Transcription of genes encoding trans-acting factors required for rRNA maturation/ribosomal subunit assembly is coordinately regulated with ribosomal protein genes and involves Rap1 in \textit{Saccharomyces cerevisiae}

Keita Miyoshi, Chiharu Shirai and Keiko Mizuta*

Department of Bioresource Science and Technology, Graduate School of Biosphere Science, Hiroshima University, Kagamiyama 1-4-4, Higashi-Hiroshima 739-8528, Japan

Received November 25, 2002; Revised and Accepted February 6, 2003

ABSTRACT

We demonstrate that the genes encoding trans-acting factors essential for pre-rRNA processing/ribosomal subunit assembly are responsive to various kinds of stresses such as heat shock, nitrogen deprivation and a secretory defect, in coordination with ribosomal protein genes in \textit{Saccharomyces cerevisiae}. The \textit{rap1-17} mutation, which produces the C-terminally truncated protein of a transcriptional factor Rap1p, affects transcriptional repression of the trans-acting factor genes due to a secretory defect as shown previously for both ribosomal protein and rRNA genes.

INTRODUCTION

Rapidly growing cells of \textit{Saccharomyces cerevisiae} produce a large number of ribosomes and therefore the regulation of ribosome synthesis is important for economy of the cells (1). In fact, ribosome synthesis is controlled in response to various nutritional conditions. Furthermore, yeast cells have a regulatory system to maintain balanced syntheses of ribosome and the plasma membrane; a defect in the secretory pathway leads to significant repression of ribosome synthesis (2). It was proposed that plasma membrane stretching triggered this effect (3). A yeast ribosome consists of four rRNAs and 78 ribosomal proteins (RPs). All four rRNAs are encoded by a 9.1-kb rDNA unit that is tandem repeated 100–200 times on chromosome XII. Three (25S, 18S, 5.8S) rRNAs are transcribed as a long precursor by RNA polymerase I, whereas 5S rRNA gene is by RNA polymerase III. A secretory defect causes transcriptional repression of genes for rRNA (2), 5S rRNA (3) and RPs by RNA polymerase II (2). It is unknown how the three sets of RNA polymerase systems are controlled coordinately. We demonstrated previously that Rap1p was one of key regulators in this regulatory system (4,5). Rap1p is a relatively abundant transcriptional activator of many genes including most RP genes and also has roles in silencing at telomeres and mating-type loci (6). The C-terminal region of Rap1p is implicated in the interaction with various kinds of proteins and is important for silencing and maintenance of telomere length (6–8). We showed that the C-terminal region of Rap1p was required for transcriptional repression of two types of RP genes, with and without Rap1p-binding sites, and rRNA genes due to a secretory defect. These results suggest that Rap1p is implicated in the repression caused by a secretory defect, but a Rap1p-binding site is not required within the upstream activating sequence (4,5).

Pre-ribosomal subunits are assembled in the nucleolus through many steps including pre-rRNA processing, RNA modification and binding of RPs to pre-rRNA. Assembly of ribosomal subunits and processing of pre-rRNA are intimately connected, because many RPs are assembled on pre-rRNA that is not yet processed (9,10). An increasing number of trans-acting protein factors that have roles in pre-rRNA processing/ribosomal subunit assembly have been identified by mass spectrometry analysis following tandem-affinity purification (TAP; 11) of TAP-tagged proteins (12–16). Two consensus DNA sequences were proposed for regulation of their expression by the use of computational techniques (17–19), but the details remain unknown.

In this report, we have studied the transcriptional regulation of genes encoding trans-acting factors essential for pre-rRNA processing/ribosomal subunit assembly. Each is regulated in response to a secretory defect, mild heat shock and nitrogen deprivation, in coordination with RP genes. Moreover, the \textit{rap1-17} mutation, producing the C-terminally truncated version of Rap1p, results in deregulation of transcription of the genes encoding the factors in response to a secretory defect as shown previously for both RP and rRNA genes (4,5).

MATERIALS AND METHODS

Yeast strains and media

The yeast strains used in this study are listed in Table 1. Yeast cells were grown in synthetic complete medium containing 2% glucose (SC) or SC dropout medium depending on the

*To whom correspondence should be addressed. Tel/Fax: +81 824 24 7926; Email: kmizuta@hiroshima-u.ac.jp
RESULTS

Coordinated regulation of transcription both of trans-acting factor genes and RP genes in response to various kinds of stresses

