MOLECULAR AND CELLULAR BIOLOGY, Apr. 1993, p. 2586–2592
0270-7306/93/042586-07$02.00/0

Vol. 13, No. 4

Genetic Evidence for a Role for MCM1 in the Regulation of Arginine Metabolism in *Saccharomyces cerevisiae*

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Received 13 July 1992/Returned for modification 25 November 1992/Accepted 15 January 1993

ARGRI, ARGRII, and ARGRIII regulatory proteins control the expression of arginine anabolic and catabolic genes in *Saccharomyces cerevisiae*. We have shown that MCM1 is part of the ARGR regulatory complex, by in vitro binding experiments, at the *ARGR5,6* promoter. The participation of MCM1 in the regulation of arginine metabolism is confirmed by the behavior of an *mcm1-gcn4* mutant, which is affected in the repression of arginine anabolic genes. In this *mcm1* mutant, synthesis of the catabolic enzymes is rather constitutive, but this derepression requires the integrity of the ARGR system and of the target sequences of these proteins in the *CAR1* promoter. Our in vitro binding experiments confirm the presence of MCM1 in the protein complex interacting with the promoters of the catabolic *CAR1* and *CAR2* genes. This is the first in vivo transcription role ascribed to MCM1 other than its role in the transcription of cell-type-specific genes.

Three proteins, called ARGRI, ARGRII, and ARGRIII, regulate the expression of genes involved in the metabolism of arginine in *Saccharomyces cerevisiae*. In the presence of arginine, they are required to repress the synthesis of five anabolic enzymes and to induce that of two catabolic enzymes. Mutations in any of the three *ARGR* genes lead to constitutive expression of the anabolic genes and to an absence of induction of the catabolic genes, preventing growth on arginine and ornithine as sole nitrogen sources (1, 30, 31). ARGRI and ARGRII are specific regulators of the arginine system, while ARGRIII is involved in other cellular processes (27). The promoters of two anabolic genes (*ARG3* and *ARG5,6*) and two catabolic genes (*CAR1* and *CAR2*) have been analyzed by creating internal deletions and point mutations (6, 8–10, 12, 14, 22). To define precisely the target of the ARGR proteins, these studies have been complemented by gel retardation assays and DNase I footprinting analysis (23). The regulation of these genes by arginine and the ARGR proteins requires at least two regions, called box A and box B, located downstream of the TATA element in the *ARG3* and *ARG5,6* promoters and upstream of the TATA sequence in the *CAR1* and *CAR2* promoters.

Interestingly, box A has homology to the P box, which is the target of MCM1 protein, in mating-type-specific genes (2). It is also noteworthy that MCM1 and ARGRI proteins share significant similarity; within a stretch of 80 amino acids, 55 are identical (26).

The mechanism of binding of the ARGR proteins to the *ARG5,6* promoter has been more thoroughly investigated in vitro. We have shown that the three ARGR proteins are required to observe a protein-DNA complex with this promoter (16). This specific binding is stimulated by arginine and overexpression of ARGRII. One of the deletions created in the ARGRII protein (Δ145–165) leads to the formation of a protein-DNA complex with faster mobility, which suggests but does not prove that ARGRII is present in this complex (28). The participation of ARGRI and MCM1 in the protein-DNA complex was demonstrated by the use of antibodies raised to an ARGRI-specific peptide or to a peptide of MCM1 not homologous to ARGRI (16). Although ARGRIII protein is indispensable for the interaction of these proteins with the *ARG5,6* promoter in vitro as well as in vivo, we could not demonstrate its direct participation in the protein-DNA complex.

We have previously shown that the overexpression of ARGRII protein and the presence of arginine in the in vitro binding assay are also necessary to observe the formation of a protein complex with the *ARG3*, *CAR1*, and *CAR2* promoters. Moreover, competition experiments indicated that these promoters were probably bound by the same protein complex as was the *ARG5,6* promoter (23). Thus, it is likely that MCM1 is a component of the protein-DNA complex at arginine anabolic and catabolic genes.

In this report, proof of the participation of MCM1 in the regulation of arginine metabolism is provided by the study of an *mcm1* mutant.

