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Ribosomal Precursor RNA Metabolism and Cell Division in the Yeast *Saccharomyces cerevisiae*

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Summary. When shifted from 23° C to 36° C, cells of a non-temperature-sensitive strain of yeast arrest transiently in G1 before continuation of the cell division cycle. When shifted to 36° C, cells harboring a temperature-sensitive *rna* mutation behave similarly. Others have shown that temperature shift transiently decreases the rates of production and processing of ribosomal precursor RNA (rpreRNA). Production of rpreRNA is soon restored to normal levels in these strains, but normal processing of these rpreRNA transcripts is restored only in non-temperature-sensitive strains. Therefore these experiments serve to eliminate from cell cycle considerations the involvement of processing of rpreRNA, while maintaining the established correlation between cell cycle behavior and rpreRNA production.

Regulation of cell division in the yeast *Saccharomyces cerevisiae*, as in most other eukaryotes, occurs in the G1 portion of the cell cycle (Hartwell, 1974). Hartwell and his colleagues (Hartwell, 1974; Unger and Hartwell, 1977; Johnston et al., 1977) have defined a period within G1 referred to as "start". The "start" event is operationally defined by the response of cells to the mating pheromone α-factor; haploid cells of the *a* mating type when treated with α-factor arrest cell division at "start" (Bücking-Throm et al., 1973). When cells of yeast are deprived of required nutrients (Hartwell, 1974) or limited for growth (Hartwell and Unger, 1977), they also G1 arrest at the "start" event.

We are interested in investigating the molecular basis for G1 arrest. One approach has been to identify procedures which cause cells to arrest in G1 (at "start") and correlate this arrest with alterations in metabolism. An instructive example has been provided by Hartwell and Unger (1977), who demonstrated that cycloheximide causes accumulation of cells in G1. Affecting the process of protein synthesis thus affects cell cycle regulation. In a similar fashion, we have shown that certain compounds which decrease the rate of rRNA production, without accompanying effects on the process of protein synthesis, also cause cells of *S. cerevisiae* to arrest in G1 at the "start" event (Johnston and Singer, 1978; Singer and Johnston, 1979; Singer et al., 1978). For all treatments leading to G1 arrest examined we noted a decrease in the rate of production of the 35S ribosomal precursor RNA (rpreRNA), as well as in the maturation of rpreRNA to mature rRNA (Johnston and Singer, 1978; Singer et al., 1978). Here we examine cell cycle regulation using temperature shift experiments, to further refine correlations between cell cycle arrest and rRNA production.

Warner and Udem (1972) found that upon shift from 23° C to 36° C yeast displayed a transient decrease in the rate of synthesis of rpreRNA. When we examined the rates of labelling both of RNA and of protein following such a shift up in the haploid yeast strain GR2 (Johnston and Singer, 1978), we found similar results (Fig. 1 a). As expected, protein synthesis continued at rates comparable to those of untreated cells; however, RNA labelling rates were decreased. Even when corrected for decreased specific activities of the UTP and CTP pools (Cashel et al., 1969; Table 1), our results suggest that an absolute decrease in rates of RNA synthesis occurs upon temperature shift.

As shown in Fig. 1 b, when cells of the non-temperature-sensitive strain GR2 were shifted from 23° C to 36° C, they became transiently arrested in G1 (cells in G1 can be identified by the absence of a bud [Slater et al., 1977]). Before the temperature shift approximately 45% of cells in the randomly growing population were unbudded and in G1. By 60 min after shift

