Functional analysis of the transcription factor XlnR involved in the regulation of cellulases- and hemicellulases-encoding genes in *Aspergilli*

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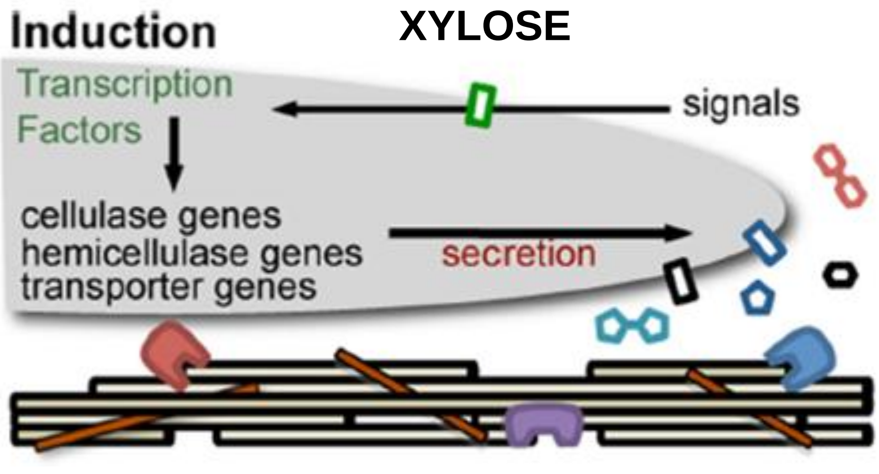
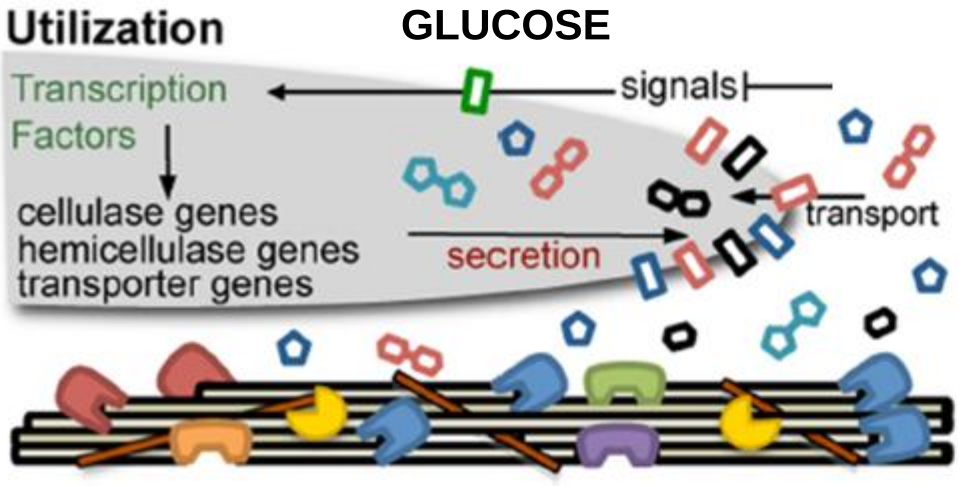
Model of plant cell wall deconstruction by fungi



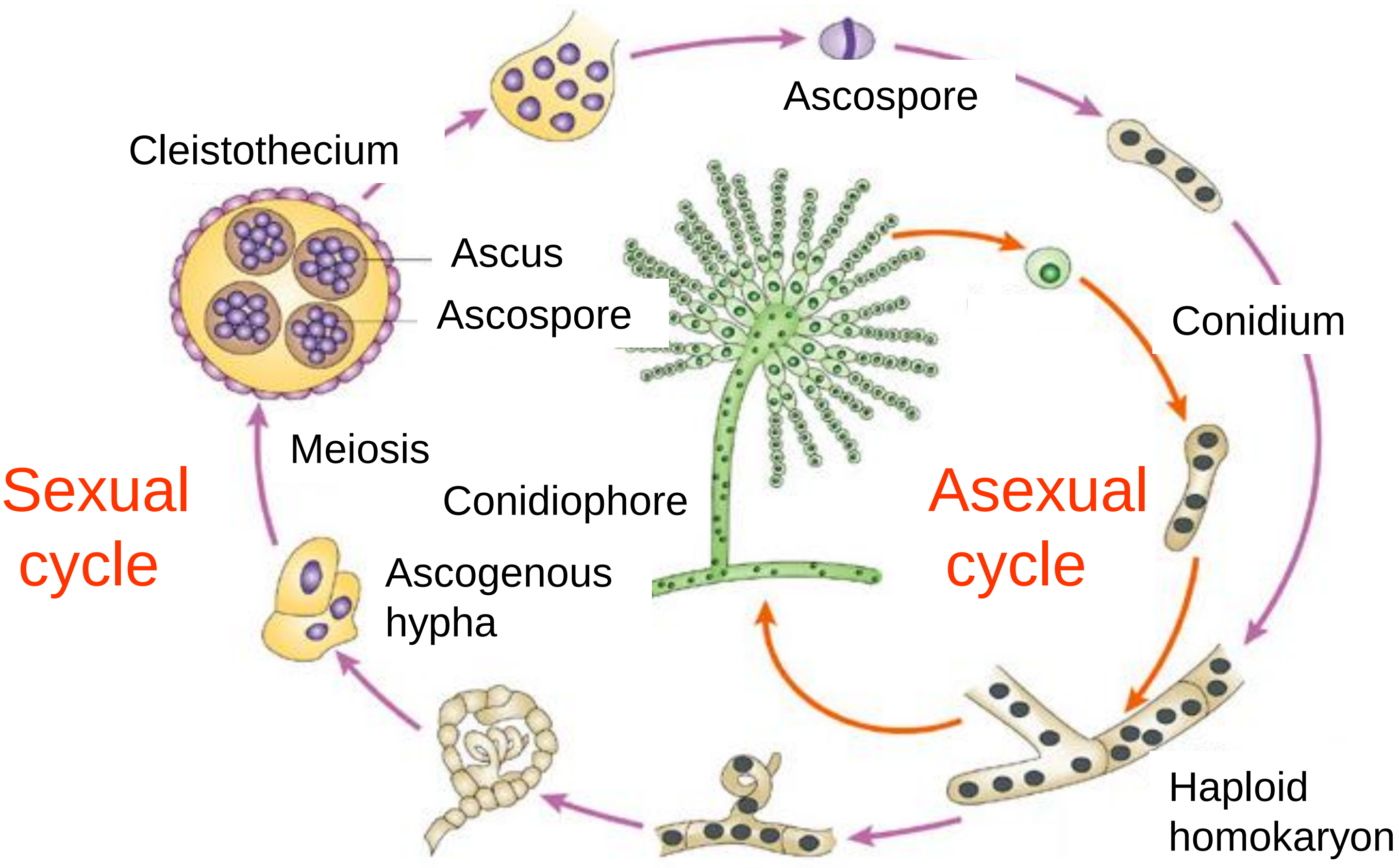
***Induction**: Extracellular enzymes expressed at low levels generate metabolites that signal the fungus to dramatically increase the expression level of genes encoding plant cell wall degrading enzymes.*



***Utilization**: Extracellular enzymes and transporters specific for translocation of cell wall degradation products enable the fungus to use plant cell material for growth. Some extracellular proteins may generate metabolites that modulate gene expression of cellulases and hemicellulases during the utilization phase.*

