

## Metabolite Repression and Inducer Exclusion in the Proline Utilization Gene Cluster of *Aspergillus nidulans*

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**The clustered *prnB*, *prnC*, and *prnD* genes are repressed by the simultaneous presence of glucose and ammonium. A derepressed mutation inactivating a CreA-binding site acts in *cis* only on the permease gene (*prnB*) while derepression of *prnD* and *prnC* is largely the result of reversal of inducer exclusion.**

All genes involved in the utilization of proline in *Aspergillus nidulans* are clustered in chromosome VII (Fig. 1). *prnB* encodes the specific proline permease, *prnD* encodes proline oxidase, and *prnC* encodes L- $\Delta^1$ -pyrroline carboxylate dehydrogenase (11). *prnA* encodes a Zn-binuclear cluster transcriptional activator mediating proline induction (5, 14). *prnX* is a gene of unknown function whose inactivation does not affect proline utilization (8).

The structural genes of the proline utilization cluster are subject to specific induction by proline and to metabolite repression. Carbon repression is mediated by CreA, a negative-acting zinc finger protein (4, 7). Nitrogen derepression is mediated by AreA, a positive-acting transcription factor belonging to the GATA family (1, 12). It was shown many years ago that these genes are repressed significantly only when both repressing carbon and nitrogen sources are present, i.e., in the simultaneous presence of ammonium and glucose (8). We have shown that this process operates at the level of the steady state of the cognate mRNAs (5, 16) (Fig. 2A). Other data indicate that the repression process operates at the level of transcription rather than at the level of mRNA stability (6). We have shown that repression operates directly on the *prnB* gene and that the expression of the *prnA* gene is not affected by repression (5, 6, 16). A model accounting for these findings has been presented (9). Mutation in any or both of two specific CreA binding sites (*prn<sup>d</sup>*), located between the *prnD* and *prnB* intergenic region, results in derepression of *prnB*, *prnC*, and *prnD* expression (5, 6, 16) (Fig. 2B).

*A. nidulans* strains lacking *areA* cannot utilize proline as a nitrogen source in the presence of glucose as the sole carbon source. The *prn<sup>d</sup>* mutations suppress this phenotype. Arst and collaborators have taken advantage of this phenotype to show that a mutation in what we now know to be one of the two physiologically essential CreA-binding sites (6) is *cis* dominant and *trans* recessive in relation to *prnB* but is *cis* and *trans* dominant in relation to *prnC* and *prnD* (3). The *cis-trans* test was carried out by checking the growth on proline of a homozygous *areA* diploid mutant carrying a *prn<sup>d</sup>* mutation placed either *cis* or *trans* to mutations in each of the structural genes. There are two ways to explain the results of the *cis-trans* test. The first explanation is that there is no direct repression of *prnD* and/or *prnC*, but that the apparent repression of these

genes is the result of inducer exclusion due to the repression of the *prnB*-encoded permease by glucose, and their apparent derepression is the result of reversal of inducer exclusion, acting via the derepression of *prnB*. The second is that the CreA-binding sites in the *prnD-prnB* intergenic region directly affect the expression of *prnD* and/or *prnC*, but that the only limiting step for growth in proline under repressing conditions is the expression of the permease coded by the *prnB* gene. In this note, we demonstrate that inducer exclusion is largely and perhaps exclusively responsible for *prnD* and, to a lesser extent, *prnC* repression.

