The CreA Repressor Is the Sole DNA-binding Protein Responsible for Carbon Catabolite Repression of the alcA Gene in Aspergillus nidulans via Its Binding to a Couple of Specific Sites*

Cristina Panozzo‡, Emmanuel Cornillot, and Béatrice Felenbok§

From the Institut de Génétique et Microbiologie, Université Paris-Sud, URA CNRS D 2225, Centre Universitaire d’Orsay, Bâtiment 409, F-91405 Orsay Cedex, France

Carbon catabolite repression is mediated in Aspergillus nidulans by the negative acting protein CreA. The CreA repressor plays a major role in the control of the expression of the alc regulon, encoding proteins required for the ethanol utilization pathway. It represses directly, at the transcriptional level, the specific transacting gene alcR, the two structural genes alcA and aldA, and other alc genes in all physiological growth conditions. Among the seven putative CreA sites identified in the alcA promoter region, we have determined the CreA functional targets in AlcR constitutive and derepressed genetic backgrounds. Two different divergent CreA sites, of which one overlaps a functional AlcR inverted repeat site, are largely responsible for alcA repression. Totally derepressed alcA expression is achieved when these two CreA sites are disrupted in addition to another single site, which overlaps the functional palindromic induction target. The fact that derepression is always associated with alcA overexpression is consistent with a competition model between AlcR and CreA for their cognate targets in the same region of the alcA promoter.

Our results also indicate that the CreA repressor is necessary and sufficient for the total repression of the alcA gene.

Aspergillus nidulans is a versatile organism capable of growing on a wide variety of nutrients by expressing enzymes and permeases involved in specific utilization pathways. When ethanol or a related carbon source is added to a growing culture of the fungus A. nidulans, the alc genes are expressed. They comprise not only the genes necessary for ethanol degradation, namely alcA and aldA, encoding alcohol dehydrogenase I (ADHI) and aldehyde dehydrogenase, respectively (1, 2), but at least four other alc genes (alcO, -M, -S, and -U), recently identified (3), which are clustered in the same locus in chromosome VII, as are alcA and alcR. This latter gene encodes the specific transactivator of the ethanol utilization pathway, AlcR, a zinc binuclear cluster protein of the C6 class, with features that differentiate it from the other proteins of this class (4, 5).

The AlcR protein is able to bind in vitro to specific DNA single sites occurring as either direct or inverted repeats (6, 7). An interesting aspect of AlcR activity is that in vivo, only repeated sites mediate specific transcriptional induction (6, 8). The alcR gene itself is subjected to a positive feedback control (9) via the binding of the AlcR protein to targets in the alcR promoter (6). The strong inducible alcA promoter encompasses three clustered AlcR targets (10), which are responsible for the synergistic activation of the alcA gene (8).

When a favored carbon source such as glucose is added to the culture medium, transcription of the alc regulon is severely reduced (11). This phenomenon is mediated by CreA, the repressor responsible for carbon catabolite repression in A. nidulans (12). CreA contains two Cys2-His2 fingers and is related to Mig1, the repressor responsible for glucose repression of several genes in Saccharomyces cerevisiae, and to the mammalian Krox20/Egr and Wilm’s tumors proteins (13). CreA counterparts have also been found in other fungal species such as Aspergillus niger and Trichoderma reesei (14, 15). All of these proteins bind to a GC-rich motif. Binding sites for CreA, whose consensus sequence is 5’-(C/G)YGGRG-3’, have been identified in the alc regulon (11, 16, 17) and in the proline cluster (18). The repressor CreA exerts transcriptional repression on the transacting gene alcR and independently on structural genes alcA and aldA (17). A great number of CreA binding sites have been identified in the upstream regions of the alcR and alcA genes, and these sites are localized in the same region as the specific AlcR binding sites (16). In the alcR promoter region, it has been shown that for at least one of the CreA binding sites overlapping the AlcR inverted repeat target, direct competition between the two regulators occurs both in vivo and in vitro. More generally, under nonrepressing physiological growth conditions (induced and noninduced), it was shown that both regulators control the expression of the alc genes (19, 20). The interplay between these two circuits therefore regulates the expression of the ethanol regulon genes.

