Nucleic Acids Research, 1995, Vol. 23, No. 9 1475–1480

Transcription activation of yeast ribosomal protein genes requires additional elements apart from binding sites for Abf1p or Rap1p

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Received February 13, 1995; Revised and Accepted March 20, 1995

ABSTRACT

All ribosomal protein (rp) gene promoters from *Saccharomyces cerevisiae* studied so far contain either (usually two) binding sites for the global gene regulator Rap1p or one binding site for another global factor, Abf1p. Previous analysis of the rp*S33* and rp*L45* gene promoters suggested that apart from the Abf1 binding site, additional *cis*-acting elements play a part in transcription activation of these genes. We designed a promoter reconstruction system based on the β-glucuronidase reporter gene to examine the role of the Abf1 binding site and other putative *cis*-acting elements in promoting transcription. An isolated Abf1 binding site turned out to be a weak activating element. A T-rich sequence derived from the rp*S33* proximal promoter was found to be stronger, but full transcription activation was only achieved by a combination of these elements. Both in the natural rp*L45* promoter and in the reconstituted promoter, a Rap1 binding site could functionally replace the Abf1 binding site. Characteristic rp gene nutritional control of transcription, evoked by a carbon source upshift or by nitrogen re-feeding to nitrogen starved cells, could only be mediated by the combined Abf1 (or Rap1) binding site and T-rich element and not by the individual elements. These results demonstrate that Abf1p and Rap1p do not activate rp genes in a prototypical fashion, but rather may serve to potentiate transcription activation through the T-rich element.

INTRODUCTION

The rate of synthesis of ribosomal components in *Saccharomyces cerevisiae* depends on the physiological state of the cell, which determines the need for protein biosynthetic capacity (1,2). Control of ribosome formation, implying a balanced production of all ribosomal constituents under a variety of growth conditions, occurs primarily at the level of transcription of the various ribosomal genes (3). The coordinate regulation of transcription of ribosomal protein (rp) genes is in particular manifest during alterations of growth conditions, such as occur upon a nutritional upshift, when glucose is added to a yeast culture growing on a non-fermentable carbon source (4,5) or upon re-addition of nitrogen to nitrogen starved cells (this paper).

Study of the transcriptional regulation of a large number of rp genes has led to the identification of *cis*-acting promoter elements, among which the binding site for the global regulator of gene expression Rap1p (6,7) most frequently occurs. The Rap1 binding sites (also designated RPG boxes, displaying the consensus sequence ACACCCATACATTT) proved to be essential for efficient transcription of several rp genes (reviewed in 8). Most of these promoters also contain a T-rich element downstream of the Rap1 binding site(s) and in some cases this motif has been shown to play a role in transcriptional activation (9).

More recently it became apparent that a subset of rp genes, e.g. those encoding S33 (10), L45 (11), L2 (12) and L3 (13), does not share this general promoter structure. The respective upstream regions contain, instead of Rap1 binding sites, one binding site for another global regulator of gene expression, protein Abf1p. Characterization of the promoters of the rp*S33* and rp*L45* genes has shown that, in addition to the Abf1 binding site (having the split consensus motif RTCRYYYNNNACG; 14,15), other *cis*-acting elements are likely to play a role in transcriptional activation (10,11). So far it is not yet clear how transcription activation of the two subsets of rp genes controlled by different *trans*-acting factors, Rap1p or Abf1p, is coordinated. The primary structures of Rap1p and Abf1p show some homology, which may reflect partial functional similarity (16). One hypothesis is that Rap1p and Abf1p may recruit common, as yet unidentified, factors which specifically regulate transcription of rp genes. These factors might also be the mediator of growth-related regulation of rp gene transcription.

Here we describe a set of experiments aimed at elucidating the contribution of Abf1p and Rap1p to transcription activation and at identifying additional *cis*-acting elements in the Abf1-regulated rp*S33* promoter. We obtained evidence that the function of Abf1p in transcription activation of rp genes most likely is to promote transcription in concert with an additional T-rich

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promoter element. Combination of the Abf1 binding site and the T-rich *cis*-element turned out to be required for proper regulation of transcription in response to carbon or nitrogen upshifts. The Abf1 binding site could be replaced by a Rap1 binding site in the context of the reconstituted rp*S33* promoter without any change in the level of transcription activation or the ability to respond to a nutritional shift.

