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The C-terminal silencing domain of Rap1p is essential for the repression of ribosomal protein genes in response to a defect in the secretory pathway

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ABSTRACT

We have previously shown that a functional secretory pathway is essential for continued ribosome synthesis in *Saccharomyces cerevisiae*. When a temperature-sensitive mutant defective in the secretory pathway is transferred to the non-permissive temperature, transcription of both rRNA genes and ribosomal protein genes is nearly abolished. In order to define the *cis*-acting element(s) of ribosomal protein genes sensitive to a defect in the secretory pathway, we have constructed a series of fusion genes containing the *CYH2* promoter region, with various deletions, fused to *lacZ*. Each fusion gene for which transcription is detected is subject to the repression. Rap1p is the transcriptional activator for most ribosomal protein genes, as well as having an important role in silencing in the vicinity of telomeres and at the silent mating-type loci. To assess its role in the repression of transcription by the defect in the secretory pathway, we have introduced *rap1* mutations. The replacement of wild-type Rap1p by Rap1p truncated at the C-terminal region caused substantial attenuation of the repression. Furthermore, we have demonstrated that the Rap1p-truncation affects the repression of *TCM1*, encoding ribosomal protein L3, which has no Rap1p-binding site in its upstream regulatory region. These results suggest that the repression of transcription of ribosomal protein genes by a secretory defect is mediated through Rap1p, but does not require a Rap1p-binding site within the UAS.

INTRODUCTION

The biosynthesis of ribosomes consumes an extraordinary proportion of the macromolecular economy of *Saccharomyces cerevisiae*. In a rapidly growing cell, ribosomal RNA transcription represents ~60% of total transcription, and ribosomal protein synthesis ~15% of total translation. Thus, precise and coordinate regulation of the components of ribosomes is critical for the economy of the cell (1). During our study of the mechanism of this regulation, we found that a temperature-sensitive (ts) mutation in *SLY1*, whose gene product is involved in the ER–Golgi trafficking (2,3), caused the transcriptional repression both of ribosomal RNA and of ribosomal protein genes (4). Analysis of a variety of mutants showed that a defect anywhere in the secretory pathway, from a step prior to insertion of the nascent peptide into the ER to a step involved in the formation of the plasma membrane, prevents the continued synthesis of the components of the ribosome (4,5). Furthermore, most of the ts mutants in which transcription of ribosomal protein genes is temperature-sensitive appear to be defective in secretory pathway (5). These results suggest an important coupling of plasma membrane biosynthesis and ribosome biosynthesis. We propose the existence of a signal transduction pathway from plasma membrane to nucleus, recognizing that other models are possible.

In order to elucidate the molecular mechanism of the regulation of transcription of ribosomal protein genes, we attempted to identify *cis*-acting elements that mediate this repression, in the promoter region of the ribosomal protein gene, *CYH2*. However, any construct in which detectable transcription occurred was subject to repression by a failure of the secretory pathway.

The UAS regions of most ribosomal protein genes have two sites for the DNA-binding protein Rap1p, which is responsible for most of the transcriptional activity of the gene (6–11). Rap1p is a particularly interesting transcription factor. Not only does it promote transcription of ribosomal protein genes, as well as genes of the translation factors and of the glycolytic pathway, but it also plays a key role in the silencing of the silent *HM* loci and of genes adjacent to telomeres (12–15). We asked whether Rap1p could be responsible for the repression of the ribosomal protein genes as well. The experiments to be reported below demonstrate that the Rap1p C-terminal region, which has been shown to be essential for telomere position effect and silencing at the *HM* loci, is also important for the repression of ribosomal protein genes. Furthermore, we have demonstrated that Rap1p is also responsible for the repression of *TCM1*, encoding ribosomal protein L3, transcriptional activity of which is not dependent on Rap1p. The results suggests that the repression does not necessarily require a Rap1p binding site within the UAS.

