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The divergently transcribed genes encoding yeast ribosomal proteins L46 and S24 are activated by shared RPG-boxes

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ABSTRACT

Transcription of the majority of the ribosomal protein (rp) genes in yeast is activated through common *cis*-acting elements, designated RPG-boxes. These elements have been shown to act as specific binding sites for the protein factor TUF/RAP1/GRF1 *in vitro*. Two such elements occur in the intergenic region separating the divergently transcribed genes encoding L46 and S24. To investigate whether the two RPG-boxes mediate transcription activation of both the L46 and S24 gene, two experimental strategies were followed: cloning of the respective genes on multicopy vectors and construction of fusion genes. Cloning of the L46 + S24 gene including the intergenic region in a multicopy yeast vector indicated that both genes are transcriptionally active. Using constructs in which only the S24 or the L46 gene is present, with or without the intergenic region, we obtained evidence that the intergenic region is indispensable for transcription activation of either gene. To demarcate the element(s) responsible for this activation, fusions of the intergenic region in either orientation to the galK reporter gene were made. Northern analysis of the levels of hybrid mRNA demonstrated that the intergenic region can serve as an heterologous promoter when it is in the 'S24-orientation'. Surprisingly, however, when fused in the reverse orientation the intergenic region did hardly confer transcription activity on the fusion gene. Furthermore, a 274 bp FnuDII-FnuDII fragment from the intergenic region that contains the RPG-boxes, could replace the naturally occurring upstream activation site (UAS_{rpg}) of the L25 rp-gene only when inserted in the 'S24-orientation'. Removal of 15 bp from the FnuDII fragment appeared to be sufficient to obtain transcription activation in the 'L46 orientation' as well. Analysis of a construct in which the RPG-boxes were selectively deleted from the promoter region of the L46 gene indicated that the RPG-boxes are needed for efficient transcriptional activation of the L46 gene. We conclude that all promoter elements for the S24 gene are located within the intergenic region, where the RPG-boxes are the most likely UAS-elements. However, the intergenic region (including the RPG-boxes) is required but not sufficient to confer transcription activity on the L46 gene.

INTRODUCTION

Most ribosomal protein genes (rp-genes) in yeast share upstream DNA elements, so-called RPG-boxes [1], which act as transcription-activating sites [2–5]. RPG-boxes represent binding sites for a general transcription factor, TUF [6,7], also designated RAP1 [8] or GRF1 [9,10].

Previous studies using synthetic oligonucleotides carrying the consensus sequence ACACCCATACATTT have revealed several characteristics of these TUF-responsive elements [3,11]. RPG-boxes display a rather high variation in nucleotide sequence [reviewed in 12;13] and function at variable distances from the respective genes [11]. In most genes, multiple elements are present in a tandem arrangement and evidence has been obtained that these elements can act in a cooperative fashion [7,11]. In addition, RPG-boxes function in both orientations though with a slight difference in efficiency [3,11]. The orientation-

independence is particularly relevant in the cases in which the extended promoter regions of two rp-genes may overlap as is the case for the genes encoding L46 and S24. The translation startsites of these divergently transcribed genes are 630 bp apart [14]. Almost in the middle of this intergenic region two RPG-like elements have been identified by gel retardation analyses and footprinting [7]. The question therefore arises whether these boxes can mediate transcription activation of both genes.

Though most rp-genes in yeast are scattered over the genome [reviewed in Ref. 15], the close proximity of the L46 and S24 genes is not the only example of such an arrangement. The genes for rp29 and L32 display a similar close linkage, being separated by a short intergenic region harbouring both promoters [16]. The genes for rp28 and S16A are also located near each other, but in this case the genes are arranged in a head-to-tail fashion [17].

To determine whether the promoters of the L46 and S24 genes share *cis*-acting elements, we constructed fusions of the intergenic region in either orientation with the galK-reporter gene. We present evidence that indeed the two RPG-boxes are required for transcription activation of both genes. Whereas sequences within the intergenic region are sufficient for transcription of the S24 gene, however, (an) additional element(s) seem to be required for transcription of the L46 gene.

Note: Yeast ribosomal protein L46 is structurally related to rat liver ribosomal protein L39 [18].

Yeast ribosomal protein S24 has a structural relationship with *E.coli* protein S8 [19].

MATERIALS AND METHODS

Recombinant plasmids

Plasmid pBMCY135 consists of a HindIII-generated DNA fragment carrying the genes for ribosomal proteins L46 and S24 [14]. Plasmid YCpR6Δ12H, kindly provided by Dr. R. Zitomer, is an *E. coli*-yeast shuttle vector carrying the *E. coli* galK gene under control of the cyc1-promoter [20]. Plasmid pEMBLYe23R is an multicopy *E.coli*-yeast shuttle vector [21].

Preparation and Northern analysis of RNA

Cells were broken with glass beads essentially as described by Bromley *et al.* (22). Samples containing 10 μg of total cellular RNA were fractionated on 1.5% agarose gels after denaturation in 1 M glyoxal and 50% dimethylsulfoxide [23]. The gels were blotted onto nylon filters (Hybond, Amersham). GalK, S10 and S33 gene-specific probes were labelled according to the procedure of Hu and Messing [24]. We used an EcoRI-generated fragment from YCpR6 [galK; Ref.20], a (TaqI+BglII)-generated fragment of pBMCY113 [S10; Ref.25], a (HindIII+PvuII)-generated fragment of pBMCY76-3 [S33; Ref. 26], or gene-specific oligonucleotides as indicated in the text.

Miscellaneous

Recombinant DNA techniques, propagation and purification of plasmids as well as labelling of oligonucleotide probes were performed by standard methods [27]. Restriction enzyme digestions were performed using conditions recommended by the supplier (BRL).

