Volume 219, number 1, 254-258 FEB 04894 July 1987

A TY1 element is inserted in the CYR1 control region of *Saccharomyces cerevisiae* strain AB320

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Received 25 April 1987

Southern blotting using a 5'-proximal probe of the *Saccharomyces cerevisiae* CYR1 gene has revealed heterogeneity in laboratory strains. It is demonstrated that strain AB320 contains a Ty1 element inserted in the promoter region of CYR1. The Ty1 orientation suggests that transcription of CYR1 is initiated downstream from the insertion region.

cyclic AMP; Insertion element; Promoter

1. INTRODUCTION

Cyclic nucleotides play an essential role in controlling many of the activities of eukaryotic and prokaryotic cells. In the yeast *Saccharomyces cerevisiae,* cAMP is required for growth [1]. The regulation of adenylate cyclase, the enzyme that converts ATP into cAMP, is therefore of central importance in cellular physiology and has been the subject of intensive research. In yeast, the main results that have been obtained to date are that the catalytic center of the enzyme is encoded by the CYR1 gene, found to be identical to the CDC35 gene. It has also been found that the corresponding gene product possesses enough catalytic activity to overcome the cAMP defect in an *Escherichia coli* strain, when overproduced from a muticopy plasmid [2,3]. Uno et al. [4], however, have shown that when the yeast gene was expressed at a low level in *E. coli* it could be activated in the presence of the RAS gene product. Finally, it could be observed that two major transcripts were synthesized in *S. cerevisiae,* depending upon the nature of the growth medium [3]. This suggested the existence of several promoters in the CYR1 gene, and prompted us to investigate the upstream promoter region at the molecular level.

We report here that in the strain used for cloning (strain AB320), a Ty1 control element is inserted in an orientation suggesting that transcription initiation of the CYR1 can proceed efficiently downstream from the insertion region.

Ty1 elements consist of an internal 5.3 kb fragment of DNA (epsilon DNA) flanked by direct repeats (deltas) of about 0.3 kb. There are approx. 30 copies of the complete element and over 100 copies of Ty1-associated and solo delta sequences in the haploid *S. cerevisiae* genome of laboratory strains, although the number and distribution vary considerably from strain to strain. These elements are transcribed into polyadenylated RNA species which are initiated in one direct repeat and terminated in the other. In the case of the cloned CYR1 gene, DNA sequencing enabled us to find both the insertion site of the element and its

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The nucleotide sequence presented has been submitted to the EMBL/GenBank database under the accession number Y00682

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

presumed orientation of transcription (from sequence comparison with other known Ty1 sequences).

2. MATERIALS AND METHODS

2.1. *Yeast strains, bacterial strains and growth media*

The yeast strains used here were AB320 (HO ade2-1 lys2-1 trp5-2 leu2-1 can1-100 ura3-1 and/or ura1-1 met4-1) [5], RH218 (a trp1 gal2 mal suc2 cup1) and GX3067-6.4 (trp1 cdc35) [6]. Strain AB320 has been used for the construction of a yeast DNA library [5], from which the CYR1 gene was obtained [6].

2.2. *DNA sequencing*

Plasmids were derivatives of pBR322 and a variant of the expression plasmid pDIA9204 [7,8], where a *Bam*HI-*Sal*I linker had been introduced at the end of the *lac*Z gene (pDIA9224). The donor of the CYR1 DNA segment was plasmid pCEY710