Tian *et al.*, 2009. *PNAS*, 106: 22157–22162

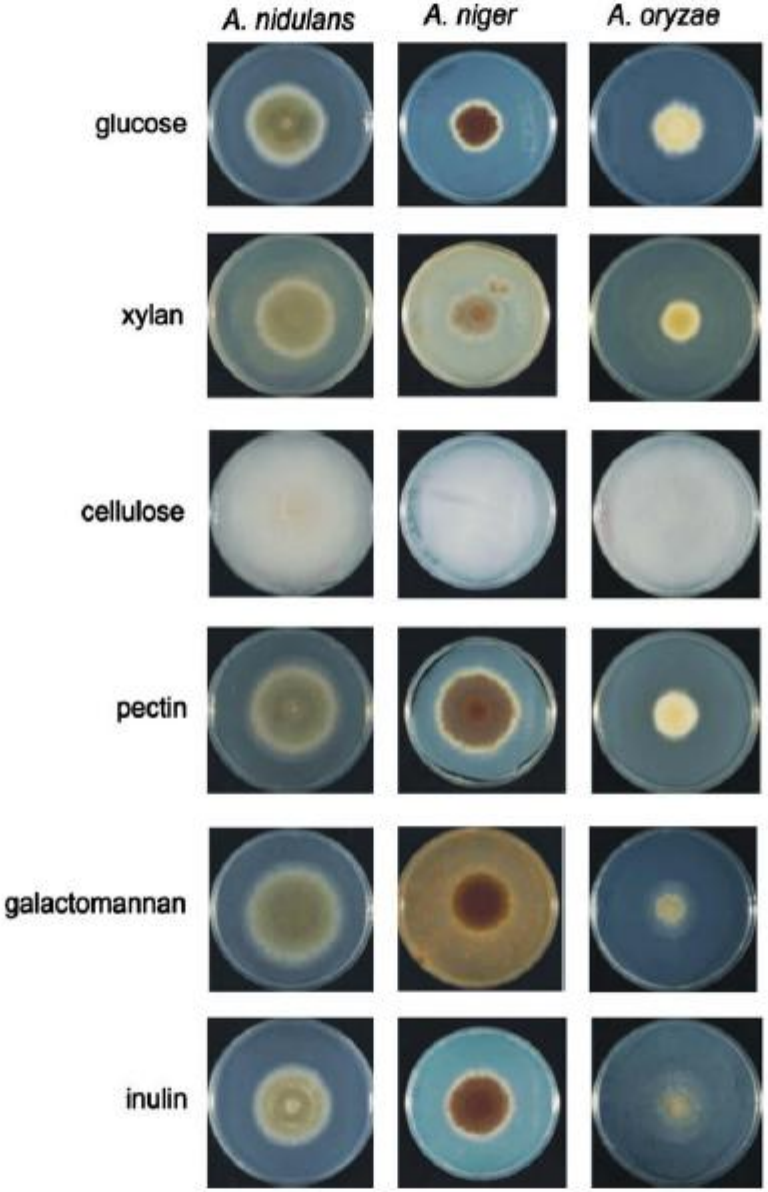
Life cycle of *Aspergillus nidulans*



Genome assembly size:
30,068,514 bp
Number of genes:
10,284 (Version III)

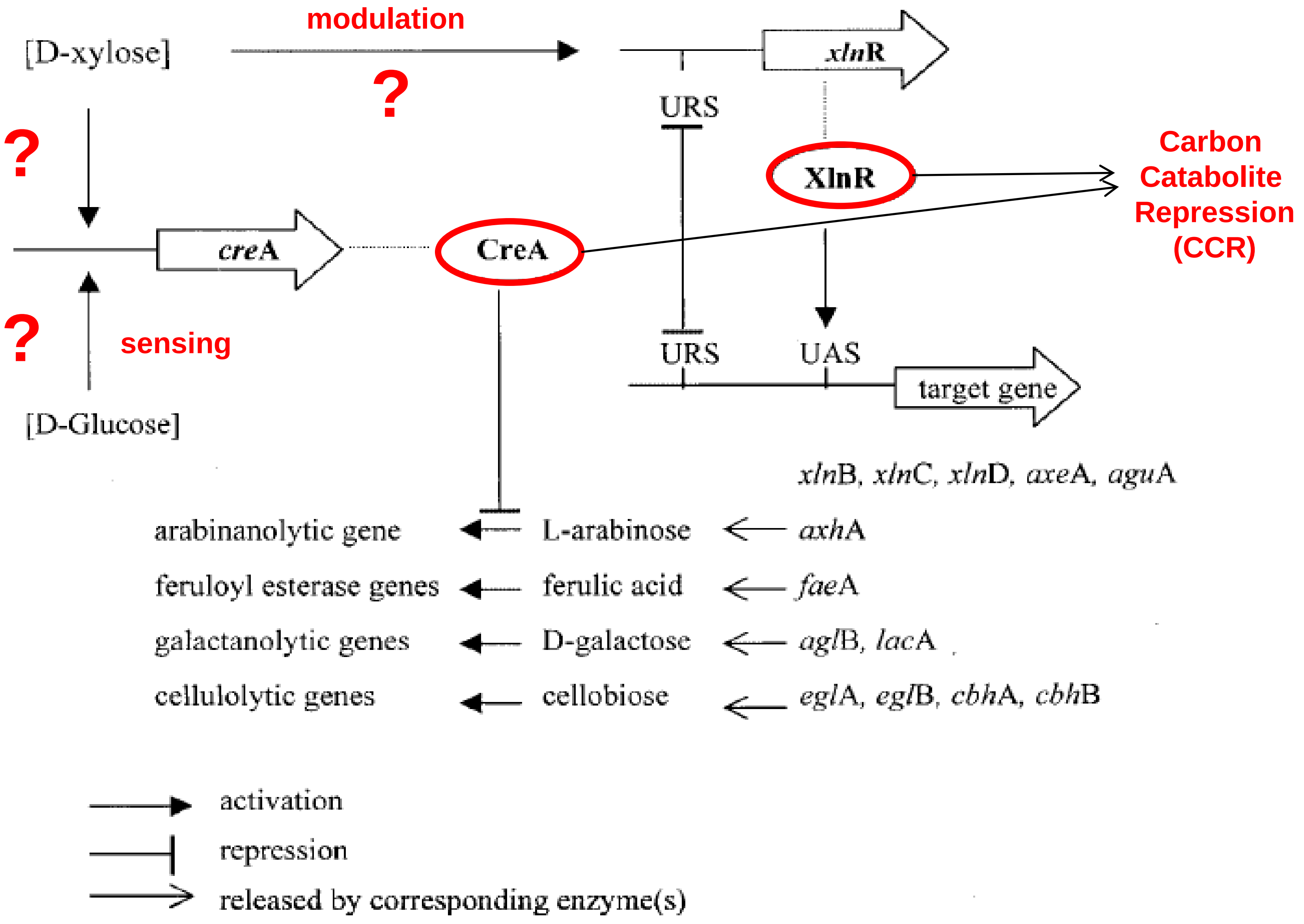
Casselton & Zolan (2002) *Nature Rev.* 3:683-97

Growth of *A. nidulans*, *A. niger*, and *A. oryzae* on polysaccharides in comparison to growth on glucose



