Characterization of the Promoter of *PRS1* in *Saccharomyces cerevisiae* Identifies Three Regions Potentially Involved in Control of Expression

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The transcription initiation site of the *Saccharomyces cerevisiae PRS1* gene was mapped at –179 bp. Measurement of β -galactosidase activity of the successively deleted *PRS1* promoter linked to *lacZ* and integrated at the *ura3* locus defined three DNA regions involved in the control of *PRS1* expression. Gel shift analysis confirmed the data.

The enzyme 5-phosphoribosyl-1(α)-pyrophosphate synthetase (PRS) (ATP:D-ribose-5-phosphate pyrophosphotransferase; EC 2.7.6.1) acts at a key junction in intermediary metabolism. PRS transfers the pyrophosphate moiety released from ATP to ribose-5-phosphate, thus giving rise to phosphoribosyl pyrophosphate (PRPP). This reaction directs ribose-5-phosphate from energy generation by the pentose phosphate pathway to the essential biosynthetic intermediate PRPP, a precursor in the production of purine, pyrimidine, and pyridine nucleotides and the amino acids histidine and tryptophan (13).

PRS genes have been identified for more than 20 organisms, including bacteria, protozoa, plants, and mammals (6). The *PRS* gene family in *Saccharomyces cerevisiae* consists of five genes, each of them located on a different chromosome (2, 8). The five polypeptides each contain the characteristic motifs of PRS polypeptides: a divalent cation nucleotide binding site and a PRPP binding site (1, 11). Disruption analysis showed that none of the *PRS* genes is essential and, although all of them are involved in PRPP biosynthesis, the contributions of each to the cell's metabolism do not appear to be equal (3, 8, 9).

In order to define the promoter region of *PRS1*, we mapped the 5' end of its transcript. Total RNA from exponentially growing YN94-1 cells (9) was prepared, and a *HindIII/PstI* DNA fragment containing 762 bp corresponding to the region located between –807 and –45 nucleotides (nt) from the start codon of *PRS1* (see Fig. 2) was used for S1 nuclease mapping (17). As shown in Fig. 1A (lanes 3 and 4), the main product protected from the S1 digestion was a triplet of fragments 133 to 135 nt long corresponding to a transcription initiation between positions –180 to –178 from the *PRS1* translation initiation codon. To confirm this result, primer extension analysis was carried out using primer P10 (5'-TTAAGACTATTAAA CGGT-3') complementary to the *PRS1* mRNA sequence between positions –18 and –35 (Fig. 1B). This produced a triplet

of extension products of sizes ranging from 159 to 161 nt, which places the *PRS1* transcription start site between nt –179 and –177 from the *PRS1* AUG codon (Fig. 1A, lanes 1 and 2). Both techniques placed the *PRS1* mRNA start site at the nt –179 position (which corresponds to an A nucleotide in Fig. 1B), 311 nt downstream of the stop codon of the preceding gene (14). There are three TATA-like elements, one canonical, located 203 nt upstream of the *PRS1* mRNA initiation site, and two noncanonical, located at 175 and 65 nt upstream of the *PRS1* mRNA initiation site.

To define the extent of the region required for the expression of *PRS1*, a translational fusion was constructed by inserting the 898-bp *HindIII/BsrGI* fragment containing the first 88 bp of the coding region of *PRS1* plus 810 bp upstream thereof (2) into the appropriately restricted plasmid YEp356R (10). A linker (*HindIII-BamHI-SphI-SmaI-XbaI-HindIII*) was inserted at the *HindIII* site at –810 to create pSS13. Linearization with *SphI* and *XbaI* provided a DNA template for creating unidirectional deletions by exonuclease III-mung bean nuclease treatment. The extent of each deletion was determined by DNA sequencing. We obtained seven derivatives of pSS13, and from each of these plasmids the *HindIII/NsiI* fragment spanning the promoter and the *lacZ* reporter cassette was cloned into appropriately restricted YIp352 (16). The integrative plasmids obtained were linearized at the *NcoI* site of the *URA3* gene to target them to the *ura3* locus of YN94-1, thus creating the strains listed in Table 1. Yeast transformations were performed according to the method of Elble (5).