MATERIALS AND METHODS

Strains and media. Strain 8534-8c (*his4 leu2 ura3*) and the isogenic mutant mcm1-gcn4/DE(Q) are described by Christ and Tye (7). Strains 10R34d-II (*ura3 argRII::CAR1*), 02431a (*ura3 leu2 argRII::CAR1*) (28), 10R32b (*ura3 leu2 argRI*), and 10R41b (*ura3 leu2 argRIII*) were used as recipient strains for plasmids containing either the *ARGRI* or *ARGRII* gene. Strains 02613c [*argRII::CAR1 mcm1-gcn4/DE(Q) ura3*] and 02613d (*argRII::CAR1 ura3*) were obtained by crossing 10R34d-II and mcm1-gcn4/DE(Q). Strain 02578b [*cargRI mcm1-gcn4/DE(Q) leu2 his4 ura3*] was obtained by crossing mcm1-gcn4/DE(Q) and 02322c (*cargRI ura3*). Strains 02591d (*car1 ura3*) and 02586a (*car1 mcm1-gcn4 ura3 leu2 his4*) were obtained by crossing 02356d (*car1 ura3*) with mcm1-gcn4/DE(Q) and were used as recipient strains for plasmids pCV7 and pCV40. Since the *MAT*α strain mcm1-gcn4/DE(Q) is sterile, it was transformed with plasmid pMCM1 prior to mating with the different mutant strains. Once the zygotes were formed, loss of plasmid pMCM1 was selected prior to sporulation.

All yeast strains were grown on minimal medium containing 0.02 M $(NH_4)_2SO_4$ as the nitrogen source (M. ammonia), supplemented with 3% glucose or 2% galactose, vitamins, and trace minerals (22). L-Arginine (1 mg/ml) or L-lysine (60 μg/ml) was added as indicated to M. ammonia. In M.

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TABLE 1. Effect of the *mcm1-gcn4* mutation on the expression of several genes encoding metabolic enzymes

Strain	Medium[a]	Sp act (μmol of product formed/h/mg of protein)[b]								
		OTCase	Acetyl-glutamate kinase	OTase	Arginase	CPSase	Argininosuccinase	Product of *LYS9*	NADP glutamate dehydrogenase	NAD glutamate dehydrogenase
8534-8c	M. ammonia	23	0.085	0.41	27	0.56	1.7	1.1	31	1.2
	M. ammonia + Arg	5	0.025	1.20	80	0.13	1.3			
	M. ammonia + Lys							0.25		
	M. glutamate								24	61
mcm1-gcn4/DE(Q)	M. ammonia	31	0.110	1.15	134	0.47	1.4	1	36	1.9
	M. ammonia + Arg	23	0.088	1.56	239	0.13	1.1			
	M. ammonia + Lys							0.25		
	M. glutamate								21	70
mcm1-gcn4/DE(Q)/ pMCM1	M. ammonia	27		0.32	26					
	M. ammonia + Arg	5		1.12	94					

[a] Since strains 8534-8c and mcm1-gcn4/DE(Q) are *ura3 his4 leu2*, 25 μg each of uracil and histidine and 50 μg of leucine per ml were added to the growth medium. Where noted, 1 mg of arginine or 60 μg of lysine per ml was added to M. ammonia medium.

[b] Each value is the mean of two to five independent measurements which did not differ by more than 20%.

glutamate medium, the nitrogen source was L-glutamate (1 mg/ml). Where mentioned, 20 μg of uracil or L-histidine or 50 μg of L-leucine per ml was also added to M. ammonia.

Plasmids. Plasmids YpED1 (pBR322, *ARGRII*, *URA3*, 2μm), YEP34 (p*GAL10-ARGRII*, *LEU2*, 2μm), and YEP52-RI (p*GAL10-ARGRI*, *LEU2*, 2μm) are described by Dubois (3, 15, 28) and Messenguy (15, 28). Plasmid pCV7 results from insertion of a 5.8-kb *Bam*HI-*Bam*HI DNA fragment from the *CAR1* gene into the monocopy plasmid pFL38 described in reference 5. Plasmid pCV40 results from insertion in plasmid pFL38 of a 5.8-kb *Bam*HI-*Bam*HI DNA fragment deleted of box A and box B in the *CAR1* promoter (−210 to −202 and −184 to −168, +1 being at the first ATG), as described in reference 23. Plasmid pMCM1 results from insertion of a 3.4-kb *Sph*I-*Sph*I fragment carrying the *MCM1* gene into the multicopy plasmid pFL1 (pBR322, *URA3*, 2μm). Plasmid pED30 results from insertion of a 5-kb *Hin*dIII-*Hin*dIII DNA fragment containing the *CARGRI* gene in plasmid pFL44 (pUC19, 2μm, *URA3* [5]).

