the nucleic acids they surround are valuable real estate for factor function. It seems likely that access of factors to these surfaces and the dynamics of their association and exchange are subject to active regulation. Understanding the interplay between RNAP-bound factors and their mechanisms of action once bound will be required for a complete view of the transcription process in vivo.

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Gimme Phospho-Serine Five! Capping Enzyme Guanylyltransferase Recognition of the RNA Polymerase II CTD

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In this issue of *Molecular Cell*, two papers on the structure of murine capping enzyme guanylyltransferase (Ghosh et al., 2011) and yeast studies of the recognition of the RNA polymerase II CTD (Schwer and Shuman, 2011) describe the mechanism of recruitment of the capping apparatus to nascent pre-mRNAs.

Capping of eukaryotic pre-mRNAs proceeds via a sequential three-step process, involving (1) removal of the 5′ γ-phosphate group by an RNA triphosphatase (RTase), (2) addition of GMP to the 5′ β-phosphate by a GTP-dependent guanylyltransferase (GTase), and (3) addition of a methyl group at position 7 of the 5′ guanine (derived from GMP) by a methyltransferase, yielding the 7-methyl-G(5′)ppp(5′)N cap structure (where N is any nucleotide). GTases are conserved among taxa and targeted to nascent pre-mRNAs via interactions with the serine-phosphorylated repeated heptapeptide $Y_1S_2P_3T_4S_5P_6S_7$ that comprises the C-terminal domain (CTD) of the large subunit of RNA polymerase II (reviewed in Ghosh and Lima, 2010). Earlier work in *Saccharomyces cerevisiae* established that CTD recognition by GTase requires phosphorylation of Ser5 (West and Corden, 1995). Two papers published in this issue of *Molecular Cell* demonstrate how GTase recognition of the CTD depends on the presence of $Ser5_P$ (where $_P$ denotes phosphorylation). Lima and coworkers determined the structure of a CTD phospho-peptide bound to murine GTase (Ghosh et al., 2011), thereby revealing the molecular mechanism responsible for recognition. Intimate contacts with $Ser5_P$ support enzyme recruitment and are essential for maximal enzyme function in vivo in a *S. cerevisiae* complementation model. Schwer and Shuman (2011) describe experiments aimed at discerning a "CTD code" in *Schizosaccharomyces pombe*, documenting that the requirement for Ser_5 phosphorylation can be bypassed by delivering the capping enzyme to the transcription elongation complex by an alternative means.

X-ray crystallographic studies of murine GTase bound to a CTD phospho-peptide (Ghosh et al., 2011) (PDB code: 3RTX) and parallel work on the apo form of human GTase (Chu et al., 2011) (3S24) bring important new insights to pre-mRNA capping. Both enzymes are structurally similar to their *Candida albicans*

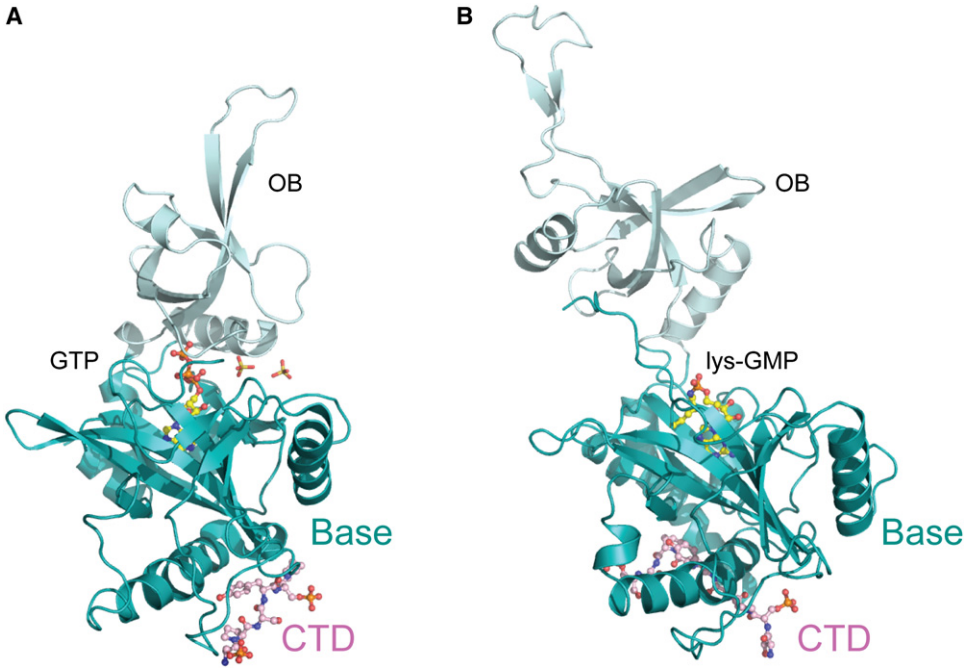

Figure 1. Phospho-CTD Recognition by Mammalian and Fungal Capping Enzyme Guanylyltransferases
(A) Model of the full-length mammalian capping enzyme GTase interacting with GTP (stick figure), $S_{5P}P_6S_7Y'_1S'_{2P}P'_3$ (ball-and-stick figure), and two sulfate anions (stick figure), prepared by overlaying the murine and human GTase nucleotide transferase domains (rmsd = 1.2 Å for 255 common α-carbon atomic pairs) with their respective bound oligopeptide and bound sulfate ions and docking GTP into the resulting composite structure in the location of the nucleotide binding site identified in the structure of PBCV1 capping enzyme reported by Håkansson et al. (1997). The OB domain was not visible in the murine structure.
(B) Structure of *Candida* capping enzyme GTase covalently linked to GMP and recognizing $T_4S_{5P}P_6S_7Y'_1S'_{2P}P'_3T'_4S'_{5P}$ (Fabrega et al., 2003). Both protein structures are presented as color-coded ribbon representations (NTase domain, blue; OB fold domain, gray). Figure courtesy of C.D. Lima.

