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RNA Polymerase II Subunit Composition, Stoichiometry, and Phosphorylation

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RNA polymerase II subunit composition, stoichiometry, and phosphorylation were investigated in *Saccharomyces cerevisiae* by attaching an epitope coding sequence to a well-characterized RNA polymerase II subunit gene (*RPB3*) and by immunoprecipitating the product of this gene with its associated polypeptides. The immunopurified enzyme catalyzed α-amanitin-sensitive RNA synthesis in vitro. The 10 polypeptides that immunoprecipitated were identical in size and number to those previously described for RNA polymerase II purified by conventional column chromatography. The relative stoichiometry of the subunits was deduced from knowledge of the sequence of the subunits and from the extent of labeling with [^{35}S]methionine. Immunoprecipitation from ^{32}P-labeled cell extracts revealed that three of the subunits, RPB1, RPB2, and RPB6, are phosphorylated in vivo. Phosphorylated and unphosphorylated forms of RPB1 could be distinguished; approximately half of the RNA polymerase II molecules contained a phosphorylated RPB1 subunit. These results more precisely define the subunit composition and phosphorylation of a eucaryotic RNA polymerase II enzyme.

Eucaryotic nuclear RNA polymerases I, II, and III are responsible for the synthesis of rRNA, mRNA, and small stable RNAs, respectively. These enzymes were originally defined by their ability to catalyze RNA chain initiation and elongation on nonspecific DNA templates and were distinguished by their chromatographic properties and their differential sensitivity to α-amanitin (26, 36). It was subsequently shown that purified RNA polymerases I, II, and III can selectively initiate transcription in vitro in the presence of appropriate templates and associated factors (19, 22, 27, 30, 39).

While the factors that provide specificity for the initiation of transcription are under intensive scrutiny, the RNA polymerases remain poorly understood. In contrast to their procaryotic counterparts, whose components have been well defined genetically and biochemically (13, 42), eucaryotic RNA polymerase subunits have been described largely as the polypeptides that copurify with nonspecific transcriptional activity. These eucaryotic RNA polymerases consist of 10 to 14 polypeptides that range from 220 to 10 kilodaltons (26, 31, 36). It is not clear whether these proteins are the complete set of RNA polymerase II subunits in vivo, whether they are present in equimolar amounts, and how they contribute to the function of the enzyme.

The components of *Saccharomyces cerevisiae* RNA polymerases have been a focus of attention because RNA polymerases appear to be highly conserved among eucaryotes and because of the relative ease of genetic and biochemical experimentation in *S. cerevisiae*. Yeast RNA polymerases are perhaps the most thoroughly studied eucaryotic RNA polymerases, largely because of the work of Sentenac and his colleagues (for a review, see reference 36). The genes that encode the 10 putative RNA polymerase II subunits have been isolated and sequenced (2, 23, 38, 40a, 41, 41a; Woychik and Young, unpublished data). The two largest subunits of RNA polymerase II, RPB1 and RPB2, are homologs of the β′ and β subunits of procaryotic RNA polymerase (2, 38). These two large subunits are essential for mRNA synthesis (28, 35) and are able to bind nucleoside triphosphates and DNA (12, 14, 32). The smaller RNA polymerase II subunits (RPB3, RPB4, RPB5, RPB6, RPB7, RPB8, RPB9, and RPB10) have no obvious procaryotic counterparts. Nonetheless, at least some of these proteins have roles in transcription. Mutations in the genes for RPB3 and RPB4 can cause defects in mRNA transcription or in RNA polymerase II assembly in vivo (23, 41). In addition, three of the polymerase II subunits, RPB5, RPB6, and RPB8, are shared by all three nuclear RNA polymerases (10, 40a).

With the genes for the putative RNA polymerase II subunits in hand, we sought to reinvestigate the subunit composition of this enzyme and to determine the stoichiometry and phosphorylation state of each of its components. To do this, a nine-amino-acid epitope recognized by an influenza hemagglutinin monoclonal antibody was added to the amino terminus of RPB3, a subunit previously shown to be essential for RNA polymerase II assembly and function in vivo (23). The immunoprecipitation and immunopurification of RPB3 with its associated proteins have allowed us to define more precisely the composition and phosphorylation of RNA polymerase II.