Yeast transformation. The procedure was performed as described by Hinnen et al. (17).

Gel retardation assays. Extract preparation and the binding assays were performed as described by Dubois and Messenguy (16). For binding studies with the *CAR1* promoter, a 320-bp *Eco*RV-*Bgl*II or a 167-bp *Tha*I-*Bgl*II fragment from pED22 was used. For binding studies with the *CAR2* promoter, a 135-bp *Hha*I-*Hae*III fragment from pED23 was used (23). The fragments were end labeled with [γ-^{32}P]ATP (Amersham) by using polynucleotide kinase according to the standard method (21). Preparation of anti-ARGRI and anti-MCM1 antibodies is described by Jarvis et al. (18) and Dubois and Messenguy (16).

Enzyme assays. Ornithine carbamoyltransferase (OTCase; EC 2.1.3.3), carbamoylphosphate synthetase (CPSase; EC 2.7.2.9), argininosuccinase (EC 4.3.2.1), acetylglutamate kinase (EC 2.7.2.8), arginase (EC 3.5.3.1), ornithine transaminase (OTase; EC 2.6.1.13), saccharopine dehydrogenase (NADP, glutamate forming; product of *LYS9*; EC 1.5.1.10), and NAD and NADP glutamate dehydrogenases (EC 1.4.1.2 and EC 1.4.1.4) were assayed as described in references 25, 20, 11, 19, 25, 14, 29, and 4, respectively. Proteins were determined by the Folin method.

RESULTS

Effect of a mutation in *MCM1* on the specific control of arginine-regulated genes. As the *MCM1* gene is essential for cell viability, it is difficult to test its role in arginine regulation in vivo. We have already reported that the *mcm1-1* mutant (point mutation), which is α sterile and impaired in minichromosome maintenance (26), is unaffected in its regulation by arginine (16). Christ and Tye have produced a new *mcm1* mutant in which amino acids 98 to 152 are replaced by amino acids 85 to 150 from GCN4 (containing the acidic activation domain of GCN4) (7). This mutant is also affected in the control of α-specific genes and minichromosome maintenance, but in contrast to the strain carrying the *mcm1-1* point mutation, it has partially lost the repression of a-specific genes in α cells. This mutant is also impaired in the repression of arginine anabolic genes by arginine and is affected in the expression of the two catabolic genes (Table 1). OTCase and acetylglutamate kinase represent two anabolic enzymes whose synthesis is repressed by the ARGR proteins in the presence of arginine. Their specific activities are higher in the mutant strain grown on M. ammonia and on M. ammonia-arginine media than in the isogenic wild-type strain, showing a reduction of the repression of their synthesis. Arginase and OTase are the two catabolic enzymes whose synthesis is induced by arginine and the ARGR proteins. In the *mcm1* mutant strain, the specific activities of both enzymes are very high, even in the absence of exogenous arginine. When strain mcm1-gcn4/DE(Q) is transformed with the multicopy plasmid pMCM1, OTCase, arginase, and OTase activities are again comparable to those of the wild-type strain.

We also tested the effect of the *mcm1-gcn4* mutation on the expression of other metabolic genes, two of which belong to the arginine anabolic pathway but are not regulated by the ARGR proteins. Argininosuccinase is only under general amino acid control (11), and CPSase A is regulated by arginine at the translational level (24, 32). As shown in Table 1, their specific activities are not significantly affected by the *mcm1-gcn4* mutation, whether the strains are grown in the presence or absence of arginine in the growth medium. The product of the *LYS9* gene (glutamate-forming saccharopine dehydrogenase) is normally repressed by lysine in the

TABLE 2. Effect of the *mcm1-gcn4* mutation in *argRII* and *cargRI* mutant strains

Strain	Genotype	Plasmid	Sp act (μmol of product formed/h/mg of protein)[a]					
			OTCase		Arginase		OTase	
			M. am	M. am + Arg	M. am	M. am + Arg	M. am	M. am + Arg
8434-8c	Wild type		23	5	27	80	0.41	1.20
mcm1-gcn4/DE(Q)	*mcm1-gcn4*		31	23	134	239	1.15	1.56
02613d	*ARGRII::CAR1*		39	40	11	11	0.05	0.07
02613c	*ARGRII::CAR1 mcm1-gcn4*		53	55	6	5	0.06	0.05
02578b	*mcm1-gcn4 cargRI*				221		2.7	
02578b	*mcm1-gcn4 cargRI*	pCARGRI			143		1.5	
02578b	*mcm1-gcn4 cargRI*	pMCM1			50		0.76	
02322c	*cargRI*				41		0.7	