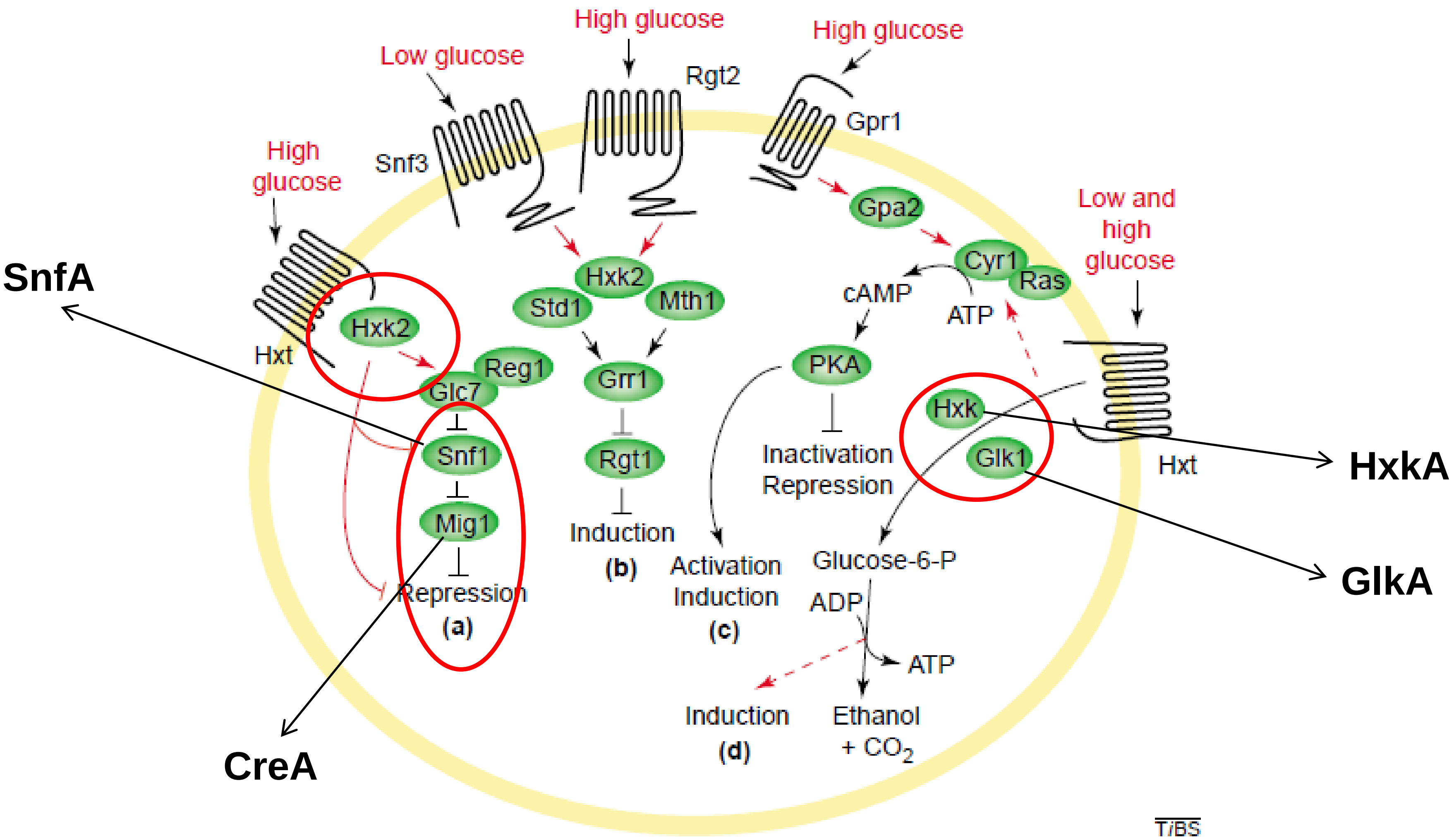
Coutinho *et al.*, 2009. Fungal Genet Biol. 46 Suppl 1:S161-S169.

Model for the role of XlnR and CreA in the genetic regulation of the genes encoding (hemi)cellulose-degrading enzymes by *Aspergilli*



de Vries and Visser, 2001. Microbiol Mol Biol Rev., 65: 497-522

Glucose sensing in yeast



Rolland *et al.*, 2001, TIBS, 26: 310-317

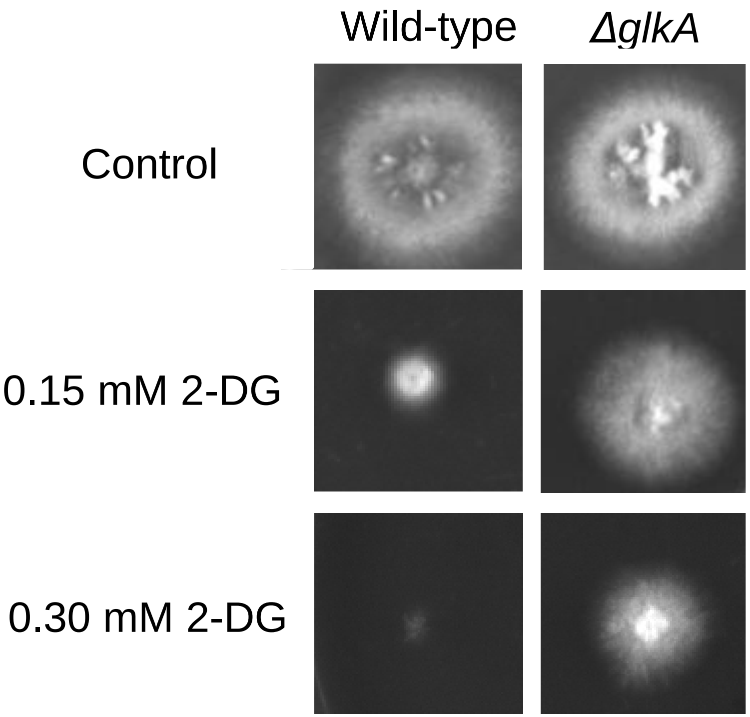
Outline

- Molecular characterization of genes involved in glucose sensing in *A. nidulans*

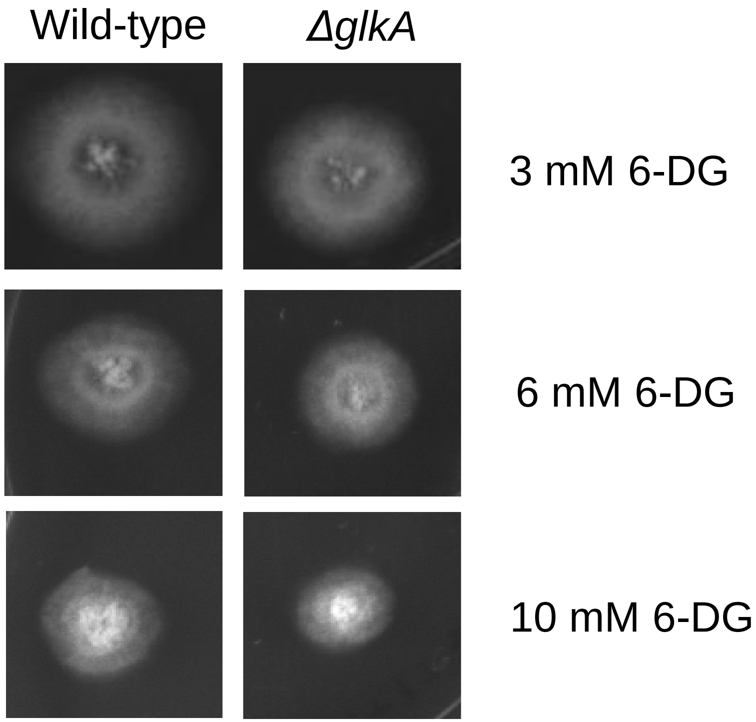
- Identification of an *A. nidulans* gene encoding an F-box protein involved in xylanase induction