In order to investigate whether or not the expression of the trans-acting factor genes is regulated in a similar manner to RP genes, we analyzed mRNA levels of the trans-acting factor genes in cells under various kinds of stresses. In recent reports, 90S and different 60S pre-ribosomal particles were purified by TAP based on simultaneous tagging of two components and it was suggested that the factors required for 40S ribosomal subunit assembly were predominantly distinct from those required for 60S subunit assembly in S. cerevisiae (reviewed in 25). Thus, we selected factors that have functions at the various stages in ribosome biogenesis, including proteins associated with snoRNA U3 [Nop1p, Nop56p, Nop5p/Nop58p (16,26,27)], other factors required for 18S rRNA synthesis [Krr1p (28), Nsr1p (29)] and for 25S rRNA synthesis [Rps21p (23,30), Ebp2p (31), Nog2p (32)], and rRNA pseudouridine synthase, Cbf5p (33,34). In order to determine if transcription of the factor genes is responsive to a secretory defect, we used a temperature sensitive sly1 mutant strain, which is defective in membrane trafficking from the endoplasmic reticulum to Golgi apparatus (2). As shown in Figure 1A, mRNA levels of the trans-acting factor genes were decreased when sly1 cells were shifted to the restrictive temperature. It was observed that when the cell culture was shifted to 36°C from 25°C, mRNA levels of RP genes were temporarily decreased even in wild-type cells due to a mild heat shock (35). It is noted that the temporary heat shock effect was also observed in mRNA levels of the trans-acting factor genes (Fig. 1A, lane 2). To ascertain further the repression of the factor genes by a secretory defect, northern analysis was performed by using cells treated with tunicamycin, which interferes with the secretory pathway by inhibiting the glycosylation of proteins in the endoplasmic reticulum. As shown in Figure 1B, transcription of the factor genes was repressed by addition of tunicamycin. Changes in the averages of mRNA levels of the factor genes and RP genes were compared. Although the extent of repression of the factor genes was lower than that of RP genes, mRNA levels of the two sets of genes declined similarly; transcription of both the sets of genes was fully repressed 3 h after tunicamycin treatment and half repressed after 1–1.5 h (Fig. 1C).

As it is known that ribosome synthesis is also regulated by limitation and re-addition of nitrogen source (36), we next examined if this kind of stress also affected transcription of the factor genes. As shown in Figure 2, transcription of each of the factor genes was repressed 1 h after the shift of cells to N-starved medium and the addition of ammonium chloride to N-starved medium resulted in recovery of the transcription of both the factor genes and RP genes (lanes 1–9). These results suggest that transcription of the factor genes is coordinately regulated with that of RP genes in response to heat shock, a secretory defect and N-starvation.

The rap1-17 mutation cancels transcriptional repression of the trans-acting factor genes due to a secretory defect

Most of the RP genes are regulated by a transcriptional activator, Rap1p. We showed previously that the rap1-17 mutation, which produces C-terminally truncated proteins, resulted in attenuation of transcriptional repression of RP genes in response to a secretory defect (4). However, Rap1p-binding sites in upstream regions of RP genes were not necessary for the function of Rap1p on the secretory response (4). In addition, transcriptional repression of the rRNA gene due to a secretory defect was also affected by the rap1-17 mutation (5). This prompted us to examine whether the rap1-17 mutation had any effect on the repression of the factor genes as well, even though the genes had no Rap1p-binding site upstream. For each of the factor genes, the rap1-17 mutation attenuated the transcriptional repression due to a secretory defect caused by either the temperature shift-up of the sly1 mutant cells or tunicamycin treatment (Fig. 3). Thus, the rap1-17 mutation leads to attenuation of the repression of
the RP genes, rRNA genes and also the factor genes. In contrast, the transcriptional regulation of the factor genes by N-starvation and re-addition of nitrogen source was not affected by the rap1-17 mutation (Fig. 2, lanes 10–18), as is the case for the RP genes (5).

Figure 1. Transcription of trans-acting factor genes is repressed in response to both mild heat shock and a secretory defect. (A) Yeast W303a and KM003 (sly) cells were grown in SC medium to log phase (OD\textsubscript{600} = 0.4–0.6) at 25°C. The cultures were shifted to 36°C and, at the indicated times, the cells were harvested for northern analysis. (B) Wild-type (W303a) cells were grown to log phase in SC medium at 30°C and treated with tunicamycin (2.5 μg/ml). RNAs from cells harvested at the indicated times were analyzed by northern blotting. (C) Averages of mRNA levels for trans-acting factor genes (closed triangles) and RP genes (open squares) and mRNA level of ACT1 (open circles) were calculated from (B).

Figure 2. Transcription of the factor genes is responsive to N-starvation. Strains KM328 (RAP1) and KM329 (rap1-17) were grown to OD\textsubscript{600} = 0.3–0.4 in SC medium at 25°C, harvested by filtration and resuspended in SC–N medium. The culture was incubated at 25°C until >95% of the cells were unbudded (for 8 h), at which time ammonium chloride was added to a concentration of 5 g/l. Cells were harvested at the indicated times after shifting of the cells to SC–N medium and after nitrogen addition. RNAs were prepared and analyzed by northern blotting.

