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Regulation of Arginine Metabolism in *Saccharomyces cerevisiae*: a Network of Specific and Pleiotropic Proteins in Response to Multiple Environmental Signals

Francine Messenguy and Evelyne Dubois*

Institut de Recherches Microbiologiques J. M Wiame, 1, Avenue Emile Gryzon
and Laboratoire de Microbiologie de l'Université Libre de Bruxelles, 1070 Brussels, Belgium

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Summary

In *Saccharomyces cerevisiae*, the expression of the genes involved in the synthesis and degradation of arginine is modulated by multiple specific and pleiotropic factors, acting as repressors or activators as a function of the availability of amino acids, of nitrogen source, and the presence or absence of arginine. Four proteins (Arg80, Arg81, Mcm1 and Arg82) coordinate the expression of arginine metabolic genes, by repressing the biosynthetic genes and by inducing the catabolic genes, in response to arginine. Arg80, Arg81 and Mcm1 form a complex interacting with DNA sequences called »arginine boxes« present in the promoters of arginine co-regulated genes. Binding of arginine to Arg81 allows the interaction of the complex with DNA. The role of Arg82 is to stabilize the Mcm1 and Arg80 proteins. The synthesis of one of the subunits of carbamoylphosphate synthetase encoded by *CPA1* gene is also repressed by arginine. However, this results from a translational control involving a 25 amino acid peptide encoded by the messenger of *CPA1*. Expression of the catabolic genes *CAR1* and *CAR2* is repressed, as long as exogenous nitrogen is available, by the regulatory complex Ume6-Sin3-Rpd3 exhibiting histone deacetylase activity. Expression of *CAR1* but not of *CAR2* is activated by Gln3 and Nil1 when cells are grown on poor nitrogen sources.

Key words: yeast, arginine metabolism, nitrogen control

Introduction

In *Saccharomyces cerevisiae*, the synthesis of arginine has three main components: the synthesis of ornithine, the synthesis of carbamoylphosphate and the conversion of these two compounds into arginine. The catabolic pathway consists of the hydrolysis of arginine to ornithine and urea, the breakdown of urea to ammonia and carbon dioxide, and the conversion of ornithine to glutamate through the proline anabolic and catabolic pathways (*1*).

The five anabolic enzymes catalyzing the conversion of glutamate to ornithine are mitochondrial, and are encoded by *ARG2* (acetylglutamate synthase), *ARG5,6* (acetylglutamate kinase and acetylglutamyl-P reductase), *ARG8* (acetylornithine transaminase) and *ARG7* (acetylornithine-glutamate acetyltransferase). The *ARG5,6* locus encodes a single translation product with two enzyme activities. The precursor polypeptide is cleaved into two mature enzymes upon entry into the mitochondria (*2*). Conversion of ornithine to arginine requires three cytosolic enzymes, ornithine carbamoyltransferase (OTCase), argininosuccinate synthetase and argininosuccinate lyase, encoded by *ARG3*, *ARG1* and *ARG4*, respectively. The synthesis of carbamoylphosphate (CP), the other

* Corresponding author; Phone: ++32 2 5267 277; Fax: ++32 2 5267 273; E-mail: fanarg@resulb.ulb.ac.be

substrate of OTCase, is catalyzed by the cytosolic carbamoylphosphate synthetase A (CPS-A), which is specific for the arginine pathway, whereas the CPS-P is specific for the pyrimidine pathway. CPS-A consists of a small and a large subunit, encoded by the unlinked genes *CPA1* and *CPA2*. An additional gene, *ARG11*, is required for the biosynthesis of arginine. This gene encodes a mitochondrial integral inner membrane protein, which could participate in the export of ornithine from the mitochondria into the cytosol (*3*). The first two steps involved in arginine degradation take also place in the cytosol, and are catalyzed by arginase and ornithine transaminase encoded by *CAR1* and *CAR2*, respectively.

Since ornithine is a common intermediate in anabolic and catabolic pathways, the degradation of newly synthesized arginine to ornithine has to be avoided. The establishment of a futile ornithine cycle, when exogenous arginine is provided, is prevented by multiple control mechanisms leading to the exclusion of the non-required pathway. Adaptation to the shift from anabolism to catabolism, or the reverse, needs efficient and versatile controls of gene expression and enzyme activity. Arginine feedback inhibits the activity of the first two enzymes of the biosynthesis (*4*,*5*), and an unusual mechanism (epiarginase control) leads to inhibition of OTCase by arginase, in the presence of ornithine and arginine (*6*). Both the anabolism and the catabolism of arginine are subjected to a specific control in response to arginine availability, and to more global regulations integrating this metabolism in the cell response to amino acid availability (anabolism) and nitrogen availability (catabolism). Most of these controls are absent in other yeasts such as *S. pombe*. In this organism there is no epiarginase control on OTCase and no significant repression of the arginine biosynthetic enzymes in the presence of arginine, but synthesis of catabolic enzymes is induced (*7*). In *S. pombe* as in *N. crassa*, an efficient compartmentation of enzymes and metabolites seems to be sufficient to control the arginine metabolic flux. In these two organisms OTCase and carbamoylphosphate synthetases are mitochondrial, while arginase is cytoplasmic (*8*).

This review aimed at summarizing the work of several groups during a few decades, that led to establish a complex network of regulatory circuits necessary to coordinate the synthesis and degradation of one amino acid, arginine.