In the alcA promoter, several CreA binding sites have been identified by sequence analysis. Three are close to or overlap AlcR binding sites. The alcA promoter is one of the strongest inducible genes in A. nidulans. Synergistic transcriptional activation by AlcR is mediated by three sets of AlcR repeat sites, which were shown to be functional in vivo and represent alcA upstream activating sequences (8). The transcriptional induction of the alcA gene is absolutely dependent upon the presence of AlcR. In glucose-repressed conditions, the alcA gene is totally repressed, and as a consequence (by a cascade mechanism), alcA is not expressed (9).

The aim of this work was to understand the mechanism of carbon catabolite repression in the alcA promoter. We have addressed several questions: (i) which CreA binding sites are functional in vivo; (ii) whether CreA is the only repressor
Fig. 1. A, the alcA promoter region. The AlcR binding sites (a, b, and c) are represented by horizontal arrows. The CreA binding sites identified by footprint experiments (B₁, C, D₁, D₂, and E) are represented by triangles. B, CreA binding sites and flanking regions in the alcA promoter.

EXPERIMENTAL PROCEDURES

Strains, Media, and Growth Conditions—The A. nidulans strain used as the host for transformation was alcA argB uaZ (aclA4951, argB2, pabaA1, yA2, uaZ111). Media and supplements were as described by Cove (21). The mycelia for Northern analyses and ADHI activities were grown for 7 h at 37 °C on 0.1% fructose as the sole carbon source. Induction was achieved by adding the gratuitous inducer ethyl methyl ketone (50 mM) or ethanol (50 mM). Cells were harvested after a further 2.5 h (induced conditions). Under repressed conditions, 1% glucose was used as the host for transformation was

Plasmid Construction and Transformant Selection—The constitutive and derepressed gpdA:alcR:argB plasmid (hAN8) was constructed as follows. The alcR coding sequence was amplified by polymerase chain reaction from the SoI–SalI (−1064 to +2848) fragment of the alcR gene, introducing an EcoRI site at the 5′-end and an NcoI site at the ATG. The EcoRI–SalI fragment (−5 to +2848) was cloned into the EcoRI–SalI sites of pBluescript SK+, resulting in plasmid hAN6. The EcoRI–NcoI fragment corresponding to the gpdA promoter was cloned into the EcoRI–NcoI sites of hAN6, resulting in hAN7. The XhoI–XhoI fragment (made blunt end), of the argB gene was cloned into the EcoRI site (made blunt end) of plasmid hAN7, resulting in plasmid hAN8.

The pyrG:alcR:argB (hAN10) plasmid was constructed as follows. The ppyG promoter was amplified by polymerase chain reaction introducing an EcoRI site at the 5′-end and an NcoI site at the 3′-end. The EcoRI–NcoI fragment was cloned into the EcoRI–NcoI sites of hAN6, resulting in plasmid hAN9. The argB gene was then cloned, resulting in hAN8.

The alcA argB:uaZ strain was transformed with hAN8 or hAN10. Southern blot analysis of the selected argB transforms showed that the plasmids were integrated in a single copy at the uaZ locus (data not shown).

Isolation of RNA and Quantitative Analyses—Total RNA was isolated from A. nidulans as described by Lockington et al. (23), separated on agarose gels after denaturation by glyoxal (24) and blotted on Hybond-N membranes (Amersham Corp.). Hybridization was carried out in 0.5 x sodium orthophosphate, pH 7, 1 mM EDTA, 7% SDS, and 1% bovine serum albumin at 65 °C for at least 14 h. The probes used were the entire genes either of alcR or alcA, cloned into Bluescript plasmids (1, 25) and labeled with [32P]dCTP using random hexanucleotide primers (Amersham). The membranes were also hybridized with a BamHI–KpnI restriction fragment containing the actin gene, isolated from the pgS7 plasmid (26), as an internal control to monitor the amount of mRNA loaded. Autoradiograms were developed at various times to avoid saturation of the film. Densitometric scanning was performed with a system Biosoft-Orkis. Experiments were repeated at least three times. The amount of specific mRNA was calculated relative to the actin signal. Observed variations among the various hybridizations were 20–30%.

