THE MOLECULAR AND CELLULAR BIOLOGY OF THE YEAST *SACCHAROMYCES*

GENE EXPRESSION

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Paperback Front Cover: Immunolocalization of α-factor pheromone receptors to the region of morphogenesis in α-factor-treated *MAT***a** *S. cerevisiae* cells. The cells were treated with 10^{-7} M α-factor for 90 min and then stained with anti-Ste2 antibodies to detect the α-factor receptors as described by Jackson et al. (*Cell 67:* 389 [1991]). The green color indicates Ste2 staining that was detected with FITC-conjugated goat anti-rabbit IgG. The cells were counterstained red with Evans Blue dye in order to visualize cell morphology. (Photograph courtesy of J. Konopka, State University of New York, Stony Brook.)

Paperback Back Cover: The **a**-pheromone receptor is synthesized and transported to the cell surface where it resides only transiently. Rapid, ligand-independent endocytosis leads to internalization of surface receptor and its subsequent delivery to the vacuole for *PEP4*-dependent degradation. Experimental support for the conclusion that the vacuole is the final destination for the receptor is shown in the three panels. *myc*-epitope-tagged receptor was expressed from the *GAL1* promoter in *MAT*α *pep4*Δ cells. After 4 hr, new receptor synthesis was halted by addition of glucose, and 30 min later, the cells were prepared for immunostaining. The position of the receptor as revealed by indirect immunofluorescence microscopy (*middle panel*) coincided with the position of the vacuole as revealed by Nomarski microscopy (*top panel*) or by indirect immunodetection of the vacuolar membrane protein, alkaline phosphatase (*bottom panel*). (Photograph courtesy of N. Davis and G.F. Sprague, Jr., University of Oregon, Eugene.)

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Preface

In this second volume of the three-volume set *The Molecular and Cellular Biology of the Yeast* Saccharomyces, the emphasis is on gene expression. A continuing theme of these monographs has been the extent to which basic biological processes have been conserved in the eukaryotic world during evolution. This conservation is particularly striking in those facets of yeast biology reviewed in this volume: the transcriptional machinery, tRNA processing, mRNA splicing, stress and chaperone proteins, ubiquitin-associated functions, the trimeric G-protein-based pheromone response pathway, and chromosomal imprinting. This is an impressive list. At the time of the original monograph, we did not even know that some of these processes occurred in yeast, and for most of them, we had no clue that the molecular underpinnings of these cellular phenomena would resemble so closely those found for similar processes in larger eukaryotes. Even more striking is the fact that these resemblances extend not merely to the molecular outline of the processes, but also to the molecules themselves: Many of the key players in these pathways or complexes show cross-complementation across biological kingdoms.

The circuitry involved in regulating metabolic pathways in yeast is, of course, that required for the yeast life style—hanging out on grapes probably requires a particular focus on regulating carbon and nitrogen metabolism and involves a feast or famine existence. The particular molecular mechanisms effecting this control, however, incorporate the same tried and true eukaryotic themes used elsewhere, namely, activator proteins, acidic blobs for activation domains, helix-loop-helix proteins, leucine

zippers, zinc fingers, and combinatorial regulation. And, of course, these regulatory proteins, here and elsewhere, retain the capacity to interact with the basic transcriptional apparatus of diverse eukaryotic cells.

We are no longer quite so surprised as we used to be to find that the time required from conception to completion of a volume or monograph greatly exceeds that planned. We are nevertheless grateful to a number of our author/colleagues for the forgiveness they have shown us and for the grace with which they have updated manuscripts that had lain far too long because they had met our early, too optimistic, deadlines. We are especially grateful to Alan Hinnebusch, Mark Johnston, and Marian Carlson, of whom we required a large effort twice over.

We thank Nancy Ford, Dorothy Brown, and Joan Ebert for their valuable assistance in editing the manuscripts and for making this publication a reality and Miriam Braunstein for compiling the index. We thank each of the authors of the papers in this volume. It is their efforts that have made these exciting research fields accessible to us all.

Elizabeth W. Jones
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Yeast Nuclear RNA Polymerases

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INTRODUCTION

All eukaryotes analyzed so far contain three distinct nuclear DNA-dependent RNA polymerases, each transcribing a different set of genes.

