Macromolecule Synthesis in Temperature-sensitive Mutants of Yeast

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Approximately 400 temperature-sensitive mutants of *Saccharomyces cerevisiae* were isolated. The mutants were unable to form colonies on enriched media at 36°C, but grew normally, or nearly so, at 23°C. The mutants were tested for loss of viability, change in morphology, increase in cell number, and the ability to synthesize protein, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) after a shift from 23 to 36°C. Mutations were found which resulted in a preferential loss of ability to carry out protein synthesis, RNA synthesis, DNA synthesis, cell division, or cell-wall formation. Diploid cells heterozygous for the temperature-sensitive mutations were constructed and tested for their ability to form colonies at 36°C. Four mutations dominant to their wild-type allele were identified.

Horowitz and Leupold (6) first suggested that the isolation of temperature-sensitive mutants might provide a technique for obtaining mutations in indispensable (i.e., nonauxotrophic) genes. The work of Edgar and co-workers with bacteriophage T4 (2, 4) demonstrated that temperature-sensitive mutants can be used effectively to gain information about indispensable gene functions. These studies demonstrated that temperature-sensitive mutations can occur in many different genes in an organism. For instance, 39 genes were identified in bacteriophage T4 by amber mutations; 26 of these were shown to be able to carry mutations leading to a temperature-sensitive phenotype. Neidhardt described the isolation of temperature-sensitive mutants of *Escherichia coli* and some of the patterns of macromolecule synthesis that result when the mutants are shifted to the nonpermissive temperature (9). The biochemical lesion in three of these mutants has been identified as being due to thermolabile valine-activating enzyme, phenylalanine-activating enzyme (3), and fructose 1,6-diphosphate aldolase (1). An extensive investigation of 400 temperature-sensitive mutants of *E. coli* has recently been published by Kohiyama et al. (7). Lesions which preferentially affect cell division, cell wall formation, and the synthesis of protein, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) were identified through a study of macromolecule synthesis and changes in morphology after the mutants were shifted to the nonpermissive temperature.

Temperature-sensitive mutants should provide a useful probe into the poorly understood processes of macromolecule biosynthesis and its regulation in the eucaryotic cell. The organism of choice for this type of study should fulfill two conditions. It should have the smallest amount of genetic material that is compatible with the complexity of a eucaryotic cell. It should possess a genetic system that allows one to study complementation and dominance versus recessiveness of mutations.

*Saccharomyces cerevisiae* appears to fulfill both of these criteria. It is one of the simplest eucaryotic organisms, having only 2.2 × 10^-8 µg of DNA per haploid nucleus (10). If one assumes that an average protein contains 500 amino acids, this DNA content corresponds to enough genes to produce 13,000 different proteins. From the same assumption, an *E. coli* cell is able to produce 3,000 different proteins. Thus, the eucaryotic yeast cell is only about four times as complex as one of the simpler procaryotic cells. Genetic studies of yeast have reached a high level of sophistication (for a review, see 8). The yeast genetic system is ideally suited for study of the complementation patterns and determination of the dominance or recessiveness of mutations.

This paper reports the isolation and preliminary characterization of about 400 temperature-sensitive mutants of yeast. The mutants form colonies at 23°C but are unable to form colonies at 36°C on an enriched medium containing a mixture of vitamins, yeast extract, peptone, adenine, uracil, glucose, and succinate; the wild type can grow at either temperature. The mutants
were grown up at 35 C, shifted to 36 C, and tested for loss of viability, change in morphology, increase in cell number, and the ability to synthesize protein, RNA, and DNA. Each mutation was examined to determine whether it was dominant or recessive with respect to the wild-type allele. F. LaCroute (personal communication) has isolated a large number of temperature-sensitive mutants in yeast and is carrying out studies similar to those reported here.

