

## Thermosensitive Mutations Affecting Ribonucleic Acid Polymerases in *Saccharomyces cerevisiae*

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Among 150 temperature-sensitive *Saccharomyces cerevisiae* mutants which we have isolated, 15 are specifically affected in ribonucleic acid (RNA) synthesis. Four of these mutants exhibit particularly drastic changes and were chosen for a more detailed study. In these four mutants, RNA synthesis is immediately blocked after a shift at the nonpermissive temperature (37 C), protein synthesis decays at a rate compatible with messenger RNA half-life, and deoxyribonucleic acid synthesis increases by about 40%. All the mutations display a recessive phenotype. The segregation of the four allelic pairs  $ts^-/ts^+$  in diploids is mendelian, and the four mutants belong to three complementation groups. The elution patterns (diethylaminoethyl-Sephadex) of the three RNA polymerases of the mutants grown at 37 C for 3.5 h show very low residual activities. The in vitro thermodenaturation confirms the in vivo results; the half-lives of the mutant activities at 45 C are 10 times smaller than those of the wild-type enzymes. Polyacrylamide gel electrophoresis shows that the synthesis of all species of RNA is thermosensitive. The existence of three distinct genes, which are each indispensable for the activity of the three RNA polymerases in vivo as well as in vitro, strongly favors the hypothesis of three common subunits in the three RNA polymerases.

In eukaryotic cells, multiple forms of ribonucleic acid (RNA) polymerase are distinguished from one another by their intracellular localization, chromatographic behavior, sensitivity to inhibitors, template specificity, or divalent cation requirement. In yeast, three or perhaps four RNA polymerases have been demonstrated and termed I<sub>A</sub>, I<sub>B</sub>, II, and III (or A, B, C) (1). Mammalian RNA polymerase I is a nucleolar enzyme. Yeast polymerase I has been purified by Buhler (2), and indirect evidence has suggested that this enzyme is involved in ribosomal RNA (rRNA) synthesis. Cramer et al. (4) showed that, in vitro, RNA polymerase I preferentially transcribes the light strand of  $\gamma$  deoxyribonucleic acid (DNA). This strand contains sequence coding for rRNA, 5S RNA, and a small proportion of transfer RNA (tRNA). Nucleoplasmic RNA polymerase II activity is sensitive to  $\alpha$ -amanitin, and, in mouse liver cells, synthesis of the nuclear precursor of messenger RNA (mRNA) is strongly inhibited by this drug. In yeast, RNA polymerase II has been purified by Dezelee and Sentenac (5). In mouse myeloma cells, Weinmann and Roeder (21) have demonstrated that RNA polymerase III synthesizes (precursor) 4S RNA and a 5S RNA species.

It seemed to us that selection of thermosensitive mutants of *Saccharomyces cerevisiae* could prove to be a powerful tool for the characterization of each RNA polymerase form. So far, Hartwell et al. have identified 10 genes that play an essential role in the formation or maturation of ribosomes in yeast (10) and one mutant ( $ts136$ ) affected in mRNA synthesis (12). The RNA polymerase activities did not seem to be affected in all of these mutants.

In the present work, we report the isolation and characterization of three mutants in which all three distinct RNA polymerase activities are thermosensitive in vivo as well as in vitro. In these mutants, synthesis of all species of RNA is strongly inhibited at the nonpermissive temperature.

### MATERIALS AND METHODS

**Strains.** The yeast strain used in this work (1278b [ $\alpha$ ]) is a haploid strain of *S. cerevisiae*. It has been described by Grenson et al. (7). Strains 3962c and 12079d are of the *a* mating type and isogenic with 1278b.

**Media.** Medium 863 is a complex medium containing, per liter of distilled water: yeast extract, 10 g; peptone, 10 g; glucose, 20 g. Agar plates (medium 868) were prepared by adding 2% agar to medium 863.

Minimal medium 149 contains 3% glucose and has been previously described (7). Medium 150 has the same composition, but without the nitrogen source. Solidified minimal medium (156) is medium 149 with 1% agar.