[a] Since strains 8534-8c and mcm1-gcn4/DE(Q) are *ura3 his4 leu2*, 25 μg each of uracil and histidine and 50 μg of leucine per ml were added to the growth medium. Where noted, 1 mg of arginine was added to M. ammonia (M. am) medium. Each value is the mean of three independent measurements which did not differ by more than 20%.

mcm1-gcn4 strain, and the expression of the NAD and NADP glutamate dehydrogenases is not affected either (Table 1). Thus, the *mcm1-gcn4* mutation impairs the regulation only of genes whose expression is controlled by the ARGR proteins. In the *mcm1-gcn4* mutant, repression of OTCase and acetylglutamate kinase synthesis is reduced, but the effect is partial compared with the behavior of an *argR* mutant (02613d) (shown only for OTCase in Table 2). Other functions controlled by MCM1 are also only partially impaired by the *mcm1-gcn4* mutation (7). In the *mcm1-gcn4 argRII::CAR1* double mutant (02613c), OTCase activity is consistently higher than in the strain bearing an *argRII* mutation (Table 2). This additional derepression is not observed in the *argRI argRII argRIII* triple mutant (11), suggesting either that MCM1 and the ARGR proteins operate differently in enabling arginine-mediated repression or that the transcription activation domain of the gcn4 moiety boosts the overall transcription of the OTCase gene. The *mcm1-gcn4* and *argR* mutations have opposite effects on induction of the arginine catabolic genes. In an *argR* mutant (02613d), arginase and OTase synthesis is not inducible, whereas in the *mcm1-gcn4* strain, the expression of *CAR1* and *CAR2* genes is rather constitutive. One possibility is that wild-type MCM1 is a repressor of transcription of catabolic genes and that the ARGR products act to overcome this repression in the presence of arginine. If this is so, an *mcm1-gcn4 argR* double mutant should show constitutive expression of the catabolic genes. However, arginase and OTase levels in an *argRII mcm1-gcn4* strain were comparable to those seen in an *argRII* strain (Table 2), ruling out the possibility that the ARGR proteins act solely to overcome MCM1-mediated repression.

We also investigated the relationship between MCM1 and other regulators of *CAR1* (arginase) and *CAR2* (OTase) through double-mutant studies. Mutation of CARGRI (CAR80), CARGRII (CAR81), or CARGRIII (CAR82) leads to constitutive synthesis of arginase and OTase, and in this case, constitutive synthesis is seen even in *cargR argR* double mutants (13, 14). As shown in Table 2, the *cargRI mcm1-gcn4* double mutant exhibited elevated arginase and OTase activities compared with the same strain transformed with a plasmid bearing the CARGRI gene (pED30) and compared with the *mcm1-gcn4* single mutant. As above, the increased expression of *CAR1* and *CAR2* may indicate that MCM1 and CARGRI function by different means or may simply reflect a contribution to transcription activation by mcm1-gcn4.

To determine whether MCM1 functions through the same target as do the ARGR proteins, we measured arginase activity in an *mcm1-gcn4 car1* strain transformed with monocopy plasmids containing either the wild-type *CAR1* gene (pCV7) or the *CAR1* gene carrying a deletion of box A and box B (deletion of −211 to −202 and −184 to −168), defined as the target of the ARGR proteins (23). As shown in Table 3, the constitutive expression of *CAR1*, as a result of the *mcm1-gcn4* mutation, requires the box A and box B sequences. All of these results support the idea that MCM1 regulates arginine catabolic genes by acting together with the ARGR proteins through box A and box B sequences. We next used in vitro experiments to test whether formation of the ARGR complex with the catabolic promoters is modified in the *mcm1-gcn4* mutant.