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Table 1. Yeast strains used in this study

Strain	Genotype	Source or reference
W303	*MATa/α his3-11,15/his3-11,15 ade2-1/ade2-1 ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100*	Rothstein, R.
W303α	*MATα his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100*	Rothstein, R.
J1003.1D	*MATα trp1-1 ade2-1 ura3-1 leu2-3, 112 can1-100*	(13)
J1003.1E	*MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 can1-100*	(13)
KM001[a]	*MATα trp1-1 ade2-1 ura3-1 leu2-3, 112 can1-100 sly1*	(2)
KM007	*MATa/α his3-11,15/+ ade2-1/ade2-1 ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/+ can1-100/can1-100 sly1/sly1*	This study
KM009	*MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100 rap1::LEU2 sly1* pURA3CEN4-RAP1	This study
KM010	*MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100 rap1::LEU2* pURA3CEN4-RAP1	This study
KM011	*MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100 rap1::LEU2 sly1* pHIS3CEN6-RAP1	This study
KM012	*MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100 rap1::LEU2 sly1* pHIS3CEN6-RAP1-12	This study
KM013	*MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100 rap1::LEU2 sly1* pHIS3CEN6-RAP1-17	This study
KM014	*MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100 rap1::LEU2* pHIS3CEN6-RAP1	This study
KM015	*MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100 rap1::LEU2* pHIS3CEN6-RAP1-12	This study
KM016	*MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100 rap1::LEU2* pHIS3CEN6-RAP1-17	This study

[a]KM001 was isolated by two back-crosses of the original *ts* mutant obtained from a screen of the library of *ts* mutants (4,16).

MATERIALS AND METHODS

Strains, media and plasmids

The yeast strains used in this study are W303 and its derivatives, as listed in Table 1. Yeast cells were grown in either yeast extract–peptone–dextrose (YPD) or minimal medium supplemented with 2% glucose as a carbon source. Plasmids containing *RAP1* (17) and *PGK* (18) were kindly provided by D. Shore and S. M. Kingsman, respectively. The plasmids pHIS3CEN6-RAP1 and pURA3CEN4-RAP1 were constructed by insertion of the 3.7 kb fragment including *RAP1* in pRS313 and YCp50, respectively.

Plasmid-shuffling mutagenesis

Site-directed mutagenesis of pHIS3CEN6-RAP1 was performed *in vitro* using the U. S. E. Mutagenesis kit (Pharmacia). The DNA sequences were confirmed on a DNA sequencer. Plasmid shuffling was employed to introduce plasmids with *rap1* mutation in *rap1*-disrupted strain as follows (Fig. 1). Diploid W303 and KM007 (*sly1/sly1*) cells were transformed with pURA3CEN4-RAP1 and induced to undergo meiosis. Among the progeny, haploid KM009 and KM010 in which *RAP1* was disrupted and pURA 3CEN4-RAP1 was transformed were recovered. KM009 and KM010 were transformed with pHIS3CEN6-RAP1, pHIS3CEN6-RAP1-12 or pHIS3CEN6-RAP1-17. Transformants losing pURA 3CEN4-RAP1 were selected by streak on a 5-FOA-containing plate (KM011–KM016).

Other methods

Telomere-tract lengths were measured by Southern blot analysis of *Xho*I-digested genomic DNA using poly[d(G–T)]·poly[d(C–A)] as a probe (19). Northern blot analysis was carried out using 1.5% agarose gel in formaldehyde and Nytran membrane, as described