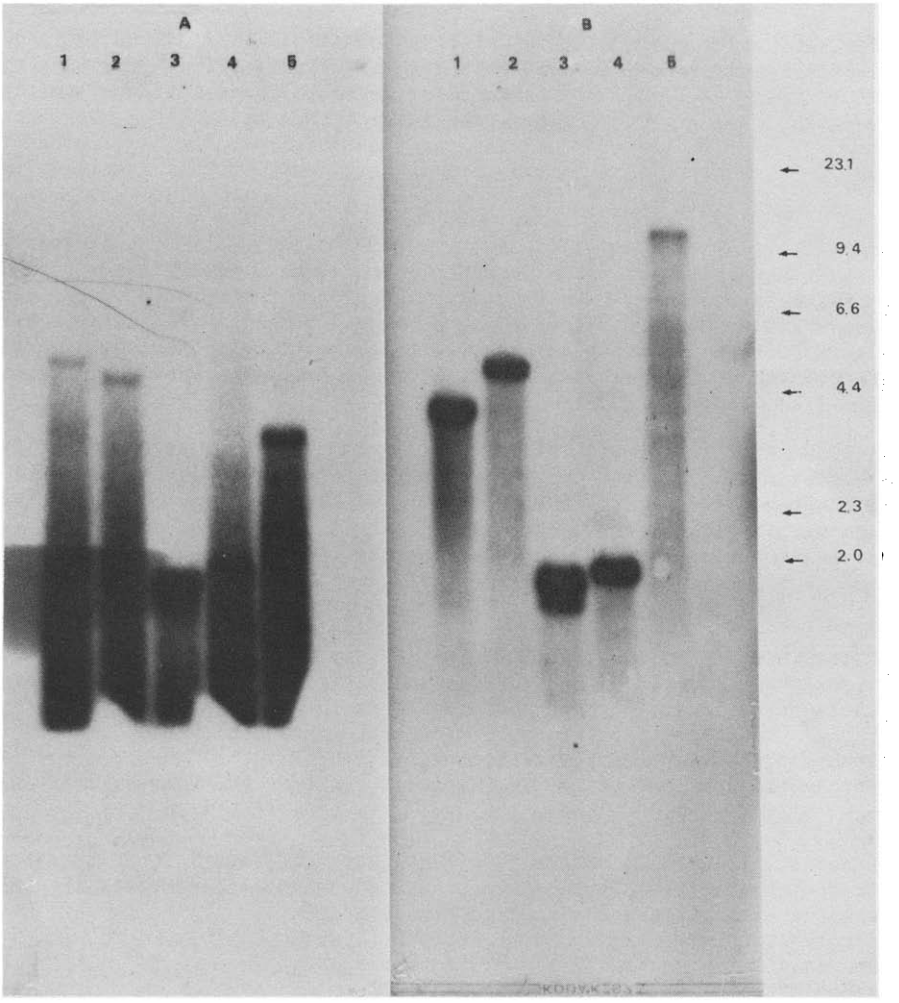

Fig.1. Southern hybridization of DNA from strain AB320 and RH218. Total yeast DNA from strain AB320 (A) and RH218 (B) was extracted and digested respectively by *Bgl*II (1), *Pvu*II(2), *Pst*I(3), *Hin*dIII(4) and *Xho*I(5). After electrophoresis, DNA was transferred to a nylon membrane (Hybond N, Amersham) using the method of Southern. Hybridization was carried out at 65°C in 3×SSC. The 0.8 kb *Eco*RI fragment from plasmid pCEY710 was nick translated. λ DNA fragments digested by *Hin*dIII (length indicated in kb) were used as markers.

containing the whole CYR1 region. A fragment encompassing the promoter region was cloned into M13 for DNA sequencing experiments. Plasmid preparation, transformation of yeast and *E. coli* cells, were performed as described by Maniatis et al. [9].

3. RESULTS AND DISCUSSION

It was noticed previously that a repetitive sequence was present on the fragment of *S. cerevisiae* (strain AB320/DNA containing the adenylate cyclase gene [6]). Hybridization of yeast

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-------------------- pBR322 --------------------------------
TCGCTTCGCTACTTGGAGCCACTATCGACTACGCGATCATGGCGACCACACCCGTCCTGTGGATCTTGATTTGTGTGGAC
gagttagccttagtggaagccttatcatattcttgaattttggaagctgaaacgtctaacggatcttgatttgtgtggac   YSCTY1DC


TTCCTTAGAAGTAACCGAAGCACAGGCGCTACCATGAGATATATGTGGGTAATTAGATAATTGTTGGGATTCCATTATTG
ttccttagaagtaaccgaagcacaggcgctaccatgagaaatgggggaatgttgagataattgttgggattccattgttg   YSCTY1DC
                                  tgagatatatgtgggtaattagataattgttgggattccattgttg   YSCTY1
                                  tgaagattgggtgaattttgagataattgttgggattccattttta   YSCTY9173
                                  tgaaaagtgggtgaattttgagataattgttgggattccatttta   YSCTY9175
                                  tgagaaatgggtgaatgttgagataattgttgggattccattgttg   YSCTYADA1


