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Nitrogen Catabolite Repression of Arginase (*CAR1*) Expression in *Saccharomyces cerevisiae* Is Derived from Regulated Inducer Exclusion

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Expression of the *Saccharomyces cerevisiae* arginase (*CAR1*) gene is regulated by induction and nitrogen catabolite repression (NCR). Arginine was demonstrated to be the native inducer. *CAR1* sensitivity to NCR has long been accepted to be accomplished through a negative control mechanism, and *cis*-acting sites for it have been hypothesized. In search of this negatively acting site, we discovered that *CAR1* sensitivity to NCR derives from regulated inducer (arginine) exclusion. The route of catabolic entry of arginine into the cell, the general amino acid permease (GAP1), is sensitive to NCR. However, *CAR1* expression in the presence of sufficient intracellular arginine is NCR insensitive.

Arginine is both an anabolic substrate for protein synthesis and a catabolic nitrogen source in *Saccharomyces cerevisiae* (4). Concomitant with the multiple metabolic roles fulfilled by arginine, Middlehoven reported that arginase production was regulated in two ways (22–25). Production increased when arginine was added to the culture medium (induction) and decreased when cells were provided with readily used nitrogen sources such as asparagine or glutamine (nitrogen catabolite repression [NCR]) (2, 22–25).

Whitney and Magasanik identified arginine itself or the arginine analog homoarginine as the small molecule signal for induced production of arginase (36). Isolation of a *cis*-dominant mutation resulting in inducer-independent production of arginase (*CAR1-0⁻* mutation) provided the first indication that induced arginase production might be regulated at the level of transcription (37). We and others subsequently cloned the arginase gene (*CAR1*) and demonstrated that steady-state *CAR1* mRNA levels significantly increased and decreased in response to induction and NCR, observations consistent with transcriptional control (9, 15, 29). The most convincing evidence for transcriptional control, however, was provided by identifying the molecular lesion of the *CAR1-0⁻* mutation as a C-to-G transversion at *CAR1* upstream position −153 and the finding that all of the *cis*-acting elements required for normally regulated *CAR1* expression were situated upstream from the start of transcription (8, 30–34).

The *CAR1* promoter consists of four *cis*-acting elements in the order 5′-UAS (upstream activation sequence)$_{C1}$-UAS$_{C2}$-UAS$_{I}$-URS1 (upstream regulatory sequence 1)-3′ (17–20). Both UAS$_{C1}$, consisting of two ABF-1 and two RAP1 protein binding sites, and UAS$_{C2}$ function in an inducer-independent fashion (17–19). In contrast, the operation of UAS$_{I}$ possesses an absolute requirement for arginine (19). URS1, the site of the *CAR1-0⁻* mutation (32), is the binding site for a general transcription factor participating in the regulated expression of many yeast genes (20). Mutation of three unlinked loci, *ARG80*, *ARG81*, and *ARG82* (ARGRI, ARGRII, and ARGIII) have been shown to result in loss of induced arginase production (1, 37). The region at which one of these proteins (*ARG81* ARGRII) putatively acts upstream of *CAR1* has been proposed. This conclusion was based on the demonstration of protein binding to a 55-bp region by gel shift and footprinting experiments using long (311- and 219-bp) DNA fragments. This 55-bp region contained sequences that were similar to those contained upstream of the biosynthetic *ARG3* and *ARG5,6* genes, which have been more thoroughly studied (21). More detailed studies of protein binding to small *CAR1* DNA fragments contained within the 311- and 219-bp fragments used in the earlier report demonstrate the presence of seven to nine specific protein binding sites (17–20).

Studies of the *CAR1* gene's NCR sensitivity have been formally similar to those addressing induction. Steady-state levels of *CAR1*-specific mRNA decreased to undetectable levels when cells were grown with a readily used nitrogen source such as asparagine, an observation consistent with the hypothesis that NCR was exerted at transcription (29). This hypothesis was further and more strongly supported by two observations: (i) reporter gene expression driven by the *CAR1* 5′ region (cloned into an expression vector) was sensitive to NCR, and (ii) *CAR1* expression in cells containing the *CAR1-0⁻* mutation at position −153, upstream of the *CAR1* transcription site, was significantly resistant to NCR (50% of the derepressed level of expression was observed under repressive growth conditions) (31–34).

Together, these observations not only were consistent with the suggestion that NCR of *CAR1* expression was exerted at transcription but also raised the possibility that NCR might be a negatively acting regulatory process operating through URS1. Although this explanation was coherent within the framework of *CAR1* expression, it could not be easily reconciled with data obtained through studies of allantoin degradative gene expression and its sensitivity to NCR (7). All of the allantoin degradative genes in *S. cerevisiae* contain multiple copies of UAS$_{NTR}$ (3, 27, 38). This element, which contains the sequence 5′-GATAA-3′ at its core and requires a wild-type GLN3 protein for operation, has been shown to be absolutely necessary for expression of the *DAL5* and *DAL7* genes and to be sufficient for sensitivity to NCR (6, 7). A corollary of the allantoin system studies

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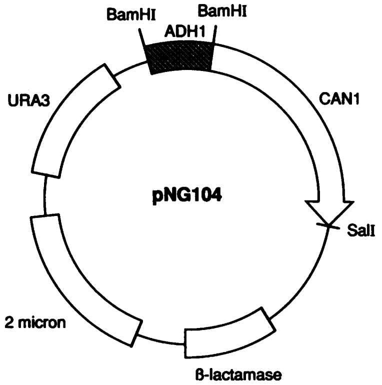

FIG. 1. *ADH1-CAN1* promoter fusion plasmid used for the experiment shown in Table 1. The construction and characterization of this plasmid are described elsewhere (11, 13). The plasmid contains the *ADH1* 5′ regulatory region fused to the *CAN1* gene. As a result of this fusion, *CAN1* expression is placed under *ADH1* control. The derived *CAN1* expression is not responsive to induction by arginine or NCR sensitive.

was that NCR operates through a positively acting rather than a negatively acting process (6, 7).