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In the past decade, studies with reconstituted RNA-polymerase-dependent systems, combined with genetic evidence obtained mainly in yeast, have established that RNA polymerase I (or A) synthesizes ribosomal RNA precursors, RNA polymerase II (or B) transcribes protein-encoding genes, and RNA polymerase III (or C) produces 5S rRNA and tRNAs. Most of the small nuclear RNAs (snRNAs) are transcribed by enzyme II or, in some cases, by enzyme III. Each enzyme is directed for promoter selection and correct initiation by a set of general and gene-specific transcription factors. The characterization of yeast RNA polymerases, transcription factors, and regulatory proteins has greatly contributed to our overall understanding of eukaryotic transcription.

In this chapter, we provide an overview of the molecular structure of the three yeast nuclear RNA polymerases as derived from biochemical and immunological studies, present recent progress made in characterizing the genes encoding the polymerase subunits, and discuss the specific in vitro transcription systems now available for all three yeast RNA polymerases, with special emphasis on the RNA polymerase I and III systems. The regulatory aspects of RNA-polymerase-II-dependent transcription are presented by Guarente (this volume).

II. MOLECULAR STRUCTURE OF NUCLEAR RNA POLYMERASES: AN OVERVIEW

A. Enzyme Purification

Yeast RNA polymerases are large multisubunit proteins ranging in size from 500 kD to 600 kD (Sentenac and Hall 1982; Sentenac 1985 and references therein). Their general physical properties and affinity for nucleic acids or other polyanionic polymers were exploited in conventional large-scale purification methods, usually including a sizing step. The three enzymes can be resolved on heparin-substituted matrices or on DEAE-Sephadex, which was first used to show the existence of multiple forms of RNA polymerase in eukaryotes (Roeder and Rutter 1969). The unusual adsorption behavior of RNA polymerase III on DEAE-Sephadex is probably related to its special aptitude for engaging in nonionic interactions (Gabrielsen et al. 1984). Purification methods that yield milligram amounts of pure enzyme are available for RNA polymerases I (Buhler et al. 1974; Huet et al. 1975; Valenzuela et al. 1976c), II (Dezélée et al. 1976), and III (Valenzuela et al. 1976b; Huet et al. 1985). More recently, affinity chromatography with monoclonal antibodies directed to the carboxy-terminal domain of the largest subunit has provided a new and powerful means to purify RNA polymerase II selective-

ly (Edwards et al. 1990). Together with the use of phosphatase and protease inhibitors, dissociation of the enzyme-antibody complexes under conditions maintaining the physical and functional integrity of the enzyme allowed the rapid purification of highly active RNA polymerase II with no detectable degradation of the largest polypeptide, thus solving a hitherto major problem in enzyme purification (see Sentenac 1985). Methods for small-scale purification of yeast RNA polymerases are also available (Sentenac and Hall 1982). Immunoadsorption with polyclonal subunit-directed antibodies has been used to purify enzymes I, II (Huet et al. 1975; Bréant et al. 1983b), and III (Huet et al. 1985). A more sophisticated approach made use of monoclonal antibodies directed to epitope-tagged enzyme II (Kolodziej et al. 1990). Combined with a mild elution procedure, this method should be useful for functional analysis of mutant RNA polymerases. Rapid and selective immunoprecipitation of enzymes I and II from crude or partially purified extracts, which yields essentially the intact forms of the enzymes, has been used to analyze the phosphorylation state of enzyme subunits (Bréant et al. 1983a; Kolodziej et al. 1990).

B. Specific Inhibitors

There are large differences in the sensitivities of the three RNA polymerases to inhibitors, the best known of which is the mycotoxin α-amanitin. Yeast enzymes II, I, and III require 1, 300, and >>1000 μg/ml, respectively, of α-amanitin for 50% inhibition. Yeast enzyme II is at least 100-fold less sensitive than the corresponding mammalian enzyme, whereas yeast enzyme III differs from the mammalian polymerase III in its resistance. α-amanitin blocks chain elongation by interfering with the translocation process, but it does not prevent the formation of the first phosphodiester bond (de Mercoyrol et al. 1989).

Tagetitoxin is a strong and fairly selective inhibitor of RNA polymerase III at a concentration of about 1 μM, which also inhibits vertebrate and insect pre-tRNA synthesis as well as chloroplast or bacterial transcription (Steinberg et al. 1990). Tagetitoxin's mode of action is unknown, but its structure (Mitchell et al. 1989) suggests that it functions as a nucleotide analog. Finally, the nucleotide analog 3′dATP differentially inhibits RNA polymerases by blocking chain elongation. Yeast enzymes II, III, and I require 0.3, 3, and >3 μM, respectively, of 3′dATP for 50% inhibition (Horowitz et al. 1976). This differential sensitivity is intriguing and could reflect differences in the proofreading aptitudes of the enzymes for removal of the chain terminator. For information on the effects of other inhibitors on the yeast enzymes, see Sentenac and Hall (1982).