ATAAAGGCTATAATATTAGGTATACAGAATATACTAGAAGTTCTCCTCGAGGATATAGGAATCCTCAAAA TGGAATCTA
ataaaggttataatattgggtatacggaatatactagaagttttcctcaaggatataggaatcctcaaaa tagaattta
ataaaggctataatattaggtatacagaatatactagaagttctcctcgaggatataggaatcctcaaaaatggaatcta
at aaggcaataatattaggtatgtaga tatactagaagttctcctcgaggatttaggaatccataaaa gggaatctg
at aaggcaataatattaggtatgtagaatgtactagaagttctcctcgaggatttaggaatccataaaa gggaatctg
ataaaggctataatattaggtatacagaatatactagaagttctcctcgaggatttaggaatccataaaa gggaatctg


TA TTTCTACATACTAATATTACGATTATTCCTCATTCCGTTTTATATGTT     TCATT ATCCTATTACATTATCAA
ta tttctacatactaatattacgattatt ctcattccgttttatatgtttatactcattgatcctactacattatcaa
tattttctacatactaatattacgattattcctcattccgttttatatgtttatattcattgatcctat acattatcaa
caatttctacacaattctataaattattat  cat  cattttatatgttaatattcattgatcctat acattatcaa
caatttctacacaattctataaattattat  cat  cattttatatgttaatattcattgatcctat acattatcaa
caa ttctacacaattctataaattattattatcat cgttttatatgttaatattcattgatcctattacattatcaa


TCCTTGCACTTCAGCTTCCTCTAACTTCGATGACAGCTTCTCATAACTTATGTCATCATCTTAACACCGTATATGATAAT
tcc tgcgtt cagcttcctctaacatcgatgacagcttctcatagcttatgccatcatcttaacaccgtatatgataat
tccttgcgtttcagcttcctctaacatcgatgacagcttctcataacttatgtcatcatcttaacaccgtatatgataat
tccttgcgtttcagcttccactaatttagatgactatttctcatcatttgcgtcatcttc taacaccgtatatgataat
tccttgcgtttcagcttccactaatttagatgactatttctcatcatttgcgtcatcttc taacaccgtatatgataat
tccttgcgtttcagcttccactaatttagatgactatttctcatcatttgcgtcatcttc taacaccgtatatgataat


                                                  -------- CYR1 ------------
ATATTGATAATATAACTATTAGTTGATAGACGATAGTGGATTTTTATTCCAACACTTACTTTCTCCTCTATCAAACCTAA
atattgataatataactattagttgatagacgatagtgaatttttattccaacaatgaccaacctttgaaattgggtaat
atattggtaatataactattagttgatagacgatagtggatttttattccaaca
atactagtaacgtaaatactagttagtagatgatagttgatttttattccaaca
atactagtaacgtaaatactagtaagtagatgatagttgatttttattccaaca
atactagtaacgtaaatactagttagtagatgatagttgattt
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Fig.2. Nucleotide sequence of the CYR1 promoter region in strain AB320. Comparison with other sequences (from Genbank) reveals an extensive homology with Ty1 sequences present in various yeast genes: this demonstrates that a Ty1 element is inserted upstream from the CYR1 gene in strain AB320. The sequence in capitals is the sequence determined in this work, comparisons are made with five Ty1 sequences, going from the upper to the lower sequence: YSCTY1DC [14]; YSCTY1 [15]; YSCTY9173 [15]; YSCTY9175 [15] and YSCTYADA1 [12].

genomic DNA extracted from original strain AB320 and strain RH218 was carried out according to Southern [10], using the *Eco*RI fragment of 0.8 kb (present in the upstream region of the CYR1 ORF) as a probe [6]. Chromosomal DNA preparations were respectively digested with *Bgl*II, *Pvu*II, *Pst*I, *Hin*dIII and *Xho*I. As can be seen in fig.1, the probe hybridized differently on DNA preparations from strain AB320 and RH218, when digested by *Bgl*II and *Xho*I. Moreover, the *Xho*I digestion on AB320 DNA gave rise to a fragment of 3.1 kb having the same length as the corresponding one present on the isolated plasmid. Other strains such as Gx3067.64 present the same pattern of hybridization as RH218 (not shown).

These results indicate that the upstream region of the adenylate cyclase gene of the original strain AB320 is modified, when compared to other laboratory strains. In order to characterize this modification, the nucleic acid sequence of the region situated upstream from the CYR1 gene in strain AB320 was established (fig.2). As can be seen from the figure this sequence displays remarkable homologies with the 3′-distal end of a Ty1 element, including the well-conserved delta inverted repeat [11]. The insertion region is located 319 bp upstream from the putative translation start of adenylate cyclase.