The *A. nidulans ΔglkA* mutant strain is more resistant to 2-DG

Xylose 1%

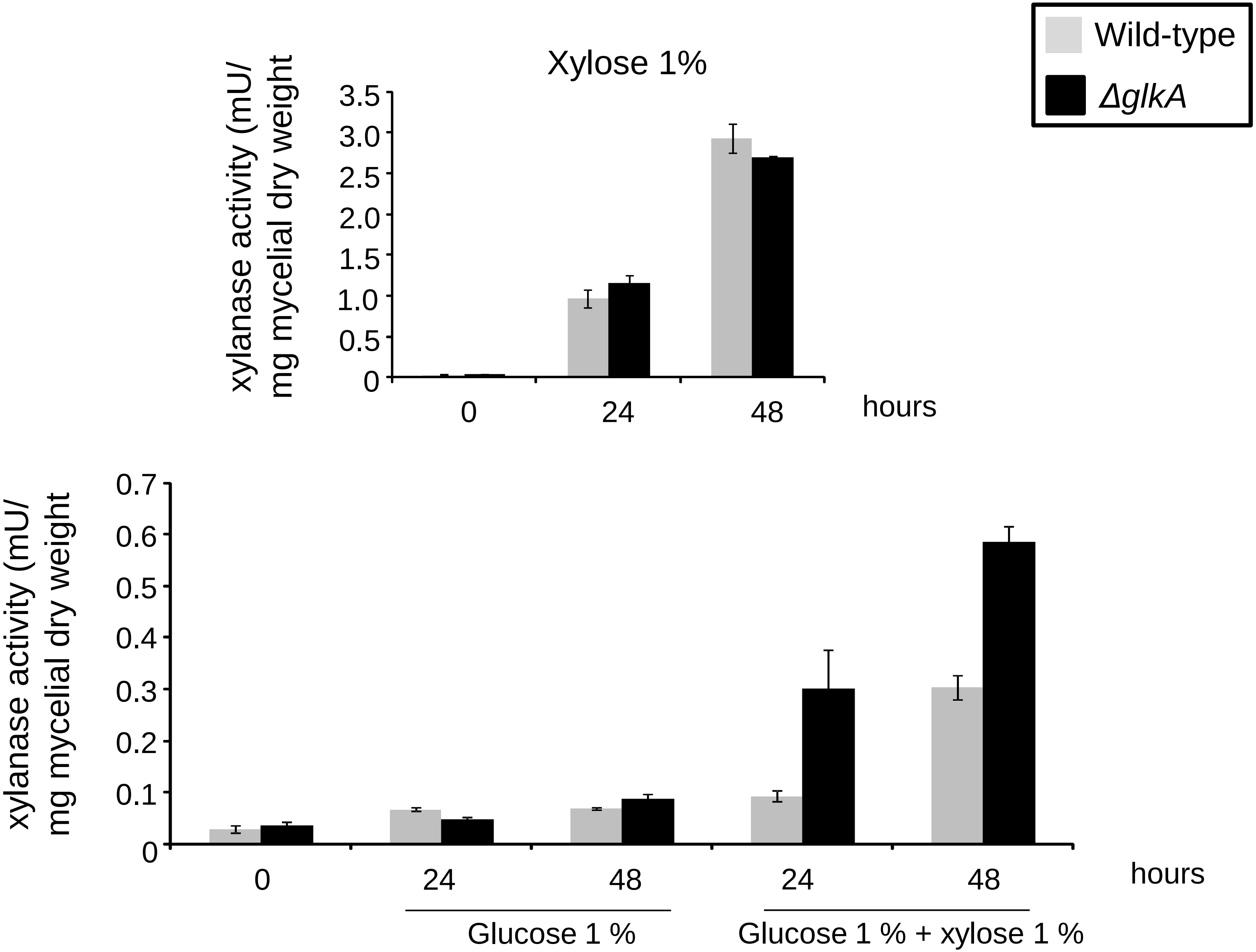


2-DG = phosphorylated but not metabolized further

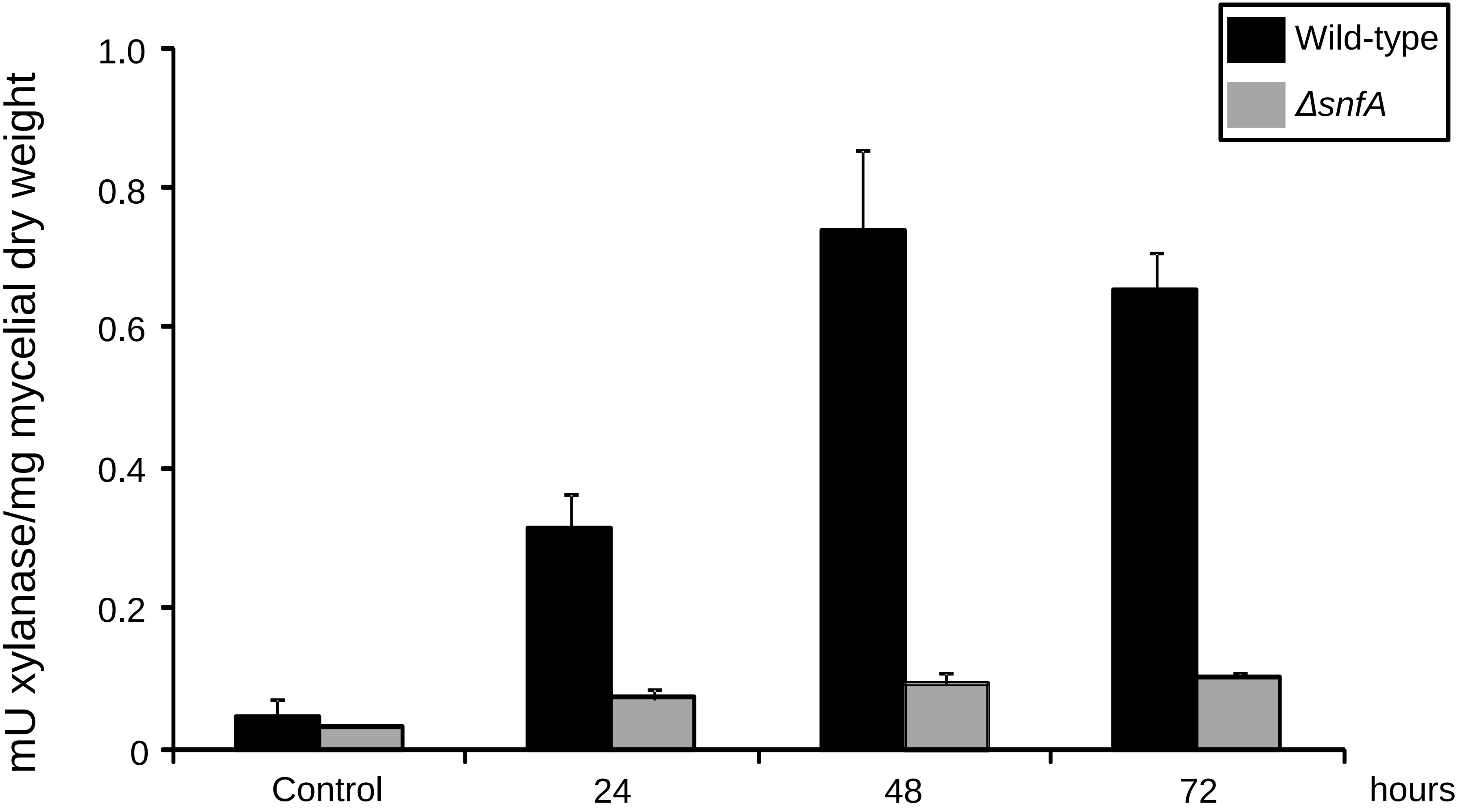


6-DG = transported but not phosphorylated

The xylanase production in *A. nidulans ΔglkA* mutant strain is partially resistant to catabolite repression by glucose