Table 1 Subunits and genes of the three nuclear RNA polymerases of *S. cerevisiae*

RNA polymerase I (A)									
						Mutants			
Subunit	M_r[a]	Approximate stoichiometry	pI	Gene	Chromosome	null	ts or leaky	Homologs	Notable features
A190	186	1	7.3	*RPA190*	XV	lethal	yes	β′ (*E. coli*)	phosphorylated, zinc binding
A135	136	1	8.9	*RPA135*	XVI	lethal	yes	β (*E. coli*)	zinc binding
A49	46	1[b]	9.6 (9)	*RPA49*		condi-tional			RNase H activity?
A43	38	1[b]	7.4 (5.1)	*RPA43*	XV				phosphorylated
AC40	38	1	5.6 (5.5)	*RPC40*	XVI	lethal	yes	B44, α (*E. coli*)	
A34.5	27	1[b]	7.3 (7.1)	*RPA34*		viable			phosphorylated, basic C-end
ABC27	25	2	10.2 (9.2)	*RPB5*	II	lethal		archaebacteria, human	
ABC23	18	1	5.2 (4.5)	*RPB6/RPO26*	XVI	lethal			phosphorylated
AC19	16	1	4.5 (4.5)	*RPC19*	II	lethal		α (*E. coli*)?	phosphorylated
ABC14.5	17	1	4.2 (4.6)	*RPB8*	XV	lethal			
A14		1	(5.2)	not cloned					
A12.2	13.7	1	8.0 (6.2)	*RPA12/RRN4*		condi-tional			zinc binding
ABC10α	7.7	1	10.1	*RPC10*	VIII	lethal			zinc binding
ABC10β	8.3	1	8.1	*RPB10*	XV	lethal			zinc binding

RNA polymerase II (B)									
B220	190	1	5.3	*RPO21/RPB1*	IV	lethal	yes	β′ (*E. coli*)	zinc binding phosphorylated CTD
B150	139	1	6.3	*RPB2*	XV	lethal	yes	β (*E. coli*)	zinc binding
B44	38	2	4.3 (4.5)	*RPB3*	IX	lethal		AC40, α (*E. coli*), hRPBP33 (human)	
B32	25	1[b]	4.6 (4.9)	*RPB4*	X	condi- tional			
ABC27	25	2	10.2 (9.2)	*RPB5*	II	lethal		archaebacteria, hRPB23 (human)	
ABC23	18	1	5.2 (4.5)	*RPB6/RPO26*	XVI	lethal			phosphorylated
B16	18	1[b]	4.2	*RPB7*	XII	viable			
ABC14.5	17	1	4.2 (4.6)	*RPB8*	XV	lethal			
B12.6	14	1	9.6	*RPB9*	VII	condi- tional		*Drosophila*	zinc binding
B12.5				*RPB11*	XV	lethal			
ABC10α	7.7	1	10.1	*RPC10*	VIII	lethal			zinc binding
ABC10β	8.3	1	8.1	*RPB10*	XV	lethal			zinc binding
RNA polymerase III (C)									
C160	162	1	8.3	*RPC160/RPO31*	XV	lethal	yes	β′ (*E. coli*)	zinc-binding domain
C128	129	1	8.7	*RET1*	XV	lethal	yes	β (*E. coli*)	zinc-binding domain
C82	75	1[b]	9.0	*RPC82*	XVI	lethal			
C53	47	0.7[b]	9.2	*RPC53*	IV	lethal	yes	BN51 (human)	sensitive to proteolysis
AC40	38	1	5.6 (5.5)	*RPC40*	XVI	lethal	yes	B44, α (*E. coli*)	
C37		0.5[b]		not cloned					
C34	36	1[b]	5.5	*RPC34*	XIV	lethal	yes		

Continued on following page.

