The Neurospora crassa arg-2 Locus

STRUCTURE AND EXPRESSION OF THE GENE ENCODING THE SMALL SUBUNIT OF ARGinine-SPECIFIC CARBAMoYL PHOSPHATE SYNTHETASE*

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We have characterized genomic and cDNA clones for arg-2, the gene encoding the small subunit of the Neurospora crassa arginine-specific carbamoyl phosphate synthetase (CPS-A), and examined its transcriptional regulation. The polypeptide's predicted amino acid sequence (453 residues) is 56% and 36% identical with the sequences of the homologous polypeptides of Saccharomyces cerevisiae and Escherichia coli, respectively. The ARG2 polypeptide has an additional unimodular domain with the hallmark features of a mitochondrial signal sequence. The arg-2 mRNA also encodes a 24-residue peptide in the segment upstream of the coding region for the ARG2 polypeptide. This upstream open reading frame (uORF) strongly resembles the uORF in the homologous S. cerevisiae transcript. Northern analyses indicate that arg-2 mRNA levels are reduced by arginine supplementation and increased by amino acid limitation. The large increase in arg-2 mRNA levels that occurs in response to amino acid limitation is not observed in a strain containing the cpc-1 mutation, indicating that the cross-pathway control system participates in arg-2 regulation. Four copies of the sequence TGACTC, the binding site for GCN4, the DNA binding protein that mediates the S. cerevisiae general control response (Hinnebusch, 1988a). In addition, as in Neurospora, synthesis of CPA1, the gene encoding the small subunit of CPS-A, is specifically regulated by arginine (Hinnebusch, 1988b). With regard to arginine-specific regulation of CPA1 synthesis, both cis and trans regulatory mutations have been obtained (Hinnebusch, 1988b, Werner et al., 1987). Analysis of the cis-acting mutations that abolish arginine-specific regulation demonstrated that CPA1 expression is regulated translationally by a peptide encoded in the upstream region of CPA1 mRNA (Werner et al., 1987).

In this report, we present the sequences of cloned genomic and cDNA copies of the N. crassa gene arg-2, which encodes the small subunit of CPS-A. We also describe our initial analyses of arg-2 regulation.

MATERIALS AND METHODS

Neurospora Strains—Wild-type strain 74-OR23-1A (74A) was obtained from D. Perkins, Stanford University. Strain 45a [arg-2(2CD80) pyr-3(DFC3) caa-2(M246) aro-2(M6-11) in1(89601) A] was constructed by W. Schneider (Orbach et al., 1988). The cpc-1CD19A strain was obtained from the Fungal Genetics Stock Center, Dept. of Microbiology, University of Kansas Medical Center, Kansas City, KS 66103.

Plasmids—pAR201 contains a 4.9-kb BamHI fragment of N. crassa DNA that encodes arg-2 (Fig. 1) inserted into the BamHI site of pUC18 (Norrander et al., 1985). pAR23 contains the 4.0-kb SacI fragment of pAR201 inserted in pUC18 (Vieira and Messing, 1981). pAR24 was constructed in two steps to generate a plasmid that has unique restriction sites with 5' and 3' protrusions for digestion by, and protection from, EcoRI, respectively. First, the 3.7-kb PstI fragment of pAR201 that contained arg-2 was inserted into pUC18. Then, from an appropriately oriented clone, a 3.0-kb BamHI-ScaI fragment (containing the PstI-ScaI arg-2 Insert) was isolated and inserted into pUC18. Clones for sequencing were generated from plasmids pAR23 and pAR24 using the progressive deletion method described by Henikoff (1984). The plasmid pAN2 contains the BamHI-PstI fragment of pAR201 inserted into BamHI-HindIII-digested Bluescript plasmid pKS (Short et al., 1988).
Plasmid pAU1, which contains an intronless version of arg-2, was constructed in several steps. The 3.1-kb SacI-PstI fragment derived from pAR201 was inserted into SacI-PstI-digested pDSP72 to construct pAU1. pDSP72, a modified version of vector pSP72 (Promega Biotec), was constructed by removal of the small XhoI-HindIII fragment to create a vector which contains no PvuII sites. To form pAU1, PvuII was digested at unique PvuII and Ncol sites to release an 1867-nt fragment which contained all three introns; this fragment was replaced with the intronless 1387-nt PvuII-Ncol fragment from cDNA clone pARG228. pARG228 was obtained by probing a λ ZAP cDNA library representing the mRNA of germinating conidia with 32P-labeled arg-2 DNA purified from pAR241.

