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The adenylyl cyclase-encoding gene from *Saccharomyces kluyveri*

(Recombinant DNA; yeast; evolution; amino acid sequence comparison)

Dallan Young, Kathy O'Neill, Daniel Broek and Michael Wigler

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 (U.S.A.)

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SUMMARY

The gene encoding adenylyl cyclase (CYR) from *Saccharomyces kluyveri* has been cloned. Comparison of the predicted amino acid sequence of this protein with the *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* CYRs revealed homology between different structural and putative functional domains that suggest a high degree of conservation in the function and regulation of these proteins.

INTRODUCTION

Cyclic AMP (cAMP) plays an important role as a second messenger in signal transduction pathways in many, if not all, eukaryotic organisms. Yet the regulation and effector pathways of cAMP appear to have diverged between species. To explore the evolution of the structure and regulation of CYR we have cloned and compared the genes encoding CYR from three different yeasts. The sequences of the *CYR1* genes from the budding yeast *S. cerevisiae* (Kataoka et al., 1985) and the distantly related fission yeast *Sc. pombe* (Young et al., 1989; Yamawaki-Kataoka et al., 1989) have been previously reported. The regulation of these two proteins appears to be different: the *S. cerevisiae* CYR activity is stimulated by RAS proteins, while the *Sc. pombe* CYR activity is not (Young et al., 1989; Yamawaki-Kataoka et al., 1989; Toda et al., 1985). The aims of this study were to clone the *CYR1* gene from the budding yeast *S. kluyveri* and compare the aa sequence of the encoded protein with the two other identified yeast CYRs.

Correspondence to: Dr. M. Wigler, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724 (U.S.A.)
Tel. (516)367-8377; Fax (516)367-8381.

Abbreviations: aa, amino acid(s); cAMP, cyclic AMP; CYR, adenylyl cyclase; *CYR*, gene encoding CYR; *S.*, *Saccharomyces*; *Sc.*, *Schizosaccharomyces*.

EXPERIMENTAL AND DISCUSSION

(a) Cloning the *Saccharomyces kluyveri CYR1* gene

The *S. kluyveri CYR1* gene was first detected by Southern-blot hybridization to a DNA probe derived from the *S. cerevisiae CYR1* gene. To clone the *S. kluyveri CYR1* gene, libraries were constructed from size-fractionated *S. kluyveri* genomic DNA in the vector pUC119. Plasmids containing the *CYR1* gene were detected in these libraries by colony-filter DNA hybridization to the *S. cerevisiae* DNA probe. We have determined the nt sequence of the coding and flanking regions of the *S. kluyveri CYR1* gene and have submitted this sequence to the EMBL data base (accession No. X56042). This gene encodes a 1839-aa protein (Fig. 1).

(b) Comparison of yeast CYRs

We have compared *Sc. pombe*, *S. kluyveri* and *S. cerevisiae* CYRs (Figs. 1 and 2). Previous genetic and bio-

Fig. 1. Comparison of the *Sc. pombe* and *S. cerevisiae* adenylyl cyclase protein sequences by the FASTDB program. The deduced aa sequences of the *Sc. pombe* (S.p.) and *S. kluyveri* (S.k.) CYRs were compared and aligned with the *S. cerevisiae* (S.c.) CYR sequence by the Fast Pairwise Comparison of Sequences program (FASTDB, Intelligenetics, Inc., Mountain View, CA, 1990) using a PAM-250 similarity matrix (Brutlag et al., 1990). The parameters used were: K-tuple = 1; threshold level of similarity = 40%; mismatch penalty = 1; gap penalty = 0.65; gap size penalty = 0.35; joining penalty = 1; window size = 500. Dashes represent gaps that were inserted during the alignment process. Numbers on the left indicate the aa positions. Vertical lines are between identical aa and colons are between conserved aa.

