INDUCED MITOTIC RECOMBINATION IN *ASPERGILLUS*

by

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INDUCED MITOTIC RECOMBINATION IN *ASPERGILLUS* STRAINS DIFFERING IN SENSITIVITY TO ULTRAVIOLET LIGHT

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ABSTRACT

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N-methyl-N'-nitro-N-nitrosoguanidine (NTG) and 5-fluorodeoxyuridine (FUDR) have been used to induce mitotic segregation in germinating conidia of *Aspergillus nidulans*. Since a significant increase of visible twin spots could be detected after these treatments, it was concluded that reciprocal mitotic crossing-over had been induced. It seems likely that these agents increase mitotic crossing-over by inducing damages in the DNA which can be corrected by a system of repair enzymes, some of which are involved in recombination.

Two recessive UV sensitive mutants have been isolated, but testing showed that both were alleles of the same gene. The effect of this gene on mitotic and meiotic recombination has been studied. In diploid strains homozygous for *uvs1*, both the spontaneous and induced frequencies of mitotic crossing-over are increased. There was no observed effect of this gene in meiotic crosses. The best interpretation of these preliminary results seems to be that *uvs1* possesses excess exonuclease activity.

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Introduction

The mechanism of intergenic recombination in fungi has been widely studied from two points of view. These include an examination of spontaneous and induced mitotic crossing-over in heterozygous diploid strains, and an analysis of the effect of recombination-negative mutants on mitotic and/or meiotic crossing-over.

Spontaneous mitotic crossing-over (which can be easily detected by homozygosis of a colour marker) is a rare event, but numerous physical and chemical agents have been shown to increase this frequency many-fold. In general, these agents may be subdivided into two classes:

1. Agents which are mutagenic as well as recombinogenic (e.g. ultraviolet light and N-methyl-N'-nitro-N-nitrosoguanidine, James, 1954, 1955; James and Lee-Whiting, 1955; Holliday, 1961, 1962, 1965b, 1967; Hurst and Fogel, 1964; Käfer and Chen, 1964; Wood, 1967; Wood and Käfer, 1968; Zimmerman, Schwaier and von Laer, 1966).

2. Agents which Holliday (1964) labels "recombinogens" because they increase the frequency of mitotic crossing-over at concentrations at which they are not mutagenic (e.g. 5-fluorodeoxyuridine and Mitomycin C, Esposito and Holliday, 1964; Holliday, 1964).

In Aspergillus nidulans, two systems of paired visible markers located on the same chromosome arm have been isolated (Wood, 1967; Wood and Käfer, 1967). When these markers are placed into diploid strains in repulsion to one another, a mitotic cross-over event between the centromere and the more proximal marker produces "twin spots" of mutant colour. This terminology was first used by Stern in Drosophila melanogaster (1936) in his

elegant analysis of yellow body/singed bristle twin spots on the abdomen of heterozygous flies. The term "twin spot" is expanded in Aspergillus to describe reciprocal paired colour sectors in a single colony. These sectors are usually small if of spontaneous origin, but may each represent a quarter or half of a colony if occurring during the first or second mitotic division (Käfer, 1961).

The three visible markers of these two selective systems are incorporated into one diploid for this study, together with several biochemical markers on the same chromosome arm. This permits an examination of the frequency and distribution of mitotic crossing-over induced by different agents. Several types of twin spots can be visually identified, and, in addition, the mutagenic effects of different agents can be examined by comparing percentage survival and abnormal colonies produced (Käfer, 1963, 1968). These abnormal colonies are mainly mutations and deletions which have been concurrently induced by the agent used to induce the recombination event (Käfer, 1968).

UV sensitive mutants have also been used to gain insight into recombination mechanisms, since the discovery that recombination-negative mutants are UV light sensitive (Clark and Margulies, 1965), and UV light sensitive mutants may be recombination-negative (van de Putte, Zwenk and Rörsch, 1966; Howard-Flanders and Theriot, 1966; Holliday, 1967; Lanier, Tuveson and Lennox, 1968). Thus, it has been suggested that the enzymes involved in the degradation and repair of recombining DNA might also be active in the excision and repair of UV-induced lesions (Howard-Flanders and Boyce, 1964). As recombination-

negative mutants are difficult to isolate, the effect of UV sensitive mutants on recombination (conjugation or transformation in bacteria, van de Putte, Zwenk and Rörsch, 1966; Howard-Flanders and Theriot, 1966; Okubo and Romig, 1966; and mitotic or meiotic crossing-over in fungi, Holliday, 1967; Lanier, Tuveson and Lennox, 1968) has been examined in many micro-organisms. In addition, the sensitivity of these mutants to other mutagens has been tested, since the existence of a generalized repair mechanism, capable of correcting different types of DNA damage, has been postulated (review, Hanawalt and Hayes, 1967). The types of damage produced by these agents can often be inferred from their cross-sensitivities.

In the present study, two UV sensitive mutants have been induced, and their effect on recombination has been examined on mitotic crossing-over in heterozygous diploid strains using the above selective system of paired visible markers. Meiotic crosses have also been examined. Finally, the UV sensitive mutants have been tested to determine whether or not they were NTG sensitive.

Literature Review

The effect of ultraviolet light on DNA: Light and dark repair mechanisms

The lethal and mutagenic effects of ultraviolet light (UV) on micro-organisms has long been known. These effects were presumed to be the result of direct action of the UV on DNA since it has been reported that DNA strongly absorbs UV light. In particular, the absorption spectrum of DNA and the action spectrum of UV on micro-organisms have been observed to be similar. Wavelengths of approximately 2600 Å are found to be most effective (Deering, 1962).

Early studies on solutions of each of the four nitrogenous bases commonly found in DNA gave no clue to the possible distortions produced by the UV light. The first major breakthrough came with the discovery that in UV-irradiated frozen solutions - unlike in liquid ones - thymine is converted into a substance with a characteristic absorption at lower wavelengths than the original thymine (Beukers, Ijlstra and Berends, 1960). This led to the hypothesis that *in vivo*, the arrangement of the four nitrogenous bases within the double-stranded DNA helix might juxtapose near-by thymidines so that the UV light would exert its effect in the same manner as in frozen solutions of thymine. Further work by this group, including crystallographic studies and molecular weight determination *in vitro* led to a proposed structure: the thymine dimer (Beukers and Berends, 1960). Its presence in DNA would be expected to produce distortion of the phosphodiester backbone and interfere with normal hydrogen-bonding of bases (Howard-Flanders, 1964). Since this time, other pyrimidine photoproducts of UV-irradiated

DNA have been recognized (Setlow, 1966). These include homodimers of cytosine and heterodimers of thymine and cytosine. More recently, Varghese and Wang (1967) have identified another thymine photoproduct by the use of different chromatographic procedures than had previously been used.

Numerous *in vivo* and *in vitro* studies on pyrimidine dimers followed the early studies of Beukers and colleagues. Wacker (1963) was the first to extract thymidine dimers from DNA of UV-irradiated bacteria. Setlow and co-workers obtained thymidine dimers using poly-T *in vitro* (Deering, 1962). As single-stranded DNA was used, this experiment demonstrated the presence of intrastrand dimers. Interstrand dimers were recognized by Marmur and Grossman (1961) in *Diplococcus pneumococci* DNA.

In vivo pyrimidine dimers produced by irradiation caused reduced biological activity of transforming DNA of *Hemophilus influenzae* (Setlow and Setlow, 1962). As well, the ability of heat denatured DNA to act as a primer in a calf thymus polymerase system was sharply reduced after irradiation with UV light (Bollum and Setlow, 1963).

Repair of the damage produced by UV light irradiation is now understood to follow one of two distinct enzymatic processes:

1. In the light, a repair system is activated whereby pyrimidine dimers are broken. This "*photoreactivation*" phenomenon was first noted by Kelner (1951). An enzymatic fraction capable of photoreactivating UV-damaged transforming DNA of *Hemophilus influenzae* in the presence of visible light has been isolated in extracts of *E. coli* B (Rupert

Goodgal and Herriot, 1958), and Bakers yeast (Rupert, 1960). Wulff and Rupert (1962) have used this fraction successfully *in vitro* to break pyrimidine dimers of irradiated DNA extracted from *E. coli*. Essentially the same results were obtained by Marmur and Grossman (1961) in transforming DNA of *Diplococcus pneumococci* and *Bacillus subtilis*.

2. The mechanism of *dark repair* (pyrimidine dimer excision and repair replication without light) has received much attention since 1964. In a now classical experiment, Setlow and Carrier (1964) observed that radioactively-labelled thymidine dimers produced in bacterial DNA by exposure to UV light became acid-soluble when incubated in the dark. Thus, a process different from photoreactivation (which has been shown to just *split* dimers in the DNA) must be involved (Setlow, Swenson and Carrier, 1963). Essentially the same results were obtained in *E. coli* K-12 (Boyce and Howard-Flanders, 1964a). In an equally elegant experiment, Pettijohn and Hanawalt (1964) used density centrifugation to show that after UV irradiation, feeding bacterial cells the heavy thymine analogue 5-bromouracil (5-BU) resulted in non-conservative incorporation of this heavy label into the DNA. This was followed by a normal semi-conservative round of replication. Thus, early DNA synthesis following irradiation must involve "short single-stranded segments distributed at random throughout the genome".

These results implied that at least four enzymatic steps are involved in the dark repair process (Holliday, 1967):

1. Excision of pyrimidine dimers from the DNA.

2. Degradation along a single strand at the point of excision.

3. Re-synthesis of degraded segments of DNA by complementary base pairing with the opposite strand.

4. Rejoining of the phosphodiester bond to produce an intact DNA molecule.

The enzymatic nature of this process has been examined *in vitro* in extracts of *Micrococcus lysodeikticus*. (This micro-organism is remarkably resistant to UV light, and is, therefore, thought to have a highly efficient dark repair process - Setlow, 1966). These extracts have been used to re-activate UV-irradiated replicating-form DNA of bacteriophage ØX174 (Rörsch, van der Kamp and Adema, 1964) and transforming DNA of UV inactivated *Hemophilus influenzae* (Elder and Beers, 1965).

Other researchers have isolated enzymes capable of performing a specific step in the excision-repair mechanism *in vitro*. Strauss, Searashi and Robbins (1966) have identified a nuclease specific for excising UV induced photoproducts in DNA. An exonuclease has been purified which can degrade double-stranded DNA at the 3' end but stops before breaking the molecule into two pieces (Richardson, Lehman and Kornberg, 1964). Mead (1964) obtained condensation of oligonucleotides with polydeoxyribonucleotides with his oligodeoxyribonucleotide transferase. Finally, working with T4 infected *E. coli*, Weiss and Richardson (1967) have identified a ligase capable of catalyzing covalent joining of two segments of an interrupted strand of DNA.

Ultraviolet light sensitive mutants

The first mutant showing a changed response to the lethal action

of UV light was a resistant derivative of E. coli B (called E. coli B/R) isolated by Witkin (1946). The mutant was observed to be "stable and heritable" (Witkin, 1947).

Over a decade later, an ultraviolet light sensitive mutant was isolated, again in E. coli B, and was labelled B_{S-1} (Hill, 1958). Different UV sensitive mutants were later reported by the same group (Hill and Simson, 1961; Hill and Feiner, 1964). Ellison, Feiner and Hill (1960) noted that UV-irradiated phage T_1 were "reactivated" to a much smaller extent when plated on the mutant UV sensitive strain than was observed on the normal wild-type E. coli B. This phenomenon of "host cell reactivation" - hcr - (Sauerbier, 1962) was first noted by Garen and Zinder (1955) with Salmonella typhimurium and phage P22. Originally, phage reactivation was interpreted to imply partial homology between phage and bacterial genomes. However, more recent work on this topic, particularly the observations that UV sensitive mutants can also be hcr mutants as well (Ellison et al., 1960), have shown it to be enzymatic in nature (Sauerbier, 1961; Stahl, Crasemann, Okun, Fox and Laird, 1961; Harm, 1963).

In 1964, when the first insight into the dark repair process was gained, much attention was focussed on these mutants in an attempt to determine for which enzymatic step they might be mutant. Setlow and Carrier (1964) found the ability of E. coli B_{S-1} to excise thymidine dimers was sharply reduced. The same was found for uvrA, the UV sensitive mutant isolated by Boyce and Howard-Flanders (1964a) in E. coli K-12.

These results were followed by isolation and detailed analysis of UV sensitive mutants in many organisms. In E. coli they have been isolated and mapped by two groups of researchers. Both groups have used E. coli K-12, since strain B (into which the original mutants were induced) is self-sterile. Howard-Flanders devised a selection method for hcr⁻ mutants. He plated irradiated E. coli K-12 on media containing UV-irradiated phage T_1. Any bacteria capable of growing must be either hcr⁻ or phage resistant. Indeed, 14 of the 26 bacteria forming colonies on this medium were found to be UV sensitive (Howard-Flanders and Theriot, 1962). A detailed analysis of 23 mutants followed. Conjugation and transduction mapping showed that all mutants fell into one of three positions on the map - labelled uvrA, B and C (Howard-Flanders, Boyce, Simson and Theriot, 1962; Howard-Flanders, Boyce and Theriot, 1966). Similar conclusions have been reached by van de Putte and co-workers. Their UV sensitive mutants (called dar_1-dar_6 - dark repair mutants) have been isolated by a non-selective technique. Their six mutants fall into the same three discrete map positions found by Howard-Flanders and colleagues (van de Putte, van Sluis, van Dillewijn, and Rörsch, 1965; Mattern, van Winden and Rörsch, 1965).

In fungi, the best analyzed system is the one studied by Holliday in Ustilago maydis (Holliday, 1965, 1967). Three different mutants have been isolated and called uvs-1, uvs-2 and uvs-3. All are recessive when in the heterozygous state in diploids, and all are strongly photoreactivable. The mutants are distinguishable by their survival ability to x-rays: uvs-3 is x-ray resistant; the other two mutants are x-ray sensitive, but uvs-2 is more sensitive than uvs-1.

UV sensitive mutants have also been isolated in numerous other micro-organisms. In bacteria these include Bacillus subtilis (Mahler, 1965; Reitter and Strauss, 1965; Okuba and Romig, 1965); Micrococcus lysodeikticus (Feiner, 1967); Pseudomonas aeruginosa (Holloway, 1966); Hemophilus influenzae (Setlow, Brown, Boling, Mattingly and Gordon, 1968); and Salmonella typhimurium (Wing, Levine and Smith, 1968). In fungi, Jansen has isolated a UV sensitive mutant in Aspergillus nidulans (Dorn, 1967). In the same organism, Lanier and Tuveson (1965) have reported a nitrous acid sensitive mutant that is also UV sensitive. In Aspergillus rugulosus, four UV sensitive mutants have been identified (Lennox and Tuveson, 1967) of which at least one has been shown to behave like a single gene. They also noted that the colour of the strain influenced the expression of sensitivity to UV light. Chang and Tuveson (1967) have isolated UV sensitive mutants in Neurospora crassa. One of them segregates in crosses as a single gene; another is considerably more complicated. Similar results have been obtained in Schizosaccharomyces pombe (Haefner and Howry, 1967). Of four mutants isolated, two behave as single genes, while two others appear more complicated and await further study. In Saccharomyces cerevissiae, UV sensitive mutants have also been isolated (Snow, 1967; Nakai and Matsumoto, 1967). All of Snow's six mutants are recessive, and all combinations complement in diploids. Nakai and Matsumoto obtained three recessive mutants, two of which are highly UV-sensitive. A third mutant is only slightly UV sensitive but highly x-ray sensitive.

Thus, in all the well analyzed bacterial and fungal systems, at least three different loci for UV sensitivity exist. All the bacterial

UV sensitive mutants behave as single recessive genes. However, in fungi, some are more complex and are thought to be cytoplasmically inherited. Mutants can further be characterized and often distinguished from one another by their ability to repair x-ray damage, UV-damaged phage, or by their ability to excise thymidine dimers. This type of classification can suggest for which step(s) the strain might be mutant.

Recombination deficient mutants

Insight into the physical mechanism of phage recombination was first gained from crosses between density-labelled λ bacteriophage (Meselson and Weigle, 1961; Kellenberger, Zichichi and Weigle, 1961). Both have demonstrated that parental DNA can be present among recombinant phage progeny, and thus that breakage of parental DNA was involved in the recombination process. A more detailed examination of the same system followed (Meselson, 1964). Analysis of the distribution of isotopic label among recombinant phage showed that genetic recombination must have occurred by breakage and rejoining of the double-stranded phage DNA. As well, the appearance of light shoulders on fully and three-quarter labelled recombinants implied that a small amount of the DNA (of the order of 5-10%) is degraded and re-synthesized during the course of recombination.