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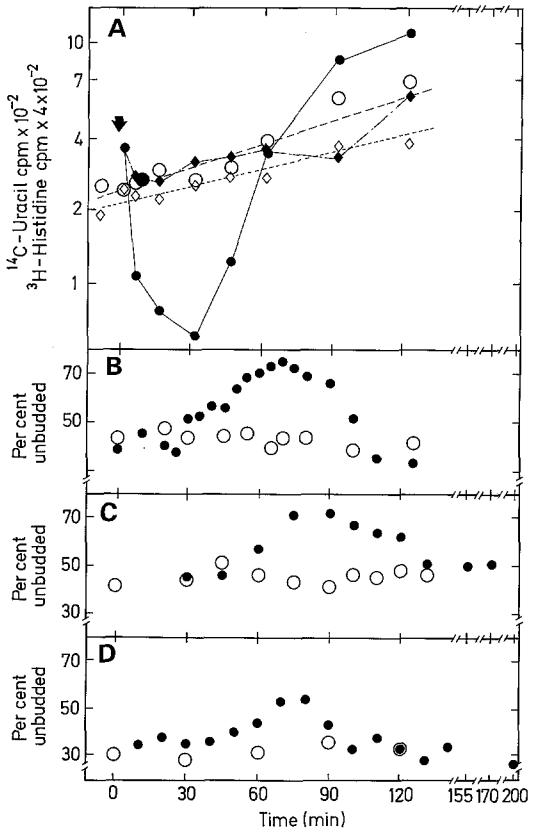

Fig. 1. Effects of temperature shift on growth. Cells were shifted from 23° C to 36° C at time zero. Panel *A* – rates of incorporation of histidine and uracil in cells of strain GR2 (Johnston and Singer, 1978). At intervals, samples were removed to tubes containing [^{3}H]-histidine (10 μCi/ml) and [^{14}C]-uracil (1 μCi/ml) for 5 min incubations at the same temperature as the parent culture. Incubation was stopped and incorporated label collected and counted as described previously (Johnston and Singer, 1978). Symbols: (○), uracil incorporation at 23° C; (●), uracil incorporation at 36° C; (◇), histidine incorporation at 23° C; (◆), histidine incorporation at 36° C. Panel *B* – cell cycle effect of temperature shift of strain GR2. Panel *C* – cell cycle effect of temperature shift of strain *ts*368 (*rna*2). Panel *D* – cell cycle effect of temperature shift of strain *ts*166 (*rna*6). Symbols: (○), culture at 23° C; (●), culture at 36° C

to 36° C the proportion of cells in G1 had risen to 75%. Cells arrested by the temperature shift were at or before the "start" event as shown by the response of these cells to the mating pheromone α-factor. After incubation at 36° C for 60 min, cells placed in the presence of α-factor remained arrested in G1 and displayed the distinctive morphology characteristic of α-factor treated cells. In the absence of α-factor cells at the high temperature resumed budding (data not shown).

Temperature shift undoubtedly causes many changes in macromolecular metabolism, including changes in rRNA metabolism. As noted above, Warner and his colleagues (Warner and Udem, 1972) have

Table 1. Nucleoside triphosphate pools after temperature shift

		23° C	36° C
15 min	UTP	1.0	0.51
	CTP	0.49	0.11
30 min	UTP	1.0	0.32
	CTP	0.63	0.13

Cells of strain GR2 were labelled for at least 6 generations with ^{14}C-uracil. At the times indicated after shift from 23° C to 36° C, samples were labelled for 6 min with ^{3}H-uracil. Nucleoside triphosphate pools were extracted with 1 M formic acid overnight at 4° C, and resolved by chromatography on PEI plates as described by Cashel et al. (1969). Spots of UTP and CTP comigrating with the extracts were scraped off the plates, hydrated with H_2O, and counted in Aquasol.

Values shown are ^{3}H/^{14}C ratios normalized to UTP ratios at 23° C for each time, and are the averages from two experiments. The amounts of ^{3}H in UTP spots were approximately 5-fold those in corresponding CTP spots; thus changes in UTP pool specific activity have far greater effects on label incorporation

extensively characterized the effects of temperature shift on rRNA metabolism in yeast. In addition to causing a transient decrease in the rate of rpreRNA production, temperature shift was also shown to cause transient decreases in the rates of synthesis of mRNA for ribosomal proteins (Warner and Gorenstein, 1977; Gorenstein and Warner, 1977). Since ribosomal proteins are required for normal processing of rpreRNA to mature rRNA (Gorenstein and Warner, 1976), decreased rates of synthesis of mRNA for ribosomal proteins would decrease rates of rpreRNA processing. Thus temperature shift causes in wild type strains decreases both in rpreRNA production and in its proper processing.