Fig. 1 summarizes different regulations involved in the control of the expression of arginine anabolic and catabolic genes.

Coordination between arginine biosynthesis and degradation is achieved through the involvement of the same regulatory elements in the control of both pathways. In the presence of arginine, four proteins Arg80 (ArgRI), Arg81 (ArgRII), Mcm1 and Arg82 (ArgRIII) are required to repress the synthesis of five anabolic enzymes (*9*) and to induce the synthesis of two catabolic enzymes (*10*). Expression of gene *CPA1* is controlled independently at the translational level by arginine and a 25 amino acid peptide (*11*). In addition, all the anabolic genes are subjected to the »general control« exerted by Gcn4 in response to amino acid starvation. One or several copies of the Gcn4 binding site are present in the promoters of these genes (*12*). The utilization of arginine as a nitrogen source is repressed if better nitrogen compounds such as ammonia, asparagine or glutamine are present. This is termed as »Nitrogen catabolite repression«. Only the expression of the *CAR1* gene is prone to the effect of this repression, and in the presence of a poor nitrogen source, its release is mediated by GATA sequences associated with Gln3 and Nil1 transcription activators (*13–15*). On the other hand, *CAR2* expression is induced by allophanate, the last intermediate of the allantoin-degradative pathway and the two positive regulators Dal81 and Dal82 (*16*,*17*). This induction by the degradative compound of urea is of particular physiological significance when the cells are grown on arginine as sole nitrogen source. More recently, we have shown that the *CAR1* and *CAR2* genes are repressed by the Ume6-Sin3-Rpd3 complex presenting histone deacetylase activity, as long as nitrogen is available in the growth medium (*18*).

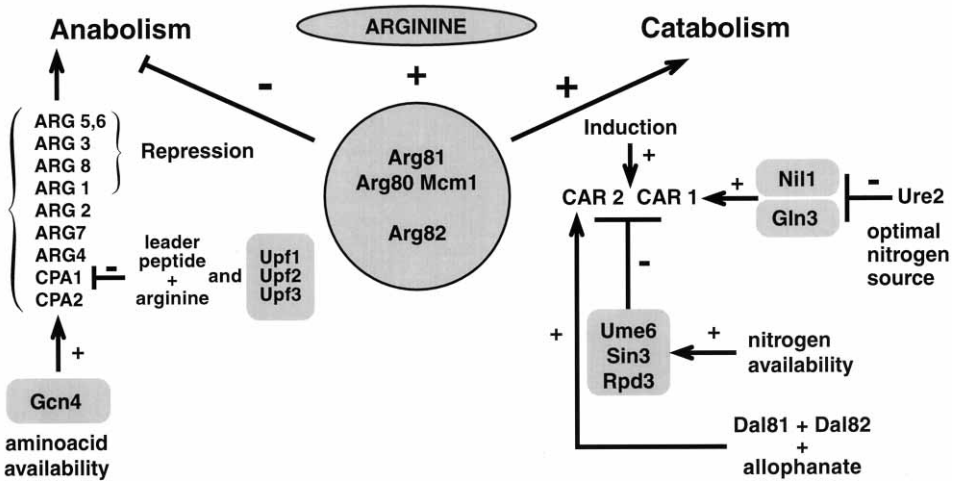

Fig. 1. Network of regulatory circuits controlling the arginine anabolic and catabolic genes in response to nitrogen signals. For details see text

Regulation of Arginine Anabolic and Catabolic Genes by the Arg80-Arg81-Mcm1 Complex in Response to Arginine

Features and role of the regulatory proteins

Mutations in genes *ARG80*, *ARG81*, *ARG82* and *MCM1* lead to constitutive production of the arginine biosynthetic enzymes encoded by the *ARG1*, *ARG3*, *ARG5,6* and *ARG8* genes and to the loss of ability to induce the synthesis of the two catabolic enzymes, products of the *CAR1* and *CAR2* genes, in the presence of arginine (*9*,*10*,*19*). Consequently the growth of these regulatory mutants on arginine or ornithine as nitrogen sources is strongly reduced, allowing the cloning of the cognate regulatory genes (*20*,*21*). Gene *MCM1* was cloned independently (*22*).

Arg80 and Arg81 are specific regulators, whereas Arg82 and Mcm1 are global regulators. These four proteins are nuclear and they do not control each other's expression (*23*).

The Arg80 protein of 177 amino acids and the Mcm1 protein of 286 amino acids belong to the »MADS--box« family of eukaryotic transcription factors, including human SRF (Serum Response Factor) and plant AG (Agamous) and DEFA (Deficiens) (*21*,*22*,*24*). The MADS--box consists of a region of about 60 amino acids showing extensive sequence homology with more than 60 regulatory proteins. The highly basic region of the MADS-box is the major determinant of DNA binding specificity and the hydrophobic C-terminal region is necessary for dimerization. Both N- and C-terminal regions are also implicated in protein-protein interactions (*25*). The region of similarity between Arg80, Mcm1 and SRF is sufficient to ensure their respective functions (*26*–*28*).

Arg81 is 880 amino acids long and belongs to the Zn_2Cys_6 binuclear cluster protein (*29*). This protein contains a region of identity with various retroviral and pancreatic RNases (aa133–203), which is indispensable for the repression function of the protein (*30*). Comparison of the amino acid sequence of Arg81 with those of several bacterial arginine repressors reveals that two regions of Arg81 located between aa 89 and 114 and aa 563–587 share identity with the C-terminal domain of these bacterial repressors (Fig. 2). Different studies showed that this domain contained an arginine binding site involved in oligomerization (*31*,*32*). We have shown that Arg81 is the sensor of arginine and that the main site required for response to arginine lies between aa 89 and 114 (see below).

