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Evolutionary conservation of transcriptional machinery between yeast and plants as shown by the efficient expression from the CaMV 35S promoter and 35S terminator

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Summary. Complementation of fission yeast mutants by plant genomic libraries could be a promising method for the isolation of novel plant genes. One important prerequisite is the functioning of plant promoters and terminators in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Therefore, we studied the expression of the bacterial β-glucuronidase (GUS) reporter gene under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter and 35S terminator. We show here that *S. pombe* initiates transcription at exactly the same start site as was reported for tobacco. The 35S CaMV terminator is appropriately recognized leading to a polyadenylated mRNA of the same size as obtained in plant cells transformed with the same construct. Furthermore, the GUS-mRNA is translated into fully functional GUS protein, as determined by an enzymatic assay. Interestingly, expression of the 35S promoter in the budding yeast *S. cerevisiae* was found to be only moderate and about hundredfold lower than in *S. pombe*. To investigate whether different transcript stabilities are responsible for this enormous expression difference in the two yeasts, the 35S promoter was substituted by the ADH (alcohol dehydrogenase) promoter from fission yeast. In contrast to the differential expression pattern of the 35S promoter, the ADH promoter resulted in equally high expression rates in both fission and budding yeast, comparable to the 35S promoter in *S. pombe*. Since the copy number of the 35S-GUS constructs differs only by a factor of two in the two yeasts, it appears that differential recognition of the 35S promoter is responsible for the different transcription rates.

Key words: *Schizosaccharomyces pombe* – *Saccharomyces cerevisiae* – CaMV 35S promoter – CaMV 35S terminator – Heterologous expression

Introduction

At present several approaches can be used to isolate plant genes with regulatory functions in plant growth or development. One method requires the construction of cDNA libraries from different developmental stages of a plant organ. Differential screening of these cDNA banks allows the isolation of stage-specific genes which are, however, usually not the regulatory genes themselves. Another method makes use of knowledge obtained from studies on other organisms, such as animals or lower eukaryotes. Specifically, heterologous probes, consisting of either gene fragments or synthetic oligonucleotides, are hybridized to plant gene banks. A third approach employs an isolation procedure that selects for functional, rather than for sequence, homology. Since the function of a protein appears to be much more conserved during evolution than its sequence, this method may be particularly attractive for the isolation of genes which cannot be obtained by cross-hybridization methods. Specific temperature-sensitive yeast mutants are complemented with either genomic or cDNA expression libraries. Yeast is ideally suited for this purpose, since many mutants are available and the respective genes have, in many cases, been biochemically defined. Furthermore, transformation and screening of large quantities of cells is experimentally easy. This method has been successfully employed for the isolation of the human CDC2 homologue (Lee and Nurse 1987). For this purpose, a human cDNA bank was cloned into an expression vector which contained the SV40 promoter. As the SV40 promoter is strongly recognized in *S. pombe* (Jones et al. 1985) the human cDNA clones could be expressed and complemented the mutant fission yeast cdc2 gene. For successful complementation with genomic plant libraries, several requirements must be fulfilled. These are proper transcription (i.e. selection of start site, processing of introns, termination and polyadenylation) and translation (including post-translational modifications) of the plant genes. Although the budding yeast *S. cerevisiae* has been shown to recognize some, but not all, of several plant promoters (Langridge et al. 1984; Cramer et al. 1985; Coraggio et al. 1986), this yeast seemed unsuitable for heterologous complementation, because of the unusual branch point requirement of a TACTAAC box in the introns.

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In contrast, the evolutionarily highly diverged fission yeast, *S. pombe*, does not have this strict requirement and was shown to process an animal intron correctly (Käufer et al. 1985). However, no proper analysis of the expression of plant genes in *S. pombe* has been carried out. Therefore, we investigated the expression of the CaMV 35S promoter and 35S terminator in *S. pombe*. These elements were selected as they have been used extensively for expression studies of foreign genes in different plant species. The promoter is recognized in a wide range of species, both monocots and dicots (On-Lee et al. 1986; Fromm et al. 1986; Ow et al. 1987; Nagata et al. 1987; Ecker et al. 1986) and is one of the most active promoters in plants, giving rise to equally high expression rates as the chlorophyll a/b binding protein or the small subunit of the ribulose bisphosphate carboxylase promoters (Fluhr et al. 1986; Jones et al. 1985). The bacterial β-glucuronidase (GUS) gene was put under the control of the 35S promoter and terminator and inserted into a yeast episomal plasmid. Transformation into both budding and fission yeast resulted in functional expression of enzymatically active GUS protein, though at highly different levels. Concentrating on *S. pombe*, we could show that both the 35S promoter and 35S terminator are properly recognized leading to transcript levels comparable to that of the strong alcohol dehydrogenase (ADH) promoter from *S. pombe*.