Figure 3. The rap1-17 mutation affects the transcriptional repression of the factor genes due to a secretory response. (A) Strains KM014 (SLY1 RAP1), KM016 (SLY1 rap1-17), KM011 (sly1 RAP1) and KM013 (sly1 rap1-17) were grown to log phase (OD\textsubscript{600} = 0.5–0.6) in SC–His medium at 25°C. A third of the culture was shifted to 33 or 36°C for 90 min. (B) Strains KM014 (SLY1 RAP1) and KM016 (SLY1 rap1-17) were grown in SC–His medium at 25°C. Half of the culture was treated with tunicamycin at a final concentration of 1 μg/ml for 4 h at 25°C.
DISCUSSION

We have explored the regulation of *trans*-acting protein factors that are implicated in pre-rRNA processing/ribosomal subunit assembly. Nsr1p and Krr1p are required for maturation of 18S rRNA and 40S ribosomal subunit assembly, and Rrs1p, Ebp2p and Nog2p are for maturation of 25S and 60S ribosomal subunit assembly. Nop1p, Nop5p and Nop56p are proposed to be members of U3 snoRNP complex (SSU processome) (16) and Cbf5p is rRNA pseudouridine synthase (33). CBF5, NOP5, NOP1 and NSR1 are expressed with high transcriptional frequency (http://web.wi.mit.edu/young//expression/halflife.html), whereas KRR1, KRS1 and EBP2 are not highly expressed, but might be important for growth control (28,30,31). Nsr1p is homologous to mammalian nucleolin.

It was calculated that RP genes represent ~50% of the total RNA polymerase transcription events (1). We estimate that 4% of the total transcription is for the factors (http://web.wi.mit.edu/young//expression/halflife.html). The average half-life of mRNAs for *trans*-acting factors is 12 min, which is similar to that of mRNAs for RPs and relatively short compared with the average half-life of all mRNAs (19 min) in the same database.

Two consensus sequences termed RRPE and PAC have been proposed for promoter elements of many genes implicated in ribosome biogenesis (17,18). However, mutation analysis did not provide clear insight into the function of these motifs; site-directed mutagenesis suggested that the promoter elements might be important for the regulation of the *trans*-acting factor genes after release from alpha factor arrest, whereas it is unclear if the elements are required for the other stress response (18). Recent systematic analysis using a set of yeast gene deletion strains suggests that ribosome biogenesis is intimately linked to cell size through Sfp1p (19), a predicted zinc finger transcription factor (37). However, there are three concerns that lead us to question the linkage. (i) Although the set of Sfp1p-regulated genes is enriched for the elements mentioned above, each gene containing the elements upstream is not necessarily regulated by Sfp1p (19). For example, *EBP2* has the two motifs in its promoter region (18), but the expression is not regulated by Sfp1p (19). In our experiments, the genes with and without the RRPE and/or PAC elements are transcriptionally co-regulated, suggesting that some other factor(s) than Sfp1p are required for the transcriptional regulation of the factor genes. (ii) Although many genes of ribosome biogenesis appeared to be induced by overexpression of *SFP1*, deletion of *SFP1* did not necessarily cause repression of the genes (19). However, in yeast cells, ribosome synthesis is regulated by transcriptional repression in response to various kinds of stresses, such as heat shock, secretory defect, N-starvation and amino acid starvation. (iii) Genome-wide location analysis for binding of 106 transcriptional regulators showed that Sfp1p binding was not detected in promoter sequences of the nine factor genes that we analyzed (38) (http://web.wi.mit.edu/young/regulator_network/). No common transcription factor was found in the promoter sequences, except that Ab1p was bound to promoters of *EBP2* and *NOP5*.

We show that the *rap1-17* mutation affects transcriptional repression of the factor genes without Rap1p-binding sequences. This suggests that Rap1p is essential for the repression in response to a secretory defect, but that Rap1p-binding sites are not necessary as *cis*-acting element for the repression. This is consistent with our previous results showing that transcriptional repression of both *RPL28* (Rap1p-driven) and *RPL3* (Abf1-driven) was diminished by the C-terminally truncated rap1-17 mutation (4). We also demonstrated that the *rap1-17* mutation attenuated transcriptional repression of rRNA genes (5). However, it remains to be learned how Rap1p functions in the repression. It is unclear whether there is a single regulatory mechanism affecting all the genes for RPs, rRNA and *trans*-acting factors. As mRNA levels of *trans*-acting factor genes declined similarly to that of RP genes after tunicamycin treatment (Fig. 1C), it is unlikely that the repression of *trans*-acting factor genes is a secondary effect caused by the repression of RP genes. It is possible that Rap1p is a master regulator affecting secondary signaling substances that have their own mechanism for controlling the three sets of genes.

It is interesting that the mechanism of the repression due to a secretory defect is different from other regulations such as heat shock and N-starvation; *rap1-17* affects only the repression due to a secretory defect. There may be several signal transduction pathways to control ribosome synthesis through transcriptional regulation of rRNA, RP and *trans*-acting factor genes.

ACKNOWLEDGEMENTS

We are grateful to J. R. Warner for critical reading of the manuscript. This research was supported by research grants from the Human Frontier Science Program and from the Naito Foundation to K. Mizuta. K. Miyoshi is the recipient of a Japan Society for the Promotion of Science (JSPS) Pre-doctoral Research Fellowship.

REFERENCES