Arg82 (355 aa) is a pleiotropic factor required for mating, cell growth and sporulation (*21*,*33*). Contrarily to Arg80, Mcm1 and Arg81, Arg82 does not present features of DNA binding proteins. Recently, two independent groups have shown that this protein has inositol polyphosphate multikinase activity (*34*–*36*). It phosphorylates Ins(*1*,*4*,*5*)P3 to Ins(*1*,*3*,*4*,*5*)P4 and Ins(*1*,*4*,*5*,*6*)P4 both of which are then converted by Arg82 to Ins(*1*,*3*,*4*, *5*,*6*)P5. Although Odom *et al.* (*36*) suggested that this kinase activity could be required for its role in the control of arginine metabolism, this appears unlikely. Mutations in the conserved residues of the IP3 binding site abolish the kinase activity, without affecting the repression of arginine anabolic genes and the induction of catabolic genes (unpublished data).

Pairwise interactions between Arg81, Arg80 and Mcm1 were identified using the two-hybrid system (Fig. 3). Arg80 and Mcm1 interact also with Arg82 (*37*). None of these interactions requires the presence of arginine. The putative α helix present in the MADS-box domain of Arg80 and Mcm1 is their major region of interaction with Arg82, whereas the first 180 amino acids of Arg81 are sufficient to interact with Arg80 and Mcm1 (*38*).

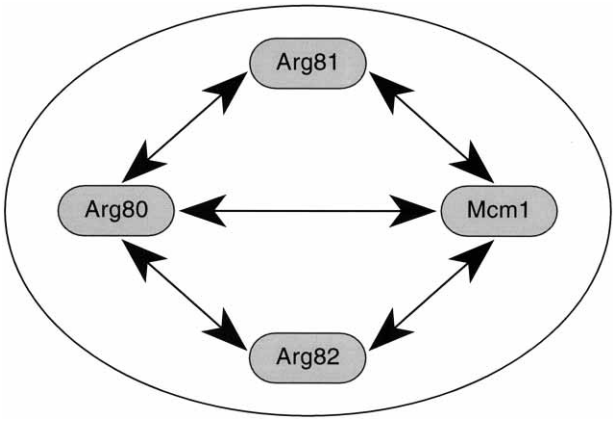

Fig. 3. Pairwise interactions between the four regulatory proteins of the arginine metabolism

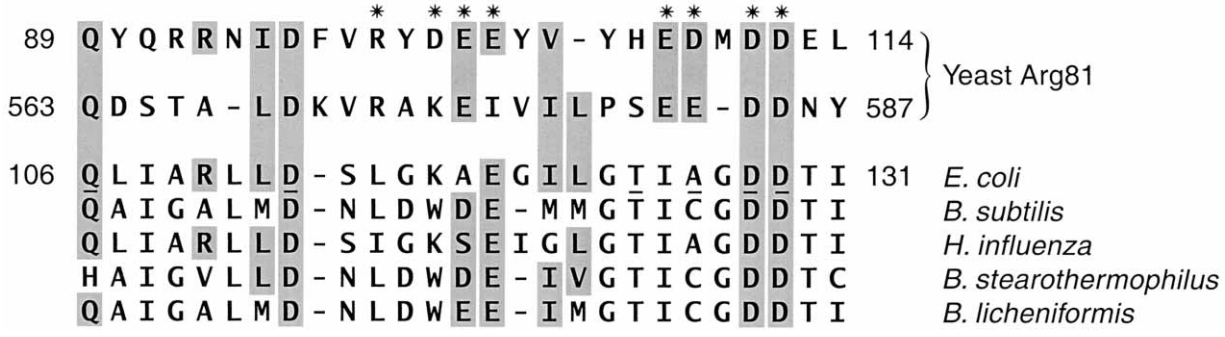

Fig. 2. Amino acid aligment between yeast Arg81 and the arginine repressor from *E. coli*, *B. subtilis*, *H. influenza*, *B. stearothermophylus and B. licheniformis* (32). The conserved amino acids between yeast Arg81 and bacterial repressors are shaded. Amino acids in the *E. coli* sequence contacting arginine are underlined. * indicates the amino acids required for Arg81 arginine-dependent function

The requirement for Arg82 in the control of arginine metabolism can be bypassed *in vitro* as well as *in vivo* by overexpression of Arg80 or Mcm1. We have shown that the impairment of arginine regulation in an *arg82* deleted strain results from the lack of stability of Arg80 and Mcm1 (*37*). The role of Arg82 in this regulatory mechanism is to recruit the two MADS-box proteins leading to their stabilization in the nucleus.