The present work, therefore, consisted of two objectives. The first was to identify the *cis*-acting element(s) through which NCR sensitivity of *CAR1* expression was exerted and to determine whether the regulatory process was negatively acting as previously suggested by Wiame (37) or was positively acting as observed for the allantoin degradative genes (6, 7). The second objective was to reconcile conclusions derived from studies of the *DAL* and *CAR* genes and their sensitivity to NCR. In other words, was NCR of *DAL* and *CAR* gene expression exerted through different mechanisms?

MATERIALS AND METHODS

Strains and culture methods. *S. cerevisiae* RH218 (*MAT***a** *trp1 CUP1 gal2 SUC2* Mal$^-$) and M1682-19b (*MAT***a** *ura3-52 trp1-289*) were used throughout this work. The *Escherichia coli* strains used for cloning were HB101 (*hsd20 levB supE44 ara14 galK2 lacY1 proA2 rpsL20 xyl5 mtl1 recA13 mcrB*) and SURE (*recB recJ sbcC201 urvC umvC mcrA mcrBC mrr lac hsdRM5 endA1 gyrA96 thi-1 relA1 supE44* [F′ *proAB lacI*^{q} *Z*ΔM15 Tn*10*]) (Stratagene). Yeast cultures for β-galactosidase assays were grown in medium containing 0.17% Difco yeast nitrogen base without amino acids or ammonium sulfate, 2% glucose, and 0.1% arginine, 0.1% glutamate, 0.1% asparagine, or 0.26% (0.02 M) ammonia.

Transformations, cotransformations, and β-galactosidase assays. *E. coli* and yeast transformation procedures were described earlier (19). Yeast transformations were performed by the spheroplast procedure, using strain RH218 as the recipient (12). Cotransformation of yeast cultures with two plasmids was performed by the lithium acetate technique (14), using strain M1682-19b as the recipient. This strain was transformed to the Trp$^+$ Ura$^+$ phenotype by two plasmids, one carrying *TRP1* and the other carrying *URA3*. Plasmid pNG104 (Fig. 1), containing an *ADH1-CAN1* promoter fusion, was a generous gift from Neil Green (11, 13). Since all plasmids used in this work contained an autonomously replicating sequence, we took precautions to avoid problems that might result from a varying copy number. These precautions were described in great detail earlier (3, 19, 35, 37). The effect of varying copy number has been evaluated in the past by comparing results with *CEN* and *ARS* plasmids and found not to be an influencing factor (3, 35).

Plasmid constructions. The *CAR1* 5′ deletion plasmids, the *CAR1* URS1-containing plasmids, and plasmid pRS124 were described earlier (32, 33). Similarly, all of the plasmids used for the experiments shown in Fig. 4 to 6 were described in detail elsewhere (19).

β-Galactosidase assays. The β-galactosidase assay was described earlier (19). Enzyme activity was expressed in units defined by Miller (26), with the modification that 25-ml cultures were collected for assay.

RESULTS

Deletion analysis of the *CAR1* 5′ region. A mechanistic understanding of *CAR1* sensitivity to NCR requires identification of the sequences required for that sensitivity. To meet this requirement, we conducted a 5′ deletion analysis of *CAR1*. The deletion plasmids used in this experiment were derived from a fusion plasmid in which the entire upstream region of *CAR1* was fused to the *E. coli lacZ* gene (19, 31–34). These plasmids were used as sources of DNA to transform wild-type strain RH218. Transformants were grown in media containing either arginine alone or arginine plus asparagine as the nitrogen source and assayed for β-galactosidase activity. Growth in minimal glucose-arginine medium resulted in a profile of β-galactosidase activities that was dependent on the array of *cis*-acting elements that each of the constructions possessed (Fig. 2). These observations, their interpretation, and substantiation through identification of the various transcription factor binding sites have been discussed in detail elsewhere (19). For our present objectives, the important finding was that every plasmid that supported reporter gene expression on arginine alone did so in a manner that was sensitive to NCR (Fig. 2). The values observed when transformants were grown in arginine-plus-asparagine medium were 20- to 80-fold lower than those observed in arginine medium (Fig. 2).

These observations could be interpreted in either of two ways. One possibility was that the sequences required for NCR sensitivity were situated 3′ to position −190. A possible corollary of this hypothesis was that NCR was a negatively acting regulatory process, with URS1 acting as the negative site mediating NCR. Alternatively, NCR might be mediated through one or more of the three UAS elements previously shown to be situated between *CAR1* positions −160 and −516. According to this hypothesis, UAS function would be expected to be sensitive to NCR. The second possibility would further suggest that NCR of *CAR1* expression was not a negative process as previously reported.

