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Gene cloning from yeast chromosome-specific mini-library

Isolation of the SRP1-related DNA sequence located on chromosome XV

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We have isolated and purified chromosome XV DNA molecules from the yeast *S. cerevisiae* using contour-clamped homogeneous electric field (CHEF) gel electrophoresis. A chromosome-specific mini-library was constructed and the element of the SRP1 (serine-rich protein) related sequence family located within chromosome XV was isolated by in situ colony hybridization with an SRP1 probe. Results indicated that (i) a single-copy sequence homologous to SRP1 is present within chromosome XV; (ii) this sequence lies within the 2.3 kb *Hin*dIII fragment of the plasmid pXVAc6; (iii) the lack of a specific transcript from this SRP1-related element suggests that it could be considered as a pseudogene.

Pulsed field electrophoresis; Chromosome library; Gene family; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

The recent development of pulsed field electrophoresis, allowing resolution of large DNA molecules [1-3], has opened the way for new cloning strategies. Organisms like *Saccharomyces cerevisiae* are particulary well adapted for electrophoretic karyotypes; not only direct information on the karyotype, or number and size of chromosomes, is available, but separated chromosomes can then be isolated and subjected to restriction mapping or manipulation for construction of mini-libraries. Thus, a chromosome-specific library couldd be very useful for cloning the different members of a gene family when they are localized on distinct chromosomes.

We report here an example of this gene-cloning strategy, i.e. isolation of the SRP1 homologous copy located on *S. cerevisiae* chromosome XV. The SRP1 gene is a glucose-inducible gene which is highly expressed (1-2% of total poly(A)$^+$ RNA) and whose function remains still unknown. The encoded protein (M_r 24 875) contains eight tandem repeats, 12-amino-acid long, particularly rich in serine residues [4,5]. Recently we have shown that a DNA-binding protein was able to interact specifically within the 3'-end of the gene coding region [6]. Beside the normal location of this gene on chromosome V, 12 other chromosomes carry SRP-related sequences with various degrees of homology. Copies located within chromosomes XV, VII and XI could be detected under stringent conditions of hybridization (T_m -20°C) while those on chromosomes I-IV, VI, VIII, X-XII were observed only under low stringency (T_m -40°C). Cloning and sequencing of several SRP1-related sequences would be of interest for the study of this family of repetitive DNA.

2. MATERIALS AND METHODS

Escherichia coli K12 strain TG1 (Δlac-pro, supE, thi, hsdΔ5/F' traD36, proA$^+$B$^+$, lacIq, lacZΔM15) was employed as the host for the propagation of *E. coli*/yeast shuttle vectors. Growth and transformation of *E. coli* cells were carried out ac-

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cording to standard procedures [7]. The yeast strain YP148 (a generous gift from Dr P. Hieter) was grown at 28°C on YPD medium (1% yeast extract, 2% Bactopeptone, 2% dextrose) [5].

S. cerevisiae chromosomal DNA samples were prepared as reported by Schwartz and Cantor [1] with slight modifications. For preparative electrophoresis, cells were resuspended to a concentration of 5×10^8 cells/ml of agarose block. The DNA samples were subjected to CHEF gel electrophoresis, using a hexagonal array of electrodes as described by Chu et al. [3], through a 1% agarose gel (Pharmacia NA) at 12°C. The 17 chromosomes of strain YP148 were well separated after 48 h at 6 V/cm in 0.25×TBE (1×TBE: 90 mM Tris, 90 mM boric acid, 2.5 mM EDTA) and with a 100 s pulse time. After staining for 1 h with ethidium bromide (0.5 μg/ml), the gel was washed overnight in water. Following electrophoresis, DNA fragments were transferred onto nitrocellulose mebranes as in [5].

In situ hybridization of bacterial colonies was performed according to Hanahan and Meselson [9]. High (T_m −20°C) and low (T_m −40°C) stringency hybridizations were carried out as described [5]. DNA probes were oligolabeled according to Feinberg and Vogelstein [10]. The SRP1 probe was the 0.57 kb *Pvu*II fragment isolated from plasmid pR21A [4] while the probe *Hin*dXV, specific of the SRP homologous sequence located on chromosome XV, was the 2.3 kb *Hin*dIII fragment purified from plasmid pXVAc6. HIS3 and URA3 DNA probes were the 1.7 kb *Bam*HI and 1.1 kb *Hin*dIII fragments isolated from cloned HIS3 [11] and URA3 [12] yeast genes, respectively.