DNA targets of the Arg80-Arg81-Mcm1 protein complex

Cis-acting sequences upstream of the *ARG5,6*, *ARG3*, *CAR1* and *CAR2* genes have been defined by the analysis of *cis*-dominant mutations obtained *in vivo* (*39–41*). Cloning and sequencing of the wild type and mutated genes revealed that for the *ARG3* gene, the two mutations leading to constitutive expression of ornithine carbamoyltransferase are located downstream of the TATA box but about thirty nucleotides apart (*42*). In *ARG5,6*, the point mutation impairing repression by arginine is located in a region well conserved between the two anabolic promoters (*43*). Further analysis of all the arginine co-regulated promoters by creation of deletions and point mutations allowed the identification of two regions homologous to the target of Mcm1 which is called Pbox (Fig. 4). These regions were named »arginine boxes«. They are located upstream of the TATA box in the *CAR1*, *CAR2* and *ARG1* promoters, and downstream of the TATA box in the *ARG5,6*, *ARG3* and *ARG8* promoters (*44–47*). Thus the position of the »arginine boxes« relatively to the TATA box is not the key element leading to induction or repression in response to arginine (Fig. 5). As shown in Fig. 4, these »arginine boxes« are not perfectly well conserved, and the *ARG8* promoter contains only one box. There is no correlation between the efficiency of the regulation and the degree of sequence identity with the Pbox consensus. The intervening sequences between the two »arginine boxes« which are G-C rich, especially for *CAR1* and *CAR2* promoters, are also required for proper regulation (*47*). The two »arginine boxes« and the G-C rich element are named UASarg or URSarg in Fig. 5. The importance of these sequences was confirmed for the *ARG5,6*, *ARG3*, *CAR1* and *CAR2* genes by DNAse I footprinting experiments (Fig. 5, *44*). The effect of mutations in the »arginine boxes« of these promoters are not additive with the mutations in the *ARG81* gene, suggesting that these sequences are the target of this regulator.

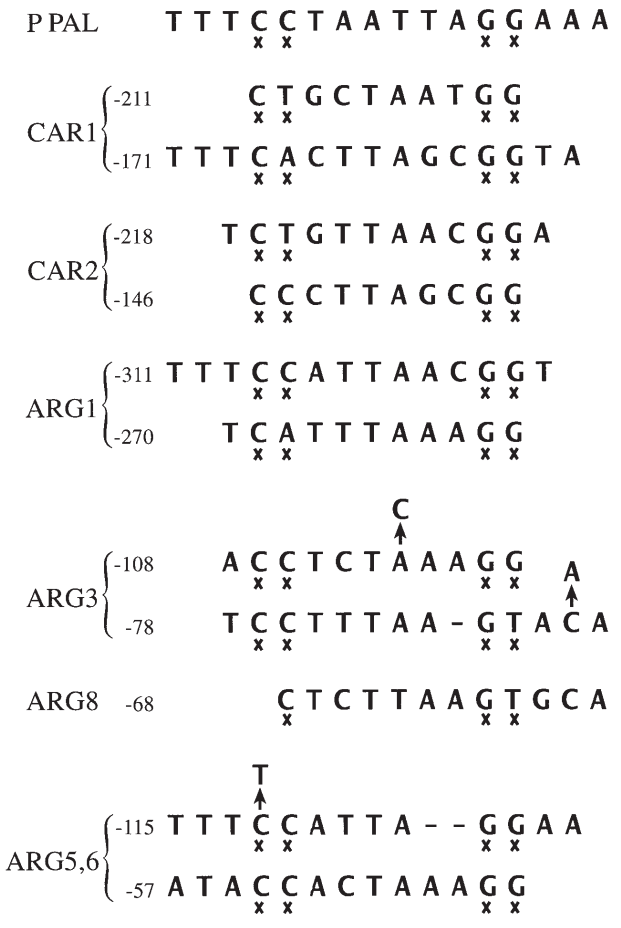

Fig. 4. Nucleotide sequence aligment of the UASarg of the *CAR1* and *CAR2* genes, the URSarg of the *ARG1*, *ARG3*, *ARG8* and *ARG5,6* genes and the binding sites of Mcm1. PPAL is the perfect palindrome sequence. The numbers indicate the limits of each region (+1 at first ATG). Arrows refer to mutations impairing repression by arginine and isolated *in vivo* (according to *39,40,42,43*)

The location of the control regions of different anabolic and catabolic coregulated genes, upstream of the mRNA initiation sites, except for *ARG5,6* and *ARG8*, suggested that the regulation of these genes by arginine occurs at the level of transcription. However, steady state levels of mRNA transcripts of anabolic genes measured by Nothern blot assays under varying growth conditions suggested that arginine specific regulation was not restricted to the transcriptional level (*48,49*). Moreover, S1 mapping experiments showed that the 5′ ends of the *ARG5,6* transcripts are different when the yeast cells are grown on medium with or without arginine, indicating post-transcriptional modification (*43*).

Interaction of Arg80-Arg81-Mcm1 with UASarg and URSarg

By gel retardation assays using yeast extracts, we demonstrated that Arg80, Arg81 and Mcm1 form a complex binding to the UASarg and URSarg, in an arginine-dependent manner (*50*). At least two DNA/protein complexes were formed, both enhanced when Arg81 was overexpressed. Under these conditions, the first 180 amino acids of Arg81 were sufficient to form a regulatory complex with Arg80 and Mcm1, with arginine-dependent DNA-binding activity (*38*). The presence of Arg80 and Mcm1 in these DNA/protein complexes was shown using antibodies raised against Arg80 and Mcm1. Although each one of these proteins contains a putative DNA-binding motif, separately none interacts with the target, since no binding was observed with extracts from mutant strains (Fig. 6A). Mobility shift studies using various combinations of purified recombinant GST-Arg80, GST-Arg81$_{2–180}$ and GST-Mcm1 proteins produced in *E. coli* showed that these three proteins are sufficient to bind to the UASarg or URSarg, in the presence of

