# From Model Organism to Industrial Workhorse:

## Analysis of genes in *Aspergillus nidulans* and disruption of *cre2* for *Trichoderma reesei* strain improvement.

By

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Genetics Discipline

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## Abstract

Carbon catabolite repression is a regulatory system whereby an organism can sequentially utilise carbon sources based on their available energy. This system results in the repression of genes encoding enzymes responsible for the utilisation of poorer carbon sources when preferable ones are available. Carbon catabolite repression has been extensively studied in the filamentous fungus *Aspergillus nidulans*. Repression is mediated via CreA, a zinc finger DNA binding protein, which is in turn, either directly or indirectly, regulated by an ubiquitination / deubiquitination system involving CreB, CreC and CreD.

Previous work demonstrated that the *A. nidulans* genome contains a CreD homologue, ApyA, and that both of these proteins interact with an ubiquitin ligase, HulA. This relationship was proposed to be similar to Rod1p and Rog3p and their interaction with the ubiquitin ligase Rsp5p in *Saccharomyces cerevisiae*. Both *apyA* and *hulA* were targeted for disruption to facilitate phenotypic analysis and the study of epistatic interactions. Deletion of *hulA* was shown to be lethal in an *A. nidulans* haploid, but viable as a heterozygote in an *A. nidulans* diploid. The only detectable phenotypes of this deletion in a heterozygous diploid were increased sensitivity to molybdate and acriflavine. A strain containing a disruption of *apyA* did not demonstrate any detectable phenotypes, however, the *apyA* disruption allele showed epistatic interactions with mutations in *creB*, *creC* and *creD*. The disruption of *apyA* partially suppressed the phenotype of sensitivity to allyl alcohol in the presence of glucose displayed by strains containing mutations in *creB* and *creC*. However, the level of suppression exhibited by the disruption of *apyA* was not as strong as that shown by the *creD34* mutation. A strain containing mutations in both *creD* and *apyA* demonstrated severe morphological deficiencies on minimal media as well as stronger resistance to acriflavine than *creD34* alone, and resistance to molybdate.

Bioinformatic analysis of CreD and CreD-like proteins, including ApyA, from sequenced members of the Aspergilli and Rod1p, Rog3p and related proteins from members of Saccharomycetes suggested that the arrestin-like proteins, a group to which these belong, are subject to frequent gene duplication events. The number and range of sequenced fungal genomes also allowed a bioinformatic examination of the conservation of proteins involved in the carbon repression mechanisms across the fungal kingdom. A homologue of CreA was identified only within the members of Ascomycota that were examined, but putative homologues of CreB and CreC were identified across the fungal

kingdom. The Saccharomycetes were an exception to this as a CreC homologue was not indentified and the CreB homologue was highly divergent or absent.

The filamentous fungus, *Trichoderma reesei* is an important source of cellulases for use in the textile and alternative fuel industries. Previous studies have suggested a benefit for the manipulation of carbon catabolite repression for strain improvement, as the industrially significant strain RUTC-30 contains a mutation in *cre1*, the *T. reesei creA* homologue. The *T. reesei* orthologue of the *A. nidulans creB* gene, designated *cre2*, was shown to be functional in carbon repression through complementation of a *creB* mutation in *A. nidulans*. This gene was targeted for disruption in *T. ressei* as disruption in *A. nidulans* leads to carbon derepression of some systems without the severe morphological effects of strains containing *creA* mutations. A *T. reesei* strain containing a *cre2* disruption exhibited phenotypes similar to the *A. nidulans creB* mutant strain on solid media and had elevated cellulase levels.

## Declaration

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Jai A. Denton

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"Disruption of *Trichoderma reesei cre2*, encoding an ubiquitin C-terminal hydrolase, results in increased cellulase activity" by JA Denton and JM Kelly. (In Submission)

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# Abbreviations

**General Abbreviations**

| | |
|---|---|
| ATP | adenosine triphosphate |
| ATCC | American Tissue Culture Collection |
| aa | amino acid |
| bp, kb, mb | basepair, kilobase pairs, megabase pairs |
| CCR | carbon catabolite repression |
| °C | degrees Celsius |
| DIG | Digoxigenin |
| DNA | deoxyribonucleic acid |
| DUB | deubiquitinating enzyme |
| FGSC | Fungal Genetics Stock Center |
| GPCRs | G protein-coupled receptors |
| gDNA | genomic deoxyribonucleic acid |
| g, mg, µg, ng | gram, milligram, microgram, nanogram |
| GFP | green fluorescent protein |
| JGI | Joint Genome Initiative |
| l, ml, µg | litre, millilitre, microlitre |
| M, mM | molar, millimolar |
| NCBI | National Center for Biotechnology Information |
| nt | nucleotide |
| PCR | polymerase chain reaction |
| RNA | ribonucleic acid |

**Nucleotide abbreviations**

| | | | |
|---|---|---|---|
| A | adenine | S | guanine or cytosine |
| G | guanine | W | adenine or thymidine |
| T | thymidine | B | not adenine |
| C | cytosine | D | not cytosine |
| R | adenine or guanine | H | not guanine |
| Y | cytosine or thymidine | V | not thymidine |
| M | adenine or cytosine | N | any nucleotide |
| K | guanine or thymidine | | |

**Protein Domains**

Arrestin: Domains mirrored at either end of a protein with Ig-like beta sandwich fold that are involved in protein-protein interactions.

C2: A calcium dependent membrane targeting module.

DUB: An ubiquitin hydrolase domain.

HECTc: A C-terminal ubiquitin-transferase domain.

PEST: Proline, glutamic acid, serine and theronine rich regions involved in protein turnover.

PPXY & PXY: One or two proline residues next to any residue followed by a tyrosine.

WW: Two highly conserved tryptophan residues involved in protein-protein interactions.

Zinc finger: Involved in DNA or RNA binding. Use zinc ions to stabilise their folding.