**Mutant isolation.** A culture of *S. cerevisiae* strain 1278b was grown overnight from a small inoculum in medium 863 at 29 C. The mutagenesis technique has been described by Lindgren et al. (13). A 0.3-ml amount of ethyl methane sulfonate, 9.2 ml of 0.2 M phosphate buffer (pH 8.0), and 0.5 ml of 40% glucose solution in distilled water were mixed in a sterile test tube with  $10^8$  cells harvested in log phase. This suspension was shaken at 29 C. After 70 min, aliquots of 0.2 ml were withdrawn, and ethyl methane sulfonate was inactivated by the addition of 9.8 ml of 6% sodium thiosulfate solution. After 10 min, cells were centrifuged and washed twice with distilled water. The pellets were suspended in medium 863 and incubated at 29 C. After 18 h, cells were collected and washed twice with 10 ml of 150 medium. They were then incubated in 150 medium (pH 4.2) for 24 h (for nitrogen starvation). Cells were then washed twice in 150 medium (pH 4.2), suspended in 149 medium (pH 4.2), and incubated at 37 C. In the exponential growth phase, 100 U of nystatin per ml was added. After 1 h, cells were washed, spread on 868 medium, and incubated at 29 C. The nystatin selection technique is a modification of the Thouvenot and Bourgeois procedure (19). When colonies appeared, plates were replicated twice into 868 medium and minimal agar; one of each was incubated at 37 C and the others were incubated at 29 C.

**Genetic techniques.** The techniques for the isolation and tetrad hybrids were those of Mortimer and Hawthorne (14).

**Incorporation tests.** Protein synthesis was measured by the incorporation of [ $^3\text{H}$ ]leucine and [ $^3\text{H}$ ]lysine. Nucleic acid synthesis was measured by the incorporation of [ $^8\text{-}^{14}\text{C}$ ]adenine. The mutants were grown in liquid culture at 29 C in 149 medium with 0.2  $\mu\text{Ci}$  of [ $^8\text{-}^{14}\text{C}$ ]adenine (specific activity, 53.5 mCi/mM) and 20  $\mu\text{g}$  of cold adenine per ml or in 149 medium with 0.4  $\mu\text{Ci}$  each of [ $^3\text{H}$ ]leucine and [ $^3\text{H}$ ]lysine and 100  $\mu\text{g}$  each of cold leucine and lysine per ml. When in log phase, the cultures were shifted from 29 to 37 C. Growth was followed by measuring the optical density at 540 nm. At intervals, the amount of [ $^8\text{-}^{14}\text{C}$ ]adenine incorporated into RNA was determined. One milliliter of culture was precipitated by the addition of 5 ml of cold 5% trichloroacetic acid. The precipitate was collected on membrane filters (Millipore Corp., type HA, 0.45- $\mu\text{m}$  pore size), washed five times with 3 ml of 5% cold trichloroacetic acid, and counted in a Packard scintillation counter. As reported earlier (9), total [ $^{14}\text{C}$ ]adenine incorporation is a good measure of RNA synthesis, since less than 2% of this labeled adenine is incorporated into DNA. The amount of [ $^{14}\text{C}$ ]adenine incorporated into DNA in our experiments was determined by incubating 1.0 ml of culture in 7 ml of NaOH at a final concentration of 1 N for 24 h at room temperature, followed by precipitation with 7 ml of 33% cold trichloroacetic acid. The procedure solubilizes all RNA counts (9).

Precipitates were collected on membrane filters (Millipore Corp., type HA, 0.45- $\mu\text{m}$ ) and counted.

Protein synthesis was estimated by [ $^3\text{H}$ ]amino acid incorporation. At intervals, 1 ml of culture (149 medium, supplied with amino acids) was boiled for 30 min in 5 ml of 10% trichloroacetic acid. Precipitates were collected on membranes filters and counted.

**RNA polymerase extraction.** Cells were grown in medium 149 and harvested in log phase. The pellets were suspended at 4 C in 1 ml of extraction buffer, per g of wet cells [0.10 tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.9; 20% (vol/vol) glycerol; 0.02 M  $\text{MgCl}_2$ ; 0.6 M  $(\text{NH}_4)_2\text{SO}_4$ ; 1.0 mM ethylenediaminetetraacetate [EDTA]; and 1.0 mM dithiothreitol]. Phenyl methylsulfonyl fluoride was present at a concentration of 1 mg/ml of extraction buffer. Cells were disrupted in a French pressure cell at 1,000 kg/cm $^2$ . The crude extract was centrifuged for 10 min at 10,000  $\times g$ . The supernatant was then spun at 100,000  $\times g$  for 90 min at 2 C. The resulting supernatant was diluted with TGED buffer (0.05 M Tris-hydrochloride, pH 7.9; 25% [vol/vol] glycerol; 0.5 mM EDTA; 0.5 mM dithiothreitol) and adjusted to a final concentration of 0.05 M  $(\text{NH}_4)_2\text{SO}_4$ .

