Hastings et al. 1976).

uvsI;uvsC double mutant strains, when tested for spontaneous mutation, resembled *uvsC* in showing mutator effects for both types of forward mutation, while causing drastic reduction of reversion for *choA1* and *pabaA1*, similar to findings for *uvsI* strains. This implies that a large portion of the mutations produced in *uvsC*, i.e., mutator, strains is independent of the *uvsI* function. In contrast, production of a certain type(s), presumably a very small fraction, of mutations in *uvsC* strains may require a functional *uvsI* gene product. Furthermore, considering the additive effects of *uvsI* and *uvsC* in double mutant strains with regard to the complete lack of spontaneous *choA1* reversion, residual *choA1* revertants shown in *uvsI* strains may be due to a functional *uvsC* product which presumably generates mutations in different ways.

The effects of *uvsI* and of *uvsC* on induced-mutagenesis after UV and EMS treatment appeared also to be distinct. The *uvsI* gene was responsible only for specific types of induced revertants, while *uvsC* mutation caused considerable reduction of induced mutations in all test systems. Such specific effects of *uvsI* was apparent when compared with EMS-induced reversion of *choA1* to that of *pabaA1* (see Fig. 4.8 and Table 4.2). In the case of *uvsI;uvsC* double mutants, the pattern of UV- and EMS-induced forward mutagenesis was like that of *uvsC*, but revertants of *choA1* were totally absent. Probably, *uvsI* and *uvsC* compete with each other in different manners for different types of premutagenic DNA lesions which are induced after UV and EMS treatment. Overall, it is clear that the role of *uvsI* on mutagenesis is qualitatively very different from that of *uvsC*.

EMS is well known to induce preferentially G:C to A:T transitions presumably by mispairing of thymine opposite O^6-alkylguanine adduct during DNA replication both *in vitro* (Abott and Saffhill 1979; Dodson et al. 1982) and *in vivo* (Loechler et al. 1984). Such bias of G:C to A:T transition was well documented when EMS-induced mutations were sequenced. Namely, the proportion of G:C to A:T transitions was more than 98% among *lacI*$^-$ mutations induced by EMS in *E. coli* (Burns et al. 1986), > 96% in yeast (among *SUP4-0* mutations, Kohalmi and Kunz 1989), and 76% in *Drosophila* (*vermilion* mutations, Pastink et al. 1991). In addition, to a small extent, A:T to G:C transitions as well as transversions (mainly from G:C site) were detected. A:T to G:C transitions may be the result of misinsertion of guanine at O^4-alkylthymine lesions during DNA replication (Hall and Saffhill 1983; Preston et al. 1986, 1987). On the other hand, misincorporation of adenine or guanine across from apurinic (AP) sites could generate G:C to T:A or G:C to C:G transversions (Sagher and Strauss 1983; Randall et al. 1987). Alkylations of guanine, i.e., N^7 or N^3-alkylguanine, stimulate destabilization of the *N*-glycosylic bonds and, in consequence, produce AP sites in the DNA (Loeb and Preston 1986).

Considering the very high ratio of G:C to A:T transitions in EMS mutagenesis, EMS-induced *sC* mutations in *uvs*$^+$ strains collected on selenate media most likely contain mutated A:T base pairs which cause the sulfite requirement. When EMS-induced reversion tests for these six different *sC* alleles were carried out, in all cases, the reversion frequencies in *uvsI* mutant strains were considerably reduced compared to those found for the same *sC* alleles in *uvs*$^+$ strains (Table 4.3.C). This result may well reflect a reduction of A:T to G:C transitions in *uvsI* strains, if such reversions were considered as true reversions (i.e., restored original DNA sequence). In addition, in all cases except one, the frequencies of EMS-induced reversion of four *sC* alleles originally isolated in *uvsI* genetic background with EMS treatment were reduced in *uvsI* strains (Table 4.3.C). The exceptional case (*sC309*) may reflect that this mutation presumably occurred by transversion rather than G:C to A:T transition when isolated originally with EMS treatment (see below, for the possibility of increases of transversions in *uvsI* strains).

When considering a very large number of cells (~10^8) plated for selecting revertants, relative high increases of EMS-induced reversion frequencies for some *sC* alleles are perhaps not surprising, even though A:T to G:C transitions are the minor event in EMS mutagenesis (especially for *sC311*, ~1.5 x 10^3 fold increase compared to the frequency found for spontaneous reversion; see Table 4.3.A). Similarly, in V79 hamster cells, high reversion frequencies for *N*-ethyl-*N*-nitrosourea (ENU)-induced reversion of *hprt* mutations originally isolated as 6-thioguanine resistance with EMS treatment have frequently been observed (i.e., in several cases, ~10^3-fold increases compared to the frequency found for spontaneous reversion were observed; Brown et al. 1986). In the case of ENU mutagenesis, G:C to A:T transition was shown to be also the predominant type of mutation (Richardson et al. 1987; Burns et al. 1988).