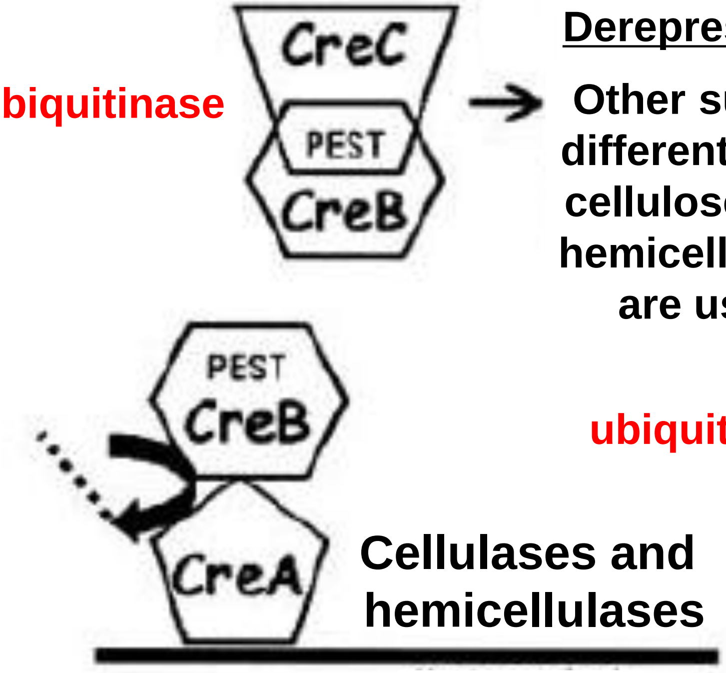
The *ΔsnfA* mutant has decreased xylanase activity



How is CreA repression regulated ?

Carbon catabolite repressing conditions

Deubiquitinase



Derepression:

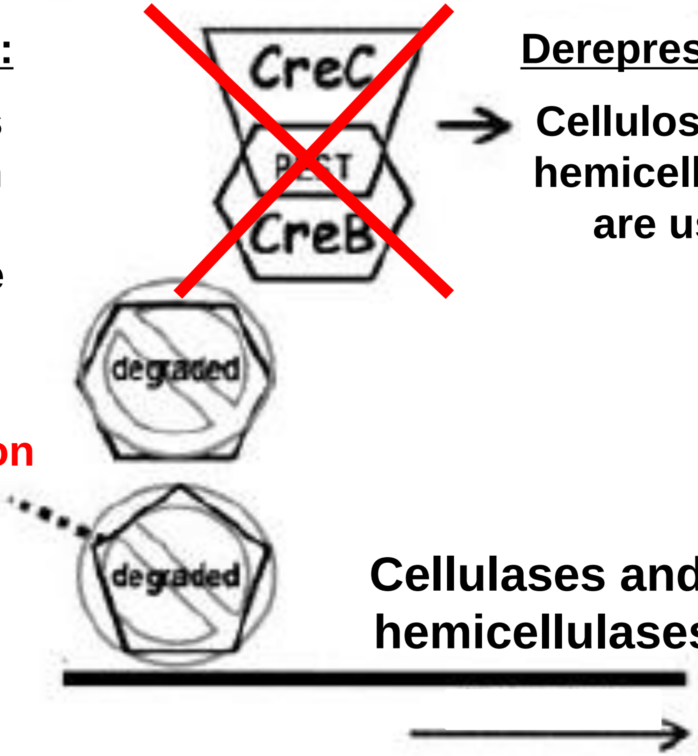
Other sugars different from cellulose and hemicellulose are used

Cellulases and hemicellulases

Not transcribed

ubiquitination

Carbon catabolite derepressing conditions



Derepression:

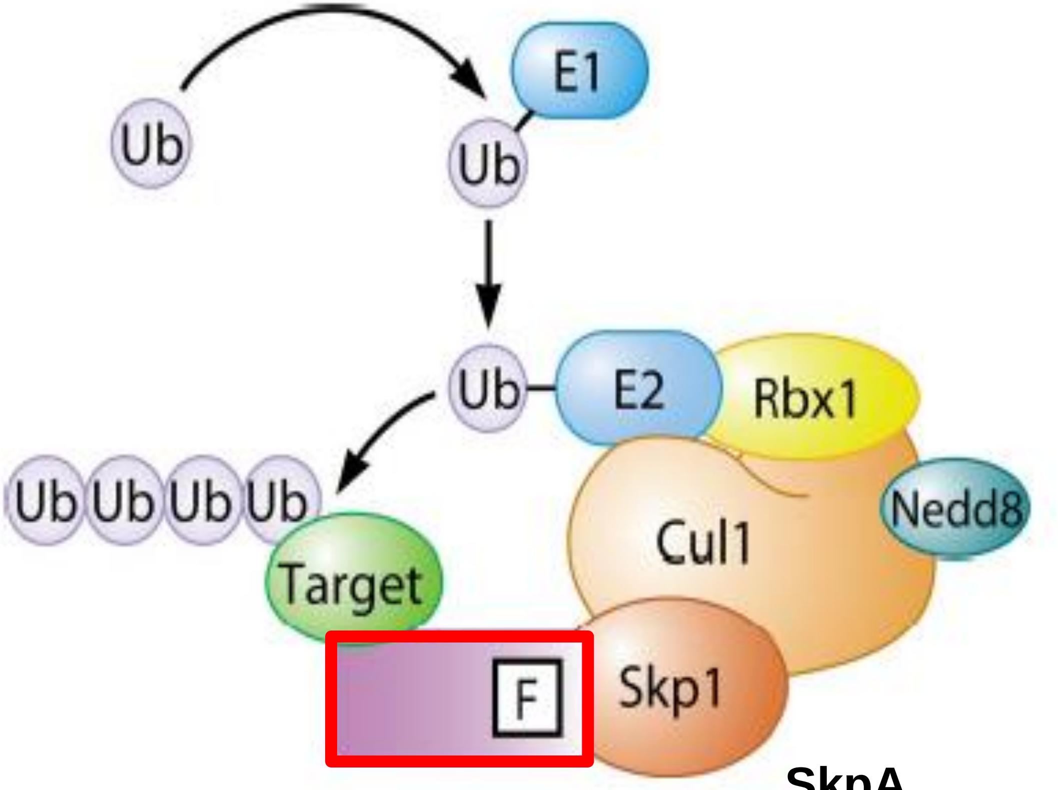
Cellulose and hemicellulose are used

Cellulases and hemicellulases

Transcribed

How is CreA ubiquitinated ?