(Fabrega et al., 2003) (1P16) and *S. cerevisiae* (Gu et al., 2010) (3KYH) counterparts, consisting of an N-terminal nucleotide transferase domain (NTase, with Hinge and Base subdomains) and a C-terminal oligonucleotide/oligosaccharide binding or OB fold domain that forms a lid over the active site (compare Figures 1A [mammalian] and 1B [*Candida*]). For known GTases, pairwise amino acid sequence identities range from ~95% (murine versus human) to 30%–35% for mammalian versus fungal and 35%–40% among the fungi. Human GTase crystallized with seven independent copies of the enzyme in the asymmetric unit, thereby revealing considerable variation in the hinge bend angle between the N-terminal and OB domains. The positioning of the OB domain relative to the NTase domain observed across seven copies of the human enzyme may recapitulate interdomain movements essential for single-stranded RNA binding and capping. Presumably, this enzyme utilizes an induced fit mechanism during catalysis, as suggested by substantial interdomain movements seen in X-ray structures of the *Paramecium bursaria chlorella* virus 1 (PBCV1) capping enzyme (Håkansson et al., 1997). Although neither mammalian GTase was crystallized with GTP or a nascent RNA substrate, a line of bound sulfate ions in the human enzyme structure marks a basic groove in which the RNA chain almost certainly lies during catalysis.

As with each previously determined structure of a protein bound to a CTD peptide (Ghosh and Lima, 2010), murine GTase demonstrates a distinct molecular mechanism of CTD recognition. Cocrystallization of the enzyme with an 18 amino acid CTD phospho-peptide revealed intimate interactions of the base subdomain with an extended β-conformation of $S_{5P}P_6S_7Y'_1S'_{2P}P'_3$ (where ′ denotes a successive heptapeptide repeat). Together, $Ser5_P$ and Tyr′1 account for most contacts between the enzyme and the CTD, which involve residues conserved among metazoans. Such conservation does not extend to fungi, and the structure of the *Candida* enzyme bound to a similar CTD phospho-peptide shows interactions with an entirely different portion of the Base subdomain (compare Figures 1A [mammalian] and 1B [*Candida*]). The surface features responsible for CTD binding by *Candida* GTase are, however, conserved among GTases from *S. cerevisiae* and *S. pombe* (Schwer and Shuman, 2011).

Notwithstanding the distinct molecular mechanisms underpinning CTD recognition in fungi and metazoans, both the murine and the *Candida* GTases appear to rely almost exclusively on contacts with $Ser5_P$ and Tyr1. We are therefore presented with an intriguing combination of divergent and convergent evolution. The cellular GTases comprise a family of essential eukaryotic enzymes that diverged from a common ancestor and catalyze the same reaction on the same substrate. Yet the molecular mechanisms by which fungal and metazoan members of this family are recruited to nascent

pre-mRNAs by the CTD appear to be the product of convergent evolution.

The importance of interactions between capping enzyme GTases and $Ser5_P$ of the RNA polymerase II CTD in vivo is bolstered by the results of yeast genetics experiments. Schwer and Shuman (2011) engineered pan Ser5 → Ala mutations into the CTD of *S. pombe* and showed that the presence of Ser5 is essential for vegetative growth. The deleterious effect of the pan Ser5 → Ala substitution can be overcome by covalently tethering the capping enzyme to the CTD directly, thereby documenting that capping enzyme recruitment is a chief function of CTD Ser5 in vivo in fission yeast. In parallel, Ghosh et al. (2011) exploited murine GTase substitution for the essential *S. cerevisiae* GTase Ceg1 (Yue et al., 1997) to verify the importance of crystallographically observed interactions between the mammalian enzyme and the CTD of the budding yeast. After performing detailed biophysical evaluations of various single and multiple alanine point mutations in murine GTase that confirmed disruption of side chain-$Ser5_P$ contacts (as judged by reduced binding affinity for CTD phospho-peptides and decreased stimulation of guanylylation in vitro), Ghosh et al. (2011) demonstrated impaired growth of the corresponding *S. cerevisiae* strains in vivo.

While the important role played by phosphorylation of Ser5 within the CTD has been elucidated with this elegant combination of X-ray crystallography, biophysical studies, and yeast genetics experiments coming from three research teams, we lack detailed knowledge of the molecular mechanisms underpinning catalysis and enzyme regulation in higher eukaryotes. Further crystallographic studies of yeast and/or metazoan GTases bound to GTP and substrate both with and without bound CTD would be highly desirable. Tackling the issue of CTD stimulation of mammalian enzyme activity may prove somewhat more challenging. In the murine GTase-CTD cocrystal structure, the CTD phospho-peptide does not interact directly with the enzyme active site, and we are limited at present to a "Rube Goldberg"-type explanation for the phenomenon. Appeal to additional complementary research tools will almost certainly be required in the push to a more detailed mechanistic explanation. Nuclear magnetic resonance spectroscopy studies of protein motion and computational molecular dynamics simulations both come to mind as potentially useful avenues of attack. Given the importance of pre-mRNA capping, work along these lines would appear well worth the effort.

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