When revertants were selected, suppressor mutations rather than true reversions cannot be ruled out. In reversion of *sC* alleles, however, indirect intergenic suppressions, i.e., circumventing of defects due to the mutation by modification of

alternative pathways, is not likely to be an explanation, since no revertants, i.e., no such suppressions, were found in reversion tests of *sC* alleles which contain deletions in the *sC* gene (data not shown). On the other hand, EMS-induced G:C to A:T transitions could cause specific forward mutations which would lead to intergenic suppression (e.g., at the tRNA level) or intragenic suppression, i.e., another mutations in the *sC* gene which could restore functional *sC* protein. If this is the case and, therefore, a reduction of G:C to A:T transition is a major effect of the *uvsI* mutation, *sC* mutations obtained initially in *uvsI* mutants after EMS treatment may contain mutated G:C base pairs other than A:T base pairs with a relatively high frequency. In that case, high reversion frequencies for these *sC* alleles in *uvs*$^+$ strains would be expected after EMS treatment. However, the results shown in Table 4.3.C do not support this hypothesis, since in *uvs*$^+$ strains the absolute frequencies for EMS-induced reversion of *sC* alleles induced originally in *uvsI* mutants were much lower than those seen for *sC* alleles induced initially in *uvs*$^+$ strains. Thus, the simplest explanation for base pair-specific effects of *uvsI* is a reduction of A:T to G:C transitions.

Based on such a hypothesis, it could be imagined that increases of transversion(s) at the sites of A:T base pairs in *uvsI* mutants would lead to enhanced spontaneous mutability, as seen for several *sC* alleles (Table 4.3.C). However, such mutator effects of *uvsI* are found only for *sC* mutations induced initially in *uvs*$^+$ background, at least among ten *sC* mutations tested here. Assuming that A:T to G:C transitions are required for true back mutation of these *sC* alleles, restoration of wild-type phenotype through transversions at mutated A:T site would depend on the codon changes required for functional amino acid at the site of original mutations. In other words, local DNA sequence at the mutated site would affect the frequencies of reverse mutation. However, distribution of lesions induced by EMS, i.e., spectra of mutation, in the *sC* gene may not be different in *uvsI* compared to *uvs*$^+$ strains, since in *uvs*$^+$ strains spontaneous mutation frequencies for *sC* alleles induced originally in *uvsI* strains did not deviate from those

found for *sC* alleles isolated initially in *uvs*$^+$ background (Table 4.3.A). Furthermore, it has been shown that EMS does not exhibit "site specificity" of G:C to A:T transition in *E. coli* (Burns et al. 1986), yeast (Kohalmi and Kunz 1986), or human systems (Ingle and Drinkwater 1989) in contrast to alkyl-nitrosoguanidines and alkyl-nitrosoureas which predominantly cause such transition at guanidines preceded by a purine base (i.e., 5'-RG-3' site: Burns et al. 1987, 1988; Richardson et al. 1987, 1988). Thus, such bias seen for mutator effects of *uvsI* only in *sC* alleles obtained originally in *uvs*$^+$ strains presumably resulted from a difference in the molecular nature of the *sC* mutations induced originally in the *uvsI* background compared to those induced in *uvs*$^+$ strains. In other words, DNA sequence context at mutation sites may not the cause of the observed bias. Nevertheless, large differences in the frequencies of EMS-induced *sC* reversions among various *sC* alleles in *uvs*$^+$ background imply that A:T to G:C transitions induced with EMS may be affected by neighboring bases (see Table 4.3.A). Comparable data also exist for ENU-induced reversion of *hprt* mutations produced originally with EMS treatment in V79 hamster cells (Brown et al. 1986). In fact, in mutational spectra of ENU, A:T to G:C transition mutation occurs mainly at thymidines preceded by a purine base (i.e., 5'-RT-3' site: Burns et al. 1988).

The relative frequencies of UV-induced reversion of *sC* mutations in *uvsI* mutants were not consistently lower compared to those found for the same *sC* alleles in *uvs*$^+$ strains (Table 4.3.C). This was not unexpected, because UV is believed to have no mutagenic specificity (Coulondre and Miller 1977; Cupples and Miller 1989). On the other hand, tests for the opposite directional mutations, i.e., A:T to G:C transitions, base analogues which have such mutagenic specificity, e.g., 2-aminopurine or 5-bromodeoxyuridine, were considered but could not be included, because these analogues are known to be very weak mutagens for *Aspergillus* (Calvori and Morpurgo 1966) as well as for *Neurospora* (Brockman et al. 1987).

Based on the proposed base pair-specific effect of the*uvsI* mutation, it is likely that the *choA1* mutation was caused by a mutant A:T base pair when first induced with UV irradiation by Pontecorvo and coworkers (Kafer 1958), since lower reversion frequencies for *choA1* were obtained in *uvsI* mutants than found in wild type strains after EMS treatment (Fig. 4.8). Among each of ten *choA1*$^+$ revertants from *uvs*$^+$ or *uvsI* strains, no suppressor mutations were ever recovered (data not shown). On the other hand, the molecular nature of mutation which led to *pabaA1* presumably differs from that of *choA1*, since *uvs*$^+$ and *uvsI* both showed induction with similar frequencies of *pabaA1* revertants after EMS treatment (Table 4.2).