3. RESULTS

3.1. *Isolation and purification of chromosomal DNA*

The karyotype of *S. cerevisiae* (AB 972) described by Carle and Olson [13] shows 12 resolved bands, after separation by CHEF gel electrophoresis, which correspond to nine singlets and three comigrating doublets. These doublets are readily separated in the yeast strain (YP148) constructed by Dr P. Hieter, and under our electrophoretic conditions, chromosome XII is also able to enter the gel; thus the full set of yeast chromosomes is completely resolved (fig.1A). Previous studies in this laboratory [5] have shown that an SRP1 probe revealed a set of at least 12 homologous sequences, the SRP copies with the

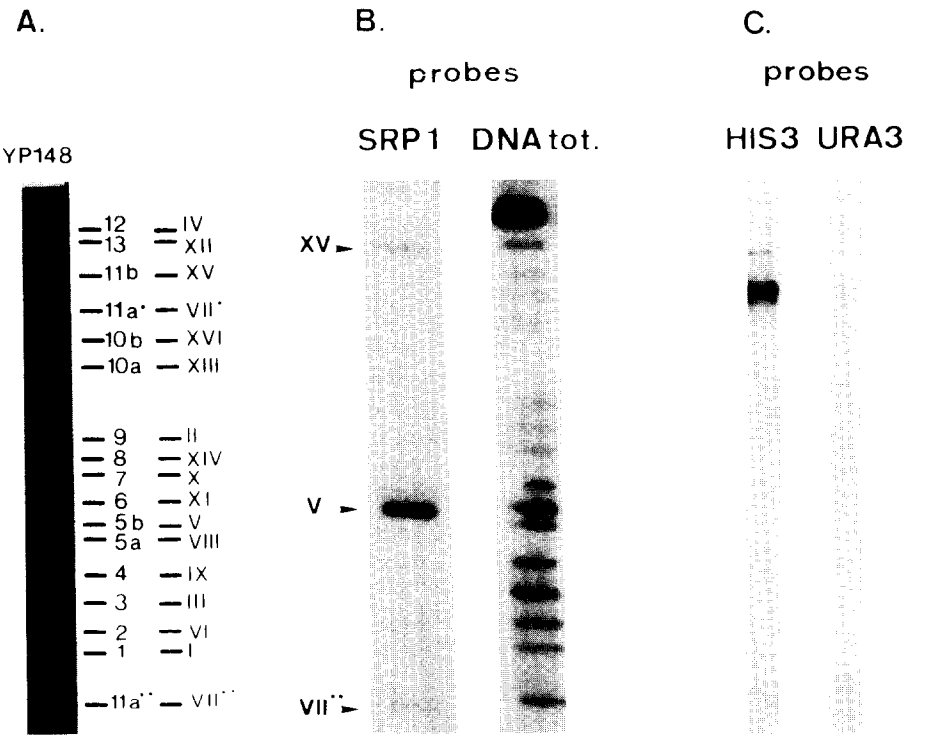

Fig.1. Isolation of *S. cerevisiae* chromosome XV. (A) Karyotype of *S. cerevisiae* strain YP148 obtained by CHEF gel electrophoresis using a hexagonal array of electrodes. Arabic and Roman numerals correspond to the assignments of Carle and Olson and the chromosome number, respectively. (B) Identification of the chromosomes bearing an SRP1 strongly homologous sequence. (C) Specificity of chromosome XV purification. Hybridizations were carried out with probes HIS3 and URA3 specific for chromosomes XV and VII, respectively.

