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The rRNA Enhancer Regulates rRNA Transcription in *Saccharomyces cerevisiae*

BERNICE E. MORROW, STEWART P. JOHNSON,† AND JONATHAN R. WARNER*

Department of Cell Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461

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In *Saccharomyces cerevisiae*, the rRNA genes are organized as a tandem array of head-to-tail repeats. An enhancer of rRNA transcription is present just at the end of each transcription unit, 2 kb away from the next one. This enhancer is unusual for *S. cerevisiae* in that it acts both upstream and downstream of, and even across, genes. The role of the enhancer in the nutritional regulation of rRNA transcription was studied by introducing a centromere plasmid carrying two rRNA minigenes in tandem, flanking a single enhancer, into cells. Analysis of the transcripts from the two minigenes showed that the enhancer was absolutely required for the stimulation of transcription of rRNA that occurs when cells are shifted from a poor carbon source to a good carbon source. While full enhancer function is provided by a 45-bp region at the 3′ end of the 190-bp enhancer, some activity was also conferred by other elements, including both a T-rich stretch and a region containing the binding sites for the proteins Reb1p and Abf1p. We conclude that the enhancer is composed of redundant elements and that it is a major element in the regulation of rRNA transcription.

One hundred and forty copies of the rRNA genes are present in a single tandem array within the genome of the yeast *Saccharomyces cerevisiae*. Their transcription by RNA polymerase I is subject to control by an enhancer element, located just downstream of one 35S rRNA gene and 2 kb upstream of the next. The enhancer is required for maximal levels of rRNA transcription in vivo (9, 10). Its effect is relatively independent of position and orientation (10). Unlike enhancers of RNA polymerase II in *S. cerevisiae*, this enhancer is active even when downstream of its target gene (15).

The enhancer, located within a 190-bp restriction fragment, contains several recognized protein binding and/or activation sites, including one for Reb1p (26, 27) (also known as GRF2 [5] or QBP [3]) and a weak one for Abf1p (formerly REB2 [26]). In addition, there is a thymidine-rich region (5, 23, 36) which may work either through an inferred binding factor or through its own influence on DNA conformation. Combinations of these three elements are also present within transcriptional control regions of a number of genes transcribed by RNA polymerase II; however, they are usually within 500 bp of the site of transcription initiation (4, 5, 30, 36). The potential involvement of these factors in transcription of both rRNA and mRNA is underscored by the recent finding that the TATA-binding factor is implicated in transcription by all three nuclear RNA polymerases (6, 8, 33).

In *S. cerevisiae*, the number of ribosomes per cell is proportional to the growth rate, which is itself a function of the carbon source in the growth medium (39). Cells grow on glycerol plus ethanol (G/E) at about one-third the rate of cells growing on glucose and have about one-half the ribosome content (19). Addition of glucose to a culture growing on G/E leads to a rapid increase in the transcription of rRNA and in the synthesis of ribosomal proteins prior to any change in total cellular protein synthesis (19). As a consequence, the concentration of ribosomes per cell, and ultimately the growth rate, increases substantially.

Little is known about the mechanism of this regulation of 35S rRNA transcription. To investigate whether the enhancer, or a portion of it, plays a role, we constructed a plasmid in which a single rRNA enhancer was placed between two differentially marked rRNA genes in a tandem array, as in vivo (15). A series of deletions within the enhancer were prepared. In each case, the levels of transcription of both the upstream and the downstream gene were measured in a culture growing in G/E and after addition of glucose.

We found that the enhancer has some activity during growth on a nonfermentable carbon source but is absolutely required for the activation of rRNA transcription induced by glucose. A 45-bp element at the 3′ end of the enhancer is both necessary and sufficient for full enhancer activity. The enhancer, however, contains redundant elements. The T-rich region confers partial glucose activation of both minigenes. The 5′ portion of the enhancer, including the Reb1p and the Abf1p sites, can stimulate transcription in G/E but is only slightly responsive to further activation by glucose.