Allele-specific defects in mutation induction are not uncommon in *rev* genes of yeast, even though their molecular basis is not clear (reviewed by Lawrence 1982). Interestingly, the postulated base pair-specific effect of *uvsI* is comparable to that found for antimutator mutants of the bacteriophage T4 DNA polymerase (i.e., *ts*CB87 and *ts*CB120, temperature sensitive alleles of gene 43; Drake and Allen 1968). The antimutator effects of these alleles were practically limited to A:T to G:C transitions, and barely affected G:C to A:T transitions (Drake and Allen 1968). Moreover, certain types of transversions were moderately increased in strains carrying the same antimutator polymerase alleles (Ripley 1975). Biochemical study of the DNA polymerase enzyme from these antimutator strains suggested that accuracy of 3' to 5' exonuclease proofreading function of DNA polymerase was enhanced (Muzyczka et al. 1972). Several other antimutators also have been shown to be the result of mutations in DNA polymerases (e.g., Halls et al. 1985, for DNA polymerase mutant in Herpes simplex virus; Schaaper and Cornacchio 1992, antimutator allele of the *dnaE* of *E. coli*; Morrison et al. 1989, non-essential DNA polymerase encoded by *rev3* in yeast; see section 1.2.1.b, for details). Considering the properties of antimutator mutant strains, it is of interest to determine whether the *uvsI* gene has homology with some sequenced DNA polymerase genes.

The *uvsI* gene is very closely linked to the *sC* gene (see Table 3.1). Thus, a cosmid clone (CSA2) which contains the *sC* as well as the *sA* genes, and a continuous sequence extending for a large distance in the direction of the *uvsI* gene (A. M. Bailey, personal communication) might include the *uvsI* gene or help in its cloning by means of overlapping cosmids. Furthermore, to test base pair-specific effects of *uvsI*, particular *sC* alleles and their revertants should be sequenced. For this project, various deletion mutants of *sC*, which were found after bleomycin treatment, would be useful. Specific break points in the *sC* gene will aid in mapping of the *sC* alleles of interest to subregions of the *sC* gene, which would facilitate their sequencing by polymerase chain reaction.

CHAPTER 5

CONCLUDING REMARKS

The work described in this thesis pertains to the genetic characterization of the UV-sensitive *uvsI* mutants in *Aspergillus nidulans*.

The growth phenotype, i.e., colonial morphology, growth rate and conidiation, of *uvsI* is very similar to wild type when mutagens are not applied. The *uvsI* mutants showed very high sensitivity specifically to UV light and 4-NQO, a UV-mimetic chemical agent. However, killing effects of these mutagens were mainly limited to quiescent conidia of *uvsI* mutant strains. A high increase of UV-survivals (~100x) was observed during growth of this mutant strain. In addition, when conidia were plated on 4-NQO containing CM, colonial growth rate and morphology of *uvsI* strains were indistinguishable from wild type (unpublished observation). In the latter case, all other 4-NQO-sensitive *uvs* mutants exhibited growth restrictions on such a medium. On the other hand, *uvsI* showed wild type levels of sensitivities to simple alkylating agents, such as MMS, EMS, and MNNG, when both quiescent and growing conidia were treated. Thus, the *uvsI* gene product is mainly involved in a DNA repair process for UV- and 4-NQO-induced DNA lesions and presumably required before cells enter the G2 cell stage.

Effects of *uvsI* on mutagenesis were dependent on mutational assay systems which select different types of mutations. Such a dependence was postulated to be due to base pair-specific effects of *uvsI* with regard to the mutations produced. Reduced A:T to G:C transition mutagenesis in *uvsI* mutant may cause reduction of reversion frequencies for certain mutant alleles, while frequencies of forward mutations which select all types of mutations were not affected. In addition, an increase of transversions from A:T base pair was proposed based on the observation of mutator phenotype and enhanced reversion frequencies for certain *sC* alleles in *uvsI* mutant strains. On the other hand, *uvsC* mutation caused a high reduction of mutation frequencies after mutagen treatment in all systems used. However, the *uvsC* gene may not be required for the processing of all

types of premutagenic DNA lesions to stable mutations. Introduction of other mutations, i.e., *uvsI* or *musN*, into *uvsC* mutant strain caused an increase of UV-induced selenate resistant mutation frequencies compared to those in *uvsC* single mutant strains. In the case of *uvsC;musN* double mutant strains, enhanced selenate resistant mutation frequencies after UV irradiation compared to those in *uvsC* strains were largely due to the active *uvsI* gene products, since in triple mutants of *uvsC;musN* with *uvsI*, the mutation frequencies were reduced compared to those in *uvsC;musN* double mutant strains and were similar to those in *uvsC;uvsI* double mutant strains (see Fig. 5.1. in APPENDIX II, for the frequency in triple mutant strains; see Fig. 4.4. for *uvsI;uvsC* double mutant strains). On the other hand, a slight increase of UV-induced selenate-resistant mutation frequencies in *uvsI;uvsC* double mutant strains may suggest an existence of other mutagenic DNA repair pathway(s).

Overall, it is unlikely that the *uvsI* gene is a member of a subgroup in the UvsC epistatic group, since the effect of *uvsI* on mutagenesis was qualitatively very different from that of *uvsC*. More likely, the *uvsI* gene may play a role in an alternate mutagenic DNA repair pathway. This proposal was also supported by the observation of synergism between *uvsI* and *uvsC* mutant strains to the killing effects of all the various mutagens tested. In *Aspergillus nidulans*, multiple mutagenic DNA repair pathways presumably operate to increase cell survival after DNA damage.

The phenotype of the *uvsI* mutant strains is unique and was not previously observed among other *uvs* mutant strains of *Aspergillus*. In fact, the *uvsI* mutants defined a fourth epistatic groups on the basis of sensitivity of all possible pairs of *uvsI* with other *uvs* mutant strains to UV light, 4-NQO, and MMS. These four groups, i.e., UvsF, UvsC, UvsI, and UvsB, may well represent four different functional groups or DNA repair processes, since all groups exhibited distinct characteristics.