MATERIALS AND METHODS

β-glucuronidase reporter gene

The reporter system used for quantitative transcription assays consists of the proximal region of the rp*L25* gene promoter (namely the region flanked by the *Nsi*I and the *Sau*3A sites at positions –118 and –12, respectively; 7) fused to the *Escherichia coli* β-glucuronidase (*GUS*) gene. This region contains the major rp*L25* transcription initiation sites, but not the respective natural UAS (which consists of two Rap1 binding sites located around –400). The transcription termination region of the *rpL25* gene (a 260 bp *Hpa*I–*Pst*I fragment from plasmid pBMCY138; 7) was inserted downstream of the *GUS* gene. Upstream of this basic GUS transcription cassette, a set of cloning sites (*Sac*I, *Xho*I, *Nhe*I, *Nsi*I, *Pst*I and *Sal*I) [GAGCTCCACTCGAGCAGC-TAGCCAATGCATCACTGCA GCAGTCGAC] was incorporated, which allowed insertion of oligonucleotides representing *cis*-acting elements to be tested for their transcription activating potency. The oligonucleotides used to construct the different promoter sequences are as follows (see Figs 1B and 2): Abf1 binding site in RN5 and RN9, CCGTCTAGAGTGACCAC; Abf1 binding site in RX5, RX7 and RG7, GGTACAGACGCGG-CCGTCTAGAGTGACCAGCACG; T-rich region in RG5 and RG7, CTAGAGTGACCACGCACTTTTTTGATAAATTTTTT-TTTCTTGGTCGTTGAACT; T-rich region in RXT, TTTTT-TTTTCTTCTAG; T-rich region in RPG3, CGCACTTTTTTGA-TAAATTTTTTTTTCTTGGTCGTTGAACT; Rap1 binding site (RPG box) in RPG1, RPG2, RPG3 and RPG5, ACACCCATA-CATTT. Correct insertion of the *cis*-acting elements was verified by sequencing.

Fusion of the entire rp*S33* promoter to the *GUS* gene (construct RS3 in Fig. 1B) has been achieved by creating a *Nco*I site at the ATG of the rp*S33* gene, which allowed fusion of the 1 kb *Hin*dIII–*Nco*I promoter fragment of the rp*S33* gene to the *GUS* gene, making use of the *Nco*I site present around the ATG of the *GUS* gene.

The fusion reporter genes were inserted in the integrative vector pRS306 (17), which contains the *URA3* selection marker. The resulting plasmid, digested with *Stu*I to direct integration into the *ura3* locus, was used to transform strain BJ2168. After selection of single copy integrants by Southern blot analysis, GUS activity was measured (see below).