RESULTS AND DISCUSSION

The genes encoding yeast ribosomal proteins L46 and S24 are closely linked on chromosome X(this laboratory, unpublished). Whereas the L46 gene occurs in a single copy on the yeast genome, for the S24 gene a second copy is present (see Fig.1). The

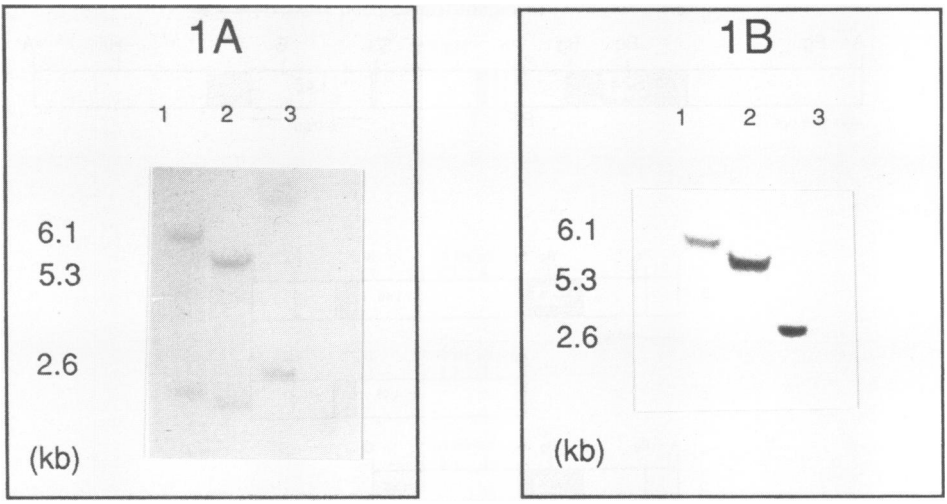

Fig. 1. Copy number of L46 and S24 genes on the yeast genome.
Total DNA was digested with HindIII (lanes 1), EcoRI (lanes 2) and Asp 718 (lanes 3) respectively, and probed with an S24 gene specific probe (encompassing nucleotides 1 to 381; A) or an L46-gene specific oligonucleotide (encompassing nucleotides 53 to 87 from the large exon; B).

L46 and S24 genes are divergently transcribed from an intergenic region of 630 bp [14]. About midway between both genes two previously identified [1] nucleotide elements, designated RPG-boxes, are present (Fig. 2A). In previous studies these RPG-boxes were shown to enhance the transcription activity of rp-genes [2−5] by binding a transacting factor, called TUF [7]. In addition in this intergenic region a T-stretch is found, which has been suggested to contribute to the expression of rp-genes [2]. In this paper we address the question whether the elements in the intergenic region could mediate transcription activation of both the L46 and the S24 gene.

The linked genes for L46 and S24 are transcriptionally active

First of all we wanted to establish whether both genes are transcriptionally active. Therefore, we cloned the Asp718-Asp718 fragment carrying both genes (*cf.* Fig. 2B) in the multicopy vector Ye23R [21]. Analysis of the transcript levels by Northern hybridization clearly shows overproduction of both L46 and S24 mRNAs (*cf.* Fig.3 lanes 1−2 and 13−14). The relative increase of the mRNA levels amounted to about a factor 3, which is lower than expected from the copy numbers of the various recombinant plasmids (see Table I). This result is in agreement with the outcome of previous gene dosage experiments performed with other rp-genes [reviewed in Ref. 12]. Increasing the copy number of the L46 gene does not result in the accumulation of unspliced precursor mRNA, as has been observed in the case of the L32 gene [28]. We were not able to detect any overproduction of the respective ribosomal proteins in the yeast transformants. Therefore, we assume that excess L46 and S24 is subject to rapid degradation, as has been documented for several other ribosomal proteins [29−31].

The intergenic region is required for transcription activation of both genes

To establish whether the intergenic region is essential for transcription activation of both the L46 and the S24 gene, we cloned several fragments of the original insert of clone

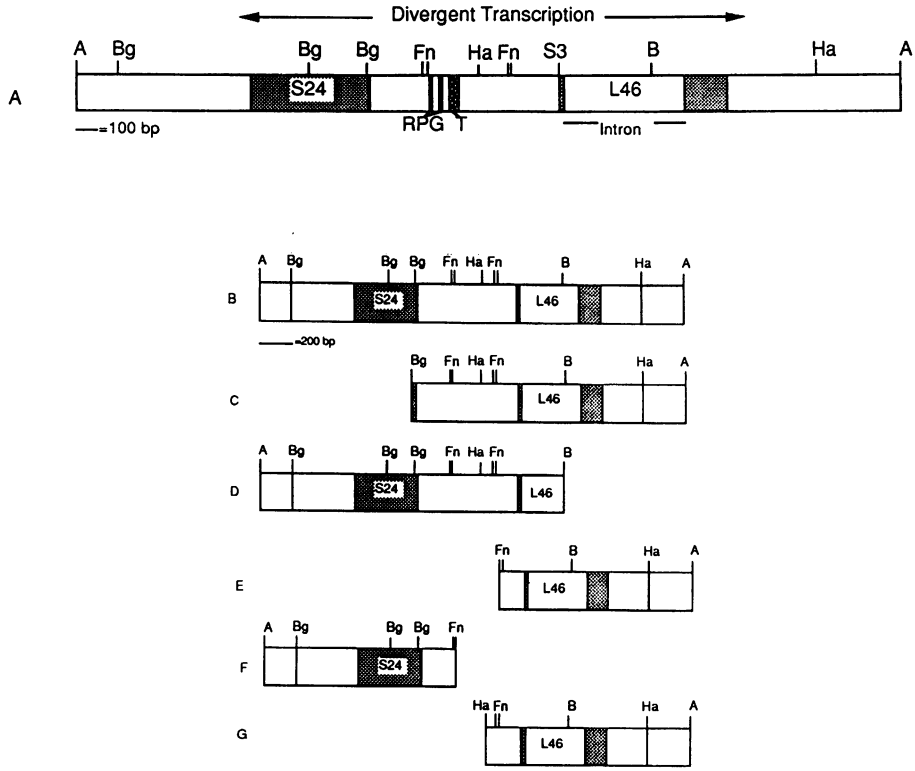

Fig. 2. Maps of DNA-fragments carrying the S24 and L46 genes. In A the genomic organization of the two genes is depicted, whereas B–G represent the DNA fragments that have been cloned in the multicopy yeast vector Ye23R. The positions of the two RPG-boxes and the T-stretch are indicated in A. The DNA fragments schematically drawn in A to G are derived from clone pBMCY135, a HindIII-generated yeast DNA fragment in pBR322; see Ref.14. A = Asp718, H = HindIII,X= XhoI, E = EcoRI, B = BamHI, Bg = BglII, Fn = FnuDII, Ha = HaeIII, S3 = Sau3AI.