MATERIALS AND METHODS

Media. YPD, low sulfate synthetic medium (LSM), no sulfate medium, and low phosphate medium have been described elsewhere (20, 37). Synthetic complete media used in strain construction has been previously described (29). The ammonium sulfate concentration in LSM is 26 mg/liter; the potassium phosphate concentration in low phosphate medium is 30 mg/liter.

Plasmids. Plasmid pY2413 is a *CEN URA3 RPB3* plasmid (23). Plasmid pY2435 is a *CEN* vector carrying the *RPB3-1*::*3′LEU2* allele, in which the 12CA5 epitope coding sequence (40) has been added to the amino-terminal coding sequence

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TABLE 1. Yeast strains

Strain	Genotype	Alias	Source
Z242	*MATa lys2-128δ ura3-52 leu2-3,112 HIS4-912δ rpb3Δ1::LYS2*(pY2413)		This laboratory
Z270	*MATa ade1 his2 leu2-3,112 trp1-1*	MDMX1-1A	M. Mendenhall
Z271	*MATa ade1 his2 leu2-3,112 trp1-1 cdc28-4*	MDMX1-2A	M. Mendenhall
Z275	*MATa lys2-128δ ura3-52 leu2-3,112 HIS4-912δ RPB3-2*		This laboratory
Z277	*MATa lys2-128δ ura3-52 leu2-3,112 HIS4-912δ RPB3-1*		This laboratory
Z296	*MATa his3Δ200 ura3-52 leu2-3,112 rpb4Δ1::HIS3*		This laboratory
Z376	*MATa his3Δ200 ura3-52 leu2-3,112 rpb4Δ1::HIS3*(pY2435)		This laboratory
Z377	*MATa ade1 his2 leu2-3,112 trp1-1*(pY2435)		This laboratory
Z378	*MATa ade1 his2 leu2-3,112 trp1-1 cdc28-4*(pY2435)		This laboratory

of *RPB3* to create *RPB3-1* and the *LEU2* gene has been inserted 3′ to the *RPB3* open reading frame (23). The plasmid pY2438 contains the *RPB3-2::3′LEU2* allele, in which the 9E10 epitope coding sequence (16) has been added to the amino-terminal coding sequence of *RPB3* to create *RPB3-2* and the *LEU2* gene has been inserted 3′ to the *RPB3* open reading frame. The *RPB3-1::3′LEU2* and *RPB3-2::3′LEU2* alleles complement an *rpb3Δ1* deletion.

Yeast strains. Yeast strains are shown in Table 1. All transformations were performed by the lithium acetate method (24). A *Pst*I DNA fragment bearing the *RPB3-1::3′LEU2* allele was excised from pY2435 and used to transform strain Z242, which contains a replacement of *RPB3* with *rpb3Δ1::LYS2* (23). Leu$^+$ transformants were screened for Lys$^-$ phenotypes, indicating replacement of *rpb3Δ1::LYS2* with *RPB3-1::3′LEU2*. Cells which had lost the plasmid pY2413 were selected in the presence of 5-fluoro-orotic acid (7), and one such isolate was called Z277. Integration was confirmed by Southern analysis. The *rpb3Δ1::LYS2* allele in Z242 was replaced with the *RPB3-2::3′LEU2* allele, and the plasmid pY2413 was removed by 5-fluoro-orotic acid selection to generate strain Z275. The *rpb4Δ1::HIS3* strain Z296 (41) and the isogenic *CDC28* and *cdc28-4* strains Z270 and Z271 were transformed with pY2435 to yield the strains Z376, Z377, and Z378.

Cell labeling. Cells (5×10^7) grown in LSM were harvested by centrifugation at 2,000 rpm for 5 min, suspended in 1 mCi of [^{35}S]methionine (Dupont, NEN Research Products; 686 Ci/mmol) and 12 μl of 5× no sulfate medium, and incubated for 5 min at the appropriate temperature. One milliliter of LSM was added, and the culture was transferred to a disposable 125-ml Erlenmeyer flask. After incubation with shaking for 20 min, 10 ml of LSM was added and growth continued for 95 min. Phosphate labeling was performed by growing 5×10^7 cells in low phosphate medium containing 100 to 250 μCi of [^{32}P]orthophosphate per ml (Dupont, NEN Research Products; 8,500 to 9,120 Ci/mmol) for 12 to 16 h at the appropriate temperature.