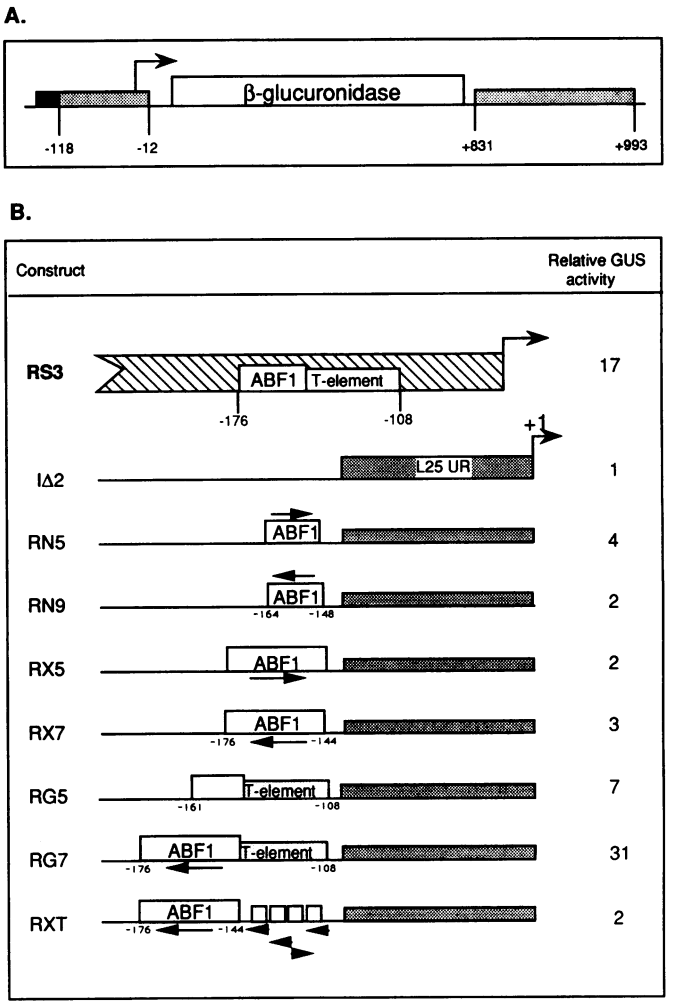

Figure 1. (**A**) The reporter system used for the quantitative transcription assays consists of the *E.coli* β-glucuronidase (*GUS*) gene flanked by the proximal transcription initiation and termination regions of the rp*L25* gene at its 5′- and 3′-ends respectively. Upstream of this basic *GUS* transcription cassette, a polylinker was inserted, as indicated by the black box (see Materials and Methods for details). The coordinates refer to the distance to the rp*L25* start codon. The sequence between the transcription start sites and the ATG of the *GUS* gene is 5′-CTAAACAAAGAAGATCCCCGGGAATTCATCGATC-CATG (transcription start sites indicated in bold). (**B**) Transcriptional activity of reconstructed rp gene promoters (see Materials and Methods for details). Construct RS3 is a fusion of the complete rp*S33* promoter to the *GUS* reporter gene. Coordinates refer to the positions of the different elements relative to the translation start site of the rp*S33* gene. The open box in construct RG5 represents the sequence corresponding to the 3′-part of the Abf1 binding site. The activity measured for the UAS-less promoter (IΔ2) was arbitrarily set at 1. Arrows indicate the orientation of the oligonucleotides (for construct RXT the arrow pointing to the left indicates that the T-rich strand runs 5′→3′).

Plasmids, strains and media

Plasmid pRAJ275 encoding the *GUS* gene was a gift of Dr Michel Haring (Department of Molecular Genetics, BioCentrum Amsterdam). Strain BJ2168 (MATa, *leu2*, *trp1*, *ura3-52*, *prc1-407*, *prb1-1122*, *pep4-3*) was used to express the *GUS* constructs. Yeast cells were grown on minimal medium containing 2% (w/v) glucose, 0.67% (w/v) yeast nitrogen base and the necessary supplements. In medium upshift experiments cells were first grown on either 2% (v/v) ethanol/0.05% (w/v) glucose or 5% (v/v) glycerol. Glucose was subsequently added to a final concentration of 2% (w/v). Nitrogen starvation and refeeding was performed as follows. Exponentially growing yeast cells cultivated on minimal medium were transferred to medium containing 0.67% (w/v) YNB without amino acids and ammonium sulphate, 2% glucose. After overnight starvation, ammonium sulphate was added to a final concentration of 30 mM. GUS reporter strains used in these experiments were made prototrophic by co-transformation of these cells with vectors containing the respective marker genes.

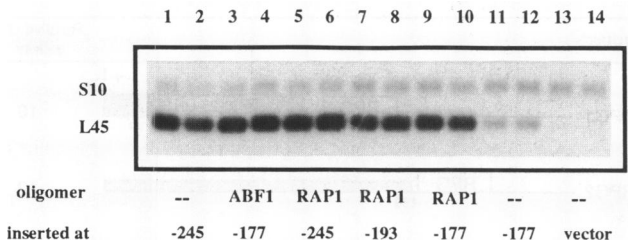

Figure 2. Transcriptional activity of the rp*L45* gene carrying a Rap1 binding site inserted at different positions in the promoter region. Two oligomers having sticky *Sph*I and *Pst*I ends were used. One contains the Abf1 binding site as it occurs in the rp*L45* promoter (encompassing nucleotides –234 to –205) and the other contained a Rap1 binding site matching the consensus sequence. The oligomers were inserted in plasmids containing various deletion constructs cut by *Sph*I (at –1800 in the polylinker) and *Pst*I (at –375) (see 11). In this way the Abf1 oligomer was inserted in the Δ-177 construct. The Rap1 oligomer was inserted in the Δ-245, Δ-193 and Δ-177 constructs. The copy number did not differ much between the various transformants (result not shown). For each construct two independent transformants were tested. For the Northern analyses rp*L45* and rp*S10* probes were used; actin mRNA was used as a loading control.

