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CARBON CATABOLITE REPRESSION IN THE REGULATION OF β-GALACTOSIDASE ACTIVITY IN *ASPERGILLUS NIDULANS*

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Introduction

For a vast array of microbes, catabolism of glucose results in severe repression of genes encoding enzymes for the degradation of alternative carbon sources. There are important reasons to study the mechanisms involved in carbon regulation in filamentous fungi such as *Aspergilli* or *Trichoderma* species, since they are used for the production of a large number of enzymes and recombinant proteins whose synthesis is repressed by glucose. Evidence is now available for a key role of the *creA/cre1* gene in *A. nidulans* and several other fungi [1]. It encodes a C_2H_2-type zinc finger protein which binds to a 5' SYGGRG 3'consensus motif, thereby conferring carbon catabolite repression.

The disaccharide lactose (1,4-0-ß-D-galactopyranosyl-D-glucose) is a byproduct of cheese production accumulating to amounts of 300 000 tons per year worldwide, of which 15 % is used as a carbon source for various microbial fermentations [2], such as cellulase production by *Trichoderma reesei* or penicillin production by *Penicillium chrysogenum*. At present, lactose is the only derepressing carbon source used on

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industrial scale. Nevertheless, little is known about the regulation of its metabolism in filamentous fungi. Lactose is metabolized slowly, and some important fungi such as *A. niger* cannot use it at all. A more detailed knowledge on the rate-limiting steps would be helpful to improve its industrial application.

The first step in the lactose metabolism is its hydrolysis to glucose and galactose by either an extracellular β-galactosidase and / or a combination of a lactose permease and an intracellular ß-galactosidase [3]. In *A. nidulans*, only a single intracellular activity appears to be present [4]. The subsequent galactose metabolism involves the coordinate operation of at least five enzymes, namely galactokinase, galactose-1-phosphate uridylyl transferase, UDP-glucose epimerase, UDP-glucose pyrophosphorylase and phosphoglucomutase (Leloir-pathway; see Figure 1). The resulting glucose-6-phosphate is further oxidized via the glycolytic pathway.

Here we report data on the carbon regulation of the activity of the intracellular ß-galactosidase of *A. nidulans*.

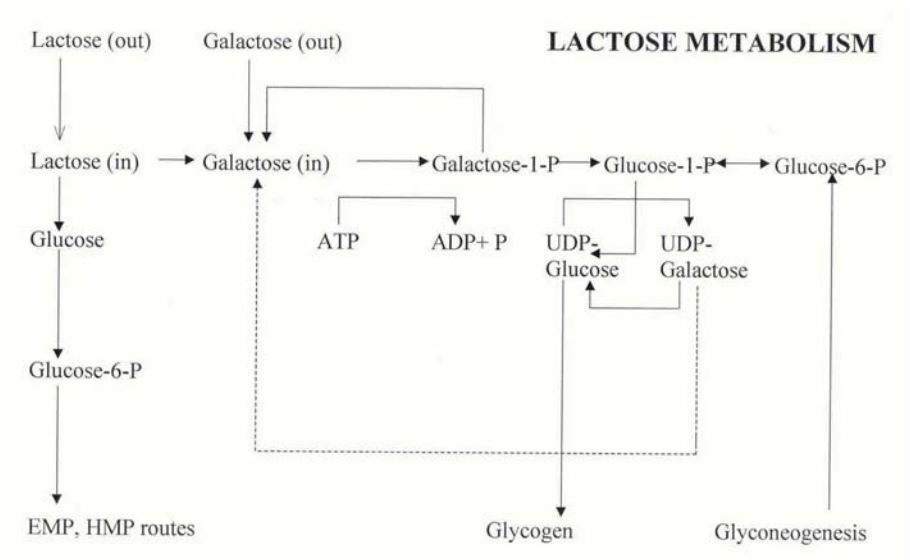

Figure 1. Lactose metabolism

Results and discussion

Wild-type *A. nidulans* did not exhibit any β-galactosidase activity on glucose or glycerol, while it was clearly present in mycelia grown on lactose. No extracellular or cell-bound β-galactosidase activity was detected. The time-profile of activity essentially paralleled growth and lactose consumption, and declined after exhaustion of the carbon source. Essentially similar results were detected in pH controlled (pH 6.5) and non-controlled conditions (during which the pH fell to 3.5). The β-galactosidase activity of mycelia growing on lactose could further raised by addition of galactose, suggesting that enzyme formation is not maximal during growth on lactose.

The formation of β-galactosidase on lactose or D-galactose, but not on glucose or glycerol suggested that its biosynthesis may be under carbon catabolite control. Addition of glucose to cultures growing on lactose immediately decreased ß-galactosidase activity and within a few hours it had declined completely. Uptake of lactose was also suspended as long as glucose was present in the medium. After the consumption of glucose, lactose appeared to be consumed again and also β-galactosidase activity was reinitiated.

These results demonstrate an immediate inhibition of the activity of β-galactosidase by glucose, but do not distinguish between inducer exclusion and true carbon catabolite repression. In order to study the potential role of the carbon catabolite repressor CreA in this process, we used an *A. nidulans* mutant strain Δ4, in which the *creA* locus had been deleted [5]. When the glucose pulsing experiment was repeated with this strain, both lactose consumption and ß-galactosidase activity remained unaffected by glucose, indicating that lactose uptake is not inhibited but repressed by glucose.