**Materials and Methods**

*Yeast strains.* The yeast strains used in this work were haploid strains of *S. cerevisiae.* The parent from which the 400 temperature-sensitive mutants were isolated was obtained from Rochelle Esposito and is designated A364A (a ade-l* ura-l* gal-l* tyr-l* his-2* leu-2* thr-4* met-2* trp-5*). Strain X1069-2D (a ade-l* ura-l* his-4* leu-2* thr-4* met-2* trp-5*) was obtained from Robert K. Mortimer. The following abbreviations for genetic markers are used: a and a indicate mating types; ade-, ura-, his-, lys-, trp-, leu-, thr-, and met* indicate inability to synthesize adenine, uracil, histidine, lysine, tryptophan, leucine, threonine, and methionine, respectively; gal* indicates inability to ferment galactose; ts* indicates inability to grow at 36 C.

*Media.* YM-1 medium contained the following components (grams per liter): yeast extract, 5; peptone, 10; yeast nitrogen base (filter-sterilized), 6.7; adenine, 0.01; uracil, 0.01; succinic acid, 10; sodium hydroxide, 6; glucose, 10; final pH, 5.8. YM-3 medium was the same as YM-1, except that the concentrations of peptone and yeast extract were reduced fivefold. YEPD-TAU plates contained (grams per liter): yeast extract, 10; peptone, 20; glucose, 20; threonine (filter-sterilized), 0.04; adenine, 0.04; uracil, 0.04; agar, 20. Sporulation plates contained (grams per liter): potassium acetate, 2; raffinose acetate, 0.22; yeast extract, 10; and agar, 20.

*Chemicals.* Yeast extract, peptone, yeast nitrogen base, malt extract, Brain Heart Infusion, and agar were all obtained from Difco Laboratories (Detroit, Mich.). N-methyl-N'-nitro-N-nitrosoguanidine was obtained from Aldrich Chemical Co. (Milwaukee, Wis.). All radioactive compounds were the products of New England Nuclear Bio Research Inc. (Orangeburg, N.Y.). Glusulase is a commercial preparation of snail digestive juice, obtained from Endo Laboratories (Garden City, N.Y.).

*Mutant isolation.* A culture of *S. cerevisiae,* strain A364A, was grown from a small inoculum overnight at 30 C in YM-1. While still growing logarithmically, 50 ml of culture was shifted to 35 C and 0.2 ml of a solution containing 4 mg (per ml) of N-methyl-N'-nitro-N-nitrosoguanidine was added. Samples containing 0.5 ml of the culture were immediately distributed to a large number of tubes. The tubes were then rotated for a period of 5 hr (survival, 0.2 to 1.0%), after which time samples were removed, diluted, plated on YEPD-TAU plates, and incubated at 30 C. When colonies appeared, the pattern was replicated onto two plates, the first of which was incubated at 30 C and the second of which was incubated at 35 C. Colonies which grew up on the latter but not the former (approximately 1% of the total number of colonies) were picked, diluted with water, and streaked onto two YEPD-TAU plates, which were again incubated at the two temperatures. Clones which formed approximately 1,000 colonies on the 35 C plate and no colonies on the 35 C plate were picked from the low-temperature plate and designated as ts* mutants. Only one or two mutants were isolated from a single mutagen-treated culture tube.

*Genetic techniques.* Haploid cultures were mating by mixing cultures of opposite mating type on YEPD-TAU plates, and the resulting diploid was isolated by the prototypic selection method of Pomper and Burbanker (11). The products of meiosis were analyzed by a random-spore technique. Diploids were sporulated on sporulation medium. The ascus was digested with glusulase, and the spores were separated by intense sonic oscillation. Microscopic counts indicated that this technique resulted in a preparation consisting of (80 to 90%) single spores, with the remainder being spore aggregates. Unsporulated cells were destroyed by the digestion and sonic procedure.