Miscellaneous

DNA manipulations were performed according to standard methods. GUS activity was measured essentially as described by Schmitz and co-workers (18). Fresh exponentially growing cells were used to prepare the lysates and protein concentration was determined as described by Bradford (19). A typical reaction mixture contained 10–20 μg total protein and 1 mM 4-methyl umbelliferyl glucuronide in a total volume of 250 μl. The formation of the fluorescent product, 4-methyl umbelliferone (MU), was monitored over the course of time. Fluorescence of the samples was measured in a Titertek Fluorscan. The GUS activities shown are the average of at least three independent transformants.

Northern blot analyses were performed as previously described (11), using *GUS*, rp*L25*, rp*S33*, rp*S10*, rp*L45* and actin gene-specific probes. The results were quantified by phosphorimaging (Molecular Dynamics 425).

RESULTS AND DISCUSSION

The β-glucuronidase reporter system

In order to be able to assess the transcription activation capacities of the different putative *cis*-acting elements present in rp gene promoters, we devised a reporter system based on the *E.coli* β-glucuronidase gene, flanked by the transcription initiation and termination regions of the gene encoding yeast ribosomal protein L25. The β-glucuronidase gene has been previously shown to be suitable for transcriptional studies in yeast (18). Moreover, GUS activity can easily be measured in crude protein extracts.

This reporter gene is schematically represented in Figure 1A (designated IΔ2 in Fig. 1B). The upstream region contains the main transcription start sites of the rp*L25* gene, but not the UAS of this gene, which consists of two Rap1 binding sites located around –400. The 260 bp fragment inserted downstream of the *GUS* gene comprises the end of the rp*L25* coding region, as well as the transcription termination site. The chimeric gene is correctly expressed in yeast, as was proven by Northern analysis and enzyme assays (result not shown). Basal transcription from the minimal promoter yielded a GUS activity in total protein extracts of 2.3 nmol MU/min/mg protein when one copy of the reporter gene was integrated at the *ura3* locus. This activity was arbitrarily set at 1.0 in Figure 1. The various *cis*-acting elements to be investigated were inserted in the polylinker upstream of the basal promoter.

The Abf1 binding site is required, but not sufficient, for transcriptional activation

The rp*S33* gene promoter has previously been shown to contain an Abf1 binding site between positions –163 and –150. Deletion analysis of the rp*S33* promoter showed that removal of sequences upstream of –156 causes a drop in transcription, suggesting that the Abf1 binding site plays a major role in transcriptional activation of this gene (10). This result was subsequently confirmed by destroying the binding capacity of the Abf1 binding site in the rp*S33* promoter by a point mutation, which resulted in a dramatic loss of transcription activity (result not shown).

In order to quantitate the transcription activating capacity of a single Abf1 binding site, we inserted oligonucleotides comprising nucleotides –164 to –148 (constructs RN5 and RN9; Fig. 1B) and –176 to –144 (constructs RX5 and RX7; Fig. 1B) of the rp*S33* promoter in the polylinker of the *GUS* reporter gene (IΔ2). In constructs RX7 and RN9 the Abf1 binding site is present in the same orientation as in the natural rp*S33* promoter and in constructs RX5 and RN5 in the opposite orientation. Comparison of the GUS activity measured for these constructs with that observed for IΔ2 led to the conclusion that the Abf1 binding site itself is a poor activating element, irrespective of its orientation (see Fig. 1B). Furthermore, the presence of additional flanking sequences in constructs RX5 and RX7, as compared with RN5 and RN9, did not improve its transcription activating capacity.

A low capacity to activate transcription of a reporter gene has also been observed for the Abf1 binding sites of the *HMR* silencer (20) and the promoter of the essential *DED1* gene (21). These findings may indicate that Abf1 binding sites are only effective in combination with neighbouring, additional *cis*-acting elements.

The T-rich region is an important *cis*-acting element of UAS-*S33*

In view of the results obtained with the constructs described above, we searched for additional functional elements in the rp*S33* promoter which might contribute to transcription activation of this gene. Previously performed deletion analysis showed a further drop in transcription activity of the rp*S33* promoter lacking the Abf1 binding site when a downstream region, including a T-rich element, was also removed (10). Consistent with this result, the transcriptional activity of a promoter carrying only the T-rich region upstream of the reporter gene (construct RG5, Fig. 1B) is considerably higher than that of constructs IΔ2 (no UAS) and RX7 (Abf1 binding site only).