Table 1 (Continued)

RNA polymerase III (C)									
Subunit	M_r[a]	Approximate stoichiometry	pI	Gene	Chromosome	Mutants: null	Mutants: ts or leaky	Homologs	Notable features
C31	28	1	4.5	*RPC31*	XIV	lethal	yes		highly acidic tail
ABC27	25	2	10.2 (9.2)	*RPB5*	II	lethal		archaebacteria, human	
C25		0.4[b]		not cloned					
ABC23	18	1	5.2 (4.5)	*RPB6/RPO26*	XVI	lethal			phosphorylated
AC19	16	1	4.5 (4.5)	*RPC19*	II	lethal		α (*E. coli*)?	phosphorylated
ABC14.5	17	1	4.2 (4.6)	*RPB8*	XV	lethal			
C11		1		not cloned					
ABC10α	7.7	1	10.1	*RPC10*	VIII	lethal			zinc binding
ABC10β	8.3	1	8.1	*RPB10*	XV	lethal			zinc binding

Sources: *Gene cloning* (see Mosrin and Thuriaux 1990; Young 1991; Sentenac et al. 1992; and references therein); *RPA43* and *RPA34* (J.M. Buhler et al., unpubl.); *RPC82* (Chiannilkulchai et al. 1992); *RPC53* (Mann et al. 1992); *RPC34* (Stettler et al. 1992); *RPA12/RRN4* (Nogi et al. 1991a and in prep.). The predicted size and pI of ABC10β were calculated after correction of the published *RPB10* sequence (D. Lalo, unpubl.).

Stoichiometry: Hager et al. (1976, 1977); Huet et al. (1985); Kolodziej et al. (1990).

Isoelectric points: Calculated from the amino acid composition. The experimental values, when available (Buhler et al. 1976a), are given in parentheses.

Phosphorylation: Bell et al. (1976, 1977); Buhler et al. (1976b); Kolodziej et al. (1990).

Zinc binding: Treich et al. (1991); Werner et al. (1992).

[a] Molecular weight x 10^{-3} (calculated from the open reading frame). Correction of the published *RPB10* sequence (Woychik and Young 1990) expanded the size of the protein from 46 to 70 amino acids (D. Lalo, unpubl.).

[b] Denotes subunits that are absent from certain preparations or can be dissociated from the purified enzyme (see text for details).

C. Subunit Structures

Each of the three yeast enzymes is more complex in terms of polypeptide content than is the single bacterial RNA polymerase. The yeast enzymes are heteromultimeric proteins composed of two large subunits and a collection of smaller polypeptides, some of which are shared between two or all three of the enzymes. In previous reviews (Sentenac and Hall 1982; Sentenac 1985), we used a nomenclature in which subunits were identified by their apparent sizes derived from SDS-PAGE and by the letter A, B, or C corresponding to the enzyme. (Subunits common to enzymes I[A] and III[C] or to all three enzymes were designated by AC or ABC.) Now that most of the corresponding genes have been sequenced, it appears that the predicted masses are often smaller than the values derived from SDS-PAGE (Table 1), but we retain the initial nomenclature for convenience and consistency.

The formation of two-dimensional crystals on positively charged lipid layers has been exploited for reconstructing the three-dimensional shapes of RNA polymerases on the basis of electron microscopy data. This important technique has been applied to the *Escherichia coli* RNA polymerase (Darst et al. 1989) and to yeast enzymes I and II (Edwards et al. 1990; Schultz et al. 1990; Darst et al. 1991). Figure 1 shows such a three-dimensional image reconstruction for yeast enzyme I. The bacterial and yeast enzymes are strikingly similar in their general architecture, with a deep cleft across the enzyme that may well accommodate the double-stranded DNA template.

All three yeast RNA polymerases contain tightly bound zinc atoms. Earlier estimates of one to two zinc atoms per enzyme molecule (Auld et al. 1976; Lattke and Weser 1976; Wandzilak and Benson 1978) must be reevaluated, because the two largest subunits of each enzyme, the two common subunits ABC10α and ABC10β, and the specific subunits B12.6 and A12.2 all bind radioactive zinc in vitro (Treich et al. 1991; Werner et al. 1992). In agreement with the latter observations, DNA sequencing has revealed a presumptive zinc-binding motif involving cysteine residues in each of these subunits (see Table 1), except for the ABC10β subunit, in which the zinc-binding motif was less obvious. In the case of the largest subunits, small domains encompassing the putative zinc-binding motif were expressed in *E. coli* and shown to bind ^{65}Zn in vitro (Treich et al. 1991; Werner et al. 1992). Mutational substitutions of the cysteine residues abolished this zinc-binding property. The zinc atoms might conceivably generate DNA-binding structures akin to the "zinc fingers" of the RNA polymerase III transcription factor TFIIIA (Miller et al. 1985) and some RNA polymerase II *trans*-activators (for review, see Berg 1990). However, this function seems unlikely in the