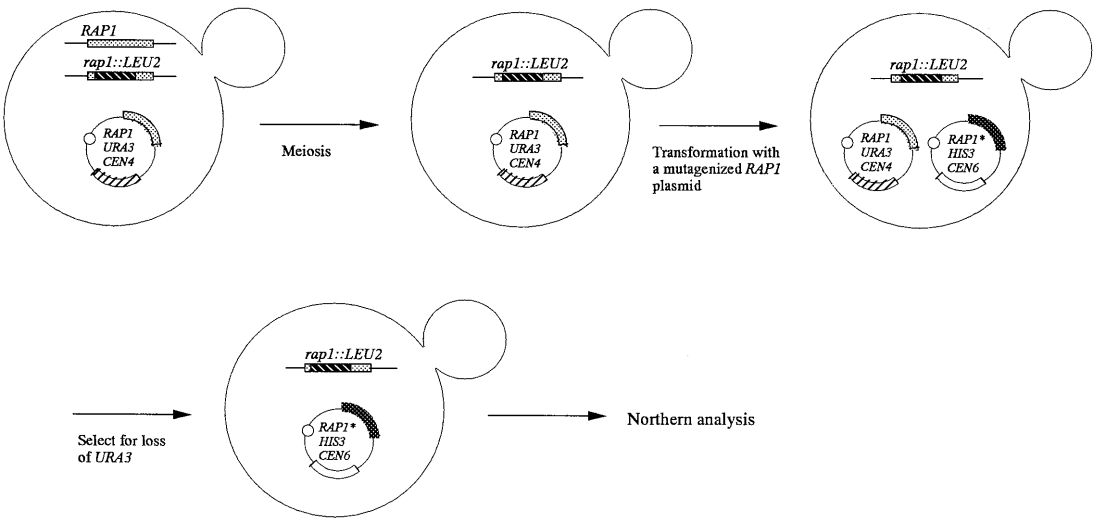

Figure 1. Scheme of plasmid shuffling mutagenesis employed to isolate Rap1p mutants.

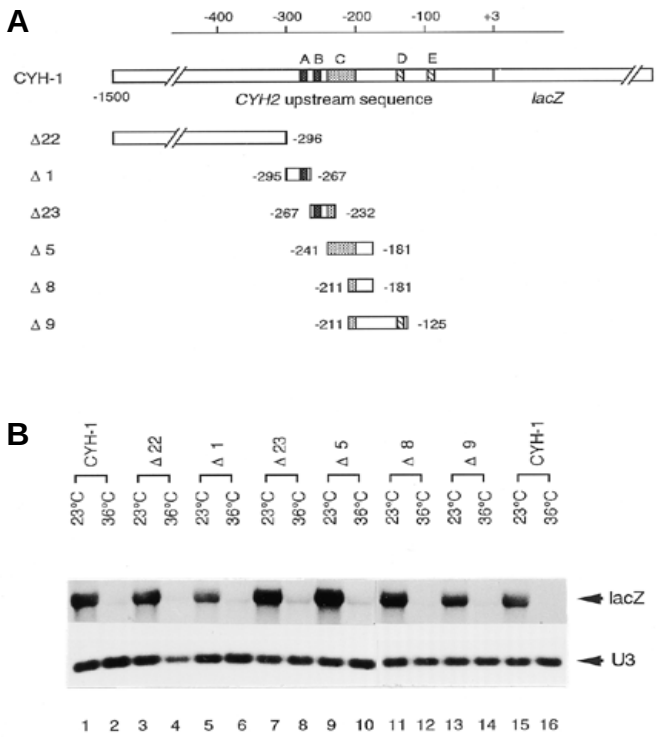

Figure 2. Effects of promoter deletions on the repression due to a secretory defect. (**A**) Deletions in the *CYH2* promoter. The top bar indicates the region of *CYH2* from –1500 to +3 (numbering is from the initiation site of translation), fused with *lacZ*. The positions of Rap1p-binding sites (A and B), T-rich region (C) and TATAs (D and E) are indicated. The remaining bars indicate the portion of the sequence deleted in each of the six deletions. In the wild-type *CYH2* gene, there are a number of sites of initiation of transcription spread between –25 and –12. The most prominent is the pair at –16 and –17 (8). (**B**) Northern analysis using various deletions in the *CYH2* promoter. Yeast *sly1* strains containing the plasmids with various deletions shown in (A) were grown at 23°C. Half of the culture was shifted to 36°C. After 90 min, the cells were harvested and total RNA was prepared. The Northern blot was probed with ^{32}P-labeled DNA fragment from *lacZ*.

previously (4,20). Yeast cells were transformed by the lithium acetate method (21). Western blotting followed standard techniques, and signals were visualized by Enhanced Chemiluminescence (Amersham). The anti-Rap1 antibody (22) was a kind gift of S. M. Gasser.

RESULTS

No sequence specifically responsible for the repression was observed in the UAS of *CYH2*