Materials and methods

Construction of yeast expression plasmids. The 2.7 kb *Hin*d III fragment of the plasmid pDH51/GUS (Pietrzak et al. 1986) was inserted into the *Hin*d III site of the yeast vector pDB262 (Beach et al. 1982) to give the plasmid pDB262/35S-GUS (Fig. 1 A). The 2.2 kb *Bam* HI/*Hin*d III fragment of the vector pDH51/GUS was inserted into the yeast expression vector pEVP11 (Russel and Nurse 1986) to give the plasmid pEVP11/ADH-GUS (Fig. 1 B).

Yeast strains and media. For transformation, *S. cerevisiae* DL1 (*leu2, his3, ura3*) and *S. pombe leu1-32*, h^- were used. Yeasts were grown in medium containing 2% glucose, 0.67% Yeast Nitrogen Base and limiting amounts of auxotrophic requirements with and without leucine. Transformation was performed as described by Ito et al. (1983).

RNA preparation and Northern analysis. PolyA$^+$RNAs were prepared from total RNA by hybridization to oligo(dT)-cellulose (Aviv and Leder 1972). Denaturation of RNA, agarose gel electrophoresis, transfer to nitrocellulose and hybridization were carried out as described by Thomas (1980). Blots were probed with gene fragments that were ^{32}P-labelled by random-primed labelling (Feinberg and Vogelstein 1983).

RNase protection assay. 10 µg of total *S. pombe* RNA were hybridized to 15 000 cpm (Cerenkov) of antisense RNA and analyzed as described by Wiebauer et al. (1988).

Construction of plasmids used for in vitro transcription. The constructs shown in Fig. 2 A and B were obtained in the following way. The 2.7 kb *Pst* I fragment, containing the GUS gene under the control of the CaMV 35S promoter and terminator (from pDH51/GUS), was ligated into the multiple cloning site of pBLUESCRIPT KS$^+$. In order to obtain the vector used to transcribe probe C (Fig. 2 C), the GUS gene sequences between the *Nru* I site and the *Kpn* I site were deleted by cutting and blunt end religation of the vector shown in Fig. 2 A.

In vitro transcription. 3 µg of template DNA were transcribed in vitro as described by Müller et al. (1988), except that T7 RNA polymerase was used instead of T3 RNA polymerase.

Primer extension. Total RNA (15 µg) from transformed *S. pombe* cells was annealed with 5 ng of a 5′-^{32}P-labelled 22-nt primer (Jefferson 1987) in 200 µl of a solution containing 10 mM Tris-HCl (pH 7.8), 2 mM EDTA and 1% SDS. The sample was heated for 3 min at 86 °C, cooled down slowly to 60 °C and incubated 15 min at this temperature. After annealing at a T_m of 58 °C for 30 min, the sample was incubated at 37 °C for 60 min. After precipitation and washing, the sample was dried carefully and resuspended in 3 µl of restriction-buffer A (BRL). After addition of 0.2 µl DTT (200 mM) and 4 µl dNTP-mix (0.5 mM each), the sample was incubated at 42 °C, and five units of reverse transcriptase were added. The reaction was performed at the same temperature for 20 min. After RNase-digestion the products were analyzed on a 7.5% polyacrylamide gel. Sequencing of the plasmid pDH51/GUS was carried out as in Chen and Seeburg (1985) with the above described oligonucleotide. The reaction products were analyzed on the same gel as the primer extension reaction products.

Southern analysis. Preparation of yeast DNAs has been described by Durkacz et al. (1986). Southern analysis and manipulations of DNA were performed according to Maniatis et al. (1982).

Fluorimetric GUS assay. Yeast cells were grown in 10 ml liquid medium until they reached an OD of 1.0. After harvesting by centrifugation and resuspension in GUS extraction buffer, they were frozen for 5 min at −70 °C. Then, the cells were thawed and sonicated for 20 sec on ice. After centrifugation for 10 min at 10 000 rpm in a microcentrifuge at 4 °C, the supernatant was transferred to new tubes and stored at −20 °C. Samples were then analyzed by a fluorimetric β-glucuronidase assay as described by Jefferson (1987). With each sample, a kinetic assay was performed for up to 120 min. As controls, *S. pombe* or *S. cerevisiae* cells transformed with pDB262 were incubated in parallel and used for standardization. Final GUS enzymatic activities were calculated in [micromol methyl-umbelliferone/minute × mg total protein].