**Diethylaminoethyl-Sephadex A-25 chromatography.** The crude extract was loaded onto a diethylaminoethyl-Sephadex (A-25) column (1.6 by 12 cm) equilibrated with 0.05 M  $(\text{NH}_4)_2\text{SO}_4$  in TGED buffer (see above). After loading, the column was washed with 1 volume of 0.05 M  $(\text{NH}_4)_2\text{SO}_4$  in TGED buffer and eluted with a 200-ml linear gradient of 0.05 to 0.425 M  $(\text{NH}_4)_2\text{SO}_4$  in TGED buffer. The flow rate was kept at 12 ml/h, and 3-ml fractions were collected.

**Assay of RNA polymerase activity.** The incubation mixture (0.1 ml) contained: Tris-hydrochloride (pH 7.9), 0.05 M;  $\text{MnCl}_2$ , 1.6 mM; adenosine 5'-triphosphate, cytidine 5'-triphosphate, and guanosine 5'-triphosphate, 0.5 mM of each; [ $^3\text{H}$ ]uridine 5'-triphosphate (12 Ci/mmol), 5  $\mu\text{Ci/ml}$ ; native or denatured calf thymus DNA, 100  $\mu\text{g/ml}$ ; enzyme fraction, 50  $\mu\text{l}$ . The resulting mixture was incubated at 30 C for 30 min. The reaction was stopped by rapid cooling to 0 C, followed by addition of 0.1 ml of bovine serum albumin (1 mg/ml) and 1 ml of cold 10% trichloroacetic acid (0.12 M) in sodium pyrophosphate. The precipitates were collected on Whatman GF/C filters, washed five times with 4 ml of 5% trichloroacetic acid (0.06 M sodium pyrophosphate), and counted.

**Labeling of cells for RNA analysis.** Cultures of the parent and mutant strains grown in 149 medium at 29 C were divided into two portions. One was shifted to 37 C and the other was kept at 29 C. After 0.5 h, [ $^3\text{H}$ ]uracil (10  $\mu\text{Ci}/25\text{ ml}$ ) (specific activity, 53 Ci/mM) was added to each culture. After 3 h, the cultures were collected by centrifugation, washed twice with cold water, and kept frozen at -25 C.

**RNA extraction.** All operations were performed at 4 C. The pellets were suspended in 9 ml of extraction buffer (Tris-hydrochloride, 0.05 M; NaCl, 0.1 M; EDTA, 0.001 M). The cells were disrupted in a French pressure cell at 1,500 kg/cm $^2$  and mixed immediately in 9 ml of water-saturated phenol. Sodium dodecyl sulfate was adjusted to a final concentration of 0.5%.

The mixture was then stirred for 30 min at room temperature. The emulsion was broken by centrifugation at  $10,000 \times g$  for 10 min; the aqueous phase was re-extracted once with 9 ml of water-saturated phenol and 1 ml of bentonite prepared according to the procedure of Fraenkel-Conrat et al. (6). The second aqueous phase was mixed with one-tenth volume of 1.5 M sodium acetate (pH 5.2) and 2 volumes of cold absolute ethanol and kept overnight at 4 C. The precipitates were pelleted by centrifugation at  $10,000 \times g$  for 15 min and dissolved in 0.3 ml of electrophoresis buffer (0.004 M Tris; 0.02 M sodium acetate; 0.001 M EDTA; 0.02% sodium dodecyl sulfate, adjusted to pH 7.2 with acetic acid).

**Discontinuous polyacrylamide gel electrophoresis (2.4%/7%).** Total nucleic acid was fractionated by electrophoresis in polyacrylamide gels. Discontinuous polyacrylamide gel electrophoresis was run according to the procedure of Petri (15). The gels were 2.4% in acrylamide for the first 45 mm and 7% in acrylamide for the remaining 45 mm. After pre-electrophoresis for 90 min at 5 mA per tube, RNA samples were applied to the gels, and electrophoresis at 6 mA per tube was carried out at room temperature for 110 min.

Gels were scanned at 260 nm, frozen in dry ice, and sliced into 1-mm fractions with a Mickel gel slicer. Each slice was directly transferred into a scintillation vial, covered with 0.5 ml of 25% ammonia solution, and kept overnight at room temperature. Ten milliliters of scintillation fluid [600 ml of toluene, 400 ml of 2-methoxyethanol, 4 g of 2,5-diphenyloxazole, and 0.2 g of 1,4-bis-2-(5-phenyloxazolyl)benzene] were then added, and radioactivity was measured in a scintillation counter.