Assay of *CAR1* URS1 element function for NCR sensitivity. To test the first hypothesis mentioned above, we determined whether URS1 function was sensitive to NCR. This was done by assaying the ability of URS1 to repress operation of an expression vector-borne heterologous UAS (UAS$_{CYC1}$) carried in cells grown in media containing repressive (asparagine) and nonrepressive (arginine) nitrogen sources. All of the constructions used in this experiment were the same ones originally used to demonstrate the URS1 element's function (32). As shown in Fig. 3, reporter gene (*lacZ*) expression was repressed 28-fold by the presence of URS1 in cells growing in minimal asparagine medium (compare plasmids pRS53 and pRS185); β-galactosidase production was repressed 68-fold in cells provided with arginine. One might

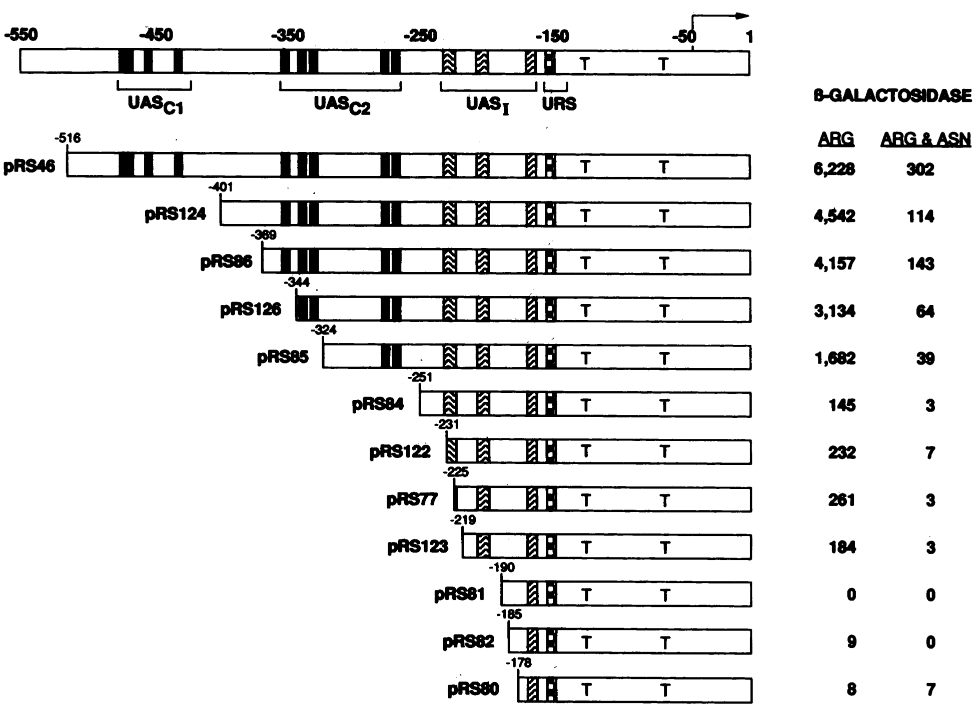

FIG. 2. Reporter gene expression supported by various *CAR1* 5′ deletion plasmids in cells grown under derepressive (arginine [ARG]) or repressive (arginine-plus-asparagine [ARG & ASN]) conditions. Areas designated UAS$_{C1}$, UAS$_{C2}$, UAS$_I$, and URS as indicated at the top have been described previously. They contain the sequences shown to be responsible for regulated expression of *CAR1* (17, 19, 20, 32). T's indicate the positions of TATA sequences. The number at the left of each plasmid construct indicates the 5′ end of the remaining *CAR1* DNA in the *CAR1-lacZ* fusions. Coordinates in this and all subsequent figures are relative to the start of translation. All of the values reported here and in Fig. 2 of reference 19 were obtained from the same experiment.

conclude that the threefold difference observed between the two results was significant. This is probably not the case, first because the validity of this interpretation depends on a very small difference between two of the lowest values, both of which are below the background levels of expression observed with a vector devoid of a UAS element (plasmid pRS179). Second, the in vivo difference between the levels of *CAR1* expression observed under repressive and derepressive conditions has been reported to range from 20- to 100-fold (29, 37) (Fig. 2), not 2- to 3-fold. Similarly, the levels of NCR-sensitive, UAS$_{NTR}$-mediated reporter gene expression that occur under conditions of severe and weak NCR are vastly different (7). In no case was the extent of URS efficacy in the control of UAS$_{CYC1}$ operation correlated with the degree of NCR. In fact, the results observed were just the opposite of those expected if URS1 was the site mediating NCR, thereby arguing against the possibility of URS1 being the *cis*-acting site of NCR control. Parenthetically, the difference between the arginine and asparagine values observed with these plasmids (compare values for plasmid pRS53, for example) is a result of the fact that asparagine is a better nitrogen source than arginine and supports a higher overall synthetic capacity in the cell, as has been noted elsewhere (19).

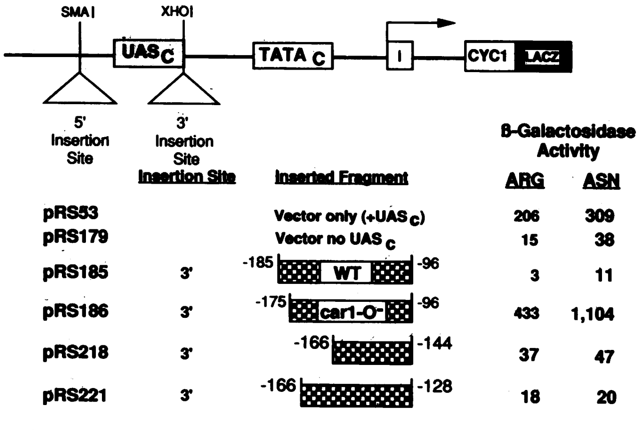

FIG. 3. Repression of the UAS$_{CYC1}$ operation mediated by DNA fragments carrying wild-type and mutant forms of the *CAR1* URS1 element in cells growing in minimal medium containing either a repressive (asparagine [ASN]) or derepressive (arginine [ARG]) nitrogen source. Construction and structures of the plasmids have been described in detail elsewhere (33).