Results

Yeast plasmid construction

To determine whether the plant DNA regulatory sequences responsible for CaMV expression in tobacco might function in *S. pombe*, we constructed the yeast plasmid pDB262/35S-GUS (Fig. 1 A) containing the bacterial GUS (β-glucuronidase) gene under the control of the 35S promoter and the 35S terminator derived from the plasmid pDH51/35S-GUS (Pietrzak et al. 1986). The yeast/*E. coli* shuttle vector pDB262 (Beach et al. 1982) has the special feature that it can be propagated in both of the evolutionarily highly diverged yeasts, *S. cerevisiae* and *S. pombe*.

RNase protection analysis of 5′ and 3′ ends of 35S-GUS transcripts in S. pombe

To determine the primary structure of the GUS transcripts in *S. pombe*, both the 5′ and 3′ ends of the *S. pombe* transcripts were mapped. An RNase protection assay was performed (Fig. 2) with two different probes for the 3′ end (Fig. 2 A and B) and one probe for the 5′ end (Fig. 2 C). In the case of the 3′ end, two major and

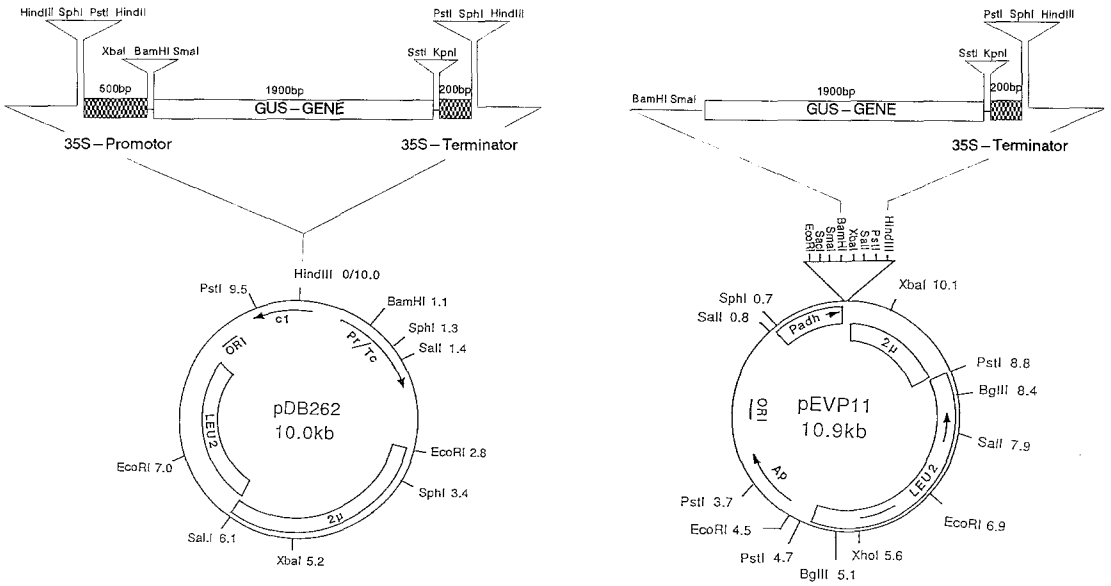

Fig. 1. Construction of yeast expression plasmids. **A** The 2.7 kb *Hin*dIII fragment, containing the 35S promoter-GUS gene-35S terminator of the vector pDH51-GUS (Pietrzak et al. 1986), was inserted into the yeast vector pDB262 (Beach et al. 1982). **B** The 2.2 kb *Bam*HI/*Hin*dIII fragment, containing the GUS gene-35S terminator of the vector pDH51-GUS, was inserted into the yeast vector pEVP11 (Russel and Nurse 1986), which contains the *S. pombe* ADH promoter

one minor transcription termination sites were observed. The two major sites could be mapped to coincide with the polyadenylation site of plants, the sequence AAUAAA at the end of the 35S terminator. The minor termination site was mapped to lie approximately 100 nucleotides upstream, still being part of the 35S terminator, to a sequence with the motif AAUUAA.