One possible model for the repression of ribosomal protein genes in response to a defect in the secretory pathway is that an unidentified repressor binds to a *cis*-acting element in the promoter region. In order to search for such a *cis*-acting element, we chose to analyze the *CYH2* gene encoding ribosomal protein L29. The transcriptional features of *CYH2*, which have been thoroughly documented (Fig. 2A) (8), are similar to those of most, but not all ribosomal protein genes (9,10,23–25). A number of mutant *CYH2* genes, with deletions in the UAS as shown in Figure 2A, were fused with *lacZ*. Temperature-sensitive *sly1* mutant which is deficient in the secretory pathway (2–4) was transformed with the plasmids containing the fusion genes. Figure 2B shows a Northern analysis of the resulting strains at the permissive temperature and after 90 min at the non-permissive temperature. Examination of the odd-numbered lanes, reflecting transcription at the permissive temperature, shows that none of the deletions had a drastic effect on transcription. As shown previously, there is substantial redundancy in the UAS elements of ribosomal protein genes. On the other hand, deletion of both Rap1p sites leads to almost complete loss of transcription (data not shown). The even-numbered lanes demonstrate clearly that whatever transcription occurs is remarkably repressed in cells with a defect in the secretory pathway. Quantitative analysis of Figure 2B shows that at the non-permissive temperature there is <15% of the *lacZ* mRNA compared with permissive temperature in each construction (data not shown). Thus, in the region from –1500 to beyond a TATA element we can find no sequence element that appears responsible for the repression of transcription in response to a defect in the secretory pathway.

Construction of yeast strains with *rap1* mutations

As deletion of both Rap1p-binding sites abolishes basal transcriptional activity of the *CYH2* gene at 23°C, each fusion gene shown in Figure 2A by necessity contained at least one Rap1p-binding site. As we have found no *cis*-acting element for the repression of ribosomal protein genes over the range covering the upstream region from the TATA box except Rap1p-binding site(s), we have next examined the role of Rap1p on the repression. Rap1p is an unusual transcription factor for it is involved in both activating and silencing genes. A silencing domain has been identified in Rap1p (12,13,28). To examine the possibility that Rap1p might have some role in the repression of ribosomal protein genes, we constructed plasmids containing either of two *rap1* mutations, *rap1-12* (12) and *rap1-17* (13), that have been found to affect the silencing of genes. A diagram of Rap1p shows the locations of the mutations and the phenotypes of the mutants (Fig. 3). The *rap1-12* gene has two missense mutations at amino acids 726 and 727. The *rap1-17* gene has a missense mutation at amino acid 661 and a frameshift mutation creating a stop codon at amino acid 663. Their phenotypes on the silencing of mating-type loci and on the telomere position effect are quite different: *rap1-17* mutation leads to loss of telomere position effect, whereas this is rather increased in a *rap1-12* strain (28). In *rap1-17* cells, *HMLα*, but not *HMRa*, is partially derepressed. On the other hand, in *rap1-12* cells, the *hmr-ΔA::TRP1* locus is completely derepressed. Each was used to replace *RAP1* by plasmid shuffling, as shown in Figure 1. Both mutations have been reported to cause telomere lengthening (12,13). To confirm that the mutant *rap1* genes were functioning as predicted, we measured the average length of telomeres from mutant strains. As shown in Figure 4, the mutants display a significant increase in the average length of telomeres as compared with the wild-type strain, indicating that the mutant cells are controlled with mutated *rap1*. Western blot analysis shows that the truncated version of Rap1p is produced at a similar level to the full-length Rap1p (Fig. 5). Although full-length Rap1p has a predicted molecular

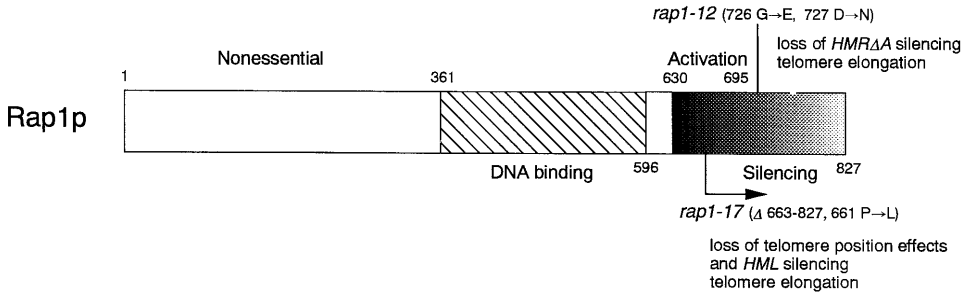

Figure 3. A diagram of Rap1p structure. The DNA binding (26), activation (27) and silencing (28) domains are shown. Also shown are the positions of the *rap1-12* (12) and *rap1-17* (13) alleles and the effect of these alleles.

weight of 93 kDa, its anomalous mobility on SDS–polyacrylamide gels has been observed previously (17).