Polyacrylamide Gel Electrophoresis and ADHI Activity—Gel electrophoresis and activity staining of ADHI were carried out according to the method described by Sealy-Lewis and Fairhurst (27). Protein concentration was measured according to Bradford (28). Concentrations of protein from different extracts were equalized before loading onto a gel and verified by Coomassie staining. Densitometric scanning was performed. Variations of 20–30% were observed between experiments.

RESULTS

Identification of CreA Binding Sites in the alcA Promoter—Fig. 1 shows that seven CreA binding sites are localized in the alcA promoter. Two of them, A and B₁, have been identified by footprint analysis (16), and others, B₂, C, D₁, D₂, and E, have been identified by sequence analysis through searching for the same consensus motif, 5′-(G/C)YGGRG-3′. We have shown previously that in vivo CreA is able to bind to any of these sites even when the flanking DNA sequence is completely unrelated to A. nidulans, such as the polylinker region of a Bluescript plasmid (16). This was confirmed by in vivo analysis of CreA binding to the penicillin biosynthetic gene in A. nidulans, which is not subject to CreA control in vivo (29) and was also suggested by Cubero and Sczzaczecchi (18) with the

All of the plasmids were used to transform the gpdA:alcR (Tg) strain, resulting in the TgmA strain transformed by pUA, the TgmB₁ strain transformed by pUB₁, the TgmB₂ strain transformed by pUB₂, the TgmB₁B₂ strain transformed by pUB₁B₂, and the TgACD₁D₂E strain transformed by hAN50. The pUB₁B₂ and pUB₂B were also used to transform the argB:pyrG:alcR (Tg) strain, resulting, respectively, in TpmB₁B₂ and TpmAB₁B₂ transformants strains.

Southern blot analysis of the selected uaZ : transformants showed that the plasmids were integrated in a single copy at the uaZ locus (data not shown).

The CreA repression of the alcA gene was achieved by introducing an additional mutation in the activator AlcR and the repressor CreA binding sites identified by footprint experiments (A, B₁, B₂, B₁B₂, and AB₁B₂) are represented by horizontal arrows. The CreA binding sites identified by footprint experiments (B₁, C, D₁, D₂, and E) are represented by triangles. B, CreA binding sites and flanking regions in the alcA promoter.
prn cluster. The same conclusions were drawn after in vitro localization of MIG1 binding sites, the repressor responsible for glucose-repressed genes in *S. cerevisiae* (30).

It has been shown previously that the alcA structural gene is under the direct control of CreA (17). Therefore, it was important to determine which of the putative CreA sites are actually functional in the alcA promoter.

**alcA Expression Is Dependent upon the Level of the AlcR Protein**—The analysis of the mechanism of carbon catabolite repression of the alcA promoter has to be performed in an alcR derepressed background. The reason already mentioned is the absolute dependence for alcA transcription on the presence of an active AlcR protein that is repressed in glucose growth medium. A constitutively derepressed alcA strain was constructed using the promoter of *gpdA*, encoding glyceraldehyde-3-phosphate dehydrogenase, upstream of the full-length *alcR* gene with its own transcriptional termination signal, unlike our previous construct in which the first six amino residues and the termination signal of *AlcR* were deleted (4, 17).

The *gpdA* promoter is very strong and drives the expression of a high amount of *alcR* gene. Under repression growth conditions, *alcR* transcription is substantially derepressed (50%) as a consequence of AlcR synthesis. Since the level of *alcR* expression is dependent on that of *alcR* (17), it was important to set up glucose-repressing conditions in which *alcR* expression could be monitored with suitable accuracy. It has been shown previously (31) that total alcohol dehydrogenase activity in crude extracts varies considerably, depending upon the alcohols and ketones used as inducers. The best inducer was shown to be the gratuitous inducer butan-2-one (also called ethyl methyl ketone (EMK)). Transcriptional analyses of *alcR* and *alcA* and semiquantitative measurements of ADHI activity, estimated by specific staining of gels after native electrophoresis, of the *gpdA:alcR* strain were compared with the wild type strain after the addition of two different external inducers, ethyl methyl ketone and ethanol. Northern blots, presented in Fig. 2A, show that both these external inducers have about the same transcriptional inducing ability for *alcR* in the wild type strain and in the *gpdA:alcR* strain, in which a higher steady state amount of *alcR* mRNA is also observed. As a result, an increase in *alcA* transcription is observed in the *gpdA:alcR* strain compared with the wild type strain, which, in the presence of the inducer ethanol, is 4-fold higher and in the presence of EMK is 8-fold higher.