The RNase protection analysis of the 5′ end of the GUS transcripts in *S. pombe* is shown in Fig. 2C, mapping to a site which corresponds to the position at which tobacco was found to initiate transcription (Odell et al. 1985). Confirmation of this result was obtained by primer extension, as shown in Fig. 3 for *S. pombe*. The transcription start site was located to lie exactly 26 nucleotides downstream of the TATAA box, to the same nucleotide which was reported to be used as a transcription start in plants (Odell et al. 1985). During the preparation of the manuscript, the same transcriptional start site was also reported by Pobjecky et al. (1990).

These results show that fission yeast recognizes both types of plant transcriptional control elements, the 35S promoter and the 35S terminator.

35S-GUS and ADH-GUS steady state transcript levels in fission and budding yeast

To investigate the GUS transcripts in the two yeasts, a Northern analysis was performed with pDB262/35S-GUS transformed *S. pombe* and *S. cerevisiae* cells. To ensure that equal amounts of RNA were applied to the gel, the spectroscopically determined concentrations of total RNAs were checked by ethidium bromide staining of the ribosomal RNAs. As shown in Fig. 4A, the level of 35S-GUS transcripts in *S. pombe* (lanes 4 to 6) is at least hundred times higher compared to *S. cerevisiae* (lanes 7 to 9). In fact, the level in budding yeast is so low, that it could not be detected at the indicated exposure time.

One reason for the variation of the GUS activities in these organisms could be due to different stabilities of the transcripts. For this reason a fragment, consisting of the GUS gene fused to the 35S terminator, was inserted into the yeast/*E. coli* vector pEVP11 (Russell and Nurse 1986) which contains the *S. pombe* ADH (alcohol dehydrogenase) promoter (Fig. 1B). Analysis of the ADH-GUS transcripts of both fission and budding yeast (Fig. 4, lanes 1 to 3 and lanes 10 to 12, respectively) showed similar expression levels. In *S. pombe,* the ADH-GUS and 35S-GUS transcript levels differ only by a factor of three. However, in *S. cerevisiae* transformants, the difference is at least hundredfold. Therefore, transcript stability cannot be the determining factor for the enormous difference in 35S-GUS expression in the two yeasts. Furthermore, we investigated whether the 35S-GUS transcripts are found in the poly A^+ fraction of the RNAs in the two yeasts. As is shown in Fig. 4B, the 35S-GUS transcripts seem to be polyadenylated in both yeasts. However, *S. pombe* cells (1 μg in lane 1) contain about hundred times more GUS transcripts than *S. cerevisiae* cells (ten times the amount was applied, i.e. 10 μg in lane 2). Both yeasts produce GUS transcripts of the same length (2.2 kb) as was found in *N. tabacum* cells which were transformed with the same construct (data not shown).

Taken together, the results indicate that the differences in GUS activities between the two yeast species appear to be mainly due to different transcription rates of the GUS gene.