Assay of *CAR1* UAS element function for NCR sensitivity. To test the second hypothesis, we assayed each of the UAS elements previously demonstrated to be responsible for *CAR1* expression. As shown in Fig. 4, all of the plasmids containing the *CAR1* UAS$_{C1}$ element or portions of it supported more reporter gene expression when cultures were grown with asparagine than when they were grown with arginine; i.e., there was no sensitivity to NCR. A similar result was observed with all of the plasmids containing the *CAR1* UAS$_{C2}$ element (Fig. 5). The fact that both 5′ and 3′ deletions were contained among the constructions analyzed argued against an undetected negatively acting site being located in these regions. If such were the case, one of the deletions would have been expected to result in a marked

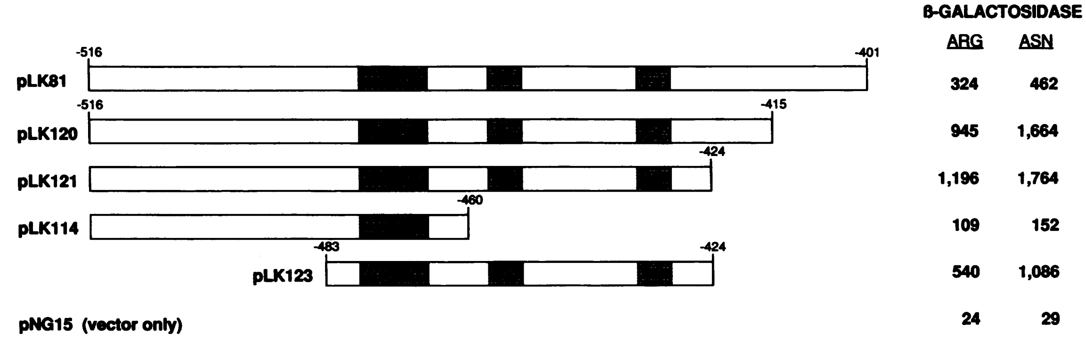

FIG. 4. β-Galactosidase production supported by plasmids containing the *CAR1* UAS_{C1} regulatory region. The plasmids were generated by cloning *CAR1* upstream fragments into the expression vector pNG15. The 5′ and 3′ termini of the inserts are as indicated in the figure and have been described in detail earlier (19). Transformants containing these plasmids were cultured in either minimal arginine (ARG) or asparagine (ASN) medium.

increase in activity when the putative negatively acting element was deleted.

A different response was observed when this form of analysis was conducted with constructions containing the *CAR1* UAS_I element. β-Galactosidase production exhibited significant NCR sensitivity. As shown in Fig. 6, reporter gene expression supported by the *CAR1* UAS_I element decreased from 813 U, when a culture containing plasmid pLK40 was grown in minimal glucose-arginine medium, to 233 U when the glucose-arginine-asparagine medium was used instead. In an analogous experiment using plasmid pLK49, the corresponding values were 252 and 55 U, respectively.

Insensitivity of *CAR1* expression to NCR when arginine uptake is constitutive. The simplest interpretation of the data presented in Fig. 6 was that *CAR1* UAS_I functioned only under conditions of minimal NCR. However, this interpretation harbors the caveat that inducer exclusion from the cell under conditions of NCR did not affect the results. This caveat derived from the fact that operation of *CAR1* UAS_I