Thus it has been suggested that enzymes involved in degradation and repair of recombining DNA might be the same as those involved in excision and repair of UV induced lesions (Howard-Flanders and Boyce, 1964). This has been strongly supported by the discovery in E. coli K-12 of

recombination deficient mutants (rec^-) that are also UV sensitive, (Clark and Margulies, 1965). Reversion studies implied a single gene was responsible for reduced recombination ability and UV sensitivity. One of these mutants has been analyzed in detail and it was observed to degrade its DNA excessively (Clark, Chamberlin, Boyce and Howard-Flanders, 1965), and has therefore been called "wreckless". A different rec^- mutant ("cautious") shows less than normal degradation of its DNA (Howard-Flanders, Theriot and Stedeford, 1966). Van de Putte *et al.* (van de Putte, Zwenk and Rörsch, 1966; van de Putte and Rörsch, 1966) obtained recombination mutants in *E. coli* CR34 by isolating x-ray and UV sensitive mutants. Of seven such mutants, four were classed as recombination deficient by their reduced ability to conjugate with suitable strains. The position of the mutant on the *E. coli* map was determined by checking for the appearance of stable recombinants in a cross of Hfr rec^+ x F^- rec^-. These stable recombinants could not appear until the allelic rec^+ marker of the donor strain has entered the recipient.

Howard-Flanders and Theriot (1966) have isolated five x-ray sensitive mutants in *E. coli* K-12 that appear to be able to accept genetic material from a suitable donor strain, but are unable to incorporate it into the recipient chromosome. The mutants were also found to be sensitive to UV light.

Working with his three UV sensitive mutants in *Ustilago maydis*, Holliday (1967) has examined their influence on spontaneous and UV-induced mitotic and meiotic recombination in diploids either heterozygous or homozygous for each of those mutants. *Uvs-1*, an x-ray sensitive

mutant, is not recombination deficient: on the contrary its effect, when present in a homozygous condition in diploids, is to increase spontaneous and UV-induced mitotic segregation. This is thought to arise as a result of excessive exonuclease activity following pyrimidine dimer excision. However, uvs-2 is recombination deficient since it completely blocks meiosis and decreases the frequency of mitotic crossing-over. Holliday suggests that such results might be expected from a mutant unable to accomplish the final rejoining of the phosphodiester backbone. Finally, uvs-3, appears to have little influence on recombination. Since it is not x-ray sensitive, it may be deficient in its ability to excise pyrimidine dimers which are not produced by x-irradiation.

In Aspergillus nidulans, a nitrous acid sensitive mutant (which is also UV sensitive) has been found which results in an aborted meiosis when this gene is present in a homozygous condition (Lanier, Tuveson and Lennox, 1968). However, a UV sensitive gene in Aspergillus rugulosus appears to have no effect on recombination (Tuveson and Lennox, 1968).

These types of analyses have therefore supported the hypothesis that recombination involves breakage and rejoining of the DNA by one or more of the enzymes involved in the repair of UV-damaged DNA.

(a) Somatic segregation and

(b) Induced mitotic crossing-over

(a) Spontaneous segregation in most organisms was first thought to be restricted to germ cells undergoing a meiotic division. The first instance of somatic or mitotic crossing-over was the elegant demonstration

in Drosophila melanogaster of yellow body/singed bristle twin spots at low frequencies in heterozygous flies of genotype $\frac{y+}{+sn}$ (Stern, 1936).

The same process was observed in Aspergillus nidulans soon after the discovery that heterozygous diploid strains may be produced by the rare event of fusion of two genotypically different haploid nuclei in a heterocaryotic mycelium (Roper, 1952, Pontecorvo and Roper, 1953). These diploids may be easily selected by their vigorous growth on minimal media on which neither haploid strain alone could survive. Analysis showed that these diploids are stable, but do give rise to rare mitotic segregants of several types (Pontecorvo et al., 1953; Pontecorvo, Tarr Gloor and Forbes, 1954). The process producing the majority of diploid segregants is mitotic crossing-over of the type described by Stern for Drosophila melanogaster. This results in a diploid segregant which is homozygous for the markers on one (or more) chromosome arm(s). All markers on other chromosome arms remain heterozygous (Pontecorvo et al., 1953; Pontecorvo, Tarr Gloor, and Forbes, 1954; Pontecorvo and Käfer, 1958). Mitotic crossing-over is understood to take place at the four-strand stage, followed by normal segregation of the centromeres. Products of the type of segregation in which both reciprocal cross-over strands go to the same nucleus have been isolated and identified resulting both from inter-allelic and inter-genic mitotic crossing-over (Roper and Pritchard, 1955; Käfer, 1961). Käfer (1961) has also identified two twin spots. These are formed as a result of the second type of centromere segregation in which reciprocal cross-over strands go to different nuclei. Wood and Käfer (1967) have now worked

out two systems of paired visible markers located on the same chromosome arm. When placed in repulsion to one another, mitotic crossing-over produces twin spots which are easily visually identified.

Diploids, also spontaneously, give rise to a variety of segregants (haploids, aneuploids, and non-disjunctional diploids) resulting from a second process - chromosomal segregation (Käfer, 1961). Since coincidence with mitotic crossing-over is rare, haploid segregants show free recombination between markers, on the eight different chromosomes, and complete linkage for markers located on the same chromosome. They have thus been used to locate new markers and to establish the eight linkage groups (Käfer, 1958).

(b) As spontaneous mitotic crossing-over occurs with low frequency, methods of increasing this frequency would be useful, if these segregants are to be used for genetic analysis. Many physical and chemical agents have been found to increase this frequency many-fold.

In fungi, ultraviolet light has been used successfully. In Saccharomyces cerevissiae, both inter- and intragenic recombination frequencies increase with treatment with UV light (James, 1954; 1955; James and Lee-Whiting, 1955; Roman and Jacob, 1958; Sherman and Roman, 1963; Hurst and Fogel, 1964). The same has been observed in Ustilago maydis (Holliday, 1961; 1962; 1965b; 1966; 1967) and in germinating conidia of Aspergillus nidulans (Käfer and Chen, 1964; Jansen, 1966; Wood, 1967).

Holliday has observed that certain chemical agents are useful for increasing mitotic crossing-over without increasing mutation rates, and

proposed that such chemicals be called recombinogens (Holliday, 1964). These agents would be very useful for studying induced mitotic crossing-over as there would be a minimum of mutation-produced homozygosis in the heterozygous diploid. Both 5-fluorodeoxyuridine (FUDR) and Mitomycin C (MC) are classed as recombinogens because they increase the frequency of mitotic segregation in heterozygous diploids of Ustilago maydis without increasing the mutation frequency in haploid strains treated under the same conditions (Holliday, 1964; Esposito and Holliday, 1964).

FUDR is thought to exert its effect via thymidylate starvation of cells. Its phosphorylated derivative, 5-fluorodeoxyuridylate inhibits the enzyme thymidylate synthetase (Cohen, Flaks, Barner, Loeb and Lichtenstein, 1958) which converts deoxyuridylate to thymidylate in extracts of normal and phage-infected E. coli (Flaks and Cohen, 1959). Cells of Vicia faba roots forced to synthesize DNA in the presence of FUDR show lesions at anaphase. These disappear if thymidine is supplied at least an hour before anaphase (Taylor, Haut and Tung, 1961). Beccari, Modigliani and Morpurgo (1967) have increased the frequency of intergenic, but not intragenic, crossing-over with both FUDR and fluorouracil (FU) using heterozygous and heteroallelic diploids of Aspergillus nidulans. Gallant and Spottswood (1965) found FUDR stimulated recombination between chromosome and episome in E. coli.

Mitomycin C also inhibits DNA synthesis in E. coli (Shiba, Tirawaki, Taguchi, and Kawamata, 1959) and is thought to act by cross-linking DNA strands (Szybalski and Iyer, 1964; Matsumoto and Lark, 1964; Shaw and Cohen, 1965). However, in bacteria, MC has been observed to be mutagenic as well as recombinogenic (Iijima and Hagiwara, 1960).

Increased frequency of mitotic crossing-over has also been observed with nitrous acid treatment (NA) in Saccharomyces cerevissiae (Zimmerman, Schwaier and von Laer, 1966) under conditions where it is mutagenic as well (Zimmerman et al., 1966; Siddiqi, 1962). Alkylating agents have also proved to be effective in Aspergillus nidulans (Fratello, Morpurgo and Sermonti, 1960; Morpurgo, 1963) and in Saccharomyces cerevissiae (Zimmerman et al., 1966; Zimmerman and Laer, 1967). Finally, x-rays have also been observed to slightly increase the frequency of mitotic crossing-over in Aspergillus nidulans (Morpurgo, 1962). However, aberrations and lethals are induced much more frequently (Tector and Käfer, 1962; Käfer, 1963), and the effect on recombination is therefore difficult to measure.

It would therefore appear that two types of agents are capable of inducing mitotic crossing-over:

1. Those agents which are powerful mutagens (e.g. UV, NTG, NA). Presumably, these agents act via the induction of errors in the DNA, which are repaired by an enzymatic repair mechanism. As some of these enzymes are involved in recombination as well, crossing-over is induced at high frequencies.

2. Those agents which inhibit DNA synthesis, but are not strongly mutagenic (e.g. FUDR, MC). Perhaps these agents act by increasing the chance of occurrence of spontaneous events (Putrament, 1967).

Cross-sensitivity of UV sensitive mutants to various chemical damages: A generalized repair mechanism

Many researchers have hypothesized a generalized repair mechanism

as an efficient method of correcting damage to DNA (Hanawalt and Hayes, 1967). Analysis has shown cross-sensitivity of specific UV sensitive mutants to numerous physical and chemical agents. This has both lent support to a generalized repair mechanism and given insight into the nature of the enzymatic steps involved.

Cross-sensitivity of UV sensitive mutants to x-rays has been noted in many organisms (e.g. Hill, 1958 in E. coli B_{s-1}; Howard-Flanders et al., 1966 in E. coli K-12 uvrA, B and C; van de Putte, Zwenk and Rörsch, 1966 in E. coli K-12 rec^-; Holliday, 1964 in Ustilago maydis uvs-1 and uvs-2; Nakai and Matsumoto, 1967 in Saccharomyces cerevissiae UV^s). However, UV sensitive mutants have also been found which are x-ray resistant (Holliday, 1967; Nakai and Matsumoto, 1967; Snow, 1967). Presumably, the latter mutants are unable to excise pyrimidine dimers, as this step would not be needed to repair damage produced by x-irradiation. This idea has therefore been successfully used in the isolation of recombination deficient mutants, since such mutants would also not need an enzyme excising pyrimidine dimer. Thus a mutant which is both x-ray and UV sensitive has a greater chance of being recombination deficient than one which is UV sensitive alone (Howard-Flanders and Theriot, 1966; van de Putte, Zwenk and Rörsch, 1966; Holliday, 1967).

The mutant B_{s-1} isolated by Hill has been found sensitive to various alkylating agents (Haynes, Patrick and Baptist, 1964; Lawley and Brookes, 1965; Kohn, Steigbigel and Spears, 1965; Bridges and Munson, 1966; Papirmeister and Davidson, 1964). In general, it has been shown that the cross-links produced by these alkylating agents disappear with

incubation in the resistant strain of E. coli (B/R), whereas they will not in the sensitive strain (B_{s-1}). Hanawalt and Hayes (1965) used a heavy 5-bromouracil pulse to detect repair replication (by the method of Pettijohn and Hanawalt, 1964) in wild-type E. coli after treating with the alkylating agent nitrogen mustard. No such repair replication was found in a UV sensitive derivative, E. coli TAU-bar.

Bridges and Munson (1966) also compared the action of methyl-methane sulfonate-MMS (which produces single-stranded breaks in DNA) and nitrogen mustard - HN_2-on an x-ray resistant hcr^- strain and on B_{s-1} (which is x-ray and UV sensitive). The hcr^- x-ray resistant strain was MMS resistant and HN_2 sensitive; B_{s-1} was sensitive to both agents. Essentially the same data were obtained with uvr^- and mms^- strains of Bacillus subtilis (Reitter and Strauss, 1965; Searashi and Strauss, 1965).

Boyce and Howard-Flanders (1964b) have found all three UV sensitive mutants were also sensitive to the antibiotic Mitomycin C (MC) which also cross-links DNA. A MC sensitive mutant that is also UV sensitive has been isolated in Bacillus subtilis (Okubo and Romig, 1966). However, Otsuji (1968) has isolated a Mitomycin C sensitive mutant of E. coli K-12 which is not UV sensitive.

Chang and Tuveson (1967) found that their two UV sensitive mutants in Neurospora crassa were both nitrous acid sensitive as well. However, only one mutant was sensitive to N-methyl-N'-nitro-N-nitrosoguanidine (NTG). Repair replication following NTG damage was recognized by density labelling in E. coli B, but not in E. coli B_{s-1} (Cerdà-Olmeda and Hanawalt, 1967). In yeast, Snow (1967) found four of his six UV

sensitive mutants were also nitrous acid-sensitive to different degrees. However, he did not find a parallel ranking of UV and nitrous acid sensitivity. Finally, Lanier and Tuveson (1965) have isolated a nitrous acid-sensitive mutant in Aspergillus nidulans (NAS-1) which is also UV sensitive and recombination negative (Lanier et al., 1968).

The existence of a highly efficient generalized repair mechanism has thus been supported by isolating strains mutant for sensitivity to one agent and testing them for sensitivity to other agents which are thought to have similar effects on the DNA. Inter-relating the ability of a specific mutant to repair damage induced by different agents can give information on the kind of damage induced and the enzymes needed to repair it.

Materials and Methods

Strains

All strains used were descendents of the same wild-type haploid strain used by Pontecorvo and co-workers in Glasgow (Pontecorvo, Roper, Hemmons, MacDonald and Bufton, 1953). Table I lists the haploid strains taken from Montreal stock, and gives their origin and stock number. Care was taken to use only translocation-free strains (Käfer, 1965). Figure 1 gives the pedigree explaining the origin of the new UV sensitive mutants and the crosses performed with these. The genotypes of the diploids used in these experiments are given in Figure 2.

Media

Standard *Aspergillus* media were used (Pontecorvo *et al.*, 1953, modified by Käfer, 1958; details published in Barratt *et al.*, 1965).

Minimal media (MM) were supplemented with the appropriate solutions of single growth factors for the testing of biochemical markers. Resistance to acriflavin was identified on complete media (CM) supplemented with stock solution of acriflavin (Roper and Käfer, 1957). To identify alkaline phosphatase mutants, KH_2PO4 was omitted from the MM, DL β-glycerophosphate was added at a concentration of 12 gm./l. and the pH was adjusted to 8.2 (β-glycerophosphate media, βGP; Dorn, 1965). Acetate mutants were identified on MM in which dextrose was replaced with sodium acetate (8 gm./l.) and the pH was adjusted to pH 6.1 (Acetate media, AM; Apiron, 1965).

In replication experiments using velvet pads, sodium desoxycholate was added to the media at a concentration of 0.8 gm./l. as a growth

TABLE I : Genotypes and origin of stock strains used for crosses

Montreal stock number	Genotype*	Origin
1265	ribo y	C 630 (Figure 1, Barratt, Johnson and Ogata, 1965)
1231	paba; w2	C 639 (Figure 1, Barratt et al., 1965)
1314	suad20 lu lys88 paba y ad20; s3; cha	C 842/
1344	ribo ad20 bi; Acr; fw2	C 795/
1449	ad20 bi; Acr; fw2	C 795/
1426	ad20; Acr; fw2 pal87	C 814/
1427	suad20 paba y ad20 bi; w2; ni21 facB101 ribo2 cha	C 908/
1428	suad20 paba y ad20; fw2 ribo2; cha	C 816/
1409	ribo ad20; Acr w2; ni21 pal87	C 815/

* Unless otherwise stated, mutant isolation number is 1. Map position and explanation of markers is given in Dorn (1967) with the following exceptions:
  lys88 - allele of lys51 (Pees, unpublished)
  fw2 - allele of fw1 (Clutterbuck, unpublished)
  ni21 - allele of ni50 (Dorn, personal communication)

/ These crosses have been done by S. Wood, and are unpublished. All can be traced back through a few crosses, UV induced and spontaneous mutants of stock strains to published data.