SCF complex (E3 ubiquitin ligase) and ubiquitination of target proteins

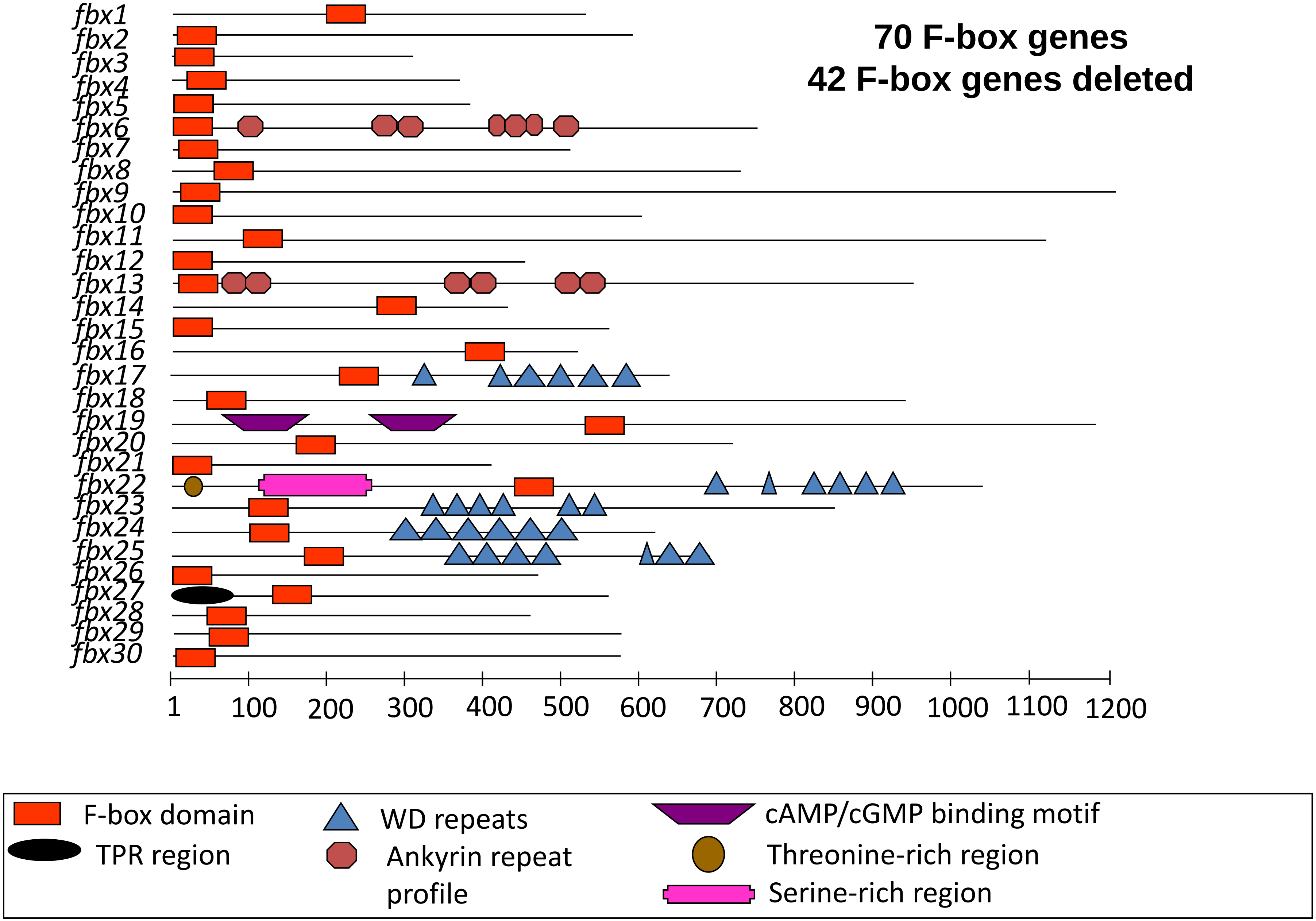


SkpA

E3
ubiquitin
ligase

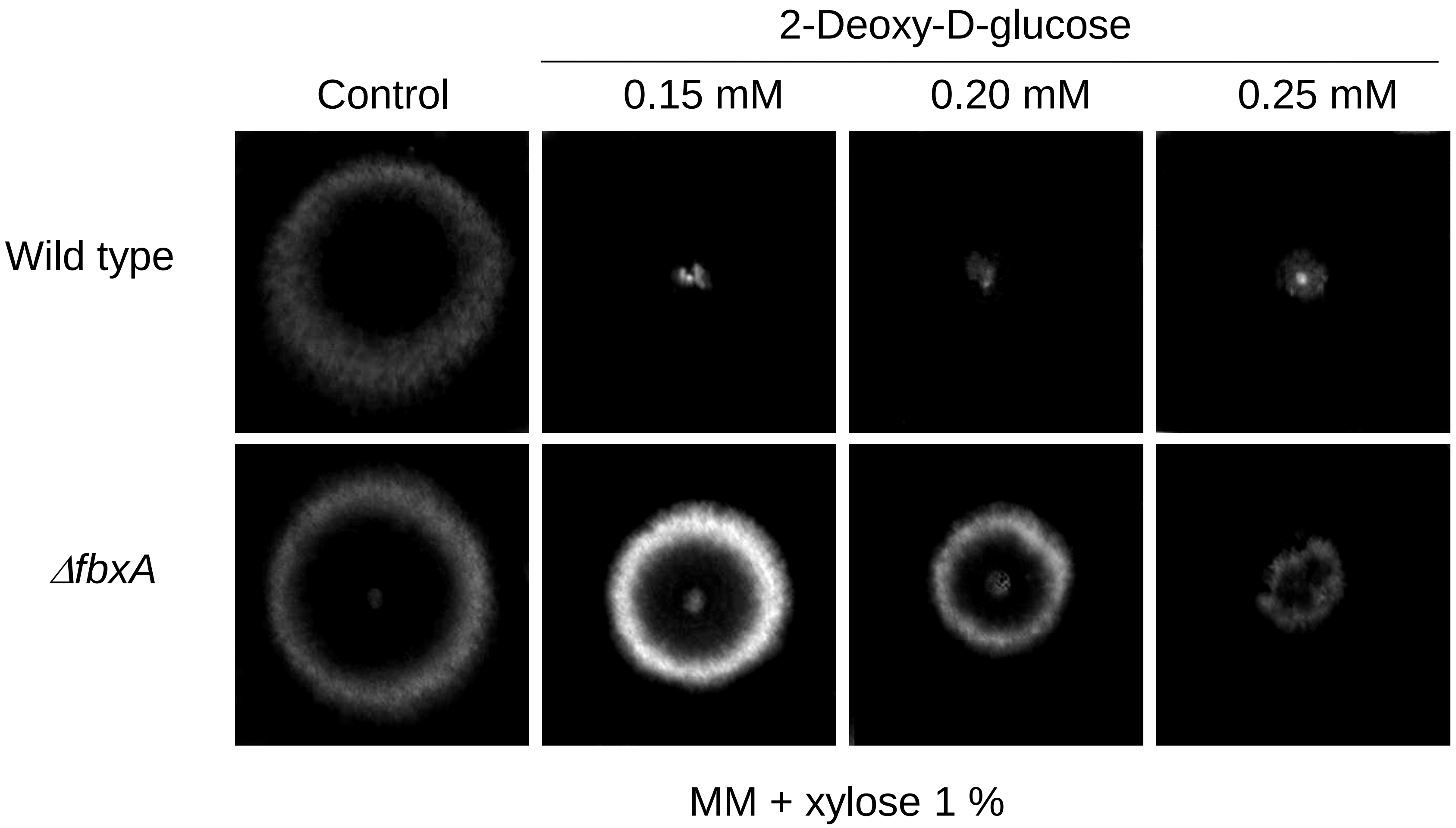
The F-box hypothesis = F-box-containing proteins act as scanvengers in the cell, collecting “junk” proteins to deliver to a “waste processor” called the SCF complex, to which they dock through their F-box domain.

Is there an F-box for CreA ubiquitination ?