Because temperature shift may cause many other changes in cellular metabolism, temperature shift experiments may not be entirely appropriate for identifying major correlations between macromolecular metabolism and cell cycle behavior. Such is not the object here, since we have already identified correlations between rRNA production and cell cycle regulation (Johnston and Singer, 1978; Singer et al., 1978; Singer and Johnston, 1979). Now we can draw upon the large body of information on rRNA metabolism and temperature shift provided by Warner, and the characterization of certain mutant strains as discussed below, to *eliminate* from cell cycle considerations aspects of rRNA metabolism *not* correlated with cell cycle arrest.

To this end we examined the response to temperature shift of the *rna* strains (Hartwell et al., 1970). Strains carrying the temperature sensitive *rna* mutations, when transferred from 23° C to 36° C, become permanently defective in the production of mature

rRNA (Hartwell et al., 1970), but are still proficient in the transcription of rRNA genes (Shulman and Warner, 1978). The defects of 7 of the 11 different *rna* mutations with this phenotype have been determined; *rna*2, *rna*4, *rna*5, *rna*6, *rna*9, *rna*10 and *rna*11 all restrict the synthesis of ribosomal proteins (Gorenstein and Warner, 1976, and C. Gorenstein, personal communication). Thus in the continued absence of ribosomal protein synthesis, processing of the initial high molecular weight rpreRNA transcript does not occur properly and the rpreRNA is degraded, even after its rates of production return to normal. It is this discoordination between rpreRNA production and processing in these temperature-sensitive *rna* strains which we have exploited.

The kinetics of cell cycle arrest caused by temperature shift were determined for strains carrying *rna* mutations. We chose to examine strains carrying the *rna*2 and *rna*6 mutations since after shift to 36° C these strains displayed significant increases in cell number, greater than 1.5-fold. (Strains with other *rna* mutations arrested growth and cell division so abruptly that kinetics of bud initiation could not be determined). When the strains carrying *rna*2 or *rna*6 were shifted to the non-permissive temperature, they too exhibited transient G1 arrest with kinetics similar to those for the non-temperature-sensitive strain GR2. More importantly, the proportions of unbudded cells then returned to the range characteristic of a growing population (Figures 1c and 1d). Subsequent to this, there was little cell number increase as cells arrested randomly in the cell cycle.

Thus cell cycle behavior was in all strains still correlated with rpreRNA production, but not correlated with its processing. Temperature shift, which initially affects both rpreRNA production and processing, caused identical cell cycle effects in both wild type and temperature-sensitive strains. In contrast, the temperature-sensitive strains showed that with the resumption of rpreRNA production, but in the absence of normal rpreRNA processing, there was release of cells from G1 arrest and resumption of normal cell cycling. The observation reported here, coupled with earlier experiments (Johnston and Singer, 1978; Singer et al., 1978; Singer and Johnston, 1979) leads us to conclude that the aspect of rRNA metabolism correlated with cell cycle regulation is the production of rpreRNA.

This consistent correlation of rpreRNA production with cell cycle regulation (Johnston and Singer, 1978; Singer et al., 1978; Singer and Johnston, 1979) further leads us to speculate about those processes to which cell cycle regulatory machinery is sensitive. Several workers have established that the cell cycle regulatory machinery is sensitive to alterations in growth (Johnston et al., 1977; Hartwell and Unger, 1977; Johnston et al., 1979) and nutrient supply (Hartwell, 1974; Unger and Hartwell, 1979). Central to cell growth are processes such as protein synthesis and rpreRNA production leading to ribosome formation. As suggested previously (Unger and Hartwell, 1977; Hartwell and Unger, 1977; Johnston and Singer, 1978; Singer et al., 1978; Singer and Johnston, 1979), perturbations in the macromolecular biosynthesis processes may well cause G1 arrest although the detailed mechanism by which this G1 arrest is brought about remains to be delineated. In considering such a mechanism for G1 regulation, two aspects of yeast metabolism should be considered: (1) perturbations in production of rpreRNA, but not in its processing, appear to be correlated with G1 arrest; (2) yeast are stringent (Warner and Gorenstein, 1978), linking protein synthesis with the production of rpreRNA. These two characteristics suggest to us that the production of rpreRNA may be monitored in cell cycle regulation as a means of integrating information concerning both protein and RNA synthesis.

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