## RESULTS

**Selection and screening of mutants.** One hundred and fifty temperature-sensitive mutants were selected after ethylmethane sulfonate mutagenesis, followed by nystatin treatment at 37 C (the nonpermissive temperature). The efficiency of the selection was increased by treating the cells with nystatin after a nitrogen starvation period. The selected mutants did not form colonies at 37 C either on minimal medium (151) or on rich medium (868), but did form colonies at 29 C on the same media. The wild-type strain grew as well at the permissive (29 C) as at the nonpermissive temperature (37 C).

Among the 150 mutants isolated, four distinct classes could be recognized by measuring the rates of protein, RNA, and DNA synthesis in exponentially growing cells before and after a shift from 29 to 37 C: (i) mutants showing a simultaneous decay in protein, RNA, and DNA synthesis, the major class; (ii) mutants with a specifically impaired DNA synthesis; (iii) mutants showing low incorporation of RNA and DNA precursors but retaining a subnormal rate of protein synthesis; (iv) mutants specifically

affected in RNA synthesis. Among the 15 representatives of the last class, four mutants (ts1564, ts4472, ts4572, and ts4573) with particularly drastic changes were chosen for a more detailed study.

The results of the incorporation of labeled precursors into protein, DNA, and RNA in the mutant ts4472 are presented in Fig. 1. They show that, after the shift to 37 C, RNA synthesis was immediately blocked, protein synthesis was decaying at a rate compatible with the half-life of mRNA (12), and DNA synthesis increased by about 40%. The results are similar for the three other mutants. At 29 C, on minimal medium, ts4572, ts4573, and ts1564 grew at the same rate ( $\mu$ ), 0.29 generation/h, ts4472 grew at 0.40, and the wild strain (1278b) grew at 0.46. At the permissive temperature, incorporation of amino acids and adenine increased proportionally to the optical density.

**Genetic analysis.** All the mutants are of the  $\alpha$  mating type; mutants of the opposite mating type ( $a$ ) have been constructed by crossing with the isogenic ( $a$ ) wild-type strain (3962c). All the mutations display a recessive phenotype, but the heterozygotes  $ts^-/ts^+$  have a partially affected growth rate.

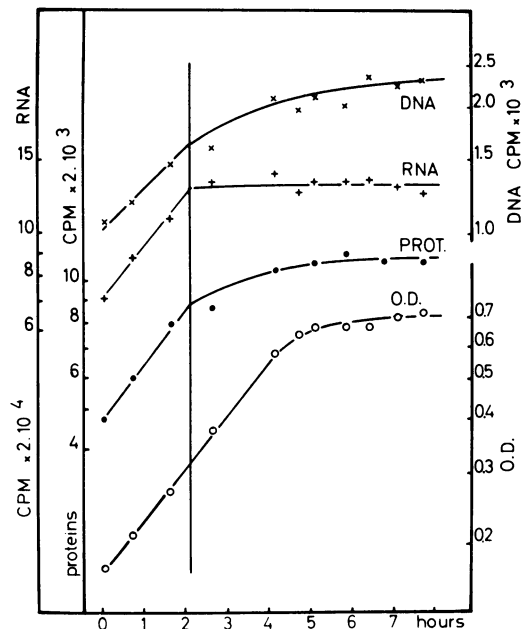


FIG. 1. Culture of the mutant ts4472. Optical density (540 nm) and incorporation of labeled precursors into protein, RNA, and DNA at 29 C and after a shift from 29 to 37 C. Incorporations were measured as described in Materials and Methods.

The results of Table 1 show: (i) that the segregation of the four allelic pairs  $ts^-/ts^+$  in diploids is mendelian, thus showing the monogenic character of each mutant; (ii) that the four mutants belong to three complementation groups (mutations  $ts4572$  and  $ts4573$  are allelic); and (iii) that the three mutations ( $ts4472$ ,  $ts1564$ , and  $ts4573$ ) are unlinked.

The sporulation of  $ts^-/ts^+$  diploids is normal, but the viability among the ascospores may be very poor (less than 10% of complete tetrads).

**Assay of RNA polymerases.** Figure 2 shows the results of diethylaminoethyl-Sephadex A-25 chromatography of a crude extract of a wild-type strain grown at 29 C [elution by a linear gradient of  $(NH_4)_2SO_4$  (0.05 to 0.425 M)]. Four peaks of activity are resolved:  $I_A$ ,  $I_B$ , II, and III. The relative amounts of enzymes  $I_B$ , II, and III varied only slightly from run to run; for enzyme  $I_A$ , however, the variability was high and thus  $I_A$  activity is not taken into account in the following discussions. This variability has also been tested by Adman et al. (1).