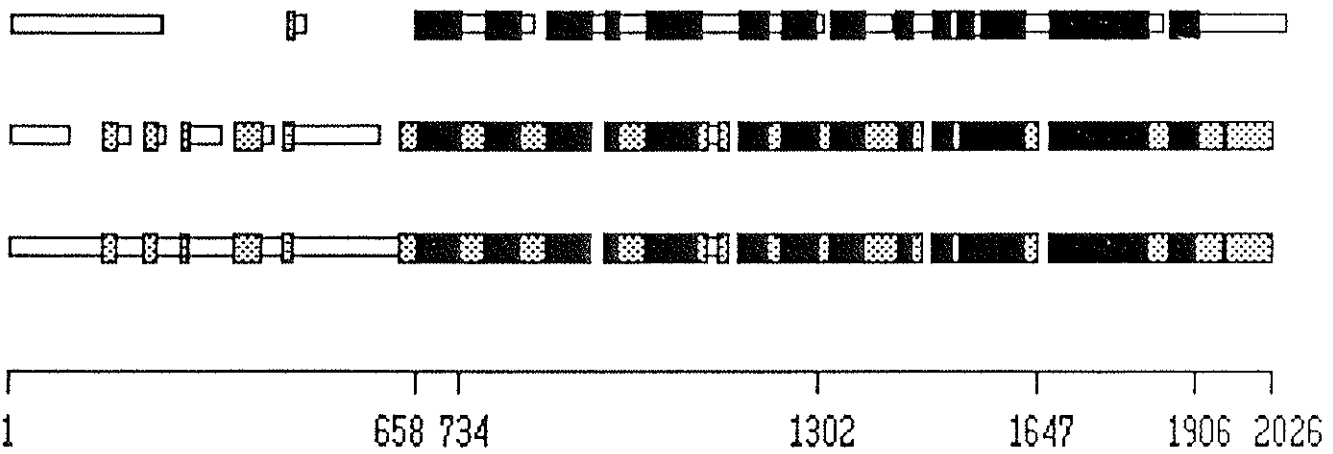

Fig. 2. Schematic comparison of the *Sc. pombe*, *S. kluyveri* and *S. cerevisiae* adenylyl cyclases by the MACAW program. Each of the three yeast CYRs, *Sc. pombe* (top), *S. kluyveri* (middle) and *S. cerevisiae* (bottom), are represented by a set of noncontiguous rectangular segments. Breaks between segments were made to align homologous regions. Each segment is aligned with segments, above or below, that represent homologous sequences in the other two proteins. Shaded regions represent sequences that are homologous with either one (stippled) or both (black) of the other two proteins. The numbers at the bottom indicate the position of aa in the *S. cerevisiae* protein that define the boundaries of different functional and structural domains of this protein (see section b). The alignment of these proteins was performed using the Multiple Alignment Construction and Analysis Workbench (MACAW) program (Schuler et al., 1991).

chemical analyses have defined different functional and structural domains of the 2026-aa *S. cerevisiae* protein. A sequence comprising 259-aa (residues 1647–1905), is sufficient for the catalytic conversion of ATP to cAMP (Kataoka et al., 1985; Suzuki et al., 1990). A 345-aa sequence (1302–1646) separates this catalytic domain from a 568-aa region (734–1301) consisting of tandem repeats that are rich in Leu residues. In *S. cerevisiae*, CYR activity is stimulated by RAS proteins (Toda et al., 1985). Deletion analysis has revealed that the N-terminal domain (aa 1–657) and a short sequence in the C-terminal region (aa 1933–1959) are not required for this stimulation, while deletions elsewhere destroy RAS responsiveness (Colicelli et al., 1990; Suzuki et al., 1990). The function of the N-terminal domain remains unknown.

Significant homology between the three yeast CYRs is found only between the C-terminal two thirds of the proteins, with the strongest homology between the catalytic domains. The N-terminal domains are variable in length an have almost completely diverged during evolution. Significant homology begins very near the N terminus of the minimal protein that is RAS responsive in *S. cerevisiae* (Colicelli et al., 1990). The *S. kluyveri* protein is more closely related to the *S. cerevisiae* protein (68% identity) than is the *Sc. pombe* protein (25% identity) in the conserved region. Both the *S. kluyveri* and *Sc. pombe* proteins, like the *S. cerevisiae* CYR, have Leu-rich repeat structures in the conserved region, and share an identical consensus repeat sequence. The conservation of the region required for RAS responsiveness suggests that the mechanisms for regulating CYRs are conserved in the related budding yeasts and may be conserved, to a lesser degree, in the distantly related fission yeast. In contrast, the striking divergence of the N-terminal domains suggest that the role of these domains is not conserved.

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REFERENCES

Brutlag, D.L., Dautricourt, J.-P., Maulik, S. and Relph, J.: Sensitive similarity searches of biological sequence databases. CABIOS 6 (1990) 237–245.

Colicelli, J., Field, J., Ballester, R., Chester, N., Young, D. and Wigler, M.: Mutational mapping of RAS-responsive domains of the *Saccharomyces cerevisiae* adenylyl cyclase. Mol. Cell. Biol. 10 (1990) 2539–2543.

Kataoka, T., Broek, D. and Wigler, M.: DNA sequence and characterization of the *S. cerevisiae* gene encoding adenylyl cyclase. Cell 43 (1985) 493–505.

Schuler, G.D., Altschul, S.F. and Lipman, D.J.: A workbench for multiple alignment construction and analysis. Proteins: Struc. Funct. Genet. 9 (1991) 180–190.

Suzuki, N., Choe, H.-R., Nishida, Y., Yamawaki-Kataoka, Y., Ohnishi, S., Tamaoki, T. and Kataoka, T.: Leucine-rich repeats and carboxyl terminus are required for interaction of yeast adenylate cyclase with RAS proteins. Proc. Natl. Acad. Sci. USA 87 (1990) 8711–8715.

Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K. and Wigler, M.: In yeast,

RAS proteins are controlling elements of the adenylyl cyclase. Cell 40 (1985) 27–36.

Yamawaki-Kataoka, Y., Tamaoki, T., Choe, H.-R., Tanaka, H. and Kataoka, T.: Adenylate cyclases in yeast: a comparison of the genes from *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 86 (1989) 5693–5697.

Young, D., Riggs, M., Field, J., Vojtek, A., Broek, D. and Wigler, M.: The adenylyl cyclase gene from *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. USA 86 (1989) 7989–7993.