highest homology being localized on chromosomes XV and VII (Fig.1B). In order to clone specifically these SRP copies we first purified chromosome XV. The DNA bands were cut under UV light (302 nm) from a preparative gel after staining with ethidium bromide and the DNA molecules were electroeluted from the agarose blocks in dialysis bags. The electrophoretic conditions were the same as for the separation, i.e. the dialysis bags were put in the center of the hexagonal array of electrodes and the elution was run for 96 h (6 V/cm, 100 s switching interval, 0.25 × TBE, 12°C). The elution buffer was then dialyzed for 3 h vs 1 l TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and the DNA was precipitated with a small amount of glycogen as a carrier. The DNA was gently resuspended with TE buffer without pipeting or vortex-mixing (4 h) and was then used for restriction and cloning. We verified that the isolated chromosomal DNA was free from contamination of DNA belonging to the adjacent chromosomes by demonstrating that the purified DNA from chromosome XV hybridized with the HIS3 probe but not with the URA3 probe (fig.1C), which is able to detect the largest fragment of the split chromosome VII located immediately below chromosome XV (fig.1A). An alternative method of purification of chromosomal DNA molecules was also used with similar results. The CHEF hexagonal electrophoresis was run through a 0.5% low-melting-point agarose gel, then the bands were cut and after 1 h equilibration with TE buffer the agarose blocks were melted by heating at 65°C for 5 min, twice diluted with 2 × restriction enzyme buffer and digested for 4 h at 37°C. The mixture was then allowed to solidify on ice and the agarose plug subjected to standard electroelution.

3.2. *Mapping and cloning of the SRP1 homologous sequence belonging to chromosome XV*

The specific genomic environment of the SRP sequence within the chromosome XV was studied in order to determine what fragment would be suitable for cloning. Southern blots of chromosome XV DNA digests were hybridized under low stringency conditions (T_m −40°C) with the SRP1 probe and are illustrated in fig.2A. *Hin*dIII and *Pst*I digestions displayed only one band 2.3 kb and 7.2 kb long, respectively. These results indicated

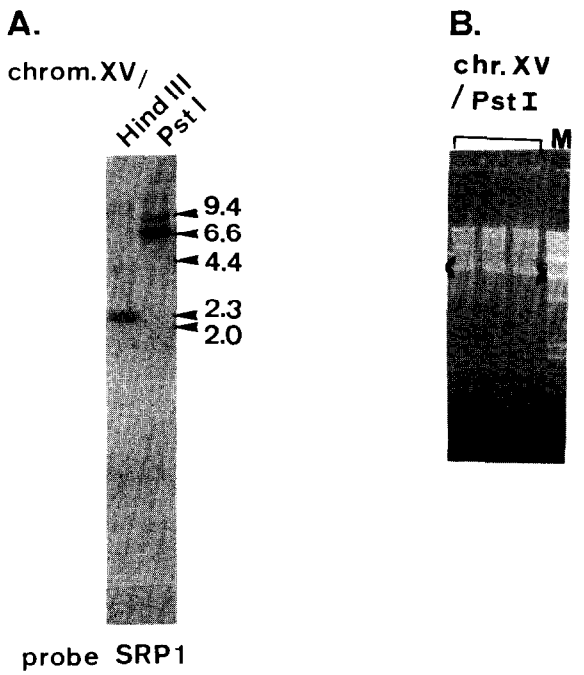

Fig.2. (A) Restriction mapping of chromosome XV for the SRP1 homologous sequence. Chromosome XV DNA was digested with *Hin*dIII and *Pst*I and hybridization was performed with the SRP1 probe at T_m −40°C. (B) Preparative purification of chromosome XV *Pst*I DNA fragments. The eluted fraction is indicated with brackets. M: λ DNA digested by *Hin*dIII.

that probably only one sequence homologous to the SRP1 gene was present within chromosome XV.

To clone the chromosome XV copy of the SRP1 gene, DNA fragments (5–8 kb long) were isolated from a *Pst*I digest of chromosome XV DNA molecules following electrophoretic separation (fig.2B). Note that discrete digestion products were clearly visible in contrast to the usual smear obtained with total genomic DNA digestion. The DNA fragments were inserted in the *Pst*I site of the pAT153 vector. 285 recombinant clones were obtained after transformation of *E. coli* cells and one positive clone (pXVAc6) was found among 96 plasmids analyzed by in situ colony hybridization with the probe SRP1 under low stringency conditions.

