Onset of Carbon Catabolite Repression in *Aspergillus nidulans*: Parallel Involvement of Hexokinase and Glucokinase in Sugar Signalling*

Michel Flipphi ad, Peter J.I. van de Vondervoort bde, George J.G. Ruijter bf, Jaap Visser bg, Herbert N. Arst, Jr. c, and Béatrice Felenbok ah

From the a Institut de Génétique et Microbiologie, CNRS UMR 8621, Université Paris-Sud XI, Centre d'Orsay, Bâtiment 409, F-91405 Orsay Cedex, France, the b Section Molecular Genetics of Industrial Microorganisms, Wageningen University, Dreijenlaan 2, NL-6703 HA Wageningen, The Netherlands, and the c Imperial College of Science, Technology and Medicine, Department of Infectious Diseases and Microbiology, DuCane Road 150, London UK-W12 0NN, United Kingdom

Running Title: Signalling of Carbon Catabolite Repression in *A. nidulans*

Additional Key Words: Hexose Phosphorylation – Hexose Kinase Mutants – Repression of Induced Transcription – CreA – Sugar Transport

* This work was supported by the Centre National de la Recherche Scientifique (UMR 8621), the Université Paris-Sud XI and the European Community, grants BIO4-CT96-0535 andQLK3-CT99-00729.

d These authors (MF & PJIvdV) contributed equally to this work.

e Present address (PJIvdV): Laboratory of Phytopathology, Wageningen University, Binnenhaven 5, NL-6709 PD Wageningen, The Netherlands.

f Present address (GJGR): Department of Clinical Genetics, Leiden University Medical Centre, PO Box 9600, NL-2300 RC Leiden, The Netherlands.

g Present address (JV): FGT Consultancy, PO Box 396, NL-6700 AJ Wageningen, The Netherlands.

h To whom correspondence should be addressed (BF). Tel.: 33 1 69156328; Fax: 33 1 6915 7808; E-mail: felenbok@igmors.u-psud.fr

The abbreviations used are: 2DOG, 2-deoxy-D-glucose; CCR, carbon catabolite repression; Glk, glucokinase; Hxk, hexokinase
SUMMARY

The role of hexose phosphorylating enzymes in the signalling of carbon catabolite repression was investigated in the filamentous fungus Aspergillus nidulans. A D-fructose non-utilizing, hexokinase-deficient \((hxkA1 – \text{formerly designated } frA1)\) strain was utilized to obtain new mutants lacking either glucokinase \((glkA4)\) or both hexose kinases \((hxkA1 \text{ glkA4})\). D-Glucose and D-fructose phosphorylation is completely abolished in the double mutant which consequently cannot grow on either sugar. The glucokinase single mutant exhibits no nutritional deficiencies. Three repressible diagnostic systems – ethanol utilization \((alcA \& alcR \text{ genes})\), xylan degradation \((xlnA)\) and acetate catabolism \((facA)\) — were analysed in these hexose kinase mutants at the transcript level. Transcriptional repression by D-glucose is fully retained in the two single kinase mutants, while the hexokinase mutant is partially derepressed for D-fructose. Thus, hexokinase A and glucokinase A compensate each other for carbon catabolite repression by D-glucose in the single mutants. In contrast, both D-glucose and D-fructose repression are severely impaired for all three diagnostic systems in the double mutant. Dissimilar to the situation in Saccharomyces cerevisiae, the hexose phosphorylating enzymes play parallel roles in glucose repression in A. nidulans.
INTRODUCTION

To survive among competing microorganisms in an environment with limited resources saprophytic filamentous fungi such as *Aspergillus nidulans* and *Aspergillus niger* adapt rapidly to changing nutrient conditions. Two major control circuits, specific induction and general carbon catabolite repression (CCR), enable considerable versatility in utilizing a wide range of carbon sources while preferentially consuming readily available substrates of high nutritional value before less accessible ones. In *A. nidulans*, CCR is ultimately mediated by the DNA-binding transcriptional repressor CreA, which prevents activation of the catabolism of less preferred carbon sources if a more favorable growth substrate is available (1; reviewed by 2–4). CreA function somewhat resembles that of Mig1p, the main mediator of D-glucose repression in *Saccharomyces cerevisiae* (5; reviewed by 6). The presence of a high concentration of a repressing growth substrate, not restricted to D-glucose, is necessary to trigger the CCR response.

Much is known about the targeting of transcriptional control of nutrient utilization in *A. nidulans*, the molecular means by which induction and repression are imposed on the promoter regions of genes subject to catabolic regulation. In the inducible ethanol utilization pathway, the functional *cis*-acting elements conferring induction and repression, the target sequences of the DNA-binding activator AlcR and the general CCR-repressor, have been identified in the regulatory *alcR* gene encoding the pathway-specific activator and the structural genes for alcohol dehydrogenase I and aldehyde dehydrogenase, *alcA* and *aldA*, respectively (reviewed by 4). In this model system, various mechanisms by which induction and repression of induction are mediated were evidenced and a subtle interplay between the two regulatory circuits was shown to fine tune the expression of each of these three genes in distinct ways (7–12). Functional regulatory target sites have also been identified in two other catabolic systems, the xylanase gene *xlnA* and the proline permease gene *prnB* (13–16).

Far less is known about the means by which the CreA repressor becomes functional in response to repressing carbon sources and how the repressional regulatory circuit adapts to changing nutrient conditions, e.g. upon exhaustion of a preferable carbon source. It has been shown that transcription of the *creA* gene itself is negatively autoregulated in response to repressing carbon sources, leading to a reduced steady-state *creA* transcript level (17). However, the CCR-repressor function appears to be mainly controlled at the post-

---

1 The abbreviations used are: 2DOG, 2-deoxy-D-glucose; CCR, carbon catabolite repression; Glk, glucokinase; Hxk, hexokinase.
transcriptional or post-translational level (17–20). In *A. nidulans*, strains mutant in two additional genes, *creB* and *creC*, exhibit some derepressed characteristics similar to those observed in loss-of-function *creA* mutants but also show a number of phenotypes not related to CCR (21, 22). These two genes have been characterized recently and interestingly, *S. cerevisiae* does not appear to harbor any close homologues (23, 24).