The elution patterns obtained with the extracts of the three mutants  $ts1564$ ,  $ts4472$ , and  $ts4572$  grown at the permissive temperature were identical to that of the wild-type strain (data not shown). To determine the in vivo thermosensitivity of the mutant RNA polymerase activities, extracts were prepared from cells grown at 29 C and shifted to 37 C for 3.5 h. During this period, the cell numbers did not increase, but optical density of the culture increased by about 50 to 100%. The elution patterns of extracts of the three mutants grown in these conditions showed very low residual activities for the three enzymes (Fig. 3). Under the same conditions, the wild-type strain showed an elution pattern identical to that

TABLE 1. Tetrad analysis of different  $ts^- \times ts^+$  and  $ts^- \times ts^-^a$

Mutants ( $\alpha \times a$ )	Segregation $ts^-/ts^+$				
	4:0	3:1	2:2	1:3	0:4
$ts1564 \times 3962c$	0	0	5	0	0
$ts4572 \times 3962c$	0	0	13	0	0
$ts4573 \times 3962c$	0	0	10	0	0
$ts4472 \times 3962c$	0	0	6	0	0
$ts4572 \times 4573 3c$	22	0	0	0	0
$ts4472 \times 4573 3c$	1	5	0	0	0
$ts1564 \times 4573 3c$	3	6	5	0	0
$ts4472 \times 1564 2b$	0	8	4	0	0

<sup>a</sup> Mutant 3962c = wild type. Mutants 4573 3c, 1564 2b, and 4472 1a were of mating type a and isogenic, respectively, with ts 4573, ts 1564, and ts 4472.  $ts^-$  means inability to grow at 37 C.

obtained at the permissive temperature (data not shown). These results are interpreted as indicating the presence, in each mutant, of a thermosensitive element necessary to the activities of the three RNA polymerases.

**In vitro thermosensitivity of the RNA polymerases.** An extract from cells grown at 29 C was partially purified by adsorption on diethylaminoethyl-Sephadex A-25. The column (1.6 by 12 cm) was then washed with 1 volume of 0.05 M  $(NH_4)_2SO_4$ -TGED buffer, and the three RNA polymerases were simultaneously eluted with 0.425 M  $(NH_4)_2SO_4$ -TGED buffer. The enzymatically active fraction (4 ml) was dialyzed for 4 h against  $4 \times 250$  ml of TED buffer (0.05 M Tris-hydrochloride, 0.5 mM EDTA, and 0.5 mM dithiothreitol) to remove glycerol and ammonium sulfate. Forty percent of the activity was lost during this dialysis. This active fraction was incubated at 45 C. Aliquots of 0.1 ml were taken at various times and mixed with 0.1 ml of cold 0.425 M  $(NH_4)_2SO_4$ -TGED buffer containing 50% glycerol. Enzyme assays were conducted at 30 C as described in Materials and Methods. The final concentration of  $(NH_4)_2SO_4$  in the incubation mixture was 0.106 M.

Figure 4 shows the in vitro thermodenaturation kinetics for the wild type and the three mutant strains. The half-lives of the enzymatic activities in these conditions were 33, 4, 3, and 2 min for the wild-type strain and the mutants  $ts4472$ ,  $ts1564$ , and  $ts4572$ , respectively. These results show a significant increase in the thermosensitivity of the RNA polymerase activities of the three mutants.

**Analysis of the RNA synthesized at the restrictive temperature.** Cultures of parent and mutant strains grown at 29 C were divided into two portions. One of them was shifted to 37 C and the other was kept at 29 C. After 30 min, [<sup>3</sup>H]uracil was added to each culture and cells were harvested after 3 h. RNA was extracted and analyzed by polyacrylamide gel electrophoresis.

The results are shown in Fig. 5 for mutant  $ts1564$ . It is clear that the synthesis of all RNA species is inhibited at 37 C. A similar analysis was carried out on the two other mutants with the same results. When the cells were grown at 29 C, the patterns were identical for parent and mutant strains.