Effect of the *mcm1-gcn4* mutation on the formation of a protein-DNA complex at the arginine coregulated promoters. Since the *mcm1-gcn4* strain is impaired in arginine regulation, we tested whether a protein extract from this strain had retained its arginine-dependent DNA binding activity at the *ARG5,6*, *CAR1*, and *CAR2* promoters. When these promoters are incubated with extracts from control (8534-8c) and mutated [*mcm1-gcn4/DE(Q)*] strains transformed with

TABLE 3. Effect of deletion of the arginine boxes in the *CAR1* promoter on induction by mcm1-gcn4 mutant protein

Strain	Genotype	Plasmid	Arginase sp act in M. ammonia (μmol of product formed/h/mg of protein)[a]
02591d	*car1*	pCV7 (wild-type *CAR1* gene)	18
		pCV40 (*CAR1* gene deleted of box A and box B)	4
2586a	*car1 mcm1-gcn4*	pCV7	254
		pCV40	5

[a] Each value is the mean of two independent measurements which did not differ by more than 20%.

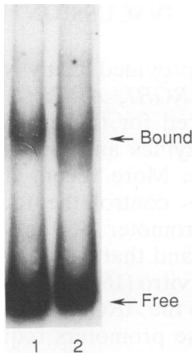

FIG. 1. Effect of the *mcm1-gcn4* mutation on the formation of a protein-DNA complex with the *CAR1* promoter. The ^{32}P-end-labeled 167-bp *Tha*I-*Bgl*II DNA fragment was incubated with yeast extracts (10 μg) from strains 8534-8c (*MCM1 ura3 his4 leu2*; lane 1) and mcm1-gcn4/DE(Q) (*mcm1-gcn4 his4 ura3 leu2*; lane 2) transformed with plasmid YpED1 (pBR322, *URA3*, 2μm, *ARGRII*) and grown on M. ammonia-glucose-arginine plus 25 μg of histidine and 50 μg of leucine per ml.

p*ARGRII* on the multicopy plasmid pFL44 (pUC19, *URA3*, 2μm; gift from F. Lacroute [5]), we observe the formation of the same protein-DNA complex with unaffected mobility. Figure 1 shows only the data obtained for the *CAR1* promoter. In lanes 1 and 2, extracts were prepared from wild-type (8534-8c) and mutated [*mcm1-gcn4/DE(Q)*] strains, in which ARGRII expression is under its own promoter. In each case, we observe the formation of only one complex, with comparable efficiency and mobility in the wild-type and mutant strains. We also confirmed that the binding activity present in the mutant extract is still dependent on the presence of arginine in the binding assay (data not shown).

These results demonstrate that the *mcm1-gcn4* mutation has no detectable effect on the formation of the protein complex with arginine anabolic and catabolic promoters in vitro, but no portion of MCM1 required for DNA binding is missing in the mutant protein (7). It is noteworthy that most of the deletions created in vitro in the ARGRII protein, outside of the zinc finger which is its putative DNA binding domain, do not affect the mobility or the formation of a protein complex with arginine control regions (reference 28 and unpublished data).

Composition of the protein complex binding the *CAR1* and *CAR2* promoters. Competition experiments had suggested that the *ARG5,6*, *ARG3*, *CAR1*, and *CAR2* promoters were bound by the same protein complex. For the *ARG5,6* gene, binding activity required the integrity of ARGRI, ARGRII, and ARGRIII proteins, and gel shift assays in the presence of antibodies confirmed the participation of ARGRI and MCM1 in the protein-DNA complex (16). However, since the *mcm1-gcn4* mutation affects expression of the *CAR1* and *CAR2* genes in an opposite way than *argR* mutations, it seemed worthwhile to determine whether a complex of different composition interacts with *CAR1* and *CAR2* promoters in vitro. By gel retardation assays, we have already shown that strong binding to *CAR1* and *CAR2* promoters is observed only in the presence of 5 mM L-arginine and with proteins from cells in which the *ARGRII* gene is overexpressed, suggesting the participation of ARGRII in the protein-DNA complex (16). We have also checked the participation of MCM1, ARGRI, and ARGRIII in the formation of a protein-DNA interaction with these promoters.

