G-protein/cAMP signaling pathway-mediated repression of genes encoding polysaccharide-degrading enzymes in filamentous fungi

Emi KUNITAKE

Graduate School of Bioresources, Mie University

Report: Carbon catabolite repression (CCR) of cellulase and hemicellulase genes is regulated by cAMP signaling factors independent of the transcription factor CreA in *Aspergillus nidulans*. The degree to which each factor contributes to CCR varies depending on culture conditions (plate or submerged culture, and the type of inducer and/or repressing carbon source added to growth media).

Research aims

Fungal cellulases and hemicellulases are important industrial enzymes for effective biomass utilization, and there is strong demand for improved productivity. Since expression of cellulase and hemicellulase genes in filamentous fungi is induced by sugars derived from cellulose and hemicellulose, cellulosic biomass-derived substrates are used as carbon sources for production of these enzymes. However, monosaccharides, the degradation products of inducer substrates, can cause carbon catabolite repression (CCR). Since increased enzyme productivity can induce CCR, CCR-defective strains become essential to further improve enzyme productivity. In the model fungus *Aspergillus nidulans*, it has long been known that the transcriptional repressor CreA mediates CCR, whereas we found that CCR of cellulase genes is mainly regulated via cAMP-dependent protein kinase (PkaA), independently of CreA. We also identified GanB as the main G protein α subunit involved in CCR. The aim of this study is to clarify the CCR mechanisms mediated by CreA and PkaA/G proteins.

Methods

In addition to *creA*, *pkaA*, and Gα (*ganA*, *ganB*, *fadA*) gene deletion strains (Δ*creA*, Δ*pkaA*, Δ*ganA*, Δ*ganB*, and Δ*fadA*, respectively), double deletion strains Δ*creA*/Δ*pkaA* and Δ*creA*/Δ*ganB* have been constructed. Cellulase production by each deletion strain was examined by plate assay for plate culture and by zymography of culture supernatants for submerged culture. Carboxymethyl cellulose (CMC) and ball-milled cellulose (BMC) were used as inducing substrates, respectively. Cellulase genes were subjected to RT-qPCR-mediated transcriptional analysis using RNA extracted from mycelia grown on the inducers, cellobiose or BMC. Glucose, fructose, mannose, xylose, and 2-Deoxyglucose (2DG) were used as repressing carbon sources. Xylanase and mannanase production were

analyzed by plate assay using AZCL-xylan and locust bean gum (LBG) as enzyme substrates, respectively. Zymography using Azo-xylan and Azo-carob galactomannan was performed for supernatants of cultures with xylose and LBG. For transcriptional analysis, xylose and β-mannobiose were used to induce xylanase and mannanase gene expressions, respectively.

Results

De-repression of cellulase production by CCR was observed by plate assay in Δ*pkaA* and Δ*ganB* but not Δ*creA* strains, suggesting that cAMP signaling is the main mediator of CCR of cellulase genes in plate culture. To clarify the relationship between CreA and PkaA/GanB, Δ*creA*/Δ*pkaA* and Δ*creA*/Δ*ganB* strains were also examined (Fig. 1). Cellulase production in the presence of glucose was higher in Δ*creA*/Δ*pkaA* and Δ*creA*/Δ*ganB* than in Δ*pkaA* and Δ*ganB*, indicating that CreA is partially involved in CCR independently of PkaA/GanB. In contrast with results obtained on agar plates, in submerged culture using BMC as inducer and glucose as the repressing carbon source, cellulase gene expression and enzymatic activity were detected in Δ*creA* but not in Δ*pkaA* and Δ*ganB* strains. In the double deletants, both gene expression and enzyme activity were detected earlier than in the Δ*creA* strain. Transcriptional analysis using cellobiose as an inducer and 2DG as the repressing carbon source revealed nearly equal involvement of CreA and PkaA in CCR. Double deletion of *creA* and *pkaA* caused stronger de-repression, confirming the presence of cAMP-signaling dependent and CreA-independent CCR of cellulase genes (Fig. 2). Because other carbon sources also repress expression of the cellulase genes, we further examined CCR by fructose, mannose, and xylose in addition to glucose. CCR by hexoses was partially abolished in Δ*creA* and Δ*pkaA* strains, as observed when glucose was the repressing carbon source. By contrast, CCR by xylose was completely abolished by the *creA* deletion, whereas the *pkaA* deletion yielded very minor de-repression if any (Fig. 2). These observations imply that CCR caused by hexoses is mediated by the additive actions of CreA and PkaA, while xylose-induced CCR is mainly CreA mediated. Interestingly, higher de-repression was obtained with Δ*ganB* than Δ*pkaA*, implying that GanB, independent of