## DISCUSSION

One hundred and fifty temperature-sensitive mutants of *S. cerevisiae* were isolated. The  $ts^-$  phenotype is probably not due to a lesion in the

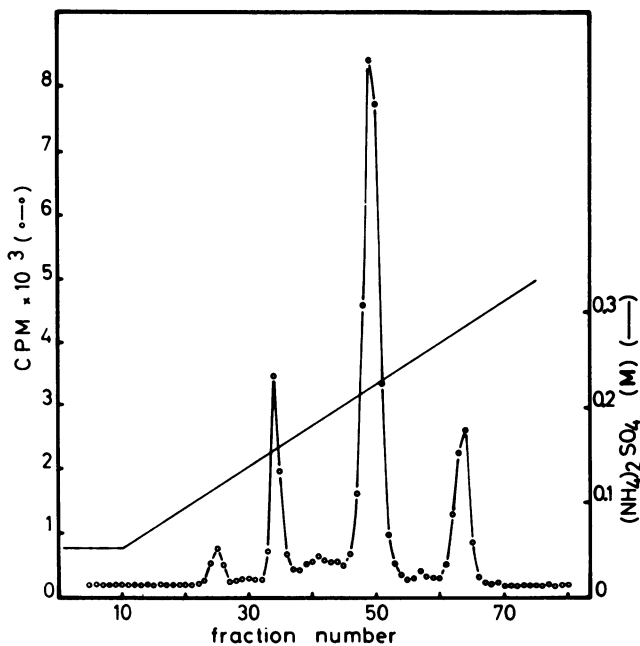


FIG. 2. Resolution of yeast RNA polymerase activities by diethylaminoethyl-Sephadex A-25 chromatography of an extract of the wild-type strain grown at 29 C. [ $^3\text{H}$ ]uridine 5'-triphosphate incorporation was measured as described in Materials and Methods.

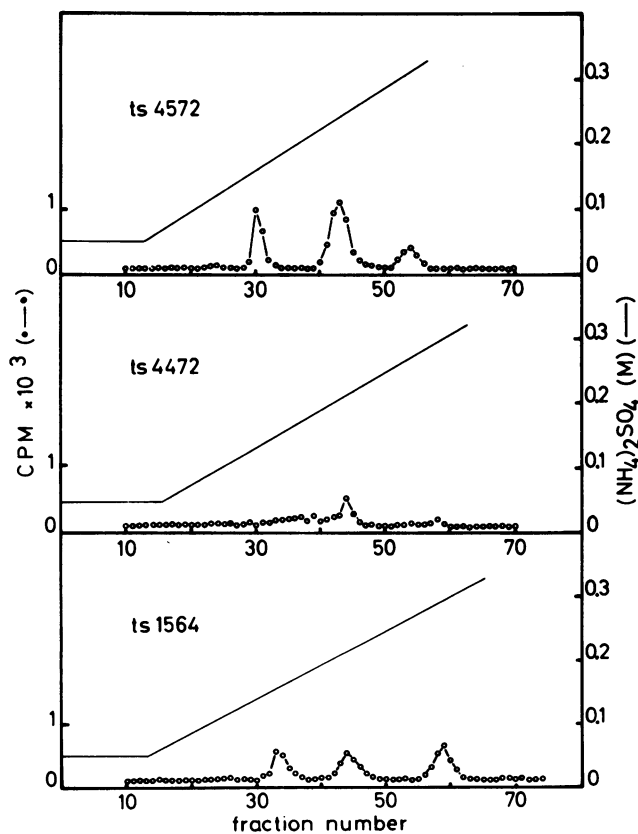


FIG. 3. Resolution of yeast RNA polymerase activities for the three mutants. Cells were grown at 29 C and shifted to 37 C for 3.5 h.

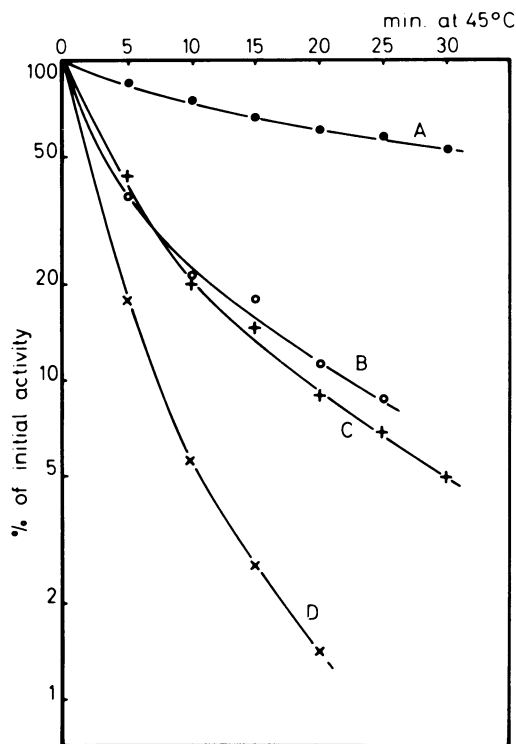


FIG. 4. *In vitro* thermodenaturation kinetics at 45 C of partially purified extracts from cells grown at 29 C (A, 1278b; B, 4472; C, 1564; D, 4572).

ability to synthesize small molecules such as vitamins, amino acids, purine, and pyrimidine derivatives, since the mutants were isolated on a complex medium.