To test whether β-galactosidase in itself is also affected by CreA-dependent carbon catabolite repression, mutant strain Δ4 was grown on glucose and glycerol, and the β-galactosidase activity was measured. Mycelia formed a clear, albeit low β-galactosidase activity, corresponding to approx. 18 % of the levels observed on lactose. This indicates that part of the β-galactosidase formation is indeed subject to CreA-dependent carbon catabolite regulation, but it is the induction that accounts for the major part of the activity. In fact, β-galactosidase was effectively induced by the addition of D-galactose. Simultaneous addition of glucose and D-galactose did not inhibit the uptake of D-galactose, but reduced the β-galactosidase activity by about 30%, thus indicating some effect of glucose also on the induction process. The involvement of CreA in this effect was proven by the use of *A. nidulans* mutant strain Δ4, in which D-galactose induction of β-galactosidase was completely unaffected.

Our data show that the effect of glucose occurs at least at three levels, which were all dependent on the carbon catabolite repressor CreA: (a) a blockade of constitutive β-galactosidase formation; (b) a partial interference with induction, such that in a CreA-negative background 150% of the wild-type levels of induction were obtained; and (c) a repression of lactose uptake by glucose. Interference of CreA-dependent carbon catabolite regulation with gene expression at multiple regulatory levels have been reported in *A. nidulans* [6]. However, interference of CreA with inducer transporting permeases (in this case with the lactose permease) has not yet been shown.

In order to identify which stage of D-galactose metabolism is essential for ß-galactosidase formation, we made use of an *A. nidulans* mutant strains defective in galactokinase and UDP-glucose-galactose-1-phosphate transferase activity. The galactokinase mutant still formed β-galactosidase activity upon addition of D-galactose with much lower inducer concentrations required for maximal activities. Similar results were obtained in the wild-type strain by fucose, a non-metabolizable D-galactose analogue. These data indicate that D-galactose metabolism is dispensable for β-galactosidase induction by D-galactose.

A mutant defective in galactose-1-phosphate uridylyl transferase activity, however, exhibited a different behaviour: in this strain a constitutive level of β-galactosidase activity was formed even on glucose or glycerol, which was approximately twice as high as that in the CreA Δ4 mutant and could not be further induced by D-galactose. The time course of the formation of β-galactosidase activity displayed a strictly growth-associated pattern in this mutant, while growth of the mutant was comparable to that of the wild-type, thus ruling out that the ß-galactosidase formation could be the result of differences in the growth rate. This effect is similar but clearly not identical to the effect of the corresponding GAL7 mutation in *K. lactis* [7]. In *K. lactis*, constitutive β-galactosidase activity has been claimed to be due to the internal accumulation of low D-galactose concentrations during idiophase [7]. If this is applied to *A. nidulans*, it would be difficult to understand why the low level of constitutive formation would not be further inducible by D-galactose. Also, in *A. nidulans*, the constitutive formation of β-galactosidase occurs throughout the whole growth phase and not only during the idiophase as in *K. lactis*. Thus, in *A. nidulans* it appears more likely that galactose-1-phosphate uridylyl transferase acts as a repressor of β-galactosidase biosynthesis, and the inducer D-galactose is mainly needed to antagonize this repression, although such a model does not explain why derepression of the β-galactosidase would only be partial. Further studies using *A. nidulans* strains conditionally expressing the GAL7 homologue will be required to answer these questions.

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References

1. Dowzer,C.E., Kelly,J.M.: Analysis of the *creA* gene, a regulator of carbon catabolite repression in *Aspergillus nidulans*. Mol Cell Biol **11**, 5701 (1991).
2. Roelfsema,W.A., Kuster,F.M., Pluim,H.: Lactose and derivatives. In: Elvers,B., Hawkins,S., Schulz,G. (eds.) Ullmann's Encyclopedia of Industrial Chemistry, 4th ed. VCH Weinheim FRG, 1990, pp 107–114
3. DeVries,R.P., van den Broek,H.C., Dekkers,E., Manzanares,P., de Graaff,L.H., Visser,J.: Differential expression of three β-galactosidase genes and a single β-galactosidase gene from *Aspergillus niger*. Appl Environ Microbiol **65**, 2453 (1999).
4. Roberts,C.F.: Enzyme lesions in galactose non-utilizing mutants of *Aspergillus nidulans*. Biochim Biophys Acta **201**, 267 (1970).
5. Shroff,R.A., O'Connor,S.M., Hynes,M.J., Lockington,R.A., Kelly,J.M.: Null alleles of *creA*, the regulator of carbon catabolite repression in *Aspergillus nidulans*. Fungal Genet Biol **22**, 28 (1997).
6. Mathieu,M., Felenbok,B.: The *Aspergillus nidulans* CREA protein mediates glucose repression of the ethanol regulon at various levels through competition with the ALCR-specific transactivator. EMBO J **13**, 4022 (1994).
7. Cardinali,G., Vollenbroich,V., Jeon,M.S., de Graaf,A.A., Hollenberg,C.P.: Constitutive expression in gal7 mutants of Kluyveromyces lactis is due to internal production of galactose as an inducer of the Gal/Lac regulon. Mol Cell Biol **17**, 1722 (1997).