MATERIALS AND METHODS

Strains and media. The host strain of *S. cerevisiae* is W303a (*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*), which was obtained from R. Rothstein (Columbia University). Strain W303a was usually cultured on defined minimal medium lacking uracil to select for the YCp50-derived plasmids, with a carbon source of either 2% glycerol–2% ethanol (G/E) or 2% glucose.

Plasmid constructs. All starting plasmids have been described previously (15). Each of the constructs described below and diagrammed in Fig. 3B was sequenced to verify the fusion joints. Nucleotide coordinates refer to Fig. 3A.

* Corresponding author.
† Present address: Department of Pathology, Duke University Medical Center, Durham, NC 27710.

YCpRR89. Plasmid pRR82B (15) was cleaved with *Bgl*II and *Sal*I. The 3.2-kbp fragment containing the T7B rRNA minigene with the intact enhancer was ligated to *Bam*HI-*Sal*I-cleaved YCpRR8 (9).

YCpRR93 (deletion of entire enhancer). Plasmid pRR83, lacking the enhancer, was digested with *Bgl*II and *Sal*I, and the 3.0-kbp fragment was isolated and ligated to *Bam*HI-*Sal*I-cut YCpRR8.

YCpRR128 (deletion of Reb1p-binding sites). The two REB1 sites within the rRNA spacer were deleted. The Reb1p-binding site within the rRNA enhancer from positions 14 to 19 (Fig. 3A) was deleted by site-directed mutagenesis (20). Similarly, the Reb1p-binding site, TACCCGG, from position −210 to −203 upstream from the transcription initiation site of the T7B gene was removed. The resulting plasmid was named YCpRR128.

YCpRR106 (deletion of the 3′ half). The 71-bp *Eco*RI-*Fok*I fragment from the enhancer was isolated, blunt ended with Klenow fragment, and ligated into pRR82B that had been digested with *Eco*RI and *Hin*dIII to remove the wild-type enhancer and blunt ended with Klenow fragment. The resulting plasmid, pRR82 (*Eco*RI-*Fok*I), was digested with *Bgl*II and *Sal*I and cloned into YCpRR8 as described above.

YCpRR133 (deletion of Reb1p and Abf1p sites). Two oligonucleotides, GGAAGATCTATGATAGTGTGTAAGAGT (containing a *Bgl*II restriction site fused to the enhancer sequence beginning 61 bp downstream from the *Eco*RI site) and CGGGAACGTTTGTGAAAGCCCTTCTCT (containing the *Hin*dIII site), were used to prime the polymerase chain reaction to create an enhancer mutant lacking the 5′ 61 bp. The polymerase chain reaction product was isolated and digested with *Bgl*II and *Hin*dIII and cloned into pRR82B cut with *Bgl*II and *Hin*dIII. The resulting plasmid, pRR133, was sequenced for verification, digested with *Bgl*II and *Sal*I, and cloned into YCpRR8 to form YCpRR133.

YCpRR87 (deletion of 3′ region). Plasmid pRR87 contained a deletion from positions 153 to 207 downstream from the *Eco*RI site of the enhancer. This plasmid was cut with *Bgl*II and *Sal*I and cloned into YIpRR8 to form YIpRR87. The 7-kbp *Cla*I-*Sal*I fragment from YIpRR87 was isolated and cloned into YCpRR50 (10) to form YCpRR87.

YCpRR130 (3′ end alone). The complementary oligonucleotides (see Fig. 3A)

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          149
          |
5′ GATCTTTCGTTGCAAAGATGGGTTGAAAGAGAAGGGCTTTCACAA     3′
3′     AAAGCAACGTTTCTACCCAACTTTCTCTTCCCGAAAGTGTTTCGA 5′
```

containing *Bgl*II-*Hin*dIII ends were annealed and ligated to plasmid pRR82B that had been cut with *Bgl*II and *Hin*dIII to replace the wild-type enhancer. Following sequence analysis for verification, the resulting plasmid, pRR130, was cut with *Bgl*II and *Sal*I and ligated to *Bam*HI-*Sal*I-cut YCpRR8.

YCpRR116 (deletion of T-rich region). The plasmid pRR82D6 was constructed by a double-primer mutagenesis of M13-RD1 with oligonucleotide CATCTTTGCAACGGAC CAAATACTAA and the M13 universal primer. Following the extension-ligation reaction, the double-stranded DNA was cut with *Eco*RI and *Hin*dIII, and the resulting fragments were ligated to pRR82B that had already been cut with *Eco*RI-*Hin*dIII and treated with phosphatase. The mutants were sequenced within the enhancer region for verification. The resulting plasmid, pRR82D6, was cut with *Bgl*II and *Sal*I and cloned into YCpRR8 as described above.

YCpRR131 (T-rich region alone). The complementary oligonucleotides containing the T-rich region of the enhancer

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          121
          |
5′ GATCTCTTTTTATTTTTTATTTTTTTTTTTTTTTCGTTA      5′
3′     AGAAAAATAAAAAATAAAAAAAAAAAAAAAAAGCAATTCGA 3′
```