Possibly, the UvsB group genes which may be required for DNA double strand break repair are functionally separate from a recombinational DNA repair pathway mediated by UvsC group genes. In *E. coli* and yeast, DNA double strand break repair is believed to be carried out by genes of the recombinational repair pathway. Alternatively, the UvsB group may represent a novel DNA repair pathway. On the other hand, the UvsC group genes presumably act on both recombinational DNA repair and mutagenic DNA repair processes, which differs from the properties of the RAD52 group in yeast. Moreover, the UvsC group genes may play a major role at the G2 cell stage. A nucleotide excision repair process is possibly mediated by genes in the UvsF group, but remains to be proved. The UvsI group gene may be involved in a minor mutagenic DNA repair process which plays an important role in cell survival after UV and 4-NQO treatments at the G1 cell stage.

It is clear that the phenotypes of each of the four epistatic groups in *Aspergillus nidulans* (as discussed in chapter 2) do not agree well with those represented for the three epistatic groups of radiation DNA repair in the yeast, *Saccharomyces cerevisiae*, nor those found in the three major DNA repair processes in *E. coli*. However, for further direct evidence of functional groups, especially for excision types, biochemical analysis for excision capacity in *uvs* mutant strains is required. In addition, molecular cloning of *uvs* genes of interest or "cross-species-complementation tests" with cloned DNA repair genes may help to elucidate their functions on DNA repair processes.

APPENDIX II

1. ASPERGILLUS MEDIA

Minimal medium (MM): for 1 liter of solid medium use:

(A)	Agar	12.5 g; Steam in 750 ml of demineralized or distilled water
(B)	Salts	50 ml of "20x Salt mix - $MgSO_4$"
	$MgSO_4$	0.52 g
	C-source	10 g of Glucose
	TE	1 ml of Trace element stock solution (see next page)
	Water	950 ml

Mix in 2 l flask pH will be ca. 6.4, no adjustment

Mix (A) with(B), dispense, supplement as needed, autoclave, and pour plates, or store at room temperature until needed.

Complete medium (CM); for 1 liter of solid CM:

To (B) of minimal medium, add the following (before agar):

Difco-Bacto Peptone	2 g
Difco Bacto yeast Extract	1 g
Casamino Acids	1 g
Vitamin Solution (see next page)	1 ml

[plus extra riboflavine(0.5 mg), amino acids(lysin-Hcl, 36.5 mg; L-methionine, 5 mg), purines (adenine-Hcl, 15 mg) or pyrimidins, if optimal growth of such mutants is required]

Mix with (A) of MM, dispense to flasks, autoclave and pour as needed.

Acetate Medium: for 1 liter of solid medium

Ammonium acetate	12 g
NaCl	2 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KH_2PO_4	3 g
Trace Element	1 ml
Agar	12.5 g
Water	up to 1 liter

Adjust pH to 6.1 by 4N-HCl, and autoclave

"MM - Dextrose"

Make MM but leave out detrose and autoclve.

To add 1% of galactose, lactose, sorbitor, or maltose

Salt Mix - $MgSO_4$ (20x stock)

To make up 1 liter, dissolve in 800 ml distilled water

$NaNO_3$ (sodium nitrate)	120.0 g
KCl (potassium chloride)	10.4 g
KH_2PO_4 (potassiun monobasic)	16.3 g
K_2HPO_4 (phosphate dibasic)	20.9 g

Hutner's Trace Element Solution: use 1 ml per liter of medium

To make up 100 ml, dissolve in 80 ml distilled water (in sequence, one at a time)

$ZnSO_4 \cdot 7H_2O$ (Zinc sulfate)	2.2 g
H_3BO_3 (boric acid)	1.1 g
$MnCl_2 \cdot 4H_2O$ (Manganous chloide)	0.5 g
$FeSO_4 \cdot 7H_2O$ (Ferrous sulfate)	0.5 g
$CoCl_2 \cdot 6H_2O$ (Cobaltous chloride)	0.16 g
$CuSO_4 \cdot 5H_2O$ (Cupric sulfate)	0.16 g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Ammonium molybdate)	0.11 g
EDTA, tetrasodium salt	6.5 g
EDTA, disodium salt	0.77 g

Fill up to 100 ml, autoclave and store at 4° C; unadjusted pH will be 6.5

Vitamin Solution: use 1 ml per liter of medium

To make up 200 ml, dissolve in 160 ml distilled water

Pyridoxin-HCl	0.02 g
Thiamin-HCl	0.03 g
p-aminobenzoic acid	0.15 g
Nicotinic acid	0.5 g
Riboflavin	0.5 g
Choline-HCl	4.0 g
Biotin (use conc. stock: 50 mg/ 100 ml DW)	10 ml

Autoclave and store at 4° C

2. STOCK SOLUTIONS

1x : use 1 ml in 100 ml MM;
10x : use 0.1 ml in 100 ml MM;
100x : dilute 1/10 to get 10x.