**Analytical methods.** Macromolecule synthesis was studied in the basic types of experiments. In the pulse-label experiment, protein synthesis was measured by the incorporation of a mixture of 14C-labeled amino acids in one culture flask, and RNA and DNA synthesis was measured by the incorporation of adenine-8-14C in another culture flask. In the uniform-label experiment, protein was labeled with 14H-lysine in the same culture flask in which RNA and DNA were labeled with adenine-8-14C. The amount of adenine-8-14C incorporated into RNA was determined by precipitating a portion of culture in an equal volume of cold 10% trichloroacetic acid, collecting the precipitate on membrane filters (Millipore, type HA), and counting the filters in a gas-flow counter. Little or no 14C-adenine was incorporated into protein, as evidenced by the fact that boiling labeled cells in 5% trichloroacetic acid reduced the precipitable counts by greater than 99%. Base hydrolysis of 14C-adenine-labeled cells and chromatography of the hydrolysate revealed 95% of the counts as guanosine-3'-monophosphate and adenosine-3'-monophosphate. (Only 2% of the counts were in DNA, since *S. cerevisiae* has an RNA-DNA ratio of about 2:1.) In samples of 14C-adenine-labeled cells from a uniform-label experiment, the protein was also labeled with 14H-lysine. Owing to the poor counting efficiency of 14H in the gas-flow planchet counter, the contamination of samples being counted for RNA with protein counts was always less than 0.05%. The sedimentation pattern of 14C-adenine counts from a new medium containing sulfamate-treated cell lysate in a sucrose density gradient displayed the three peaks expected for soluble RNA and the two ribosomal RNA components when long labeling times were employed.

The amount of adenine-8-14C incorporated into DNA was determined by the incubation of 1.0 ml of culture in 1 n NaOH at room temperature for 16 hr, followed by precipitation with cold trichloroacetic
acid to a final concentration of about 10%. This procedure solubilizes all the RNA counts. The precipitates were again collected on membrane filters and counted in a gas-flow counter. Treatment of a cell lysate with deoxyribonuclease solubilized about 70% of these NaOH-resistant counts. Depurination of the DNA with 0.02 N HCl at 100 C for 1 hr, followed by paper chromatography of the hydrolysate, allowed the recovery of 95% of the NaOH-resistant counts as guanine and adenine. When protein was also labeled with 3H-lysin, the contamination of DNA counts with protein counts was less than 2%.

When the protein was labeled with a mixture of 14C-amino acids, a portion of the culture was boiled in 5% trichloroacetic acid for 30 min and the precipitate was collected on a membrane filter. The filters were counted in a gas-flow planchet counter. In the uniform-label experiments, when 3H-lysin was used as a label for protein, a portion of the culture was boiled in 10% trichloroacetic acid for 30 min to solubilize the 14C-adenine counts in RNA and DNA. The precipitate was collected on a membrane filter, which was treated in a scintillation vial with 0.4 ml of formic acid. The radioactivity was then counted in a toluene-ethyl alcohol scintillation mixture (5) in a scintillation counter. Although the efficiency of counting is low for 3H in this scintillation mixture (about 3%), the amount of quenching is influenced only slightly by variations in the number of cells on the filter.

**RESULTS**

**Isolation of ts - mutants.** Approximately 400 temperature-sensitive (ts -) mutants of *S. cerevisiae* were isolated by a replica-plating technique. The mutants do not form colonies at 36 C but do form colonies at 23 C; the wild type forms colonies at both temperatures. At least 90% of the mutants are known to be of independent origin, since they were obtained from independent samples of mutagen-treated cultures. No direct selective step was employed in the isolation procedure. Since the mutant clones have never been exposed to the nonpermissive temperature, mutants which lose viability at high temperature have not been selected against. The mutants have been assigned numbers from 92 to 489.

The ts - phenotype is probably not, in most cases, due to a lesion in the ability to synthesize small molecules such as vitamins, amino acids, purines, or pyrimidines, since the mutants were isolated on a complex medium containing a vitamin supplement, adenine, uracil, yeast extract, peptone, and two carbon sources, glucose and succinate. Furthermore, 160 of the mutants have been checked for temperature sensitivity on an enriched medium containing malt extract and Brain Heart Infusion in addition to yeast extract and peptone. All of the mutants except for one was ts - on this medium as well.

**Morphology and viability of ts - mutants.** Microscopic examination of the surface of agar plates indicated that most of the mutants formed less than 32 cells per clone after 3 days at 36 C, and that approximately 10% of the mutants underwent morphological alteration during their exposure to the nonpermissive temperature. Some of the mutants became larger or smaller than normal, and others appeared to lyse. Ten mutants underwent an extreme alteration in which the cells became very long and branched, resulting in a mycelial type of growth (Fig. 8).