and containing *Bgl*II-*Hin*dIII ends were annealed and inserted into pRR82B cut with *Bgl*II and *Hin*dIII to replace the wild-type enhancer to form pRR131. Plasmid pRR131 was cut with *Bgl*II-*Sal*I and ligated to YCpRR8 that had been cut with *Bam*HI and *Sal*I to form YCpRR131.

Transformation of yeast cells. Plasmids YCpRR87 to -133 were used to transform yeast strain W303a by the lithium acetate method (12).

Isolation of RNA. Homogenization of the cells and isolation of RNA were performed as previously described (9).

Preparation of RNA probes and Northern (RNA) blot analysis. Northern blot analysis of total RNA was performed as previously described, with antisense RNA hybridization probes for the T7A and T7B rRNA minigenes (15). Antisense actin RNA was radiolabeled and used as a probe for a loading control.

Quantitation of Northern blot analysis. Each autoradiogram was scanned by using a Molecular Dynamics Computing Densitometer and the Quantity One program of *pdi* Inc. The area of each band was determined for each experiment and normalized to the level of actin mRNA, which was used as a control for loading of RNA. (Figure 2, as well as our other data, suggests that the level of actin mRNA is relatively independent of the carbon source.) Two independent Northern blots were quantitated from a minimum of two separate experiments, and the mean and standard deviation were determined. Thus, each value in Table 1 derives from four independent determinations. In addition, since YCpRR89 and YCpRR93 were always used as a positive and negative control, respectively, there was a minimum of six separate experiments, each analyzed twice by Northern blot hybridization for these constructs.

RESULTS

Construction of tandem rRNA minigenes. Most of the internal coding regions of the test rRNA genes were replaced with reporter sequences from bacteriophage T7 DNA to detect plasmid-specific rRNA transcription (15). The 5′ and 3′ ends of the rRNA genes were left intact to provide initiation and termination signals (10). Within a yeast centromere plasmid, two rRNA minigenes (A and B, marked with distinct sequences from bacteriophage T7 DNA) were inserted in tandem (Fig. 1) in order to mimic the arrangement of rRNA genes in vivo (15). A single enhancer element was placed in its normal location just downstream from the A minigene and 2 kb upstream from the B minigene (Fig. 1).

The rRNA enhancer is required for activation of rRNA transcription by glucose. *S. cerevisiae* strain W303a grows at 30°C, with doubling times of 4.5 h on G/E and 1.5 h on 2% glucose. *S. cerevisiae* containing either plasmid YCpRR93 (no enhancer) or plasmid YCpRR89 (including the enhancer) was grown to mid-log phase on either G/E or glucose. The RNA was isolated from each culture and subjected to Northern blot analysis to determine the levels of rRNA minigene transcription (Fig. 2). The results are summarized in the first two lines of Table 1, where all values have been calculated relative to that of YCpRR89 in glucose as 100 (boxed in Table 1). (Note that strictly speaking, Fig. 2 and Table 1 report accumulation, not transcription; however, there is little reason to suspect that they are different.)

