Short Communication

Ribosomal RNA Transcription in a Mutant of *Saccharomyces cerevisiae* Defective in Ribosomal Protein Synthesis

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Summary. In *Saccharomyces cerevisiae*, the transcription of ribosomal precursor RNA is severely inhibited in the absence of protein synthesis. However, such transcription is not dependent on the synthesis of ribosomal proteins, nor on the synthesis of mRNA for ribosomal proteins, nor on the processing of ribosomal precursor RNA.

The ribosome is a complex assembled from three or more RNA molecules and seventy or more proteins. How does the cell coordinate the synthesis of these different macromolecules? In bacteria, inhibition of protein synthesis by deprivation of an amino acid brings about a drastic reduction in the transcription of ribosomal RNA (reviewed by Gallant and Lazzerini, 1976). When protein synthesis is partially inhibited, the capacity for synthesis of ribosomal proteins is differentially reduced (Dennis and Nomura, 1974) due to a deficiency of mRNA for ribosomal proteins (Dennis and Nomura, 1975). In a mutant strain which exhibits relaxed control over RNA accumulation (Stent and Brenner, 1961), the transcription of both ribosomal RNA and of mRNA for ribosomal proteins is no longer regulated by amino acid deprivation (Dennis and Nomura, 1975).

In *Saccharomyces cerevisiae*, the transcription of ribosomal RNA is also inhibited when protein synthesis is suppressed by deprivation of an amino acid (Gross and Pogo, 1974; Shulman, Sripati and Warner, 1977) or by the addition of cycloheximide (de Kloet, 1966). We now ask if this effect on transcription is due specifically to the lack of a supply of new ribosomal proteins. This possibility can be examined by using temperature sensitive mutants of yeast (Hartwell, McLaughlin and Warner, 1970) which are defective in the synthesis of ribosomal proteins. In these mutants, at the restrictive temperature of 36°C, the synthesis of mRNA for ribosomal proteins and hence the synthesis of ribosomal proteins themselves is differentially depressed by more than 90 percent (Gorenstein and Warner, 1976). Although previous work employing a five minute pulse (Warner and Udem, 1972), suggested that ribosomal precursor RNA synthesis might be affected by such mutations, recent results raised the concern that newly synthesized RNA might have been degraded in those experiments. Therefore, we have repeated the measurement of RNA synthesis using a very brief pulse.

The transcription of ribosomal RNA under these conditions is measured as described in Figure 1. Wild type (A364A) or mutant (ts 368) cells were labeled for several generations with 14C uracil. They were then pulse-labeled for 30 seconds with 3H [methyl] methionine after one hour of incubation at 36°C, the nonpermissive temperature for the mutant. Methionine was used to pulse-label the RNA because the labeling of the pool of S-adenosyl methionine is far less sensitive to culture conditions than is the labeling of the pools of nucleoside triphosphates (Warner, Morgan and Shulman, 1976; Shulman, Sripati and Warner, 1977). Even under severely non-physiological conditions, the incorporation of methyl groups into newly transcribed RNA appears to occur normally (Shulman, Sripati and Warner, 1977). Thus C5H5 incorporation is an accurate measure of transcription.

Figure 1 shows the results of such an experiment. The 14C uniformly labeled 25S and 18S ribosomal RNA serve as an internal control to monitor the
Fig. 1. Analysis of methyl-labeled ribosomal RNA Saccharomyces
cerevisiae, A364A (ATCC No. 22244), and a temperature sensitive
mutant, ts 368, derived from it, which is unable to accumulate
ribosomes at the restrictive temperature, have been described (Hart-
well et al., 1970; Warner and Udem, 1972). The cells were grown
as previously described (Udem and Warner, 1972; Warner, 1971).
Cells were grown overnight at 23°C to 1 x 10^6/ml in synthetic me-
dium (SC) containing 0.05 μCi/ml [14C] uracil. The cultures were
shifted to the restrictive temperature (36°C) and held for 1 hr before
commencing the experiment. The cells were pulse-labeled for 30 s
with 100 μCi/ml L-[methyl 3H] methionine as previously described
(Udem and Warner, 1972; Shulman et al., 1977). The pulse was
terminated by pouring the culture onto ice. The cells were collected
by centrifugation, washed once with cold H2O, suspended in 1/10
volume of cold 1 M D-sorbitol containing 2 μg/ml zymolase (Kirin
Brewery) and allowed to spheroplast at 4°C. The spheroplasts were
collected by centrifugation, washed once with cold 1 M D-sorbitol
and lysed in a solution containing 0.1 M lithium chloride, 0.01 M
EDTA, 10 mM Tris pH 7.4, and 1% SDS. The RNA was phenol
extracted (Udem and Warner, 1972), and analyzed by electrophoresis
in 2.75% gels (Shulman, Sripati and Warner, 1977), which
were sliced and counted (Udem and Warner, 1972). Migration
is from left to right. A, A364A; B, ts 368. [3H/14C]ts368/[3H/14C]A364A = 0.84

Table 1. Synthesis of ribosomal RNA

<table>
<thead>
<tr>
<th></th>
<th>Total 3H cpm</th>
<th>Total 14C cpm</th>
<th>3H/14C</th>
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<tbody>
<tr>
<td>A364 A</td>
<td>7090</td>
<td>7427</td>
<td>0.96</td>
</tr>
<tr>
<td>ts 368</td>
<td>5260</td>
<td>6521</td>
<td>0.81</td>
</tr>
</tbody>
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The [3H/14C]ts368/[3H/14C]A364A = 0.84

yield of RNA through preparation and analysis. In the wild type, strain A364A, the 3H is found mainly
in 35S precursor molecules, but some processing to
27S and 20S intermediates has occurred, even during
a pulse of 30 s (Udem and Warner, 1972). In the
mutant, there is substantial incorporation of 3H into
35S molecules, but little if any processing occurs,
preumably due to the absence of ribosomal proteins
(Gorenstein and Warner, 1976).

The data of Figure 1 are analyzed quantitatively
in Table 1. At the restrictive temperature, cells of
ts 368 transcribed ribosomal precursor RNA at 84% of
the wild type rate. In a number of experiments
conducted with minor variations, values of 60% to
95% were obtained. Ribosomal precursor RNA
does not accumulate, however, (Warner and Udem,
1972) implying that it is degraded rapidly in the ab-
sence of ribosomal proteins.

Under the conditions of Figure 1, cells of ts 368
have transcribed no mRNA for ribosomal proteins
for nearly sixty minutes and have synthesized riboso-
mal proteins at an exponentially decreasing rate which
is only five to ten percent of normal at the time
of the pulse (Gorenstein and Warner, 1976; Warner
and Gorenstein, 1977). Thus the results of Figure 1
and Table 1 demonstrate that the transcription of ri-
bosomal precursor RNA in yeast is not dependent
on concurrent synthesis of ribosomal proteins or of
their mRNAs. Therefore, the inhibition of transcrip-
tion of ribosomal RNA which occurs when cells are
deprived of an amino acid (Shulman et al., 1977)
is caused not by a lack of ribosomal proteins but
by some factor dependent on protein synthesis in gen-
eral. Unlike the stringent response in E. coli, the key
factor does not appear to be uncharged tRNA, since
cycloheximide is almost as effective as amino acid
starvation in inhibiting transcription of ribosomal
RNA (Shulman et al., 1977).

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