

# Effects of CreA phosphorylation sites on carbon catabolite repression in *Aspergillus nidulans*

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## Abstract:

Saprobic microorganisms, such as filamentous fungi of the genus *Aspergillus* are of particular interest for hydrolytic enzyme secretion as some species are already vastly used in industry where their natural capacity to secrete plant cell wall-degrading enzymes, that are known as carbohydrate-active enzymes (CAZy), is exploited. The genomes of *Aspergillus spp.* encode several hundred CAZy and the corresponding genes are under tight transcriptional control. The presence of easily metabolizable sugars such as glucose, whose concentrations increase during plant biomass hydrolysis, results in the repression of CAZy-encoding genes, in a process known as carbon catabolite repression (CCR). To date, the C<sub>2</sub>H<sub>2</sub> transcription factor CreA has been described as the major CC-repressor in *Aspergillus spp.* In the presence of glucose, CreA translocates to the nucleus where it represses target genes; whereas, in the presence of plant biomass polysaccharides, it translocates to the cytoplasm, relieving gene repression. CCR is a highly conserved process in ascomycetous fungi, and its onset is dependent on the sensing and identification of extracellular nutrients. In terms of evolution, CCR allows the fungus to use the energetically most favorable carbon source required for niche colonization whereas it is undesired for the purpose of large-scale enzyme production. This work shows the effect of CreA point mutations, that mimic hypo-phosphorylation, through replacing serine (S) by alanine (A), on CCR. Using phospho-proteomics, a phosphorylation site on serine S262 was found when glucose was added to the cultures. Mass spectrometry data identified CkiA (casein kinase A) and GskA (glycogen synthase kinase A) as CreA interaction partners. Indeed, when mutating the predicted CkiA (S262A and S268A) and GskA (S308A) phosphorylation sites on CreA, cellular localization of the latter was aberrant. As a result, xylanase secretion increased significantly in the phosphomutants, suggesting that CCR was defective. Addition of the GSK3 inhibitor (GskA inhibitor) also prevented CreA nuclear translocation in the presence of glucose, confirming that GskA-mediated phosphorylation is important for CreA localization and function. In conclusion, these results suggest that phosphorylation-mediated regulation of CreA is essential for CCR and presents an interesting target for biotechnological strain manipulations without the need to delete essential genes which can result in undesired side effects.

Keywords: CreA, *Aspergillus*, phosphorylation, GskA, CkiA.

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