Still less is known about the sensing of repressing compounds and the means by which such compounds trigger the CCR response in filamentous fungi. In *S. cerevisiae*, an important role in the glucose-sensing process has been ascribed to hexokinase Hxk2p, an enzyme catalysing the first step in glycolysis and glucose fermentation, phosphorylation of d-glucose at C6 (reviewed by 6). Baker’s yeast actually specifies three enzymes capable of this phosphorylation, hexokinases (Hxk: EC 2.7.1.1) Hxk1p and Hxk2p, and glucokinase (Glk:EC 2.7.1.2) Glk1p (25). Any one suffices for growth on glucose, but Hxk2p is the main activity for phosphorylating glucose as it is predominantly expressed during fermentation (26, 27). Concomitantly, only this isozyme is essential for repression of catabolism of alternative carbon sources as sucrose and maltose: loss-of-function *hxk2* mutants are defective in glucose repression mediated by Mig1p (28; reviewed by: 6, 29). The mechanism by which Hxk2p participates in glucose repression remains obscure to date. In general, a strong correlation is found between the capacity of mutant Hxk2p to phosphorylate glucose or fructose and CCR by these two sugars (30, 31). However, catalytic activity of Hxk2p might not be essential for glucose repression but rather signal transmission might be linked to Hxk2p conformational changes induced by the sugar and ATP (32–34).

In the filamentous fungus *A. nidulans*, hexose phosphorylation was previously studied in the d-fructose non-utilizing *frA1* ("fructokinase") mutant (35, 36). This mutant lacks Hxk activity but, unlike the situation in *S. cerevisiae*, its glucose CCR of ethanol and 1-arabinose catabolism appeared fully functional. Here, we have utilized three carbon utilization systems — ethanol (*alcA* and *alcR* genes), xylan (*xlnA*) and acetate (*facA*) — to investigate the role of hexose kinases in CCR at the transcript level. The previously mentioned Hxk mutant (*frA1* renamed herein *hxkA1*) as well as newly obtained Glk (*glkA4*) and hexose kinase double (*hxkA glkA4*) mutants were studied. We show that the two hexose kinases play parallel roles in glucose repression in the model organism *A. nidulans*.
EXPERIMENTAL PROCEDURES

Aspergillus nidulans Strains, Media and Growth Conditions—A. nidulans strains used in this study are listed in Table I. The references refer to the mutations relevant to this work. Other markers are in standard use (37). Media composition, supplements and basic growth conditions at 37°C were as described by Cove (38), using di-ammonium tartrate (5 mM) as the nitrogen source and the various carbon sources at 1 % (w/v or v/v), unless stated otherwise. Conidiospores were obtained on solidified complete medium with either glycerol or sodium D-gluconate as the carbon source. Mycelia for enzyme assays were grown on glycerol minimal medium for 16 h and then transferred to fresh minimal medium containing 1 % (v/v) ethanol and 1 % (w/v) D-glucose or D-fructose, and incubated for another 4 h prior to harvesting. Mycelia for the analysis of alc and facA transcription were grown for 10 – 12 h in minimal medium with glycerol as the carbon source and urea (5 mM) as the nitrogen source. For the analysis of xlnA transcription, sodium D-gluconate replaced glycerol and the incubation time was extended to 40 h. Induction was achieved by the addition of the inducer compounds specific for the three diagnostic systems examined – 2-butanone to 50 mM (final concentration) for alc, D-xylose to 50 mM for xlnA or sodium acetate (pH 6.8) to 10 mM for facA, respectively – and cultures were harvested after 2.5 h of further incubation (inducing conditions). For repressed conditions, D-glucose or D-fructose was added simultaneously with the inducer to a final concentration of 1 % (w/v) (i.e. 55 mM). Noninduced mycelia were grown in the initial growth media during the induction period.

Mutagenesis and Genetic Techniques—Conventional genetic techniques were employed (39). Following UV mutagenesis of a suspension of 10^6 conidiospores of G092 per ml, the survival rate was ~25 %. Selection for resistance to 50 mg/l 2-deoxy-D-glucose (2DOG) was done in the presence of 1 % (v/v) glycerol as carbon source and 0.08 % (w/v) sodium desoxycholate to reduce colony size. Resistant colonies were allowed to develop for 6 days at 37°C and mutants were purified using the same medium. Wild type strain C62 was used to cross out the hxxA1 (frA1) translocation in NW298 to yield single glucokinase (glkA4) mutants NW299 and NW300. Genetic mapping of the glkA4 mutation was only possible in a Hxx-deficient background (see “Results” section). 2DOG-resistant strain NW193 (glkA4 hxxA2) was crossed to CEA54 to exchange auxotrophic markers to facilitate the formation of a diploid between the progeny strain NW303 and the tester strain NW301, utilized in parasexual analysis to localize the glkA4 and hxxA2 mutations (see Table I).
Enzyme Assays—Cell-free extracts were prepared from frozen mycelia in liquid nitrogen as described previously (36). All enzyme assays were done at 25°C. Glucose- and fructose-phosphorylating activities in crude extracts were determined as described by Ruijter et al. (36). To distinguish better between in vitro Hxk and Glk activities, assays were performed in both the absence and presence of the Hxk-specific inhibitor trehalose-6-phosphate (40). At 1 mM, the inhibitor reduced the measured Hxk activity to about 1/10 of its actual value (see Table II, compare to activities in the Glk mutant glkA4).