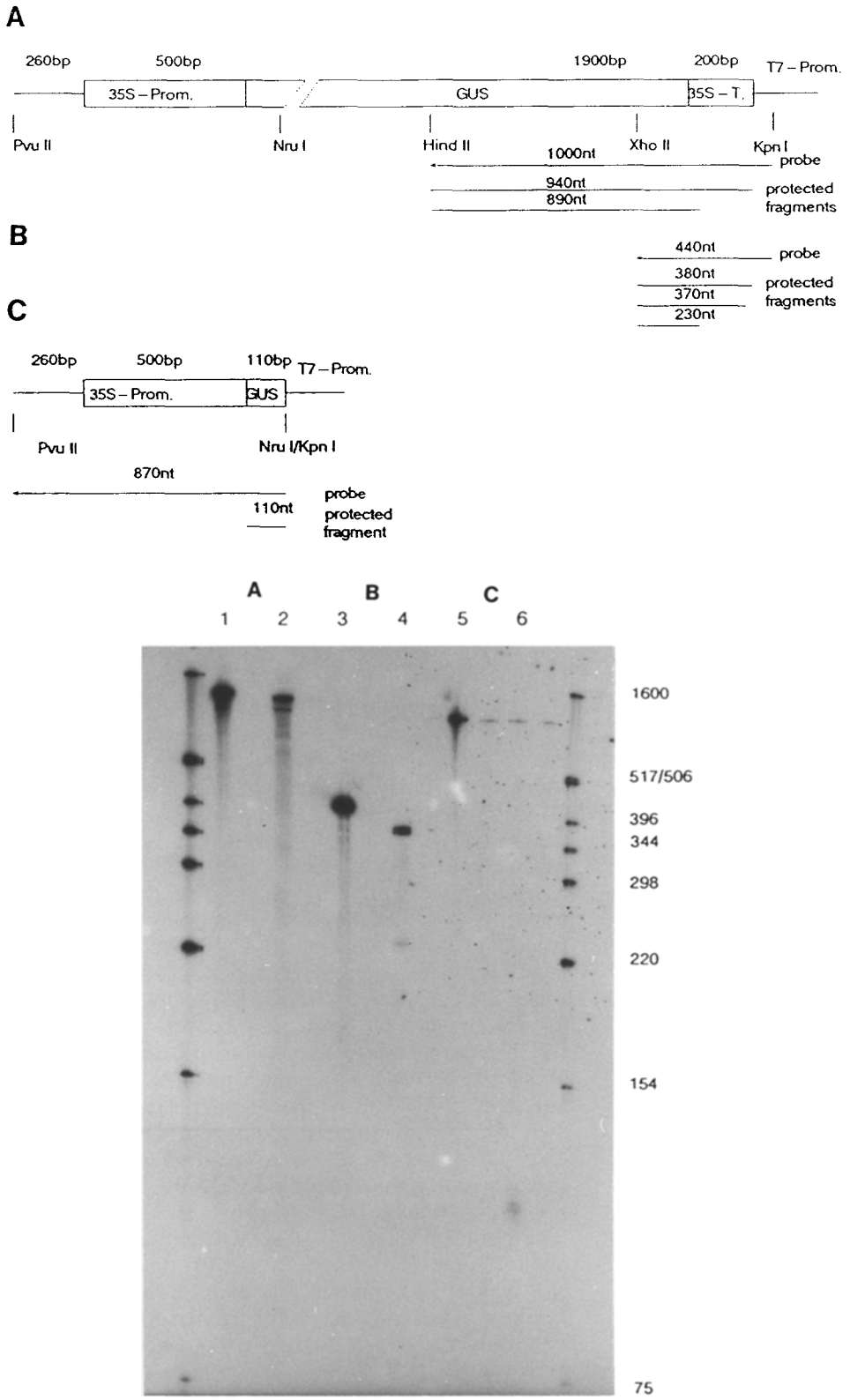

Fig. 2A–C. (top) Schematic maps of the antisense RNA probes and of the RNase-protected fragments. Relevant restriction sites are indicated. Radioactive antisense RNAs were transcribed from the T7-promoter of pBLUESCRIPT KS$^+$. For in vitro transcription, vectors were cut with: **A** *Hin*dII, **B** *Xho*II, **C** *Pvu*II. In order to obtain the vector used to transcribe probe C, the sequences between the *Nru*I site and the *Kpn*I site 3′ of the 35S terminator (as shown in Fig. 2A) were deleted by cutting and blunt end religation of the vector. **A–C** (autoradiogram) RNase protection mapping of the 3′ and 5′ ends of the GUS transcripts in *S. pombe* cells transformed with pDB262/35S-GUS. Sizes and structure of the probes and of the protected fragments are indicated. *Lanes 1, 3 and 5:* undigested antisense RNA probes. *Lanes 2, 4 and 6:* protected fragments. *Lanes 1 to 4:* mapping of the 3′ end with antisense probes as depicted in Fig. 2A and 2B, respectively. *Lane 5 and 6:* mapping of the 5′ end with an antisense probe as depicted in Fig. 2C. *Hin*fI-digested end-labelled pBR322 DNA was denaturated and used as a size marker