The striking result is that in the absence of the enhancer the carbon source has no influence on the transcription of

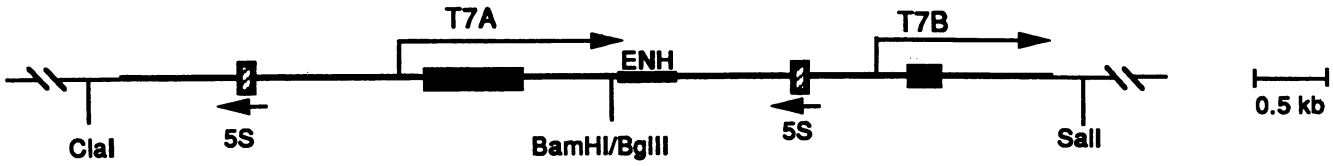

FIG. 1. Diagram of rDNA minigenes in YCp50. Two rDNA minigenes were inserted between the *Cla*I and *Sal*I sites of the yeast shuttle vector YCp50 (15). Most of the internal coding region of 35S rDNA has been replaced with different bacteriophage T7 sequences (black boxes) to distinguish them from the chromosomal rDNA genes and from each other. The 5S rRNA genes (hatched boxes) are present within each spacer region and are transcribed in the orientation opposite to that of the A and B rDNA genes as indicated. A single enhancer (ENH) has been inserted in its normal position between the two minigenes. This construct has been named YCpRR89. The identical construct lacking the enhancer is YCpRR93.

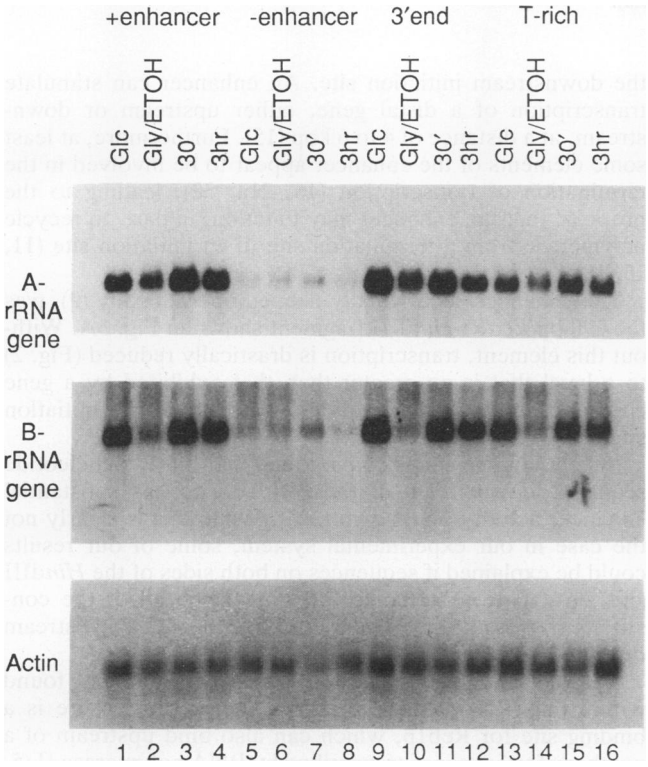

FIG. 2. Northern blot analysis of rDNA minigenes A and B. Cultures grown either in glucose or in G/E were harvested at an optical density at 600 nm of 1.0. After an aliquot was removed from the culture grown in G/E, glucose was added to a concentration of 2% and additional aliquots were harvested after 30 min or 3 h. Total RNA was prepared from yeast strains containing plasmid YCpRR89, YCpRR93, YCpRR130 (3′ end), or YCpRR131 (T-rich region [Fig. 3B]). Five micrograms of total RNA was subjected to denaturing gel electrophoresis (15). The blot was probed sequentially first for the B gene (because its intensity was lower, since the transcript is shorter) and then for the A gene. The blot was counterprobed for actin. Actin mRNA levels were not affected by the growth medium. A series of six independent experiments was performed, with two Northern blots each, for constructs YCpRR89 (+enhancer) and YCpRR93 (−enhancer). These results were subjected to quantitation (Table 1). The twofold increase observed with the B rRNA gene in lane 7 was not consistent with results from the other experiments. Experiments with the 3′ end construct (YCpRR130) and the T-rich construct (YCpRR131) were performed twice with two independent Northern blots each.

either test gene (Fig. 2, lanes 5 and 6; Table 1, lines 1 and 2). Thus, it is the enhancer element, and not the sequences in the vicinity of the promoter elements, that participates in the response of rRNA synthesis to the carbon source. A second observation is that the response of the upstream gene differs from that of the downstream gene. Transcription of the latter is only marginally stimulated by the presence of the enhancer in G/E medium (2-fold), while such stimulation is about 10-fold in glucose. The upstream gene, on the other hand, is stimulated more than fourfold in G/E but is stimulated only twofold further when glucose is added. We conclude that the enhancer is the DNA element largely responsible for the coupling of rRNA transcription to the carbon source.