To examine the effect of a CDC28 mutation on the phosphorylation of the RPB1 carboxy-terminal repeat domain, cells were maintained in either LSM (for [^{35}S]methionine labeling) or low phosphate medium (for [^{32}P]orthophosphate labeling) at 24°C or were shifted to 36°C for 30 min prior to the addition of the label. Otherwise, labeling was carried out as described above except incubation with [^{32}P] orthophosphate occurred for only 2 h.

Immunoprecipitation of RNA polymerase II. Immunoprecipitation with the 12CA5 antibody was performed as described previously (23), except that immunoprecipitates were washed twice with buffer B (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid]–NaOH [pH 7.9], 5% glycerol, 10 mM EDTA) containing 0.4 M ammonium sulfate and once with buffer B containing 0.05 M ammonium sulfate, prior to suspension in Laemmli loading buffer (25). Immunoprecipitates were examined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and fluorography as previously described (23). The gels were 12.5% acrylamide, 0.125% bis-acrylamide. Phosphatase and protease inhibitors were present in lysis buffer as previously described (P. A. Kolodziej and R. A. Young, Methods Enzymol., in press).

RNA polymerase subunit stoichiometry. The relative molar amounts of RNA polymerase II subunits were estimated by scanning Dupont XAR film exposures (see Fig. 2) with an LKB 2202 Ultrascan laser densitometer. Four independent immunoprecipitation experiments with [^{35}S]methionine-labeled RNA polymerase II were carried out, and multiple lanes were scanned with the densitometer. The signal produced by each of the subunits was divided by the number of methionine residues predicted by the gene sequence (RPB1, 44 [2]; RPB2, 38 [38]; RPB3, 11 [23]; RPB4, 3 [41]; RPB5, 9 [40a]; RPB6, 4 [40a]; RPB7, 5 [unpublished data]; RPB8, 3 [40a]; RPB9, 3 [unpublished data]; RPB10, 1 [unpublished data]) to obtain the relative molar ratio of the proteins. For the purposes of this estimate, it was assumed that each of the subunits retained the N-terminal methionine residue; microsequence data from RPB10 confirm that this subunit does retain the N-terminal methionine. If this assumption is not correct for all of the RNA polymerase II subunits, the interpretation of the relative stoichiometry of the proteins, except for RPB9, would not be different. For any one of the subunits other than RPB9, the loss of a single methionine would not substantially alter its methionine content. If RPB9 does not retain its N-terminal methionine, then it exists in three copies per RNA polymerase II molecule.

Immunoaffinity purification of RNA polymerase II. RNA polymerase II-containing epitope-tagged RPB3 was purified as described for adenylate cyclase (18). RNA polymerase assays were performed as previously described (33).

RESULTS

Polypeptide composition of RNA polymerase II. To investigate RNA polymerase II subunit composition and stoichiometry, the polypeptides that coprecipitate with an epitope-tagged RPB3 protein were investigated (Fig. 1). RPB3 was selected as a target for immunoprecipitation because it has been demonstrated to copurify with RNA polymerase II activity in vitro and to be involved in mRNA synthesis in vivo (23). Moreover, the addition of a nine-amino-acid epitope to its amino terminus does not adversely affect cell growth, and thus, RNA polymerase II function (23). The epitope-tagging approach was used because it has several advantages over immunoprecipitation with conventional anti-subunit antibodies for investigating the subunit composition of a complex multisubunit enzyme. These include the