Vitamins

Aneurine (=Thiamine-HCl)	0.15 g in 100 ml of DW	= 100x
Biotin	0.03 g	100x
Choline-HCl	2.0 g	10x
Nicotinic acid	0.3 g	10x
p-aminobenzoic acid	0.1 g	10x
Ca-pantothenate	10.0 g	10x
Putrescine	0.4 g	10x
Pyridoxin-HCl	0.05 g	100x
Riboflavine	0.03 g	1x

Amino acids: 1x usually 0.1 M

L-Arginine-HCl	2.1 g in 100 ml of DW	= 1x
(for argB weigh out 0.3 g - 0.6 g per liter MM or CM)		
L-Cysteine-HCl	0.2 g	1x
Histidine	1.0 g	1x
Isoleucine	0.3 g	1x
Leucine	0.3 g	1x
L-Lysine	3.7 g	1x
L-Methionine	0.5 g	1x
L-Ornithine-HCl	1.7 g	1x
Phenylalanine	0.5 g	1x
L-Proline	1.0 g	1x
L-Tryptophane	weigh out: 0.6 g per liter MM or CM	

3. INHIBITORS

Acriflavin:

For the stock solution, dissolve 0.5 g in 100 ml DW. Acriflavin is light sensitive, thus autoclave in brown bottle and store at 4°C.
Add 0.5 ml of stock solution to 100 ml hot CM, mix well and pour 4 plates. Such plate can be stored at 4°C for several weeks. Higher levels of acriflavin (up to 0.75 ml / 100 ml CM) are needed for media containing extra adenine (if tests include *ad*$^+$ strains; *ad*$^-$ strains are slightly more sensitive).
Distinction between wild type and *Acr* strains is usually possible after incubation for 2 days at 37°C, i.e., practically no growth of sensitive, and normal growth of resistant strains are found; normally, heterzygous diploids show clearly reduced growth (and dark colour on the back of plates) but may produce resistant sectors.

Actidione (= Cycloheximide):

Actidione is difficult to dissolve; weigh out amounts required and dissolve in sterile hot CM by stirring on hot plate or heating in steam bath. Do not autoclave.
Use 0.125 - 0.15 g per 100 ml CM to test haploids (heterozygous crosses), more up to 0.55 g per 100 ml for heterozygous vs. homozygous diploids. Plates can be stored at 4°C for several weeks.
Results can be read after 2 days at 37°C in the case of crosses, but heterozygous diploids can be recognized more easily by reduced growth at the higher concentration and such plates may need 3 days of incubation (sectors may be found for *Act* / + types, but is less clear than in the case of acriflavin).

Oligomycin: 5 mg in 1.5 ml methanol plus 9 ml DW (for 1X). Keep at 4°C. Add 1 ml per 100 ml hot CM

***p*-Fluorophenylalanine** (for test of resistance): 9.2 mg / ml DW (for 1/2 x). Keep at 4°C. Add 2 ml per 100 ml MM

Sulfanilamide: weigh out 2.2 g per 400 ml hot CM (0.0375 M)

4. SELENATE MEDIUM (SEM)

For 1 liter of solid SEM

(A)	188 ml	DW
	50 ml	MM-NO_3 Salt solution (20X)
	1 ml	Hunter's trace element
	10 g	Dextrose
	0.4 g	Urea
		Adjust pH 6.5 with 3N NaOH
(B)	750 ml	DW
	12.5 g	Bacto Agar
		Dissolve separately

Mix (A) with (B), dispense, and autoclave

Add supplements before use

1. 2 ml / 400 ml medium D-Methionine (1X) ($2x10^{-4}M$)
2. 8 ml / 400 ml medium Selenate stock solution ($1x10^{-3}M$)
3. Supplements, as required by nutritional markers

Stock Solution

1. D-Methionine (1X); 0.5 g/100 ml DW
2. Selenate (1/4 X); 0.99 g Na_2SeO_4 anhydrose / 100 ml DW

MM-NO3 salt solution (20x)

To make up to 100 ml, dissolve the followings one at a time

NaCl	8.25 g
KCl	1.04 g
MgSO4.7H2O	1.04 g
KH2PO4	3.04 g

5. CHROMATE MEDIUM (CHM)

For 1 liter of solid CHM

(A)	188 ml	DW
	50 ml	MM-NO_3 Salt solution (20X)
	1 ml	Hunter's trace element
	10 g	Dextrose
	0.4 g	Urea (5mM)

Adjust pH 6.5 with 3N NaOH

(B)

750 ml	DW
12.5 g	Bacto Agar

Dissolve separately

Mix A with B, dispense, and autoclave

Add supplements before use

1. 0.6 g /400 ml medium L-Methionine($1x10^{-2}M$)
2. 4 ml /400 ml medium Chromate stock solution ($5x10^{-3}M$)
3. Supplements, as required by nutritional markers

Stock Solution

Chromate (1/4 X); 8.1 g $Na_2CrO_4 \cdot 4H_2O$ / 100 ml DW

6. STRAINS USED FOR EPISTASIS-TEST

A. Single *uvs* mutants

1. **uvs$^+$**; 2892/ (2600.1.15)
   pabaA1; AcrA1; ActA1; choA1; riboB2, chaA1

2. **uvsA101**; 3935/ (3138.1.9)
   uvsA101; pyroA4; choA1; chaA1

3. **uvsB110**; 3570/ (2608.1.8)
   pabaA1; AcrA1; ActA1; uvsB110; choA1; riboB2, chaA1

4. **uvsC114**; 3571/ (2606.2.3)
   pabaA1; choA1; uvsC114, chaA1

5. **uvsD153**; 3811/ (3109.2.18)
   pabaA1; pyroA4; uvsD153; choA1; chaA1

6. **uvsE182**; 3733/ (3110.1.8)
   pabaA1; ActA1; uvsE182; choA1; riboB2, chaA1

7. **uvsF201**; 3812/ (3121.3.3)
   uvsF201, pabaA1; AcrA1; ActA1; nicA2; choA1; riboB2, chaA1