Role of individual elements within the enhancer. The enhancer (Fig. 3A) contains sites for the binding of two abundant DNA-binding proteins, Reb1p (bp 13 to 28) and Abf1p (bp 41 to 60). In addition, it has a T-rich region (bp 116 to 156) that has been implicated in the transcription of many genes by RNA polymerase II (4, 28, 31, 34), although no binding protein has yet been identified.

To ascertain whether any of these elements plays a role in the regulation of rRNA transcription by the carbon source, we constructed partial deletions within the enhancer (Fig. 3B). Analysis of the levels of RNA derived from the reporter genes adjacent to these altered enhancers is summarized in Table 1. Deletion of the Reb1p site (YCpRR128) or of both the Reb1p and the Abf1p sites (YCpRR133) does not reduce the transcription levels of either the upstream or the downstream minigene in glucose or in G/E. Thus, we conclude that neither of these sites is necessary for the enhancement of transcription or for the response to glucose. On the other hand, a construct in which the enhancer is replaced by a fragment including only these two sites (YCpRR106) does show slight enhancer activity following glucose supplementation, but this activity is transient. Cells growing continuously on either carbon source show no difference in the level of transcription (Table 1).

In YCpRR128, the Reb1p site was also deleted from both the enhancer and the promoter regions of the B gene. The lack of effect shown in Table 1 confirms the results reported previously (21), suggesting that Reb1p does not play an obvious role in initiation of RNA polymerase I transcription.

The role of the T-rich element was explored by using constructs YCpRR116 and YCpRR131. When the T-rich element is deleted (YCpRR116), we find minimal enhancer activity in G/E medium but substantial activity in glucose. When the enhancer element was replaced with the T-rich element by itself (YCpRR131), there was increased transcriptional activity in both carbon sources, although less

TABLE 1. Quantitation of Northern blots of rDNA enhancer mutants under different nutritional conditions[a]

Construct	rRNA gene A accumulation[b] (mean ± SD) when grown in:				rRNA gene B accumulation[b] (mean ± SD) when grown in:			
	Glucose	G/E	G/E, then glucose for 30 min	G/E, then glucose for 3 h	Glucose	G/E	G/E, then glucose for 30 min	G/E, then glucose for 3 h
YCpRR89	[100]	60 ± 26	116 ± 24	92 ± 22	[100]	21 ± 10	106 ± 48	75 ± 27
YCpRR93	13 ± 7	14 ± 10	20 ± 9	10 ± 4	11 ± 5	10 ± 6	13 ± 7	11 ± 7
YCpRR128	86 ± 10	32 ± 16	82 ± 16	71 ± 25	103 ± 67	18 ± 16	88 ± 58	86 ± 62
YCpRR106	28 ± 11	29 ± 20	46 ± 5	37 ± 1	20 ± 13	17 ± 12	39 ± 21	30 ± 23
YCpRR133	152 ± 26	95 ± 10	139 ± 24	121 ± 32	206 ± 67	44 ± 9	213 ± 64	118 ± 45
YCpRR87	33 ± 6	24 ± 9	37 ± 14	40 ± 11	28 ± 12	12 ± 5	32 ± 16	31 ± 8
YCpRR130	145 ± 6	97 ± 1	135 ± 41	85 ± 6	153 ± 17	35 ± 8	127 ± 14	70 ± 14
YCpRR116	55 ± 36	19 ± 10	23 ± 5	24 ± 4	44 ± 27	10 ± 4	20 ± 9	22 ± 8
YCpRR131	75 ± 4	52 ± 22	80 ± 4	55 ± 25	64 ± 20	27 ± 6	66 ± 17	43 ± 25

[a] Northern blots such as those shown in Fig. 2 were probed for the upstream (A) and downstream (B) genes and quantitated (see Materials and Methods). The means and standard deviations were determined. For YCpRR89 and YCpRR93, the data are the averages of 6 (T7A) or 12 (T7B) independent determinations; for the others, they are averages of four independent determinations (see legend to Fig. 2).