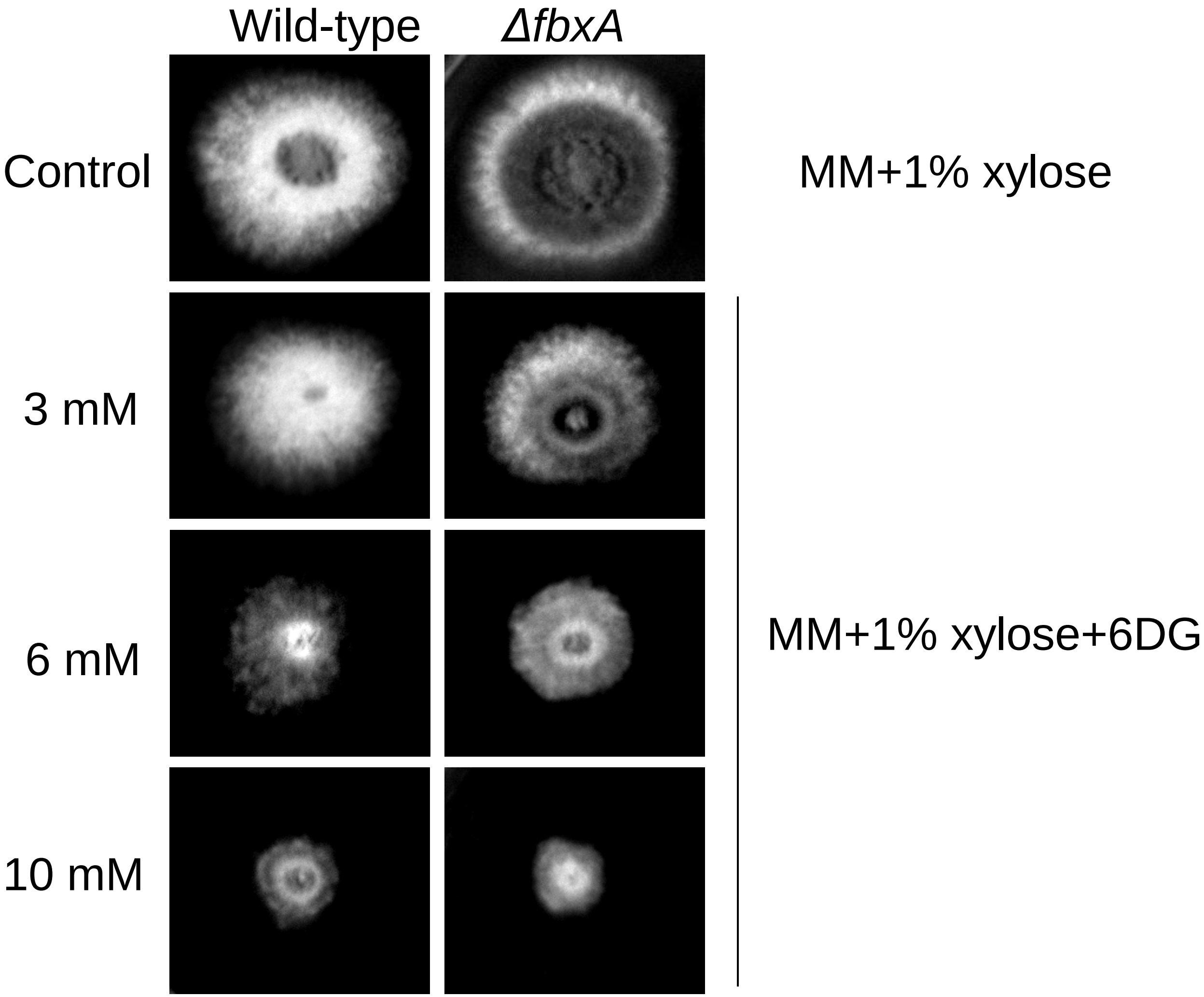


70 F-box genes
42 F-box genes deleted

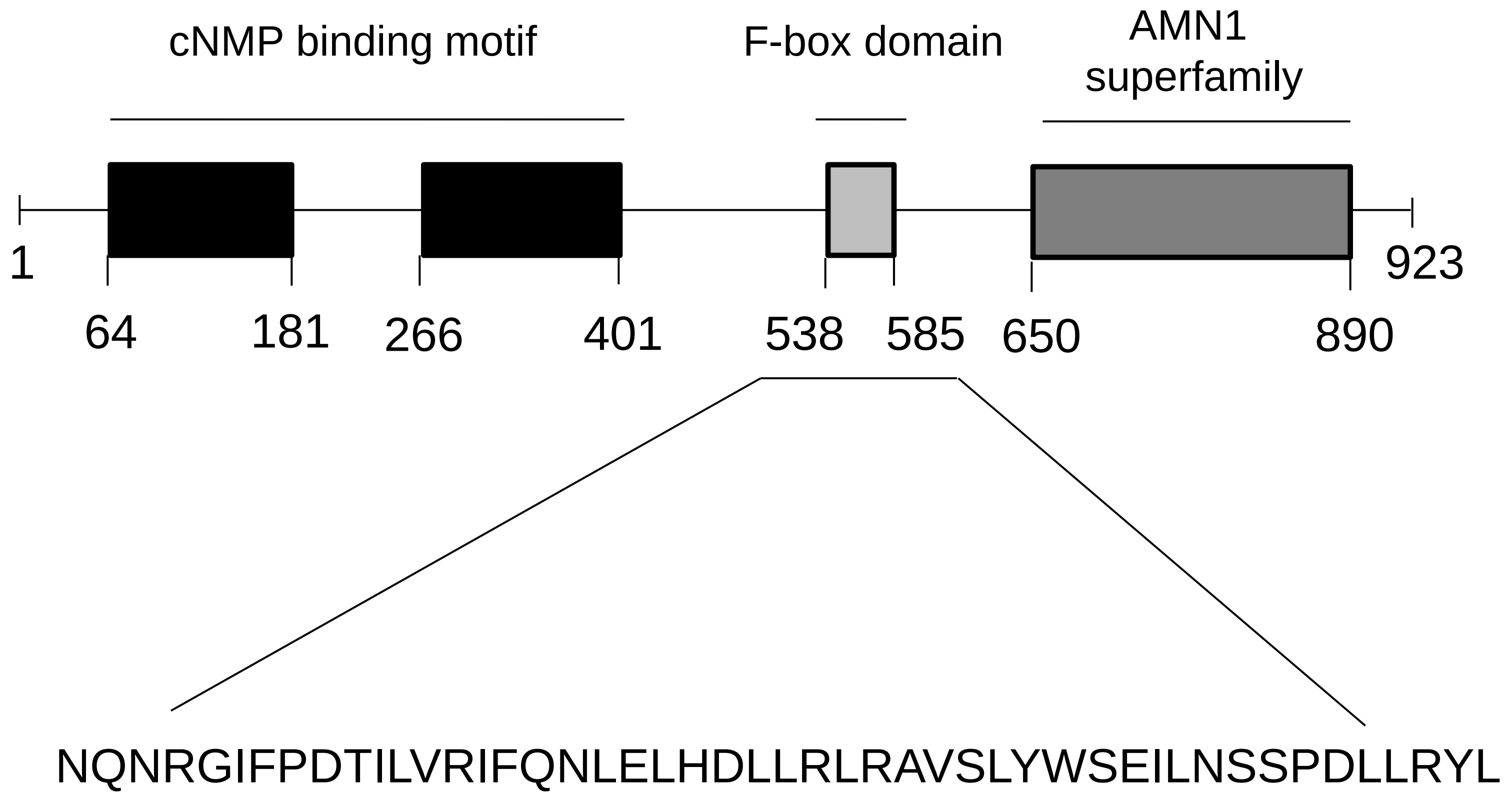
The *A. nidulans ΔfbxA* mutant strain is more resistant to catabolite repression in the presence of xylose