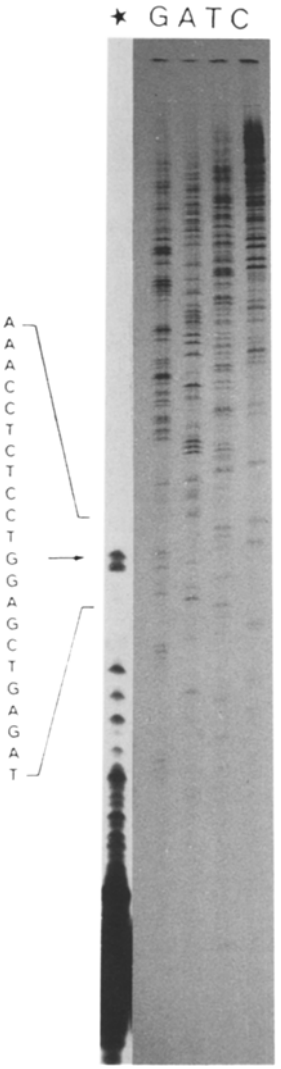

Fig. 3. Primer extension analysis of 5′ end of GUS transcripts in *S. pombe* transformed with the plasmid pDB262/35S-GUS. 15 µg of total *S. pombe* RNA were used. 5 ng (20 000 cpm) of a ^{32}P-labelled 22 nt long primer was used for the reverse transcription (see Materials and methods). The products were analyzed on a denaturing 7.5% polyacrylamide gel. In parallel, the plasmid pDB262/35S-GUS was sequenced with the same cold primer used for the primer extension reaction. The sequence around the 5′ end of the trancript (indicated with an *arrow*) is shown for clarity

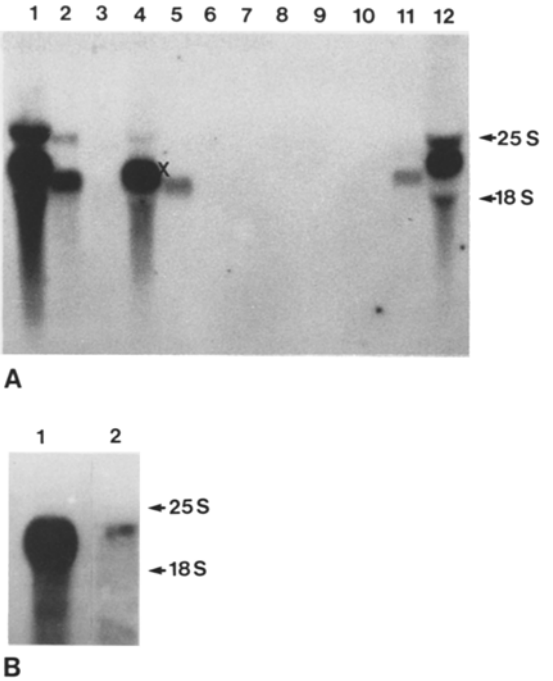

Fig. 4. **A** Northern analysis of steady state levels of GUS transcripts in *S. pombe and S. cerevisiae* cells transformed with pDB262/35S-GUS and pEVP11/ADH-GUS. *Lanes 1 to 3:* 10, 1 and 0.1 µg total RNA of ADH-GUS transformed *S. pombe* cells. *Lanes 4 to 6*: 10, 1, and 0.1 µg total RNA of 35S-GUS transformed *S. pombe* cells. *Lanes 7 to 9:* 10, 1 and 0.1 µg total RNA of 35S-GUS transformed *S. cerevisiae*. *Lanes 10 to 12:* 0.1, 1 and 10 µg total RNA of ADH-GUS transformed *S. cerevisiae*. **B** polyA$^+$ RNA of pDB262/35S-GUS transformed *S. pombe* and *S. cerevisiae* cells. *Lane 1: S. pombe,* 35S-GUS, 1 µg; *lane 2*: *S. cerevisiae,*. 35S-GUS, 10 µg

Determination of copy numbers and integrity of 35S-GUS constructs in budding and fission yeast

Since the copy number of one and the same plasmid may differ considerably in the two yeasts (Heyer et al. 1986), it is conceivable that different amounts of 35S-GUS plasmids are responsible for the observed expression pattern. Therefore, we performed a Southern analysis with the pDB262/35S-GUS transformed yeast cells. The radioactively labelled probes consisted of the 35S promoter-GUS gene-35S terminator and the LEU2 gene. The hybridization pattern of the *Hin*d III-digested DNAs (*S. cerevisiae* transformants, lanes 1–4; *S. pombe* transformants, lanes 7–10) is shown in the dilution series in Fig. 5. In both yeasts, only two bands of the expected sizes of 2.5 kb (35S promoter-GUS-35S terminator) and 10.0 kb (LEU2 of pDB262) showed hybridization, indicating that the constructs did not undergo any obvious rearrangements. As controls, untransformed yeast DNAs were also digested with *Hin*d III and analyzed in Fig. 5, lanes 6 (*S. cerevisiae*) and 12 (*S. pombe*). As expected, the LEU2 probe did not cross-hybridize to the *S. pombe* DNA but rather to the chromosomal, approximately 11 kb long, *Hin*d III fragment in *S. cerevisiae* DNA. Apparently, in *S. cerevisiae* the plasmid is present at a 1.5–2 fold higher copy number than in *S. pombe* cells. In comparison to the single copy LEU2 gene in the wild type *S. cerevisiae*, the copy number of the pDB262/35S-GUS plasmids is 4–5 copies for *S. pombe* and 7–9 copies for *S. cerevisiae*. Undigested transformed pDB262/35S-GUS yeast DNAs showed typical plasmid bands [Fig. 5, lanes 5 (*S. cerevisiae*) and 11 (*S. pombe*)], indicating the episomal status of the constructs.