3.3. *Characterization of the plasmid pXVAc6*

The *Pst*I insert was 7.2 kb long and matched the size of the fragment detected by Southern blot

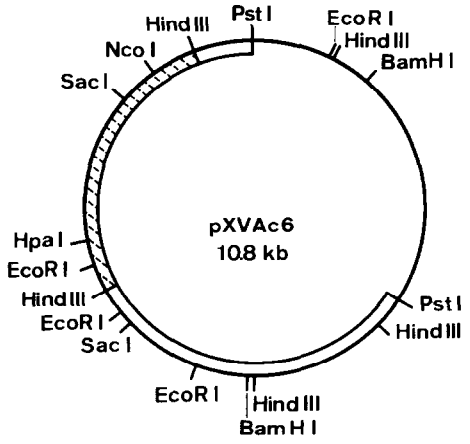

Fig.3. Restriction map of the recombinant plasmid pXVAc6. The thick line indicates the 7.2 kb *Pst*I insert, the region homologous to the SRP1 gene being cross-hatched.

analysis of chromosome XV DNA (figs 2A,3). Analysis of the region (within this fragment) homologous to the SRP1 gene was carried out by Southern blotting. The results (fig.4A) showed this sequence to be located in the 2.3 kb *Hin*dIII fragment. Using this fragment as a probe (*Hin*dXV),

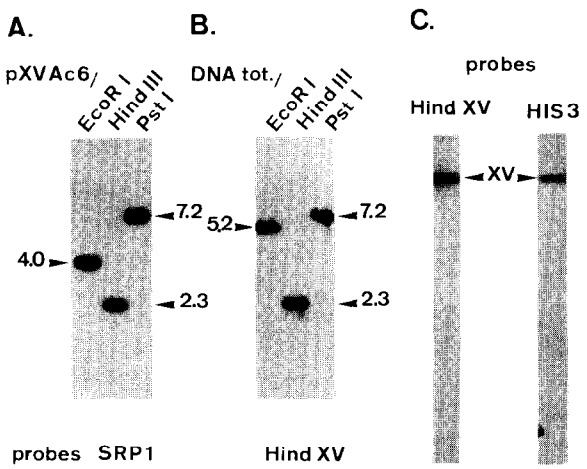

Fig.4. Characterization of the SRP1 homologous region in pXVAc6. (A) Plasmid pXVAc6 was digested by *Eco*RI, *Hin*dIII and *Pst*I. Hybridization performed with the SRP1 probe at $T_m - 40°C$. (B) Genomic DNA from S288C strain was digested by *Eco*RI, *Hin*dIII and *Pst*I. Hybridization performed with the *Hin*dXV probe at $T_m - 20°C$. (C) Chromosomal assignment of the cloned SRP1 homologous copy. Southern blot of YP148 DNA fractionated by CHEF gel electrophoresis and analyzed with the *Hin*dXV and the HIS3 probes at $T_m - 20°C$.

we tested yeast genomic DNA digested with *Eco*RI, *Hin*dIII and *Pst*I under high stringency hybridization conditions. The results indicated that for *Pst*I and *Hin*dIII the flanking genomic environment was the same for both chromosome XV and total yeast DNA and similar to the plasmid restriction map which also explains the *Eco*RI digestion pattern (fig.4B). Other results (not shown) indicated that (i) the sequence similarity with the SRP1 gene lay within the 0.4 kb *Sac*I/*Nco*I fragment; (ii) the 30 bp region bearing the recognition sequence for the SRP1 DNA-binding factor, located at the 3′-end of the SRP1 coding region, was also present within the cloned *Pst*I fragment [6].

The chromosomal origin of the cloned SRP1 homologous copy was directly identified by hybridization to a blot of chromosomal DNAs fractionated by CHEF gel electrophoresis. Under high stringency conditions only one band was detected using the 2.3 kb *Hin*dIII probe purified from the pXVAc6 plasmid; that band was also revealed by an HIS3 probe and then identified to the chromosome XV (fig.4C).

Finally, the in vivo expression of the chromosome XV SRP1 homologous sequence was examined by Northern blot analysis. RNAs prepared from wild-type and SRP1-deleted strains were hybridized with the *Hin*dXV probe after electrophoretic fractionation and blotting onto nitrocellulose membrane. No transcript was detected with total RNA or the poly(A)$^+$ fraction.