8. **uvsH77** ; 3730/ (3108.1.5)
   pabaA1; ActA1; uvsH77, pyroA4; choA1; chaA1

9. **uvsH304**; 3572/ (2602.1.4)
   pabaA1; AcrA1; ActA1; uvsH304, pyroA4; nicA2; choA1; riboB2, chaA1

10. **uvsI501**; 3547/ (3113.4.13)
    pabaA1; ActA1; uvsI501; choA1; riboB2, chaA1

11. **uvsJ1**; 3938/ (3141.6.10)
    pabaA1; pyroA4; uvsJ1; choA1; chaA1

B. Double *uvs* mutants

1. **uvsI501 & uvsA101**; 3934/ (3138.4.21)
   uvsA101; cnxH3, uvsI501; pyroA4; choA1; chaA1

2. **I501 & B110**; 3814/ (3122.1.5)
   pabaA1; AcrA1; uvsI501; uvsB110; choA1; riboB2, chaA1

3. **I501 & C114**; 3630/ (3104.11.11)
   pabaA1; uvsI501; (±ssbA1, P=1/4); choA1; uvsC114, chaA1

4. **I501 & D153**; 3809/ (3109.3.4)
   pabaA1; cnxH3, uvsI501; pyroA4; uvsD153; choA1; chaA1

5. **I501 & E182**; 3736/ (3110.2.8)
   pabaA1; uvsI501; uvsE182; choA1; riboB2, chaA1

6. **I501 & F201**; 3923/ (3123.1.13)
   uvsF201, pabaA1; uvsI501; (±ssbA1, P=1/4); nicA2; choA1; riboB2, chaA1

7. **I501 & H77**; 3731/ (3108.2.4)
   pabaA1; uvsI501; uvsH77, pyroA4; choA1; chaA1

8. **I501 & J1**; 3939/ (3142.6.4)
   pabaA1; cnxH3, uvsI501; pyroA4; uvsJ1; choA1; chaA1

9. **H304 & F201**; 2720/ (2605.1.21)
   uvsF201, pabaA1; AcrA1 ; uvsH304, pyroA4; nicA2 ; riboB2, chaA1

10. **E182 & C114**; 3926/ (3125.3.11)
    pabaA1; ActA1; uvsE182; choA1; uvsC114, chaA1

11. **D153 & C114**;3920/ (3120.11.13) -- poor conidiation
    pabaA1; ActA1; (±ssbA1, P=1/4); uvsD153; choA1; uvsC114, chaA1

12. **D153 & B110**; 3927/ (3126.3.2)
    pabaA1; uvsB110; uvsD153; choA1; chaA1

13. **A101 & F201**; 4037/ (3013.2.25)
    uvsF201, uvsA101; choA1; riboB2, chaA1

14. **A101 & B110**; 3931/ (3135.1.11)
uvsA101; AcrA1; uvsB110; choA1; riboB2, chaA1

15. **A101 & C114**; 3932/ (3136.3.2)
uvsA101; choA1; uvsC110, chaA1

16. **A101 & H77**; 3933/ (3137.1.16)
uvsA101; uvsH77, pyroA4; choA1; chaA1

17. **uvsJ1 & uvsB110**; 4036/ (3141.12.20)-very poor conidiation
pabaA1; uvsB110; uvsJ1; sD85 ,fwA2

18. **uvsJ1 & uvsF210**; 3936/ (3139.3.19)
uvsF201, pabaA1; uvsJ1; choA1; riboB2, chaA1

C. Single *musN* mutants

1. **musN227**; 3550/ (3114.2.7)
pabaA1; AcrA1; ActA1; musN227; riboB2, chaA1

D. double *musN;uvs* mutants

1. **musN227 & uvsB110**; 3808/ (3122.1.22)
pabaA1; AcrA1; ActA1; uvsB110; musN227; riboB2, chaA1

2. **N227 & C114**; 3632/ (3104.4.21)
pabaA1; AcrA1; ActA1; (±ssbA1, P=1/4); musN227; uvsC114, chaA1

3. **N227 & D153**; 3924/ (3124.2.24)
pabaA1; ActA1; uvsD153; musN227; chaA1

4. **N227 & E182**; 3737/ (3110.1.14)
pabaA1; ActA1; uvsE182; musN227; riboB2, chaA1

5. **N227 & H77**; 4034/ (3107.17.3)
pabaA1; ActA1; uvsH77,pyroA4; nicA2; musN227; chaA1

6. **N227 & I101**; 3634/ (3103.5.10)
   pabaA1; uvsI501; nicA2; musN227; riboB2, chaA1

7. **N227 & J1**; 3937/ (3140.1.4)
   pabaA1; (±ssbA1, P=1/4); uvsJ1; musN227; sD85, fwA2

E. Triple *musN;uvs;uvs* mutants

1. **musN227 & uvsI501 & B110**; 3815/ (3122.1.14)
   pabaA1; AcrA1; uvsI501; uvsB110; musN227; riboB2, chaA1

2. **N227 & I501 & C114**; 3638/ (3104.5.21)!
   pabaA1; uvsI501; (±ssbA1, P=1/4); musN227; uvsC114, chaA1

3. **N227 & I501 & E182**; 3738/ (3110.4.7)
   pabaA1; uvsI501; uvsE182; musN227; riboB2, chaA1