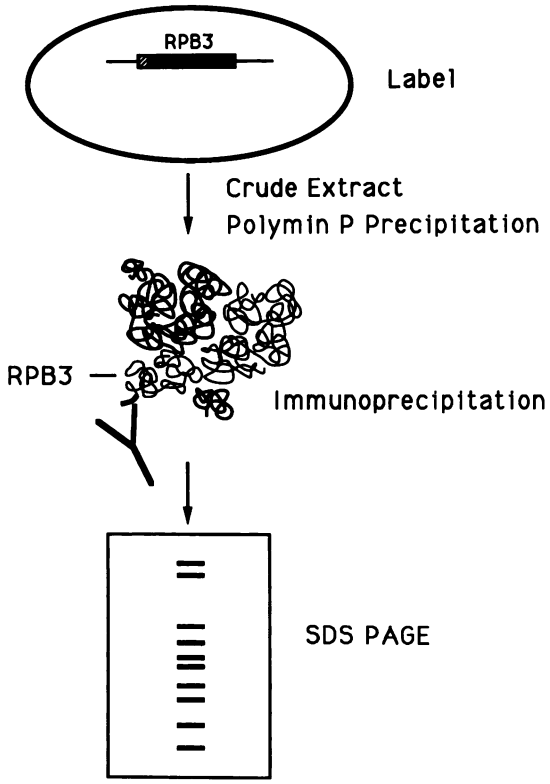

FIG. 1. Analytical purification of RNA polymerase II. The RPB3 subunit was modified by the addition of a nine-amino-acid sequence recognized by a monoclonal antibody. Extracts from cells labeled with [^{35}S]methionine or ^{32}P were fractionated by polymin P precipitation, followed by immunoprecipitation with a monoclonal antibody specific for the epitope tag.

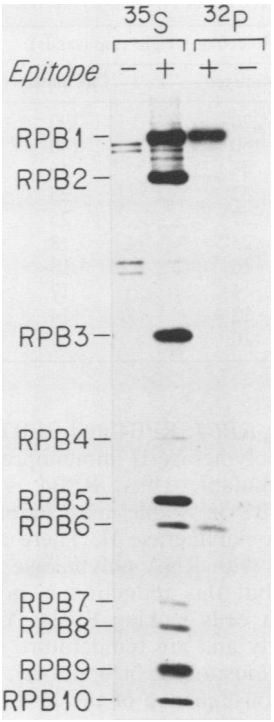

FIG. 2. RNA polymerase II defined by polypeptides that coprecipitate with RPB3. The figure shows an autoradiograph of an SDS-polyacrylamide gel containing the [^{35}S]methionine and ^{32}P-labeled proteins that are immunoprecipitated from cells that contain RPB3 with (+) or without (−) the epitope tag recognized by monoclonal antibody 12CA5.

ability to prepare a negative control from cells that do not carry the epitope-tagged protein and the capacity to detect the protein of interest with a well-characterized monoclonal antibody.

RPB3 and associated proteins were immunoprecipitated from a polymin P fractionated extract of ^{35}S-labeled Z277 cells with the monoclonal antibody 12CA5. A total of 10 polypeptides are immunoprecipitated from extracts containing the epitope-tagged subunit (Fig. 2). None of these are immunoprecipitated from control extracts of Z275 that lack epitope-tagged RPB3. The numbers and sizes of these proteins are identical to those of yeast RNA polymerase II subunits purified by conventional procedures. The elimination of the polymin P fractionation step does not alter the appearance of the RNA polymerase II proteins but results in an increase in the nonspecific background. When RNA polymerase was affinity purified from a polymin P fractionated extract with a 12CA5 antibody column, it had a subunit composition identical to that of RNA polymerase II purified by conventional column chromatography. Moreover, the activity and α-amanitin sensitivity of the affinity-purified enzyme was comparable to conventionally purified enzyme, as measured in a nonspecific transcription elongation assay (33).

Stoichiometry of RNA polymerase II subunits. The 10 yeast RNA polymerase II subunit genes have been isolated and sequenced, and the amino acid sequence predicted for each subunit matches multiple peptide sequences obtained from gel-purified subunits of conventionally isolated RNA polymerase II (2, 23, 38, 40a, 41, 41a; Woychik and Young, unpublished data). The ability to immunoprecipitate [^{35}S]methionine-labeled RNA polymerase II subunits, coupled with knowledge of the relative methionine content of each of the sequenced subunits, allowed us to deduce the relative molar ratio of each of the subunits of the enzyme from densitometry of the protein bands from four independent immunoprecipitation experiments (Fig. 2) (see Materials and Methods for details). The apparent molecular weights of the immunoprecipitated RNA polymerase II subunits, their actual molecular weights deduced from the gene sequences, and their relative molar ratios are summarized in Table 2. These data indicate that the average RNA polymerase II molecule is composed of one copy each of RPB1, RPB2, RPB6, RPB8, and RPB10 and two copies of RPB3, RPB5, and RPB9. The results also show that, under these immunoprecipitation conditions, the RPB4 and RPB7 subunits are present at less than one copy on the average. Identical results were obtained when the enzyme was immunoprecipitated with a monoclonal antibody directed against the RPB1 carboxy-terminal repeat domain, when detergent was omitted from the immunoprecipitation procedure or when the polymin P fractionation was eliminated (data not shown).