β -galactosidase activity was determined according to the method of Guarente (7), and the values obtained for the series of integrated deletions are shown in Fig. 2. There was a doubling of β -galactosidase activity when sequences between –434 (YN98-1-90) and –346 (YN98-1-21) (region A) (Fig. 2), which correspond to the transcription termination signals of the *FAS1* mRNA (14), were deleted. Deletion of sequences between –346 (YN98-1-21) and –282 (YN98-1-55) caused a 50% loss of β -galactosidase activity, suggesting that this region (region B) is required for maximum activity of the promoter (Fig. 2). There was a further reduction of activity when se-

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FIG. 1. Mapping of the 5' end of *PRS1* mRNA. (A) Primer extension analysis was performed using 150 or 30 ng of RNA from exponentially growing YN94-1 (lanes 1 and 2, respectively) with primer P10. S1 mapping was performed using the same total RNA as for the primer extension hybridized at 37°C in formamide buffer (lane 3) or Na-tris-tris buffer (lane 4). Lane O contains a ³²P-labeled *Hinf*I digest of Φ x174 DNA, and lanes T, C, G, and A contain sequence reaction products of M13mp18. A 6% polyacrylamide–50% urea sequencing gel was used. Positions corresponding to the 5' end of transcripts are indicated by arrowheads. (B) Upstream sequence of *PRS1*. The *PRS1* transcription initiation site is at –179 (▼). The position of the P10 primer is indicated by a dashed line. The TATA box-like sequence located within C-21 is indicated in bold. The sequences corresponding to oligonucleotides A-213, B-311, and C-21 giving rise to DNA-protein complexes are underlined.

quences between –282 (YN98-1-55) and –205 (YN98-1-52) were deleted, indicating that this region (region C) is required for basal expression of the gene (Fig. 2). In strain YN98-1-11, the transcription initiation site has been eliminated, and the β -galactosidase activity was reduced to that of the promoterless *lacZ* gene, YN98-1-10.

The three regions defined above may contain regulatory sequences involved in the control of *PRS1* expression. Each of these regions was examined for DNA-protein binding by electrophoresis mobility shift assays (EMSA). These assays were performed as described in reference 19. For region A, we used a 115-nt PCR product (A-1) (positions –443 to –328) as a

probe in an EMSA with cell extracts from YN94-1. A specific retarded band (Fig. 3A, arrowhead) whose intensity increased with increasing amounts of protein extract was observed. This complex was sequence specific because it could be completed by excess amounts of unlabeled probe. A-1 was divided into two overlapping products, A-2 (–443 to –377) and A-3 (–399 to –328), and only A-2 successfully competed with the complex. A-2 was further divided into the overlapping fragments A-21 (–443 to –406) and A-23 (–416 to –377); of these two, A-21 was the better competitor of the lower A-2 DNA-protein complex (Fig. 3B). Interestingly, a further specific, larger band is also obtained with A-2 which is in competition with A-21 but not with A-23 (Fig. 3B, asterisk), also indicative of a protein binding within A-21. Dissection of A-21 into A-211 (–439 to –419) and A-213 (–428 to –406) showed that A-213 successfully competed with the A-21 complex (Fig. 3C). The latter results are confirmed in Fig. 3D, and the position of A-213 in the *PRS1* promoter is shown in Fig. 1B.

Region B is required for maximum expression of *PRS1* and spans the region between nt –346 and –282. B-1 (–358 to –254), which overlaps with A-1, gave rise to a retarded band (Fig. 4A) which was sequence specific and did not compete with excess amounts of unlabeled A-1, indicating that the factors binding to regions A and B are different (data not shown). Using the same approach as for region A, B-1 was subdivided into B-2 (–358 to –299), B-3 (–316 to –253) (Fig. 4A and B), B-31 (–312 to –293), B-33 (–292 to –258) (Fig. 4C and D), B-311 (–312 to –293), and B-313 (–303 to –282) (Fig. 4D and E). B-311 is the smallest fragment which produces a retardation complex (Fig. 1B and 4E). Furthermore, B-3 gives rise to a second larger complex (Fig. 4B and C, asterisk) which competes with B-1, B-3, and B-31 and appears to be due to protein binding between –299 and –292.

Finally, we tested whether region C, extending from –282 to –205 (Fig. 2), could give rise to a DNA-protein complex. We performed EMSAs with C-1 (–279 to –168), overlapping with B-1 (–358 to –254), and a retarded complex was found (Fig. 5A) which was not in competition with B-1 (data not shown). Using C-2 (–279 to –215), C-3 (–238 to –168), C-21 (–256 to –228), and C-23 (–237 to –216), we have shown that C-21 (cf. Fig. 1B), the smallest fragment and which contains the postulated TATA box, is responsible for this DNA-protein complex (Fig. 5A and B).