Figure 1: Pedigree explaining the origin of new UV sensitive mutants and the subsequent crosses performed in these experiments.

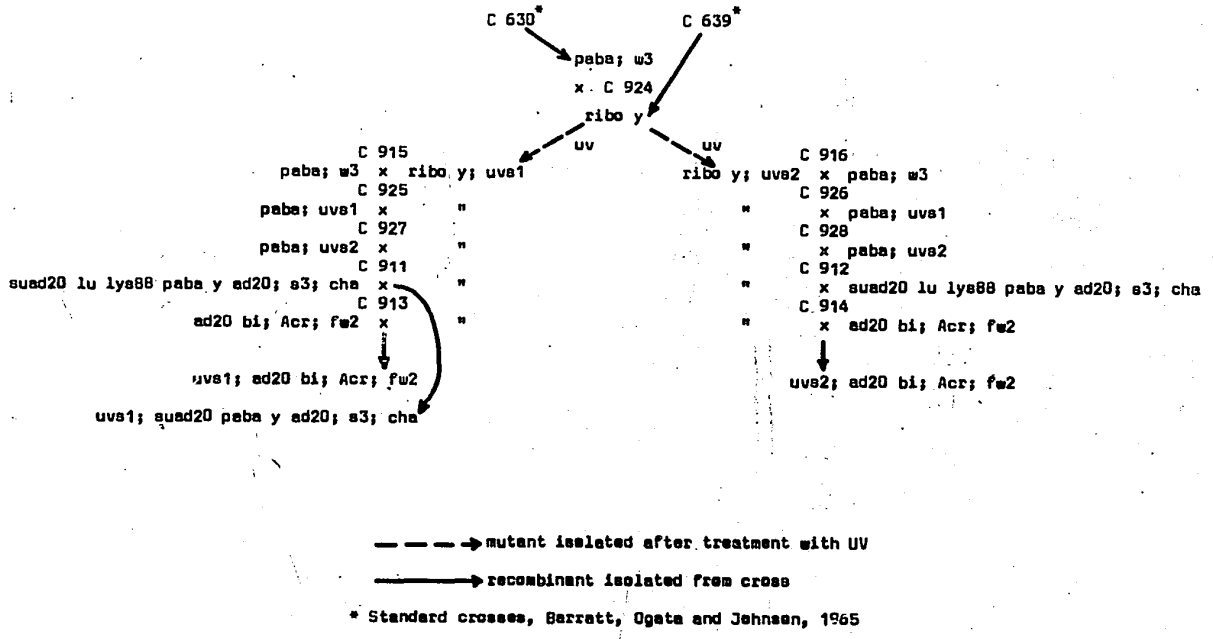
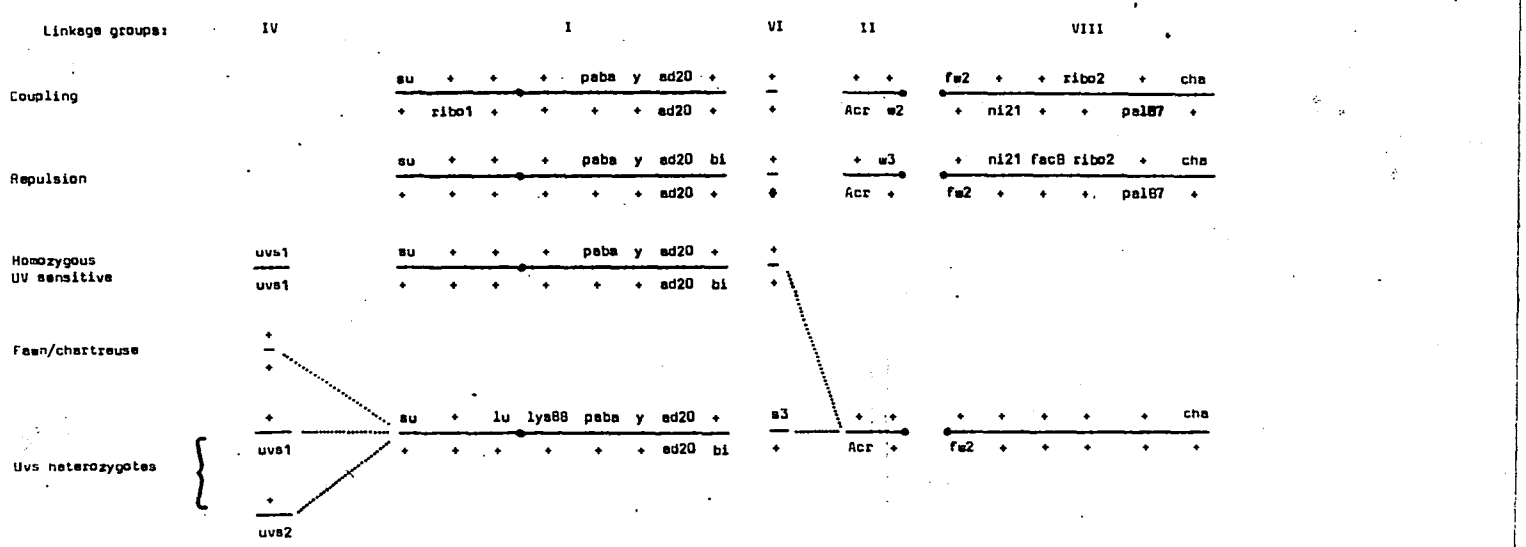

C 630*
C 639*
paba; w3
x C 924
ribo y
uv
uv
C 915
paba; w3 x ribo y; uvs1
C 925
paba; uvs1 x "
C 927
paba; uvs2 x "
C 911
suad20 lu lys88 paba y ad20; s3; cha x "
C 913
ad20 bi; Acr; fw2 x "
uvs1; ad20 bi; Acr; fw2
uvs1; suad20 paba y ad20; s3; cha
ribo y; uvs2
C 916
x paba; w3
C 926
x paba; uvs1
C 928
x paba; uvs2
C 912
x suad20 lu lys88 paba y ad20; s3; cha
C 914
x ad20 bi; Acr; fw2
uvs2; ad20 bi; Acr; fw2
mutant isolated after treatment with UV
recombinant isolated from cross
* Standard crosses, Barratt, Ogata and Johnsen, 1965

Figure 2: Genotypes of diploid strains


Linkage groups:
IV
I
VI
II
VIII
Coupling
su + + + paba y ad20 +
+ ribo1 + + + + ad20 +
+ + Acr w2
fw2 + + ribo2 + cha
+ ni21 + + pal87 +
Repulsion
su + + + paba y ad20 bi
+ + + + + + ad20 +
+ w3 Acr +
+ ni21 fac8 ribo2 + cha
fw2 + + + pal87 +
Homozygous
UV sensitive
uvs1
uvs1
su + + + paba y ad20 +
+ + + + + + ad20 bi
Fawn/chartreuse
Uvs heterozygotes
uvs1
uvs2
su + lu lys88 paba y ad20 +
+ + + + + + ad20 bi
a3
+ +
Acr +
+ + + + + cha
fw2 + + + + +

retardant (Mackintosh and Pritchard, 1963). Parafluorophenylalanine (pfp) was added to completed media at a concentration of 0.07 gm/l. to induce haploidisation of diploids (Lhoas, 1961, 1967; Morpurgo, 1961; McCully and Forbes, 1965).

Techniques

Crosses

Mixed inocula crosses were carried out as described by Pontecorvo et al. (1953). "Perithecium" analysis was used in all cases (Hemmons Pontecorvo and Bufton, 1953).

Diploids

A heterocaryotic mycelium was obtained by incubating conidia of two different haploid strains together in liquid complete media. Spontaneously occurring diploid sectors were selected by transferring small pieces of this mycelium onto minimal media (supplemented when necessary for markers present in a homozygous condition; Roper, 1952; Pontecorvo and Roper, 1953).

Location of new uvs mutants into linkage groups

Diploids were synthesized as above between mutant strains and a tester strain which had markers on all eight linkage groups. Haploid segregants were isolated on complete media supplemented with parafluorophenylalanine, and were tested for recombination between the new mutant and the markers located in the different linkage groups. Absence of recombination between the mutant and the marker of a specific linkage group, and free recombination between the new mutant and markers on all seven other groups was taken as evidence that the mutant was located on that chromosome (Käfer, 1958).

Ultraviolet light irradiation

(a) Survival curves

In these and most of the following experiments, conidia were treated in suspension in saline with Tween 80 added, unless otherwise stated. These suspensions were prepared at concentrations of 3×10^5 conidia/ml. from streaks which were three days old (two days of incubation at 37° C, followed by one day at room temperature). This was consistently followed to obtain conidia with minimum differences in age and physiological condition (Mishra and Nandi, 1962).

Irradiations were carried out in a closed, windowless room with constant overhead illumination. A General Electric 15 watt bulb, emitting 95% of its radiant energy in the region of 2537 Å, was used at a distance of 50 cm. After equilibration time was allowed, the intensity of the UV light at the point of irradiation was 16 ergs/mm.^{2}/sec, as read on a Blakray UV intensity meter.

Ten ml. samples of the original suspension were irradiated for different time intervals in glass petrie dishes. A control was similarly treated, except the irradiation step was omitted. One hour of repair was allowed under the lighting conditions described above. Dilutions were performed in saline, and the appropriate volumes were pipetted into warm complete media (40° C) which was then poured into petrie dishes. After incubation for 1-1/2 to 2 days at 37° C, total counts were made for each set of plates. The percentage survival of the experimental series was calculated using the value from the set of control plates as a base line.

(b) Induction and identification of uvs mutants

For the isolation of mutants, colonies were used from UV treatments giving less than 10% survival (irradiation of 5 or more minutes). Master plates were prepared by transferring up to 26 of these colonies onto minimal media containing desoxycholate and supplements satisfying all the requirements of the strain used. After two days of incubation at 37° C the plates were left at room temperature to conidiate. Each plate was then replicated onto two plates of the same media as described above, using sterile damp velvet (Lederberg and Lederberg, 1952). This was repeated for each master plate, using a fresh piece of velvet, so that two pairs of plates were obtained, and could be used as experimental plates each with its own control. Four to six hours incubation was allowed before irradiation to increase the differential between wild-type and mutant colonies (Jansen, 1967). One of the two experimental plates was irradiated for one minute, the other for three minutes as described above. The two control plates were treated identically except the irradiation step was omitted. After sixteen hours of further incubation at 37° C, the two experimental plates were compared to their own controls. Any colony showing reduced growth on both experimental plates, or distinct reduced growth after three minutes of irradiation, was rechecked by repeating the same replication technique. Such colonies were found with a frequency of about 1%. If reduced growth after irradiation was consistent, the possible mutant colony was tested for reduced survival after irradiation in suspension as previously described (Haefner and Howry, 1967; Nakai and Matsumoto, 1967; Lanier, Tuveson and Lennox, 1968).

Treatment of haploid conidia with N-methyl-N'-nitro-N-nitrosoguanidine (NTG)

Samples of conidial suspensions were added to tris-maleic buffer (.05 M, pH 6.0; Clutterbuck and Sinha, 1966) containing 0.5 mg. NTG (final concentration of NTG was 0.05 mg/ml.). At specific time intervals, a hundred-fold dilution was carried out into phosphate buffer (0.1 M, pH 7.0; Chang and Tuveson, 1967). This was followed by plating, incubation and survival counts of experimental and control samples of conidia.

Treatment of germinating diploid conidia with NTG

Germinating conidia were obtained by incubating quiescent conidia for a period of 4 to 4-1/2 hours in liquid minimal media (supplemented when necessary for markers present in a homozygous condition) at 37° C on a shaker. NTG was dissolved in a tris-maleic buffer, and conidial suspensions were added to give a final concentration of NTG of 0.5 mg./ml. (When the homozygous UV sensitive diploid was used, the NTG concentration was lowered to 0.05 mg./ml. in order to obtain comparable survival levels, as this diploid was also NTG sensitive). After appropriate treatment, a hundred-fold dilution into phosphate buffer was carried out, followed by plating, incubation, survival counts and examination of coloured sectors of treated and control samples.

Treatment of germinating conidia with 5-fluorodeoxyuridine (FUDR)

Conidial suspensions were added to liquid minimal media (supplemented when necessary for homozygous markers) containing FUDR and uridine. The uridine was added to compete against any 5-fluorouracil which may be

formed (Taylor et al., 1964). Final concentration of FUDR was 100 µg./ml., while that for uridine was 20 µg./ml. (Esposito and Holliday, 1964).

Conidia were treated throughout germination time since FUDR probably exerts its effect via DNA synthesis inhibition. In addition, a lower temperature was used to increase the effective time of exposure of the conidia to the FUDR, without increasing the number of nuclear divisions. A shaking water bath, holding a constant temperature of 28°C, was used. Under these conditions, control suspensions (containing neither FUDR nor uridine) germinated after 8-10 hours, while experimental suspensions germinated after 11-14 hours.

At the end of this treatment, a thousand-fold dilution was carried out, and followed by plating, incubation, survival counts, and examination of coloured sectors of treated and control samples.

Recording and analysis of coloured sectors from control and treated diploids

Coloured sectors originating from the centres of normal colonies in control and experimental plates were purified by streaking out on complete media. Needle replication onto supplemented minimal media was used to test for the presence of biochemical markers. Haploids were eliminated by visual identification (conidiation is denser and conidial heads are more regularly shaped in haploid strains) and by testing for the presence of markers which could be detected in the heterozygous state (Acr/+, and su1ad20/+ in ad20 homozygotes). Fawn and chartreuse non-disjunctional diploids could not be distinguished from diploids produced by mitotic crossing-over since no markers were present on the left arm

of linkage group VIII. However, an estimate of the frequency of the occurrence of non-disjunction could be obtained from an examination of yellow diploids which could be tested for the presence of the marker su1ad20 in the homozygous condition.

A classification dividing sectors into "large" or "small" was used (where any sector smaller than an eighth of a colony was considered "small"), as there appeared to be a large gap between these two. It was therefore felt that the majority of large sectors had been chemically induced, while most of the small sectors were due to spontaneous events.

Experiments and Results

Chemical induction of mitotic crossing-over

The purpose of these experiments was two-fold. Firstly, an attempt was made to induce mitotic crossing-over in heterozygous diploid strains of Aspergillus with various chemical agents which have been shown to be effective in other fungi. These frequencies of coloured sectors could then serve as controls against the frequencies obtained from homozygous UV sensitive diploids to determine the effect of this mutant on spontaneous and induced mitotic recombination.

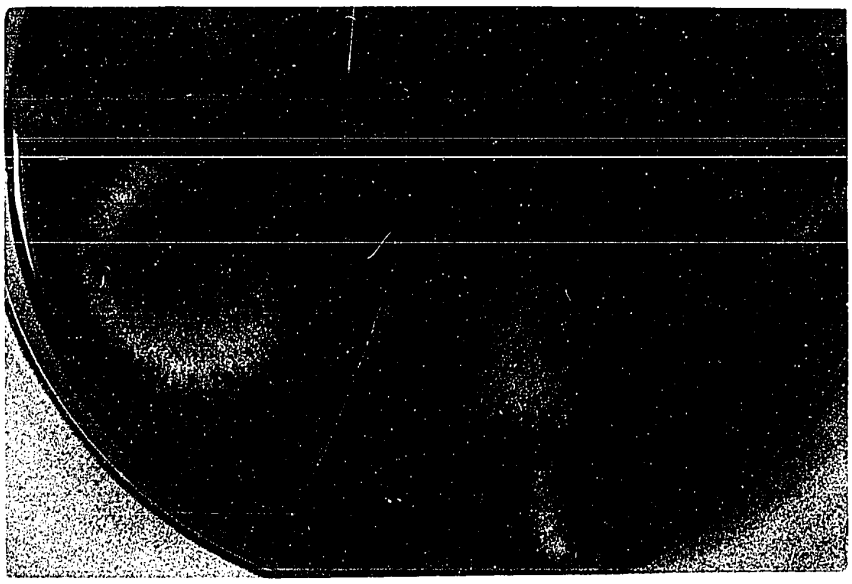
To obtain these values, the fawn/chartreuse diploid (uvs^+/uvs^+, Figure 2) was used because the reciprocal products of mitotic crossing-over - fawn/chartreuse twin spots - are visually easily identifiable. Also, no biochemical markers are located in linkage group VIII and so both products are equally viable, barring mutational events induced concurrently by the chemical used. Photographs of some of the twin spots and coloured sectors obtained by chemical treatment are given in Figure 3. Table II gives the frequencies of fawn/chartreuse, chartreuse, and fawn segregants for this diploid after three minutes treatment with 0.5 mg. NTG/ml. Corresponding values for FUDR-treated conidia (12 hours at 100 µg./ml.) are given in Table III.