Finally, in construct RG7 (Fig. 1B) the rp*S33* promoter sequences between nucleotides –176 and –108, encompassing both the Abf1 binding site and the T-rich region, were inserted upstream of the reporter gene. This region, which we designated UAS-*S33*, appeared to represent a powerful upstream activating sequence (UAS), which can account for the full transcriptional activity of the rp*S33* promoter. In fact, GUS activity measured for the construct carrying the reconstituted UAS-*S33* is even higher than the activity corresponding to the entire *S33* promoter fused to the *GUS* gene (see Fig. 1B). This may be due to the different transcription start sites used, resulting in leader sequences with

different stability or translatability. However, it might also reflect the presence of a repressing element in the natural *S33* promoter. The observed level of activation by reconstituted UAS-*S33* is considerably higher than the sum of the separate activities of the Abf1 binding site and the T-rich region, indicating a synergistic interaction between the two elements.

T-rich regions occur in the promoters of a broad spectrum of yeast genes and have often been found to contribute to transcriptional activation. The mechanisms underlying this transcriptional enhancement are as yet unknown, but two hypotheses have been put forward. The first one predicts that T-stretches essentially play a structural role; they may keep promoters nucleosome-free and in doing so facilitate the access of the transcriptional machinery to the promoter (22). The second hypothesis predicts the existence of (a) T-stretch binding protein(s), which may play a direct role in transcriptional activation (21). For instance, in the context of the *DED1* promoter the Abf1 binding site has been reported to activate transcription synergistically with a T-rich element (21). This T-rich element bears no obvious sequence homology with the one found in the rp*S33* promoter, except for the high content of thymidine. It has been suggested that the transcription activation effect of poly(dA–dT) stretches is proportional to their thymidine content, which may prevent nucleosome formation and confers a peculiar 'kinky' structure to the DNA (22). In order to find out whether a high content of thymidine might be sufficient to bring about synergistic activation with the Abf1 binding site, we inserted an oligonucleotide containing the 3′-half of the T-rich region of UAS-*S33* between the Abf1 binding site and the transcription start site in construct RX7, generating construct RXT (Fig. 1B). Four copies of the oligonucleotide are present in this construct, creating an extended T-rich region. However, the relatively low transcriptional activity observed for RXT demonstrates that this 'artificial' T-rich region failed to produce the synergistic effect observed for construct RG7. The observed transcriptional activity is comparable with that observed for construct RX7 (Abf1 binding site alone). We conclude that the role played by the T-rich region in transcriptional activation of the rp*S33* gene is not determined only by its high thymidine content. Rather, it seems likely that within this T-rich region, specific sequences are present that are responsible for binding an as yet unidentified factor.

A Rap1 binding site can replace the Abf1 binding site in UAS-*L45* and -*S33*

As mentioned in the Introduction, the most common *cis*-acting element occurring in rp gene promoters is the binding site for Rap1p. These sites occur, usually in a tandem arrangement, in all rp gene promoters studied so far which do not contain an Abf1 binding site (8). Making use of the previously generated deletion of the rp*L45* gene promoter (11), the naturally occurring Abf1 binding site was replaced by a consensus binding site for Rap1p. The results of the Northern analyses presented in Figure 2 demonstrate that approximately equal amounts of L45 mRNA are present in the various transformants. Therefore, in the context of the rp*L45* promoter the Abf1 binding site can be functionally replaced by a Rap1 binding site. Subsequently, the β-glucuronidase reporter system was used to compare the activation capacity of the Rap1 binding site with that of the *cis*-acting elements from the rp*S33* promoter. The results are shown in Figure 3. For the first promoters shown (constructs RPG1 and RPG2), which both

