Regulation of the RNA Polymerase I and III Transcription Systems in Response to Growth Conditions*

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To better understand the mechanisms that regulate stable RNA synthesis, we have analyzed the RNA polymerase I and III transcriptional activities of extracts isolated from cells propagated under a variety of conditions. Under balanced growth conditions, the levels of both RNA polymerase I- and III-specific transcription increased proportionally with growth rate. Upon nutritional starvation, RNA polymerase I transcription rapidly declined, followed by S S rDNA and eventually tDNA transcription. Transcriptional activities in extracts were restored when the non-growing cultures were suspended in fresh medium, although growth did not resume. The differential expression of S S rDNA and tDNA genes in extracts prepared from cells subjected to partial starvation was traced to a S S rDNA-specific inhibitor and not to a defect in any RNA polymerase III transcription factor. Characterization of this inhibitor indicated that it was not S S rRNA. It was sensitive to phenol extraction and resistant to RNase, and its target did not appear to be transcription factor IIIA. Not all treatments that slowed or stopped growth down-regulated the stable RNA transcription apparatus. Cells that have been subjected to either energy starvation or cycloheximide treatment still retain the ability to synthesize stable RNA in vitro, suggesting the presence of alternative regulatory mechanisms.

It has been appreciated for a number of years that organisms adjust their translational capacity to meet, but not exceed, the need for protein synthesis. A central aspect of this regulation is the control of stable RNA (tRNA and rRNA) production. In procaryotes the three RNA genes are cotranscribed with a number of tRNA genes by the same RNA polymerase, providing a simple target for regulation, initiation of transcription. In eucaryotes three RNA polymerase complexes are responsible for stable RNA synthesis. RNA polymerase I produces the 35 S rRNA molecule that is processed into the three largest rRNAs, while the smallest rRNA and tRNAs are produced by RNA聚合ase III. In vivo analyses of Saccharomyces cerevisiae under a variety of treatments clearly establish a direct link between translational load, stable RNA synthesis, and ultimately ribosome biogenesis. Under some conditions the coordinate synthesis of both rRNA and tRNA is observed. For example, cells with slower balanced (constant) growth rates, have decreased levels of both rRNA and tRNA synthesis, although tRNA synthesis is decreased to a lesser extent. Similar coordinated regulation is observed during some unbalanced, transitory, growth conditions. Upon nitrogen starvation, both rRNA and tRNA synthesis are quickly shut off. Likewise, in response to a nutritional upshift, the synthesis of both rRNA and tRNA rapidly increases, although rRNA at a faster rate.

Regulatory events involved in transcriptional upshift are likely to be different in unbalanced growth (13–15). In these cases, this response is due to the inactivation of either RNA聚合ase I or a tightly associated factor. This factor, known as C*, TIFI-A, or TFIC (13, 16–18), is necessary for formation of the initiation complex and is inactivated early in the transcription cycle (16, 19, 20). Although the modulation of RNA聚合ase I has been the best studied regulatory response, several lines of evidence suggest the presence of other regulatory mechanisms, including the modification of RNA聚合ase I transcription factor (21–23) or the accumulation of specific inhibitors (24, 25). Less is known about the molecular basis of RNA聚合ase III regulation. During cessation of growth and mitosis, tRNA synthesis declines due to reduced activity of the transcription factor TFIIIB (26–31). A transcriptional inhibitor that interacts with the TATA-binding protein in TFIIIB has been identified, although its function in regulation is not clear (32). In contrast, viral infection and serum factors have been shown to alter the activity of the TFIIIC fraction (33, 34). Recently the differential expression of the 5 S rRNA and tRNA genes during encystment in Acanthamoeba castellanii has been attributed to the disappearance of the 5 S rRNA-specific transcription factor TFIIIA (35).