Isolation of RNA and Northern blot Analysis—Total RNA was isolated from about 250 mg mycelial powder, obtained by grinding mycelia in liquid nitrogen, with RNA Plus extraction solution (Qbiogene) following the manufacturer’s instructions. It was further purified by precipitation in 3 M sodium acetate, pH 6.0, for 2 h at –20°C. The precipitate was collected by centrifugation and residual salt was removed by conventional alcohol precipitation. Northern analysis was carried out with 15 μg samples of glyoxal-treated total RNA (41) using Hybond N membranes (Amersham Pharmacia Biotech). Membranes were hybridized with 32P-labelled probes synthesized from DNA fragments from the cloned A. nidulans genes alcA (10), alcR (42), xlnA (43), facA (44) and acnA (γ-actin) (45). Autoradiographs were exposed for various time periods to avoid film saturation. Intensities of the hybridization signals were quantified using a PhosphorImager (Molecular Dynamics). The γ-actin gene was used to normalize the data from a single membrane. For alc and facA, panels A and C of Figs 1 and 4 – 7 originate from a single membrane, enabling direct comparison among all principal strains. For xlnA, panels B of Figs 1 and 4 – 6 originate from a single membrane, allowing direct comparison between wild type and the three different hexose kinase mutants. All expression experiments were repeated at least twice.
RESULTS

Only Mutants Impaired in Both Glucokinase (GlkA) and Hexokinase (HxkA) Activity Cannot Phosphorylate Glucose—To investigate the involvement of hexose phosphorylation in the signalling of D-glucose repression in filamentous fungi, we obtained new mutants lacking either Glk or both Glk and Hxk by classical means. D-Fructose non-utilizing Hxk-deficient hxkA1 (frA1) mutant strains are unable to phosphorylate fructose but grow quite well on glucose by virtue of Glk activity (36). The selection of Glk mutations in a hxkA1 strain was based on increased resistance to the toxic antimetabolite 2-deoxy-D-glucose (2DOG) in the presence of glycerol as sole carbon source (see “Experimental Procedures” for details). This glucose analogue is phosphorylated by either hexose kinase but cannot be catabolized any further (46).

Among the 2DOG-resistant mutants, several were unable to grow on glucose. The glucose non-utilizing mutant exhibiting the lowest residual glucose phosphorylating activity was chosen for further analysis. Fructose-utilizing (hxkA+) progeny from an outcross grew normally on glucose and were tested for hexose phosphorylating activities in crude extracts as compared to wild type, the 2DOG-resistant glucose non-utilizing parent and the single hxkA1 mutant. Some of the glucose- and fructose-utilizing progeny clearly lacked Glk activity (see Table II). The mutation resulting in the Glk lesion was designated glkA4. Other glucose and fructose non-utilizing mutants were selected for 2DOG resistance in an outcrossed glkA4 single mutant. Parasexual analysis of one such strain allocated glkA4 to chromosome III and the newly selected hxkA2 mutation, as expected, to chromosome IV (results not shown).

We can conclude that only hexose kinase double mutants are unable to grow on glucose as sole carbon source. In contrast to the fructose non-utilizing Hxk mutants, the Glk mutant strains do not show any obvious nutritional deficiencies. Table II shows high levels of glucose and fructose phosphorylating activities in a glkA4 strain which are decreased drastically in the presence of the Hxk inhibitor trehalose-6-phosphate. This strongly suggests that Hxk can compensate the lack of Glk activity, allowing glkA4 mutants to grow normally on glucose. Similarly, Glk appears to compensate the absence of Hxk in hxkA1 for glucose phosphorylation and growth as reasonable levels of glucose phosphorylation were measured in the absence and presence of trehalose-6-phosphate (Table II). In agreement with nutritional phenotypes, only the double sugar kinase double mutant (hxkA1 glkA4) is unable to phosphorylate either hexose (Table II).
Defining the Conditions to Trigger CCR Using Three Different Catabolic Systems—

Ethanol Utilization—The ethanol utilization (alc) pathway is convenient for studying the signalling of CCR because of its highly inducible structural (alcA) and regulatory (alcR) gene expression, the marginal levels of non-induced (constitutive) expression and the high repressibility by CreA in the presence of repressing carbon sources like glucose, fructose and D-xylose (Fig. 1A). Competition between the pathway-specific activator AlcR and the general repressor CreA occurs in the alcA and alcR genes under all conditions of growth while alcR expression is, in addition, subject to direct repression by CreA (7, 8, 10, 11). A further advantage is the availability of an efficient gratuitous inducer, 2-butanone (47).

Xylan Degradation—The gene encoding xylanase A (X22) in A. nidulans, xlnA (43), was chosen as the second system (Fig. 1B). xlnA is one of the few genes in which a functional target site for CreA has been identified (13). In addition, the xlnR regulatory gene, encoding the pathway-specific activator, is most likely subject to CreA-mediated repression (48). For expression of xlnA, induction is absolutely required. The simplest inducer, D-xylose (the monomer of xylan), is highly metabolizable and is strongly repressing at high concentrations (1). This pentose requires specific transport (see below). The xylanase system is extremely sensitive to repression. D-Gluconate as carbon source for growth allows reproducible glucose-repressible, xylose-inducible expression of xlnA in wild type, but as will be shown below, nevertheless exerts significant repression.

Acetyl-CoA Synthetase—The third system chosen is the facA gene, encoding acetyl-CoA synthetase catalysing the first step of acetate catabolism in A. nidulans (44, 49). This gene features expression characteristics, completely different to those of xlnA (Fig. 1C). Glycerol was used as growth substrate. As on many other carbon sources, facA is constitutively expressed to considerable levels on glycerol, but wild type strains remain clearly inducible by acetate and glyoxylate (to between two- and threefold the basal level). We utilized 10 mM acetate (pH 6.8) as inducer, as higher concentrations reduce general transcription efficiency as characterized by lower γ-actin transcript levels (47). The true inducer of facA expression is neither acetate nor glyoxylate but more likely acetyl-CoA, a key intermediate of cellular metabolism (50). In contrast to the basal level, the acetate-induced

---

2 As confirmed by transcript analyses in alc (M. Flipphi, and B. Felenbok, unpublished data).
3 And M. Flipphi, and B. Felenbok, unpublished data.
facA expression appears completely repressible by glucose and fructose in the wild type. Although repression of induced facA expression appears to depend on CreA (see below), no functional analysis of CreA target sites has been reported.