pBMCY135 into the multicopy yeast vector Ye23R (*cf.*Fig.2). Constructs C and D contain the L46 and S24 gene respectively, flanked by the entire intergenic region. Construct E contains the L46 gene preceded by its own promoter up to the FnuDII-site,—a distance of about 160 bp—, and thus lacks the RPG-boxes. Construct F similarly contains only the S24-gene, and also lacks the RPG-boxes. All four constructs were transferred to yeast and Northern analysis was performed to estimate the L46 and S24 mRNA levels (Fig 3). Both the L46 and the S24 gene are transcriptionally inactive when flanked only by the shortened promoter region (lanes 7–8 and 9–10 respectively). However, when the entire intergenic region is present both genes do display transcription activity (lanes 3–4 and 5–6). Obviously, two transcripts of different size are produced from the S24 gene. The difference in size is probably due to extra trailer sequences in the longer transcript [14]. The results of the Northern analysis were quantified by laser scanning densitometry and corrected for differences in copy number between the various transformants as determined by Southern blotting (data not shown). The data presented in Table I clearly indicate that

Table I. Relative levels of L46 and S24 mRNA in yeast cells transformed with various recombinant plasmids. RNA and DNA were isolated from the same aliquots of the various yeast cultures all collected at the same optical density. The autoradiograms showing the results of Northern hybridizations (Figs. 3 and 11) or Southern hybridizations were analyzed densitometrically using an LKB laser-scanner. The numbers are calculated relative to the values measured for untransformed cells (control). The mRNA levels in the fourth and fifth column were calculated by subtracting the chromosomal signal from the plasmid encoded signal and dividing them by the copy numbers. The resulting figure representing the relative amount of mRNA per gene is higher for the chromosomal signal than for the plasmid encoded signals because the mRNA overproduction does not increase in concert with the copy number.

Construct[1)] copynumber	Relative level of mRNA L46	S24	Copy-number	level of mRNA after correction for copynumber L46	S24
B	2.9	3.0	10	3.7	3.2
C	3.9	–	15	3.6	–
D	–	3.1	10	–	3.3
E	1.5	–	30	0.3	–
F	–	1.4	45	–	0.2
G	2.2	–	43	0.6	–
control	1.0	1.0	1	17	16

[1] see Fig. 2

the intergenic region is indispensable for expression of both the L46 gene and the S24 gene.

The intergenic region in the L46-direction does not activate transcription of a reporter gene

To be able to demarcate the *cis*-acting elements that are responsible for transcription activation of the L46 and S24 gene , we constructed fusions between the intergenic region in both orientations and the *E.coli* galK-reporter gene in the centromere-containing vector YCpR6Δ12H. As indicated in Figure 4A we modified this vector by replacing the upstream

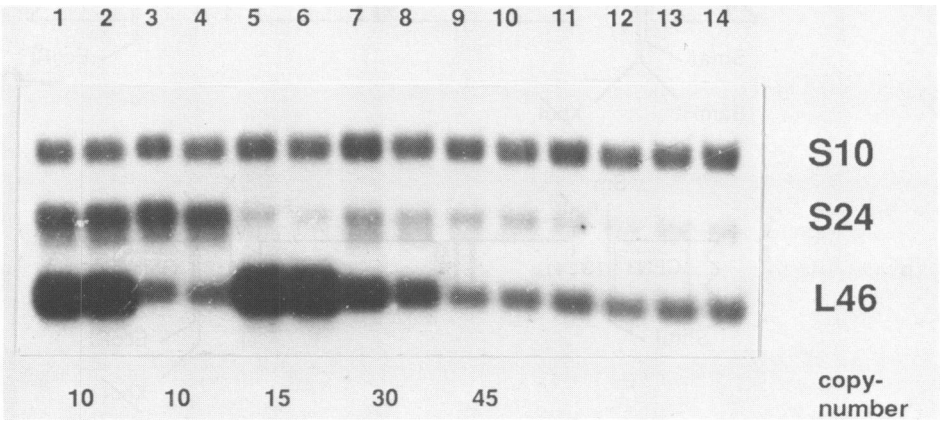

Fig. 3. Transcriptional activity of the L46 and S24 genes carrying various parts of the intergenic region. In Fig.2 the recombinant vectors are depicted that were used for transformation of the yeast cells. For Northern analysis of L46 and S24 mRNAs oligonucleotides encompassing nucleotides 73 to 108 of the S24-gene [14], nucleotides 53 to 87 of the L46-gene[14] and, as a control, nucleotides 541 to 575 of the S10-gene [25] were used as probes. Lanes 1–2: construct B; lanes 3–4: construct D; lanes 5–6: construct C; lanes 7–8: construct E; lanes 9–10: construct F; lanes 11–12: YE23R without insert; lanes 13–14: untransformaned host cells.