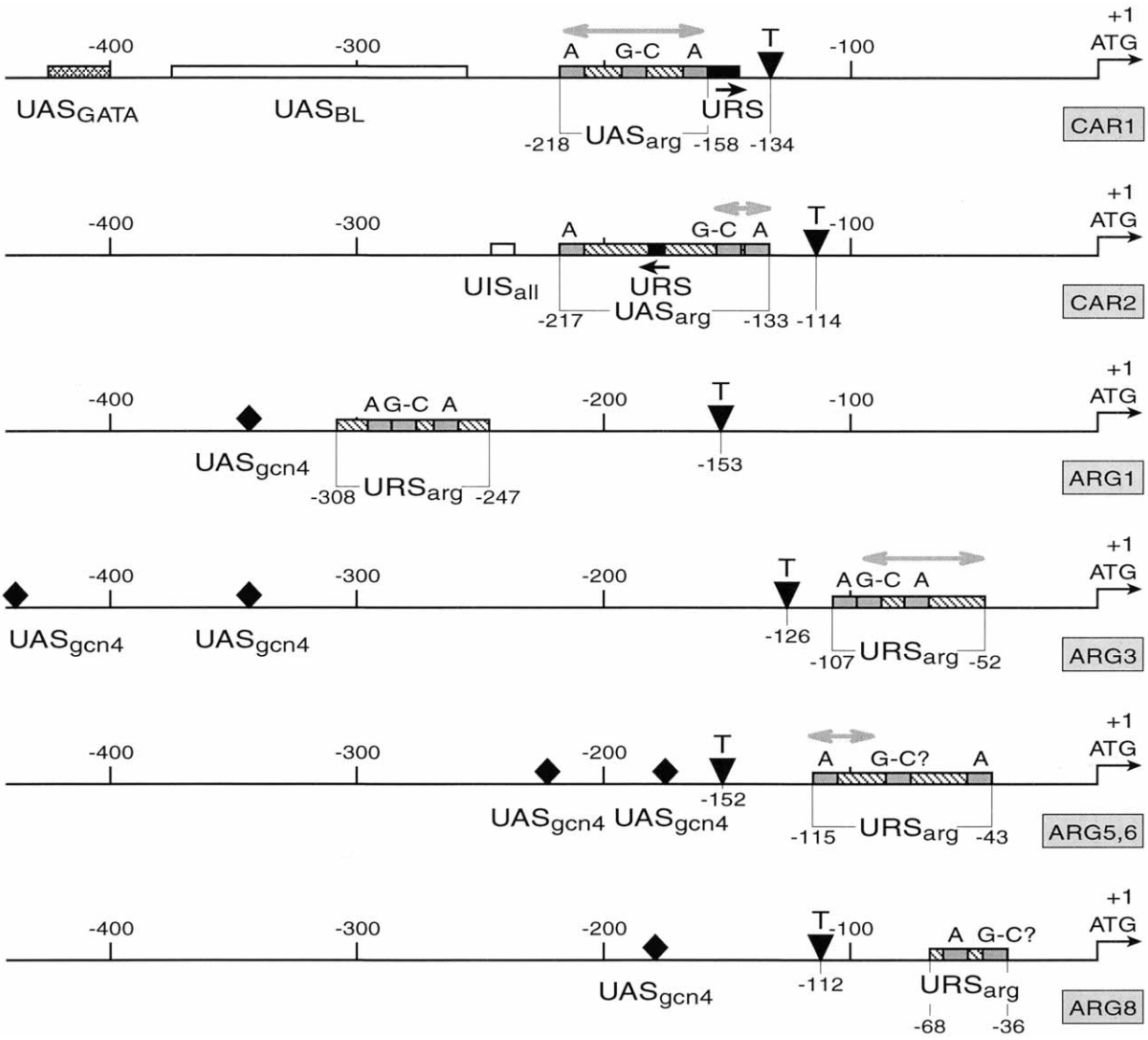

Fig. 5. Organization of the promoters of the arginine anabolic and catabolic co-regulated genes. Black triangles refer to the TATA box. Arrows indicate the region protected in DNAse I footprinting protection experiments (*44*). The shaded boxes named A represent the »arginine boxes«, targets of the MADS-box proteins and the shaded boxes named G-C, targets of Arg81, in UASarg and URSarg

arginine only, and that the individual proteins are unable to interact with these sequences (Fig. 6B, *38*).

We have also gathered evidence indicating that Arg81 is the sensor of arginine. Mutations in the N-terminal region of Arg81 presenting similarity with the arginine binding site of bacterial repressors, strongly decrease the apparent affinity of the regulatory complex for arginine (*38*).

The sequence of events leading to induction of catabolic genes and repression of anabolic genes by Arg80, Arg81, Arg82 and Mcm1 in response to arginine is presented in Fig. 7. The first step is the recruitment of the two MADS-box proteins Arg80 and Mcm1 by Arg82, leading to their stabilization in the nucleus and promoting their interaction with Arg81. When arginine is present, the protein complex is then able to interact with DNA. Since biochemical and structural studies of MADS-box proteins demonstrate that their binding to DNA provokes DNA bending (*51*), we propose that the role of Arg80 and Mcm1 would be to interact with the »arginine boxes«, and induce DNA bending. This would allow the interaction of Arg81 with the G-C rich intervening region, leading to the formation of a stable active protein/DNA complex in the presence of arginine.