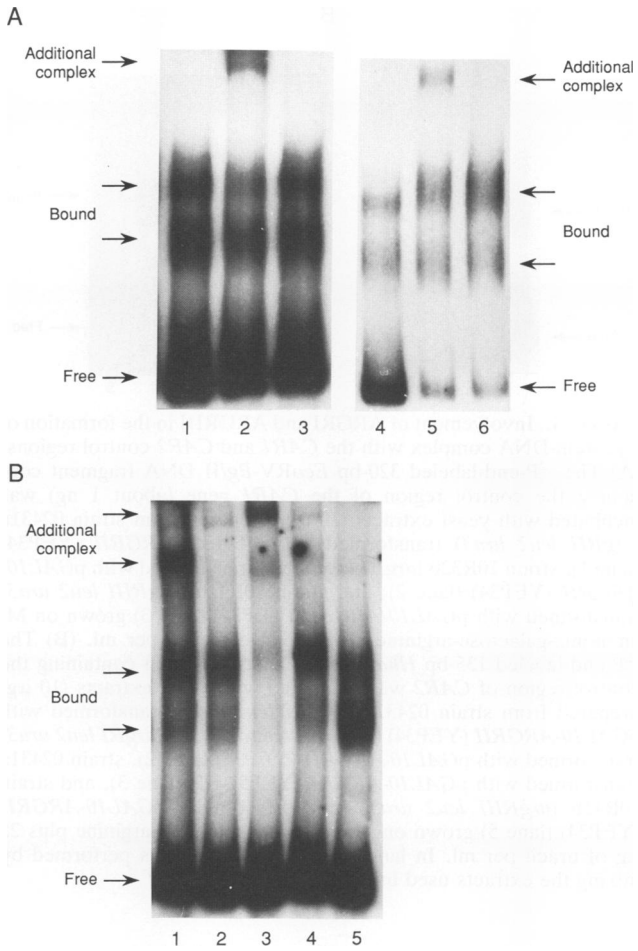

FIG. 2. Evidence that ARGRI and MCM1 are part of the protein-DNA complex with the *CAR1* and *CAR2* control regions. (A) The ^{32}P-end-labeled 320-bp *Eco*RV-*Bgl*II *CAR1* DNA fragment (about 1 ng) was incubated with a yeast extract prepared from strain 02431a (*argRII leu2 ura3*) transformed with p*GAL10-ARGRII* (YEP34) and grown on M. ammonia-galactose-arginine plus 25 μg of uracil per ml. The extract was incubated with ARGRI antibodies (lane 2) or MCM1 antibodies (lane 5) before incubation with the fragment. In lane 3, ARGRI antibodies were preincubated with ARGRI peptide before incubation with the extract. In lane 6, MCM1 antibodies were incubated with MCM1 peptide before incubation with the extract. (B) The ^{32}P-end-labeled 135-bp *Hha*I-*Hae*III *CAR2* DNA fragment (about 1 ng) was incubated with a yeast extract prepared from strain 02431a (*argRII leu2 ura3*) transformed with p*GAL10-ARGRII* (YEP34) and grown on M. ammonia-galactose-arginine plus 25 μg of uracil per ml (lane 5). The extract was incubated with ARGRI antibodies (lane 1) or MCM1 antibodies (lane 3) before incubation with the fragment. In lane 2, ARGRI antibodies were preincubated with ARGRI peptide before incubation with the extract. In lane 4, MCM1 antibodies were preincubated with MCM1 peptide before incubation with the extract.

Gel shift experiments were carried out to demonstrate the participation of MCM1 in the ARGR complex with *CAR1* and *CAR2* control regions. When antibodies raised to an internal peptide of MCM1 (a gift from G. Sprague [18]) with no homology with ARGRI (16) are added in the binding assay, an additional protein-DNA complex with reduced mobility is observed. This complex is absent when MCM1 antibodies are preincubated with MCM1 peptide prior to the