As expected with the constitutive and derepressed *gpdA:alcR* promoter, the same steady state amount of *alcR* mRNA is observed in mycelia grown in noninduced, induced, and glucose-repressed conditions. In agreement with our previous results (9, 17), no *alcA* transcription is observed in the absence of an external co-inducer. Interestingly, in the *gpdA:alcR* strain, in the presence of the inducer ethanol, *alcA* mRNA derepression is weak (10%), whereas in the presence of EMK, it reaches 50% (Fig. 2A). Semiquantitative evaluation of ADHI activity by native gel staining (Fig. 2B) shows that with ethanol, only a weak ADHI derepression is observed, whereas with EMK, it is much more efficient. Therefore, this *gpdA:alcR* strain, when induced with ethanol, is suitable to analyze the mechanism of *alcA* repression.

**Among Seven CreA Binding Sites, a Pair of Sites Is Largely Responsible for *alcA* Repression**—As seen in Fig. 1, seven putative CreA binding sites, sharing the consensus sequence, 5'- (C/G)YYGGRG-3', have been identified in the *alcA* promoter. Four of them, C, D1, D2, and E, localized in the 5′ alcA promoter region, have been deleted. The resulting deleted *alcA* plasmid, containing the selectable *uaZ* gene marker, was used to transform the *A. nidulans gpdA:alcR alcA− uaZ−* strain. Transfor-
CreA Repression of the alcA Gene

Independent of AlcR Level—

FIG. 3. Effects of individual CreA A, B1, and B2 site disruptions in the alcA promoter on ADHI activity in the gpdA:alcR strain. Growth conditions, ADHI staining, and further details were as described under “Experimental Procedures” and in the legend to Fig. 2B. Induction was achieved by adding ethanol (50 mM). A, ADHI activities of TgmB1 and TgmB2 strains compared with the control Tg strain. Each track was loaded with 30 µg of protein. B, ADHI activity of TgmA strain compared with the control Tg strain. Each track was loaded with 50 µg of protein. The scanning diagram was normalized to a value of 10, representing ADHI activity of the Tg strain induced by ethanol.

mants containing a single copy of the alcA gene, integrated in the uaZ locus, were selected. ADHI activity of the selected transformants (TAΔCD1D2E) was estimated by native gel staining after noninduced, induced, and glucose-repressed conditions and compared with the control strain carrying the entire alcA gene in the gpdA:alcR background. Similar ADHI activities were obtained in all growth conditions (results not shown). Therefore, we can conclude that CreA binding sites C, D1, D2, and E do not contribute to alcA repression.

The involvement of CreA sites A, B1, and B2 in alcA repression was tested by individual site-directed mutagenesis to leave intact all the AlcR induction sites and to retain the natural distance to the alcA transcription start.

Fig. 3B shows that mutagenesis of the CreA A binding site, localized near the AlcR b target, results in an increase in ADHI activity and a slight derepression (10%) compared with the transformation control Tg. Fig. 3A shows that individual mutations in CreA binding site B1 or B2, localized downstream of the AlcR c target, result in an increase in ADHI activities (12-fold) and a strong ADHI derepression (about 80%). Therefore, disruption of either CreA binding site, B1 or B2, is sufficient to derepress the alcA promoter.

alcA Derepression following CreA Binding Site Disruption Is Independent of AlcR Level—To know if alcA expression could be totally derepressed, the three active CreA binding sites A, B1, and B2 were disrupted. All mutagenized alcA transformants were selected as single copy integrants at the uaZ locus.