A simple streak test was used to screen mutants for loss of viability at the nonpermissive temperature. A suspension of cells was streaked onto two plates. One was placed immediately at 23 C; the other was placed at 36 C for 16 hr and then shifted to 23 C. After 4 days of growth, the colony density on the two plates was compared. Approximately 40% of the mutants underwent a greater than 90% reduction in colony-forming ability during their exposure to high temperature.

**Dominance and recessiveness of ts - mutants.** All mutants were tested for dominance or recessiveness of their ts - mutation. They were mated with a wild-type diploid of opposite mating type. The diploid which resulted was isolated on selective media and streaked on plates at high and low temperature. The vast majority of the mutants displayed a recessive phenotype; that is, the diploid carrying the ts - mutation in the heterozygous state formed approximately equal numbers of colonies at high and low temperature. Sporulation of these diploids and an examination of the resulting haploids for temperature sensitivity demonstrated that the diploids which grew at 36 C were still heterozygous for the ts - mutation. A total of four dominant ts - mutations were found in the collection of 400 mutants (numbers 344, 349, 408, and 453). The diploids harboring these mutations in the heterozygous state formed significantly fewer colonies at 36 C than they did at 23 C. The ts - phenotype is either extremely leaky or subject to a high rate of reversion in the heterozygous state, as cultures of these diploids form up to 10% as many colonies at 36 C as they do at 23 C. Haploid cultures of these four mutants had a low incidence of revertent cells, 10 -8 to 10 -7.

**Pulse labeling for macromolecule synthesis.** A simple procedure was developed for screening large numbers of mutants for protein, RNA, and DNA synthesis at the nonpermissive temperature. The mutants were grown in liquid culture at 23 C in YM-5 medium. When they were in a logarithmically growing state, a portion of the culture was diluted threefold into YM-5 medium (at 36 C) containing 14C- amino acids (reconsti-
tuted 14C-protein hydrolysate; a second portion was diluted threefold into YM-5 medium (at 36 C) containing adenine-8-14C. After 3 hr at 36 C, samples were removed for measurement of the amount of radioactive amino acids incorporated into protein and the amount of radioactive adenine incorporated into RNA and DNA. During this 3-hr period, the growth of the original culture at 23 C was followed by turbidity measurements. About 85% of the mutants grew with rates between 0.80 and 1.10 of the wild-type growth rate at 23 C. The data obtained for some of the mutants are listed in Table 1. Figure 1 is a plot of the protein value for each of the 400 mutants as a function of the RNA value for the same mutants. Lines have been drawn along axes of protein to RNA ratios (P/R) equal to 1, 2, and 4. Almost all of the mutants display P/R ratios equal to or greater than 1. This observation can probably be explained on the basis of control mechanisms which shut down RNA synthesis as growth slows down for any one of a variety of reasons. The parent strain A364A has a stringent control over RNA synthesis in that the inhibition of protein synthesis by starvation for a required amino acid results in an immediate cessation of net RNA synthesis. A large number of mutants have P/R ratios greater than 4. These will be discussed below.

**Uniform-label experiment.** The data were analyzed and the mutants further studied with the aim of finding mutations which preferentially affect the following processes: protein synthesis, RNA synthesis, DNA synthesis, cell division, and cell wall formation. Certain mutants suspected of falling into one of these five classes, on the basis of the pulse-labeling experiments, were chosen and a uniform-label experiment was performed on them. In this experiment, the mutants were grown at 23 C from a small inoculum in medium containing 14C-adenine and 3H-lysine. It should be recalled that the mutants were all derived from a strain which was auxotrophic for lysine and adenine. When the cultures had reached a sufficient density and were still growing logarithmically, they were shifted to 36 C. At the time of the shift, the cell's protein is uniformly labeled with 3H, and the RNA and DNA is uniformly labeled with 14C. Thus, any increase in radioactivity after the shift to high temperature is directly proportional to the net increase in the particular macromolecule being studied. In contrast to the pulse-labeling experiment, there is no problem with pool equilibration.