Several mutants in *S. cerevisiae* have been characterized that are altered in gene expression due to the insertion of a 5.6 kb transposable element, Ty1, upstream from the structural gene. A variety of phenotypes have been associated with this type of insertion. For instance, transposition of Ty1 to a location upstream from the HIS4 gene can produce a His^- phenotype, but insertion of Ty1 into the 5′-flanking region of the gene encoding iso-2-cytochrome *c* results in a 20-fold increase in expression of this gene. Williamson et al. [12] have presented evidence showing that constitutive expression of the glucose-repressible isozyme of alcohol dehydrogenase (ADHII) can be caused by insertion of a Ty1 element into the 5′-flanking region of the gene. In most cases where the Ty1 element has been involved in alteration of gene expression it has been shown to be related to the transcription orientation of the Ty1 major internal transcript, permitting read-through of the distal delta repetition. In the case of the CYR1 gene we find that the orientation of the Ty1 element must be opposite to that of the cyclase transcript thus indicating that normal CYR1 transcription should proceed downstream from the insertion point. This would be consistent with Northern hybridization experiments which have allowed the location of the promoter region in this very region [2,3]. Thus, it appears that the transcription start site is located between the Ty1 insertion point and the putative AUG start of adenylate cyclase. This 319 bp region has very noteworthy features: 40 bp downstream from the insertion point we find a sequence extremely rich in pyrimidines:

$$CCTTATTTTTTCACTTCCTTTTTCTTACT_{21}$$

or $_{22}$GTATTTT, immediately followed by a purine-rich sequence:

GGAAGGAAAGCAGGGGGGACTTACCAT-
AAGAAAAGCGACTCTAAAAAAGTGTA
[2].

Yeast promoters are not clearly defined, but it seems that they usually exhibit the presence of a pyrimidine-rich sequence immediately downstream from the usual TATA box, transcription starting at distances that are variable from this sequence [12]. In the present case we do not find a clear TATA box, although 24 bp downstream from the Ty1 insertion point the sequence TAAATATT might play the role of the corresponding box. No other noteworthy signal seems to be present in the region, although comparison of the translation start point with other yeast translation starts suggest a similarity with genes involved in glycolysis [12].

The insertion element we have identified has the expected features for a Ty1 element, namely the delta terminal sequence is identical in almost every position with other published delta sequences. The only variation appears in regions that are found to be more susceptible to variations (see fig.2). This provides new information on the organization of transposable elements in yeast, and substantiates the observation that such transposable elements have a tendency to insert in transcription promoter regions.

REFERENCES

[1] Matsumoto, K., Uno, I., Oshima, Y. and Ishikawa, T. (1982) Proc. Natl. Acad. Sci. USA 79, 2355–2359.
[2] Kataoka, T., Broek, D. and Wigler, M. (1985) Cell 43, 493–505.
[3] Masson, P., Lenzen, G., Jacquemin, J.M. and Danchin, A. (1986) Curr. Genet. 10, 343–352.
[4] Uno, I., Mitsugawa, H., Matsumoto, K., Tanaka, K., Oshima, T. and Ishikawa, T. (1985) Proc. Natl. Acad. Sci. USA 82, 7855–7859.
[5] Nasmyth, K. and Reed, S. (1980) Proc. Natl. Acad. Sci. USA 77, 2119–2129.
[6] Masson, P., Jacquemin, J.M. and Culot, M. (1984) Ann. Microbiol. (Inst. Pasteur) 135A, no. 3, 343–351.
[7] Leplatois, P. and Danchin, A. (1983) Biochimie 65, 317–324.
[8] Jacq, C., Banroques, J., Becamp, A.M., Slonimski, P.P., Guiso, N. and Danchin, A. (1984) EMBO J. 3, 1567–1572.
[9] Maniatis, T., Fritsch, E. and Sambrook, J. (1982) in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[10] Southern, E. (1975) J. Mol. Biol. 98, 503–517.
[11] Eigel, A., Olah, J. and Feldmann, H. (1981) Nucleic Acids Res. 9, 2961–2970.
[12] Williamson, V.P., Cox, D., Young, E.T., Russel, D.W. and Smith, M. (1983) Mol. Cell. Biol. 3, 20–31.
[13] Beachman, I., Schweitzer, B., Warrick, H. and Carbon, J. (1984) Gene 29, 271–279.
[14] Eigel, A. and Feldmann, H. (1982) EMBO J. 1, 1245–1250.
[15] Roeder, G.S., Farabough, P.J., Chaleff, D.T. and Fink, G.R. (1980) Science 209, 1375–1380.