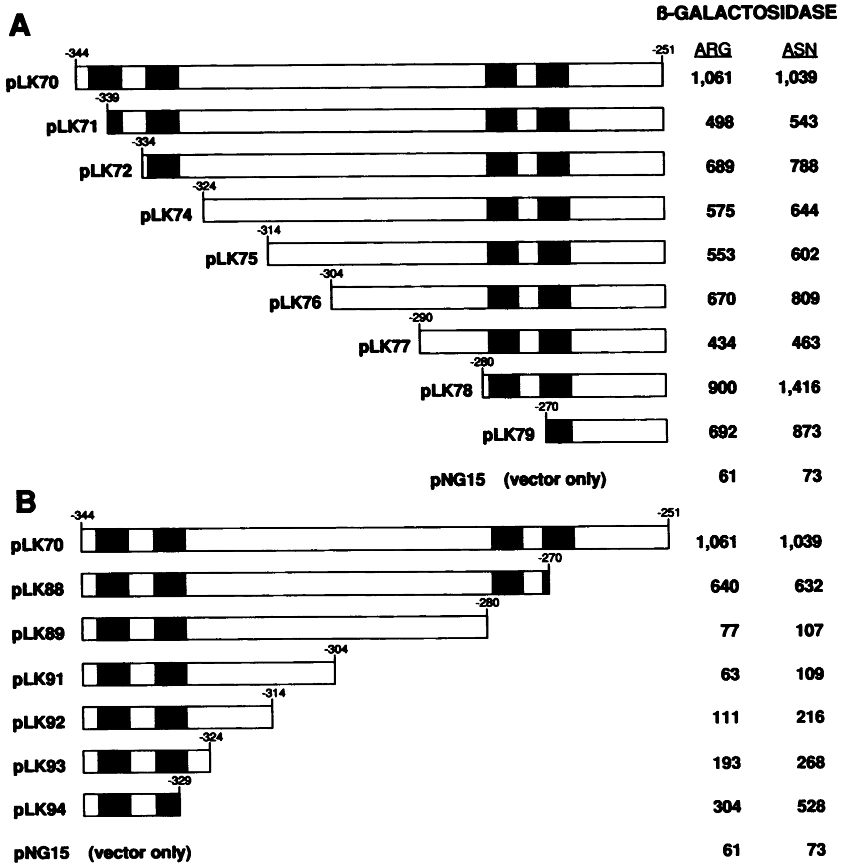

FIG. 5. β-Galactosidase production supported by plasmids containing the *CAR1* UAS_{C2} regulatory region. The plasmids used have been described earlier (19). The culture and assay conditions were as described for Fig. 4.

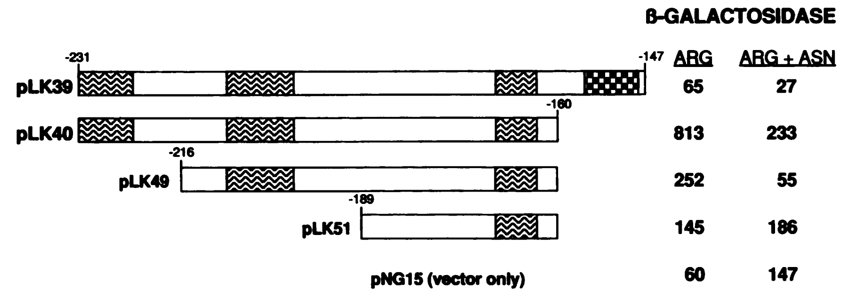

FIG. 6. β-Galactosidase production supported by the *CAR1* UAS$_I$ regulatory region. The plasmids used have been described earlier (19). Plasmid pLK39 also contains the *CAR1* URS1 site indicated (19).

had been previously shown to absolutely require the presence of an inducer, arginine, within the cell (19). Arginine enters the cell by way of a low-capacity biosynthetic transport system mediated by the *CAN1* product and a high-capacity catabolic transport system mediated by the *GAP1* product (5). In keeping with these metabolic functions, it is broadly accepted and has been shown indirectly that expression of *CAN1* is insensitive to NCR. *GAP1* expression has been recently shown to be sensitive to NCR (16). To determine whether loss of *CAR1* UAS$_I$ function, under conditions of NCR, derived from inducer exclusion rather than NCR sensitivity of *CAR1* transcription, we performed the following experiment. Wild-type strain M1682-19b (Trp$^-$ Ura$^-$) was transformed with either of two pairs of plasmids: (i) plasmid pRS124, carrying the entire *CAR1* upstream region fused to *lacZ* and a wild-type *TRP1* gene, and vector plasmid YEp24, carrying the *URA3* gene; or (ii) plasmid pRS124 along with plasmid pNG104, which is plasmid YEp24 carrying the *CAN1* gene coding region fused to the *ADH1* promoter (Fig. 1). Plasmid pNG104 expresses *CAN1* at higher levels than would normally be found in the cell (11). Therefore, inducer exclusion of arginine would be significantly diminished during measurements of the effects of NCR on *CAR1* expression in a transformant carrying plasmid pNG104. When cells that expressed *CAN1* at its normal wild-type levels (transformants containing plasmids pRS124 and YEp24) were grown in minimal arginine medium, they produced 18,390 U of β-galactosidase. Addition of glutamate, ammonia, or asparagine along with arginine to the medium resulted in 3.3-, 18.3-, and 22.5-fold repression of reporter gene expression in these cells, respectively (Table 1). When this experiment was repeated under conditions in which *CAN1* was expressed at high level (pRS124 plus pNG104), the corresponding values observed following addition of glutamate, ammonia, or asparagine were 1.0, 1.2, and 1.2, respectively. In other words, there was a strong effect of nitrogen source on reporter gene (*lacZ*) expression driven by the *CAR1* promoter in cells containing normal levels of arginine permease, the *CAN1* product. This effect of nitrogen source, and hence NCR, largely disappeared when the *CAN1* product was present at higher intracellular concentrations. The effects of plasmid pNG104 on inducer-independent, basal-level *CAR1* expression that we observed when ammonia or glutamate alone was provided as the nitrogen sources was minimal (Table 1).

DISCUSSION

Data presented in this work suggest that loss of *CAR1* expression when cultures of *S. cerevisiae* are grown in the presence of repressive nitrogen sources derives from inadequate intracellular concentrations of the inducer, arginine, rather than NCR sensitivity of *CAR1* transcription. Previous reports of *CAR1* sensitivity to NCR did not consider inducer exclusion as a possible mechanism of action (37). However, Courchesne and Magasanik have suggested that *CAR1* sensitivity to NCR might derive from inducer exclusion as an explanation of the phenotype of a mutation that they isolated, but they did not address the issue experimentally (8a). Two further deductions may be derived from these data: *CAR1* expression is perhaps more sensitive to inducer concentration than previously realized, and the rate at which arginine can enter the cell when being transported by the biosynthetic *CAN1* system alone is insufficient to support the intracellular concentrations of arginine needed to induce *CAR1* expression.