Interestingly, analysis of transformants in which B1 and B2 CreA binding sites are simultaneously mutagenized results in the same increase in induction and the same level of derepression as observed after the disruption of either B1 or B2 CreA binding sites (Fig. 4). Therefore, it seems that CreA sites B1 and B2 are working as a pair. When the three CreA binding sites A, B1, and B2 are disrupted simultaneously, as in Tgm(AB1B2), it is clear that there is a large increase in induced ADHI activity besides an almost totally derepressed ADHI activity (Fig. 4). To verify that this total derepression did not result from the high AlcR concentration driven by the strong gpdA promoter, AlcR was expressed under the control of the pyrG promoter (pyrG encodes orotidine-5′-phosphate decarboxylase). This promoter is not subject to carbon catabolite repression and is considered as a weak, noninducible promoter. A translational fusion between the pyrG promoter and the alcR coding sequence was constructed, and the argB auxotrophy gene was also inserted into the plasmid. A selected argB+ transformant, containing pyrG:alcR, was then used as the recipient strain (TgpyrG alcA uaZ) for plasmids carrying the mutagenized alcA gene. This strain contains the pyrG:alcR gene in addition to the alcR resident gene.

As shown in Fig. 5, under induced growth conditions in the pyrG:alcR strain (Tp), the ADHI activity is higher than in the wild type strain due to the presence of the pyrG:alcR gene and the chromosomal alcR gene. In this pyrG:alcR strain, when the three CreA binding sites A, B1, and B2 in the alcA promoter are disrupted, ADHI derepression is observed along with increase in ADHI activity in induced growth conditions (Fig. 6). The ADHI derepression observed in this strain can only be accounted for by the pyrG:alcR derepressed gene, whereas in induced growth conditions, alcA expression is driven by two alcR genes (3). This ADHI derepression is complete with regard to the pyrG:alcR gene. We can conclude that mutagenesis of the three CreA binding sites A, B1, and B2, in a context in which AlcR is moderately expressed, results in a totally derepressed alcA promoter. In other words, disrupted CreA sites have a dominant effect on AlcR concentration in the cell.

DISCUSSION

We have previously shown that the repressor CreA, responsible for carbon catabolite repression in A. nidulans, acts at two different levels. First, it prevents completely the transcription of the trans-acting gene alcR, which could account for the drastic decrease of transcription of the two structural genes alcA and aldA (9) and also of the other clustered alc genes (3). Second, however, direct repression of the two structural genes alcA and aldA (17) and of other alc genes such as alcS and alcO...
physiological CreA target, as in the alcR promoter, and in known MIG1 sites (in GAL1 and GAL4) involved in glucose repression, no AT-rich flanking sequence have been found. In the ipnA promoter, the in vitro CreA binding sites are not functional in vivo (29). We have to mention that in vitro CreA binding sites have been localized using a dimeric GST-CreA fusion protein. We have recently shown in our laboratory (7) that the utilization of GST, as an AlcR GST fusion protein, can introduce a serious bias in AlcR DNA recognition of AlcR sites in the alcR, alcA, and aldA promoters, probably as a consequence of the normally monomeric structure of AlcR. It is also expected that CreA is monomeric in solution, as demonstrated for the related Zif268 protein by crystallography studies. It is therefore possible that the use of a GST-CreA fusion protein will also introduce DNA recognition artifacts. It was postulated by Espeso and Peñalva (29) and by Cubero and Scaccia (18) that the dimeric nature of GST-CreA fusions could stabilize CreA binding to sites that do not fit the GC consensus site. In this regard, physiological results should also be used when defining CreA targets. Therefore, CreA targets in the alcA promoter, localized in vivo by site-directed mutagenesis, can be considered as upstream repressing sequences.

Interestingly, functional CreA binding sites are more often organized as pairs of sites directly repeated (e.g. the alcR promoter (16)) or as inverted repeats (e.g. the alcA promoter (this work) and in the prnB promoter region (18)). The single CreA A site in the alcA promoter makes only a minor contribution to glucose repression. MIG1 sites are also often organized as repeats (e.g. the promoters of GAL1, SUC2 (32)). It is well established that MIG1 recruits TUP1 and SSN6, which are general repressors, to establish repression of glucose-repressed promoters. It is possible that CreA acts in a similar way to repress the transcriptional machinery and that two CreA binding sites are necessary.