The substoichiometric levels of RPB4 and RPB7, and the observation that these two proteins dissociate from the rest of the enzyme under partially denaturing conditions (34), led us to investigate the subunit composition of RNA polymerase II in a cell lacking the *RPB4* gene. Figure 3 shows the results of immunoprecipitation of epitope-tagged RPB3 from a yeast cell containing the wild-type RPB4 gene and from a

TABLE 2. Yeast RNA polymerase II subunits

Subunit	Molecular weight (thousands)		Stoichiometry
	Estimated	Calculated	
RPB1	220	190	1.1
RPB2	150	140	1.0
RPB3	45	35	2.1
RPB4	32	25	0.5
RPB5	27	25	2.0
RPB6	23	18	0.9
RPB7	16	18	0.5
RPB8	14	17	0.8
RPB9	12	14	2.0
RPB10	10	5	0.9

yeast cell lacking *RPB4*. RPB4 and RPB7 proteins are both absent in RNA polymerase II immunoprecipitated from the *RPB4* deletion mutant. Thus, RPB4 is required for the presence of RPB7 in stable association with the other subunits of RNA polymerase II. There appears to be less RPB8 associated with RNA polymerase II in cells lacking RPB4 (Fig. 3), but this reduction is not observed in all experiments with cells lacking RPB4. Yeast cells lacking RPB4 grow slowly and are temperature sensitive and cold sensitive and are inositol auxotrophs (41). These phenotypes may thus be a consequence of the loss of both RPB4 and RPB7 from the enzyme.

Mutations in *RPB1*, *RPB2*, *RPB3*, *RPB4*, and *RPB6* have allowed us to demonstrate unequivocally that these genes encode the RNA polymerase II subunits RPB1, RPB2, RPB3, RPB4, and RPB6 (Fig. 2). Partial deletions of the RPB1 carboxy-terminal repeat domain alter the mobility of the largest polypeptide (29). *RPB2* mutations can affect the stability of the enzyme as examined by immunoprecipitation (data not shown). The addition of an epitope tag to RPB3 and

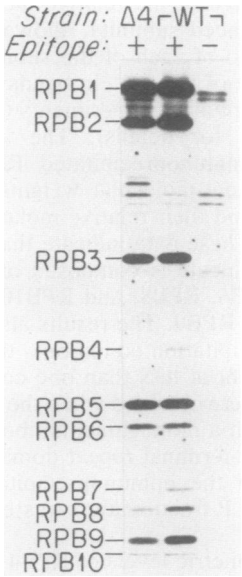

FIG. 3. RNA polymerase II immunoprecipitated from wild-type (WT) and RPB4 (Δ4) deletion mutant cells. Autoradiograph of an SDS-polyacrylamide gel containing the [^{35}S]methionine proteins that coprecipitate with epitope-tagged RPB3 (+) from cells that contain or lack the RPB4 subunit. A control was prepared from wild-type cells that lack the 12CA5 epitope (−).

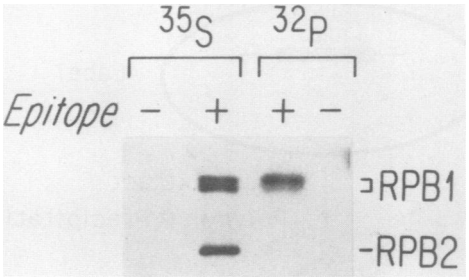

FIG. 4. Resolution of multiple forms of the largest RNA polymerase II subunit. Immunoprecipitated RNA polymerase II was prepared as described in the legend to Fig. 2 but was subjected to a long electrophoresis run to improve the resolution. Immunoprecipitates were prepared from cells that contain RPB3 with (+) or without (−) the epitope tag recognized by monoclonal antibody 12CA5.