The Onset of Induction is Faster than that of Carbon Catabolite Repression—It was important to analyze the time course of induction versus repression for the CCR-regulated systems in order to establish appropriate conditions to study the effects of hexose kinase mutations on carbon catabolite repression.

For xlnA, a short induction period appears desirable as the inducibility drops sharply with time when using a high concentration of xylose as inducer of medium-shifted mycelia (51). This feature could result from “self” CCR by the inducer compound, xylose, consistent with observations for xylanolytic genes in A. niger, for which the inducibility drops with increasing xylose concentration (52). In the absence of medium shift, the inducibility does not decline drastically with time or with increasing xylose concentrations, although the xlnA transcript levels are lower than those reported by MacCabe et al. (51) (results not shown). Fig. 2A shows that an induction period of 1 h was not sufficient to observe any repression by fructose while some xlnA repression could be observed after 2.5 h. Comparison of these two induction periods in wild type for the alc genes, clearly shows that induction by 2-butanone had been established within 1 h while repression by fructose and also that by glucose was far from complete at that time (Fig. 2B).

Induction thus occurs faster than repression. For the alc system, induction is a rather straightforward process, requiring the binding of AlcR to DNA targets in the presence of the physiological inducer, acetaldehyde (reviewed by 4). Our results suggest that onset of transcriptional repression requires several steps such as sugar uptake, formation of a physiological repressor, post-translational modification of CreA and, possibly, indirectly involved proteins such as CreB and CreC. For transcript analysis, we have chosen the longer time period of 2.5 h to properly evaluate induction and repression of all three systems.

Inducer Exclusion Accounts for One Apparent Form of CCR of xlnA Expression—A very straightforward way to prevent the expression of an inducible catabolic system is by inducer exclusion, i.e. blocking entry of the inducer. Inducer exclusion can result from direct CreA-mediated repression of the gene encoding the specific permease of the repressible catabolic system. This is the principal mechanism of repression of the structural genes of proline catabolism in A. nidulans (16, 53).
The regulation of the xlnA gene illustrates inducer exclusion. 5 mM xylose is sufficient to induce xlnA in wild type and, as expected, xlnA expression is completely prevented in the presence of 1% glucose (results not shown). This is, however, not related to any action of CreA since in the strongly derepressed mutant creA\textsuperscript{d30}, expression of xlnA is also prevented under these conditions (Fig. 3). The presence of fructose has no such effect. Furthermore, this phenomenon did not occur in the creA\textsuperscript{d30} strain when equimolar (50 mM) amounts of both xylose and glucose were present although xlnA was fully repressed in wild type under these conditions (not shown). We therefore avoided inducer exclusion by employing the higher xylose concentration (50 mM). Presumably, the inducer exclusion is caused by a direct inhibition of xylose transport by the structurally related sugar glucose. Xylose inhibition of 2DOG transport in A. nidulans has been reported (54).

The Effect of Single Hexose Kinase Lesions on CCR by Glucose and Fructose: Hexokinase is Required for Full Fructose Repression—The lack of hexokinase in the hxkA\textsuperscript{1} mutant does not prevent glucose repression in any of the three systems at the transcript level (Fig. 4). However, the Hxk-deficient strain is clearly derepressed in the presence of fructose, the sugar that is neither phosphorylated nor catabolized by this mutant. This indicates that Hxk at least plays some role in the transmission of the repression signal for fructose. In S. cerevisiae, elimination of Hxk activity, by the deletion of both the Hxk-encoding genes, HXK\textsuperscript{1} and HXK\textsuperscript{2}, likewise leads to derepression on fructose (55).

Interestingly, from the analysis in alc, clearly this derepression is far from complete (Fig. 4A). Partial derepression is consistent with the ability of hxkA\textsuperscript{1} mutants to be suitable for selection of CCR-derepressed creA mutants such as creA\textsuperscript{d30} (56). This strongly suggests that, in the absence of hexokinase, another factor, devoid of apparent fructose phosphorylating activity, can partly fulfil the regulatory function of Hxk with respect to CCR by fructose and its precursors.

One possible candidate for this regulatory factor would be glucokinase, a hexose phosphorylating enzyme with a narrower substrate range apparently constitutively produced (Table II). However, the single Glk mutant glkA\textsuperscript{4} exhibits wild-type repression by both fructose and glucose for all three systems (Fig. 5). It would thus appear that Glk integrity is not important for CCR in A. nidulans. In S. cerevisiae, Glk does not fulfil any regulatory function in either glucose or fructose repression, even when artificially overexpressed (31, 55, 57).
Derepression in Hexose Kinase Double Mutants: Hexokinase HxkA and Glucokinase GlkA Compensate Each Other for CCR by Glucose—In the hxA1 glkA4 double mutant all three systems are derepressed for both glucose and fructose (Fig. 6). The translocation-free hxA2 glkA4 double mutant gave identical results for the alc system derepression (not shown). This excludes the possibility that derepression involved a consequence of a translocation breakpoint other than the translocation associated with the hxA1 mutation. The derepression of the alcR and alcA genes on fructose is almost complete (80-100%) whereas on glucose, it is about 50%. The xlnA and facA genes are completely derepressed both on fructose and glucose. However, an unexpected observation for xlnA is that its induced expression is decreased considerably. Furthermore, for facA, the basal level expression is increased, whereas the steady state induced level remains similar to that in the wild type. The reason for this increased facA basal level is unknown.

Comparison of the derepression of the alc genes on fructose in the single hxA1 mutant and in both hexose kinase double mutants shows that the derepression in absence of both hexose phosphorylating enzymes is virtually complete while that in the single hexokinase-deficient strain is only partial (Fig. 4A and 6A and data not shown). For the signalling by the ketosugar, glucokinase appears to partially compensate the Hxk deficiency. Our results suggest that Glk is – either directly or indirectly – involved in fructose repression in A. nidulans, despite the fact that fructose is not a relevant catalytic substrate for the enzyme (cf. Table II). A direct involvement of Glk in fructose repression in A. nidulans would unambiguously distinguish the regulatory function of hexose phosphorylating enzymes in CCR-related signal transmission from the catalytic activity with regard to this sugar.