The *rap1-17* mutation leads to attenuation of the repression due to a secretory defect

The effect of *rap1* mutations on the repression of ribosomal protein genes in response to a secretory defect was determined under two conditions; a temperature up-shift of a *sly1* ts mutant to the non-permissive temperature, and treatment with tunicamycin which inhibits the secretory pathway by inhibition of glycosylation of proteins in the ER. Wild-type and *rap1* mutant strains were cultured at 23°C overnight and transferred to a water-bath at 33°C for 90 min. RNA prepared from cultures taken before and after the temperature shift was subjected to Northern analysis (Fig. 6). The blot has been probed to reveal the transcripts of ribosomal protein genes *CYH2* and *TCM1*, of a glycolytic gene *PGK*, and of *KAR2*, a gene encoding the yeast version of the chaperone BiP, that is induced in response to a defect in the ER–Golgi trafficking (30,31). SnoRNA U3 was used as a loading control. It is evident that in cells with an intact secretory pathway (lanes 7–12) the temperature shift has little effect on the level of transcripts of *CYH2*, *TCM1*, *KAR2* or *PGK*. On the other hand, in the *sly1* ts mutant, the temperature shift leads to a substantial induction of *KAR2* transcription, demonstrating that there is a defect in the secretory pathway. The concomitant repression of transcription of *CYH2* in cells carrying the *RAP1* wild-type allele is evident from lane 2. In cells carrying the *rap1-12* allele, the results are much the same (lane 4). However, lane 6 reveals that in cells carrying the *rap1-17* allele the repression of the *CYH2* gene is largely attenuated.

Although the great majority of ribosomal protein genes are driven by Rap1p-binding site(s), several are activated by a single Abf1p-binding site instead (23–25). An example is *TCM1*, encoding ribosomal protein L3. The transcription of these ribosomal protein genes is also repressed by a secretory defect (ref. 4 and Fig. 6, lane 2). Unexpectedly, however, the C-terminal deletion of Rap1p has a similar effect on the repression of *TCM1* (Fig. 6, lane 6). This result suggests that the repressive effect of Rap1p does not necessarily require a DNA binding site *in cis*. Although the transcription of glycolytic genes is under the control of Rap1p, it is not repressed through a secretory defect (4). As shown in Figure 6, the transcription of *PGK*, a glycolytic gene, is not affected by mutation of *RAP1*.

To extend this result, we asked whether the *rap1-17* allele prevented the repression of ribosomal protein gene transcription when the secretory pathway was disturbed by the presence of the drug tunicamycin. As is apparent in Figure 7, the result using tunicamycin is consistent with the result of temperature up-shift. Whether in *sly1* or in *SEC*$^+$ background, treatment with tunicamycin resulted in a substantial decrease of mRNA level of *CYH2* and *TCM1* in cells carrying the *RAP1* wild-type or the *rap1-12* allele (lanes 2, 4, 8 and 10). However, the repression of both *CYH2* and *TCM1* was greatly attenuated in cells carrying *rap1-17* (lanes 6 and 12). The transcription of *PGK* was not affected by tunicamycin treatment nor by the *rap1* mutation. The induction of *KAR2* mRNA confirms that the tunicamycin treatment caused a secretory defect in each strain. Thus, it is suggested that Rap1p is implicated in the repression of the transcription of both types of ribosomal protein genes and that the C-terminal region of Rap1p is important for this role.

The *rap1-17* mutation has little effect on the temporary repression by mild heat-shock

Mild heat-shock causes the temporary repression of transcription of ribosomal protein genes (32,33). To ask if this repression acts in the same way as that due to a failure in the secretory pathway, we have examined the effect of *rap1-17* mutation on the repression through heat-shock at 36°C. As shown in Figure 8, the ribosomal protein genes, *CYH2* and *TCM1*, are repressed 15 min after the temperature shift-up in either *RAP1* or *rap1-17* strain in the background both of *sly1* and of *SEC*$^+$ (lanes 2, 7, 12 and 17). This indicates that the *rap1-17* allele has little effect on the temporary repression of ribosomal protein genes through heat-shock. However, after the repression, in the background of *sly1*, the *rap1-17* mutation permits the recovery of the transcription (lanes 8–10), although the mRNA level is not so high compared with that at 33°C. The time course of mRNA level of *TCM1* is very similar to that of *CYH2*. Heat-shock effect is also detected at 33°C, while the *rap1-17* allele has little effect on the temporary repression (data not shown).