In order to follow the distribution of cross-over events along a chromosome arm and to analyze the coincidence of several events, NTG and FUDR treatments were also performed on two diploids with more markers on the right arm of linkage group VIII (coupling and repulsion diploids, Figure 2). In addition to fawn and chartreuse, these diploids

Figure 3: Photographs of several NTG-induced twin spots in the fawn/chartreuse diploid.

Upper photograph: A whole colony fawn/chartreuse twin spot can be observed on the right side; while a half-colony twin spot appears on the left.

Lower photograph: A half-colony twin spot can be seen at the bottom centre. Fawn and chartreuse half-colonies can be observed on the left and right, respectively.



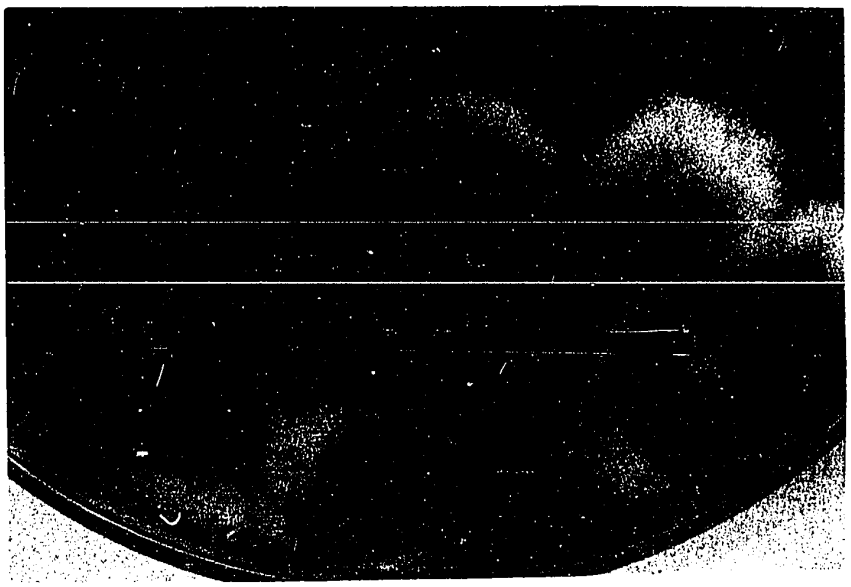


TABLE II: Effect of NTG on mitotic crossing-over in fawn/chartreuse diploid

Colour of segregant	Size	Frequency (%) Control	Experimental	Difference
fawn/chartreuse	large	-	2.24	2.24
	small	0.15	0.28	0.13
chartreuse	large	0.20	4.96	4.76
	small	0.40	1.12	0.72
fawn	large	0.15	2.10	1.95
	small	0.25	0.84	0.59
% abnormal colonies		0.10	13.07	12.97
Total number of colonies examined		1984	1431	
% survival		100*	72.4 (average) 71-76 (range)	

* set at 100%

TABLE III: Effect of FUDR on mitotic crossing-over in fawn/chartreuse diploid

Colour of segregant	Size	Frequency (%)		
		Control	Experimental	Difference
fawn/chartreuse	large	-	.94	.94
	small	-	-	-
chartreuse	large	0.24	4.00	3.76
	small	0.72	0.94	0.22
fawn	large	-	0.94	0.94
	small	-	0.47	0.47
% abnormal colonies		0.72	6.12	5.40
Total number of colonies examined		415	425	
% survival		100*	102.4	

* set at 100%

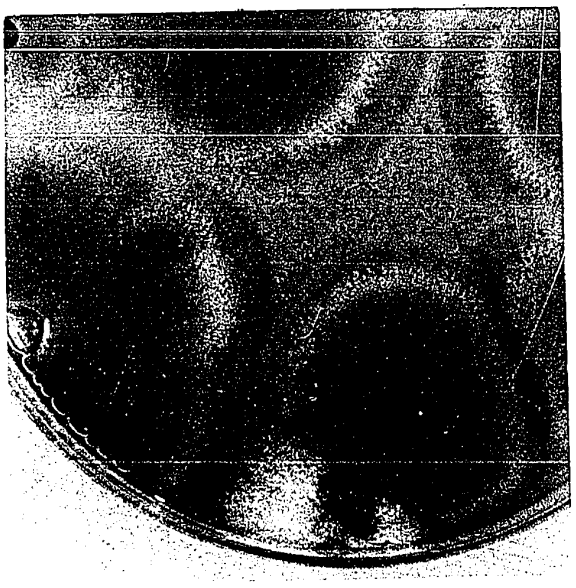
incorporate a third visible marker, palB7, also located in linkage group VIII. Homozygous palB sectors are very dark in appearance on complete medium and show reduced viability. Therefore, the palB part of these twin spots are always small and presumably some palB/chartreuse twin spots are produced in which only the chartreuse sector is detected. However, using these diploids, several different types of twin spots can be identified, and those that are detected can be further examined for segregation of the biochemical markers also located in linkage group VIII. In addition, yellow and white sectors were isolated and examined. Photographs of a few cases of twin spots induced in these diploids are given in Figure 4. Platings of control and experimental suspensions after NTG treatment are shown in Figure 5 to show the difference in the frequency of coloured sectors between these two.

Figure 4: Twin spots obtained from coupling and repulsion diploids.

Upper left photograph: A fawn/palB twin spot obtained by FUDR treatment of the coupling diploid.

Upper right photograph: A palB/chartreuse twin spot obtained by NTG treatment of the coupling diploid. Such twin spots could also be observed from platings of treated conidia of the repulsion diploid.

Lower photograph: A fawn-palB/chartreuse twin spot obtained by NTG treatment of the repulsion diploid. Because the fawn sector also carries the marker palB, it is relatively inviable.



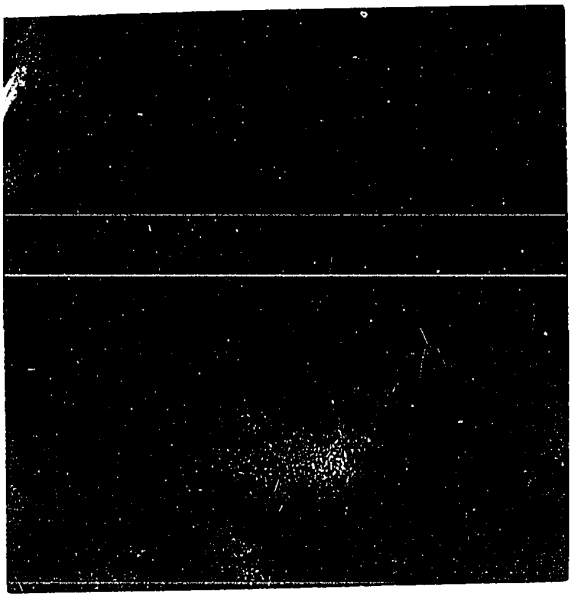


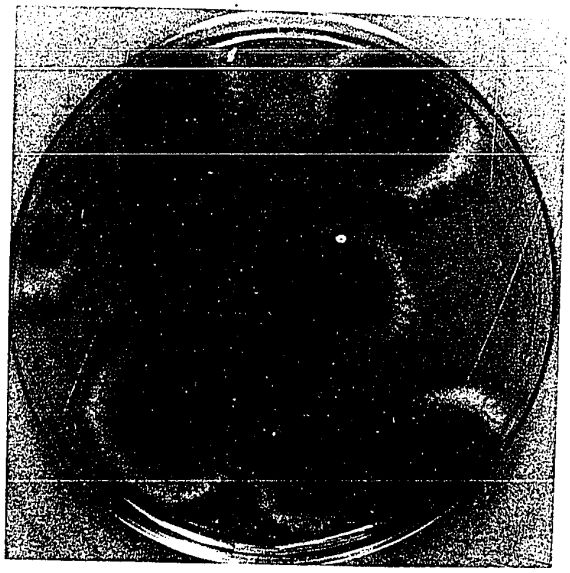
Figure 5: Comparison of platings of treated and untreated conidia of coupling and repulsion diploids.

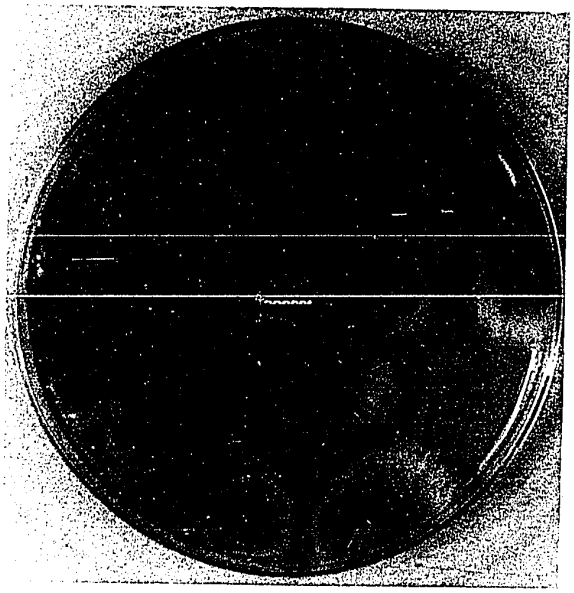
Upper left photograph: Control plating of coupling diploid.

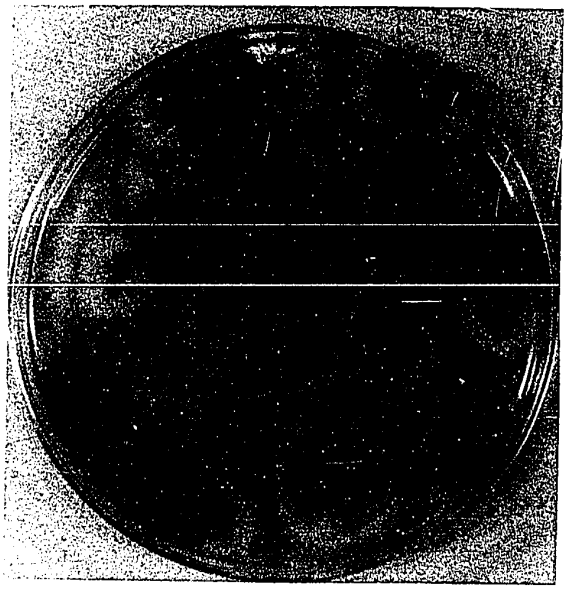
Upper right photograph: Experimental plating (after NTG treatment) of coupling diploid.

Lower left photograph: Control plating of repulsion diploid.

Lower right photograph: Experimental plating (after NTG treatment) of repulsion diploid.







Tables IV to XI give the results that were obtained from control and treated conidia of both of these diploids. The following conventions are used to represent these phenotypes:

1. Colour selected: This indicates how the colony is visually identified (i.e. the phenotypic appearance of the colony).

2. Marker: Only mutant phenotypes are given (-). All others appeared wild-type and are either heterozygous as in the parental diploid, or homozygous wild-type. In the cases of suad20 and Acr, where heterozygotes could be distinguished from either homozygote, homozygotes are reported as either (+) or (=), while the unchanged heterozygous parental-type is left blank.

3. Size: Sectors are divided into "large" and "small", where a small colour sector constitutes less than an eighth of that colony.

4. Twin spots: Wherever paired reciprocal sectors are isolated together, they are reported together and brackets are placed around them.

5. Single versus multiple events: Sectors placed under "single events" are due to a single mitotic cross-over event, while those classed under "multiple events" are probably caused by more than one cross-over event. In some cases these are not distinguishable from non-disjunctional events.

6. Epistasis of fawn over chartreuse: Assuming a minimum number of exchanges compatible with the observed phenotypes, all fawn segregants from coupling diploids are classified as homozygous cha, which is indicated as (-?).

TABLE IV: COUPLING DIPLOID: Phenotypes of segregants analyzed from control platings of NTG-treated conidia.

(a) Single events

Colour selected	Linkage group : I				II		VIII				Number observed	
											Large	Small
	Marker:	suad20	ribo1	paba	y;	Acr	w2;	fw2	ni21	ribo2	pal87	cha	
y				-	-								2	1
w						+	-						-	1
pal											-		1	-
cha												-	1	2

(b) Multiple events

none

Total number of colonies examined: 1198

TABLE V: COUPLING DIPLOID: Phenotypes of segregants analyzed from experimental platings of NTG-treated conidia.

(a) Single events

Colour selected	Linkage group : I				II		VIII				Number observed	
											Large	Small
	Marker:	suad20	ribo1	paba	y;	Acr	w2;	fw2	ni21	ribo2	palB7	cha	
y (i)				-	-								9	8
(ii)					-								6	-
w						+	-						13	3
fw								-		-		-?	16	2
fw/pal								-	-	-		-?	5	-
pal (i)									-		-		5	-
(ii)											-		4	-
pal/cha (i)									-		-	-	1	-
(ii)											-	-	2	-
cha (i)										-		-	5	4
(ii)												-	15	-

TABLE V: (continued)

(b) Multiple events

Colour selected	Linkage group:	I				II		VIII				Number observed	
												Large	Small
	Marker:	suad20	ribo1	paba	y;	Acr	w2;	fw2	ni21	ribo2	palB7	cha	
y (i)		+		-	-								1	-
(ii)		-			-								1	-
w		+				+	-						1	-
fw		+						-		-		-?	1	-
fw/cha								-		-		-	1	-

Total number of colonies examined: 786

TABLE VI: REPULSION DIPLOID; Phenotypes of segregants analyzed from control platings of NTG-treated conidia.

(a) Single events

Colour selected	Linkage group:	I				II		VIII					Number observed	
													Large	Small
	Marker:	suad20	paba	y	bi;	Acr	w2;	fw2	ni21	facB101	ribo2	palB7	cha	
y (i)			-	-	-								-	1
(ii)				-	-								-	1
w						-	-						-	4
fw-pal	-							-			-		-	1
cha (i)									-	-	-	-	4	1
(ii)												-	1	-

(b) Multiple events

cha (i)		+										-	1	-
(ii)						+						-	-	1

Total number of colonies examined: 1078

TABLE VII: REPULSION DIPLOID: Phenotypes of segregants analyzed from experimental platings of NTG-treated conidia.

(a) Single events

Colour selected	Linkage group:	I				II		VIII					Number observed	
													Large	Small
	Marker:	suad20	paba	y	bi;	Acr	w2;	fw2	ni21	facB101	ribo2	palB7	cha	
y (i)			-	-	-									10	10
(ii)				-	-									2	4
w						-	-							8	1
fw-pal								-				-		3	1
fw-pal/cha								-				-		7	2
									-	-	-		-		
pal												-		1	-
pal/cha (i)												-		3	-
										-	-		-		
(ii)												-		1	-
													-		
cha (i)									-	-	-		-	13	5
(ii)										-	-		-	4	2
(iii)													-	17	1

TABLE VII; (continued)

(b) Multiple events

Colour selected	Linkage groups: I											II		VIII						Number observed	
	Marker: suad20		paba	y	bi;							Acr	w2;	fw2	ni21	facB101		ribo2	palB7	cha	Large	Small
y	+		-	-	-																-	2
w												-									1	-
fw														-							1	-
fw/cha (i)														-						-	1	-
(ii)														-				-		-	1	-
(iii)			-	-	-									-				-		-	1	-

Total number of colonies examined: 634

TABLE VIII: COUPLING DIPLOID: Phenotypes of segregants analyzed from control platings of FUDR-treated conidia.

(a) Single events

Colour selected	Linkage group:	I				II		VIII				Number observed	
												Large	Small
	Marker:	suad20	ribo1	paba	y;	Acr	w2;	fw2	ni21	ribo2	palB7	cha	
y				-	-								2	-
w						+	-						2	-
fw								-		-		-?	1	-
cha												-	1	-

(b) Multiple events

none

Total number of colonies examined: 315

TABLE IX: COUPLING DIPLOID: Phenotypes of segregants analyzed from experimental platings of FUDR-treated conidia.