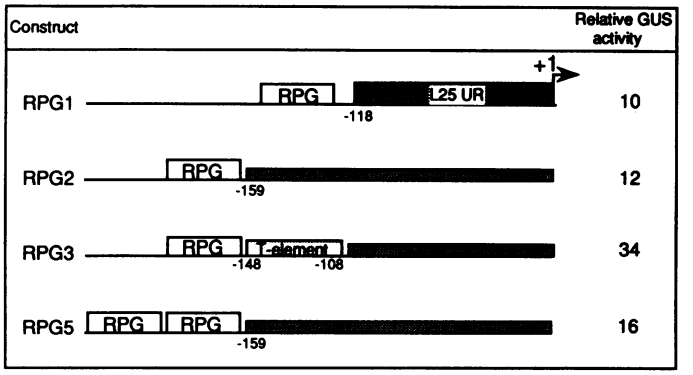

Figure 3. Transcriptional activation of the *GUS* reporter gene by promoters carrying 'consensus' Rap1 binding sites. The coordinates indicated refer to the translation start sites of either the rp*L25* gene (RPG1, 2 and 5) or the rp*S33* gene (T-rich element in RPG3). GUS activities are given relatively to the UAS-less promoter (IΔ2; see Fig. 1).

contain one Rap1 binding site but differ in their distance to the transcription start site, a significantly higher transcriptional activation was measured than for a similar construct carrying instead one Abf1 binding site (cf. RX7). This observation is consistent with results obtained in other reporter systems (20,21). However, in combination with the T-rich region of UAS-*S33* (construct RPG3; Fig. 3) a Rap1 binding site constitutes a strong promoter, resulting in the same (not higher) transcription activation as UAS-*S33*. This result again indicates that the Rap1 binding site can functionally replace the Abf1 binding site in this type of promoter.

The majority of the promoters of rp genes under the control of Rap1p (for example the rp*L25* gene; 7,23) contain two Rap1 binding sites. This raises the question of whether this pair of RPG boxes can constitute a promoter of the same strength as UAS-*S33*. Therefore, we constructed a promoter containing two identical 'consensus' Rap1 binding sites, by introducing an additional Rap1 binding site in construct RPG2, resulting in construct RPG5 (Fig. 3). The activity measured for this construct, however, was only slightly higher than for RPG2. This might be due to a less favourable position of the Rap1 binding sites, since Rap1 binding sites are usually found in rp gene promoters at positions around –400. Furthermore, evidence has previously been obtained (23) suggesting that the distance between a Rap1 binding site and the transcripton start site may be critical for its function as a UAS. An alternative explanation might be that in this promoter a T-rich element also fulfils an important role in transcriptional activation. Such a tripartite UAS has been found to function in at least one rp gene (namely *RP39*; 9) and a T-rich element is also present in the rp*L25* promoter.

Cis-acting elements involved in the transcriptional response of rp genes to growth stimuli

Upon a growth stimulus like, for instance, a nutritional upshift (by adding glucose to a yeast culture growing on a non-fermentable carbon source), rp genes display a coordinate increase in transcriptional activity (4- to 6-fold) (2). Recently, this nutritional upshift response has been studied with respect to components that might be involved in glucose sensing and subsequent signal transduction (5).

In order to find out which of the *cis*-acting elements mentioned above are necessary and/or sufficient to mediate the nutritional upshift response of rp gene transcription, we performed shift