The plasmids used to obtain probes for hybridization analyses were: pAR241 (arg-2), pH73 (sub-2; Orbach et al., 1986), and pSRCC05 (cox-5; Sachs et al., 1988).

Construction of cDNA Libraries—N. crassa poly(A) mRNA was obtained from germinating conidia, 6-h mycelia, and histidine-starved mycelial cultures (described below). RNA (5 μg) in water was heated to 65 °C for 3 min and chilled on ice. The reaction mixture for first strand cDNA synthesis was incubated for 2 h at 41 °C and contained the following components in a volume of 100 μl: 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 6 mM MgCl2, 1 mM concentration each of dATP, dCTP, dGTP, and dTTP, 100 μCi of [α-32P]dCTP (0.4 mCi dithiothreitol, 5 μg of oligo(dT)12-18, 80 units of RNasin (Promega Biotec), and 40 units of avian myeloblastosis virus reverse transcriptase (Seikagaku). The reaction was stopped by adjusting the EDTA concentration to 12 mM. Nucleic acids were precipitated by adding 20 μg of glyoxal, 100 μl of 4 M ammonium acetate, and 400 μl of ethanol, followed by freezing on dry ice. After collection by centrifugation, the nucleic acids were washed with 80% ethanol, dissolved in 10 μl of water, and chromatographed on a 1-mL Bio-Gel A-5m column. Peak fractions were pooled and precipitated with ethanol after the addition of 20 μg of glyoxal and dissolved in 10 μl of water.

The reaction mixture for second strand cDNA synthesis contained the following components in a volume of 100 μl: 1–3 μg of first strand cDNA, 100 mM HEPES-KOH (pH 6.9), 70 mM KCl, 6 mM MgCl2, 15 mM d-mercaptoethanol, 0.1 mM concentration each of dATP, dCTP, dGTP, and dTTP, 1.2 units of RNase H (Boehringer Mannheim), and 40 units of DNA polymerase I (New England Biolabs). After 2.5 h at 16 °C, the reaction was stopped by adjusting the EDTA concentration to 12 mM, followed by extraction with phenol/chloroform (1:1) and precipitation with ethanol.

The cDNA products were methylated, made flush-ended with T4 DNA polymerase, and ligated into the EcoRI site of XZap phage DNA (Short et al., 1988; phage DNA was obtained from Stratagene, San Diego, CA) as previously described (Sachs et al., 1988). Recombinant phage DNA was packaged in vitro, and amplified phage libraries were prepared as described (Short et al., 1988). The cDNA library representing mRNA from germinating conidia was estimated to contain 106 cells/ml and grown to midlog phase (0.5–1.0 mg/ml dry weight); some cultures were supplemented as described in the legend of Fig. 5.

N. crassa RNA was isolated by the method of Reinert et al. (1981) for preparing the poly(A) mRNA used in Fig. 5A or by modifications of the method of Kurtz and Lindquist (1984). The preparations of RNA used in Fig. 5B were obtained from lyophilized mycelial pads (0.1–0.05 mg dry weight). The pads were first powdered in a Waring blender. To the powder we added 20 ml of ice cold extraction buffer (100 mM Tris-Cl (pH 7.5), 100 mM LiCl, 20 mM dithiothreitol), followed by 20 μl of 50% phenol/50% CHCl3 and 1 ml of 20% sodium dodecyl sulfate. Nucleic acids were extracted from the cells by two 30-s blending pulses separated by a 3-min cooling period. The mixture was transferred to a 50-ml screwcap centrifuge tube, and the phases were mixed at 20 rpm for 15 min on a vertical rotating wheel. Phases were separated by centrifugation in a swinging bucket clinical centrifuge for 20 min at 2000 × g. The aqueous phase was transferred to a fresh 50-ml screwcap tube and was re-extracted with 20 ml of 50% phenol/50% CHCl3 using a vortex mixer. Phases were separated by centrifugation, and the aqueous phase was extracted once with 20 ml of CHCl3. RNA was precipitated from the aqueous phase by the addition of 0.1 volume of 3 M NaOAc (pH 6.0) and 2.5 volumes of ethanol and centrifuged by collection. Twenty to forty mg of RNA were recovered from 0.4 to 0.6 g (dry weight) of mycelium in typical preparations.