Translational Regulation of the *CPA1* Gene by Arginine and a 25 Amino Acid Peptide

Carbamoylphosphate (CP) is required for two major biosynthetic pathways, those of arginine and pyrimidine. Two independently regulated carbamoylphosphate synthetases (CPSases) feed interchangeable cellular CP pools. One enzyme, CPSase P, is repressed and feedback inhibited by the pyrimidines; it is encoded together with aspartate carbamoyltransferase by the complex *URA2* locus. The second enzyme, CPSase A, is encoded by the unlinked *CPA1* and *CPA2* genes, and its synthesis is repressed by arginine (*52*). Lack of CPSase P in a *ura2c*

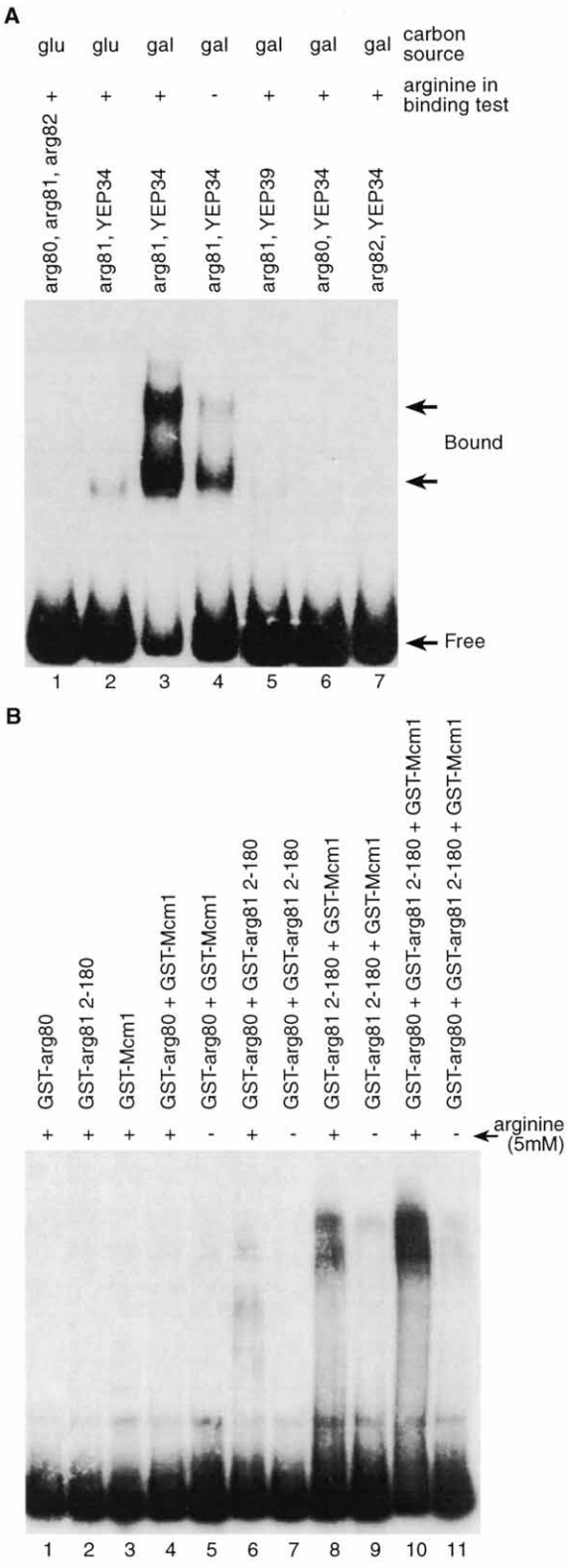

Fig. 6. Arginine-dependent binding of Arg80-Arg81-Mcm1 to *ARG5,6* DNA. A) The end-labelled *ARG5,6* DNA fragment was incubated with 10 μg of yeast extracts from different strains as indicated. YEP34 contains p*GAL10-ARG81* and YEP39 contains a deletion of the first 60 amino acids of Arg81 (*44*). **B)** The end--labelled *ARG5,6* DNA fragment was incubated with about 3 μg of purified GST-Arg80, GST-Arg82$_{2–180}$, and GST-Mcm1 (*38*). In both types of experiments, 5mM L-arginine was added in the binding assays, where indicated

mutant results in sensitivity to arginine caused by arginine-mediated repression of the *CPA1* gene. Advantage has been taken of this growth sensitivity to select two classes of mutations affecting the arginine specific control: »cpa1-O« mutations, *cis*-dominant and closely linked to the *CPA1* gene, and *cpaR*, recessive and unlinked to the structural gene (*53*). It was shown that all the »cpa1-0« mutations mapped in the leader of *CPA1* mRNA, in an uORF encoding a 25 amino acid peptide. This peptide is the essential negative *cis*-element for repression of the *CPA1* gene by arginine, because by itself it can confer arginine-dependent repression to a heterologous gene. Moreover, this translational regulation depends only on the amino acid information of the uORF. Indeed, missense mutations of particular codons derepress translation of the downstream *CPA1* mRNA. In constrast, mutations in these same codons that retain the amino acid coding information preserve the inhibiting effect (*11*,*54*). More recent studies showed that the active domain of the Cpa1 regulatory peptide extends from amino acid 6 to 23, a region which is well conserved in the messengers of *CPA1* equivalents in other fungi, suggesting the conservation of such a translational control mechanism among fungi (*55*,*56*). Recent data also showed that the repression mechanism exerted by the peptide in the presence of arginine does not modify the starts of transcription, but leads to destabilization of the 5′ ends of *CPA1* mRNA (*56*). This destabilization does not occur in *cpa1-O* nor *cpaR* mutants. It turned out that gene *CPAR* was allelic to gene *UPF1*, whose product is involved with the *UPF2* and *UPF3* gene products in nonsense-mediated destabilization of certain classes of mRNA containing premature stop codons (*57*; for a review see *58*). Ruiz-Echevarria and Peltz (*59*) confirmed that *CPA1* mRNA was indeed stabilized in *upf1* deleted strains.