The strategy we have used to this aim is shown in Fig. 1. In a strain carrying an internal deletion of *prnB*, *prnB337* resulting in a null phenotype for proline uptake (17), we have inserted a fragment of the *prn* cluster in *trans*. This fragment includes the whole wild-type *prnB* gene, the entire *prnB-prnD* intergenic region, and also a short (902-bp) segment of the *prnD* gene. Two strains were constructed, one carries the wild-type repressible promoter, the other carries a promoter containing a mutation in one of the essential CreA-binding sites, *prn<sup>d</sup>22* (6, 16). This mutation changes the canonical CreA-binding site 5'CTGGGG into 5'CTGAGG. This base pair change is sufficient to prevent all binding of this site to CreA in vitro (6). *uaZ11* is a I/VIII chromosomal translocation, which splits the *uaZ* gene (13). This null mutation results in the inability to utilize uric acid as the sole nitrogen source. Transformants able to utilize uric acid can be selected by transforming *uaZ11* strains with a plasmid containing the 3' *uaZ* moiety, including an overlap with the 5' moiety. Thus, transforming sequences are always targeted to the 5' moiety of the *uaZ* gene. The *prn* transgenes were integrated adjacent to the *uaZ* locus by transforming a *prnB337 uaZ11* strain (see the legend to Fig. 1 for the complete genotype) with plasmids pXCwt and pXC*prn<sup>d</sup>22* (Fig. 1). We can thus directly investigate the levels of expression of the *prnD* and *prnC* genes in *trans* to the promoter region driving the active *prnB* gene.

The results of this investigation are shown in Fig. 2B. (i) In spite of the presence of the complete *prnD-prnB* intergenic region, the *prnB* transgene is expressed less than the corresponding sequences at the *prn* locus. This remains unexplained. Nevertheless, the ratio between derepressed and repressed levels in the wild-type promoter and the ratios between the levels of the wild type and those of the *prn<sup>d</sup>22* mutant are the same for the gene in the cluster and for the transgene. (ii) The *prn<sup>d</sup>22* mutation in the resident cluster results in derepression of *prnB*, *prnC*, and *prnD*, as previously described by Sophianopoulou et al. (16). The same effect is seen on the *prnB* transgene. (iii) In the derepressed mutant, the steady-state levels of *prnB* are higher than in the nonderepressed (wild-type

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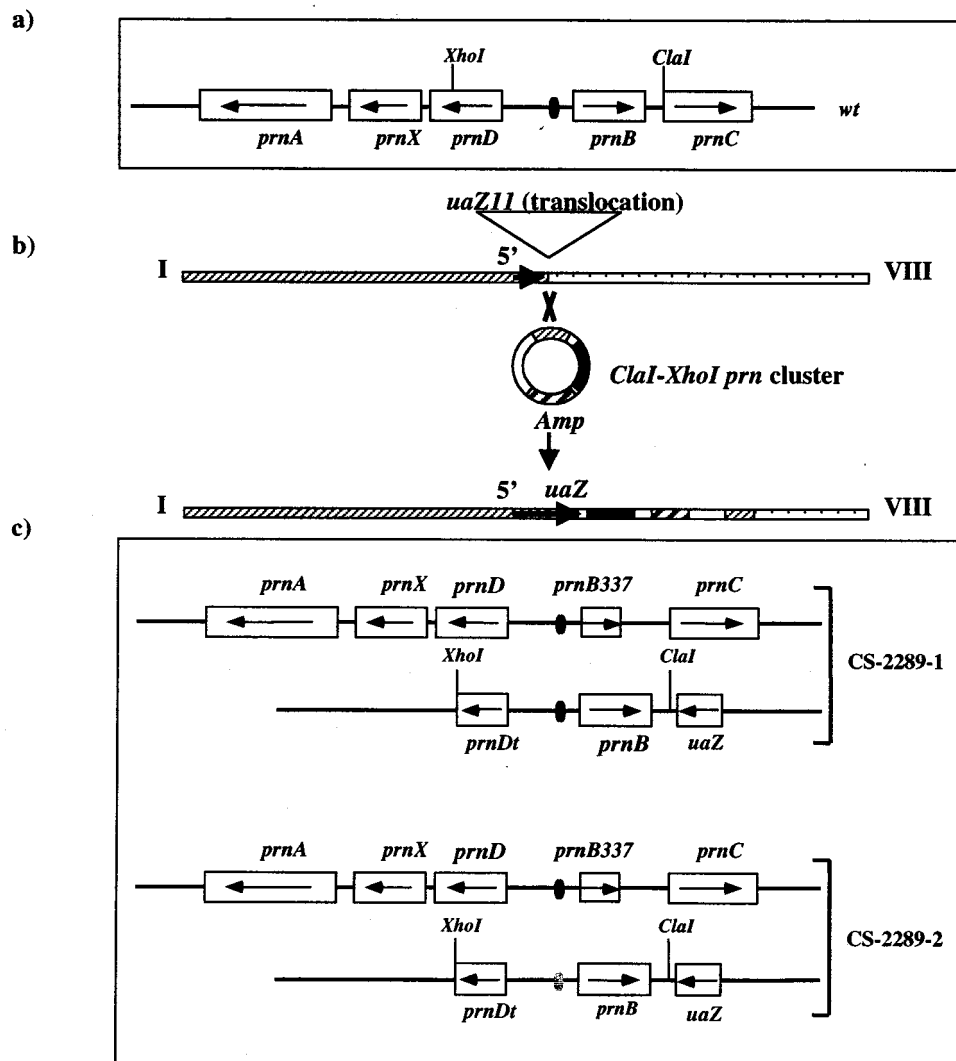