Poly(A) RNA used in Northern blot analyses was purified from cell cultures as described (Aviv and Leder, 1975). The isolation of RNA from the different N. crassa developmental stages used for the construction of cDNA libraries was described elsewhere.3

Analyses of mRNA Levels by RNA Blotting—RNA was electrophoresed on 1.5% agarose gels containing 0.6 M formaldehyde, 20 μM Mops (pH 7.0), 5 mM sodium acetate, and 1 mM EDTA, following denaturation by heating at 65 °C for 10 min in the same buffer containing 50% denatured formamide. RNA was transferred to GeneScreen membrane (Du Pont-New England Nuclear) by capillary action using 20 × SSPE as the buffer (1 × SSPE is 10 mM NaCl, 180 mM Na2CO3, 1 mM EDTA (pH 7.0)). The RNA blots were rinsed in 5 × SSPE, then baked for 2 h at 80 °C. They were incubated with agitation for at least 6 h at 42 °C in sealed bags containing 10–20 ml of prehybridization solution (50% formamide, 0.2% polyvinylpyrrolidone (molecular weight 40,000), 0.2% bovine serum albumin, 0.2% Ficoll (molecular weight 400,000), 0.05 M Tris-HCl (pH 7.5), 1 M NaCl, 0.1% sodium pyrophosphate, 1% sodium dodecyl sulfate, 10% dextran sulfate (molecular weight 500,000), 100 μg/ml denatured salmon sperm DNA). 32P-labeled DNA probes (106 cpm), prepared by the random priming method (Feinberg and Vogelstein, 1983), were added to the prehybridization mixtures; RNA blots were hybridized with probes for 14 to 18 h at 42 °C and then were washed twice for 5–10 min with 2 × SSPE at room temperature and twice for 30 min with 0.1 × SSPE. Blots were exposed at −80 °C using Kodak XAR film and x-ray intensifying screens.4

RESULTS

The Sequence of arg-2—We previously described the clonning of arg-2 genomic DNA by direct recovery in Escherichia coli (Orbach et al., 1988). The DNA sequence of a 3681-nt BamHI/PstI fragment containing the arg-2 coding and surrounding regions was obtained by sequencing both DNA strands (see “Materials and Methods”). The arg-2 coding region, present in two exons, specifies a polypeptide of 453 amino acid residues (Figs. 1 and 2). Synthesis of the ARG2 polypeptide is predicted to initiate at the second AUG codon in the mRNA (nt 1451–1453, Fig. 2) and proceed to a UAA stop codon (nt 2866–2868). The coding region is interrupted by one 56-nt intron between codons 60 and 61, based on RNA nucleotide mapping studies and comparison of cDNA and genomic sequences. The codons used to specify the ARG2 polypeptide are biased in a manner similar to other N. crassa genes (Orbach et al., 1986; Sachs et al., 1989).

Comparison of the N. crassa ARG2 Polypeptide with Other CPS Polypeptides—An alignment of the deduced amino acid

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of high identity, separated by less conserved segments. The mitochondrion (von Heijne, 1986; von Heijne et al., 1989).

The amino-terminal 45-residue domain of ARGZ conforms to the mitochondrial signal sequences.

Sequence of the N. crassa ARG2 polypeptide with that of the deduced yeast CPA1 polypeptide (Nyunoya and Lusty, 1984; Werner et al., 1985) and the E. coli CarA polypeptide (Piette et al., 1984) is shown in Fig. 3A. In this alignment, 56% of the residues are identical between Neurospora and yeast and 36% are identical between Neurospora and E. coli; there are regions of high identity, separated by less conserved segments. The greatest conservation of primary sequence is observed in the regions corresponding to residues 78–126, 171–191, 232–261, 271–284, 303–326, and 331–404 of ARG2. The rat CPSI protein (Nyunoya et al., 1985) also shows significant homology to ARG2 in these regions and can be aligned with 35% identical residues (data not shown). The relatedness of the ARG2 polypeptide to other glutamine amidotransferases is discussed in more detail below.