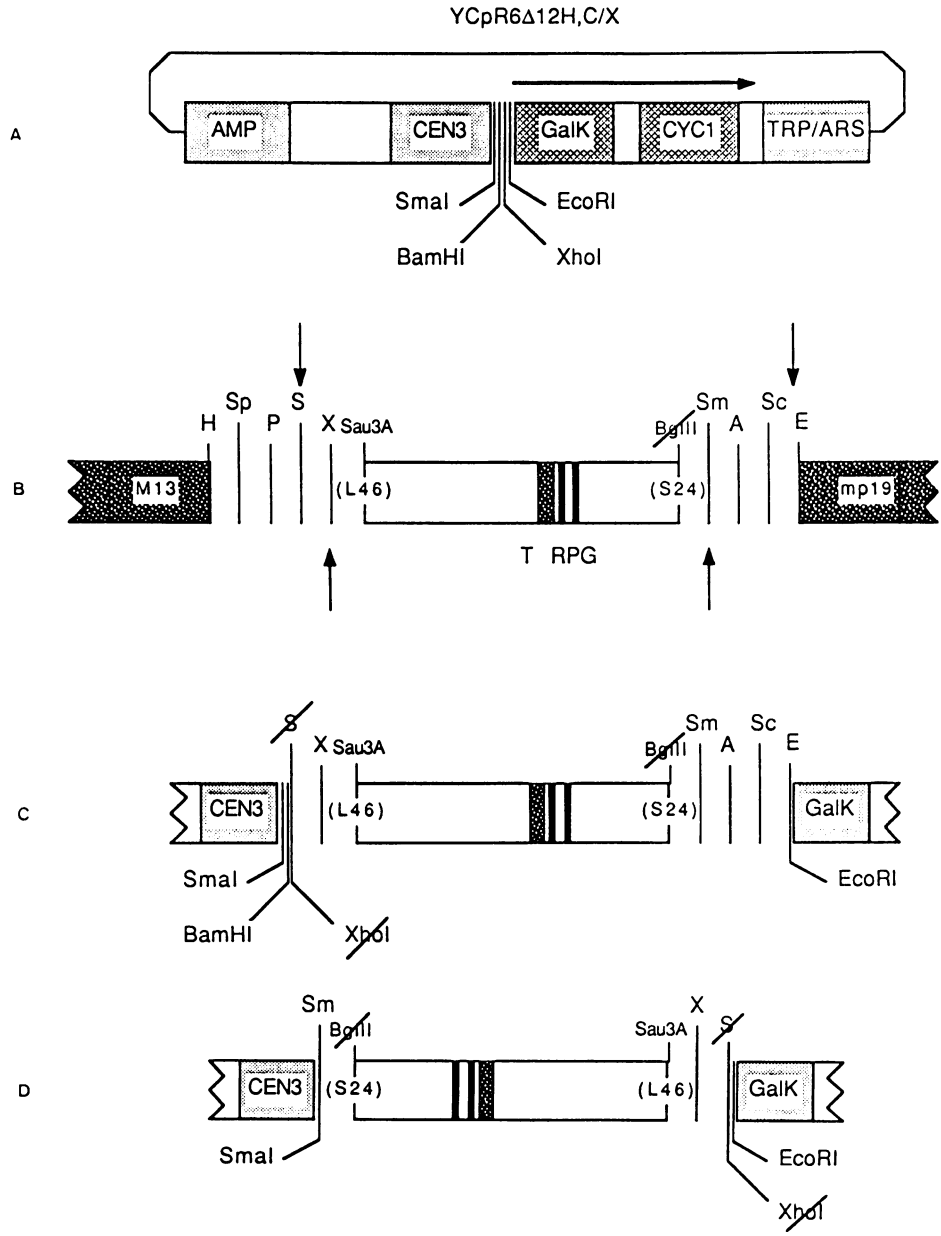

Fig. 4. Construction of GalK-fusion genes containing the intergenic region in either orientation. See text for explanation.

region of the galK gene containing the cyc1-derived transcriptional elements (a BamHI-EcoRI fragment) with a BamHI-XhoI-EcoRI oligonucleotide adapter. A BglII-Sau3AI fragment extending from the 5′-end of the S24 gene (BglII-site) to the leader of the L46

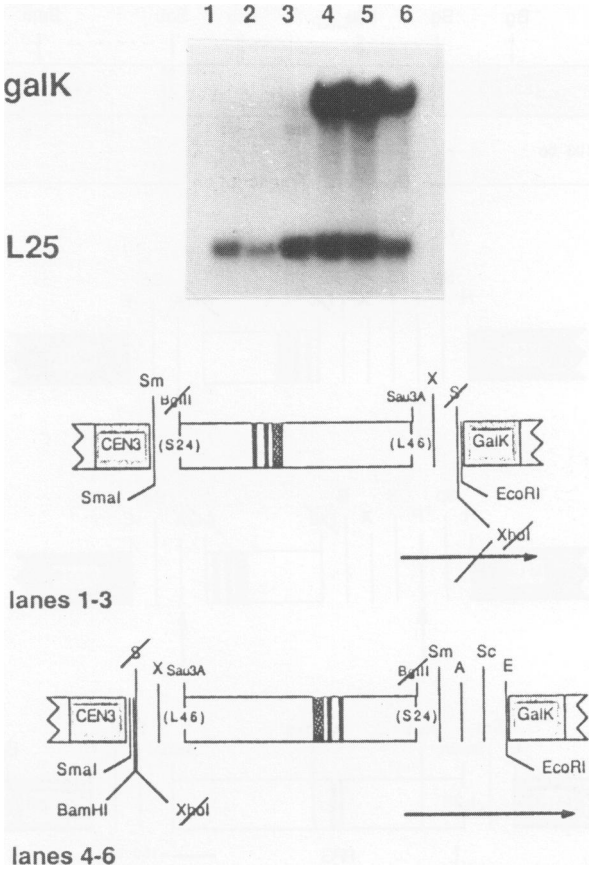

Fig. 5. Transcriptional activity of GalK-fusion genes containing the L46-S24 intergenic region in either orientation. In Fig. 4 the recombinant vectors are depicted that were used for transformation of the yeast cells. For Northern analysis of the GalK-fusion transcripts L25- and GalK-gene specific probes, described in Materials and Methods, were used. Lanes 1−3: construct 5D ('L46-orientation'); lanes 4−6: construct 5C ('S24-orientation').

gene (Sau3AI-site) was cloned in M13mp19 (see Fig. 4B). Orientation of the insert was established by sequencing. To construct the fusion with the intergenic region in the 'S24-orientation' the SalI-EcoRI fragment was then cloned in the modified YCp-vector digested with XhoI and EcoRI (Fig. 4C). The fusion in the 'L46-orientation' was obtained by cloning the SmaI-XhoI fragment in the vector that had been digested with XhoI and SmaI (Fig. 4D). Subsequently, the cellular level of the fusion transcripts was assayed by Northern hybridization. The results shown in Figure 5 demonstrate that the intergenic region functions as proper (extended) promoter in the S24 orientation (Fig.5, lanes 4−6). Obviously introducing an upstream ATG (since the BglII-site is within the coding region of the S24 gene) does not affect the transcript level. In striking contrast with the strong transcript signal observed for the S24-galK hybrid gene , only a very weak transcript signal was found for the L46-galK hybrid gene (Fig.5, lanes 1−3). The latter result does not