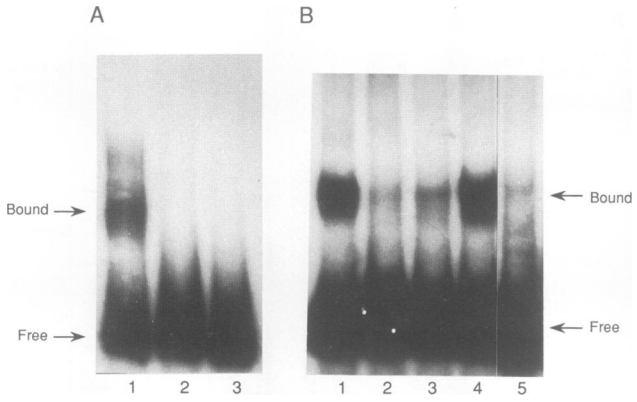

FIG. 3. Involvement of ARGRI and ARGRIII in the formation of a protein-DNA complex with the *CAR1* and *CAR2* control regions. (A) The ^{32}P-end-labeled 320-bp *Eco*RV-*Bgl*II DNA fragment containing the control region of the *CAR1* gene (about 1 ng) was incubated with yeast extracts (10 μg) prepared from strain 02431a (*argRII leu2 ura3*) transformed with p*GAL10-ARGRII* (YEP34) (lane 1), strain 10R32b (*argRI leu2 ura3*) transformed with p*GAL10-ARGRII* (YEP34) (lane 2), and strain 10R41b (*argRIII leu2 ura3*) transformed with p*GAL10-ARGRII* (YEP34) (lane 3) grown on M. ammonia-galactose-arginine plus 25 μg of uracil per ml. (B) The ^{32}P-end-labeled 135-bp *Hha*I-*Hae*III DNA fragment containing the control region of *CAR2* was incubated with yeast extracts (10 μg) prepared from strain 02431a (*argRII leu2 ura3*) transformed with p*GAL10-ARGRII* (YEP34) (lane 1), strain 10R32b (*argRI leu2 ura3*) transformed with p*GAL10-ARGRII* (YEP34) (lane 2), strain 02431a transformed with p*GAL10-ARGRI* (YEP52-RI) (lane 3), and strain 10R41b (*argRIII leu2 ura3*) transformed with p*GAL10-ARGRII* (YEP34) (lane 5) grown on M. ammonia-galactose-arginine plus 25 μg of uracil per ml. In lane 4, the experiment was performed by mixing the extracts used in lanes 2 and 3.

binding assay (Fig. 2A, lanes 4 to 6; Fig. 2B, lanes 3 to 5). Thus, a component of the complex shares an epitope with MCM1 protein.

ARGRI and ARGRIII are also required for in vitro protein binding to the *CAR1* and *CAR2* promoters. As shown in Fig. 3A (lanes 1 to 3) and B (lanes 1, 2, and 5), little or no binding occurred with extracts from either an *argRI* (10R32b) or *argRIII* (10R41b) strain transformed with a p*GAL10-ARGRII* plasmid (YEP34). An arginine-dependent complex at the *CAR2* promoter can be restored by adding an extract prepared from a strain containing overproduced ARGRI to an extract containing overexpressed ARGRII synthesized in an *argRI* mutant strain (Fig. 3B, lanes 2 to 4). A similar in vitro complementation was observed at the *CAR1* promoter (data not shown). Thus, the absence of binding with an extract from an *argRI* strain is not due to the instability of ARGRII protein in the extract. This result supports the idea that ARGRI has a direct role in formation of the arginine-dependent protein-DNA complex rather than a role in the generation of required posttranslational modifications of other proteins. The experiments presented in Fig. 2 provide additional evidence for the presence of ARGRI in the protein complex at the *CAR1* and *CAR2* promoters. Indeed, when antibodies raised against a 13-amino-acid peptide of ARGRI are added in the binding assay, an additional protein-DNA complex with reduced mobility is observed. The addition of the ARGRI peptide inhibits its formation (Fig. 2A, lanes 1 to 3; Fig. 2B, lanes 1, 2, and 5).

DISCUSSION

Genetic evidence provided many years ago (1, 30, 31) indicated that the *ARGRI*, *ARGRII*, and *ARGRIII* gene products were required for the in vivo repression of five arginine anabolic enzymes and induction of two catabolic enzymes by arginine. More recent data showed that the three ARGR proteins control the formation of a specific complex with the promoter of the *ARG5,6* gene in the presence of arginine and that MCM1 also seems to participate in this binding in vitro (16). We have also shown that the binding of proteins to the *ARG3*, *CAR1*, and *CAR2* arginine boxes present in these promoters required the presence of arginine and the overexpression of ARGRII (24). In this report, we present evidence indicating that ARGRI and MCM1 proteins are part of the protein complex interacting, in the presence of arginine, with the *CAR1* and *CAR2* catabolic promoters, as was shown previously for the *ARG5,6* anabolic promoter. ARGRIII is also required for the interaction of these proteins with their DNA targets, but we have no proof for its direct participation in the protein-DNA complex.