Enzymatically active GUS proteins are synthesized in both yeasts

In order to test the yeast transformants for expression of functional GUS protein, a fluorimetric enzyme assay was performed with the protein extracts of the yeast cells. As shown in Table 1, both yeasts seem to express GUS protein, although an approximately 100 fold higher GUS

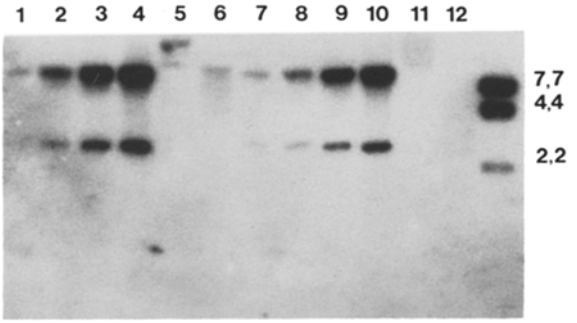

Fig. 5. Southern analysis of pDB262/35S-GUS transformed fission and budding yeast mutants. *Lanes 1 to 4* and *7 to 10:* 0.25, 0.5, 1 and 2 μg *Hin*dIII-digested DNA of pDB262/35S-GUS transformed *S. cerevisiae* and *S. pombe*, respectively. *Lane 5 and 11:* 1 μg undigested DNA of pDB262/35S-GUS transformed *S. cerevisiae* and *S. pombe*, respectively. *Lanes 6 and 12:* 1 μg *Hin*dIII-digested DNA of untransformed *S. cerevisiae* and *S. pombe*, respectively

Table 1. Fluorimetric analysis of GUS expression in *S. pombe* and *S. cerevisiae* cells transformed with the plasmids pDB262/35S-GUS, pEVP11/ADH-GUS and pDB262. Enzymatic GUS activities are expressed in units of micromol methyl-umbelliferone/min × mg total protein

Construct	*S. pombe* GUS activity	*S. cerevisiae* GUS activity
pDB262/35S-GUS	32	0.3
pDB262/ADH-GUS	48	52
pDB262	–	–

activity was recorded in the *S. pombe* transformants (32 micromol methyl-umbelliferone/min × mg total protein) compared to *S. cerevisiae* transformants (0.3 micromol methyl-umbelliferone/min × mg total protein). As controls, both fission and budding yeast cells were transformed with the plasmid pDB262 alone, but no enzymatic activity could be determined over a time period of 24 h. Fluorimetric quantification of the pEVP11/ADH-GUS transformed yeasts revealed that in *S. pombe* both the 35S and the ADH promoters are strongly expressed. The 35S promoter appears to be nearly as efficient as the strong *S. pombe* ADH promoter (Table 1). In *S. cerevisiae*, the ADH promoter also gives rise to high levels of GUS activities (52 micromol methyl-umbelliferone/min × mg total protein), resulting in a more than hundredfold higher activity compared to the 35S promoter (0.3 micromol methyl-umbelliferone/min × mg total protein).

Discussion

To determine whether genomic plant libraries can be used for complementation of fission yeast mutants, plant genes must be functionally expressed in this yeast. As a first step, we investigated the functioning of typical plant transcriptional elements, the CaMV 35S promoter and 35S terminator, which are frequently used to express various genes in a variety of plant species and which contain all sequence elements necessary for expression in plants. The bacterial β-glucuronidase (GUS) gene served as a reporter gene whose expression could be easily quantified by a sensitive fluorimetric assay (Jefferson 1987). RNase protection mapping (Fig. 2) and Northern analysis (Fig. 3) of the GUS transcripts showed that the 35S promoter is properly recognized in *S. pombe*, initiating transcription at exactly the same nucleotide as in plant cells (Odell et al. 1985). These results are confirmed by the data of Pobjecky et al. (1990), who found the same start site by S1 mapping, and also by the report of Gmünder et al. (1989), who showed that the bacterial neomycin phosphotransferase II gene is expressed in *S. pombe* when fused with the 35S promoter. We could also show that the 35S terminator produces transcripts which are predominantly polyadenylated close to the typically eucaryotic AAUAAA sequence. These findings suggest that *S. pombe* and plants contain similar transcription machineries sharing functionally homologous and highly conserved transcription and termination factors. Somehow surprising was the observation that, although *S. cerevisiae* seems to initiate and terminate at similar sites as *S. pombe* (data not shown), the steady state levels of the GUS transcripts were found to be several hundred times lower.