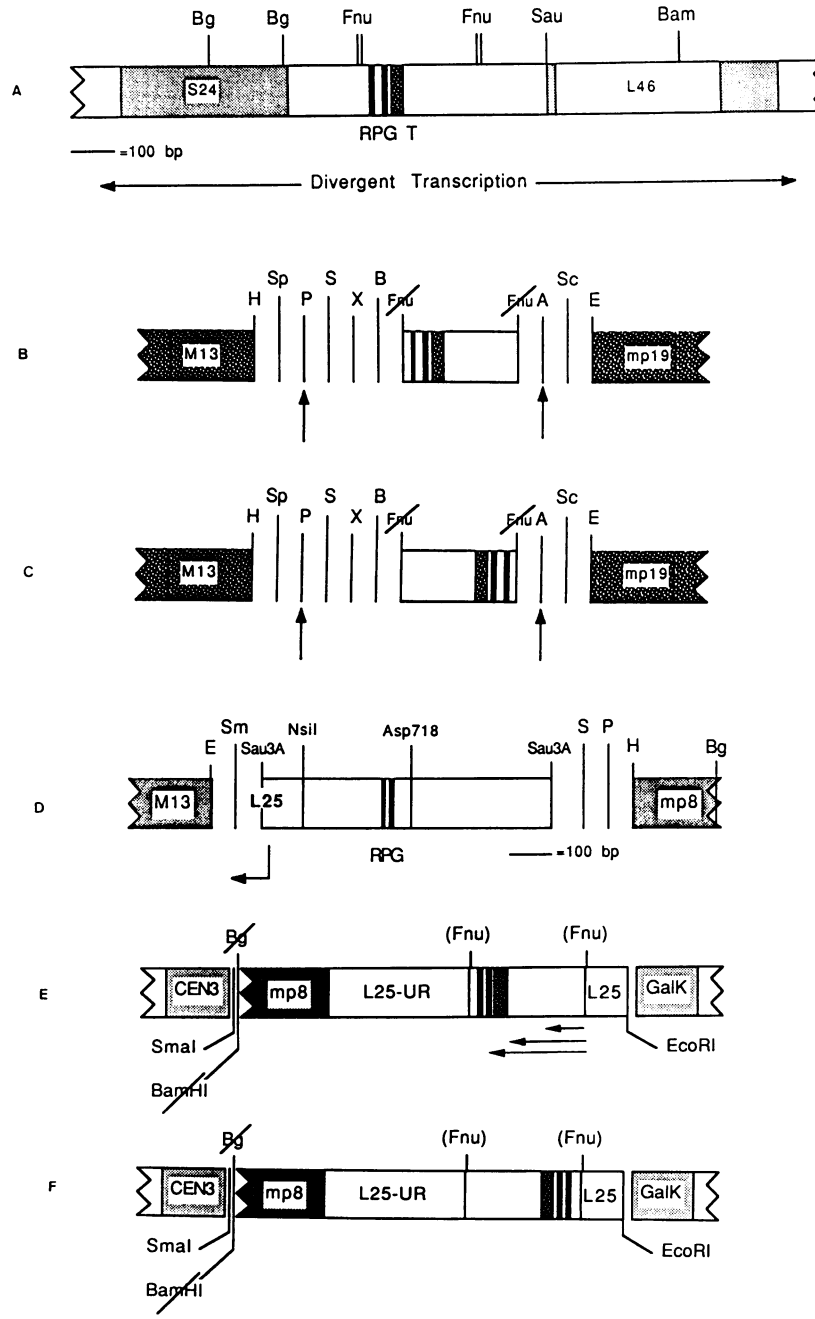

Fig. 6. Construction of GalK-fusion genes containing the FnuDII-FnuDII fragment from the L46-S24 intergenic region in either orientation. See text for explanation.

agree with the previous observation that the intergenic region acts as a promoter for the L46 gene.

The intergenic DNA fragment containing the RPG-boxes functions as heterologous UAS in only one orientation

To rule out the possibility that the orientation-dependent transcriptional activation observed for the galK fusion genes, is due to an artifact introduced by the construction of these genes, we analyzed transcriptional activation of another gene (that for yeast ribosomal protein L25) by a different fragment of the intergenic region in either orientation, namely the FnuDII-FnuDII fragment containing both RPG-boxes (*cf.* Fig.2). The FnuDII-FnuDII fragment was cloned in M13mp19 in both orientations (Fig. 6B/C). Then these fragments were used to replace the naturally occurring UAS_{rpg} of the L25-gene, in a M13mp8 subclone containing the upstream region of the L25 gene from the Sau3AI-site in the leader up to the Sau3AI-site at −710 (32; *cf.*Fig.6D). The clone was digested with NsiI and Asp718, thus deleting the UAS_{rpg} of the L25-gene, and religated in the presence of either one of the two PstI-Asp718 fragments harboring different orientations of the 'intergenic UAS_{rpg}'. The BglII-EcoRI fragment isolated from either clones was subsequently used to construct galK-fusion genes in the vector YCpR6Δ12H (Figs. 6E and 6F represent the FnuDII-FnuDII fragment in the L46- and S24-orientation, respectively). Again Northern analyses were performed to estimate the cellular levels of the fusion mRNAs. The results are shown in Figure 7. In full agreement with the previous result, in which the entire intergenic region was fused to galK in either of the two orientations, also a strong orientation-dependency of promotor activity was observed (Fig.7). The FnuDII-FnuDII fragment in the 'S24-orientation' mediates strong transcription activation of the L25-galK marker gene (lanes 4−6). In striking contrast, with the same fragment in the reverse, *i.e.* L46-orientation, hardly any transcripts can be detected (Fig.7, lanes 1−3). Thus, the remarkable unidirectional action of the intergenic region in promoting the transcription of a fusion gene is associated with the FnuDII-FnuDII fragment. Obviously the RPG-boxes, when oriented in the L46-direction, are not able to activate transcription of a fusion gene in the L46-direction. This finding raises two important questions: Firstly, why is transcription activation of these fusion genes hampered in the L46-direction, and secondly, how can we explain the paradox that the intergenic region is required for L46 gene expression whereas (in the same orientation) it can not activate transcription of the fusion genes?