Thus the regulation of *CPA1* mRNA at the level of translation is mediated by the leader peptide in two ways:

– Firstly, the peptide encoded by the uORF and arginine exert a negative effect on the translation of the downstream *CPA1* ORF, probably by decreasing the translation from downstream initiation codons.

– Secondly, the presence of a stop codon at the end of the uORF signals this mRNA to be selectively recruited by the Upf1-Upf2-Upf3 complex, independently of the presence of a high intracellular arginine concentration.

Full expression of the *CPA1* gene is only observed in the absence of a functional leader peptide, combined with a genetic *upf1/cpaR* genetic background. Both, the arginine-peptide repression mechanism and surveillance by the Upf1-Upf2-Upf3 complex are complementary mechanisms.

Additional Controls on Arginine Catabolic Genes Exerted through Global Regulators

Nitrogen catabolite control on CAR1 expression

In response to the quality of the nitrogen source, *S. cerevisiae* is able to adjust its enzymatic composition.

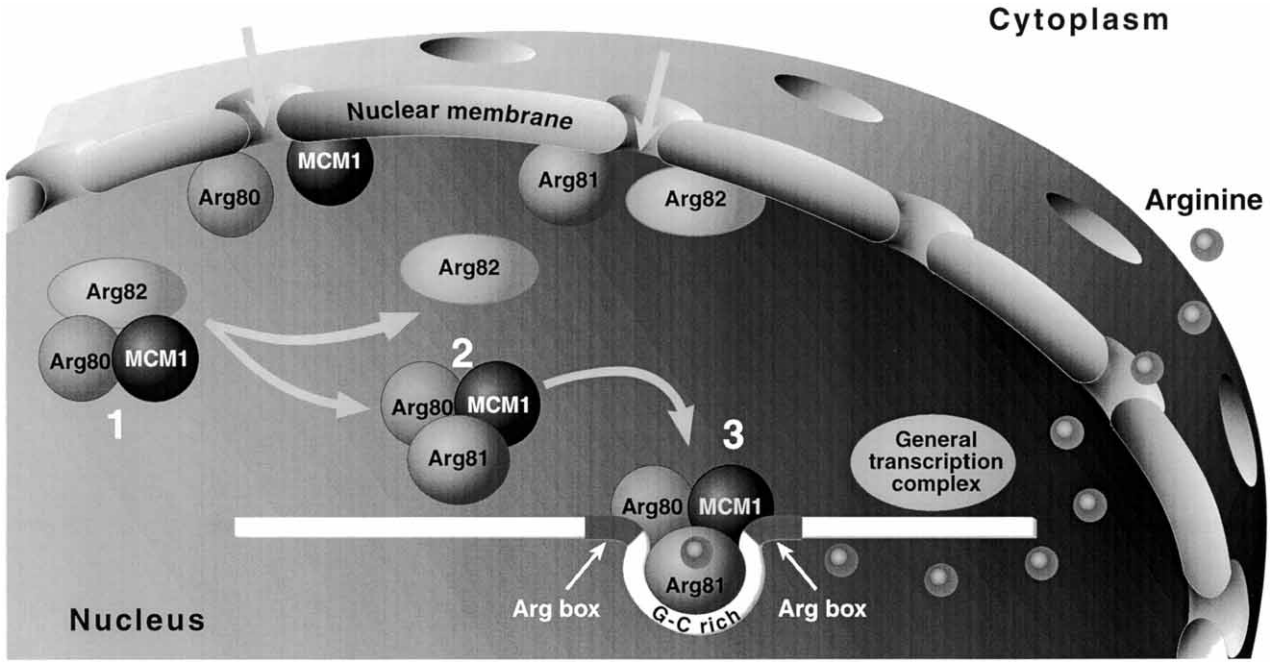

Fig. 7. Sequence of events required for regulation of arginine anabolic and catabolic genes by arginine. For details see text