Among the mutants, 15 displayed a significant preferential inhibition of RNA synthesis after the shift to 37 C, when compared to protein and DNA synthesis.

In this paper, four mutants are described. They exhibit a strong and rapid cessation of adenine incorporation after the shift to the restrictive temperature. It thus seems that the synthesis of all RNA species is stopped. This is obvious for rRNA's, major components of RNA. Conversely, a conclusion about tRNA's, a minor class of RNA, would be hazardous with such an incorporation test (18). This problem, which has been analyzed by polyacrylamide gel electrophoresis, will be discussed later. The kinetics of radioactive amino acid incorporation (in trichloroacetic acid-precipitable materials) at 37 C is consistent with an mRNA half-life of 25 min, which means that mRNA synthesis is also inhibited. The behavior of the mutant observed in the experiments reported in Fig. 1 is qualitatively very similar to the one induced by action

of daunomycin and lomofungin on a wild-type strain (3, 19). These two drugs are known to inhibit RNA synthesis.

Genetic analysis proves the monogenic character of the four mutants. All mutations are recessive and belong to three complementation groups, corresponding to three unlinked loci.

The elution pattern of the three RNA polymerases of the mutants grown at 29 C is similar to that of the wild-type strain. On the other hand, 3.5 h after a shift to 37 C the activities of the mutant RNA polymerases are strongly reduced compared to that of the wild-type strain (Fig. 3a, b, c). This result shows that there are no more active enzymes at 37 C, which implies that the enzymes present at the moment of the shift are also inactivated. Thus, it seems that each mutation affects the activity (structure) of a factor common to all three RNA polymerases. Since the three mutations are distinct and do not complement each other, we suppose that the three RNA polymerases share three common subunits.

This hypothesis is supported by the finding that RNA polymerases I and II are immunologically related and share some common antigenic factors (11). According to Buhler (2), it cannot be excluded that enzymes I and II share common subunits, since three polypeptide chains present in I and II appear to have the same molecular weight (41,000, 29,000, and 16,000). RNA polymerase III has not been purified so far; no data exist about its subunit composition.

The *in vitro* thermodenaturation curves confirm the results observed *in vivo*. In the presence of 25% glycerol and 0.425 M  $(\text{NH}_4)_2\text{SO}_4$ , conditions known to protect protein structures, it is impossible to show differences in thermostability between the wild-type strain and mutant enzymes. In the absence of both glycerol and ammonium sulfate, the half-lives of the mutant enzymatic activities at 45 C are 10 times smaller than those of wild-type enzymes. A difference in thermostability is already evident in the absence of one of these protectors.

The thermodenaturation experiments were performed on partially purified extracts containing the three RNA polymerases. This poses the problem of the salt conditions that have to be used for enzymatic assays. Indeed, the optimal conditions are not the same for the full expression of the three enzymic activities. Ammonium sulfate concentration has been chosen on the basis of the activity curves of both Adman et al. (1) and Ponta et al. (16) so that they optimize the activities for each of the three polymerases in the partially purified extract. The salt conditions [0.106 M  $(\text{NH}_4)_2\text{SO}_4$ ] used

