Arginine repression of the *Saccharomyces cerevisiae ARG1* gene

Comparison of the *ARG1* and *ARG3* control regions

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Summary. The *Saccharomyces cerevisiae ARG1* gene coding for argininosuccinate synthetase has been isolated and the nucleotide sequence of both its control region and of its amino terminal end coding region determined. The startpoint of transcription was established by S1-mapping and reverse transcriptase procedures. Northern blot hybridizations showed that whereas arginine-specific repression reduced the enzyme activity fivefold, it did not reduce the steady state level of the corresponding messenger in proportion; by analogy with the coregulated *ARG3* gene, this result suggests a post-transcriptional regulatory mechanism. In contrast, proportionally between enzyme activity and mRNA content was observed under conditions where general amino acid control (known to be transcriptional) was operating.

Comparing the 5' untranscribed domains of *ARG1* and *ARG3* revealed a first region of homology between the TATA box and the transcription startpoint. In this region a 10 bp (*ARG3*) or 11 bp (*ARG1*) central box is flanked by two segments which, by mutation, have been shown to be part of the ARG operator (Crabeel et al. 1985). The repressor is assumed to bind at this primary target site prior to establishing contacts with the proximal part of the nascent mRNA molecule (Crabeel et al. 1985).

By in vitro directed deletion mutagenesis we show that the central conserved box of *ARG3* is not essential for arginine-specific repression to occur. Another region of homology was found in the leader part of the messenger RNA; deletion of this region causes a small reduction in *ARG3* expression but also does not alter regulation. Neither of these two regions are thus part of the primary repressor target site. In addition, in terms of post-transcriptional regulation, the latter result indicates that no sequence specificity is required in the RNA recognition step.

Key words: Argininosuccinate synthetase — Arginine specific regulation — General amino acid control

Introduction

Assignment of a functional role to specific sequences in the promotion or regulation of DNA transcription results from comparisons between wild-type strains and mutant derivatives, or between coregulated genes of the same pathway. We have followed both these approaches to gain further insight in the molecular basis for the modulation of gene expression in the arginine pathway of the yeast *Saccharomyces cerevisiae*.

Two independent regulatory mechanisms influence the expression of yeast *ARG* genes: the cross-pathway or general amino acid control mechanism common to several amino acid biosynthetic pathways (Wolfner et al. 1975; Davis 1986) and the specific arginine-mediated repression of some of the *ARG* genes (Bechet et al. 1970; Davis 1986), which is the main subject of this paper. Three regulatory *argR* genes have been isolated and characterized (Messenguy et al. 1986; Dubois et al. 1987). Among the genes responding to arginine repression, *ARG3* (encoding ornithine carbamoyltransferase, OTCase, EC 2.1.3.3) is presently the only one for which both a complete structural investigation (Huygen et al. 1987) and an extensive analysis of its regulatory mode (see below) have been performed. These studies brought an unusual situation to light: whereas the localization of operator-constitutive mutations clearly defined the existence of a repressor-binding operator region in a non-transcribed portion of the DNA between the TATA box
and the transcription startpoint (Crabeel et al. 1985), previous Northern hybridization measurements had revealed a total lack of proportionality between messenger RNA levels and OTCase specific activities (Messenguy and Dubois 1983), a finding which had led the latter authors to propose a post-transcriptional mechanism for arginine-specific repression.

To explain this paradox we envisaged the following regulatory “cascade” (Crabeel et al. 1985): a component of the repressor system (presumably the ARGRII protein, see Messenguy and Dubois 1983) would first make specific contacts with the operator DNA immediately upstream from the transcriptional startpoint and then modify the nascent mRNA in such a way that either its integrity, its translational efficiency or its accessibility to the translation apparatus would be affected. Central in this “cascade model” is the question of the recognition mechanism between the mRNA and the repressor; it could be sequence-specific or it could not, the specificity being provided at the primary DNA recognition step. A sequence-specific recognition mechanism would necessarily implicate the leader (untranslated) portion of the mRNA as previous experiments had shown that most (Crabeel et al. 1983) or even all (Crabeel and Tinel, unpublished) of the ARG3 structural gene could be substituted for by the E. coli lacZ or galK genes without affecting the regulatory response to arginine.