FIG. 1. (a) Schematic representation of *pm* cluster. The direction of transcription of the genes (according to references 8, 9, 10, and 16) is indicated by arrows. (b) Strategy used for the construction of strains CS-2289-1 and CS-2289-2. Strain *uaZ11 prnB337 pabaA1 riboB2 yA2* was transformed with the plasmids pXCwt and pXC $prn^{d22}$ . Both plasmids contain the 3' end of the *uaZ* gene and the *XhoI*-*Clal* region of the *pm* cluster. pXCwt contains a wild-type region, and pXC $prn^{d22}$  contains a region carrying a mutation in an essential CreA-binding site (6). (c) Schematic representation of strains CS-2289-1 and CS-2289-2. For each strain, the upper scheme shows the resident *pm* cluster, and the lower scheme shows the *pm* sequences integrated at the *uaZ* locus. The *XhoI*-*Clal* fragment of *pm* cluster integrated at the *uaZ* locus contains a truncated copy of *prnD* gene (*prnDt*), the *prnD*-*B* intergenic region, the *prnB* gene, and the *prnB*-*C* intergenic region. The resident *pm* locus carries the *prnB337* deletion. The CreA-binding sites active in repression are represented as ovals: dark ovals for wild-type sites and a white oval for the *prn<sup>d22</sup>* mutant site.

promoter) strain even under conditions of derepression for the latter. It could be said that the *prn<sup>d22</sup>* mutation results also in an "up-promoter" effect. This is true both for the transgene and for the resident locus (16). (iv) We know from classical genetic tests that *prn<sup>d</sup>* mutations are *cis* dominant and *trans* recessive in relation to *prnB* (3). However, the level of the short *prnB* message transcribed from the resident gene carrying a deletion mutation is not repressed when in *trans* with the *prn<sup>d22</sup>* mutation. This could be expected if overexpression of the *prnB* transgene resulted in inducer accumulation and if this accumulation could partially bypass carbon and nitrogen metabolite repression. Alternatively or additionally, the small residual message may be more stable than the wild-type *prnB* message. (v) The crucial observation is that the introduction of the *prn<sup>d22</sup>* mutation results in complete derepression of *prnD* in *trans* and a considerable derepression of *prnC* in *trans*. The mRNA of the truncated *prnD* transgene follows the same re-

pression pattern as the wild-type mRNA from the resident cluster. The conclusion is that inducer exclusion accounts for the carbon and nitrogen catabolite repression of *prnD* and *prnC*. (vi) For *prnC*, however, derepression in *trans* seems less pronounced than derepression in *cis*. Northern blots provide semiquantitative estimates, and we would not wish to claim that this small difference is significant. However, a clear effect at a distance of sequences in the *prnD*-*prnB* intergenic region on the expression of *prnC* was demonstrated long ago with classical genetics (2). These results have now been confirmed at the level of the expression of the *prnC* mRNA (15) (D. Gómez and C. Scazzocchio, unpublished results). The region involved has been mapped (D. Gómez and C. Scazzocchio, unpublished results) and coincides with a region involved in the integration of carbon and nitrogen metabolite repression (9). This may suggest that while inducer exclusion is the main parameter involved in the repression of *prnD*, a moderate