Disruption of the three CreA targets, B1, B2, and A, results in totally derepressed alcA gene expression. This result indicates that CreA seems to be the only repressor acting directly on alcA during carbon catabolite repression. In S. cerevisiae, it has been shown recently that besides MIG1, another similar zinc finger repressor MIG2, which binds to the same binding sites as MIG1, is involved in glucose repression of SUC2 expression (33). In fact, disruption of MIG1 relieves most of the glucose repression of GAL1 and GAL4 expression, partially relieves SUC2 repression, and has no effect on the glucose repression of other genes (34). In A. nidulans, no carbon catabolite repressor, other than CreA, has been found. This is in agreement with our results presented here and with the complete glucose-derepressed prnB expression observed after the disruption of two CreA binding sites. In this system, it was shown recently that CreA binding prevents the activity of a positive element on the prnB promoter (35).

In the alcA promoter, CreA promoters are very close to, and even overlap with, the AlcR binding sites. It is noticeable that there is a correlation between the level of alcA derepression and that of overexpression. In agreement with our previous results (17, 19), this correlation also indicates that CreA is active under derepressing conditions. The disruption of the minor CreA A site results in a weak increase in alcA expression, whereas disruption of the pair sites, B1 and B2, playing the major role in repression, results in a drastic increase in alcA expression. The alcA promoter region encompassing the induction target c comprises direct repeats (c1–c2) and an overlapping inverted repeat (c3) separated by 16 base pairs with respect to c2. It was

---

2 M. Mathieu and B. Felenbok, unpublished results.
3 V. Gavrias and C. Scaccia, personal communication.
CreA Repression of the alcA Gene

recently shown that to reach full alcA transcriptional activation, the three sites, c1, c2, and c3, are necessary (8). The CreA B1 site overlaps the AlcR c3 site, and the CreA B2 site is

and

the degree of

mechanism whereby the relative levels of these proteins govern

sites (20). These results are in agreement with a regulatory

conditions.

alcR

should be functional in the

alcR

site. Disruption of this CreA site results in an overexpression of

positively autoregulated, the induction site overlaps a CreA

promoter. This steric competition could account for

alcA overexpression when functional CreA binding sites are

disrupted. The induction of

alcA

is correlated with the level of

alcR, as observed in contexts in which AlcR is highly (gpdA:

alcR)

or moderately (pyrG:alcR) expressed. Interestingly, when the functional CreA targets are disrupted in the

alcA

promoter, which is expected, since in the absence of CreA sites, AlcR can fully occupy its cognate targets. In the

alcR

promoter, which is positively autoregulated, the induction site overlaps a CreA site. Disruption of this CreA site results in an overexpression of

alcR

and only a partial derepression, since other CreA sites

should be functional in the

alcR

promoter. The mechanism involved is a direct competition between the two regulators

alcR

and CreA (17). Such a competition mechanism could control

the expression of other clustered

alc
genesis, such as

alcS

and

alcO,

where promoter regions contain both

alcR

and CreA sites (20). These results are in agreement with a regulatory mechanism whereby the relative levels of these proteins govern the degree of

alc

regulation under different growth conditions.

Acknowledgment—We thank David Clarke for editorial corrections to the manuscript.

REFERENCES

1. Gwynne, D. I., Buxton, F. P., Sibley, S., Davies, R. W., Lockington, R. A., Scaglione, C., and Sealy-Lewis, H. N. (1987) Gene (Amst.) 51, 205–216

2. Pickett, M., Gwynne, D. I., Buxton, F. P., Elliot, R., Davies, R. W., Lockington, R. A., Scaglione, C., and Sealy-Lewis, H. N. (1987) Gene (Amst.) 51, 217–226