Demarcation of a putative negative regulation site

In an attempt to localize the DNA-element that is responsible for the presumed repression of transcription activation of the fusion genes in the L46-direction, we undertook a deletion study. The M13mp19 subclone c (see Fig.6) was digested with HindIII and then treated with Bal31. In this way part of the sequence of the FnuDII-FnuDII fragment between the RPG-boxes and the L46 gene was removed. After recloning the blunt-BglII fragment in M13mp19 , three clones were selected in which 15, 150 and 221 bp had been deleted (Fig.6E). These fragments (in the L46-orientation) were again used to replace the natural UAS_{rpg} of the L25 gene promoter in the same way as described above. Northern hybridization (Fig.8) revealed that a deletion of 15 bp is sufficient to obtain transcription-activation of the L25-galK fusion gene (compare lanes 1−2 to lanes 5−6). The level of transcription achieved after deletion of 15 bp (Fig.8, lanes 5−6) is still lower than that observed with the FnuDII-FnuDII fragment in the S24-orientation (Fig.8, lanes 3−4), but this difference might be due to the different orientation of the actual *cis*-acting elements and/or the different nucleotide context of the UAS-region in the two hybrid promoters.

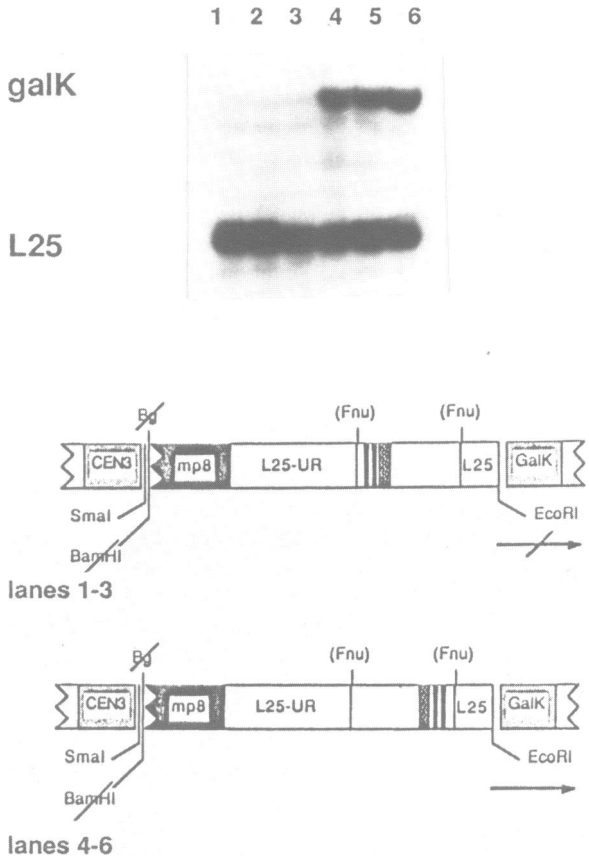

Fig. 7. Transcriptional activity of GalK fusion genes containing the FnuDII fragment in either orientation. In Fig. 6 the recombinant vectors are depicted that were used for transformation of the yeast cells. For Northern analysis of galK-fusion transcripts, L25 and GalK gene specific probes, described in Materials and Methods, were used. Lanes 1−3: construct 7E ('L46-orientation'); lanes 4−6: construct 6F ('S24-orientation').

Removal of 150 bp reduces transcriptional activation although it is still considerably higher than observed with the intact FnuDII-FnuDII fragment (compare lanes 7−8 to lanes 1−2). Supposedly, this deletion either removes some non-essential element of the promoter or results in a less efficient nucleotide context of the UAS_{rpg}. We deem it is unlikely that the reduction is due to the decreased distance between UAS_{rpg} and the transcription initiation site, since this distance is about the same as in the fusion gene having the FnuDII-FnuDII fragment in the S24-orientation. With the deletion mutant up to −221 from the FnuDII-site (Fig.8, lanes 9−10) transcription activity is reduced even below that of the control (Fig. 8, lanes 1−2). Since in this deletion mutant both the RPG-box, that was previously shown to be the most effective one in TUF factor-binding *in vitro* [7], and the T-stretch has been removed, this result once more demonstrates the importance of the RPG-boxes in transcriptional activation. With respect to the nucleotide composition in the pertinent part of the intergenic region a very high GC content around the L46-proximal

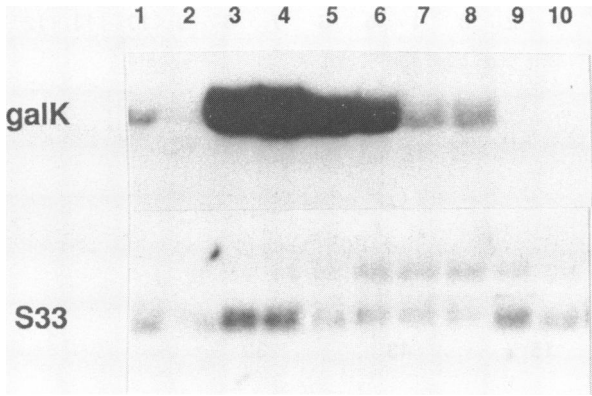

Fig. 8. Transcriptional activity of GalK-fusion genes carrying various deletions of the FnuDII-FnuDII fragment. In Fig. 6 the recombinant vectors are depicted that were used for transformation of the yeast cells. For Northern analysis of galK-fusion transcripts, S33 and GalK gene specific probes, described in Materials and Methods, were used. Lanes 1–2: construct 6E; lanes 3–4: construct 6F; lanes 5–6: deletion constuct 6E (−15 bp); lanes 7–8: −150 bp; lanes 9–10: −221 bp.