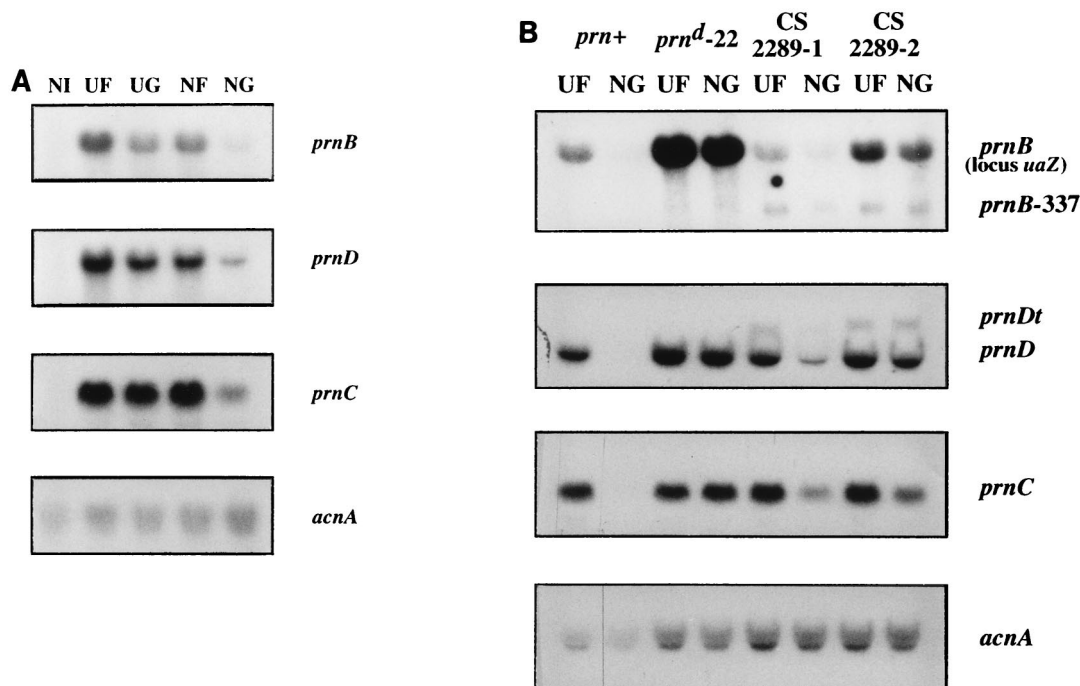


FIG. 2. Northern blot analysis of *prn* genes transcription. (A) Expression of the *prnB*, *prnD*, and *prnC* genes in a wild-type strain grown under different conditions. Noninduced levels and all possible combinations of carbon and nitrogen repressing conditions are shown. (B) mRNA levels of the *prn* genes in a strain carrying *prn<sup>d-22</sup>* and in strains CS-2289-1 and CS-2289-2. Only two conditions are shown: induced nonrepression (UF) and induced double repression (NG). mRNAs extracted from a wild-type strain grown under identical conditions are also shown. Less RNA, as shown by the *acnA* panels, has been loaded for the latter, but the strong repression afforded by the simultaneous presence of glucose and ammonium is clearly visible and can be compared with that of the equivalent mRNAs of panel A. Mycelia were grown for 8 h at 37°C in 0.1% fructose and 5 mM urea and then were either left noninduced (NI) or induced with 20 mM L-proline (UF). Simultaneously, they either were left nonrepressed (UF) or were glucose repressed (1% glucose, 5 mM urea) (UG), nitrogen repressed [20 mM diammonium D-(+)-tartrate, 0.1% fructose] (NF), or carbon and nitrogen repressed [1% glucose, 20 mM diammonium D-(+)-tartrate] (NG) for 2 h at 37°C. The membranes have been probed for *prnB*, *prnD*, *prnC*, and *acnA* (actin), the latter as a control of RNA loading. The methods used for RNA preparation were those of González et al. (9).

*cis*-acting effect of the *prnD-prnB* intergenic region might also be exerted on the expression of *prnC*.