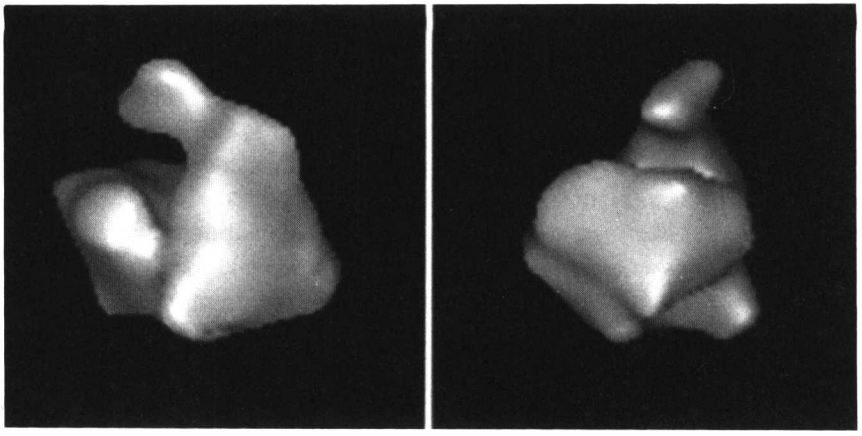

Figure 1 Surface-shaded three-dimensional representation of *S. cerevisiae* RNA polymerase I. Two-dimensional crystals of the enzyme were obtained and observed by electron microscopy as described previously (Schultz et al. 1990). The model presented here was built up from 40 tilted crystal projections. The enzyme is 10 x 11 x 15-nm in size, and a deep cleft separates two asymmetric domains that could correspond for the most part to the two largest polypeptides. (Courtesy of P. Schultz and P. Oudet, Strasbourg.)

present case. Instead, recent data favor a structural role, in which zinc binding would stabilize the subunit organization of the enzyme (Yano and Nomura 1991; Werner et al. 1992). Finally, a direct catalytic role of zinc is by no means ruled out.

1. RNA Polymerase I

Enzyme I contains 14 polypeptides (Huet et al. 1975; Hager et al. 1976, 1977; Carles et al. 1991). All but one of the corresponding genes have now been cloned (Table 1 and references therein). The association of these polypeptides into the multimeric enzyme is documented by their cosedimentation with enzyme activity, comigration in nondenaturing polyacrylamide gels, and coprecipitation by antibodies raised against the largest subunit, A190 (Huet et al. 1975). Coomassie blue staining showed that most of these polypeptides have molar ratios of about one, relative to the largest subunit, or two in the case of ABC27. However, A43 is present in submolar amount, and A49 and A34.5 are loosely associated with the enzyme and can be removed by chromatography or electrophoresis, thus generating a simplified A^* form of the enzyme with altered enzymatic properties (Huet et al. 1975, 1976; Sentenac et al. 1976; Cooper and Quincey 1979). In addition, a form of the enzyme

depleted of A49 and A43 is less active on poly[d(A-T)] than the complete enzyme (Hager et al. 1977).

A comparison of the polypeptide contents of RNA polymerases I from different yeasts indicated a polymorphism in their subunit structures (Riva et al. 1982). The enzymes from different *Saccharomyces* subspecies had the same subunit pattern, but the *Schizosaccharomyces pombe* and *Candida tropicalis* enzymes lacked components homologous to A49, A43, A34.5, A14, and A12.2. Therefore, the minimal subunit composition of enzyme I may be defined by the two large subunits, A190 and A135, plus the seven subunits common to enzymes I and III, to the exclusion of the five enzyme-I-specific polypeptides. The *S. pombe* and the *C. tropicalis* enzymes cross-react with antibodies directed to *S. cerevisiae* A190, A135, and AC40 (Fig. 2). These three subunits, which are homologous to the β', β, and, to a lesser extent, α subunits of the bacterial core enzyme, therefore constitute the most conserved part of RNA polymerase I.