[b] Relative to the value obtained for YCpRR89 grown in glucose, set as 100 (boxed).

than that which we observed when the complete enhancer was present (Fig. 2, lanes 13 to 16).

The function of the 3′ portion of the enhancer was investigated by using constructs YCpRR87 and YCpRR130. The enhancer in YCpRR87 lacks the 3′ end, yet provides some enhancer activity in G/E. However, it is poorly responsive to glucose, its response being similar to that of YCpRR106. Conversely, a construct containing only the 3′ 45 nucleotides of the enhancer (YCprR130) provides full enhancer activity and response to glucose for both the upstream and the downstream genes (Fig. 2, lanes 9 to 12).

Kinetics of the response to glucose. The two columns to the right of each side of Table 1 summarize the data from a kinetic analysis of the response to the addition of glucose to a culture growing in G/E. It is apparent that for nearly every construct the level of the test RNAs reaches within 30 min the same level that it does when cells are grown entirely in glucose, although it may decline somewhat after 3 h, perhaps because the cells have passed through the most active phase of log-phase growth (17). The rate of increase in transcription is consistent with our previous analysis of the rate of transcription of authentic rRNA (19).

One construct, however, behaves differently. Transcription of both genes of YCpRR116, which lacks the T-rich element, rises only slowly after addition of glucose. The steady-state level in glucose is not reached in the duration of the experiment. Thus, the T-rich elements may have some role in the rate of response to glucose. However, this is not apparent in construct YCpRr130, which lacks both the T-rich element and the Reb1p- and Abf1p-binding sites. A search of the sequences fused upstream of the 45-bp 3′ end of the enhancer in this construct does not reveal a fortuitous T-rich region. This result suggests that there are both positive and negative interactions among the elements in the intact enhancer, leading to the efficient, regulated transcription of rRNA.

DISCUSSION

The data summarized in Table 1 must be considered in light of the properties of the rRNA enhancer that are unusual, and perhaps unique, for *S. cerevisiae*. A single enhancer can affect two transcription units, one upstream and one downstream, and at considerable distances, of about 1.4 kbp for the upstream initiation site and about 2.0 kbp for the downstream initiation site. An enhancer can stimulate transcription of a distal gene, either upstream or downstream, at a distance of 4 to 6 kbp (15). Furthermore, at least some elements of the enhancer appear to be involved in the termination of transcription (16, 21a, 38), leading to the proposal that the enhancer may function, in part, to recycle polymerase from a termination site to an initiation site (11, 15, 18).

The enhancer, as originally defined operationally (9), was the 190-bp *Eco*RI-*Hin*dIII fragment shown in Fig. 3A. Without this element, transcription is drastically reduced (Fig. 2) to a level that is no greater than that exhibited by a gene containing only 200 nucleotides upstream of the initiation site.

Recent in vitro studies have found that the 135-nucleotide sequence downstream of the *Hin*dIII site has substantial enhancer activity on its own (32a). While this is clearly not the case in our experimental system, some of our results could be explained if sequences on both sides of the *Hin*dIII site participate in enhancer activity, since all of the constructs shown in Fig. 3B contain the sequences downstream of the *Hin*dIII site.

At least four potential activation elements are found within the rRNA enhancer shown in Fig. 3A. There is a binding site for Reb1p, which can also bind upstream of a number of yeast genes transcribed by RNA polymerase II (5, 27). There is a weak binding site for Abf1p, which acts as a transcription factor (4), a silencing factor (2), and a replication factor (30). There is a 30-bp T-rich stretch, that, either through its influence on DNA structure or through an as-yet-unidentified binding protein, can act as a transcriptional activator, especially in connection with other activators (5, 23, 31, 36). Finally, there is a region at the 3′ end of the enhancer. Although no protein-binding sites have yet been identified for this region, it contains an intriguing stretch of 20 consecutive purines interrupted only once with a T and once with a TT.