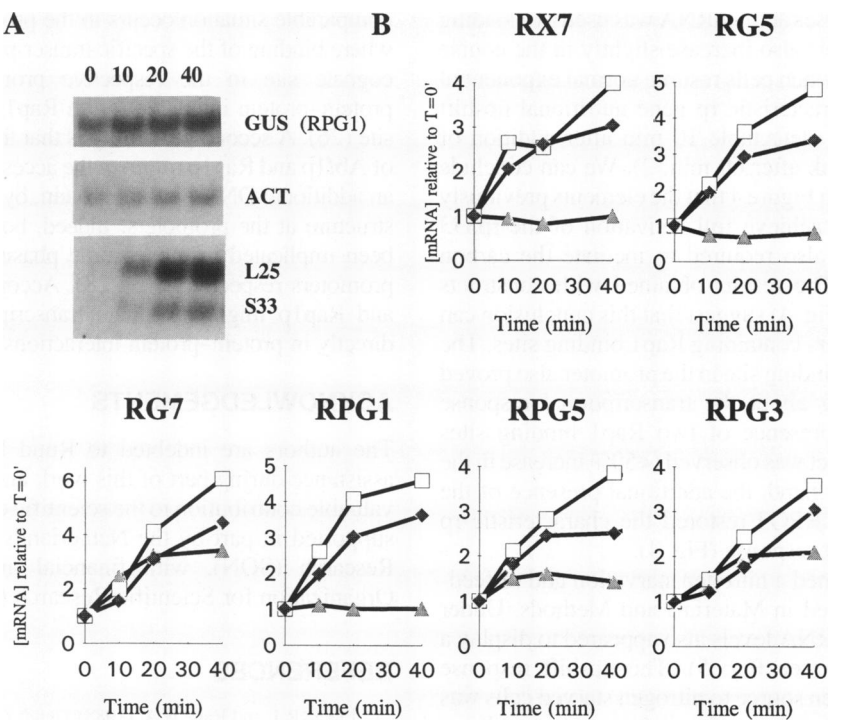

Figure 4. The capacity of the different *cis*-acting elements of the rp*S33* promoter to mediate the nutritional response upon a carbon source upshift was studied using yeast integrants carrying some of the reporter constructs shown in Figures 1B and 3. The yeast integrants were first grown on a glycerol-based medium, to which glucose was added at 0′. RNA was isolated 0, 10, 20 and 40 min after the upshift. (**A**) Northern blot of integrant RPG1 showing the changes in *GUS*, rp*L25* and rp*S33* mRNA levels after a nutritional upshift, using actin mRNA as a loading control. The corresponding quantitation data is shown in (B) (RPG1). (**B**) Quantitation of the changes in the amounts of *GUS* (triangles), rp*S33* (filled squares) and rp*L25* (open squares) mRNAs observed after the nutritional upshift for the different integrants, as indicated. The amount of radioactivity corresponding to each signal was quantified using a phosphoimager (Molecular Dynamics 425) and corrected for the amounts of RNA loaded, using the actin mRNA signal as a control. The values obtained were subsequently compared with the $t = 0'$ value, arbitrarily set at 1.

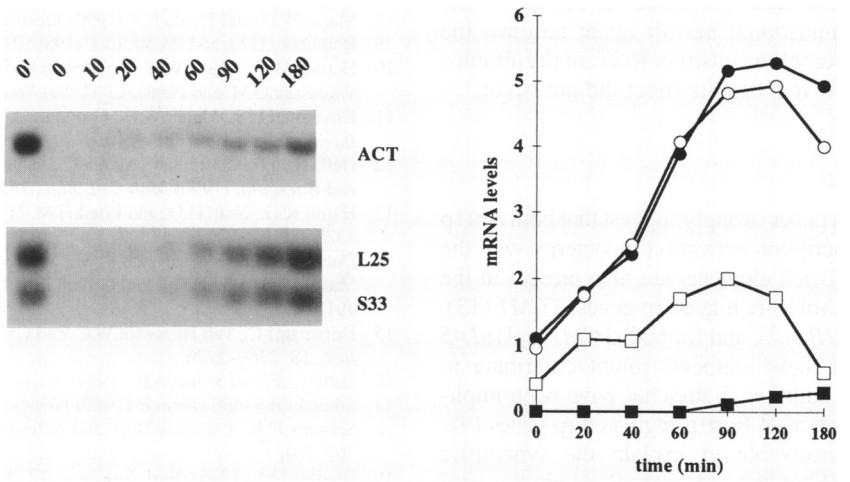

Figure 5. Change in the levels of rp mRNAs and *GUS* mRNA during a nutritional upshift induced by addition of a nitrogen source to nitrogen starved cells (see Materials and Methods), using transformants carrying constructs RX7 and RG7 and assessed by Northern blot analysis. Blots were probed with *GUS*-, rp*S33*-, rp*L25*- and actin-specific probes. On the left is shown a typical Northern blot obtained for rp*L25* and rp*S33* mRNAs. On the right is depicted a quantitation of the *GUS* (open squares for RG7; closed squares for RX7), rp*S33* (open circles) and rp*L25* (closed circles) mRNA levels relative to the levels of actin mRNA.

experiments (both carbon source and nitrogen source upshifts) using yeast integrants carrying several of the fusion genes depicted in Figures 1B and 3. The experiments in most cases were performed by monitoring both the change in the level of GUS activity in total protein extracts (results not shown) and the change in GUS mRNA levels by Northern blot analysis (see Figs 4 and 5).