Despite this progress, very little is known about the overall picture of stable RNA synthesis in any one organism, since few studies have examined both the RNA聚合ase I and III transcription complexes under a variety of conditions. There are compelling reasons to address these questions using S. cerevisiae. The ease with which yeast are cultivated in defined media and the availability of a number of genetic backgrounds facilitate the manipulation of balanced and unbalanced growth rate by altering the growth media. Despite these advantages, virtually all of the work in yeast has been restricted to in vivo analysis, largely due to the technical difficulties of isolating RNA聚合ase I and III transcription extracts from small quantities of cells. To facili-
tate the in vitro analysis of stable RNA transcription, we recently developed a method for the preparation of both RNAP I and RNAP III (5 S rDNA and tDNA) transcription extracts from less than 1 g of cells (36). This protocol minimizes the chance of inactivation due to trivial reasons, since no column chromatography is involved, and only at the last step is the RNAP I extract separated from the RNAP III extract. Here we describe the analysis of stable RNA synthesis in extracts prepared from cells that have been subjected to a variety of different growth conditions.

MATERIALS AND METHODS

Plasmids—The plasmid pDR10 linearized with EcoRV was used to assay for 35 S rRNA synthesis by RNAP I (36). The 5 S rDNA gene used in density less than 1.0 A260 unit at all times. In all cases supernatants were incubated for 20 min at 30°C with 2 fmol of probe in reaction containing 20 mM Tris acetic, pH 7.5, 200 mM potassium glutamate, 10 mM magnesium acetate, 10 mM β-mercaptoethanol, 10% (v/v) glycerol, 0.5% (w/v) polyvinyl alcohol, and 100 ng of vector DNA (pBSKSII-) in a total volume of 20 µl. Samples were digested with 0.05–0.1 units of DNase I (RNase-free; Boehringer Mannheim) for 0.5–2 min at 30°C. Digestion was terminated with the addition of 10 µl of stop mix that contained 75 mM EDTA, 0.5 mg/ml sheared salmon sperm DNA, and 1.7 M potassium acetate. Samples were extracted with phenol-chloroform and precipitated with ethanol. The pellets were resuspended in formamide load buffer and run on 10% polyacrylamide (37.5% acrylamide:10% bisacylamide) containing 8 M urea.

Growth of Cultures—The yeast strain O22 (MATa his2–1 A50–700 mM KCl gradient. The inhibitor was removed from 5 S rRNA / tRNA extracts by adjusting the extract to 500 mM KC1 and loading on a Q column (Macro-pret® high load, Bio-Rad; 10 mg of protein load per ml of resin), and the flow-through was collected and assayed. Fractions were treated with immobilized RNase (on acrylic beads; Sigma, catalog number R-7005) that had been prepared in the following manner. First, approximately 3 mg of RNase beads was extensively washed with 1 ml of water at 0°C. Protein binding sites on the beads were blocked by incubation in the presence of 50 µg of bovine serum albumin in a volume of about 100 µl at room temperature for 30 min followed by another extensive water wash. The Q-550 fraction (100 µl) was added to the moist beads and incubated at room temperature for 30 min with occasional gentle mixing. The supernatant fraction was withdrawn and passed through a small empty chromatography column to remove the residual beads. Digestion of the RNA was carried out using polyacrylamide gel electrophoresis of the treated sample. The stability of the RNase on the beads was confirmed by analyzing both the final water wash and the treated sample for the presence of RNase.

RESULTS

In the experiments described below, we have examined the RNAP I and III transcriptional capacity of extracts prepared from cells in balanced and unbalanced growth. In exponential phase, cells are in balanced growth, that is all cellular constituents are synthesized at a constant rate. In contrast, changes in environmental conditions provoke unbalanced growth conditions where the cellular components are differentially expressed, which enables the cell to adapt to the altered environment. If the new conditions permit growth, this transient phase of unbalanced growth yields to a new balanced growth phase, at a growth rate determined by the new growth conditions.