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**Table 1. Growth rate, macromolecule synthesis, and survival of colony-forming ability of some representative ts mutants as determined in the pulse-labeling experiment.**

<table>
<thead>
<tr>
<th>Mutant no.</th>
<th>Growth rate at 23 C</th>
<th>Macromolecule synthesis at 36 C</th>
<th>Survival of colony-forming ability at 36 C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>RNA</td>
<td>DNA</td>
</tr>
<tr>
<td>108</td>
<td>0.95</td>
<td>0.35</td>
<td>0.07</td>
</tr>
<tr>
<td>159</td>
<td>1.05</td>
<td>0.80</td>
<td>0.72</td>
</tr>
<tr>
<td>171</td>
<td>1.03</td>
<td>0.02</td>
<td>0.04</td>
</tr>
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<td>172</td>
<td>0.87</td>
<td>0.23</td>
<td>0.20</td>
</tr>
<tr>
<td>187</td>
<td>0.99</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>236</td>
<td>1.11</td>
<td>1.09</td>
<td>0.74</td>
</tr>
<tr>
<td>282</td>
<td>1.06</td>
<td>0.30</td>
<td>0.07</td>
</tr>
<tr>
<td>289</td>
<td>0.99</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>310</td>
<td>0.86</td>
<td>0.54</td>
<td>0.64</td>
</tr>
<tr>
<td>344</td>
<td>1.26</td>
<td>0.73</td>
<td>0.42</td>
</tr>
<tr>
<td>349</td>
<td>0.73</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>408</td>
<td>0.88</td>
<td>0.55</td>
<td>0.39</td>
</tr>
<tr>
<td>438</td>
<td>0.76</td>
<td>0.31</td>
<td>0.34</td>
</tr>
<tr>
<td>453</td>
<td>0.92</td>
<td>0.66</td>
<td>0.73</td>
</tr>
<tr>
<td>471</td>
<td>0.98</td>
<td>0.79</td>
<td>0.90</td>
</tr>
</tbody>
</table>

* Growth rate is expressed as k mutant/k wild type, where k = (ln2 B/Bo)/t.

* Protein synthesis as measured by the amount of 14C from a 14C-protein hydrolysate incorporated into protein for a 3-hr period. RNA and DNA synthesis were measured by the amount of adenine-8-14C incorporated into RNA and DNA, respectively, over a 3-hr period. All values were multiplied by (turbidity of A364A/turbidity of mutant) at the time of the shift to 36 C and then divided by the value for A364A. Thus, all values are normalized to the value of 1.0 for A364A.

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**Fig. 1.** Protein values plotted as a function of RNA values as determined in the pulse-labeling experiment.
after the shift to 36 C. Samples were taken at various times after the shift and treated to measure the radioactivity in protein, RNA, and DNA. Cell number was determined in a Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.), and colony-forming ability was determined by dilution and plating. Figure 2 presents the results of a uniform-label experiment with a culture of the wild type, A364A ($ts^+$). The doubling time of the wild type at 23 C was approximately 3 hr. Immediately after the shift to 36 C, the doubling time of all macromolecules, as well as cell number and colony-forming ability, decreased to about 1.7 hr. The slowing down of RNA synthesis at 6 hr was due to an exhaustion of adenine from the medium. In experiments with the temperature-sensitive mutants, the cell density was kept considerably below this level.

_Mutations preferentially affecting protein synthesis._ Mutants incorporating less than 10% of the radioactivity incorporated by the wild type into protein in the pulse-label experiment (Fig. 1) are suspected of being "protein synthesis" mutants. There are 21 mutants in this category. All of these mutants also show low values for RNA synthesis.

Several of these mutants were examined in a uniform-label experiment. Most of these mutants showed little or no net increase in protein after a shift to 36 C. Figure 3 presents the results of a uniform-label experiment with mutant number 187. Protein and RNA showed no net increase after the shift. DNA increased by about 35%. Cell number remained constant and there was little loss of viability. Mutant 171 is similar in phenotype to number 187. Mutants of this type are currently being examined for their ability to synthesize protein in vitro.