The 45 amino-terminal residues of ARG2 have no counterparts in CPA1 or CarA (Fig. 3A). Furthermore, the predicted size of the ARG2 primary translation product, 48,929 Da, is larger than the measured size of the mature ARG2 polypeptide, 45,000 Da (Davis et al., 1981). Because the N. crassa protein is located in the mitochondrial matrix and the yeast protein in the cytosol, the N. crassa polypeptide would be predicted to contain an amino-terminal signal sequence to target it to the mitochondrion (von Heijne, 1986; von Heijne et al., 1989).

Mitochondrial signal sequences are generally rich in serine, leucine, and basic residues, especially arginine, while acidic residues are rare (von Heijne, 1986; von Heijne et al., 1989). The amino-terminal 45-residue domain of ARG2 conforms to these biases. In addition, part of this sequence can be modelled (von Heijne, 1986) as an amphiphilic helix with a large hydrophobic moment (μH = 9.1 for residues 19–36). The amino-terminal domain of ARG2 thus contains the features of mitochondrial signal sequences.

arg-2 mRNA Contains an Upstream Open Reading Frame Interrupted by Introns—Comparison of the DNA sequences of cDNA and genomic clones containing arg-2 revealed that upstream of the arg-2 coding region there is a 24-codon uORF interrupted by two introns (Fig. 2). The predicted sequence of the uORF polypeptide strongly resembles the 25-residue uORF peptide encoded in the yeast CPA1 transcript (Fig. 3B). The yeast and Neurospora uORFs specify identical residues at 9 positions; at several other positions, there are conservative amino acid substitutions. The two uORF introns are separated by a 13-nucleotide exon (nt 1270–1282). These introns share the internal consensus and 3′ splice boundaries of other N. crassa introns, CTRA...YAG, but have atypical 5′ splice sequences, GTACGA and GTCCGC, instead of GTRNGT (Bowman et al., 1988). The third intron, located in the ARG2 coding region, possesses all three typical N. crassa consensus features.

Mapping of the Ends of the arg-2 Transcript—To map the 5′-ends of the arg-2 transcript, we used a 5′-labeled DNA fragment containing a spaced copy of nt 634-778 of the DNA sequence shown in Fig. 2 for S1 nuclelease protection experiments. This DNA fragment was annealed to N. crassa total RNA, the products were digested with S1 nucleases, and the protected fragments were analyzed on denaturing gels (Fig. 4). Lanes 3 and 4 show that the 5′-ends of arg-2 transcripts are heterogeneous. Two major classes have ends distributed near nt 752 and 809, respectively. The results of primer extension mapping of the arg-2 mRNA 5′-ends using a synthetic primer complementary to nt 1359-1375 and poly(A) mRNA were consistent with the S1 mapping results (data not shown). In addition, there were no detectable intron-containing RNA species in the preparations examined. S1 analyses using DNA probes that contained intron sequences accurately placed the 3′-boundaries of the second and third introns (data not shown).

The 3′-end of the arg-2 transcript was mapped using a uniformly labeled 540-nucleotide strand probe extending from the StyI site at nt 2706 to the end point of a pAR241 deletion clone at approximately nt 3243 (Fig. 2). S1 protection indicated that the arg-2 transcript ends in a 10–20-nucleotide region near nt 3103 (data not shown). A more precise determination of the arg-2 polyadenylation site was obtained from the sequence of a cDNA clone that contained a poly(A) tract after position 3082 (indicated in Fig. 2 by an arrowhead). The size of arg-2 mRNA determined by RNA blot hybridization agrees with the size determined by 5′- and 3′-end mapping.

Analysis of Expression of the arg-2 Gene—Synthesis of the small subunit of CPS-A, like many polypeptides subject to cross-pathway control in N. crassa (Flint and Kemp, 1981), is induced by amino acid limitation (Cybis and Davis, 1975). This induction does not occur when a strain bearing a cpc-1 mutation such as cpc-1(CD15) is subjected to amino acid limitation, indicating that arg-2 is regulated by cross-pathway control (Davis, 1986). To determine whether cross-pathway-mediated regulation of arg-2 is transcriptional, the level of arg-2 mRNA was measured in poly(A) RNA isolated from wild type mycelia grown under four conditions (Fig. 5A, arg-2 panel). The levels of arg-2 transcripts increased significantly when histidine limitation was imposed by growth in the presence of the inhibitor of histidine biosynthesis 3-amino-1,2,4-triazole (+AT). High levels of transcript were also observed in cells grown in the presence of excess tryptophan (+TRP), i.e. under conditions of amino acid imbalance. arg-2 transcript levels were lower in cultures grown in the presence of excess arginine (+ARG) relative to growth in minimal medium (MIN). Reprobing the filters with labeled Neurospora β-tubulin DNA showed that similar β-tubulin mRNA levels were present under all growth conditions used (Fig. 5A, tub-2 panel). The transcript levels for two other genes subject to cross-pathway control, trp-1 and his-3, also increased in histidine-starved cultures and in the presence of excess tryptophan (data not shown).