Derepression in Hexose Kinase Double Mutants Put in Perspective: Comparison with the Strongly Derepressed creA d30 Mutant—We compared the levels of transcriptional derepression of the three diagnostic systems in the hexose kinase double mutants and in a creA derepressed strain. Nearly complete derepression of the alc genes is achieved either in an extreme creA mutant such as creA30 (7) or by disrupting functional CreA targets in responsive promoters (10, 11). The observed "super induction" of alcA and alcR expression is a direct result of the absence of promoter binding competition between AlcR and CreA which normally occurs under all physiological conditions.

In Fig. 7, the "super induction" in the creA30 strain is evident for all three systems. In no case does the level of expression observed in the double hexose kinase mutant on glucose and fructose equal that in the creA30 strain. For facA, the basal (non-induced) level is
elevated in the creA mutant, but the gene remains inducible. The creA<sup>d30</sup> mutation virtually abolished CreA-AlcR binding competition whereas the more modest effect of the double hexose kinase deficiency suggests it does not. This indicates that hexose kinases are not involved in CreA-AlcR binding competition and thus that CreA can bind its target sites in the absence of sugar signalling.

For xlnA, "super induction" in the creA<sup>d30</sup> strain is striking, at least 100-fold greater than in the wild type (Figs. 7B and 8). Apparently, xlnA is extremely sensitive to CCR not only by repressing compounds such as glucose and fructose, but also by compounds generally considered non-repressing for other catabolic systems such as ethanol utilization, e.g. glycerol, D-gluconate and L-glutamate (1, 2; results not shown). We were in fact unable to find a completely derepressing, non-inducing carbon source for the analysis using xlnA. Gluconate was eventually selected because it allowed reproducible glucose-repressible and xylose-inducible xlnA expression in the wild type (see Fig. 1B). Hence, derepression observed in the hexose kinase double mutant appears to concern only the additional repression from glucose or fructose over the "basal" repression from gluconate and the inducer, xylose.

Reduced Inducibility of xlnA in Hexokinase Mutants: an Indication of Positive Involvement of Hexokinase in Xylose Induction—An interesting observation is the considerable reduction (three-fold) in xylose-induced expression of xlnA in the single hexokinase and hexose kinase double mutants, but not in the glucokinase mutant (Figs. 1B, 4B, 5B and 6B). Two hypotheses could explain this observation. Firstly, xlnA repression by xylose might be elevated in mutants lacking Hxk, thereby decreasing the level of expression. Alternatively, xylose induction of xlnA might be lower due to the Hxk lesion. To distinguish between these hypotheses, we analyzed xylose-induced xlnA steady-state transcription in a derepressed triple mutant (creA<sup>d30</sup> glkA4 hxkA1). If the first hypothesis were correct, the inducibility should be restored to the level observed in the creA<sup>d30</sup> single mutant, 100-fold higher than that in the wild type. In the second case, reduced inducibility should still be seen in the triple mutant. Revealingly, the xylose-induced xlnA level in the triple mutant is clearly lower than that in the single creA<sup>d30</sup> mutant (Fig. 8), although the induced level of xlnA transcript is still elevated due to the absence of functional CreA (30-fold greater than wild type) (Table III). Hence, the reduced (three-fold) inducibility observed in Hxk mutants is maintained in a truly derepressed background. This suggests that Hxk has a CreA-independent, positive role in xylose induction of xlnA.
DISCUSSION

Hexose kinase Single mutants are not affected in glucose-mediated CCR—Carbon catabolite repression in the filamentous fungus *A. nidulans* appears related to the capacity to utilize a given carbon source. The three repressible systems monitored here, ethanol catabolism (*alcA* and *alcR*), xylanase A (*xlnA*) and acetyl-CoA synthetase (*facA*), respond in a quantitatively similar manner in the different hexose kinase mutant backgrounds.

Hexokinase (*hxkA*) mutants have derepressed steady-state transcript levels for the three systems vis-à-vis fructose while being unable to utilize it. On the other hand, *hxkA* mutants grow normally on glucose while the three diagnostic systems remain repressed in its presence, in sharp contrast to the situation in *S. cerevisiae*, where Hxk2p is essential for glucose repression (but not for growth on glucose) (reviewed by 6, 29). The hexokinase mutant produces glucokinase activity in the presence of either fructose or glucose (Table II).

The single glucokinase (*glkA4*) mutant data support the argument that CCR correlates with carbon source utilization. Glk mutants do not exhibit nutritional deficiencies as they produce high levels of Hxk activity enabling phosphorylation of both fructose and glucose. The Glk mutant shows an apparently wild-type transcriptional repression for all our diagnostic systems in the presence of either sugar. The two glucose-phosphorylating enzymes thus substitute each other functionally in the single mutants, both with respect to catalytic function and in establishing glucose-mediated carbon catabolite repression. In this respect, transcript analyses of the three systems correlate with the measured hexose kinase activities.

Sugar phosphorylation is a critical step in CCR by hexose sugars—The observations in both hexose kinase single mutants are in agreement with previous work with the *hxkA1* mutant (36) and would suggest that hexokinase and glucokinase are not involved in CCR by glucose. However, both our double hexose kinase (*hxkA glkA4*) mutants refute this conclusion as they show considerable transcriptional derepression for all three systems in the presence of either glucose or fructose. Derepression in both double mutants is total for *xlnA* and *facA* whereas for *alc*, it is greater on fructose than on glucose. For these double mutants, glucose is not a carbon source and (in vitro) phosphorylation of both fructose and glucose is virtually abolished. The substantial residual repression of the *alc* genes on glucose in the double mutants might indicate involvement of a third protein, possibly capable of phosphorylating glucose in vivo although remaining undetected in vitro.
Overall, our results would implicate either glucose phosphorylation, the first step in glycolysis, or the catabolic flux initiated from it as essential for signalling glucose repression in *A. nidulans*. Two independent findings favor sugar phosphorylation as the critical step. Firstly, *A. nidulans* pyruvate dehydrogenase-deficient (*pdhA*) strains are unable to grow on glycolytic substrates (58, 59). In such glucose non-utilizing mutants, sugar phosphorylation still occurs. However, they provide a very amenable genetic background for the positive selection of derepressed *creA* mutants (2, 60). Secondly, our repression-defective hexose kinase double mutants were isolated by positive selection for resistance to 2DOG, a glucose analogue which is phosphorylated without initiating catabolic flux (54). Nevertheless, 2DOG represses induced expression of the *alc* genes at the transcript level as strongly as glucose does, as shown in Fig. 9. In *S. cerevisiae*, Hxk2p is involved in the regulation of glucose uptake and *hxk2* mutants have a different expression spectrum for hexose transporters (61; reviewed by 62). Interestingly, preliminary results show that both hexose kinase double mutants appear to have glucose uptake characteristics similar to those of wild type. This indicates that the unphosphorylated sugar is unlikely to play a direct role in signalling carbon catabolite repression in *A. nidulans*.