Up to now, it was difficult to establish the role of MCM1 in the regulation of arginine metabolism in vivo, as very few viable *mcm1* mutants were available. The *mcm1-gcn4* mutant produced by Christ and Tye (7), which is affected in expression of **a**- and α-specific genes, is impaired in the expression of arginine anabolic and catabolic genes controlled by the three ARGR proteins. An *argR* mutant expresses constitutively the anabolic pathway and is unable to induce the catabolic pathway. This *mcm1-gcn4* mutation impairs partially repression of anabolic genes by arginine and leads to high expression of the catabolic genes in the absence of arginine. The mutant behaves as a partial *argR* strain for OTCase expression, and there is a slight but reproducible cumulative effect when the *mcm1-gcn4* mutation is combined with an *argRII* mutation. The combination of *argRI*, *argRII*, and *argRIII* mutations has no such effect. The cumulative effect of the *argRII* and *mcm1-gcn4* mutations is somewhat surprising given that ARGRII is required for any protein-DNA complex formation at the OTCase gene promoter in vitro. Perhaps some complex is formed in vivo in the absence of ARGRII, or perhaps MCM1 binds at other sites. In any event, the cumulative effect of the *mcm1-gcn4* and *argRII* mutations may indicate that the MCM1 and ARGR proteins control expression of the OTCase structural gene by different means; their combined action is required to bring about complete arginine-mediated repression. Alternatively, the greater expression of OTCase in an *mcm1-gcn4 argRII* mutant than in an *argRI argRII* double mutant reflects different transcription activation potentials of wild-type and mutant MCM1. In the absence of ARGR repression, mcm1-gcn4 may be able to make a greater contribution to transcription than can wild-type MCM1. A comparison of the level of OTCase expression in the *argRI argRII* double mutant to expression of an OTCase gene deleted of the arginine boxes, elements that confer arginine-mediated repression, may help resolve which of these two possibilities is correct.

Control of expression of the two catabolic genes is more complex, since in addition to induction by arginine and the three ARGR proteins, three repressors (CARGRI, CARGRII, and CARGRIII) exert a negative control. Both *cargR* and *mcm1-gcn4* mutants exhibit expression of the catabolic genes in the absence of arginine, but the basis of expression appears to be different in the two mutants. This

conclusion follows from two observations. First, the expression that occurs in *cargR* mutants does not require the ARGR products (13, 14), but the expression that occurs in the *mcm1-gcn4* mutant does require ARGR function. Second, a *cargR mcm1-gcn4* double mutant exhibits greater expression of the catabolic genes than does the *mcm1-gcn4* single mutant. In keeping with the idea that the CARGR and the ARGR proteins play separate roles in regulating expression of the catabolic genes, the proteins appear to act at distinct sites, MCM1 at the arginine boxes and CARGRI at an adjacent sequence (reference 9 and unpublished data).

Thus, MCM1 and the ARGR proteins participate together in the induction of arginine catabolic genes by arginine. The higher expression of the *CAR1* and *CAR2* genes in the *mcm1-gcn4* mutant could be due to an increase in DNA binding affinity of the regulatory complex or to an increase of activation capacity of this complex once it is bound to DNA. As our band shift assay does not seem to show that the *mcm1-gcn4* mutation enhances the efficiency of protein binding to DNA, at least in vitro, we favor the idea that the activation capacity of the mutated complex is increased. As the acidic domain of MCM1 was replaced by the efficient acidic domain of GCN4, the activation capacity of the mutated mcm1 protein could be strongly enhanced, and the complex bound to DNA on ammonia could allow high expression of *CAR1* and *CAR2* genes. This hypothesis is supported by results presented by Christ and Tye suggesting an increase of transcription activation potential of the mutant protein (7).

Analysis of this *mcm1-gcn4* mutant provided the first genetic evidence for involvement of MCM1 in arginine regulation. However, since this mutant is quite peculiar, its behavior may not be totally representative of a loss of MCM1 function, as occurs probably for its effect on expression of arginine catabolism. At this point, it becomes necessary to generate a series of *mcm1* mutations that would not affect the role of MCM1 in cell viability but would affect its interaction with the ARGR proteins. Study of such mutants could enable identification of the domains of MCM1 that are required for regulation of arginine metabolism.

ACKNOWLEDGMENTS

We are very grateful to C. Christ and Bik-Kwoon Tye for the gift of the *mcm1-gcn4* mutant strain, to E. Joris for skillful technical assistance, and to J. P. ten Have for preparing the photographs.

Part of this work was supported by the Research Council of the Université Libre de Bruxelles.

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