FnuDII-site is notable but at present we do not know if these sequence features are relevant for the observed effect (illustrated in Fig. 9). Attempts to identify in a yeast extract a protein that putatively interacts with an oligonucleotide harboring the respective 15 nucleotides plus flanking nucleotides, so far remained negative (result not shown). To establish whether the putative negative regulation site could function likewise in a heterologous context, we inserted an oligonucleotide harboring the respective 15 nucleotides plus flanking nucleotides in the NsiI site in the upstream region of the L25 gene (Fig.6D). The distance of the oligonucleotide to the RPG-boxes was about the same as in the L46 gene. The M13mp8 subclone containing the upstream region of the L25 gene was digested with EcoRI and

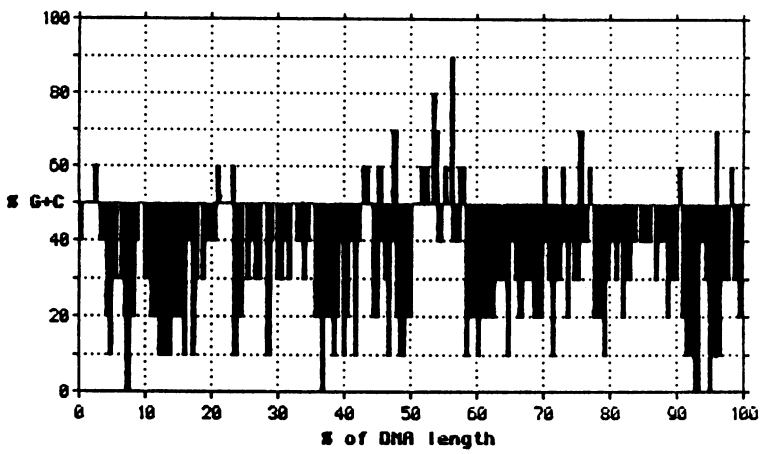

Fig. 9. Sliding base composition of the sequence encompassing 2223 nucleotides of the HindIII-generated DNA fragment carrying the L46 and S24 genes [14]. 90% GC is found around the L46-proximal FnuDII sites.

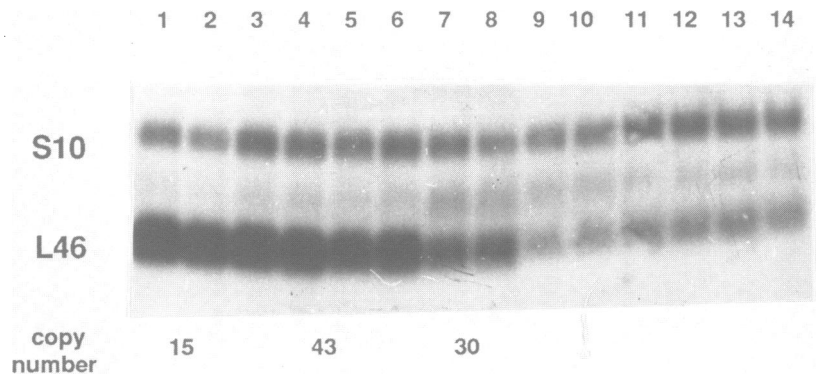

Fig. 10. Transcriptional activity of the L46 gene lacking the upstream RPG-boxes. In Fig.2 the recombinant vectors are depicted that were used to transform the yeast cells. For Northern analysis of L46 mRNA levels the same oligonucleotides were used as for Northern analysis described in Fig.3. Lanes 1–2: construct C; lanes 3–6: construct G; lanes 7–8: construct E; lanes 9–10: YE23R without insert; lanes 11–14: untransformed host cells. The copy numbers of the recombinant plasmids in the respective transformant cells, as presented in Table I, are indicated below the lanes.

BglII, followed by a ligation of the L25 upstream region in a YCpR6Δ12H,C/X vector digested with BamHI and EcoRI. Transcription activation of the resulting L25-galK fusion gene, however, was not decreased by insertion of the oligonucleotide (result not shown).

Transcription-activation of L46 gene is mediated by the RPG-boxes

The results discussed above show that the intergenic region is indispensable for efficient transcription of the L46 gene. On the other hand, analysis of a series of different fusion genes revealed the presence of a putative repression site that prevents transcription activation by the RPG-boxes in the 'L46-orientation'. This paradox could be resolved if in the natural situation the RPG-boxes primarily serve to activate transcription of the S24 gene while a distinct UAS-element operates for the L46 gene. The results for construct E presented in Table I show that a deletion of the extended promoter up to the L46-proximal FnuDII site results in an almost complete loss of transcription activity. This result implicates that a distinct UAS-element for the L46 gene cannot be located downstream of the L46-proximal FnuDII site. Hence the possibility remains that a distinct UAS-element is located between the RPG-boxes and the L46-proximal FnuDII site. To examine this possibility we cloned a HaeIII-Asp718 fragment that carries the L46 gene with its promotor extending to a position downstream of the RPG-boxes +T-stretch (see Fig.2G) in the YE23R vector. Again the cellular level of L46 mRNA was estimated by Northern hybridization. The results for construct 2G, presented in Figure 10 and Table I, rule out the possibility that a distinct UAS-element for the L46 gene exists although a slight increase in transcription activity (relative to that obtained with construct E) was detected. These results provide evidence that the RPG-boxes are essential for transcription activation of the L46 gene.

In conclusion, the data support a model in which the RPG-boxes are required for efficient transcription activation of both the S24 and the L46 gene. The S24 and L46 genes, therefore, share the same *cis*-acting elements. Functionally related genes that are divergently transcribed from a common upstream region and are controlled by common *cis*-acting elements, more often occur on the yeast genome. For instance, in the MATα locus a

promoter element has been identified, located at 120 bp and 80 bp from the 5'-ends of the α_1 and α_2 genes, respectively, which may mediate transcription of both genes [33]. Nucleotide elements between the divergently transcribed H2A and H2B genes confer cell-cycle specific regulation onto both genes [34]. In addition, midway between the clustered GAL1 and GAL10 genes a GAL-responsive region (UAS_g) occurs which about equally activates transcription of both genes [35]. An analogous situation exists for the MAL6S and MAL6T genes; transcription of these divergently transcribed genes is also co-regulated, probably through interaction of the MAL6R gene product to a common UAS_m-element in the intergenic region [36,37].

Though the RPG-boxes are necessary for efficient transcription activation of the L46 gene, the results obtained with the fusion genes suggest that (an) additional element(s) may play a part in this process. It cannot be excluded that the putative repression site was artificially generated, but we consider this possibility less likely since different fusion genes displayed similar results. We favour the interpretation that the respective element does function in the *in vivo* context, but not as a negative regulatory site. Perhaps it is involved in the constitution of a defined chromatin structure in this part of the intergenic region, that may be required to allow transcription activation through the RPG-boxes in both directions.