Balanced Growth Rate Regulation of Stable RNA Synthesis—We examined cultures growing at decreasing growth rates under steady state, balanced growth conditions. In these experiments the cell density was kept low (less than 1.0 A260 unit) by diluting the culture into fresh, warm medium. The strain O22 was cultured in a minimal medium with glucose as the carbon-energy source and ammonium sulfate, glutamine, valine, or tyrosine as the sole nitrogen source. These cultures had generation times of 1.5, 3, 5, and 8 h, respectively. The cells were harvested, and RNAP I and III transcription extracts (low salt pellets and supernatants) were prepared as described previously (36). The levels of specific RNAP I and III transcription were analyzed in vitro using a 35 S rDNA (to assay RNAP I), 5 S rDNA, or tDNA template. Extracts prepared from the cells having a reduced balanced growth rate supported reduced levels of both RNAP I and III transcription (Fig. 1), although RNAP I transcription was the most sensitive to the decreased growth rate. We have also observed similar results in response to changes in growth rate brought about by the substitution of different carbon/energy sources in a rich medium (for example see Fig. 6C). This adjustment of the RNAP I and III transcriptional activities in response to a range of balanced growth rates appears to be sufficient to account for the regulation of stable RNA synthesis observed under balanced growth conditions in vivo.

Differential Regulation of Stable RNA Synthesis during Entry into Stationary Phase—We have also examined the cellular response to the imposition of unfavorable growth conditions. Previously, we characterized inactivation of RNAP I transcription during the transition between exponential phase, when glucose is fermented and the cells grow with a generation time of 1.5 h, and stationary phase (36). In this study we have
extended this analysis by characterizing the 5 S rDNA and tDNA transcriptional activities of extracts isolated from cells during the transition phase. Three sequential samples were taken from a transition phase culture (samples A, B, and C in Fig. 2, top). RNAP III transcription extracts prepared from these samples were assayed for tDNA and 5 S rDNA transcription. Whereas in early transition phase RNAP III was equally active on both templates, as the culture progressed further into the transition phase, a striking decrease in 5 S rDNA transcription was observed (bottom). Extracts prepared from the culture in mid-transition phase (such as sample B) showed slightly decreased tDNA transcriptional activity, while 5 S rDNA activity was almost totally abolished. We have observed this differential expression in all extracts prepared from high density cultures. The persistent tRNA synthetic capacity in these slowly growing cells (generation times of greater than 24 h) in unbalanced growth is in sharp contrast to the lack of significant tRNA synthesis in extracts made from slowly growing cells in balanced growth (8-h generation time, Fig. 1).

It has been reported recently that several characteristics of stationary cells can be reversed by incubation in the presence of glucose (39). To determine if stable RNA synthesis can be restored, we replaced the spent growth medium (in which all of the glucose has been consumed) in transition phase cultures (like culture B in Fig. 2) with fresh growth medium. RNAP I transcription and 5 S rRNA synthesis in extracts, which had been turned off completely, were activated by this treatment, and tRNA synthesis was further stimulated (Fig. 3A). When cycloheximide was in the recovery medium no activation occurred (lane 3). This activation was transient, since extracts prepared from cultures that had been incubated for longer than 1.5 h had significantly reduced levels of stable RNA synthesis (Fig. 3B). No significant growth (cell division) was observed, presumably because of the high cell density, during the incubation period in fresh growth medium, and the only visible change in cell morphology was the appearance of buds, which correlated with the peak of activation. Unlike other characteristics of stationary phase cells, resuspension in a glucose solution was not sufficient to activate stable RNA synthesis. Only in the presence of glucose in a complete medium (either fresh or spent) were RNAP I and III transcription-activated.

Starvation for Essential Nutrients Regulates Stable RNA Transcription—One of the classical downshift conditions that has been extensively studied in procaryotes is starvation for an essential amino acid. The collective change in gene expression, turning off rRNA and tRNA synthesis and turning on amino acid biosynthetic genes, is termed the stringent response. To examine this response in yeast, we shifted a culture of a histidine auxotroph from minimal medium containing histidine into one lacking histidine. Under these conditions, the culture continues to grow at a 1.5-h doubling time as internal histidine pools are utilized, and then it gradually stops growing (Fig. 4, top). An extract prepared from a culture having a reduced growth rate (extract A) did not support RNAP I or 5 S rDNA transcription while tRNA synthesis continued (Fig. 4, bottom). An extract prepared from the culture after growth had ceased (extract B) was totally defective in stable RNA transcription. This response is specifically due to starvation for histidine, since supplementation of the nongrowing culture with histidine restores growth. The inactivation of the transcription we observed is sufficient to account for the noncoordinated synthesis of rRNA and tRNA in response to amino acid starvation in vivo (2, 5). Using a similar approach we also examined the effect of starvation for nitrogen on RNAP I and III transcription in extracts (not shown). Within 2 h after the growth rate changed, RNAP I transcription was turned off. Once again, when growth had ceased, all stable RNA synthesis was eliminated, paralleling what has been observed in vivo (2).