4. DISCUSSION

We have constructed a yeast mini-library from purified chromosome XV DNA molecules using a DNA fraction enriched with 5–8 kb *Pst*I fragments. The screening of about 100 clones permitted the isolation of the 7.2 kb *Pst*I fragment carrying the region similar to the SRP1 gene. The chromosomal location of that isolated fragment has been confirmed by Southern blot analysis of the yeast banding pattern obtained after CHEF gel electrophoresis. The region of similarity with the SRP1 gene was localized within the 2.3 kb *Hin*dIII fragment of the pXVAc6 recombinant plasmid. Further analysis of this second cloned SRP1 homologous sequence will enable the precise relationships between the elements of the SRP family

to be studied [5]. The absence of any detectable transcript indicates that the SRP1 homologous sequence located on chromosome XV is not functional and could be considered as a pseudogene. Since this SRP copy is found at a different chromosomal location from the expressed SRP1 gene it would fit the usual definition of a processed pseudogene. This result confirmed our previous observations on the expression of this SRP1 copy [5]. In contrast to higher eucaryotes, very few pseudogenes have been described in the yeast *S. cerevisiae*, and some suggestions have been proposed in an attempt to explain this situation [14]. Till now, only an example of a processed pseudogene has been well characterized [15] but definite conclusions about the nature of the SRP-related sequence must await knowledge of the DNA sequence.

This work shows the feasibility of mini-library construction from isolated yeast chromosome DNA molecules. Such a possibility is very useful especially for the analysis of multi-genic families because it is chromosome oriented. It facilitates the mapping of the genomic environment of homologous genes located on a particular chromosome and their cloning following screening with a homologous probe belonging to a gene lying on a distinct chromosome. Selection of genes weakly homologous is then made easier as it can be carried out without interference by the original gene used as a probe. Taking into account that chromosome XV, about 1.15 Mb long [16], is one of the larger chromosomes of *S. cerevisiae*, it would be conceivable to extend this work to any yeast chromosome. This strategy of gene mapping and cloning could possibly be of considerable help for the physical mapping of the yeast genome. Recently, techniques for cloning megabase long DNA fragments of complex genomes in yeast, with vectors able to reconstitute artificial yeast chromosomes [17], have been reported; they could probably draw great benefit from the feasibility of the chromosome-specific bank construction; specific subcloning of heterologous DNA from such yeast transformants would be considerably time-saving.

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REFERENCES

[1] Schwartz, D.C. and Cantor, C.R. (1984) Cell 37, 67–75.
[2] Carle, G.F. and Olson, M.V. (1984) Nucleic Acids Res. 12, 5647–5664.
[3] Chu, G., Vollrath, D. and Davis, R.W. (1986) Science 234, 1582–1585.
[4] Marguet, D. and Lauquin, G.J.-M. (1986) Biochem. Biophys. Res. Commun. 138, 297–303.
[5] Marguet, D., Guo, X.J. and Lauquin, G.J.-M. (1988) J. Mol. Biol., in press.
[6] Fantino, E., Marguet, D. and Lauquin, G.J.-M. (1988) in preparation.
[7] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, NY.
[8] Twigg, A.J. and Sherrat, D. (1980) Nature 283, 216–218.
[9] Hanahan, D. and Meselson, M. (1980) Gene 10, 63–67.
[10] Feinberg, A.P. and Vogelstein, B. (1984) Anal. Biochem. 137, 266–267.
[11] Struhl, K. (1985) Nucleic Acids Res. 13, 8587–8601.
[12] Chevallier, M.R., Block, J.-C. and Lacroute, F. (1980) Gene 11, 11–19.
[13] Carle, G.F. and Olson, M.V. (1985) Proc. Natl. Acad. Sci. USA 82, 3756–3760.
[14] Fink, G.R. (1987) Cell 49, 5–6.
[15] Yochem, J. and Byers, B. (1987) J. Mol. Biol. 195, 233–245.
[16] Mortimer, R.K. and Schild, D. (1985) Microbiol. Rev. 49, 181–212.
[17] Burke, D.T., Carle, G.F. and Olson, M.V. (1987) Science 236, 806–812.