4. **N227 & I501 & H77**; 4035/ (3107.14.19)
   pabaA1; uvsI501; uvsH77, pyroA4; nicA2; musN227; chaA1

5. **N227 & C114 & D153**; 3921/ (3120.9.22) --poor
   pabaA1; ActA1; pyroA4; uvsD153; musN227; uvsC114, chaA1

7. GENE SYMBOLS

A. Requiring

ad, Adenine: ***an***, Aneurine (thiamine) ***bi***, Biotine:

cho, Choline: ***meth***, Methionine: ***nic***, Nicotinic acids:

pro, Proline: ***paba***, *p*-Aminobenzoate: ***pyro***, Pyridoxin:

ribo, Riboflavin: ***sB***, Sulfate permease ***sC***, ATP sulfurylase

B. Resistance

Acr, Acriflavine: ***Act***, Actidione (cycloheximide): ***Sul***, Sulfanilamide

C. Non-utilization

sb, Sorbitol: ***ua***, Uric acid

D. Utilization

cnx, Nitrate and hypoxanthine

E. Conidial color

cha, Chartreuse: ***fw***, Fawn: ***w***, White:
y, Yellow:

F. Suppressor

suAadE, Suppressor of *adE20*: ***ssbA***, Suppressor of *sbA*

G. Sensitive to mutagen

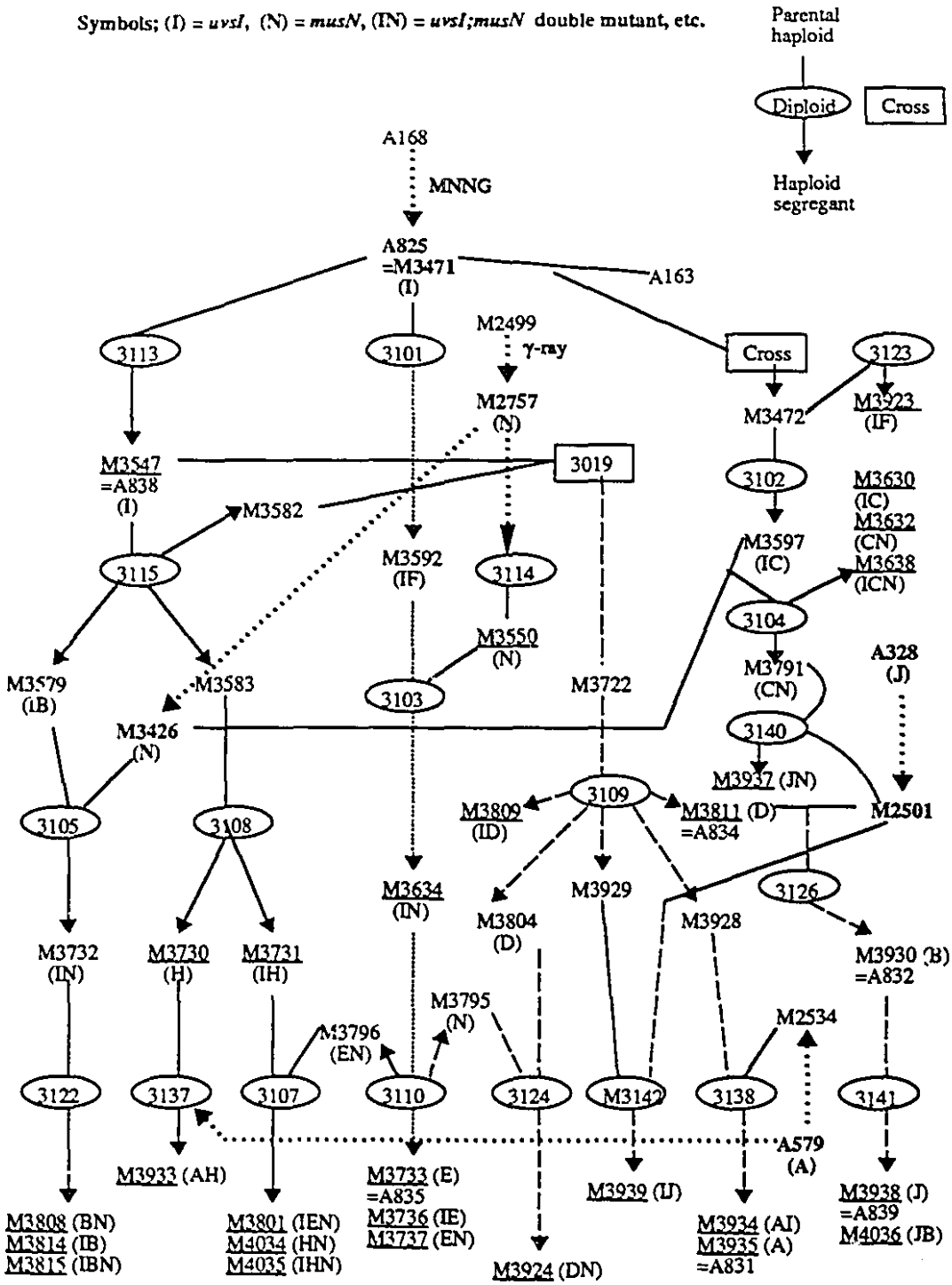
uvs, UV-sensitive ***mus***, mutagen-sensitive