In order to better define the regulatory target sites involved in arginine repression we have investigated further the regulatory role of sequences located in transcribed or untranscribed regions of ARG3. We have isolated the coregulated ARG1 gene encoding argininosuccinate synthetase (ASAsase, EC 6.3.4.5), have used the gene as a probe to quantify the cognate messenger content in various states of regulation, and compared the respective nucleotide sequences of the proximal regions of ARG1 and ARG3. This comparison brought to light the existence of (i) short homologies in their mRNA leader sequences and (ii) extensive analogies between the TATA box and the transcription startpoints not only in the two regions defined by 0° mutations in ARG3 but also in between, under the form of a central box shared by the two sequences. To test any potential role for this central box and for the leader sequences we have deleted them by oligonucleotide-directed mutagenesis and reintroduced the mutated genes into the yeast genome.

Materials and methods

Strains

Yeast strains. Σ 1278b is the reference wild-type strain to which all mutants used are isogenic. Strains 22315c (ura3–, arg1–) and MG471 (ura3–) were gifts from M. Grenson; strain BJ210 (argR1I–10) was isolated by Béchet et al. (1970); strain IC1064c (ura3–, arg3–) was received from F. Messenguy. Strain SS1 (ura3–, deletion (\textsuperscript{\textcircled{8}}) arg3–) was constructed for the purpose of this article (see below). Strain SS2 is strain SS1 in which the arg3 deletion has been substituted for by ARG3\textsuperscript{+}; it is thus equivalent to MG471. Strains SS3 and SS4 are defined below.

E. coli strains. JM103 (\textsuperscript{\textcircled{8}} prolac, thi–, rpsL, supE, endA, sbcB15, hsdR4, F\textsuperscript{\textcircled{8}} traD36, proAB, lacF, ZM15) was used for routine work with M13. Strains BMH71-18 mutS and MK30-3 were used in the gapped duplex procedure (see Kramer et al. 1984). KL16 argR– was used to test complementation with the yeast ARG1 gene.

Media

The basic minimal medium (M. medium) containing 3% glucose, vitamins and mineral traces has been described previously (Ramos and Wiame 1979). M. am medium is M. medium supplemented with 0.02 M (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} as the nitrogen nutrient. L-arginine, when added, was brought to a final concentration of 1 mg/ml. Rich medium 863 contains Difco yeast extract (10 g/l), Difco Bactopeptone (10 g/l) and glucose (20 g/l).

Enzyme assays

OTCase activity was measured as described previously (Messenguy et al. 1971). ASAsase activity (or L-citrulline:L-aspartate ligase: EC 6.3.4.5) was assayed by coupling it to the activity of argininosuccinate (ASA) lyase (C. Hennault, personal communication); by this procedure the ASA produced by ASAsase is transformed into arginine, which is then assayed by the Mickus and Stein colorimetry (1973). ASAlase synthesis is not regulated by arginine (see Messenguy and Dubois 1983). The activity of this enzyme was not limiting in the assay; indeed adding aliquots of an extract of an ASAsase– ASAlase\textsuperscript{+} strain grown in minimal medium to an extract of a strain harboring an ARG1\textsuperscript{+} multicopy plasmid (also grown in minimal medium) did not increase the amount of arginine recovered per unit of time. ASAsase activity was measured in a final reaction mixture of 1 ml in 0.1 M phosphate buffer pH 7.5, containing 10 mM each of L-citrulline, neutralized aspartic acid, neutralized ATP and MgCl\textsubscript{2}. Incubation time was 1 h at 30 °C. The reaction was started with the addition of various amounts of desalted extract (Sephadex G25 column) and stopped by addition of 1 ml trichloroacetic acid (6%); the protein precipitate was centrifuged. Supernatants were used for arginine colorimetry (see above). One blank without aspartate was run for each volume of extract. Archibald colorimetry (1945) indicated that no ureum was formed in the absence of citrulline or in the presence of 1 mM arginine, thus, no arginase activity was interfering with the assay. Proteins were measured by the Folin methods (Lowry et al. 1951).

Yeast and E. coli transformation procedures

E. coli was transformed as in Petes et al. (1978). Yeast was transformed as in Hinnen et al. (1978) with some modifications: cells were incubated 15 min with \(\beta\text{-mercaptoethanol} (1 \mu l/ml\) original culture volume) at room temperature after being concentrated to 1/10th of the culture volume in 1 M Sorbitol. After centrifugation and resuspension in fresh 1 M Sorbitol, they were treated with 1% gluconic acid/aryl sulfatase (Boehringer) to obtain 50% spheroplasts (usually after 20 min). Regeneration of the spher-