These data also afford several interesting insights into the functional integration of *cis*-acting elements mediating regulated *CAR1* transcription and the mechanisms of metabolic integration in this organism. Data in Fig. 3 demonstrated that

TABLE 1. Insensitivity of *CAR1* expression to nitrogen catabolite repression when arginine uptake is constitutive

Nitrogen source	β-Galactosidase activity (U)[a]	
	pRS124 + YEp24 (*CAR1* 5′ region)	pRS124 + pNG104 (*CAR1* 5′ region + *ADH1-CAN1*)
0.1% arginine	18,390	10,819
0.1% arginine + 0.1% Glutamate	5,648 (3.3)	11,043 (1.0)
0.1% arginine + 0.26% ammonia	1,007 (18.5)	8,879 (1.2)
0.1% arginine + 0.1% asparagine	819 (22.5)	8,765 (1.2)
0.1% glutamate	587	816
0.26% ammonia	456	445

[a] β-Galactosidase production of pRS124-YEp24 and pRS124-pNG104 cotransformants was measured on the indicated nitrogen sources in liquid medium. Inserts in the plasmids used are given in parenthesis. Values in parenthesis indicate the quotients of β-galactosidase activity observed with the indicated nitrogen source divided into that observed with 0.1% arginine alone.

the presence of the *CAR1* URS1 repressor binding site did not result in the acquisition of NCR sensitivity when placed 3′ of a heterologous UAS. Why then, did mutation or deletion of the *CAR1* URS1 element in its native location upstream of *CAR1* generate apparent partial resistance to NCR? This question comes to the heart of the functional integration of the *CAR1 cis*-acting elements and the proteins associated with them. Our present and previously reported data are consistent with the suggestion that the function of the *CAR1* URS1 repressor site and its associated protein(s) is to act as a negative balance for the two strong, constitutively functioning *CAR1* UASs, UAS_{C1} and UAS_{C2} (19, 20, 33). According to this model, inducer-independent, NCR-insensitive *CAR1* expression observed when *CAR1* URS1 is mutated or deleted derives from the action of these two UAS elements, both of whose operation has been shown to be insensitive to NCR and independent of inducer. Conversely, when URS1 is functional, the negative effects of its action on the *CAR1* transcriptional apparatus overpowers these constitutively operating *CAR1* UASs, thereby maintaining arginase at a low basal level when arginine is not provided in the medium or is not made available in significant quantities from the vacuole following nitrogen starvation (28). When intracellular arginine becomes metabolically available in adequate quantities, the inducer-dependent UAS_I operates. We suggest that the three *CAR1* UASs all functioning together overcome the negative effects of the *CAR1* URS1 site and its associated repressor proteins. Therefore, arginase production then occurs. This is not a novel concept; it is the one on which the operation of a seesaw or old-fashioned analytical balance operates. When inducer is absent, URS action outweighs the combined strength of the UAS_{C1} and UAS_{C2} elements. When inducer is present, the addition of UAS_I operation to that of the constitutive UAS_{C1} and UAS_{C2} tips the balance in the other direction and *CAR1* expression occurs. Although the regulatory proteins and their target sites upstream of the allantoin pathway genes are different, it is not surprising that the same formal regulatory interactions describe the mechanisms through which inducible *DAL* and *DUR* gene expression is accomplished (38).

Data presented in this work (Fig. 2; Table 1), those of Kovari et al. (Fig. 2) (19), and those of Dubois et al. (10) could be construed to suggest the presence of multiple mechanisms responsible for sensitivity of arginase production or *CAR1* expression to NCR. One mechanism would be the previously described one which is exerted through UAS_{NTR} and accounts for inducer exclusion of the *CAR1* inducer (7). The second one would hypothetically be a mechanism involving negatively acting elements. The pertinent observations that appear in these reports is that there is less arginase production (10) or reporter gene expression (19, 32; this work) when asparagine or ammonia is provided as a nitrogen source than there is when glutamate is used instead. Stated in another way, the basal level of *CAR1* expression exhibits characteristics expected of an NCR-sensitive process. In this case, inducer exclusion would not be a satisfactory explanation, because no inducer is added to the culture medium. Although, the existence of two mechanisms of NCR is formally conceivable, it is probably unlikely. Instead, we suggest that the decreased arginase production observed when values obtained with glutamate are compared with those obtained with asparagine as the nitrogen source derives from the effects of these two nitrogen sources on mobilization of the large vacuolar pools of arginine. This mobilization of vacuolar arginine in response to nitrogen limitation, which is what occurs when there is a shift from asparagine to glutamate, has been shown previously (28).

This work also provides an interesting insight into the metabolic integration of the biosynthetic and catabolic transport systems that mediate arginine transport into the cell. Extracellular arginine, present along with a repressive nitrogen source, enters the cell via a low-capacity biosynthetic *CAN1* transport system (5). No data have been reported to indicate that *CAN1* expression is sensitive to NCR, and indirect experiments suggest that it is not, as expected since the transport system is part of the cell's anabolic metabolism (5). When extracellular arginine is present alone and no other repressive nitrogen source is available, the high-capacity general amino acid permease mediates arginine uptake as well. This catabolic permease mediates uptake of nearly all amino acids when they are provided in quantities sufficient to serve as nitrogen sources. The latter permease is encoded by the *GAP1* gene, which has been reported to be sensitive to NCR (16). The correlations and the data presented in this work suggest that arginine flux into the cell via the anabolic *CAN1* permease is alone insufficient to support *CAR1* induction unless it is significantly overproduced, as occurred when plasmid pNG104 was transformed into the cells. Therefore, the catabolic *GAP1*-encoded permease must function in order to achieve arginine fluxes that are adequate for *CAR1* induction. Since *GAP1* expression is known to be sensitive to NCR, *CAR1* expression can be made indirectly NCR sensitive through the regulation of *GAP1* and exclusion of inducer arginine when cells are grown under nitrogen repressive conditions. In this manner, the metabolic economy of avoiding expression of the catabolic *CAR* genes when more readily used nitrogen sources are available is effectively achieved even though the *CAR* genes themselves do not possess the requisite *cis*-acting elements required for sensitivity to NCR.