The three regions of the *PRS1* promoter which influence β -galactosidase activity were each shown to be capable of binding proteins in a sequence-specific manner. The correlation

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Insert at the <i>ura3</i> locus	Reference or source
YN94-1	None	9
YN98-1-10	<i>lacZ::URA3</i>	This study
YN98-1-13	(–810 to +87) <i>PRS1''lacZ::URA3</i>	This study
YN98-1-32	(–538 to +87) <i>PRS1''lacZ::URA3</i>	This study
YN98-1-35	(–499 to +87) <i>PRS1''lacZ::URA3</i>	This study
YN98-1-90	(–434 to +87) <i>PRS1''lacZ::URA3</i>	This study
YN98-1-21	(–346 to +87) <i>PRS1''lacZ::URA3</i>	This study
YN98-1-55	(–282 to +87) <i>PRS1''lacZ::URA3</i>	This study
YN98-1-52	(–205 to +87) <i>PRS1''lacZ::URA3</i>	This study
YN98-1-11	(–41 to +87) <i>PRS1''lacZ::URA3</i>	This study

FIG. 2. Deletion analysis of the *PRS1* promoter region. The intergenic region between *FAS1* and *PRS1* is shown on the top left of the figure. $\blacktriangledown$ indicates the 5' end of the *PRS1* mRNA. Numbers to the left of each line indicate the position of the 5' end with respect to the translational ATG start codon of *PRS1*. The bars below the restriction map correspond to the three regions (A, B, and C). The β -galactosidase activities associated with the strains containing integrated *PRS1''lacZ* fusions are indicated on the right. Each value represents the average of at least three independent determinations ($\pm$ standard errors). Restriction endonuclease sites: H, *Hind*III, P, *Pst*I; and B, *Bsr*GI.

FIG. 3. In vitro DNA-protein binding analysis for region A. Increasing amounts of protein extract from strain YN94-1 (0, 10, 20, and 40 ng) were incubated with 25 ng of a 32 P-labeled A-1 probe (A). For competition assays, 100 and 200 molar excesses of unlabeled DNA corresponding to fragments A-1, A-2, and A-3 were used. Specifically competing DNA-protein complexes are indicated by arrowheads and asterisks. Protein ext., protein extract.

FIG. 4. In vitro DNA-protein binding analysis for region B. For experimental details, see the legend to Fig. 3. Protein ext., protein extract.

between the formation of these DNA-protein complexes in vitro and the function of these regions in vivo suggests that these *cis*-acting elements may be involved in the regulation of *PRS1* expression. However, the protein(s) binding to region A are more likely to be responsible for the polyadenylation and termination of *FAS1* mRNA (14), thus leaving the regions B and C to be responsible for any PRS-specific regulation. Region C contains the potential *PRS1* TATA box. It is possible that the β -galactosidase activity measured for YN98-1-55 is due to the presence of the TATA box. However, it is also possible that the activity associated with this strain might be due to the presence of another *cis*-acting regulatory element. Support for this hypothesis is that although neither fragment C-3 nor C-23 contains the putative TATA box, both compete for the DNA-protein binding activity of the C region.

A search of the *PRS1* promoter with the software RSA-tools (20) and *MatInspector Professional* (17) revealed no localization of known consensus sequences to the regions A, B, and C. Indeed, the *PRS1* promoter is markedly devoid of transcription factor binding sites. It has been reported that in a $\Delta paf1$ strain, the abundance of *PRS1* mRNA was reduced by 50% (4). Other genes whose mRNA abundance is affected by a faulty Paf1p-containing RNA polymerase II, e.g., *CYC1*, contain Rlm1p and Swi4p/Swi6p binding sites (15) in their promoters. Two *RLM1* boxes (CTAWWWTAG) are found in the *PRS1* promoter; one (-427 to -419) is contained within A-213, a region which we believe is more likely to be associated with the termination of the *FAS1* mRNA rather than the regulation of *PRS1* transcription, and the other (-391 to -383) lies between regions A-213 and B-311. An overlapping *SWI4/SWI6* binding site is found

FIG. 5. In vitro DNA-protein binding analysis for region C. For experimental details, see the legend to Fig. 3. Protein ext., protein extract.

between -126 and -119 downstream of the transcription start site, rendering it irrelevant for transcription. Extending these searches to the promoters of *PRS2* to *PRS5* revealed no similarities in the promoters.

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