The results depicted in Figure 4 show that neither the Abf1 binding site alone (construct RX7) nor the T-rich region alone (construct RG5) is able to mediate the carbon source upshift response. In contrast, the construct carrying UAS-*S33* (RG7) did show an increase in GUS mRNA levels following the addition of glucose, which parallels that observed for endogeous L25 and

S33 mRNAs. In these analyses actin mRNA was used as a loading control. Actin mRNA levels also increase slightly in the course of the upshift experiment, when cells resume normal exponential growth. However, the characteristic rp gene nutritional upshift response is much quicker (detectable 10 min after addition of glucose and reaching a peak after 60 min; 2). We can conclude from the results presented in Figure 4 that the elements previously shown to be necessary to achieve full activation of the rp*S33* promoter (see above) are also required to mediate the carbon source upshift response. The results obtained with constructs RPG1, RPG3 and RPG5 (Fig. 4) suggest that this conclusion can be extended to the promoters containing Rap1 binding sites. The presence of a single Rap1 binding site in the promoter also proved to be insufficient to bring about the transcriptional response (RPG1), whereas in the presence of two Rap1 binding sites (RPG5) a slight upshift effect was observed (<50% increase in the first 20 min). On the other hand, the additional presence of the T-rich region in construct RPG3 restored the characteristic rp gene transcriptional upshift response (Fig. 4).

Subsequently we performed a nitrogen starvation and re-feeding experiment, as described in Materials and Methods. Under this upshift condition rp mRNA levels also appeared to display a highly coordinate control (see Fig. 5). The upshift response induced by adding a nitrogen source to nitrogen starved cells was found to occur even in the presence of cycloheximide (result not shown). This finding is consistent with similar observations for a carbon source upshift and suggests that these nutritional control phenomena are based on post-translational events in which cAMP-dependent protein kinase seems to play an important role (5). Furthermore, the nitrogen response was found to be dependent on the presence of glucose; on a non-fermentable carbon source the response did not occur (result not shown). As can be concluded from the data presented in Figure 5, the response to this type of nutritional upshift again requires the composite UAS-*S33*, as present in construct RG7; in the absence of the T-rich element (RX7) this upshift effect did not occur.

Concluding remarks

The results described in this paper strongly suggest that both Abf1p and Rap1p potentiate transcription activation in synergy with the T-rich promoter element. T-rich elements are also present in the promoter regions of other Abf1p-regulated rp genes, *TCM1* (13), rp*L45* (11), rp*L2A* and rp*L2B* (12), and for both *TCM1* and rp*L45* evidence is available that these elements might contribute to transcription activation. In addition, T-stretches have been implicated in transcription activation of Rap1p-regulated rp genes (9).

Several models are conceivable to explain the synergistic interaction between the Abf1 binding site (or the Rap1 binding site) and the T-rich promoter element in UAS-*S33*. Since, so far, no protein factor has been identified which binds to this region of the rp*S33* promoter, it cannot be excluded that the role of the T-rich promoter element is simply a structural one. However, the most straightforward explanation for the available experimental data is that a novel protein factor which activates transcription synergistically with Abf1p or Rap1p is recruited to the promoter by the T-rich promoter element. Notably, both Abf1p and Rap1p have been shown to bend DNA around their recognition sequences (24,25). This may create a recognition site for a promoter binding protein, which is defined by the nucleoprotein structure formed by bound Abf1p or Rap1p, as well as by the adjacent T-rich element. A comparable situation occurs in the promoters of glycolytic genes, where binding of the specific transcriptional activator Gcr1p to its cognate site in the respective promoters is stabilized by a protein–protein interaction with Rap1p bound at a neighbouring site (26). A second possibility is that the DNA bending properties of Abf1p and Rap1p improve the accessibility of the promoters for an additional DNA binding protein, by remodelling the chromatin structure at the promoters. Indeed, both Abf1p and Rap1p have been implicated in nucleosome phasing at the *QCR8* and *HIS4* promoters respectively (27,28). According to this model, Abf1p and Rap1p might potentiate transcription without participating directly in protein–protein interactions at the promoters.

ACKNOWLEDGEMENTS

The authors are indebted to Ruud Laan for skilful technical assistance during part of this work and to Dr H. A. Raué for his valuable contribution to the scientific discussions. This work was supported in part by the Netherlands Foundation for Chemical Research (SON), with financial aid from the Netherlands Organization for Scientific Research (NWO).

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