RPB6 reduces the mobility of these two polypeptides in SDS PAGE (40a). RNA polymerase II immunoprecipitated from *RPB4* deletion mutants lacks RPB4 and RPB7 (Fig. 3). Mutations in genes encoding the remaining five subunits have not yet been obtained, and thus, we have yet to show directly that the *RPB5*, *RPB7*, *RPB8*, *RPB9*, and *RPB10* genes encode the polypeptides designated RPB5, RPB7, RPB8, RPB9, and RPB10, respectively (Fig. 2 and 3). However, all of these proteins comigrate with subunits of the conventionally purified enzyme, and multiple peptide sequences obtained from each of the isolated subunits of the conventionally purified enzyme match those predicted by each of the gene sequences (2, 23, 38, 40a, 41; Woychik and Young, unpublished data).

Phosphorylation of RNA polymerase II subunits. There are conflicting reports on the phosphorylation of *S. cerevisiae* RNA polymerase II subunits (4, 8, 9). While the results indicate that RPB6 is phosphorylated, it is unclear whether RPB1 and RPB3 are phosphorylated. We used immunoprecipitation of epitope-tagged RPB3 and associated RNA polymerase II subunits to identify the phosphorylated subunits. Figure 2 compares immunoprecipitates from cells labeled with [^{35}S]methionine and from cells labeled with [^{32}P]orthophosphate. RPB1 and RPB6 are clearly phosphorylated; long exposures indicate that RPB2 is also phosphorylated, albeit at a much lower level.

RPB1 is phosphorylated in half of the RNA polymerase II molecules. Purified mammalian RNA polymerase II contains three forms of the largest subunit, one that has a phosphorylated carboxy-terminal repeat domain (form II_o), a second subunit that lacks phosphorylation of this domain (II_a), and a third subunit that lacks the repeat domain because of proteolysis (II_b) (11, 21). *S. cerevisiae* RNA polymerase II also contains these three forms of RPB1, two of which predominate. When labeled with [^{35}S]methionine, RPB1 can be resolved as a doublet (Fig. 4). The upper band contains almost all of the phosphate and therefore appears to be equivalent to form II_o of the mammalian RNA polymerase II. The lower band contains little or no phosphate and is probably equivalent to form II_a. The separation of the two bands that compose the yeast RNA polymerase large subunit doublet is not as great as that observed with mammalian RNA polymerase II (11, 21), presumably because the mammalian carboxy-terminal repeat domain is twice the size of its yeast counterpart, while the rest of the large subunit is similar in size in mammals and in *S. cerevisiae*. In long exposures, an ^{35}S-labeled protein of approximately 180

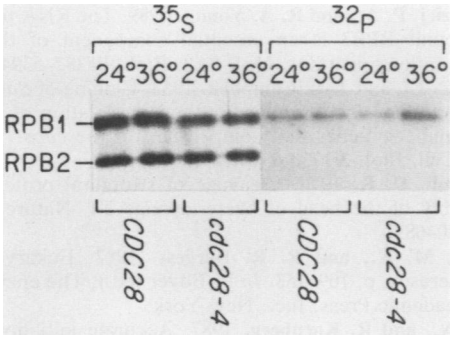

FIG. 5. Phosphorylation of RPB1 in a CDC28 mutant. RNA polymerase II was immunoprecipitated from [^{35}S]methionine and ^{32}P-labeled extracts of yeast strains that contain wild-type (*CDC28*) or mutant (*cdc28-4*) CDC28 genes. Cells were labeled at 24°C or were shifted to 36°C for half an hour prior to the addition of the label.

kilodaltons (Fig. 2) is observed that comigrates with RPB1 mutant protein that lacks a carboxy-terminal repeat domain (data not shown); this is analogous to mammalian II_b. The results shown in Fig. 4 demonstrate that essentially all of the RPB1 molecules that are assembled into RNA polymerase II in vivo contain the carboxy-terminal repeat and that approximately half of the RNA polymerase II molecules contain a phosphorylated RPB1 subunit.