2. RNA Polymerase II

Enzyme II is composed of 12 polypeptides (Bell et al. 1976; Dezélée et al. 1976; Kolodziej et al. 1990; Carles et al. 1991). The corresponding genes have all been cloned (see Table 1B and references therein). The same subunit pattern was obtained by immunoprecipitation using antibodies directed to an epitope-tagged B44 subunit (Kolodziej et al. 1990) or to the carboxy-terminal domain of B220 (Edwards et al. 1990). The molar ratios of these polypeptides were recently estimated from [^{35}S]methionine incorporation and gene sequence data (see Table 1) (Kolodziej et al. 1990). Two polypeptides, B32 and B16, are loosely associated with the enzyme and can be removed by a mild urea treatment to generate a variant form B*, which is active for the transcription of nonspecific templates (Dezélée et al. 1976). A particular mutation (*rpoB1*) in the gene encoding the largest subunit yields an RNA polymerase II defective in RNA synthesis in vitro that corresponds to form B* (Ruet et al. 1980). This mutant is viable. The B* enzyme elongates and terminates normally but is inactive in promoter-directed transcription (Edwards et al. 1991). Addition of purified B32 and B16 restores transcriptional activity. These two subunits cochromatograph and also comigrate during electrophoresis under nondenaturing conditions (Sawadogo et al. 1980; Edwards et al. 1991). A disruption of the B32-encoding gene is not lethal (Woychik and Young 1989) and leads to the concomitant loss of the B16 subunit from immunopurified enzyme II (Kolodziej et al. 1990; Edwards et al. 1991). These data strongly suggest

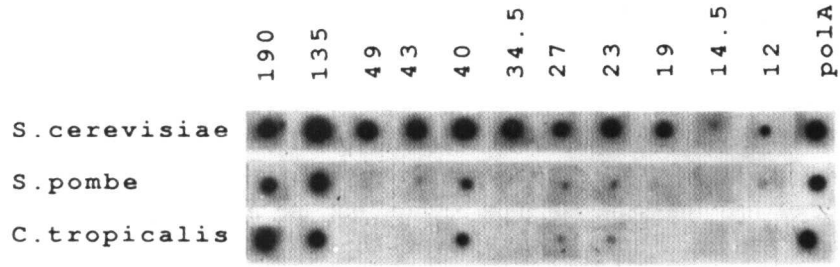

Figure 2 Antigenic cross-reactivity between RNA polymerase I prepared from the three distantly related yeasts *S. cerevisiae*. *S. pombe*, and *C. tropicalis*. Purified RNA polymerase I from each yeast was reacted with antibodies raised against individual subunits from RNA polymerase I (polA) of *S. cerevisiae*. For experimental details, see Riva et al. (1982).

that B32 and B16 directly interact and contribute to the initiation of transcription, albeit not in a strictly essential manner.

The largest subunit of RNA polymerase II, B220, is characterized by an unusual carboxy-terminal domain, hereafter abbreviated CTD (Allison et al. 1985; Corden et al. 1985). In yeast, this is a tandem repeat of the consensus heptapeptide sequence YSPTSPS, with 17 perfect matches out of 26 repeats or, in some strains, 27 repeats (Allison et al. 1985; Nonet et al. 1987a). The CTD accounts for many of the characteristic features of B220, including its phosphorylation, susceptibility to proteolysis, high antigenicity, and abnormal electrophoretic mobility. B185, the proteolytic by-product of B220 lacking the CTD, is not phosphorylated (Buhler et al. 1976b; Bréant et al. 1983a), which implies that phosphorylation of B220 is restricted to the CTD itself. Enzyme II can be further phosphorylated in vitro by a yeast kinase, generating a slower migrating form of B220 (Lee and Greenleaf 1989). Therefore, the native form of the enzyme is only partially phosphorylated, in contrast to the mammalian enzyme (see Sawadogo and Sentenac 1990 and references therein). The level of in vivo ^{32}P incorporation into B220 can actually be determined, because it is similar to that measured for A190, i.e., about six phosphate groups per molecule of subunit (see Fig. 1b in Bréant et al. 1983a). Considering that only half of the native enzyme molecules are phosphorylated, there should be an average of about 12 phosphates per molecule of phosphorylated B220 subunit, which implies that only a fraction of the 26 or 27 repeats are actually phosphorylated.

The function of the CTD remains conjectural (see Sawadogo and Sentenac 1990). It is certainly an essential component of the enzyme, because yeast mutants with extensive deletions of the CTD are nonviable, whereas limited truncations produce temperature-sensitive cells (Nonet