(a) Single events

Colour selected	Linkage group:	I				II		VIII				Number observed	
												Large	Small
	Marker:	suad20	ribo1	paba	y;	Acr	w2;	fw2	ni21	ribo2	palB7	cha	
y (i)				-	-							3	3
(ii)					-							1	2
w						+	-					-	2
fw								-	-		-?	5	5
fw/pal								-	-		-?	2	-
									-	-			
cha (i)									-		-	3	1
(ii)											-	2	2

(b) Multiple events

none

Total number of colonies examined: 273

TABLE X: REPULSION DIPLOID: Phenotypes of segregants analyzed from control platings of FUDR-treated conidia.

(a) Single events

Colour selected	Linkage groups:	I				II		VIII					Number observed	
													Large	Small
	Marker:	suad20	paba	y	bi;	Acr	w2;	fw2	ni21	facB101	ribe2	palB7	cha	
y			-	-	-									4	5
w						-	-							÷	1
fw-pal								-			-			2	2
cha (i)									-	-	-		-	1	-
(ii)										-	-		-	1	1
(iii)													-	2	3

(b) Multiple events

none

Total number of colonies examined: 1550

TABLE XI: REPULSION DIPLOID: Phenotypes of segregants analyzed from experimental platings of FUDR-treated conidia.

(a) Single events

Colour selected	Linkage group: I				II		VIII						Number observed	
													Large	Small
	Marker:	suad20	paba	y	bi;	Acr	w2;	fw2	ni21	facB101	ribo2	palB7	cha	
y (i)			-	-	-									2	2
(ii)				-	-									1	3
w						-	-							4	2
fw-pal/cha								-	-	-	-	-	-	5	-
pal											-			-	1
pal/cha											-		-	1	1
cha (i)									-	-	-		-	7	1
(ii)										-	-		-	1	2
(iii)													-	11	1

TABLE XI: (continued)

(b) Multiple events

Colour selected	Linkage groups:	I				II		VIII					Number observed	
													Large	Small
	Marker:	suad20	paba	y	bi;	Acr	w2;	fw2	ni21	facB101	ribo2	palB7	cha	
y		+	-	-	-								1	-
fw/cha		+						-	-	-	-	-	1	-
cha		+										-	1	-

Total number of colonies examined: 419

Controls

Large spontaneous twin spots are rarely observed. None were found among the 6140 colonies examined in the control platings run concurrently with these experiments on non-UV sensitive diploids. It is estimated that this occurs with a frequency of about 1/10,000 for the right arm of linkage group VIII (Käfer, personal communication). However, three small twin spots were found (Table II). All were extremely tiny, and consisted of single lines of coloured conidial heads which could only be identified as twin spots under the dissection microscope. Also, all three were fawn/chartreuse twin spots. This is an expected result since tiny twin spots in which one of the reciprocal segregants carried the marker palB should be very difficult to identify.

A comparison of the frequencies of the various coloured sectors in controls of NTG- and FUDR-treated conidia from coupling and repulsion diploids is given in Table XII. The only difference in experimental procedure between the two sets with different treatments was that the NTG controls were allowed 4-1/2 hours of germination time in liquid minimal media at 37° C, while the FUDR controls required approximately 12 hours to germinate in the same media at 28° C. If the extension in germination time had an effect on mitotic crossing-over, it would be expected to express itself as significant difference between the number of large colonies in NTG and FUDR controls. Of the eight values compared in this table, none show significant deviation at the 5% level from the tested equality of these numbers. However, in general, the comparisons among large sectors give probabilities closer

TABLE XII: Comparison of frequencies of coloured sectors from controls of NTG and FUDR treated conidia of coupling and repulsion diploids.

Treatment:	NTG		FUDR	
Size:	Large	Small	Large	Small
Colour of segregant				
yellow	0.09	0.22	0.16	0.16
white	0.04	0.09	0.16	0.05
fawn	-	0.09	0.05	0.05
chartreuse	0.13	0.22	0.21	0.11
Total number of colonies examined:	2276		1865	

to the 5% level than those comparisons among smaller sectors. Thus, a larger sample size would be preferable, but this is unfeasable at this time because of the large number of colonies that would have to be handled.

The effect of NTG and FUDR on mitotic crossing-over

The effects of these two agents on mitotic crossing-over in non-UV sensitive strains can best be observed from Tables II and III which give the experimental and net frequencies of coloured sectors after NTG and FUDR treatment of the fawn/chartreuse diploid. In this diploid, both the reciprocal products of the cross-over event have an equal probability to be picked up (barring lethal events) since they are equally viable. From the net frequencies of large twin spots and coloured sectors, it is obvious that both agents can effectively induce mitotic crossing-over. A significantly larger frequency of coloured sectors have been induced by the NTG ($P < .01$) but a significantly larger frequency of abnormal colonies have also been induced concurrently ($P < .01$).

Double crossing-over versus non-disjunction

Linkage group I has the colour marker y on the right arm of this chromosome, and suad20 on the left arm and can therefore be used to determine whether or not non-disjunction is occurring in these experiments. Table XIII gives the number of y colonies observed that were homozygous for the presence (+) or absence (-) of suad20, and compares these values with the number of single events observed. Colonies homozygous for yellow and the presence of the suppressor could

TABLE XIII: Coincident homozygosis for both arms of linkage group I among selected y/y segregants.

	Number observed	Frequency (%)
Marker: su1ad20		
+/+	4	0.21
-/-	1	0.05
+/-	66	3.45

Total number of colonies examined: 1912

either be produced by a single non-disjunctional event or by two mitotic cross-over events, one on each side of the centromere. However, colonies homozygous for yellow and the absence of the suppressor could only be produced by a mitotic cross-over event on each side of the centromere. The observed numbers in these two classes do not differ significantly from one another at the 5% level of significance ($.10 < P < .30$). Although the sample size is small, the frequency of single events is so much larger than either of these two values that it may be concluded that non-disjunctional events are not frequently induced by the agents used here.

Types of events induced by NTG and FUDR

Table XIV gives the frequencies of single and multiple events induced by NTG and FUDR on the coupling and repulsion diploids (summarized from Tables IV to XI). Control values from both diploids are summed together since they do not differ significantly from one another. The observed frequency of single events induced by NTG is significantly higher than the frequency induced by FUDR ($P < 0.01$), while the values obtained for multiple events do not differ significantly from one another at the 5% level of significance ($0.10 < P < 0.30$). In addition, multiple events are even less frequent than would be expected from the coincidence of two single events. (For NTG, the probability of two independent single events together is 1.85% (0.1359^2) which is greater than the observed frequency of 0.85%. For FUDR, the probability of two independent single events occuring together is 0.92% which is also greater than the observed frequency of 0.38%). Thus, the results

TABLE XIV: Frequencies of single and multiple events from Controls, NTG- and FUDR-treated conidia of coupling and repulsion diploids.

Treatment:	Controls		NTG		FUDR	
	Number	Frequency (%)	Number	Frequency (%)	Number	Frequency (%)
Single events	45	1.09	193	13.59	76	9.60
Multiple events	2	0.05	12	0.85	3	0.38
Total number of colonies observed	4141		1420		792	

obtained from these diploids are in agreement with those obtained from the fawn/chartreuse diploid since both indicate that NTG induces a higher frequency of mitotic crossing-over than FUDR. In addition, the data obtained from these better marked coupling and repulsion diploids indicate that all the sectors produced by both agents can be explained by the induction of independent single events.

UV sensitive mutants

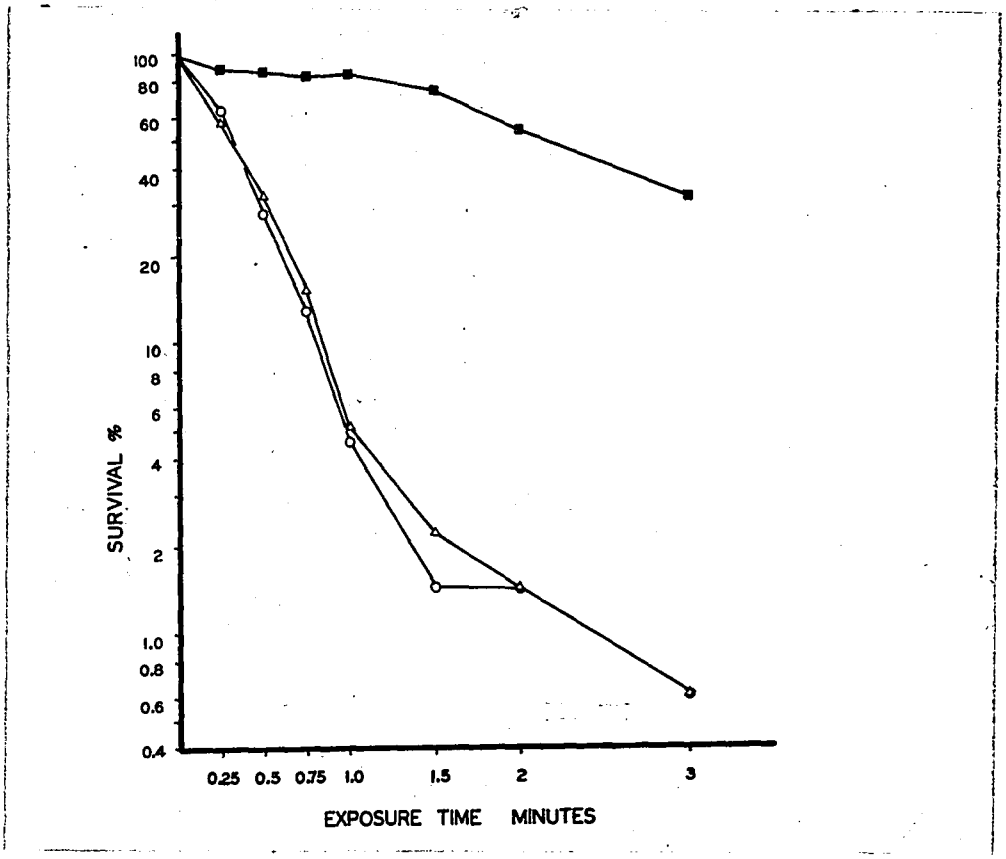
Two UV sensitive mutants were obtained from among 1500 UV irradiated conidia which were tested for marked sensitivity to UV light by the irradiation of velvet replicas. Mapping by means of para-fluorophenylalanine-induced haploidisation of a diploid strain synthesized between the mutant uvs1 strain and a tester strain marked on all eight linkage groups, showed that uvs1 was located on linkage group IV. An analysis of 104 ascospores from crosses of uvs1 x uvs2 (Crosses 927 and 928 of Figure 1) yielded no wild-type recombinants. Thus uvs1 and uvs2 were assumed to be mutants of the same gene. Also, both were determined to be recessive genes since diploid strains which were heterozygous for uvs1 and uvs2 were not more sensitive to UV light than was the wild-type homozygous diploid. At the same time, the diploid homozygous for uvs1 was markedly more sensitive.

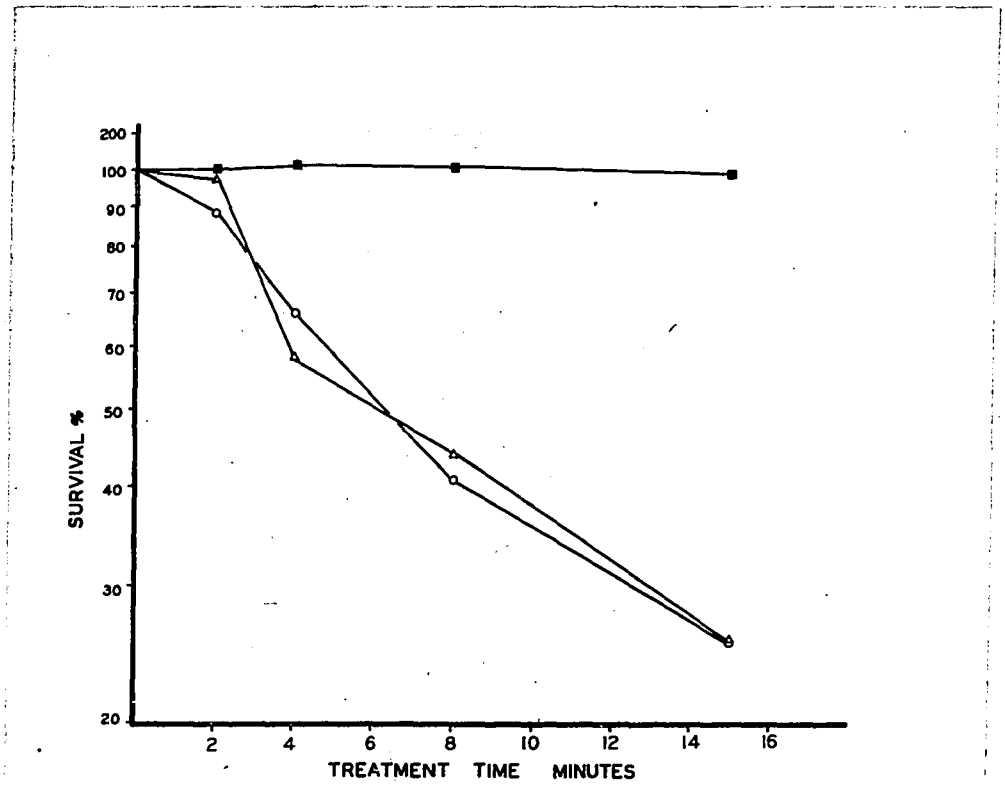
Survival curves

UV survival curves for different times of exposure of suspensions of the parent strain (ribo1 y) and its UV sensitive derivatives (ribo1 y; uvs1 and ribo1 y; uvs2) are given in Figure 6. After three minutes of irradiation at a dosage of 16 ergs/mm^2./sec. at the level of the

Figure 6: Survival curves of parental strain (*ribo1 y* -■) and its UV sensitive derivatives (*ribo1 y; uvs1* -Δ and *ribo1 y; uvs2* - O) following UV irradiation at 16 ergs/mm^2./sec.

Figure 7: Survival curves of parental strain and its UV sensitive derivatives following treatment with 0.05 mg./ml. of NTG. (Symbols same as above).


100
80
60
40
20
10
8
6
4
2
1.0
0.8
0.6
0.4
SURVIVAL %
0.25 0.5 0.75 1.0 1.5 2 3
EXPOSURE TIME MINUTES


200
100
90
80
70
60
50
40
30
20
SURVIVAL %
2 4 6 8 10 12 14 16
TREATMENT TIME MINUTES

suspension, the UV sensitive strains are about thirty times more sensitive to the UV light than the wild-type. This dosage was used to identify UV sensitive ascospores in all subsequent crosses involving these strains.

Figure 7. gives the survival curves for the same strains when exposed to NTG. Results clearly show that both UV sensitive strains are also NTG sensitive to a similar degree.

The effect of uvs1 on mitotic crossing-over

Table XV gives the frequencies of the various types of coloured sectors in control and NTG-treated (4 minutes of 0.05 mg./ml. of NTG) conidia of the homozygous UV sensitive diploid (Figure 2). The spontaneous frequencies observed in these controls are relatively high and are well above the values that have been obtained from the controls of all the other non-UV sensitive diploids in the previous experiments. In addition, a large number of sectors which do not originate from the centre of the colony are observed in control as well as in experimental platings. These are excluded from this table, but several can be seen in Figures 8 and 9. Also, abnormal colonies are observed more frequently in these controls and experimental platings than in those of experiments with non-UV sensitive diploids.