3. Fillinger, S., and Felenbok, B. (1996) Mol. Microbiol. 20, 475–488

4. Kulmburg, P., Prangé, T., Mathieu, M., Sequeval, D., Scaglione, C., and Felenbok, B. (1991) FEBS Lett. 280, 11–16

5. Sequeval, D., and Felenbok, B. (1994) Mol. Gen. Genet. 242, 33–39

6. Kulmburg, P., Sequeval, D., Lenaouvel, F., Mathieu, M., and Felenbok, B. (1992a) Mol. Cell. Biol. 12, 1932–1939

7. Lenaouvel, F., Nikolaev, I., and Felenbok, B. (1997) J. Biol. Chem. 272, 15521–15526

8. Panozzo, C., Capuano, V., Fillinger, S., and Felenbok, B. (1997) J. Biol. Chem. 272, 22859–22865

9. Lockington, R., Scaglione, C., Sequeval, D., Mathieu, M., and Felenbok, B. (1987) Mol. Microbiol. 1, 275–281

10. Kulmburg, P., Judewicz, N., Mathieu, M., Lenaouvel, F., Sequeval, D., and Felenbok, B. (1992) J. Biol. Chem. 267, 21146–21153

11. Felenbok, B., and Kelly, J. M. (1996) in The Mycota III: Biochemistry and Molecular Biology (Brandal, R., and Marzluf, G., eds) pp. 369–380, Springer-Verlag, Berlin

12. Bailey, C., and Arst, H. N., Jr. (1975) Eur. J. Biochem. 51, 573–577

13. Dowzer, C. E. A., and Kelly, J. M. (1991) Mol. Cell. Biol. 11, 5701–5709

14. Drysdale, M. R., Kolze, S. E., and Kelly, J. M. (1993) Gene (Amst.) 136, 241–245

15. Strauss, J., Mach, R., Zeilinger, S., Hartger, G., Stoffler, G., Wolschek, M., and Kubitsch, C. (1995) FEBS Lett. 376, 103–107

16. Kulmburg, P., Mathieu, M., Dowzer, C., Kelly, J., and Felenbok, B. (1993) Mol. Microbiol. 7, 847–857

17. Mathieu, M., and Felenbok, B. (1994) EMBO J. 13, 4022–4027

18. Cubero, B., and Scaglione, C. (1994) EMBO J. 13, 405–415

19. Fillinger, S., Panozzo, C., Mathieu, M., and Felenbok, B. (1995) FEBS Lett. 368, 547–550

20. Fillinger, S. (1996) Identification et Etude Fonctionnelle de Nouveaux Gènes Appartenant au Régulon Ethanol chez Aspergillus nidulans. Ph.D. thesis, University of Paris-Sud, France

21. Cove, D. J. (1966) Biochim. Biophys. Acta 113, 51–56

22. Kunkel, T. A., Roberts, D. J., and Zabour, R. A. (1987) Methods Enzymol. 154, 367–382

23. Lockington, R. A., Sealy-Lewis, H. N., Lockington, R. A., Scaglione, C., and Davies, R. W. (1985) Gene (Amst.) 33, 137–149

24. Szymbroker, J., Fritsch, E. F., and Manziatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

25. Felenbok, B., Sequeval, D., Mathieu, M., Sibley, S., Gwynne, D. I., and Davies, R. W. (1988) Gene (Amst.) 73, 355–396

26. Fidel, S., Doonan, J. H., and Morris, N. R. (1988) Gene (Amst.) 70, 283–293

27. Sealy-Lewis, H. M., and Fairhurst, V. (1992) Curr. Genet. 22, 293–296

28. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254

29. Espeso, E. A., and Penalva, M. A. (1994) FEBS Lett. 342, 43–48

30. Lundin, M., Nehlin, J. O., and Ronne, H. (1994) Mol. Cell. Biol. 14, 1979–1985

31. Creaser, E. H., Porter, R. L., Britt, K. A., Pateman, J. A., Doy, C. H. (1985) Biochim. J. 225, 449–454

32. Nehlin, J. O., Carberg, M., and Ronne, H. (1991) EMBO J. 10, 3373–3377

33. Lutfiya, L. L., and Johnston, M. (1996) Mol. Cell. Biol. 16, 4790–4797

34. Ronne, H. (1995) Trends Genet. 11, 12–17

35. Gonzalez, R., Gavrias, V., Gómez, D., Scaglione, C., and Cubero, B. (1997) EMBO J. 16, 2973–2984