Maximal growth rates are obtained with ammonia, glutamine or asparagine, and growth on these compounds leads to depression of a whole range of activities linked to the utilization of nitrogenous compounds. Under the above specified conditions, the syntheses of enzymes degrading poorer nitrogen sources, such as glutamate, proline, arginine and urea are repressed (*60*). This regulation requires two transcriptional activators, Gln3 and Nil1 acting through the GATA consensus elements, and an inhibitory factor Ure2 modulating their activity (*13–15*). The expression of the catabolic *CAR1* gene, encoding arginase, is subject to this regulation whereas the *CAR2* gene encoding ornithine transaminase is not (Fig. 1). The three GATA elements present in the promoter of *CAR1* are required for its expression in the absence of an optimal nitrogen source (*61,47*). The simultaneous deletion of Gln3 and Nil1 are necessary to abolish the derepression of *CAR1* when cells are utilizing proline as sole nitrogen source. Although expression of gene *CAR2* is not regulated by Gln3, we have found that a deletion of the *GLN3* gene strongly enhances arginase and ornithine transaminase production when cells are grown on minimal medium containing ammonia and arginine (*47*). This effect is not mediated through the GATA sequences but requires the ArgR-Mcm1 proteins. This additional role for Gln3 could be to counteract the induction by arginine when ammonia is present. Although the total arginine pool is unchanged in a *gln3* deleted strain compared to a wild type strain, the differential distribution of basic amino acids between cytoplasm and vacuole is modified. In *gln3* mutant cells grown on *M. ammonia*, the basic amino acids, arginine, lysine and histidine are more sequestred in the vacuole than in a wild type strain. On *M. ammonia* + arginine, we observe a similar distribution except for arginine whose concentration in the cytoplasm is higher than in the wild type (unpublished data). Thus the higher induction by arginine of arginase and ornithine transaminase in a *gln3* deleted strain correlates with a higher cytoplasmic arginine pool. If the effect of Gln3 on arginine compartmentation is direct or not remains unknown.

Role of histone deacetylase activity in the control of arginine catabolic genes

The growth defect of an *arg81* mutant on arginine or ornithine as nitrogen source allowed the selection of suppressor mutations falling into three complementation groups containing the *CAR80 (CARGRI)*, *CAR81 (CARGRII)* and *CAR82 (CARGRIII)* genes. Mutations in any of these genes led to overproduction of arginase and ornithine transaminase, even in an *arg81* background (*41,62*). We have shown that *CAR80* is identical to *UME6*, a gene whose product is involved in controlling the expression of early meiotic genes (*63,64*), and we have identified *CAR81* as *SIN3* and *CAR82* as *RPD3* (*18*). Ume6 is a DNA-binding protein belonging to the Zn_2C_6 family of transcription factors, interacting with DNA at a sequence named URS1 (*64*). Ume6 recruits the Rpd3-histone deacetylase complex by interacting with Sin3 (*65*). The role of the Ume6-Sin3-Rpd3 complex in the control of arginine catabolism is to block the expression of the *CAR1* and *CAR2* promoters as long as exogenous nitrogen is available. Indeed, a mutation in *UME6* abolishes completely the response of these two promoters to nitrogen depletion. Arginase and ornithine transaminase production under nitrogen starvation conditions also requires the integrity of the Arg80-Arg81--Mcm1 complex. However, differential arginine pool measurements showed that this enzyme synthesis does not result from a burst of arginine stored in the vacuole towards the cytosol. This induction results probably from an interaction between Ume6 and the specific regulators, Arg80 and Arg81, leading to a more efficient binding of the regulatory complex at the »arginine

boxes« at low arginine concentration. This hypothesis is supported by two-hybrid experiments showing an interaction between Arg80 or Arg81 and Ume6, only under nitrogen starvation conditions (*18*).

As summarized in this short review, efficient coordination between the synthesis and utilization of arginine requires the joint action of many proteins operating at the transcriptional, post-transcriptional and translational levels. One important aspect that has so far not been addressed for this metabolism is the importance of chromatin structure. Chromatin repression and chromatin remodeling are likely to play a role at least in the regulation of the two catabolic genes *CAR1* and *CAR2*, since both are controlled by the Rpd3 histone deacetylase complex, and by Rap1 and Abf1, known also to play a role in chromatin activation (*17*,*18*,*47*,*61*,*66*). A difference in the chromatin structure could explain why the same regulatory complex (Arg80-Arg81-Mcm1) is able to repress the expression of an anabolic promoter or to activate the expression of a catabolic promoter in response to arginine, regardless of the location of the DNA target sequences.

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Regulacija metabolizma arginina u *Saccharomyces cerevisiae*: mreža specifičnih i pleiotropskih proteina kao odgovor na mnogostruke signale iz okoline

Sažetak

Ekspresija gena, uključenih u sintezu i degradaciju arginina u *S. cerevisiae*, regulirana je mnogobrojnim specifičnim i pleiotropskim faktorima koji djeluju kao represori ili aktivatori, ovisno o dostupnosti aminokiselina, izvora dušika te prisutnosti ili odsutnosti arginina. Četiri proteina (Arg80, Arg81, Mcm1 i Arg82) koordiniraju ekspresiju gena za metabolizam arginina, reprimirajući biosintetske gene i inducirajući katabolitske gene u prisutnosti arginina. Arg80, Arg81 i Mcm1 stvaraju kompleks koji omogućava interakciju sa sekvencijama DNA, nazvanim »argininski slijed« (arginine box), koji se nalaze u promotorima koordinativno reguliranih gena za arginin. Vezanje arginina na Arg81 omogućava interakciju kompleksa s DNA. Uloga je Arg82 da stabilizira proteine Mcm1 i Arg80. Sinteza jedne od podjedinica karbamoilfosfat sintetaze, kodirane CPA1 genom, također je reprimirana argininom. Međutim, to je posljedica translacijske kontrole koja uključuje peptid od 25 aminokiselina, kodiran s mRNA od *CPA1*. Ekspresija katabolitskih gena *CAR1* i *CAR2* reprimirana je u prisutnosti egzogenog dušika, i to regulacijskim kompleksom Ume6-Sin3-Rpd3 koji ima aktivnost histon deacetilaze. Ekspresija *CAR1*, ali ne i *CAR2*, aktivirana je s Gln3 i Nil1 ako stanice rastu u podlozi siromašnoj izvorima dušika.