_Mutations preferentially affecting RNA synthesis._ Those mutants displaying protein-RNA ratios of greater than 4 in the pulse-labeling experiment are suspected of being mutants for RNA synthesis. There are 38 mutants in this category. A uniform-label experiment was performed on about two-thirds of these mutants. In all cases, the net increase in RNA was inhibited to a far greater extent than was the increase in protein after the shift to 36 C. Figure 4 presents the results of a uniform-label experiment with mutant 108. Total protein increased 100% over the first 3 hr after the shift, whereas RNA increases by only about 5%. DNA increased 65%, cell number increased 35%, and viability fell off slowly. About 15 mutants were found with phenotypes very similar to number 108. (Number 282 is another example.) Others show a slow, but continuous, increase in net RNA. Since ribosomal RNA is the major component of cellular RNA, it is clear that the formation of ribosomal RNA is being preferentially shut off in mutant 108. It is unclear at the present time whether messenger RNA is being shut off. Experiments are being undertaken to characterize the small amount of RNA which is made after the shift.

_Mutations preferentially affecting DNA synthesis._ The data from the pulse experiment were
examined to uncover mutants with a specific inhibition of DNA synthesis. Nineteen mutants had an RNA-DNA ratio of greater than 2 and showed a significant amount of RNA synthesis (greater than 20% of that of the wild type). These mutants were all examined in a uniform-label experiment. Only four of the mutants displayed a significant preferential inhibition of

**Fig. 4.** Values for protein (P), RNA (R), DNA (D), cell number (N), and cell viability (V) as a function of the time after a shift from 23 to 36°C for a culture of ts" mutant 108. All parameters were normalized to a value of 1 at the time of the shift.

**Fig. 5.** Values for protein (P), RNA (R), DNA (D), cell number (N), and cell viability (V) as a function of the time after a shift from 23 to 36°C for a culture of ts" mutant 172. All parameters were normalized to a value of 1 at the time of the shift.

**Fig. 6.** Values for protein (P), RNA (R), DNA (D), cell number (N), and cell viability (V) as a function of the time after a shift from 23 to 36°C for a culture of ts" mutant 236. All parameters were normalized to a value of 1 at the time of the shift.

**Fig. 7.** Values for protein (P), RNA (R), DNA (D), cell number (N), and cell viability (V) as a function of the time after a shift from 23 to 36°C for a culture of ts" mutant 289. All parameters were normalized to a value of 1 at the time of the shift.
DNA synthesis after the shift to 36°C. Figure 5 presents the data from a uniform-label experiment with one of these four mutants, number 172. The increase in DNA after the shift was much less than that of RNA or protein. However, DNA synthesis proceeded at a slow, but significant, rate after the shift.

Mutations preferentially affecting cell division. Lesions in a number of cellular processes might be expected to result in a preferential inhibition of the increase in cell number. Most entities which count as single particles in the Coulter counter consist of a cell with a bud in various stages of development. Nuclear division occurs early in bud formation (13), so most of these single particles contain two nuclei. Thus, a lesion in an "early" process such as bud initiation or nuclear division might result in an increase in cell number of as much as 100%. A lesion in a "late" process, such as cytokinesis or the separation of the bud from its parent cell, should result in little or no increase in cell number. In either case, protein synthesis should continue for some time. A number of mutants with protein values of greater than 0.6 in the pulse experiment (Fig. 1) were examined for increases in turbidity and in cell number after

Fig. 8. Dark-field, phase-contrast photomicrographs of the wild type (A364A) and three mutants after incubation for 12 hr at 36°C in YM-1 medium, all at a magnification of × 770. (a) A364A; (b) mutant 471; (c) mutant 310; (d) mutant 159.
a shift to 36 C. Many mutants showed a parallel increase in cell number and turbidity for net increases of up to 10-fold after the shift. However, some mutants displayed the anticipated phenotype, with increases in cell number of less than twofold and much larger increases in turbidity.

These mutants were further tested in a uniform-label experiment. Figure 6 presents the results of this experiment with mutant 236. The increase in cell number is consistent with our expectations for a lesion in an "early" process of cell division, showing a net increase of 70%. By 10 hr after the shift, the cells are much larger than they were at the moment of the shift and display no small buds. The content of protein, RNA, and DNA per cell has increased by factors of 6.0, 2.3, and 2.0, respectively. By 5 hr, there is a 10-fold loss of viability.