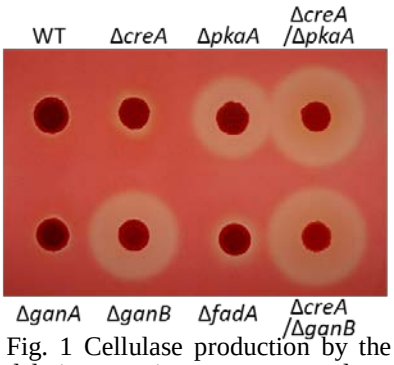

Fig. 1 Cellulase production by the deletion strains on agar plates containing CMC with glucose.

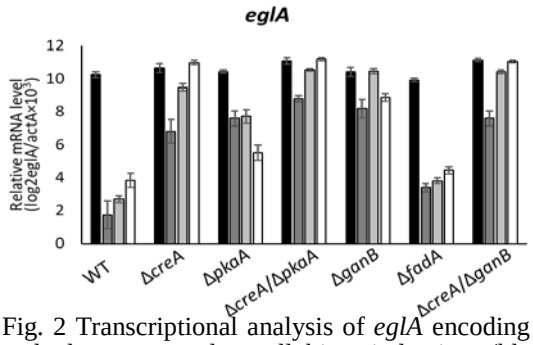

Fig. 2 Transcriptional analysis of *eglA* encoding an endoglucanase under cellobiose-induction (black) supplemented with 2DG (dark gray), glucose (light gray), and xylose (white). Relative expression levels of the genes were normalized with *actA*

PkaA, has a role in CCR of cellulase gene expression.

Expression analyses of xylanase and mannanase genes were also performed. Xylanase expression displayed CreA, PkaA, GanB, and FadA-dependent CCR, and the contribution of each factor varied among xylanase genes. Involvement of CreA and cAMP signaling factors in CCR of mannanase gene expression depended on culture conditions as was the case for cellulase gene expression, in which both CreA and PkaA/GanB regulated glucose-mediated CCR, while CreA was the main mediator of xylose-induced CCR.

As described above, GanB appears to regulate both PkaA-dependent and -independent CCR. To clarify the PkaA-independent CCR mechanism, we examined FbxA, which is an F-box protein with cNMP-binding motifs. However total cellulase, xylanase, and mannanase production in the presence of glucose were unaffected by *fbxA* deletion (Δ*fbxA*) and by mutation in its cNMP binding domain (*fbxA*ΔCNBD) on agar plates.

Conclusions

In *A. nidulans*, CCR of cellulase and hemicellulase is mediated by the CreA system as well as cAMP signaling factors, such as PkaA and GanB. Each factor plays distinct roles in CCR that are dependent on the repressing carbon source and culture conditions[1)]. FbxA, which has been reported to be involved in xylanase expression[2)], appears not to be involved in cellulase gene expression. From a biotechnological point of view, the double knockout of *creA* and *pkaA* exhibited the maximal cellulase gene expression, however, the significantly impaired strain growth would limit industrial application. Further research to identify PkaA and Gα targets is underway for deeper understanding of CCR mechanisms and for construction of a CCR-free strain with more robust growth for industrial use.

References

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2) Colabardini, A. C. et al. (2012) Molecular characterization of the *Aspergillus nidulans fbxA* encoding an F-box protein involved in xylanase induction. *Fungal Genet. Biol.* **49**: 130-140.