Total RNA was isolated from wild type (74A) and cpc-1 (CD15)A strains at several intervals following addition of AT.
Fig. 2. Sequence of the arg-2 gene and its flanking regions. The sequence of the 3681-nt BamHI-PstI fragment containing the arg-2 gene is presented with numbering starting at the first base of the BamHI site. The approximate positions of the major transcript 5'-ends are indicated by arrowheads above the sequence at nt 752 and 809; a polyadenylation site is indicated by an arrowhead at nt 3082. The deduced amino acid sequences of the uORF and the ARG2 polypeptides are indicated above the DNA sequence using one-letter amino acid abbreviations. Intron sequences are italicized. Consensus features within introns are indicated by thin underlines; a sequence repeat which is adjacent to introns 1 and 2 is indicated by double underlines. Three repeats containing the sequence CAAT are indicated by heavy underlines. The sequence TGACTC or its complement is boxed.

to cultures growing in minimal medium. RNA blot analyses (Fig. 5B, arg-2 panel) indicate that in strain 74A, arg-2 mRNA levels increase within 30 min after AT addition and remain elevated. After 60 min of growth in the presence of AT, the levels of arg-2 mRNA do not increase in the cpc-I(CD15) strain to the same extent as in the 74A strain. 74A urg-2 mRNA levels are reduced by growth in minimal medium supplemented with 1 mM arginine, and they are further reduced by growth in a rich medium (Fig. 5B). RNA samples from these cultures were also probed with ~0x5 DNA (Fig. 5B, ~0x5 panel), which encodes IV. crassa cytochrome oxidase subunit V. ~0x5 mRNA levels were similar under all growth conditions, except for a slight decrease after prolonged growth with AT.

The Minimal arg-2 Functional Unit as Defined by DNA-mediated Transformation—In N. crassa, transformation occurs predominantly by nonhomologous integration of introduced DNA (Case, 1986; Dhawale and Marzluff, 1985; Legerton and Yanofsky, 1985; Vollmer and Yanofsky, 1986). Levels of transformation to prototrophy with a truncated gene are typically 1-10% of the levels obtained with the complete gene (Case, 1986; Legerton and Yanofsky, 1985), presumably because transformation to prototrophy mainly occurs by homologous integration or gene conversion. Using
transformation frequency as an assay, we examined restriction endonuclease-digested DNA to determine the approximate end points of the arg-2 functional unit. The arg-2 strain 43a was transformed (Orbach et al., 1986) with both supercoiled and restriction endonuclease-digested plasmid pAR201, which contains the wild type arg-2 gene on a 4.9-kilobase BamHI fragment (Fig. 1). Plasmid DNA digested with BamHI, SacI, PvuII, and PstI (Fig. 1) transformed 43a to arginine prototrophy at approximately the same frequency as undigested pAR201 (greater than 10^7 transformants/μg of DNA), whereas DNA digested with NcoI, HindIII, and KpnI gave prototrophic transformants at greatly reduced frequencies (less than 5 transformants/μg of DNA). These results indicate that the arg-2 functional unit is contained within the 2.8-kb PvuII/PstI fragment of pAR201.

In an attempt to determine the extent of the upstream region required for expression of arg-2, relative transformation efficiencies were determined for a series of plasmids with deletions of upstream sequences. High transformation frequencies were observed for 5'-deletions with end points up to position 1310, while low transformation frequencies were observed for 5'-deletions with end points at position 1332 and beyond (Fig. 1). Thus, sequences important for arg-2 expression, but not necessarily its regulation, appear to be located between nt 1311 and 1332. Interestingly, this region lies within the second intron of the uORF. The explanation for high frequency transformation to prototrophy by a presumed promoter-less fragment is not known.