8. PEDIGREE OF STRAINS USED IN EPISTATIC ANALYSIS;

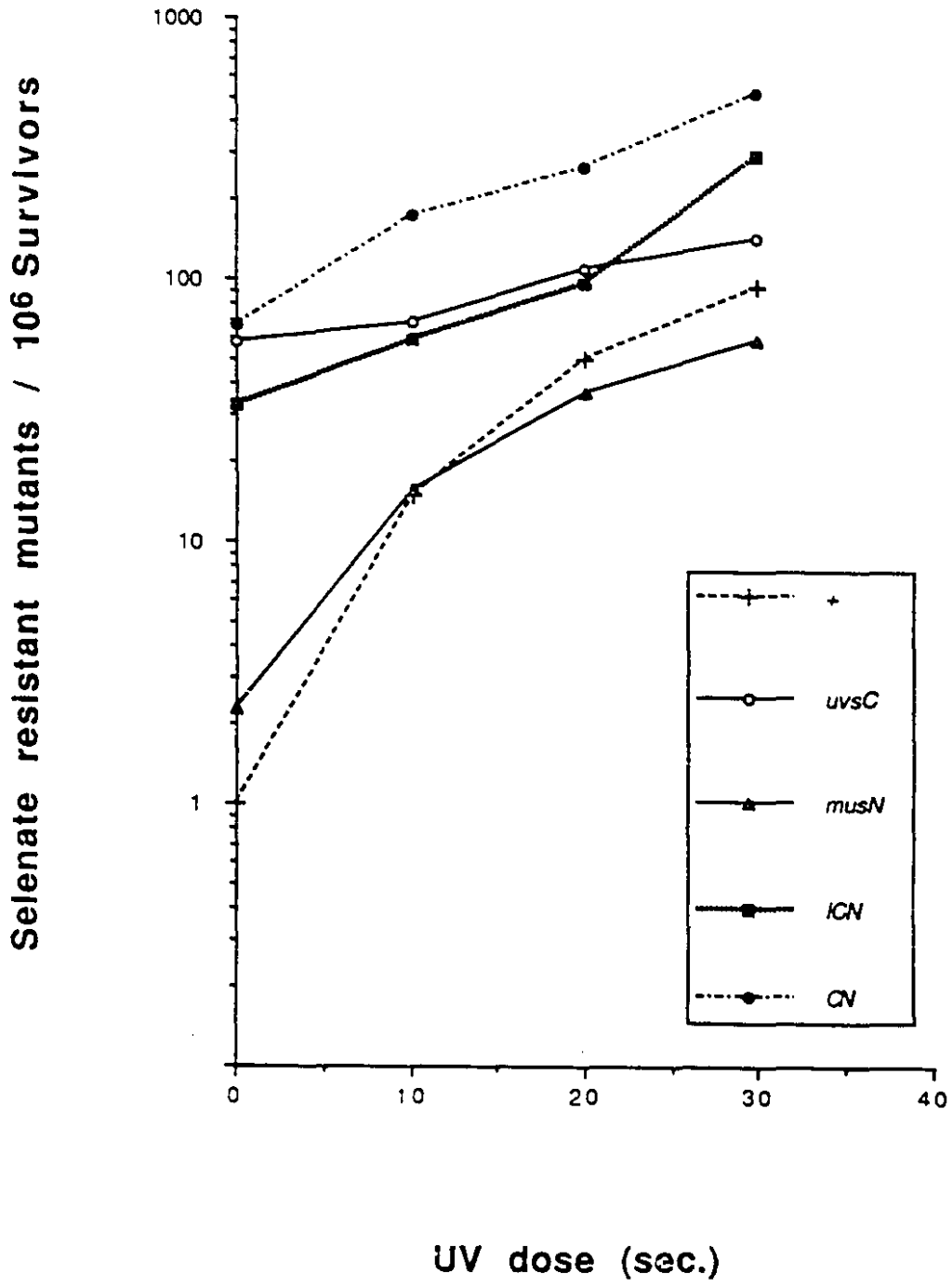
single *uvsI, J, A,* and *musN* mutants and their double mutant strains.

Genotypes of underlined haploid strains are shown in appendix II

Symbols; (I) = *uvsI*, (N) = *musN*, (IN) = *uvsI;musN* double mutant, etc.



9. Fig. 5.1. UV-induced selenate resistant mutation in *uvs*$^+$ control (+), *uvsC*, *musN* single mutant and *uvsI;uvsC;musN* triple mutant strains.



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CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

To the best of my knowledge, the following results are new and unique to this study:

There had been no reports previously to Chae and Kafer (1993 - CHAPTER 2) which demonstrated 4 epistatic groups of DNA repair for UV-, MMS-, and 4-NQO-induced DNA damage in *Aspergillus nidulans*. I was the first to map *uvsI* gene and to demonstrate that *uvsI* defined a fourth epistatic group.

This study also newly assigned *uvsJ* and *uvsA* genes to the "UvsF" and the "UvsC" epistatic groups, respectively.

This was the first study which documents antimutator and mutator effects of *uvsI* mutation. I was the first to demonstrate mutational specificity of *uvsI* mutation using several forward and reverse mutational systems. This report (CHAPTER 4) also postulated base pair-specific effect of *uvsI* on mutagenesis.

I was the first to assign *musN* to the UvsC group (CHAPTER 3). The effects of *mus* on mutagenesis were the first observed. In particular, this is the first study to identify that *musR* gene was required for certain type of reverse mutation.