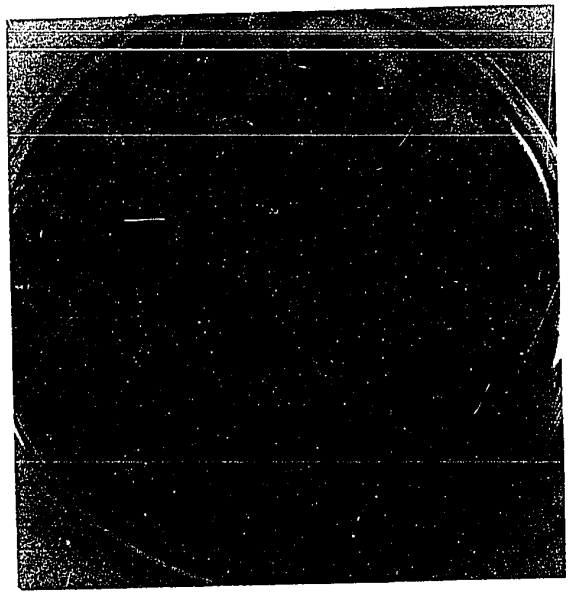
Among the colour sectors, one large twin spot was observed in the control plating. Therefore, it appeared as if these high frequencies of coloured sectors might be due to increased mitotic crossing-over. To confirm this, a larger sample was plated and examined. However, only a qualitative analysis is possible at this time. In this case,

TABLE XV: Frequencies of coloured sectors in control and experimental platings of the homozygous UV sensitive diploid.

Colour of segregant	Size	Control	Experimental
fawn/chartreuse	Large	0.21	-
	Small	-	0.50
chartreuse	Large	2.70	3.47
	Small	0.42	0.50
fawn	Large	0.42	0.50
	Small	0.62	-
% abnormal colonies		2.29	37.62
Total number of colonies analyzed		481	202
% survival		100*	42.0

* set at 100%

Figure 8: Control platings of the homozygous UV sensitive diploid showing large numbers of spontaneous colour sectors.



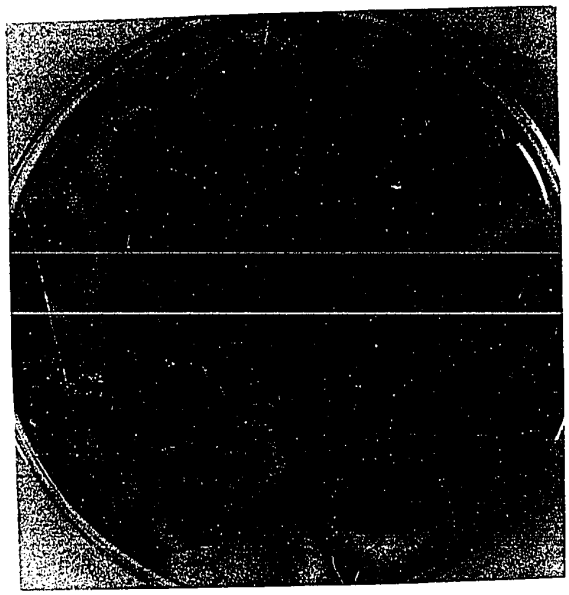
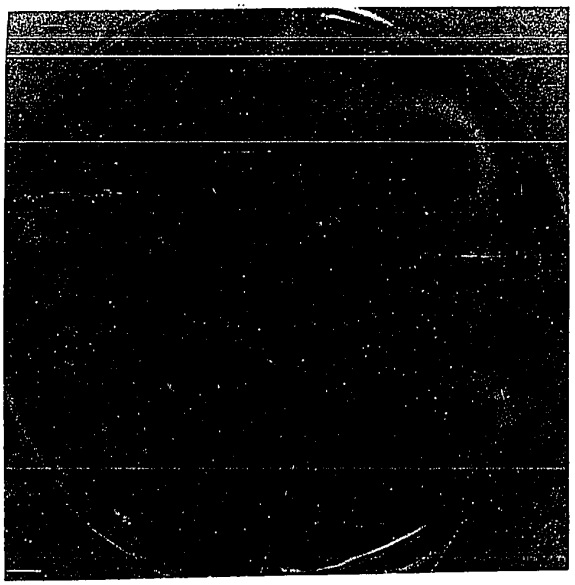
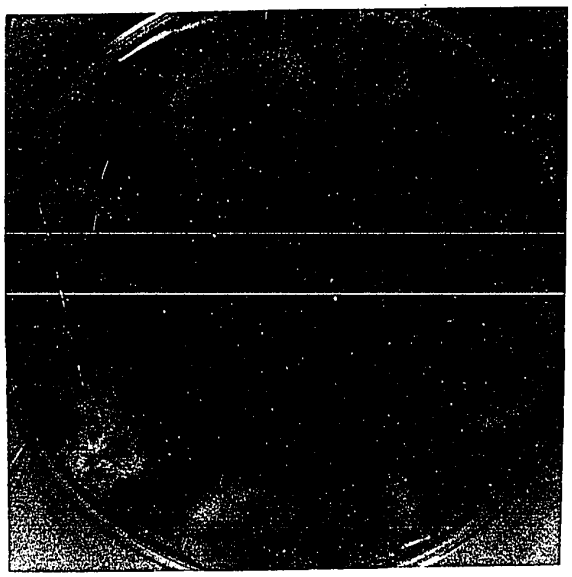
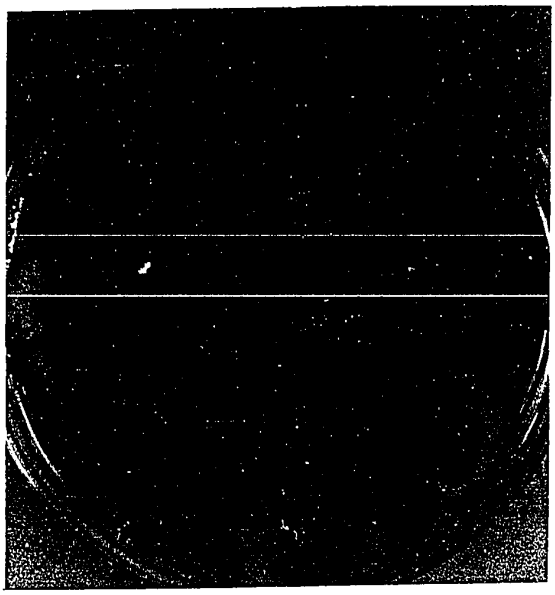


Figure 9: Experimental platings of the homozygous UV sensitive diploid following NTG treatment showing large numbers of spontaneous and induced coloured sectors and abnormal colonies.







a few additional twin spots have been found and an attempt was made to determine whether the number of observed twin spots could be explained by the coincidence of two independent events in the same part of a colony. Out of 770 in this control plating, 8 large fawn sectors were observed, while 30 large chartreuse sectors were found. Therefore the probability of producing a fawn/chartreuse twin sector by two separate events is less than 1/770. However, in this same experiment, 5 spontaneous cases of large fawn/chartreuse twin sectors were observed. Thus, it appears likely that these have been produced by a single event - one reciprocal exchange between the centromere of linkage group VIII and the more proximal marker fw.

The effect of uvs1 on meiotic crossing-over

Crosses homozygous for both uvs1 or uvs2 and heterozygous for uvs1 and uvs2 were examined together with a control cross (homozygous wild-type) to determine whether or not the UV sensitive genes had any effect on meiotic crossing-over. The results are given in Table XVI. χ^2-tests were performed to determine whether any of the observed values for the distance between paba and y differed from the expected value of 15 map units. None showed significant deviation at the 5% level of significance. In addition, the sample was found to be homogeneous at this level of significance.

TABLE XVI: The effect of uvs1 and uvs2 on meiotic crossing-over.

Cross number*	Crosses	Number of ascospores analyzed	Calculated map distance between paba and y
924	$uvs^+ \times uvs^+$	104	21.2
925	uvs1 x uvs1	104	18.3
926	uvs2 x uvs2	103	11.7
927	uvs1 x uvs2	104	9.6
928	uvs2 x uvs1	104	13.5

* details in Figure 1.

Discussion

Spontaneous and induced mitotic crossing-over

Analysis of somatic segregants has been used as a major genetic technique in *Aspergillus nidulans* since the discovery by Roper in 1952 that rare fusions of genotypically different haploid nuclei can occur to produce stable diploid strains. Spontaneously, these diploids will give rise to somatic segregants which may be picked up as coloured sectors. These segregants are produced by two distinct processes: Firstly, chromosomal segregation can produce haploids, non-disjunctional diploids, and aneuploids (Käfer, 1961). A second and independent process of mitotic crossing-over can produce diploids homozygous for the markers distal to the exchange on one chromosome arm (Pontecorvo and Roper, 1953; Pontecorvo, Tarr Gloor and Forbes, 1954; Pontecorvo and Käfer, 1958).

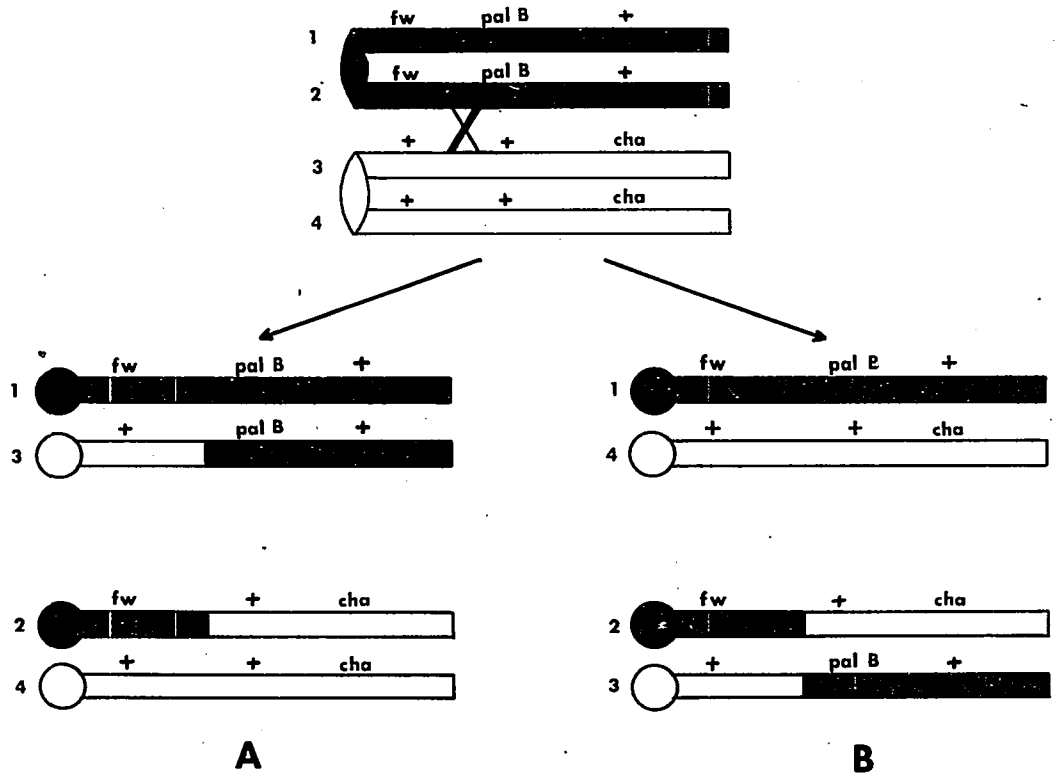
The ultimate proof of the occurence of crossing-over at the four-strand stage of mitosis rests on the ability to recover together with the reciprocal products of the cross-over event. Stern was the first to obtain these in *Drosophila melanogaster*, and he termed the yellow body/singed bristle paired reciprocal products on the abdomen of heterozygous flies "twin spots" (Stern, 1936). He interpreted the occurrence of such twin spots to mean that crossing-over had taken place at the four-strand stage of mitosis and was followed by one of the two types of normal segregation of the chromatids. Figure 10 explains this mechanism using, as an example, the most significant part of linkage group VIII of the repulsion diploid (given in Figure 2). A

cross-over event in the interval between fw (fawn) and palB (alkaline phosphatase) can lead to one of two possible results following equational mitotic segregation. In case A, the two cross-over strands segregate into different nuclei, producing two reciprocal segregants, eabh one homozygous distal to the point of the exchange. That is, a palB/chartreuse twin spot will be observed. In case B, one nucleus remains parental while the other receives both cross-over strands and appears phenotypically like the parental type. This latter type of segregation was identified in Aspergillus nidulans by Roper and Pritchard (1955), while the former type was first identified with different markers by Käfer (1961), and by Wood (1967) with this system of visible markers.

Whole colony twin spots will be observed when reciprocal cross-over events, such as that represented in Figure 10, take place during the first nuclear division. However, if crossing-over is induced in a conidium undergoing its second (or third) nuclear division, the resultant colony will consist of one-quarter (or one-eighth) one segregant, one-quarter (or one-eighth) its reciprocal, while the rest remains heterozygous parental. In all these experiments, NTG and FUDR treatments were performed on germinating conidia. These suspensions are not synchronous, and it has been estimated that from one to three nuclear divisions have taken place by germination time (Weijer and Weisberg, 1966; Wood, 1967). Thus it is expected that the majority of the segregants consisting of an eighth or more of a colony have been induced by the chemical agent used, while many of the smaller sectors

Figure 10: Schematic representation of the mechanism of mitotic crossing-over.

MECHANISM OF MITOTIC CROSSING-OVER



might be due to spontaneous events. The observed data support this: In control platings of non-UV sensitive diploids, few large coloured sectors are seen, while in experimental platings the majority of colour sectors are large, although the frequency of smaller sectors rises as well. This rise in frequency of smaller sectors in experimental platings is thought to be due to the problem of clumped conidia encountered in these experiments: Conidia tend to start sticking to one another the longer they are held in suspension, and also soon after they begin germinating. In these experiments, clumped conidia was a greater problem with FUDR treated conidia than with those treated with NTG, since in the former case germination time was extended from the normal 4-1/2 hours at 37^{o} C to 12 hours at 28^{o} C. Thus, presumably some of the smaller sectors may have been induced during the first three nuclear divisions, but appear smaller because they have clumped with heterozygous conidia. In agreement with this assumption, a higher proportion of smaller colour sectors are observed in FUDR treated conidia than in those treated with NTG.

The schematic representation of mitotic crossing-over in Figure 10 implies that mitotic crossing-over is always of a reciprocal type. The reciprocal nature of spontaneous and UV induced mitotic recombination has been conclusively shown by Haefner (1966) in Saccharomyces cerevissiae where a pedigree analysis is possible. In experiments presented here, it was possible to isolate visible twin spots in many cases after NTG and FUDR treatments of germinating conidia. There are several possible reasons why the reciprocal products would not be

isolated in every case: A chartreuse sector might be observed if crossing-over took place between the marker palB and cha in Figure 10, since the marker palB would remain heterozygous just as the marker fw remains heterozygous in the example shown. Fawn sectors might arise as a result of two cross-over exchanges, one between the centromere and the marker fw, followed be another in the region between fw and palB. In addition, only one segregant (either fawn or chartreuse) may be recovered if a lethal event has been concurrently induced by the chemical used to induce the cross-over event. Finally, either segregant may have been too small to detect visually.

The mechanism of NTG and FUDR induced mitotic crossing-over

Two classes of agents have proved effective in inducing mitotic crossing-over. These include the mutagenic agents, such as UV and NTG, and "recombinogens" (Holliday, 1964), such as FUDR and Mitomycin C which induce crossing-over at concentrations at which they are not mutagenic.

Among the most popular mutagenic agents which are capable of inducing recombination is UV light which has been shown to be effective in germinating conidia of Aspergillus nidulans (Käfer and Chen, 1964; Jansen, 1966; and Wood, 1967), as well as in other fungi (James, 1965; James and Lee-Whiting, 1955; Holliday, 1961, 1962, 1965b, 1966, 1967; Hurst and Fogel, 1964; and Sherman and Roman, 1963). In these experiments, an attempt was made to induce mitotic crossing-over with another mutagenic agent, NTG, which Zimmerman et al. (1966) have shown to be

effective in inducing recombination in diploid strains of yeast. As expected from Zimmerman's results, a large number of coloured sectors were observed after treatment of germinating conidia with this mutagen. These frequencies were close to those obtained by Wood (1967) who used UV light to induce mitotic crossing-over on the same fawn/chartreuse diploid used for part of this analysis. Just as was observed for UV light, a large number of abnormal colonies were induced concurrently. However, it appears that abnormals (which are probably mainly deletions and other mutations which reduce viability, Käfer, 1963) and mitotic cross-over events are either produced by two different processes, or by the induction of two types of damage to the DNA, only one of which can be repaired enzymatically (Käfer, personal communication). This is supported by these experiments since all the segregants which have been analyzed arose from centres of normally growing colonies. Also, it has been observed that treatment of quiescent conidia, which are not undergoing DNA synthesis, with UV light or NTG results in survival levels and frequency of abnormal colonies comparable to those found after treatment of germinating conidia, but a greatly reduced amount of mitotic crossing-over (Wood, 1967). Like UV light, NTG is thought to increase mitotic crossing-over by inducing a type of damage in the DNA which can be corrected by a system of repair enzymes, some of which have been shown to be active in recombination as well (Holliday, 1967).