**DISCUSSION**

The Structure of the ARG2 Polypeptide—The arg-2 gene of *N. crassa* encodes the small subunit of arginine-specific carbamoyl phosphate synthetase. The ARG2 polypeptide sequence, deduced from the DNA sequence shown in Fig. 2, is homologous to the sequences of the corresponding yeast and *E. coli* polypeptides (Fig. 3). These proteins, all of which function as glutamine amidotransferase subunits, contain domains which are homologous to other glutamine amidotransferases. Three of the conserved regions have been shown to be essential for the function of other glutamine amidotransferases (Amuro et al., 1985; Kawamura et al., 1978; Paluh et al., 1985; Rubino et al., 1986; Tso et al., 1980; Weng and Zalkin, 1987). These domains, which are located in the carboxyl-terminal half of the CPS subunits, are indicated in Fig. 3A, and are aligned to some other glutamine amidotransferase polypeptides in Fig. 6.

The amino-terminal halves of the CPS small subunits also show significant sequence conservation (Fig. 3A). The role(s) of these segments are unknown; one possibility is that they are responsible for association of the small and large CPS subunits. Mutations in this portion of the *E. coli* CarA polypeptide have been shown to destabilize complex formation.
FIG. 5. Northern analysis of arg-2 transcription. A. poly(A) RNA was isolated from midlog phase wild type strain 74A grown in minimal medium (M/N), in minimal medium supplemented with 1 mM arginine (+ARG), in minimal medium with histidine limitation imposed by the addition of 5 mM 3-amino-1,2,4-triazole (+AT) for 3 h, or from cultures that were transferred to fresh minimal medium containing 1 mM tryptophan for 40 min (+TRP) prior to harvesting. 

Fifteen µg of poly(A) mRNA from each sample was separated on a 1.2% agarose, 5% formaldehyde gel, transferred to nylon membranes, and probed with 32P-labeled DNA representing the N. crassa arg-2 or tub-2 genes, as indicated above the panels. B. Northern analysis of arg-2 transcription in 74A and cpc-1 (CD15). Total RNA was isolated from midlog phase cultures of strain 74A grown in minimal medium (M/N), in minimal medium containing either 1% (w/v) NZamine (RICH), or 1 mM arginine (ARG). RNA was also isolated from strain 74A grown with histidine limitation imposed by addition of 5 mM AT (+AT) for varying lengths of time, as indicated (in minutes) below the +AT arrow. Total RNA was isolated from the cpc-1 (CD15) strain grown in minimal medium (M/N) or with histidine limitation imposed by the addition of AT for 60 min. Blots containing 15 µg of total RNA from each sample were prepared and probed with poly(A) or cos5 DNA.

Fig. 6. Comparisons of conserved domains among selected glutamine amidotransferases. Shown are domains of CPS small subunits (this work; Nyunoya and Lusty, 1984; Nyunoya et al., 1985; Piette et al., 1984; Werner et al., 1985), anthranilate synthase component II polypeptides (Nichols et al., 1980; Schechtmann and Yanofsky, 1988a and b; Paluh et al., 1988, a and b; Paluh et al., 1988). The arg-2 gene does not contain an obvious TATA box, but it does contain CCAAT-like boxes in a motif repeated three times upstream of the mRNA 5'-ends, and two are downstream of the mRNA 5'-ends, one in each of the introns in the uORF. We are currently investigating the role of each of these potential target sequences in the regulation of ARG2 synthesis. Because arg-2 mRNA levels are decreased by growth in the presence of arginine (Fig. 5), arg-2 transcription or mRNA stability may also be subject to arginine-specific regulation.

DNA sequence analyses of genes from N. crassa have shown that they often are not preceded by the conserved elements involved in transcription of other eukaryotic genes, such as the TATA box and the CCAAT box (Ballance, 1986). The arg-2 gene does not contain an obvious TATA box, but it does contain CCAAT-like boxes in a motif repeated three times upstream of the arg-2 mRNA start sites (indicated by heavy underlines in Fig. 2). In other eukaryotes, the CCAAT box binds many different factors (Dorn et al., 1987; Forsburg and Guarente, 1989). Whether CCAAT binding proteins have roles in arg-2 transcription remains to be determined.

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