Growth Rate Can Be Altered Without Affecting the Activity of Components of the Stable RNA Transcription Systems—Numerous studies suggest the activities of the RNAP I and III transcription systems are directly regulated by growth rate. We have identified several conditions under which the growth rate significantly decreases without altering the integrity of any RNAP I or III transcription factors required for specific transcription in vitro.

The addition of the protein synthesis inhibitor cycloheximide to a culture in exponential phase results in the eventual cessation of cell growth. Within several hours of addition, growth slowed at a cell density considerably lower than that of un-
ences between strains.
cell density at which the cycloheximide was added or to differ-
cycloheximide treatment. This discrepancy may be due to the
two components of the RNAPII ifactor TFIIIB in response to
retained significant specific RNAP I and III transcriptional
cultures, which had been energy-starved for as long as 15 h
cultures or the cultures were aerated. Extracts prepared from
These nongrowing cells were essentially energy-starved.
immediately ceased when the glucose was exhausted (Fig. 5,
that contained limiting amounts of glucose (1%, w/v), growth
regardless of the extent of aeration. But in nonaerated cultures
implying the changes in cellular metabolism that might be en-
cultures without aeration in YEP medium containing limiting (1%, w/v) glucose (top, closed circles) or by the addition of glucosamine to an exponential phase culture growing in YEP containing 2% (w/v) glucose (top, graph, inset). Gluco-
saminewasadded(1.5%(w/v)finalconcentration)atthetime
breakage with glass beads had to be care-
the cell density at which the cycloheximide was added or to differ-
treated cultures (Fig. 5, top). To our surprise RNAP I and III
extracts prepared from these cycloheximide-treated cells were
very active, even when protein synthesis had been inhibited for
as long as 15 h (Fig. 5, bottom). Numerous extracts have been
prepared from cycloheximide-treated cultures, and as long as
the addition was made to the cells while they were in mid-
exponential phase (several generations before leaving exponen-
tial phase), the extracts were all very active. We have observed
that the cycloheximide treatment for long periods of time made
the cells much easier to break open. To preserve the transcrip-
tional activities, the breakage with glass beads had to be care-
fully monitored to avoid excessive cell lysis, which inactivates
extracts. These results with cycloheximide appear to be at odds
with those of Dieci et al. (28), who observed specific inactiva-
tion of two components of the RNAP III factor TFIIIIB in response
to cycloheximide treatment. This discrepancy may be due to the
cell density at which the cycloheximide was added or to differ-
ences between strains.

A second approach to examining the relationship between
growth rate and stable RNA transcription was the manipula-
tion of the energy source. When energy is derived from glucose
fermentation in a rich medium, cultures grow at the same rate
regardless of the extent of aeration. But in nonaerated cultures
that contained limiting amounts of glucose (1%, w/v), growth
immediately ceased when the glucose was exhausted (Fig. 5,
top), since the remaining carbon sources were nonfermentable
and there was insufficient oxygen present for respiration.
These nongrowing cells were essentially energy-starved.
Growth immediately resumed if glucose was added to these
cultures or if the cultures were aerated. Extracts prepared from
cultures, which had been energy-starved for as long as 15 h
retained significant specific RNAP I and III transcriptional
activities (Fig. 5, bottom). A second approach we used to elicit
energy starvation was to supplement a culture growing in a
rich medium containing glucose with the nonmetabolizable
glucose analog glucosamine. Glucosamine inhibits the intrac-
A–C
Regulation of Stable RNA Synthesis

FIG. 4. Stable RNA synthesis in cultures subjected to histidine
starvation. Cultures of a histidine auxotroph grown in minimal media
were deprived of histidine by dilution with fresh warm media lacking
histidine. At all times the cultures were kept at a low cell density (less
than 1.0 A