Figure 7 presents the results of a uniform-label experiment with mutant 289. This mutant displays a pattern which is consistent with a lesion in a "late" process of cell division. The increase in cell number stopped by 3 hr, but macromolecule synthesis still continued at 6 hr. Cell number increased by only 20% after the shift, whereas all macromolecules increased by large amounts. By 6 hr after the shift, the culture contained 3.8 times as much protein, 2.6 times as much RNA, and 2.3 times as much DNA per cell as it did at the time of the shift. After an initial lag, there was a slow loss of viability.

**Mutations affecting cell wall formation.** Mutations in genes which function in cell wall formation would be expected to result in the lysis of the cell or in pronounced morphological changes after extended periods at the nonpermissive temperature. Macromolecule synthesis should be relatively unaffected initially. Consistent with these expectations is the finding that few, if any, mutants with low values for protein in the pulse experiment showed observable morphological alteration after long exposure to 36 C. However, a number of mutants with large values for protein (greater than 0.5) in the pulse experiment showed extensive alterations of shape and appearance. Many of these were detected by microscopic examination of agar plates on which the mutants had been streaked at high temperature during the initial isolation procedure. Figures 8b, c, and d are dark-field, phase-contrast photomicrographs of mutants 471, 310, and 159, respectively, after 12 hr in liquid culture at 36 C. Figure 8a is of the wild-type strain A364A (ts+) growing normally at 36 C. Before the shift to 36 C, the mutants were morphologically similar to the wild type. Mutant 159 underwent extensive lysis, and much extracellular debris was present in the culture fluid; a broken cell is evident in the Fig. 8d. Mutant 310 displays elongated cells and a lack of separation between daughter cells; many of the cells in this culture are lysing. Mutant 471 has undergone extensive elongation and branching; few, if any, of the cells have undergone lysis.

**Nonspecific lesions.** Only about 10 to 20% of the mutants display patterns in a uniform-label experiment which are consistent with a lesion that preferentially inhibits protein synthesis, RNA synthesis, DNA synthesis, cell division, or cell wall formation. In the rest, a preferential effect is not evident for any of the parameters measured. Mutant 438 is one such mutant (Fig. 9). Protein, RNA, DNA, and cell number all increased several fold after the shift. However, the rate of increase in these parameters was more linear than exponential.

**DISCUSSION**

It was estimated, in the introduction, that a haploid yeast cell contains enough DNA to produce 13,000 different proteins. Watson estimated that the number of proteins needed for small-molecule metabolism in *E. coli* is 600 to 800 (12). It seems unlikely that the number of small molecules or their routes of metabolism would be vastly different in yeast and in *E. coli*, and we may therefore assume a similar number in yeast. Of course, a variety of proteins are involved in macromolecule metabolism and in the structural proteins of cells such as amino acid-activating enzymes, ribosomal proteins, polymerases, and methylating enzymes. However, the number of identified proteins in this category would total no more than a few hundred. If we assume that all of the DNA in a yeast cell is active, in the sense that it produces functional proteins, then it is evident
that the functions of the vast majority (i.e., about 90%) of the gene products in one of the simplest eucaryotic cells are unknown. The work of Edgar and Lielausis (2) indicates that a large percentage of the proteins of an organism can mutate to a temperature-sensitive form. It therefore seems reasonable to conclude that most of the 400 mutants which were selected only on the basis of a temperature-sensitive phenotype have lesions in proteins whose function in cell physiology is entirely unknown. We hope that the continued study of these mutants will lead to the identification of the roles played by the products of some of these genes in cellular processes.

In this context, it is interesting that about 80% of the mutants do not show a preferential inhibition of any of the parameters measured after the shift to 36 C. Only the determination of further variables, such as oxygen uptake, active transport, and lipid metabolism, will provide a clue as to the site of the lesion in these mutants.

In fact, of the 20% which do appear to be preferentially affected in protein synthesis, RNA synthesis, DNA synthesis, cell division, or cell wall formation, it is entirely possible that their defect is only indirectly related to the process which is inhibited. Further characterization of these mutants is continuing.

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LITERATURE CITED