The levels of derepression of the three systems in glucose-derepressed hexose kinase double mutants are less than those seen in the *creA*<sup>d30</sup> strain – widely used as a reference for derepression. These results suggest that the CreA protein normally mediates repression from other metabolites in the hexose kinase double mutant, irrespective of the sugar kinase lesions. This is most evident for *xlnA* (Table III). Several mechanisms, possibly acting in concert, might account for the 300-fold difference in induction: a total lack of repression from the carbon source gluconate and the inducer xylose, derepression of the activator-encoding *xlnR* gene in addition to that of the structural *xlnA* gene and altered xylose transport. To this latter end, we have shown that the reduced inducibility of *xlnA* in hexokinase mutants is not due to CreA-mediated repression (Fig. 8 and Table III). In the derepressed triple mutant (*creA*<sup>d30</sup> *glkA4 hxkA1*), a “superinduction” of *xlnA* is observed as expected from the *creA*<sup>d30</sup> mutation, but the wild-type xylose inducibility ratio is not restored. The positive involvement of Hxk in *xlnA* induction might occur at the level of xylose uptake by hexose transporters. The xylose-fermenting yeast *Pichia stipitis* takes up xylose and glucose with a common transport system (63). Here, we have provided evidence for inducer exclusion of xylose by glucose (Fig. 3),

---

consistent with a common transporter for xylose and glucose in *A. nidulans*. Possible regulatory functions of the hexose kinases in the catabolism of glucose and xylose are currently under study.

**CCR in A nidulans differs fundamentally from glucose repression in S. cerevisiae**—In *A. nidulans*, glucokinase and hexokinase appear to play a mere catalytic role in CCR, specific for their substrates (repressing hexose sugars). The complete absence of (*in vitro*) glucose phosphorylating activity in our hexose kinase double mutants correlates with derepression of the three diagnostic systems for glucose and fructose. In contrast to Hxk2p in *S. cerevisiae*, neither hexose kinase exhibits a unique, general regulatory function in CCR in *A. nidulans*. A possible reason could be that glucose repression in yeast is specifically related to fermentation of glucose into ethanol (64), whereas most filamentous fungi metabolize glucose almost uniquely *via* oxidative phosphorylation. Moreover, CCR in filamentous fungi can result not only from glucose, sucrose or fructose, but also from other carbon sources such as xylose and acetate (1)6. These fundamental differences might be relevant to the lack of similarity between the respective CCR-mediating repressor proteins, Mig1p and CreA, beyond the DNA-binding domains (the two Cys$_2$His$_2$-zinc fingers). CRE1, the CreA-homologue from the filamentous fungus *Sclerotinia sclerotiorum*, cannot complement a mig1 deletion in *S. cerevisiae* (65). In *A. nidulans*, CCR could be signalled independently for individual carbon sources and CreA might be the ultimate receptor of multiple converging signalling routes. In this respect, the signal transmission processes preceding transcriptional repression also seem to differ fundamentally between the two types of fungi.

**Acknowledgements**—We thank Dr. Christophe d'Enfert and Dr. John Clutterbuck who kindly provided us with several *A. nidulans* strains and Maarten Bax for technical assistance at the lab in Wageningen. This work was supported by the Centre National de la Recherche Scientifique (UMR 8621), the Université Paris-Sud XI and the European Community, grants BIO4-CT96-0535 and QLK3-CT99-00729.

5 This is the case for the *A. niger* sugar transporter MstA (P. A. vanKuyk, personal communication).
REFERENCES

FIG. 1. Transcript analyses of CRR with three inducible and carbon catabolite repressible diagnostic systems in an A. nidulans wild type strain. Mycelia were grown on either glycerol (Fig. 1A and 1C) or sodium D-gluconate (Fig. 1B) (non-induced conditions, NI) and the indicated effectors were added to cultures, as either the inducer compound only (induced conditions, I) or both inducer and repressor compounds simultaneously (repressed conditions, IG for D-glucose and IF for D-fructose), as detailed for each of the individual systems in “Experimental Procedures”. Growth culture conditions, RNA isolation and Northern blots were as described in “Experimental Procedures”. A, Northern blot analysis of the ethanol utilization pathway, i.e. the alcA and alcR genes induced by 50 mM 2-butanone. B, Northern blot analysis of xylanase A (XnA) gene (xlnA) expression induced by 50 mM D-xylose. C, Analysis of the acetyl-CoA synthetase gene (facA) expression induced with 10 mM acetate. Northern blots were hybridized with 32P-labelled probes hybridizing to alcA, alcR, xlnA and facA genes respectively, and the γ-actin gene (acnA). The latter gene served as an internal control to normalize the amounts of mRNA. Quantification of the expression levels was done with a PhosphorImager and is given underneath. The transcript level under induced conditions was set arbitrarily at 10. The relative quantitative data are given to the nearest integer while any weaker expression levels revealed are indicated as > 0. The data from two independent experiments show a maximal variation of 25 %.