FUDR has been used by Esposito and Holliday (1964) to increase recombination frequencies in diploid strains of Ustilago maydis. In

these experiments, FUDR treatments of germinating conidia resulted in increased frequencies of various coloured segregants, and these were shown to be due to mitotic crossing-over. FUDR is known to inhibit thymidylate synthesis (Cohen et al., 1958; Flaks et al. 1959). Thus its recombinogenic properties are thought to be a result of thymidylate starvation of cells (Esposito and Holliday, 1964), since a direct deprivation of thymine in bacteria has been observed to increase the frequency of recombination (Gallant and Spottswood, 1965). Putrament (1967) has shown that aminopterin (which also leads to thymine starvation) and adenine starvation induce intragenic recombination in diploid strains of Aspergillus nidulans. She also shows that a more indirect method of thymine starvation - via para-aminobenzoic acid starvation in paba-requiring strains - also induces mitotic recombination. Finally, Rolfe (1966) has shown that thymidine starvation is inhibited by concurrent inhibition of RNA and protein synthesis in bacteria.

Putrament suggests that all agents which are capable of inducing recombination, have one thing in common: all are capable of inhibiting DNA synthesis (either by damage to the DNA necessitating repair, or by direct inhibition of DNA synthesis by starvation) without concurrently affecting RNA and protein synthesis to the same major degree. Thus, although UV light damages DNA, RNA, and protein synthesis, she notes that the ability to synthesize RNA and protein is recovered first by the cell. The same has been observed for alkylating agents. Thus, it is expected that an overall depression of the metabolic rate (DNA,

RNA, and protein synthesis) by a lowering of temperature should have little effect on mitotic recombination. This was confirmed by a comparison of controls of NTG and FUDR treated conidia, in these experiments, where the only difference in experimental procedure between these two sets of values was that in the case of the FUDR treated controls, germination time was extended 7 to 8 hours above the NTG control by lowering the temperature. As no significant difference was observed, it was concluded that an overall depression of the metabolic rate has little or no effect on mitotic crossing-over.

She therefore suggests that one process is responsible for inducing recombination in all these cases, namely, an inhibition of DNA synthesis without concurrent interference with RNA or protein synthesis. However, it also appears that the repair of a certain type of damage to DNA is involved in all these cases. It has already been mentioned that UV light is thought to induce recombination by an activation of repair enzymes to correct these damages, at least one of which is involved in recombination as well. It seems reasonable to assume that NTG acts in a similar manner. Support of this theory is also obtained from the observation of non-conservative repair replication following NTG treatment similar to that observed for UV irradiated cells (Cerdà-Olmeda and Hanawalt, 1967). Other researchers have found UV sensitive mutants which are Mitomycin C sensitive, and vice versa (Boyce and Howard-Flanders, 1964b; Okubo and Romig, 1966), suggesting that Mitomycin also acts in a similar manner. Finally, Taylor, Haut and Tung (1961) have found gaps in FUDR-treated DNA of root tips of

Vicia faba, implying that discrete breaks needing repair have been induced, while Pauling and Hanawalt (1965) have observed non-conservative repair replication following thymine starvation in bacterial cells.

Thus, it seems likely that all these agents do act in one basic way. Rather than just an inhibition of DNA synthesis, this involves the induction of an enzymatically repairable type of damage in the DNA by these agents, since at least one of these enzymes has been postulated to be involved in recombination.

Generalized repair mechanisms

Many researchers have postulated a generalized repair mechanism (review, Hanawalt and Hayes, 1967) which is capable of enzymatically correcting many types of damage in the DNA. Thus it would be expected that UV sensitive mutants would show cross-sensitivity to a variety of chemical and physical agents depending on their mode of action. Indeed, this has been observed by many. In addition, both UV sensitive mutants isolated in these experiments were observed to be NTG sensitive as well. Thus, if the repair of UV- and NTG-damaged DNA have enzymatic steps in common, and, if UV is postulated to induce recombination via some of these same enzymes, then it might be expected that NTG could also be used to induce recombination. This, too, has been observed in these experiments.

The effect of uvs1 on recombination

It has been postulated that repair of the damage induced by UV light and recombination have at least one enzymatic step in common, since UV sensitive mutants have been isolated which are recombination-

negative, and recombination-negative mutants have been isolated which are UV sensitive (Clark and Margulies, 1964; van de Putte, Zwenk and Rörsch, 1966; Howard-Flanders and Theriot, 1966; Howard-Flanders, Theriot and Stedeford, 1966; Holliday, 1967; Lanier, Tuveson and Lennox, 1968). However, the UV sensitive mutant isolated in these experiments is not recombination-negative in this original sense. Rather, an increase of spontaneous and induced mitotic crossing-over is observed in diploid strains which are homozygous for uvs1. No effect on meiotic crossing-over could be detected.

A similar mutant has been isolated by Holliday in Ustilago maydis (Holliday, 1967). As in the present case, spontaneous and induced mitotic crossing-over are increased when the marker uvs1 is present in a homozygous condition in diploids, but meiotic crossing-over appears to remain unaffected. He suggests that the observed effect of this gene might be due to excessive exonuclease activity. This, he argues, might lead to terminal deletions which, in a diploid, might be expressed as "homozygosis" of terminal markers due to the uncovering of recessive genes. This type of result might follow if a repair synthesis enzyme was inactive, or if there was enhanced degradation of the DNA. Holliday prefers the latter since the mutant strain appears to be capable to digest DNA incorporated into the growth medium.

In our experiments with uvs1 of Aspergillus a largernumber of twin spots were observed than could be explained by the occurrence of two concurrent, but independent, events. Thus it is condluded that some of these cases are due to a single mitotic exchange. In addition

it appears that mitotic and meiotic recombination might be due to different mechanisms, since meiotic recombination was within the normal range for non-UV sensitive crosses.

At present these preliminary experiments suggest that the most feasible step that might be affected in this mutant of the proposed four step mechanism (excision, degradation, repair replication, and joining of phosphodiester bond) is the degradation step. However, the enhanced degradation must lead to recombinational events, and not just deletions, as Holliday proposes for his mutant. However, it is obvious that further study of this mutant is needed, both through a more detailed analysis of the types of events induced by this gene in homozygous UV sensitive diploid strains, and through an enzymatic analysis of the haploid strain.

Conclusions and Summary

NTG and FUDR have been used to induce colour sectors resulting from mitotic segregation in four different heterozygous diploid strains of Aspergillus nidulans. All diploids were marked in repulsion with visible markers of linkage group VIII. This permitted the detection of paired reciprocal colour sectors (twin spots) which provide conclusive evidence for the induction of reciprocal mitotic crossing-over.

Results from the fawn/chartreuse diploid, which had the advantage of few additional biochemical markers to reduce the viability of the products, indicate that the frequency of the various types of colour sectors is increased considerably by NTG or FUDR. Data from better marked coupling and repulsion diploids gave insight into the coincidence and distribution of events along one chromosome arm. It was concluded that NTG had a somewhat greater recombinogenic effect than FUDR at the expense of the concurrent induction of a larger number of abnormal colonies. The majority of coloured sectors induced by both agents appeared to be due to single mitotic cross-over events. Several cases of multiple events were observed as well. All of these could be explained by multiple crossing-over, and were found no more frequently than was expected from the coincidence of two single independent events.

From this work, and from related work in this and other organisms, it seems likely that NTG and FUDR induce recombination by producing a type of damage in the DNA which can be repaired by a system of repair

enzymes, some of which have been shown to be active in recombination.

Two UV sensitive mutants have been isolated, but testing showed they were mutants at the same locus. The effect of these mutants on mitotic and meiotic recombination was studied. In diploid strains homozygous for uvs1, both the spontaneous and induced frequency of coloured sectors resulting from somatic segregation are increased. Since twin spots could be identified with high frequency, it was concluded that reciprocal mitotic crossing-over was increased in the presence of this gene. In meiotic crosses homozygous for these mutants, no effect on recombination could be observed. From these preliminary results, uvs1 is best assumed to cause excess exonuclease activity, however additional experiments are needed to test this hypothesis.

References

Apiron, D. 1965 The two way selection of mutants and revertants in respect of acetate utilization and resistance to fluoro-acetate in Aspergillus nidulans. Genet. Res. 6: 317-329.

Barratt, R.W., G.B. Johnson, and W.W. Ogata. 1965 Wild-type and mutant stocks of Aspergillus nidulans. Genetics 52: 233-246.

Beccari, E., P. Modigliani, and G. Morpurgo. 1967 Induction of inter- and intragenic mitotic recombination by fluorodeoxyuridine and fluorouracil in Aspergillus nidulans. Genetics 56: 7-12.

Beukers, R., and W. Berends. 1960 Isolation and identification of the irradiation product of thymine. Biochim. Biophys.Acta 41: 550-551.

——, J. Ijlstra, and W. Berends. 1960 The effect of ultraviolet light on some compounds of nucleic acids, VI. The origin of the ultraviolet sensitivity of deoxyribonucleic acid. Rec. Trav. Chim. 79: 101.

Bollum, F.J., and R.B. Setlow. 1963 Ultraviolet inactivation of DNA primer activity, I. Effect of different wavelengths and doses. Biochim. Biophys. Acta 68: 599-607.

Boyce, R.P., and P. Howard-Flanders. 1964a Release of ultraviolet-induced thymine dimers from DNA in E. coli K-12. Proc. Nat. Acad. Sci. 51: 293-300.

——, and P. Howard-Flanders. 1964b Genetic control of DNA breakdown and repair in E. coli K-12 treated with Mitomycin C or ultraviolet light. Z. Vererbungslehre 95: 345-350.

Bridges, B.A., and R.J. Munson. 1966 Excision-repair of DNA damage in an auxotrophic strain of E. coli. Biochim. Biophys. Res. Commun. 22: 268-273.

Cerdà-Olmeda, E., and P.C. Hanawalt. 1967 Repair of DNA damaged by N-methyl-N'-nitro-N-nitrosoguanidine in E. coli. Mutation Res. 4: 369-371.

Clark, A.J., M. Chamberlin, R.P. Boyce, and P. Howard-Flanders. 1966 Abnormal metabolic response to ultraviolet light of a recombination deficient mutant of Escherichia coli K-12. J. Molec. Biol. 19: 442-454.

——, and A.D. Margulies. 1965 Isolation and characterization of recombination-deficient mutants in E. coli K-12. Proc. Nat. Acad. Sci. 53: 451-458.

Clutterbuck, A.J., and U.K. Sinha. 1966 N-methyl-N'-nitro-N-nitrosoguanidine (NTG) as a mutagen for Aspergillus nidulans. Aspergillus News Letter 7: 12-13.

Cohen, S.S., J.G. Flaks, H.D. Barner, M.R. Loeb, and J. Lichtenstein. 1958 The mode of action of 5-fluorouracil and its derivatives. Proc. Nat. Acad. Sci. 44: 1004-1012.

Chang, L., and R.W. Tuveson. 1967 Ultraviolet sensitive mutants in Neurospora crassa. Genetics 56: 801-810.

Deering, R.A. 1962 Ultraviolet irradiation and nucleic acid. Scientific American 207: 135-144.(Dec.)

Dorn, G.L. 1965 Genetic analysis of the phosphatases in Aspergillus nidulans. Genet. Res. 6: 13-26.

Dorn, G.L. 1967 A revised map of the eight linkage groups of Aspergillus nidulans. Genetics 56: 619-631.

Elder, B.L., and R.F. Beers. 1965 Nonphotoreactivating repair of UV-damaged transforming DNA by Micrococcus lysodeikticus extracts. J. Bacteriol. 90: 681-686.

Ellison, S.A., R.R. Feiner, and R.E. Hill. 1960 A host effect on bacteriophage survival after ultraviolet irradiation. Virology 11: 294-296.

Esposito, R.E., and R. Holliday. 1964 The effect of 5-fluorodeoxyuridine on genetic replication and mitotic crossing-over in synchronized cultures of Ustilago maydis. Genetics 50: 1009-1017.

Feiner, R.R. 1967 Ultraviolet radiation-sensitive mutants of Micrococcus lysodeikticus. J. Bacteriol. 94: 1270-1271.

Flaks, J.G., and S.S. Cohen. 1959 Virus-induced acquisition of metabolic function. III Formation and some properties of thymidylate synthetase of bacteriophage-infected Escherichia coli. J. Biol. Chem. 234: 2981-2987.

Fratelloy, B., G. Morpurgo, and G. Sermonti. 1960 Induced somatic segregation in Aspergillus nidulans. Genetics 45: 785-800.

Gallant, J., and T. Spottswood. 1965 The recombinogenic effect of thymidylate starvation in E. coli merodiploids. Genetics 52: 107-118.

Garen, A., and N. Zinder. 1955 Radiological evidence for partial genetic homology between bacteriophage and host bacteria. Virology 1: 347-376.

Haefner, K. 1966 Zum mechanismus der Entstehung auxotrophenzellen in heterozygoten Saccharomyces-Stammen nach UV- und Röntgenbestrahlung. Z. Vererbungslehre 98: 82-90.

——, and L. Howry. 1967 Gene-controlled UV sensitivity in Schizosaccharomyces pombe. Mutation Res. 4: 219-221.

Hanawalt, P.C., and R.H. Hayes. 1965 Repair replication of DNA in bacteria: Irrelevance of chemical nature of base defect. Biochem. Biophys. Res. Commun. 19: 462-467.

——, and R.H. Hayes. 1967 The repair of DNA. Scientific American 216 (Feb): 36-43.

Harm, W. 1963 On the relationship between host-cell reactivation and UV-reactivation in UV-inactivated phages. Z. Vererbungslehre 94: 67-79.

Haynes, R.H., M.H. Patrick, and J.E. Baptist. 1964. Comparative response of radioresistant and radiosensitive strains of E. coli to various inactivating agents. Radiation Res. 22: 194.

Hemmons, L.M., G. Pontecorvo, and A.W.J. Bufton. 1953 Perithecium analysis and relative heterothallism. Genet. Res. 5: 194-201.

Hill, R.F. 1958 A radiation-sensitive mutant of Escherichia coli. Biochim. Biophys. Acta. 30: 636-637.

——, and R.R. Feiner. 1964 Further studies of ultraviolet sensitive strains of Escherichia coli strain B. J. Gen. Microbiol. 35: 105-114.

——, and E. Simson. 1961 A study of radiosensitive and radioresistant mutants of E. coli strain B. J. Gen. Microbiol. 24: 1-14.

Holliday, R. 1961 Induced mitotic crossing-over in Ustilago maydis. Genet. Res. 2: 231-248.

——. 1962 Effect of photoreactivation on ultraviolet-induced segregation of heterozygous diploids. Nature 193: 95-96.

——. 1964 The induction of mitotic recombination by Mitomycin C in Ustilago and Saccharomyces. Genetics 50: 323-335.

——. 1965a Radiation sensitive mutants of Ustilago maydis. Mutation Res. 2: 557-559.

——. 1965b Induced mitotic crossing-over in relation to genetic replication in synchronously dividing cells of Ustilago maydis. Genet. Res. 6: 104-120.

——. 1966 Studies on mitotic gene conversion in Ustilago maydis. Genet. Res. 8: 323-337.

——. 1967 Altered recombination frequencies in radiation sensitive strains of Ustilago. Mutation Res. 4: 275-288.

Holloway, B.W. 1966 Radiation-sensitive mutants of Pseudomonas aeruginosa with reduced host-cell reactivation of bacteriophages. Mutation Res. 3: 167-171.

Howard-Flanders, P. 1964 Molecular mechanisms in the repair of irradiated DNA. Japanese J. Genet. (Suppl.) 40: 256-263.

——, and R.P. Boyce. 1964 A biochemical pathway in the repair of DNA after UV-irradiation that may have steps in common with those of genetic recombination by breakage-reunion. Genetics 50: 256-257.

Howard-Flanders, P., R.P. Boyce, E. Simson, and L. Theriot. 1962 A genetic locus in E. coli K-12 that controls the reactivation of UV-photoproducts associated with thymine in DNA. Proc. Nat. Acad. Sci. 48: 2109-2115.