Energystarvationwaselicitedbygrowingaculturewithoutaerationin
YEP medium containing limiting (1%, w/v) glucose (top, closed circles)
or by the addition of glucosamine to an exponential phase culture
growing in YEP containing 2% (w/v) glucose (top, graph, inset). Gluco-
saminewasadded(1.5%(w/v)finalconcentration)atthetime
indicated by the arrow, and the dashed line represents the 1.5-h generation time
of an exponential phase culture. Inhibition of protein synthesis was
achieved by the addition of cycloheximide to an exponential phase
culture (open circles). Cycloheximide was added to a final concentration
of 10 \( \mu \text{g/ml} \) at a cell density corresponding to 0.8 A

Extracts—

FIG. 5. The RNAP I and III transcription systems were not
regulated in response to all treatments that inhibit growth.
Energy starvation was elicited by growing a culture without aeration in
YEP medium containing limiting (1%, w/v) glucose (top, closed circles) or
by the addition of glucosamine to an exponential phase culture
growing in YEP containing 2% (w/v) glucose (top, graph, inset). Gluco-
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culture (open circles). Cycloheximide was added to a final concentration
of 10 \( \mu \text{g/ml} \) at a cell density corresponding to 0.8 A

Extracts prepared from exponential phase cells collected from a
culture grown in minimal medium containing histidine.

activity, the RNAP I nonspecific transcriptional activity, and
activity). The B activity (defined as the activity that restores specific
RNAP I transcription in the presence of the RNAP I A and C
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activities), the RNAP I nonspecific transcriptional activity, and
the ability to restore inactive extracts, have copurified. Yeast appears to regulate the response to all of these diverse environmental changes through a common mechanism, the modification of either the RNAP I enzyme itself, or a tightly associated factor.

In a similar manner, we identified the chromatographic fraction that restored specific RNAP III activity in extracts prepared from treated cells. Active RNAP III (5 S rRNA "tRNA") transcription extracts were loaded onto a Q column, which was developed with a KCl gradient. The fraction eluting in the 250 mM KCl ("Q-250" fraction) was sufficient to restore 5 S rDNA transcription in extracts from nitrogen-starved cells and slowly growing cells (Fig. 6, D and E). The synthesis of 5 S rRNA in a transition phase cell extract was also restored with this fraction to levels comparable with the tDNA transcriptional activity (Fig. 6F). All of the inactive 5 S rDNA transcription extracts examined were restored with the Q-250 fraction. This same fraction also restored tRNA synthesis to extracts prepared from slowly growing cultures (Fig. 6G). Thus, it appears that a factor(s) in the Q-250 fraction is the target of regulatory mechanisms that are responsible for the coordinate, as well as the coordinate, regulation of 5 S rRNA and tRNA synthesis, which have been observed both in our extracts and in vivo.

Identification of a 5 S rRNA-specific inhibitor—Either of two simple models could explain the selective inactivation of 5 S rDNA transcription (such as in transition phase extracts). Either a 5 S rDNA-specific factor in the Q-250 fraction becomes inactivated, or alternatively, an inhibitor interferes with the activity of a factor in the Q-250 fraction on 5 S rDNA templates. To distinguish between these possibilities, we performed extract mixing experiments. The addition of a 5 S rRNA "tRNA" extract to a 5 S rRNA "tRNA" extract resulted in decreased 5 S rRNA synthesis (Fig. 7A, lanes 2 and 3), suggesting the existence of a 5 S rRNA-specific inhibitor. This effect did not appear to be due to saturation of the transcription assay, since doubling the amount of the 5 S rRNA "tRNA" extract increased the level of 5 S rDNA transcription (lane 4).