Southern analysis (Fig. 5) revealed that the 35S-GUS construct was still intact and had not integrated into a yeast chromosome. It also showed that the copy number in *S. cerevisiae* was maximally twice as high as in *S. pombe*. Still, it could be possible that different stabilities of the GUS transcripts in the two yeasts were responsible for the different expression levels. Two observations argue against this hypothesis. First, expression analysis of a construct in which the 35S promoter was replaced by the ADH promoter from *S. pombe* resulted in similar GUS activities in both yeasts. Second, when the GUS gene was replaced by the neomycin phosphotransferase gene, Northern analysis of *S. pombe* and *S. cerevisiae* transformed cells showed the same differences in transcript levels as with the GUS gene (data not shown). Therefore, we conclude that the 35S promoter is responsible for the higher transcription rate in *S. pombe* compared with *S. cerevisiae*.

The reason for the differences in expression in the two yeasts is not known but part of the answer may lie in the differential expression pattern in the two yeasts. We have obtained evidence that the 35S promoter is repressed under rich medium conditions in *S. cerevisiae*, but not in *S. pombe*. An up to twentyfold derepression was observed in *S. cerevisiae* under starvation conditions (Hirt et al., in preparation). Taken together, it appears that the 35S promoter is diversely recognized in fission and budding yeast and is thereby effecting these highly different transcription rates.

References

Aviv H, Leder P (1972) Proc Natl Acad Sci USA 69:1408–1412
Beach D, Durkacz BW, Nurse P (1982) Nature 300:706–709
Chen EY, Seeburg PH (1985) DNA 4:165–170
Coraggio I, Compagno C, Martegani E, Ranzi BM, Sala E, Alberghina I, Viotti A (1986) EMBO J 5:459–465

Cramer JH, Lea K, Slightom JL (1985) Proc Natl Acad Sci USA 82:334–338
Durkacz B, Beach D, Hayles J, Nurse P (1986) Mol Gen Genet 201:543–545
Ecker JR, Davis RW (1986) Proc Natl Acad Sci USA 83:5372–5376
Feinberg AP, Vogelstein B (1983) Anal Biochem 132:6–13
Fluhr R, Kuhlemeier C, Nagy F, Chua N-H (1986) Science 232:1106–1112
From ME, Taylor LP, Walbot V (1986) Nature 319:791–793
Gmünder H, Kohli J (1989) Mol Gen Genet 220:95–101
Heyer WH, Sipiczki M, Kohli J (1986) Mol Cell Biol 6:80–89
Ito H, Fukuda Y, Murata K, Kimura A (1983) J Bacteriol 153:163–168
Jefferson RA (1987) Plant Mol Biol Reporter 5:387–405
Jones JDG, Dunsmuir P, Bedbrook J (1985) EMBO J 4:2411–2418
Langridge P, Eibel H, Brown JWS, Feix G (1984) EMBO J 3:2467–2471
Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Müller MW, Schweyen RJ, Schmelzer C (1988) Nucleic Acids Res 16:7383–7395
Nagata T, Okada K, Kawazu T, Takebe I (1987) Mol Gen Genet 207:242–244
Odell JT, Nagy F, Chua N-H (1985) Nature 313:810–812
On-Lee TM, Turgeon R, Wu R (1986) Proc Natl Acad Sci USA 83:6815–6819
Ow DW, Jacobs JD, Howell SH (1987) Proc Natl Acad Sci USA 84:4870–4874
Pietrzak M, Shilito RD, Hohn T, Potrykus I (1986) Nucleic Acids Res 14:5857–5868
Pobjecky N, Rosenberg GH, Dinter-Gottlieb G, Käufer NF (1990) Mol Gen Genet 220:314–316
Russell P, Nurse P (1986) Cell 45:781–782
Thomas PS (1980) Proc Natl Acad Sci USA 77:5201–5205
Wiebauer K, Herrero J-J, Fillipowicz W (1988) Mol Cell Biol 8:2042–2051

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