Table 1 shows that 45 bp of the 3′ end (YCpRR130) is both necessary and sufficient to provide full enhancer function, including the response to the addition of glucose. Indeed, this region alone appears to be slightly more active than the entire enhancer, suggesting that there are inhibitory sequences upstream. Deletion of the Reb1p and/or the Abf1p sites has little if any effect on transcription. Nevertheless,

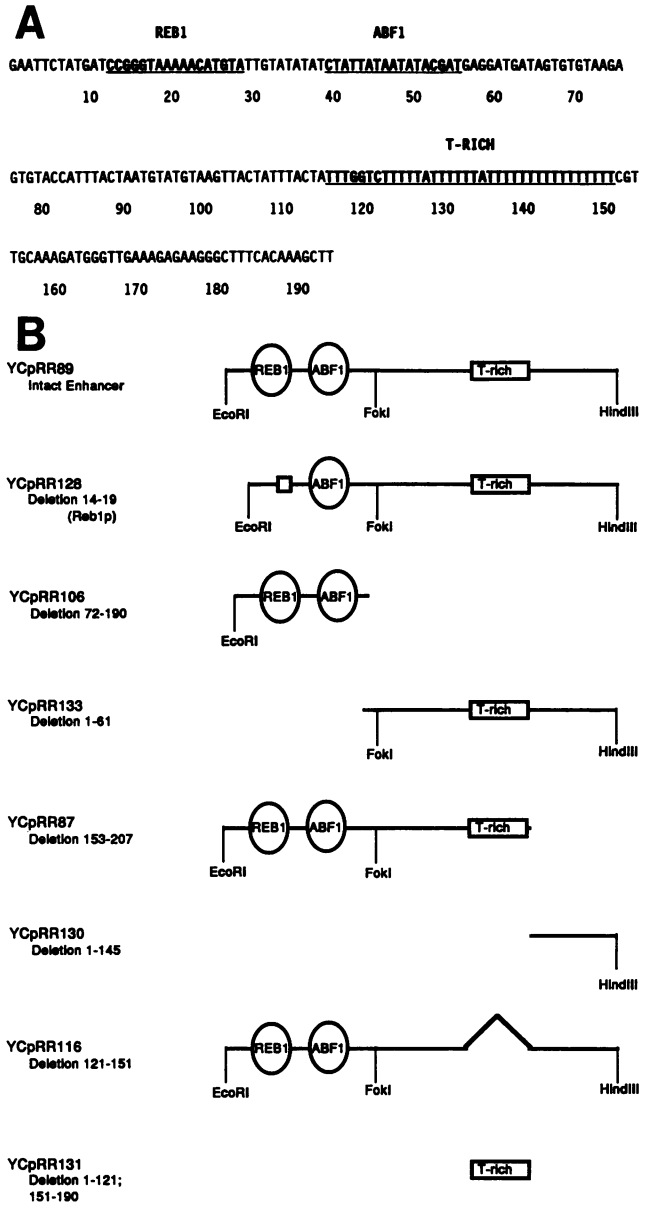

FIG. 3. (A) Sequence of the rDNA enhancer. The nucleotide sequence of the rRNA enhancer (11) between the *Eco*RI site (position 1) and the *Hin*dIII site (position 190) is shown. The Reb1p site, Abf1p site, and T-rich region are indicated (23, 26, 27). (B) Deletions within the rRNA enhancer. Deletions of regions within the enhancer were created to determine the function of individual elements. The positions of the Reb1p site, the Abf1p site, and the T-rich region are indicated (see panel A), as are the coordinates of each deletion.

the presence of those sites alone does provide some enhancer function. In addition, the T-rich region confers partial enhancer function that is responsive to glucose. Thus, there are redundant elements within the enhancer. These elements are probably interactive in some fashion, as indicated in the case of YCpRR116, in which deletion of the T-rich region decreases the level of transcription, even though the entire 3′ end is present. Our results are not entirely consistent with those of Mestel et al. (25), who found that deletions leaving only 52 nucleotides of the 3′ end of the enhancer reduced transcription by more than 80%. Their constructs differ in two respects, either of which could be relevant. On the one hand, their enhancer was placed only 210 nucleotides from the site of initiation of transcription, and may have somewhat different properties in that location. On the other hand, the 5′ end of their enhancer was fused to vector sequences; the enhancer in our construct is entirely within rDNA sequences and is subject to transcription itself from the polymerase traversing the A gene (Fig. 1).