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#### REFERENCES

- Arst, H. N., Jr., and D. J. Cove. 1973. Nitrogen metabolite repression in *Aspergillus nidulans*. *Mol. Gen. Genet.* **126**:111-141.
- Arst, H. N., Jr., and D. W. MacDonald. 1978. Reduced expression of a distal gene of the *prn* cluster in deletion mutants of *Aspergillus nidulans*: genetics evidence for a dicistronic messenger in an eukaryote. *Mol. Gen. Genet.* **163**:17-22.
- Arst, H. N., Jr., D. W. MacDonald, and S. A. Jones. 1980. Regulation of proline transport in *Aspergillus nidulans*. *J. Gen. Microbiol.* **116**:285-294.
- Bailey, C., and H. N. Arst, Jr. 1975. Carbon catabolite repression in *Aspergillus nidulans*. *Eur. J. Biochem.* **51**:573-577.
- Cazelle, B., A. Pokorska, E. Hull, P. M. Green, G. Stanway, and C. Scazzocchio. 1998. Sequence, intron-exon organization, transcription and mutational analysis of *prnA*, the gene encoding the transcriptional activator of the *prn* gene cluster in *Aspergillus nidulans*. *Mol. Microbiol.* **28**:355-370.
- Cubero, B., and C. Scazzocchio. 1994. Two different, adjacent and divergent zinc finger binding sites are necessary for CreA-mediated carbon catabolite repression in the proline gene cluster of *Aspergillus nidulans*. *EMBO J.* **13**:407-415.
- Dowzer, C. E. A., and J. M. Kelly. 1991. Analysis of the *creA* gene, a regulator of carbon catabolite repression in *Aspergillus nidulans*. *Mol. Cell. Biol.* **11**:5701-5709.
- Gavrias, V. 1992. Etudes moléculaires sur la régulation et la structure du groupe des gènes *prn* chez *Aspergillus nidulans*. Ph.D. thesis. Université Paris-Sud, Orsay, France.
- González, R., V. Gavrias, D. Gómez, C. Scazzocchio, and B. Cubero. 1997. The integration of nitrogen and carbon catabolite repression in *Aspergillus nidulans* requires the GATA factor AreA and an additional positive-acting element, ADA. *EMBO J.* **16**:2937-2944.
- Hull, E. P., P. M. Green, H. N. Arst, Jr., and C. Scazzocchio. 1989. Cloning and physical characterisation of the L-proline catabolism gene cluster of *Aspergillus nidulans*. *Mol. Microbiol.* **3**:553-559.
- Jones, S. A., H. N. Arst, Jr., and D. W. MacDonald. 1981. Gene roles in the *prn* cluster of *Aspergillus nidulans*. *Curr. Genet.* **3**:49-56.
- Kudla, B., M. X. Caddick, T. Langdon, N. M. Martínez-Rossi, C. F. Bennett, S. Sibley, R. W. Davies, and H. N. Arst, Jr. 1990. The regulatory gene *areA* mediating nitrogen repression in *Aspergillus nidulans*. Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger. *EMBO J.* **9**:1355-1364.
- Oestreicher, N., H. M. Sealy-Lewis, and C. Scazzocchio. 1993. Characterisation, cloning and integrative properties of the gene encoding urate oxidase in *Aspergillus nidulans*. *Gene* **132**:185-192.
- Sharma, K. K., and H. N. Arst, Jr. 1985. The product of the regulatory gene of the proline catabolism gene cluster in *Aspergillus nidulans* is a positive-acting protein. *Curr. Genet.* **9**:299-304.
- Sophianopoulou, V. 1989. Le groupe des gènes *prn* impliqués dans la dégradation de la L-proline chez *Aspergillus nidulans*. Ph.D. thesis. Université Paris-Sud, Orsay, France.
- Sophianopoulou, V., T. Suárez, G. Diallinas, and C. Scazzocchio. 1993. Operator derepressed mutations in the proline utilisation gene cluster of *Aspergillus nidulans*. *Mol. Gen. Genet.* **236**:209-213.
- Tazebay, U. H., V. Sophianopoulou, B. Cubero, C. Scazzocchio, and G. Diallinas. 1995. Post-transcriptional control and kinetic characterization of proline transport in germinating conidiospores of *Aspergillus nidulans*. *FEMS Microbiol. Lett.* **132**:27-37.