——, R.P. Boyce, and L. Theriot. 1966 Three loci in E. coli K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics 53: 1119-1136.

——, and L. Theriot. 1962 A method for selecting radiation-sensitive mutants of E. coli. Genetics 47: 1219-1224.

——, and L. Theriot. 1966 Mutants of E. coli K-12 defective in DNA repair and in genetic recombination. Genetics 53: 1137-1150.

——, L. Theriot, and J.B Stedeford. 1966 A second type of recombination deficient mutant of E. coli K-12. Biophys. Soc. Abstracts in Biophys. J. 6:(Suppl.): 69.

Hurst, D.D., and S. Fogel. 1964 Mitotic recombination and hetero-allelic repair in Saccharomyces cerevissiae. Genetics 50: 435-458.

Iijima, T., and A. Hagiwara. 1960 Mutagenic action of Mitomycin C on Escherichia coli. Nature 185: 395-396.

James, A.P. 1954 Relative frequencies of sectored and non-sectored mutant colonies in yeast as a function of ultraviolet dose. J. Bacteriol. 67: 237-242.

——. 1955 A genetic analysis of sectoring in ultraviolet induced variant colonies of yeast. Genetics 40: 204-213.

——, and B. Lee-Whiting. 1955 Radiation-induced genetic segregations in vegative cells of diploid yeast. Genetics 40: 826-831.

Jansen, G.J.O. 1966 UV-induced mitotic recombination in the *paba1* cistron of *Aspergillus nidulans*. Genetica 35: 127-131.

——. 1967 Some properties of the UVS_1 mutant of *Aspergillus nidulans*. Aspergillus News Letter 8: 20-21.

Käfer, E. 1958 An 8-chromosome map of *Aspergillus nidulans*. Adv. Genet. 9: 105-145.

——. 1960 High frequency of spontaneous and induced somatic segregation in *Aspergillus nidulans*. Nature 186: 619-620.

——. 1961 The process of spontaneous recombination in vegetative nuclei of *Aspergillus nidulans*. Genetics 46: 1581-1609.

——. 1963 Radiation effects and mitotic recombination in diploids of *Aspergillus nidulans*. Genetics 48: 27-45.

——. 1965 Origins of translocations in *Aspergillus nidulans*. Genetics 52: 217-232.

——. 1968 Repair sectoring due to spontaneous mitotic recombination after UV treatment of diploid conidia in *Aspergillus*. In preparation.

——, and A. Chen. 1964 UV-induced mutations and mitotic crossing-over in dormant and germinating conidia of *Aspergillus nidulans*. Microbiol. Genet. Bull. 20: 8-9.

Kelner, A. 1951 Revival by light. Scientific American 184 (May): 22-26.

Kohn, K.W., N.H. Steigbigel, and C.L. Spears. 1965 Cross-linking and repair of DNA in sensitive and resistant strains of *E. coli* treated with nitrogen mustard. Proc. Nat. Acad. Sci. 53: 1154-1161.

Kellenberger, G., M.L. Zichichi, and J.J. Weigle. 1961 Exchange of DNA in the recombination of bacteriophage λ. Proc. Nat. Acad. Sci. 47: 869-878.

Lanier, W.B., and R.W. Tuveson. 1965 A locus for nitrous acid-sensitivity in Aspergillus nidulans showing cross sensitivity to ultraviolet light. Genetics 52: 454.

——, R.W. Tuveson, and J.E. Lennox. 1968 A radiation-sensitive mutant of Aspergillus nidulans. Mutation Res. 5: 23-31.

Lawley, P.D., and P. Brookes. 1965 Molecular mechanism of the cytotoxic action of difunctional alkylating agents and of resistance to this action. Nature 206: 480-483.

Lederberg, J., and E.M. Lederberg. 1952 Replica plating and indirect selection of bacterial mutants. J. Bacteriol. 63: 399-406.

Lennox, J.E., and R.W. Tuveson. 1967 The isolation of ultraviolet sensitive mutants from Aspergillus rugulosus. Radiation Res. 31: 382-388.

Lhoas, P. 1961 Mitotic haploidisation by treatment of Aspergillus niger diploids with para-fluorophenylalanine. Nature 190: 744.

——. 1967 Genetic analysis by means of the parasexual cycle in Aspergillus niger. Genet. Res. 10: 45-61.

Mackintosh, M.E., and R.H. Pritchard. 1963 The production and replicating of micro-colonies of Aspergillus nidulans. Genet. Res. 4: 320-322.

Mahler, I. 1965 Characteristics of a ultraviolet irradiation sensitive strain of Bacillus subtilis. Biochem. Biophys. Res. Commun. 21: 384-391.

Marmur, J., and L. Grossman. 1961 Ultraviolet light induced linking of deoxyribonucleic acid strands and its reversal by photo-reactivating enzyme. Proc. Nat. Acad. Sci. 47: 778-787.

Matsumoto, I., and K.G. Lark. 1964 Altered DNA isolated from cells treated with Mitomycin C. Experimental Cell Res. 32: 192-198.

Mattern. I.E., M.P. van Winden, and A. Rörsch. 1965 The range of action of genes controlling radiation sensitivity in Escherichia coli. Mutation Res. 2: 111-131.

McCully, K.S., and E. Forbes. 1965 The use of p-fluorophenylalanine with "master strains" of Aspergillus nidulans for assigning genes to linkage groups. Genet. Res. 6: 352-359.

Mead, C.G. 1964 The enzymic condensation of oligodeoxyribonucleotides with polydeoxyribonucleotides. Proc. Nat. Acad. Sci. 52: 1482-1488.

Meselson, M. 1964 On the mechanism of genetic recombination between DNA molecules. J. Molec. Biol. 9: 734-745.

——, and J.J. Weigle. 1961 Chromosome breakage accompanying genetic recombination in bacteriophage. Proc. Nat. Acad. Sci. 47: 857-868.

Mishra, A.K., and P. Nandi. 1962 Modifying effects of environmental factors on the survival and mutation frequencies of UV-irradiated Aspergillus niger. Transactions of the Bose Research Institute 25: 89-108.

Morpurgo, G. 1961 Somatic segregation induced by p-fluorophenylalanine. Aspergillus News Letter 2: 10.

——. 1962 Increased frequency of somatic crossing-over by x-rays in Aspergillus nidulans. Microbiol. Genet. Bull. 18: 18.

——. 1963 Induction of mitotic crossing-over in Aspergillus nidulans by bi-functional alkylating agents. Genetics 48: 1259-1263.

Nakai, S., and S. Matsumoto. 1967 Two types of radiation sensitive mutants in yeast. Mutation Res. 4: 129-136.

Okubo, S., and W.R. Romig. 1965 Comparison of UV sensitivity of Bacillus subtilis bacteriophage SP02 and its infectious DNA. J. Molec. Biol. 14: 130-142.

——, and W.R. Romig. 1966 Impaired transformability of Bacillus subtilis mutant sensitive to Mitomycin C and ultraviolet radiation. J. Molec. Biol. 15: 440-454.

Otsuji, N. 1968 Properties of Mitomycin C-sensitive mutants of E. coli K-12. J. Bacteriol. 95: 540-545.

Papirmeister, B., and C.L. Davidson. 1964 Elimination of sulfur mustard-induced products from DNA of E. coli. Biochem. Biophys. Res. Commun. 17: 608-617.

Pauling, C., and P. Hanawalt. 1965 Nonconservative DNA replication in bacteria after thymine starvation. Proc. Nat. Acad. Sci. 54: 1728-1735.

Pettijohn, D., and P. Hanawalt. 1964 Evidence for repair-replication of ultraviolet damaged DNA in bacteria. J. Molec. Biol. 9: 395-410.

Pontecorvo, G., and E. Käfer. 1958 Genetic analysis based on mitotic recombination. Adv. Genet. 9: 71-104.

——, and J.A. Roper. 1953 Diploids and mitotic crossing-over. Adv. Genet. 5: 218-225.

——, J.A. Roper, L.M. Hemmons, K.D. MacDonald, and A.W.J. Bufton. 1953 The genetics of Aspergillus nidulans. Adv. Genet. 5: 141-238.

——, E. Tarr Gloor, and E. Forbes. 1954 Analysis of mitotic recombination in Aspergillus nidulans. J. Genet. 52: 226-237.

Putrament, A. 1967 On the mechanism of mitotic recombination in Aspergillus nidulans. Molec. Gen. Genetics 100: 307-320.

Reitter, H., and B. Strauss. 1965 Repair of damage induced by a monofunctional alkylating agent in a transformable UV-sensitive strain of Bacillus subtilis. J. Molec. Biol. 14: 179-194.

Richardson, C.C., I.R. Lehman, and A. Kornberg. 1964 A deoxyribonucleic acid phosphatase-exonuclease from Escherichia coli. J. Biol. Chem. 239: 251-258.

Rolfe, R. 1966 Inhibition of thymineless death in Bacillus subtilis by Actinomycin D. Biophys. Soc. Abstracts in Biophys. J. 6 (Suppl.): 61.

Roman, H., and F. Jacob. 1958 A comparison of spontaneous and UV-induced allelic recombination with reference to recombination of outside markers. Cold Spring Harbour Symp. Quant. Biol. 23: 155-160.

Roper, J.A. 1952 Production of heterozygous diploids in filamentous fungi. Experimentia 8: 14-15.

——, and E. Käfer. 1957 Acriflavine-resistant mutants of Aspergillus nidulans. J. Gen. Microbiol. 16: 660-667.

——, and R.H. Pritchard. 1955 Recovery of complementary products of mitotic crossing-over. Nature 175: 639.

Rörsch, A.C., Ca. van der Kamp, and J. Adema. 1964. Dark reactivation of ultraviolet irradiated bacteriophage DNA in vitro. Biochim. Biophys. Acta. 80: 346-348.

Rupert, C.S. 1960 Photoreactivation of transforming DNA by an enzyme from Bakers' yeast. J. Gen. Physiol. 43: 573-595.

Rupert, C.S., S.H. Goodgal, and R.M. Herriot. 1958 Photoreactivation in vitro of ultraviolet inactivated Hemophilus influenzae transforming factor. J. Gen. Physiol. 41: 451-467.

Sauerbier, W. 1961 The influence of 5-bromodeoxyuridine substitution on UV sensitivity, host-cell reactivation and photoreactivation in T-1 and P22H5. Virology 15: 465-472.

——. 1962 The bacterial mechanism reactivating UV-irradiated phage in the dark. Z Vererbungslehre 93: 220-228.

Searashi, T., and B. Strauss. 1965 Relation of the repair of damage induced by a monofunctional alkylating agent to the repair of damage induced by ultraviolet light in Bacillus subtilis. Biochem. Biophys. Res. Commun. 20: 680-687.

Setlow, J.K., D.C. Brown, M.E. Boling, A. Mattingly, and M.P. Gordon. 1967 Repair of DNA in Hemophilus influenzae: I. X-ray sensitivity of UV-sensitive mutants and their behavior as hosts to UV-irradiated bacteriophage and transforming DNA. J. Bacteriol. 95: 546-558.

Setlow, R.B. 1966 Cyclobutane-type pyrimidine dimers in polynucleotides. Science 153: 379-386.

——, and W.L. Carrier. 1964 The disappearance of thymine dimers from DNA: An error correcting mechanism. Proc. Nat. Acad. Sci. 51: 226-231.

——, and J.K. Setlow. 1962 Evidence that ultraviolet-induced thymine dimers in DNA caused biological damage. Proc. Nat. Acad. Sci. 48: 1250-1257.

——, and P.A. Swenson, and W.L. Carrier. 1963 Thymine dimers and inhibition of DNA synthesis by ultraviolet irradiation of cells. Science 142: 1464-1466.

Shaw, M., and M.M. Cohen. 1965 Chromosome exchanges in human leucocytes induced by Mitomycin C. Genetics 51: 181-190.

Sherman, F., and H. Roman. 1963 Evidence for two types of allelic recombination in yeast. Genetics 48: 255-261.

Shiba, S.A., T. Tirawaki, T. Taguchi, and J. Kawamata. 1959. Selective inhibition of formation of DNA in E. coli by Mitomycin C. Nature 183: 1056-1057.

Siddiqi, O.H. 1962 Mutagenic action of nitrous acid on Aspergillus nidulans. Genet.Res. 3: 303-314.

Snow, R. 1967 Mutants in yeast sensitive to ultraviolet light. J. Bacteriol. 94: 571-592.

Stahl, F.W., J.M. Crasemann, L. Okun, E. Fox, and C. Laird. 1961 Radiation-sensitivity of bacteriophage containing 5-bromodeoxyuridine. Virology 13: 98-104.

Stern, C. 1936 Somatic crossing-over and segregation in Drosophila melanogaster. Genetics 21: 623-630.

Strauss, B., T. Searashi, and M. Robbins. 1966 Repair of DNA studied with a nuclease specific for UV-induced lesions. Proc. Nat. Acad. Sci. 56: 932-939.

Szybalski, W., and N.V. Iyer. 1964 Cross-linking of DNA by enzymatically or chemically activating mitomycins and porfiromycins, bifunctionally "alkylating" antibiotics. Federation Proc. 23: 946-957.

Taylor, J.H., W.F. Haut, and J. Tung. 1961 Effects of fluorodeoxyuridine on DNA replication, chromosome breakage and reunion. Proc. Nat. Acad. Sci. 48: 190-198.

Tector, A., and E. Käfer. 1962 Radiation-induced chromosomal aberrations and lethal sectoring in Aspergillus nidulans. Science 136: 1056-1057.

Tuveson, R.W., and J.E. Lennox. 1968 A UV-sensitive mutant of Aspergillus rugulosus unrelated to intergenic crossing-over. Can. J. Genet. Cytol. 10: 50-53.

Wacker, A. 1963 Molecular mechanisms of radiation effects. Progress in Nucleic Acid Research 1: 369-399.

van de Putte, P., and A. Rörsch. 1966 Radiation sensitive mutants of E. coli defective in genetic recombination. Information Exchange Group 7 no. 443.

——, C.A. van Sluis, J. van Dillewijn, and A. Rörsch. 1965 The location of genes controlling radiation sensitivity in Escherichia coli. Mutation Res. 2: 97-110.

——, H. Zwenk, and A. Rörsch. 1966 Properties of four mutants of E. coli defective in genetic recombination. Mutation Res. 3: 381-392.

Varghese, A.J., and S.Y. Wang. 1967 Ultraviolet irradiation of DNA in vitro and in vivo produces a third thymine derived product. Science 156: 955-957.

Weijer, J., and S.H. Weisberg. 1966 Karyokinesis of the somatic nucleus of Aspergillus nidulans I. The juvenile chromosome cycle (Feulgen staining). Can. J. Genet. Cytol. 8: 361-374.

Weiss, B., and C.C. Richardson. 1967 Enzymatic breaking and rejoining of DNA I: Repair of single-stranded breaks in DNA by an enzyme system from E. coli infected with T_4 bacteriophage. Proc. Nat. Acad. Sci. 57: 1021-1028.

Wing, J.P., M. Levine, and H.O. Smith. 1968 Recombination-deficient mutant of Salmonella typhimurium. J. Bacteriol. 95: 1828-1834.

Witkin, E. 1946 Inherited differences in sensitivity to radiation in Escherichia coli. Proc. Nat. Acad. Sci. 32: 59-68.

——. 1947 Genetics of resistance to radiation in E. coli. Genetics 32: 221-248.

Wood, S. 1967 UV-induced mitotic crossing-over in Aspergillus nidulans. M.Sc. thesis, McGill University, Montreal.

——, and E. Käfer. 1967 Twin spots as evidence for mitotic crossing-over in Aspergillus induced by ultraviolet light. Nature 216: 63-64.

Wulff, D.L., and C.S. Rupert. 1962 Disappearance of thymine photodimer in ultraviolet-irradiated DNA upon treatment with a photoreactivating enzyme from Bakers' Yeast. Biochem. Biophys. Res. Commun. 7: 237-240.

Zimmerman, F.K., R. Schwaier, and U. von Laer. 1966. Mitotic recombination induced in Saccharomyces cerevissiae with nitrous acid, diethylsulfate, and carcinogenic, alkylating amines. Z. Vererbungslehre 98: 230-246.

——, and U. von Laer. 1967 Induction of mitotic recombination with ethyleneimine in Saccharomyces cerevissiae. Mutation Res. 4: 377-379.