One of our original reasons for studying the *CAR* gene system was to address the general question of the mechanisms through which NCR is accomplished. On the basis of information available, we considered that both allantoin and arginine degradative genes were sensitive to NCR. Therefore, by unambiguously identifying the *CAR* and *DAL* gene *cis*-acting elements required for NCR sensitivity, we would be able to determine whether or not they were the same. This would in turn provide some insight into the question of whether only a single type of *cis*-acting site was required, or alternatively whether multiple different sites could mediate NCR sensitivity. The latter situation would require that mechanistic explanations of NCR account for multiple proteins functioning in the process at the structural gene level and suggest how their action might be controlled. In the case of allantoin gene expression, we have demonstrated that UAS_{NTR}, a dodecanucleotide UAS containing the sequence 5′GATAA-3′ at its core, is necessary and sufficient for sensitivity to NCR (7). Since the *CAR1* upstream region did not contain copies of UAS_{NTR} that would be considered to be functional on the basis of previous genetic analysis of UAS_{NTR} from the *DAL5* gene (3, 27), it was possible that *CAR1* NCR sensitivity could be linked to a new *cis*-acting sequence. The potentially significant correlation to emanate from the data in this report is that the *CAR1* upstream region did not contain UAS_{NTR} sequences and was not sensitive to NCR. The *GAP1* gene, on the other hand, which our data argue is the controlling factor in apparent *CAR1* sensitivity to NCR, is sensitive to NCR, and a search of its upstream sequence (16) reveals that it contains multiple sequences that are homologous to UAS_{NTR}. This correlation and the fact that the 10 NCR-sensitive genes of the allantoin degradative system all contain multiple copies of sequences homologous to $\mathrm{UAS}_{\mathrm{NTR}}$ lead us to suggest NCR is probably mediated through a single *cis*-acting site and therefore involves a single set of regulatory proteins. It also makes the prediction that genes whose expression is truly NCR sensitive will contain multiple copies of the $\mathrm{UAS}_{\mathrm{NTR}}$ element. If *UGA1* gene expression is found to be NCR sensitive, as expected from the biological function of its product, analysis of its promoter can be expected to be a reasonable test of this prediction.

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REFERENCES

1. **Bechet, J., M. Grenson, and J. M. Wiame.** 1970. Mutations affecting the repressibility of arginine biosynthetic enzymes in *Saccharomyces cerevisiae*. Eur. J. Biochem. **12:**31–39.
2. **Bossinger, J., and T. G. Cooper.** 1977. Molecular events associated with induction of arginase in *Saccharomyces cerevisiae*. J. Bacteriol. **131:**163–173.
3. **Bysani, N., J. R. Daugherty, and T. G. Cooper.** 1991. Saturation mutagenesis of the $\mathrm{UAS}_{\mathrm{NTR}}$ (GATAA) responsible for nitrogen catabolite repression-sensitive transcriptional activation of the allantoin pathway genes in *Saccharomyces cerevisiae*. J. Bacteriol. **173:**4977–4982.
4. **Cooper, T. G.** 1982. Nitrogen metabolism in *Saccharomyces cerevisiae*, p. 39–99. *In* J. Strathern, E. Jones, and J. Broach (ed.), Molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Cooper, T. G.** 1982. Transport in *Saccharomyces cerevisiae*, p. 399–461. *In* J. Strathern, E. Jones, and J. Broach (ed.), Molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Cooper, T. G., D. Ferguson, R. Rai, and N. Bysani.** 1990. The *GLN3* gene product is required for transcriptional activation of allantoin system gene expression in *Saccharomyces cerevisiae*. J. Bacteriol. **172:**1014–1018.
7. **Cooper, T. G., R. Rai, and H. S. Yoo.** 1989. Requirement of upstream activation sequences for nitrogen catabolite repression of the allantoin system genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**5440–5444.
8. **Cooper, T. G., and R. A. Sumrada.** 1986. Dual regulation of the arginase gene, p. 37–44. *In* Shuji Tohno et al. (ed.), Immunochemotherapy of cancer. Hirosaki University School of Medicine, Hirosaki, Japan.

8a. **Courchesne, W. E., and B. Magasanik.** 1983. Ammonia regulation of ammonia acid permeases in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **3:**672–683.