DISCUSSION

We have shown previously that essentially any defect in the secretory pathway causes specific inhibition of ribosome synthesis, almost entirely at the level of transcription. We propose that this is the result of a signal transduction mechanism that maintains balanced synthesis of the components of the cell, in this case the plasma membrane and ribosomes. As a first step in identifying the components of such a signal transduction pathway, we attempted

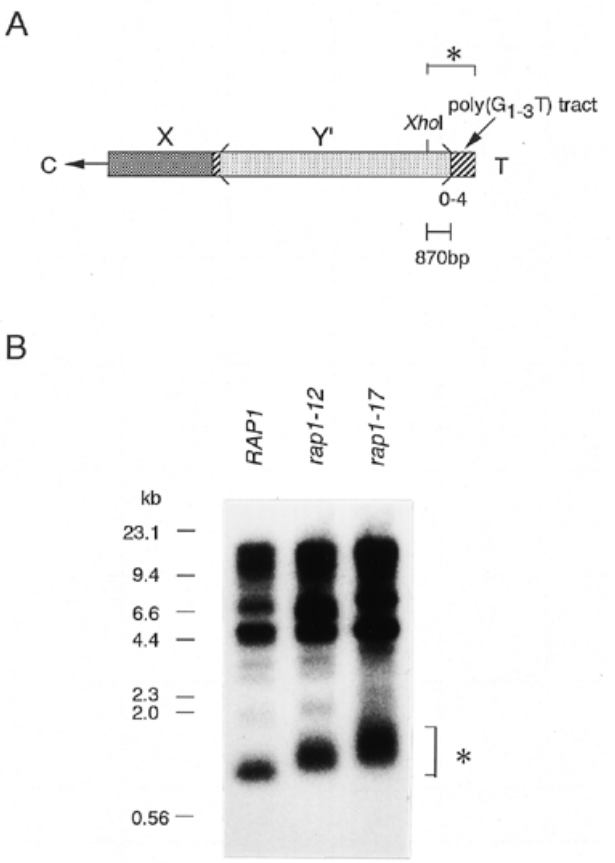

Figure 4. Elongated telomere tracts in Rap1 mutants. (**A**) Schematic representation of yeast telomeres. Many, but not all, telomeres contain the Y′ element, which has a *Xho*I site near the poly($G_{1–3}T$) tract (29). (**B**) Southern analysis using ^{32}P-labeled poly[d(G–T)·poly[d(C–A)] as a probe. Genomic DNA from *sly1* strains containing the plasmids with wild-type *RAP1* (KM011), *rap1-12* (KM012) or *rap1-17* (KM013) was digested with *Xho*I. Broad bands marked by an asterisk corresponds to the poly($G_{1–3}T$) tract as shown in (A).

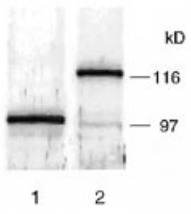

Figure 5. Western blot analysis of the truncated version of Rap1p (rap1-17) and the full-length Rap1p. Crude cell extracts prepared from *sly1* strains containing the plasmids with *rap1-17* (lane 1) or wild-type *RAP1* (lane 2) were denatured in SDS-sample buffer and heated at 95°C for 5 min. Equal amounts of protein were subjected to SDS–PAGE and Western blotting using antibodies directed against Rap1p. The positions of size markers are shown on the right.