If this specific inhibitor is solely responsible for the lack of 5 S rRNA synthesis in these cell extracts, when the 5 S rRNA "tRNA" extract is chromatographed, we should be able to 1) isolate the inhibitor in a chromatographic fraction, 2) show that the inhibitor abolishes the ability of a Q-250 fraction prepared from exponential phase cells to restore 5 S rRNA synthesis while not affecting the ability of this Q-250 fraction to rescue tRNA synthesis, 3) restore 5 S rRNA-deficient extracts with the Q-250 fraction derived from the 5 S rDNA "tRNA" extract, and 4) restore 5 S rRNA synthesis from the 5 S rDNA "tRNA" extract by removing the inhibitor. To address these points we chromatographed a 5 S rDNA "tRNA" extract on a Q column developed with a KCl gradient. Individual fractions were then assayed for the inhibitory properties of the extract from which they were derived. A fraction eluting in 550 mM KCl was found to have such an activity (Fig. 7B, lanes 2 and 3), which was not found in the Q-550 fraction prepared from transcriptional active extracts (lane 6). When the Q-550 fraction containing the inhibitor was preincubated with a 5 S rRNA "tRNA" fraction, the Q-550 fraction was no longer able to restore 5 S rRNA transcription, although it could restore tRNA synthesis (Fig. 7C). Treatment of the Q-550 fraction with RNase or phenol indicated that the inhibitor was not RNA but rather a protein (lanes 7 and 10). The most potent inhibition of 5 S rRNA transcription required preincubation of the Q-250 and Q-550 fractions before addition to the transcription assay, suggesting that this inhibitory property is the result of interactions between factors in these two fractions rather than decreasing the stability of the 5 S rRNA transcript (not shown). To determine if the presence of the inhibitor in the Q-550
KCl from a Q column developed with a salt gradient. We have determined that none of the components of the RNAP III transcription apparatus. Using reconstitution studies with extracts from exponential cultures, it was possible to rescue 5 S rRNA synthesis in extracts (Fig. 8A), suggesting that the 5 S rRNA transcription system was intact. To directly test this, we chromatographically separated the inhibitor from the RNAP III transcription apparatus. Using reconstitution studies with extracts from exponential cultures, it was determined that none of the components of the RNAP III transcription apparatus bind to a Q matrix in 500 mM KCl. To recover the RNAP III components from a 5 S rRNA-5 S rDNA extract, it was adjusted to 500 mM KCl and then chromatographed through a Q column. A significant amount of 5 S rDNA transcriptional activity was recovered in the flow-through from these 5 S rRNA-deficient extracts (Fig. 8B). These experiments are all consistent with the proposal that the selective inactivation of 5 S rRNA synthesis, which has been observed in vivo and in our extracts, is due to the accumulation of an inhibitor rather than the inactivation of a RNAP III transcription factor.

We have characterized the Q-250 fraction, as well as other fractions from the Q column, to identify the target of this inhibitor. Using DNase footprinting on a tRNA gene, we detected TFIIIC in the Q-250 fraction. This fraction did not contain a significant RNAP III activity, as measured by nonspecific transcription assays. When the Q-250 fraction was supplemented with proteins eluted from a Q column between 100 and 300 mM KCl, 5 S rRNA synthesis was restored (Fig. 9A, lanes 1 and 2). 5 S rRNA synthesis required the addition of a fraction eluting from the Q column between 100 and 300 mM KCl. This factor(s) required only for 5 S rRNA synthesis eluted in 140 mM KCl from a Q column developed with a salt gradient. We have identified TFIIIA in this fraction based on its distinctive footprint on 5 S rDNA (Fig. 9B), which is identical to previously published footprints (37). Additionally we used highly purified RNAP III transcription factors obtained from Drs. George Kasaveti and E. Peter Geiduschek to help characterize our fractions. Using these fractions, we have determined that in addition to TFIIIC our Q-250 fraction contains three of the known TFIIIB polypeptides, the TATA-binding factor, BRF, and B′.

FIG. 8. 5 S rRNA/tRNA\(^{+}\) extracts had an active 5 S rDNA transcription apparatus. A, the Q-250 fraction derived from these extracts restores both 5 S rRNA and tRNA synthesis in inactive extracts prepared from nitorgen starved cultures (Fig. 6D). B, 5 S rRNA transcription can be restored in 5 S rRNA/tRNA\(^{+}\) extracts by chromatography. A 5 S rRNA/tRNA\(^{+}\) extract (lane 1) was chromatographed through a Q column at 500 mM KCl. The flow-through (Q-FT) was collected and assayed on a 5 S rDNA template (lanes 2 and 3). The transcriptional activity of an extract from exponential cells is shown in lane 4.