9. **Cunin, R., E. Dubois, G. Vanthienen, K. Tinel, A. Jacobs, and M. Crabeel.** 1986. Positive and negative regulation of *CAR1* expression in Saccharomyces cerevisiae. Mol. Gen. Genet. **205:**170–175.
10. **Dubois, E., M. Grenson, and J.-M. Wiame.** 1974. The participation of the anabolic glutamate dehydrogenase in the nitrogen catabolite repression of arginase in *Saccharomyces cerevisiae*. Eur. J. Biochem. **48:**603–616.
11. **Green, N., and P. Walter.** 1992. C-terminal sequences can inhibit the insertion of membrane proteins into the endoplasmic reticulum of *Saccharmoyces cerevisiae*. Mol. Cell. Biol. **12:**276–282.
12. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. USA **75:**1929–1933.
13. **Hoffman, W.** 1985. Molecular characterization of the *CAN1* locus in *Saccharomyces cerevisiae*. J. Biol. Chem. **260:**11831–11837.
14. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.
15. **Jauniaux, J.-C., E. Dubois, S. Vissers, M. Crabeel, and J.-M. Wiame.** 1982. Molecular cloning, DNA structure, and RNA analysis of the arginase gene in *Saccharomyces cerevisiae*. A study of cis-dominant regulatory mutations. EMBO J. **9:**1125–1131.
16. **Jauniaux, J.-C., and M. Grenson.** 1990. *GAP1*, the general amino acid permease gene of *Saccharomyces cerevisiae*. Eur. J. Biochem. **190:**39–44.
17. **Kovari, L., and T. G. Cooper.** 1991. Participation of ABF-1 protein in expression of the *Saccharomyces cerevisiae CAR1* gene. J. Bacteriol. **173:**6332–6338.
18. **Kovari, L., and T. G. Cooper.** Unpublished data.
19. **Kovari, L., R. Sumrada, I. Kovari, and T. G. Cooper.** 1990. Multiple positive and negative *cis*-acting elements mediate induced arginase (*CAR1*) gene expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**5087–5097.

19a. **Kovari, L., H. H. J. van Vuuren, H. D. Park, and T. G. Cooper.** Unpublished data.

20. **Luche, R. M., R. A. Sumrada, and T. G. Cooper.** 1990. A *cis*-acting element present in multiple genes serves as a repressor protein binding site for the yeast *CAR1* gene. Mol. Cell. Biol. **10:**3884–4895.
21. **Messenguy, F., E. Dubois, and C. Boonchird.** 1991. Determination of the DNA-binding sequences of ARGR proteins to arginine anabolic and catabolic promoters. Mol. Cell. Biol. **11:**2852–2863.
22. **Middlehoven, W. J.** 1964. The pathway of arginine breakdown in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **93:**650–652.
23. **Middlehoven, W. J.** 1968. The derepression of arginase and ornithine transaminase in nitrogen-starved baker's yeast. Biochim. Biophys. Acta **156:**440–443.
24. **Middlehoven, W. J.** 1969. Enzyme repression in the arginine pathway of *Saccharomyces cerevisiae*. Antonie van Leeuwenhoek J. Microbiol. Serol. **35:**215–226.
25. **Middlehoven, W. J.** 1970. Induction and repression of arginase and ornithine transaminase in baker's yeast. Antonie van Leeuwenhoek J. Microbiol. Serol. **36:**1–19.
26. **Miller, J.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Rai, R., F. S. Genbauffe, R. A. Sumrada, and T. G. Cooper.** 1989. Identification of sequences responsible for transcriptional activation of the allantoin permease gene in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**602–608.
28. **Sumrada, R., and T. G. Cooper.** 1978. Control of vacuole permeability and protein degradation by the cell cycle arrest signal in *Saccharomyces cerevisiae*. J. Bacteriol. **136:**234–246.
29. **Sumrada, R., and T. Cooper.** 1982. Isolation of the *CAR1* gene from *Saccharomyces cerevisiae* and analysis of its expression. Mol. Cell. Biol. **2:**1514–1523.
30. **Sumrada, R., and T. Cooper.** 1984. Nucleotide sequence of the *Saccharomyces cerevisiae* arginase gene (*CAR1*) and its transcription under various physiological conditions. J. Bacteriol. **160:**1078–1087.
31. **Sumrada, R., and T. G. Cooper.** 1985. Targets for positive and negative regulatory elements controlling expression of an inducible eucaryotic gene, p. 291–301. *In* R. Calendar and L. Gold (ed.), Sequence specificity in transcription and translation. Alan R. Liss, Inc., New York.
32. **Sumrada, R., and T. G. Cooper.** 1987. Ubiquitous repression sequences control activation of inducible arginase gene in yeast. Proc. Natl. Acad. Sci. USA **84:**3997–4001.
33. **Sumrada, R. A., and T. G. Cooper.** 1985. Point mutation generates constitutive expression of an inducible eukaryotic gene. Proc. Natl. Acad. Sci. USA **82:**643–647.
34. **Sumrada, R. A., and T. G. Cooper.** 1987. Sequences mediating induction and repression of the nitrogen catabolic genes in

Saccharomyces cerevisiae, p. 111–123. *In* Biological research on industrial yeasts, vol. II. CRC Press, Boca Raton, Fla.

35. **van Vuuren, H. H. J., J. R. Dougherty, R. Rai, and T. G. Cooper.** 1991. Upstream induction sequence, the *cis*-acting element required for response to the allantoin pathway inducer and enhancement of operation of the nitrogen-regulated upstream activation sequence in *Saccharomyces cerevisiae*. J. Bacteriol. **173:**7186–7195.
36. **Whitney, P. A., and B. Magasaniki.** 1973. The induction of arginase in *Saccharomyces cerevisiae*. J. Biol. Chem. **248:**6197–6202.
37. **Wiame, J.-M.** 1971. The regulation of arginine metabolism in *Saccharomyces cerevisiae*. exclusion mechanism. Curr. Top. Cell. Regul. **4:**1–38.
38. **Yoo, H. S., and T. G. Cooper.** 1989. The *DAL7* promoter consists of multiple elements that cooperatively mediate regulation of the gene's expression. Mol. Cell. Biol. **9:**3231–3243.