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Abbreviations: rp-gene: ribosomal protein gene; rp-mRNA: ribosomal protein mRNA; UAS_{rpg}: upstream activation site of yeast ribosomal protein gene

REFERENCES

1. Leer,R.J., Van Raamsdonk-Duin,M.M.C., Mager,W.H. and Planta,R.J. (1985) Curr. Genet.,**9**, 273–277.
2. Rotenberg, M.O. and Woolford,J.L. (1986) Mol. Cell. Biol.,**6**, 674–687.
3. Woudt,L.P., Smit,A.B., Mager,W.H. and Planta,R.J. (1986) The EMBO J.,**5**, 1037–1040.
4. Larkin,J.C., Thompson,J.R. and Woolford,J.L. (1987) Mol. Cell. Biol.,**7**, 1764–1775.
5. Schwindinger,W.F. and Warner,J.R. (1987) J. Biol. Chem.,**262**, 5690–5695.
6. Huet,J., Cottrell,P., Cool,M., Vignais,M-L., Thiele,D., Marck,C., Buhler,J-M., Sentenac,A. and Fromageot,P. (1985) The EMBO J.,**4**, 3539–3547.
7. Vignais,M-L., Woudt,L.P., Wassenaar,G.M., Mager,W.H., Sentenac,A. and Planta,R.J. (1987) The EMBO J.,**6**, 1451–1457.
8. Shore,D. and Nasmyth,K. (1987) Cell,**51**, 721–732.
9. Buchman,A.R., Kimmerly,W.J., Rine,J. and Kornberg,R.D. (1988) Mol. Cell. Biol.,**8**, 210–225.
10. Kimmerly,W., Buchman,A., Kornberg,R. and Rine,J. (1988) The EMBO J.,**7**, 2241–2253.
11. Woudt,L.P., Mager,W.H., Nieuwint,R.T.M., Wassenaar,G.M., Van der Kuyl,A.C., Murre,J.J., Hoekman,M.F.M., Brockhoff,P.G.M. and Planta,R.J. (1987) Nucl. Acids Res.,**15**, 6037–6048.
12. Planta,R.J. and Mager,W.H. (1988) In Tuite,M.F. et al. (eds.), Genetics of Translation, NATO ASI Series. Springer Verlag, Berlin Heidelberg, series H, Vol. 14, pp. 117–129.
13. Nieuwint,R.T.M., Mager,W.H., Maurer,C.T.C. and Planta,R.J. (1989) Curr. Genet.,**15**, 247–251.
14. Leer,R.J., Van Raamsdonk-Duin,M.M.C., Kraakman,P., Mager,W.H. and Planta,R.J. (1985) Nucl. Acids Res.,**13**, 701–709.

15. Planta,R.J., Mager,W.H., Leer,R.J., Woudt,L.P., Raué,H.A., El-Baradi,T.T.A.L. (1986) In Hardesty,B. and Kramer,G. (eds.), Structure, function and genetics of ribosomes. Springer Verlag, New York, pp. 669–718.
16. Warner,J.R., Mitra,W.F., Schwindinger,W.F., Studeny,M. and Fried,H.M. (1985) Mol. Cell. Biol.,**5**, 1512–1521.
17. Molenaar,C.M.T., Woudt,L.P., Jansen,A.E.M., Mager,W.H., Planta,R.J., Donovan,D.M. and Pearson,N.J. (1984) Nucl. Acids Res.,**12**, 7345–7358.
18. Lin,A., McNally,J. and Wool,I.G. (1984) J. Biol. Chem.,**259**, 487–490.
19. Kimura,M., Arndt,E., Hatakyama,T., Hatakyama,T. and Kimura,I. (1989) Can. J. Biochem.,**35**, 195.
20. Rymond,B.C., Zitomer,R.S., Schümperli,D. and Rosenberg,M. (1983) Gene,**25**, 249–262.
21. Baldari,C.and Cesareni,G. (1985) Gene, **35**, 27–32.
22. Bromley,S., Hereford,L. and Rosbash,M. (1982) Mol. Cell. Biol.,**2**, 1205–1211.
23. McMaster,S.K. and Carmichael,G.G. (1977) Proc. Natl. Acad. Sci. USA,**74**, 4835–4838.
24. Hu,N-T. and Messing,J. (1982) Gene,**17**, 271–277.
25. Leer,R.J., Van Raamsdonk-Duin,M.M.C., Molenaar,C.M.T., Cohen,L.H., Mager,W.H. and Planta,R.J. (1982) Nucl. Acids Res.,**10**, 5869–5878.
26. Leer,R.J., Van Raamsdonk-Duin,M.M.C., Schoppink,P.J., Cornelissen,M.T.E., Cohen,L.H., Mager,W.H. and Planta,R.J. (1983) Nucl. Acids Res.,**11**, 7759–7768.
27. Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) Molecular cloning: A laboratory manual. Cold Spring Harbor Lab. Press, New York.
28. Dabeva,M.P., Post-Beittenmiller,M.A. and Warner,J.R. (1986) Proc. Natl. Acad. Sci. USA,**83**, 5854–5857.
29. El-Baradi,T.T.A.L., Van der Sande,C.A.F.M., Mager,W.H., Raué,H.A. and Planta,R.J. (1986) Curr. Genet.,**10**, 733–739.
30. Maicas,E., Pluthero,F.G. and Friesen,J.D. (1988) Mol. Cell. Biol.,**8**, 169–175.
31. Tsay,Y-F., Thompson,R., Rosenberg,M.O., Larkin,J.C. and Woolford,J.L. Jr. (1988) Genes Developm.,**2**, 664–676.
32. Leer,R.J., Van Raamsdonk-Duin,M.M.C., Hagendoorn,M.J.M., Mager,W.H. and Planta,R.J. (1984) Nucl. Acids Res.,**12**, 6685–6700.
33. Siliciano,P.G. and Tatchell,K. (1984) Cell,**37**, 969–978.
34. Osley,M.A., Gould,J., Kim,S., Kane,M. and Hereford,L. (1986) Cell,**45**, 537–544.
35. West,R.W. Jr, Yocum,R.R. and Ptashne,M. (1984) Mol. Cell. Biol.,**4**, 2467–2478.
36. Hong,S.H. and Marmur,J. (1987) Mol. Cell. Biol.,**7**, 2477–2483.
37. Solliti,P. and Marmur,J. (1988) Mol. Gen. Genet.,**213**, 56–62.

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