Phosphorylation of RNA polymerase II is unaffected by CDC28 mutants. The carboxy-terminal repeat domain of RPB1 can be phosphorylated in vitro by a purified mammalian protein kinase, a subunit of which is homologous to CDC2 (15), a kinase that controls entry into the cell cycle. We investigated whether mutations in *CDC28*, which encodes the *S. cerevisiae* homolog of CDC2 (3), affect the phosphorylation of RPB1 in vivo. RNA polymerase II was immunoprecipitated from extracts of wild-type and CDC28 temperature-sensitive (*cdc28-4*) strains that had been labeled with ^{35}S or ^{32}P at 24 and 36°C (Fig. 5). Examination of [^{35}S]methionine-labeled RNA polymerase II from these strains demonstrates that the *cdc28-4* mutation did not affect the synthesis or stability of RNA polymerase II at the restrictive temperature. Examination of ^{32}P-labeled RNA polymerase II from these strains revealed no significant effect of the mutation on RNA polymerase II phosphorylation. These results suggest that *S. cerevisiae* CDC28 does not play a major role in RNA polymerase II phosphorylation in vivo.

DISCUSSION

S. cerevisiae RNA polymerase II purified by conventional procedures contains 10 polypeptides (36). Epitope tagging of one of its components has provided an approach to investigate the in vivo subunit composition, stoichiometry, and phosphorylation of the enzyme. The results obtained by this approach indicate that the subunit composition and activity of immunoprecipitated RNA polymerase II is identical to that described for the conventionally purified enzyme.

Knowledge of the relative methionine content of each of the 10 sequenced RNA polymerase II subunits permitted an estimate of the stoichiometry of the [^{35}S]methionine-labeled proteins. The relative molar amounts of the subunits RPB1, RPB2, RPB3, RPB5, RPB6, RPB8, RPB9, and RPB10 in the immunoprecipitated enzyme are as follows: 1:1:2:2:1:1:2:1, respectively. The presence of RPB4 and RPB7 at substoichiometric levels indicates that a subpopulation of RNA polymerase II in the cells lacks RPB4 and RPB7 or that these two subunits are less stably associated with the rest of the enzyme.

Attempts to estimate the stoichiometry of RNA polymerase subunits can be made on the basis of the intensity of protein staining with Coomassie brilliant blue. While this approach is limited by the premise that the intensity of Coomassie blue stain is directly related to the amount of protein, the intensity of RPB5 and RPB9 is consistent with their presence at two copies per enzyme molecule (see Fig. 2 in reference 36). In contrast, the intensity of the RPB3 Coomassie blue signal suggests that this subunit is present at only one copy per enzyme. The [^{35}S]methionine-labeling data presented here indicate that RPB3 is present in two copies, suggesting that RPB3 does not bind Coomassie blue as well as the other subunits do.

We have investigated the phosphorylation state of RNA polymerase II subunits in vivo. The RPB1 and RPB6 subunits are clearly phosphorylated; low levels of phosphate are detected on RPB2. RPB1 appears to be present in both phosphorylated and unphosphorylated forms in vivo, with approximately half of the RNA polymerase II molecules containing one form and half containing the other form.

Characteristics of RNA polymerase II subunit composition and phosphorylation appear to be conserved among eucaryotes. RNA polymerase II purified from eucaryotes that range from *S. cerevisiae* to humans consists of polypeptides similar in size and number (31, 36). Sequence analysis of the largest two subunits of several of these enzymes indicates that they are highly conserved (1, 2, 5, 6, 17, 38). Among the smaller subunits of RNA polymerase II purified from various eucaryotes, at least three subunits appear to be common to all three nuclear RNA polymerases (31, 36). Phosphorylation of RPB1 also seems to be a conserved feature of eucaryotic RNA polymerase II; the carboxy-terminal repeat domain of the largest subunit of HeLa cell RNA polymerase II is heavily phosphorylated (11).

Immunoprecipitation of epitope-tagged constituent polypeptides provides a powerful general method for defining the composition of multisubunit enzymes. The approach permits the surveillance of a specific gene product without prior information about its function. The addition of the epitope to the end of a polypeptide minimizes the potential for disruption of the complex by antibody binding. Finally, it may help identify factors that associate with the enzyme of interest.

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