The T-rich region is clearly responsive to glucose activation of rRNA transcription (Table 1) but is less effective than the 45-bp *Hin*dIII region. A T-rich region has also been implicated in enhancing transcription by RNA polymerase II (31), and Lorch et al. (23) have suggested that the T-rich regions of rRNA and of mRNA genes are interchangeable, although the distance between the T-rich region and the promoter is far greater in the case of rRNA. How it functions, however, remains a matter of speculation. No specific binding protein has been found. The T-rich region may act simply through its effect on DNA structure, a suggestion supported by the observation that its length differs markedly among various strains of *S. cerevisiae* (14). In any case, Table 1 provides evidence that a T-rich region can be involved in the specific regulation of transcription.

The transcription of rRNA and its regulation have been studied in a number of higher organisms (for reviews, see references in 22, 29, and 35). In vertebrates, an upstream binding factor binds within 100 to 200 bp of the initiation site and facilitates the binding of factor SL1, now known to be TATA-binding factor plus several associated proteins termed TAFs (6). The search for enhancer elements has been dominated by the analysis of the 60- to 81-bp repeats found upstream of the *Xenopus* rRNA transcription unit (29). These bind upstream binding factor 1 (UBF1) and stimulate transcription. In some cases, they are associated with promoter elements that lead to transcription within the nontranscribed spacer. In mammalian cells, less-regular repeated elements found upstream of the rRNA genes also seem to stimulate transcription; a more distinct enhancer in rat cells has been reported (13).

Two paradigms have been used to explain the regulation of transcription (29). The one most frequently invoked to explain the regulation of transcription of a gene by RNA polymerase II involves alteration in the receptivity of the gene for the polymerase, usually because of the binding of new or modified stimulatory factors to the controlling elements of the gene, or, less frequently, because of the removal of inhibitory factors. The other paradigm, for which there is some evidence in the transcription of rRNA in higher organisms, suggests that the receptivity of the gene is not altered; rather, the proportion of activated RNA polymerase I molecules is altered (1, 32, 37). The view is that an active complex is permanently set up on the rRNA gene (7) but that transcription is limited by the amount of polymerase. Stimulation of rRNA transcription is effected by increasing the number of active polymerase I molecules. Stated more simply, the first paradigm suggests an excess of active polymerase searching for an active gene; the second suggests a constant supply of active genes waiting for a limiting supply of active polymerase. In the latter view, the enhancer should play a role only in helping to establish active genes. Addition of a favorable carbon source should stimulate transcription to the same degree whether or not an enhancer is present. Table 1 shows, however, that this is not the case. Since stimulation of transcription is entirely dependent on

the enhancer, we conclude that the enhancer plays a role in gene selection. Therefore, insofar as the enhancer is concerned, the regulation of rRNA transcription in *S. cerevisiae* in response to a carbon source is largely explained by the first paradigm; the enhancer increases the activity of its genes in response to glucose.

The clear conclusion is that, at the DNA level, the major element of regulation is not the promoter or adjacent sequences but is the enhancer, 2 kb distant and including or adjacent to the terminator. It is intriguing that the effect of the enhancer on transcription of the downstream (B) gene is relatively weak in G/E but is strong upon addition of glucose.

This observation extends previous suggestions that a key element, not only in the transcription of rRNA but in its regulation as well, is the functional and perhaps physical association of the end and the beginning (11, 15, 18). While such a model seems particularly appropriate for a set of tandemly repeated genes, it could apply as well to a single gene, as it appears to do for the upstream (A) gene.

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