FIG. 2. Induction is established faster than repression. Northern blot transcript analysis in a wild type strain to determine an appropriate induction and repression period. A comparison between the impacts of sugar repression after 1 h (left) and 2.5 h (right) of exposure to both inducer and repressor compounds. A, Northern blot analysis of the repression by fructose of induced xlnA expression (IF). B, Northern blot analysis of the repression by glucose (IG) and fructose (IF) of the ethanol utilization pathway (alcA and alcR). All experimental details were as described in the legend to Fig.1.

FIG. 3. Evidence for inducer exclusion of xylose in the presence of glucose as repressor. Northern blot analysis was performed in a derepressed creAΔ30 strain in the presence of two xylose concentrations: 5 mM (left) and 50 mM (right). The repressor compound, glucose or
fructose, was present at 55 mM (1% w/v). Experimental details were as described in the legend to Fig. 1.

FIG. 4. The effect of a hexokinase lesion on CCR of the three systems: wild-type repression for glucose but derepression for fructose. Transcript analyses of induction and repression of the three reporter systems in a hexokinase single mutant (hxkA1) strain. A: alcA and alcR transcripts. B: xlnA transcript. C: facA transcript. Quantification is relative to the wild type levels under induced conditions as shown in Fig. 1. Experimental details were as described in the legend to Fig. 1.

FIG. 5. Lack of glucokinase activity does not affect CCR repression characteristics of the three systems. Transcript analyses of induction and repression of the three reporter systems in a glucokinase single mutant (glkA4) strain. A: alcA and alcR transcripts. B: xlnA transcript. C: facA transcript. Details were as described in the legends to Figs. 1 and 4.

FIG. 6. Derepression of induced expression in the three systems for both fructose and glucose in a hexose kinase double mutant. Transcript analyses of a glkA4 hxkA1 strain. A: alcA and alcR transcripts. B: xlnA transcript. C: facA transcript. Details were as described in the legends to Figs. 1 and 4.

FIG. 7. Derepression and “super induction” in absence of CreA. Transcript analyses of induction and repression of the three systems in a strongly derepressed creA^d30 strain. A: alcA and alcR transcripts. B: xlnA transcript. C: facA transcript. Direct (single blot) comparison of xlnA expression in the creA^d30 strain with that in the other strains is not possible due to the considerable differences in expression level (see Table III). The relative data below the xlnA Northern were determined using a dilution range of the creA^d30 xylose-induced RNA sample. Details were as described in the legends to Figs. 1 and 4.

FIG. 8. Evidence for a positive role of hexokinase in xylose induction of xlnA. Xylose-induced xlnA expression in the derepressed creA^d30 strain (middle) was compared to that in a triple mutant carrying both hexose kinase mutations as well as creA^d30 (creA^d30 glkA4 hxkA1) (right). On the left, wild type. Details were as described in the legends to Figs. 1, 4 and 7.
FIG. 9. **2DOG represses transcription of induced alcA and alcR.** Transcript analysis of a wild type strain was performed using ethanol (50 mM) as the inducer. 2DOG and glucose were added at 1% (w/v). Details were as described in the legend to Fig. 1.
Table I. *Aspergillus nidulans* strains used in this study.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>References for characterized mutation or strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF054</td>
<td>yA2 <em>pabaA1</em></td>
<td></td>
</tr>
<tr>
<td>BF110</td>
<td><em>biA creA</em>&lt;sup&gt;30&lt;/sup&gt;</td>
<td>56, 66</td>
</tr>
<tr>
<td>C62</td>
<td><em>pabaA1</em></td>
<td></td>
</tr>
<tr>
<td>CEA54</td>
<td><em>pyrG89</em>; wA1; <em>pyroA4</em>; ∆<em>treA::neo</em></td>
<td>67</td>
</tr>
<tr>
<td>G092</td>
<td>yA2; <em>pyroA4</em> <em>hxA1</em> (T1 (IV, VIII))</td>
<td>35, 36</td>
</tr>
<tr>
<td>NW193</td>
<td><em>pabaA1</em>; <em>glkA4</em>; <em>hxA2</em></td>
<td>This work</td>
</tr>
<tr>
<td>NW298</td>
<td>yA2; <em>glkA4</em>; <em>pyroA4</em>; <em>hxA1</em> (T1 (IV, VIII))</td>
<td>This work</td>
</tr>
<tr>
<td>NW299</td>
<td>yA2; <em>glkA4</em>; <em>pyroA4</em></td>
<td>This work</td>
</tr>
<tr>
<td>NW300</td>
<td><em>pabaA1</em>; <em>glkA4</em></td>
<td>This work</td>
</tr>
<tr>
<td>NW301</td>
<td><em>pabaA1</em> <em>pyrG89</em>; wA3; <em>argB2</em> <em>galA1</em>; <em>facA</em>&lt;sup&gt;30&lt;/sup&gt;; <em>sB3</em>; <em>nicB8</em>; <em>riboB2</em></td>
<td>This work</td>
</tr>
<tr>
<td>NW303</td>
<td><em>glkA4</em>; <em>pyroA4</em> <em>hxA2</em>; ∆<em>treA::neo</em></td>
<td>This work</td>
</tr>
<tr>
<td>NW330</td>
<td><em>biA creA</em>&lt;sup&gt;30&lt;/sup&gt;; <em>glkA4</em>; <em>hxA2</em>; <em>fwA1</em></td>
<td>This work</td>
</tr>
</tbody>
</table>

NB: The trehalase gene disruption is described in (67). The diploid used for parasexual analysis was isolated from a heterokaryon of strains NW301 and NW303. Roman numerals indicate the chromosomes involved in the *hxA1*-associated translocation.
Table II. Phosphorylation of glucose and fructose in extracts of various *A. nidulans* strains.