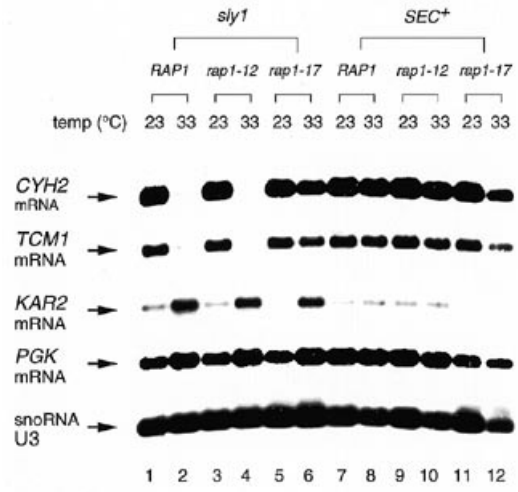

Figure 6. Northern analysis of the cells treated at 33°C. Yeast strains KM011–KM016, *sly1* (lanes 1–6) and *SEC*$^+$ (lanes 7–12) strains containing the plasmids with wild-type *RAP1* (lanes 1, 2, 7 and 8), *rap1-12* (lanes 3, 4, 9 and 10) or *rap1-17* (lanes 5, 6, 11 and 12) were grown at 23°C overnight. Half of the culture was shifted to 33°C (lanes 2, 4, 6, 8, 10 and 12). After 90 min, the cells were harvested and total RNA was prepared. The blot was probed with *CYH2* (encoding RPL29), *TCM1* (encoding RPL3), *KAR2* (encoding BiP), *PGK* and U3.

to define the *cis*-acting element on a ribosomal protein gene that is responsible for the repression of transcription, perhaps by binding a repressive element. This was unsuccessful; any construct with detectable transcription was susceptible to repression by the secretory pathway.

Transcription of most, but not all the ribosomal protein genes is under the control of the DNA binding protein, Rap1p. Rap1p is also involved in the transcriptional activation of many genes not repressed in response to a defect in the secretory pathway, e.g. *PYK1* (4), and in the transcriptional silencing of the silent mating-type loci and of genes in the vicinity of telomeres (12–15). The possibility that under certain conditions Rap1p could be acting as a silencing factor for ribosomal protein genes has led us to evaluate mutants of *RAP1* that have been described (12,13). A truncated allele of *RAP1*, *rap1-17*, missing 165 of its 827 amino acids, supports normal levels of transcription of *CYH2*. However, transcription of ribosomal protein mRNA is much less subject to repression in a *sec* mutant (Figs 6 and 7). This result suggests that the repression of ribosomal protein genes by a secretory defect is mediated through Rap1p. On the other hand, the *rap1-12* allele, which has two missense mutations at amino acids 726 and 727 has little effect on the repression. Although both *rap1-12* and *rap1-17* alleles lead to elongated telomeres, their phenotypes on the silencing of mating-type loci and on telomere position effect are different; in *rap1-17* cells, telomere position effect is lost and *HMLα*, but not *HMRa*, is partially derepressed (13). On the other hand, in *rap1-12* cells, the *hmrΔA::TRP1* locus is derepressed (12).

One model consistent with most of the data presented is that in a *sec* mutant, Rap1p is modified so that it is no longer active on ribosomal protein genes. The *rap1-17* deletion might remove such a modification site. However, we found that the transcription of *TCM1* mRNA, not dependent on Rap1p, is also repressed in response to a defect in the secretory pathway (4). Furthermore, this repression is attenuated in a *rap1-17* mutant (Figs 6–8). Another model is that modified Rap1p, or an unknown factor whose expression is regulated by Rap1p, might prevent the interaction between Rap1p or Abf1p and a component of the transcription machinery. It is possible that the transcription machinery for ribosomal protein genes might be specific and

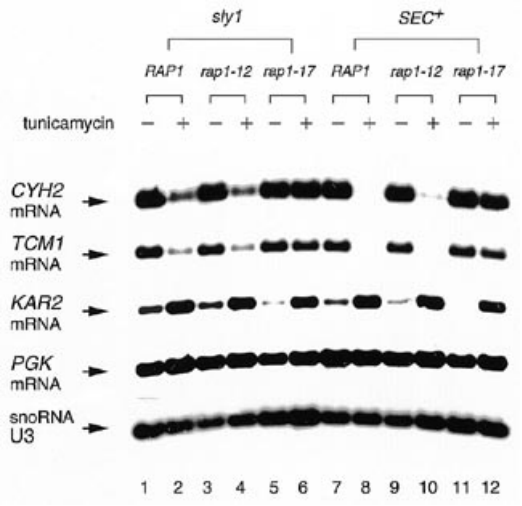

Figure 7. Northern analysis of the cells treated with tunicamycin. Yeast strains KM011–KM016, *sly1* (lanes 1–6) and *SEC*$^+$ (lanes 7–12) strains containing the plasmids with wild-type *RAP1* (lanes 1, 2, 7 and 8), *rap1-12* (lanes 3, 4, 9 and 10) or *rap1-17* (lanes 5, 6, 11 and 12) were grown at 23°C overnight. To half of the culture, tunicamycin was added (final 1.0 µg/ml; lanes 2, 4, 6, 8, 10 and 12). After 4 h at 23°C, the cells were harvested and total RNA was prepared. The blot was probed with *CYH2* (encoding RPL29), *TCM1* (encoding RPL3), *KAR2* (encoding BiP), *PGK* and U3.