Recently, Dieci et al. (28) have identified two components of the RNAP III transcription factor IIIB, BRF and B′, as the target of the regulatory response to the cessation of cell growth in response to cycloheximide treatment. Consistent with these observations, the addition of both BRF and B′ is required to restore RNA synthesis in extracts prepared from slowly growing cells in balanced growth. The addition of the same amounts of these factors (as well as TFIIIC) did not restore 5 S rDNA transcription in the same extracts (not shown). These restoration experiments and the isolation of 5 S rRNA-5 S rDNA extracts are consistent with a regulatory mechanism that enables the cell to differentially regulate RNAP III-specific transcription.

DISCUSSION

Our results indicate that the regulation of yeast stable RNA synthesis observed in vivo under a variety of balanced as well as unbalanced growth conditions is mediated by several mechanisms: the accumulation of an inhibitor that acts on 5 S rRNA synthesis, the previously observed inactivation of RNAP III transcription factors to reduce both 5 S rRNA and tRNA production (28, 29), and the control of either the RNAP I enzyme or a tightly associated protein (36). It seems reasonable that the differential regulation of 5 S rRNA and tRNA transcription, which has been observed in vivo (2, 5) and here in vitro, might involve the 5 S rDNA-specific factor, TFIIIA. In addition to binding the 5 S rDNA gene, TFIIIA binds to the gene product, the 5 S rRNA (41, 42), resulting in inhibition of transcription. In vitro experiments suggest that free ribosomal protein YL3 might prevent this sequestration of TFIIIA by forming a YL3-5 S rRNA complex (43), providing a link between a free ribosome component assembly (free YL3) and 5 S rRNA synthesis. Our experiments do not support a role for TFIIIA in this regulation. The TFIIIA-containing Q-column fractions do not rescue 5 S rRNA synthesis in 5 S rRNA/tRNA\(^{+}\) extracts. Instead, this regulation appears to be due to the accumulation of a proteinaceous inhibitor, which interferes with the function of a factor in the Q-250 fraction on 5 S rDNA. This inhibitor does not appear to be the yeast homolog of the transcriptional inhibitor DR1, since DR1 is a potent inhibitor of RNA synthesis (32). We speculate that the target of the inhibitor may be a 5 S rDNA-specific factor or activity associated with TFIIIB.

The temporal relationship of the responses to downshift experiments may provide important insight into the mechanisms of the regulation of stable RNA synthesis. RNAP I activity is most responsive to changes in growth conditions, followed by 5
S rRNA synthesis and eventually tRNA synthesis. Although both 35S rRNA and tRNA synthesis appear to be regulated in a similar manner, that is the inactivation of an essential transcription factor (or polymerase), the differences in responses suggest that they may be mediated by fundamentally different mechanisms. The persistence of tRNA activity in downshifted cells, such as in transition phase or in response to amino acid starvation, is consistent with the loss of BRF and B+ activity under these conditions occurring at the level of factor synthesis or stability and its subsequent dilution during further cell growth. A decrease in the BRF levels in down-regulated extracts has been observed (28, 29), and BRF is limiting under these conditions occurring at the level of factors synthesis. PossibletargetsmaybetheconformationoftheDNA template or nucleoside triphosphate pools. RNAP I transcription has been demonstrated to be very sensitive to the size of the intracellular nucleoside triphosphate pools (45). The nutritional upshift experiments lead to similar conclusions. The addition of glucose to dense cultures in transition phase restores RNAP I and III activity to cell extracts, although no cell division occurs. These observations enforce the notion that the "trigger" that precipitates the regulation of the transcription complex is not simply cell proliferation but is rather perhaps more narrowly defined. This work provides a basis for the further biochemical analysis of the regulation of both RNAP I and III complexes. We have demonstrated that all three of the transcription systems responsible for stable RNA synthesis are directly modified in a manner that tolerates biochemical manipulation. Identification of the conditions that provoke these regulatory responses and the initial biochemical analysis of factors involved in the regulation will facilitate a detailed analysis of the molecular mechanism of stable RNA synthesis in eucaryotes.

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