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Carbon source*</th>
<th>Phosphorylation activity [nmol.(min.mg protein)^{-1}]</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose – T6P</td>
<td>+ T6P</td>
<td>Fructose – T6P</td>
</tr>
<tr>
<td>wt</td>
<td>Glucose</td>
<td>438</td>
<td>189</td>
<td>582</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>431</td>
<td>153</td>
<td>622</td>
</tr>
<tr>
<td><em>hxA1</em></td>
<td>Glucose</td>
<td>99</td>
<td>101</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>126</td>
<td>123</td>
<td>18</td>
</tr>
<tr>
<td><em>glkA4</em></td>
<td>Glucose</td>
<td>599</td>
<td>55</td>
<td>1084</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>667</td>
<td>54</td>
<td>1054</td>
</tr>
<tr>
<td><em>glkA4 hxA1</em></td>
<td>Glucose</td>
<td>0</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>9</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

Phosphorylation of 1 mM glucose or 10 mM fructose was measured in extracts as described in “Experimental Procedures”. Activities were determined in the absence and presence of 1 mM trehalose 6-phosphate (T6P). Values are means from duplicate experiments.

* The transfer media contained ethanol (1 % v/v) in addition to the indicated sugar to avoid starvation response in the *hxA1* and *glkA4 hxA1* mutant strains. Growth conditions were as described in “Experimental Procedures”.

---

23
Table III. Relative induced levels of xlnA transcript in different mutant contexts.

<table>
<thead>
<tr>
<th></th>
<th>wt</th>
<th>hxA1</th>
<th>glkA4</th>
<th>glkA4 hxA1</th>
<th>creA&lt;sup&gt;d&lt;/sup&gt;30</th>
<th>creA&lt;sup&gt;d&lt;/sup&gt;30 glkA4 hxA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>1000</td>
<td>300</td>
<td></td>
</tr>
</tbody>
</table>

Induction by 50 mM xylose was as described in “Experimental Procedures”. The amounts of hybridized mRNA from different Northern blots (Figs. 1 and 4–8) were quantified by densitometry scanning with a PhosphorImager. Data correspond to: wild type (wt) in Fig. 1B; hxA1 in Fig. 4B; glkA4 in Fig. 5B; glkA4 hxA1 in Fig. 6B; creA<sup>d</sup>30 in Figs. 7B and 8; and creA<sup>d</sup>30 glkA4 hxA1 in Fig. 8. The values represent the amount of xlnA mRNA in each strain relative to the amount of γ-actin mRNA as an internal control. The amount of wild type xlnA mRNA was arbitrarily set at 10.
Fig. 1A

<table>
<thead>
<tr>
<th>NI</th>
<th>I</th>
<th>IG</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>alcA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>alcR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acnA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>&gt; 0</th>
<th>10</th>
<th>&gt; 0</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>alcA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1</th>
<th>10</th>
<th>&gt; 0</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>alcR</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1B

NI I IG IF

\( xlnA \)

\( acnA \)

| 0 | 10 | > 0 | 4 | \( xlnA \) |
Fig. 1C

![Image of the figure with the legend 'NI I IG IF facA acnA' and a gel with bands for 'facA' and 'acnA'.]
Fig. 2A

![Image of gel electrophoresis with bands labeled xlnA and acnA at 1 h and 2½ h.]
Fig. 2B

I  IG  IF  NI  I  IG  IF

1 h  2 1/2 h

alcA
alcR
acnA
Fig. 3

[Image of a gel electrophoresis blot with lanes labeled I, IG, IF, N, IG, IF. The lanes show bands for xl na and acnA under [5 mM] and [50 mM] conditions.]
Fig. 4A

<table>
<thead>
<tr>
<th></th>
<th>NI</th>
<th>I</th>
<th>IG</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>alcA</td>
<td>&gt; 0</td>
<td>12</td>
<td>&gt; 0</td>
<td>3</td>
</tr>
<tr>
<td>acnA</td>
<td>1</td>
<td>15</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

genes: alcA, alcR, acnA
Fig. 4B

<table>
<thead>
<tr>
<th>N</th>
<th>I</th>
<th>IG</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>xlnA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acnA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>&gt; 0</td>
<td>3</td>
</tr>
</tbody>
</table>
Fig. 4C

<table>
<thead>
<tr>
<th>N</th>
<th>L</th>
<th>G</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>10</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>

facA

acnA
Fig. 5A

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>IG</th>
<th>IF</th>
<th>alcA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>&gt; 0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>alcR</td>
</tr>
</tbody>
</table>

alcA and alcR expression levels in different conditions.
Fig. 5B

NI  I  IG  IF

\[ xlnA \]

\[ acnA \]

| 0 | 10 | 1 | 5 | xlnA |

Downloaded from http://www.jbc.org/ by guest on August 17, 2017
Fig. 5C

NI  I  IG  IF

facA

acnA

4  10  3  3 | facA
Fig. 6A

<table>
<thead>
<tr>
<th></th>
<th>N1</th>
<th>I</th>
<th>IG</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>alcA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>alcR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>acnA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>&gt; 0</th>
<th>10</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>alcA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>alcR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>
Fig. 6B
Fig. 6C
Fig. 7A
Fig. 7C

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>I</td>
<td>G</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>facA</td>
<td></td>
</tr>
<tr>
<td>acnA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>150</td>
<td>75</td>
<td>75</td>
<td>facA</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 8

- Long exposure
- Short exposure

<table>
<thead>
<tr>
<th>wt</th>
<th>creA&lt;sup&gt;d30&lt;/sup&gt;</th>
<th>glkA4 hxA1 creA&lt;sup&gt;d30&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>xlnA</td>
<td></td>
<td>acnA</td>
</tr>
</tbody>
</table>

| 10  | 0    | 1000 | 0    | 300  | xlnA |

Downloaded from http://www.jbc.org/ by guest on August 17, 2017
Fig. 9

NI  I  IG  I2DOG

\( alcA \)
\( alcR \)
\( acnA \)
Onset of carbon catabolite repression in Aspergillus nidulans: Parallel involvement of hexokinase and glucokinase in sugar signalling
Michel Flipphi, Peter J.I. van de Vondervoort, George J.G. Ruijter, Jaap Visser, Herbert N. Arst Jr, and Beatrice Felenbok

J. Biol. Chem. published online January 7, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M209443200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/early/2003/01/07/jbc.M209443200.citation.full.html#ref-list-1