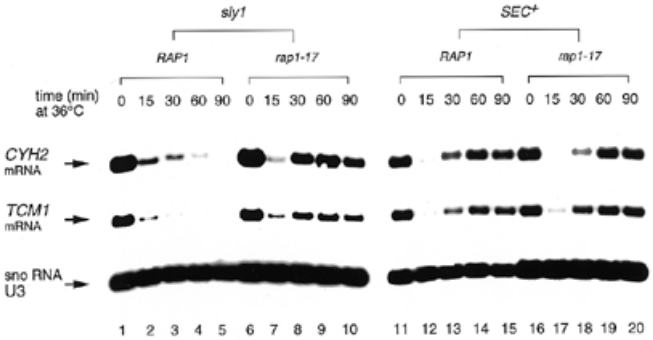

Figure 8. Northern analysis of the cells treated at 36°C. Yeast *sly1* (lanes 1–10) and *SEC*$^+$ strains (lanes 11–20) containing the plasmids with wild-type *RAP1* (lanes 1–5 and 11–15) or *rap1-17* (lanes 6–10 and 16–20) were grown at 23°C. The culture was shifted to 36°C and at the time intervals indicated, the cells were harvested and total RNA was prepared. The blot was probed with *CYH2* (encoding RPL29) *TCM1* (encoding RPL3) and U3.

common. A very recent paper (34) has suggested that the $TAF_{II}145$, a subunit of the yeast TAF_{II} complex, might be specific for ribosomal protein genes. It will be interesting to determine if Rap1p interacts directly with $TAF_{II}145$. In any case, modification of Rap1p might be important. The conversion presumably depends on the C-terminal sequences. The same C-terminal region of Rap1p has been implicated in the silencing of telomere-proximal genes, through the recruitment of many copies of Sir3p and Sir4p (35). However, neither Sir3p nor Sir4p are necessary to bring about the repression of transcription of the ribosomal protein genes (Li, Nierras and Warner, in preparation). It has been suggested that phosphorylation influences the binding and/or transcriptional activity of Rap1p (36,37), although the sites and the regulation of Rap1p phosphorylation have not been shown yet. It is also possible that phosphorylation/dephosphorylation might be important for the repression of ribosomal protein genes in response to a defect in the secretory pathway. Western blot analysis showed that the truncated version of Rap1p (rap1-17) is produced at a similar level to the normal protein (Fig. 5), indicating that the effect of *rap1-17* on the repression is not caused by higher expression.

Although the slower growth rate (1.6-fold) of *rap1-17* cells could contribute to the effect on the repression, we should note that the slow growth rate (1.2-fold) of *rap1-12* cells has no effect and that *SEC*$^+$ *rap1-17* cells, which show the attenuation of the repression, grow at a similar rate as *sly1 rap1-12*. Furthermore, we have recently identified another allele, *rap1-21* (14), that permits growth at the same rate as *rap1-12* but causes the attenuation of repression by tunicamycin treatment (Mizuta *et al*., unpublished data). These results suggest that the slow growth rate is not the main cause even if it might contribute any.

We have shown that partial inhibition of protein synthesis by cycloheximide blocks the effect of a defective secretory pathway on ribosome synthesis (4). In addition, inhibition of transcription blocks the decline of ribosomal protein mRNA in response to a *sec* mutant (Li and Warner, in preparation). These observations support the idea that an unknown protein(s) which acts as a repressor might be produced in response to a shut-off of the secretory pathway.

Interestingly, the *rap1-17* allele has little effect on the temporary repression of transcription brought about by mild heat-shock (Fig. 8), in contrast with its attenuation of the secretory response. This is the first instance in which the response to heat-shock and a secretory defect have been distinguishable. Although mechanistic details remain to be elucidated, our results strongly suggest that Rap1p has an important role in the repression of both the Rap1p- and Abf1p-regulated ribosomal protein genes in response to a secretory defect.

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