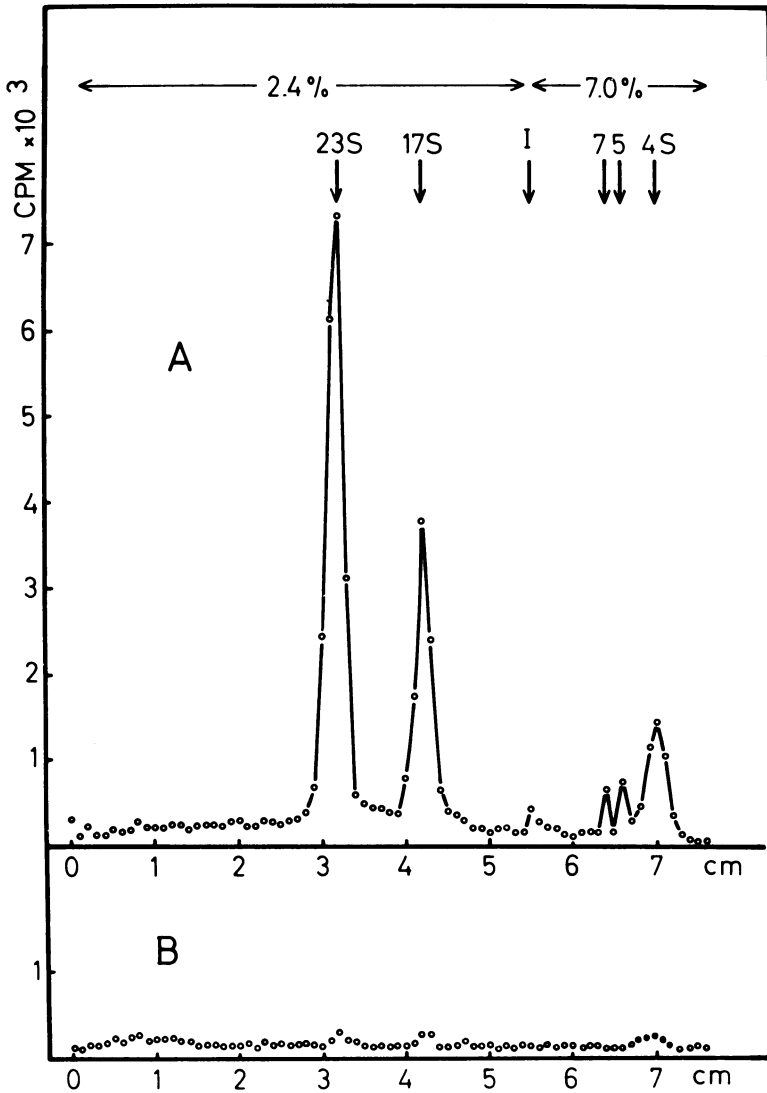


FIG. 5. Discontinuous polyacrylamide gel (2.4%/7%) electrophoresis of total RNA from the mutant *ts1564* grown at 29 C (A) and after a shift to 37 C (B). I, interface of 2.4% gel and 7% gel. The correction for losses during RNA extraction and sampling is done in the following way, which automatically includes the calculation needed to bring back the sample volumes to a given volume of cell culture. The ratio of the trichloroacetic acid-precipitable counts per minute per unit volume of culture measured at the time of harvesting for RNA extraction to the total amount of counts per minute in the gels is the factor which is used to normalize each RNA species to the unit volume of culture.

in the enzymatic assays, which were always done at 30 C, allow 15 to 40% of the I<sub>B</sub> activity, 80 to 90% of the II activity, and 60 to 80% of the III activity. Since the relative activity of RNA polymerase II is about 60% of the total activity (see Fig. 2) and the salt conditions of the enzymatic assay favor the activity of RNA polymerase II, the results reflect essentially the thermosensitivity of RNA polymerase II. Nevertheless, the values obtained after 30 min of

incubation (0.5 to 5%) exclude a thermostability of the other RNA polymerases comparable to that of the wild-type enzymes (Fig. 4).

At the restrictive temperature, the synthesis of the rRNA's (25, 17, and 5S) and tRNA's (4S) is strongly inhibited. This has been shown by polyacrylamide gel electrophoresis. In all three mutants, the synthesis of all the RNA species is inhibited more than 90%. The absence of tRNA synthesis distinguishes our mutants from mu-

tant ts136 of Hartwell (9), which, although stopping mRNA and rRNA synthesis, continues to synthesize tRNA at the nonpermissive temperature. These results confirm this *in vivo* inactivation of RNA polymerases I<sub>B</sub> and III, if RNA polymerase III is responsible for tRNA synthesis in yeast as in mouse myeloma cells.

In conclusion, it seems that in each three mutants, the three RNA polymerases are thermosensitive. The thermosensitivity of RNA polymerase I<sub>B</sub> is shown by *in vivo* inactivation and gel electrophoresis analysis. The thermosensitivity of RNA polymerase II is demonstrated by *in vivo* inactivation, *in vitro* thermodenaturation, and, if it is responsible for mRNA synthesis as it is assumed, by protein synthesis decay. The thermosensitivity of RNA polymerase III is shown by *in vivo* thermodenaturation and gel electrophoresis analysis.

All these results strongly favor the hypothesis that the three yeast RNA polymerases share three common subunits. This hypothesis is in agreement with the immunological cross-reactions of RNA polymerases I and II (11) and with the presence in RNA polymerase I and II of three subunits showing the same molecular weight (2; Sentenac, personal communication).

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