The *A. nidulans ΔfbxA* mutant is as sensitive to 6-DG as the wild-type strain

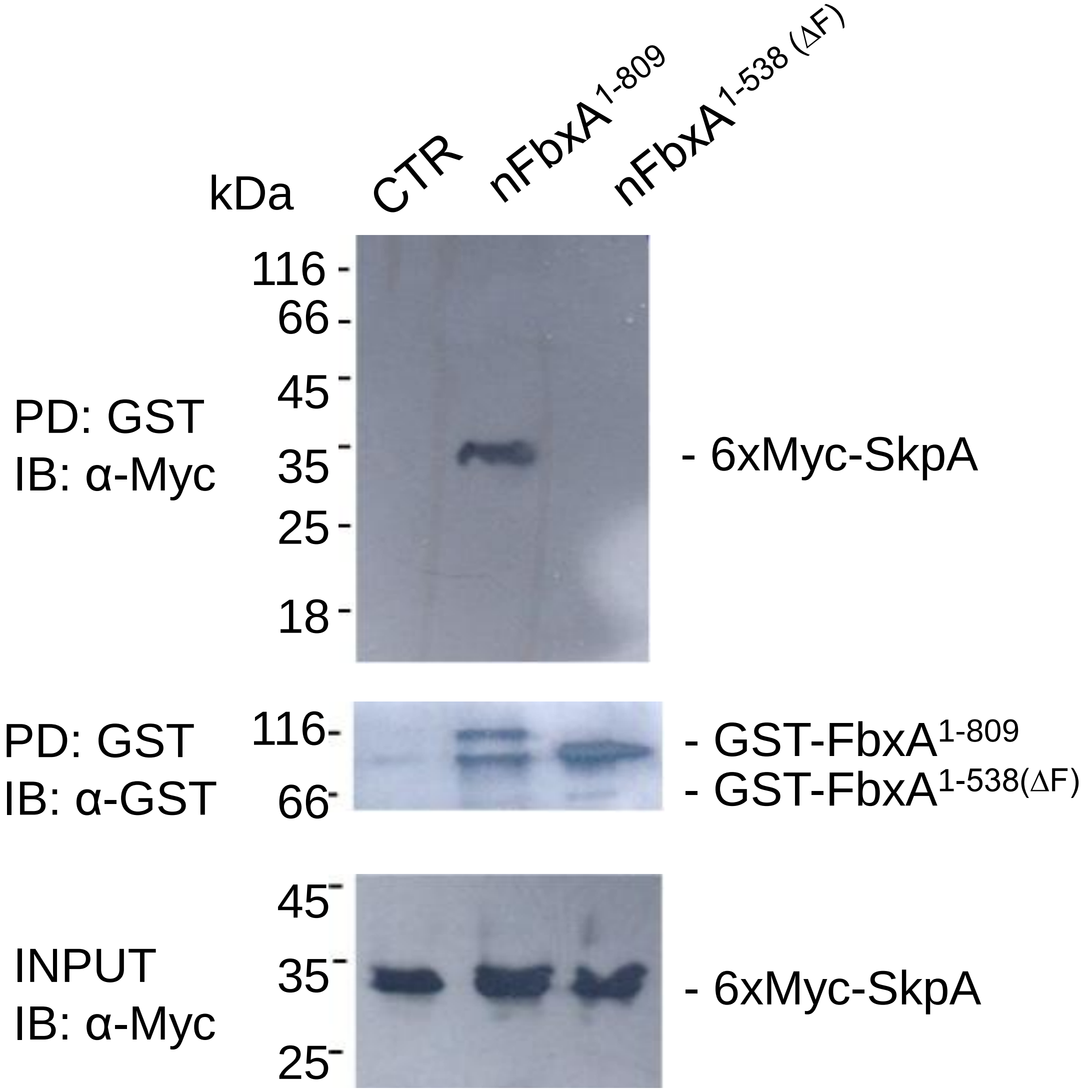


The *A. nidulans fbxA* encodes a novel protein only present in fungi

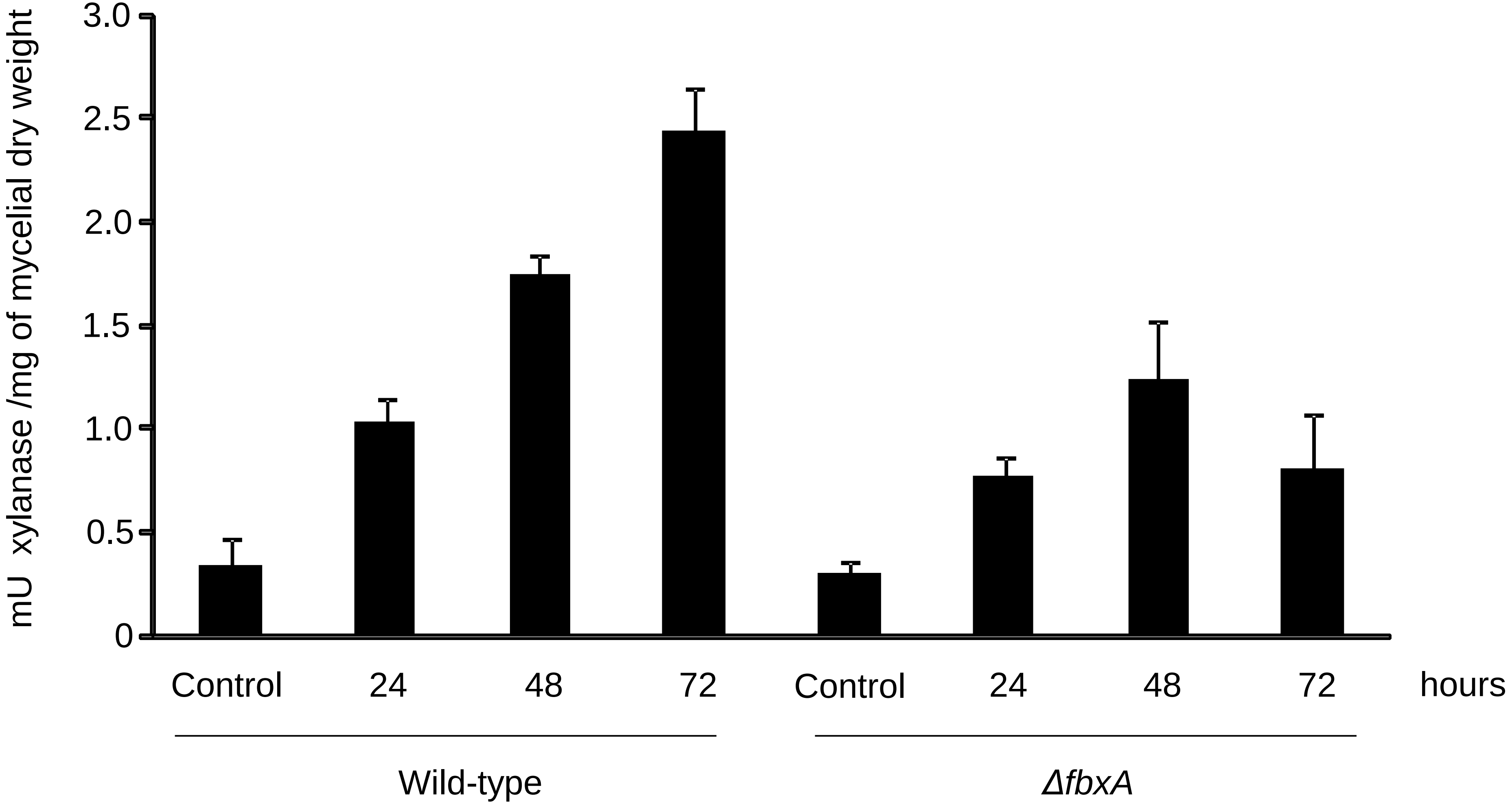


NQNRGIFPDTILVRIFQNLELHDLLRLRAVSLYWSEILNSSPDLLRYL

A. nidulans FbxA interacts with SkpA



The *A. nidulans ΔfbxA* mutant has decreased xylanase activity

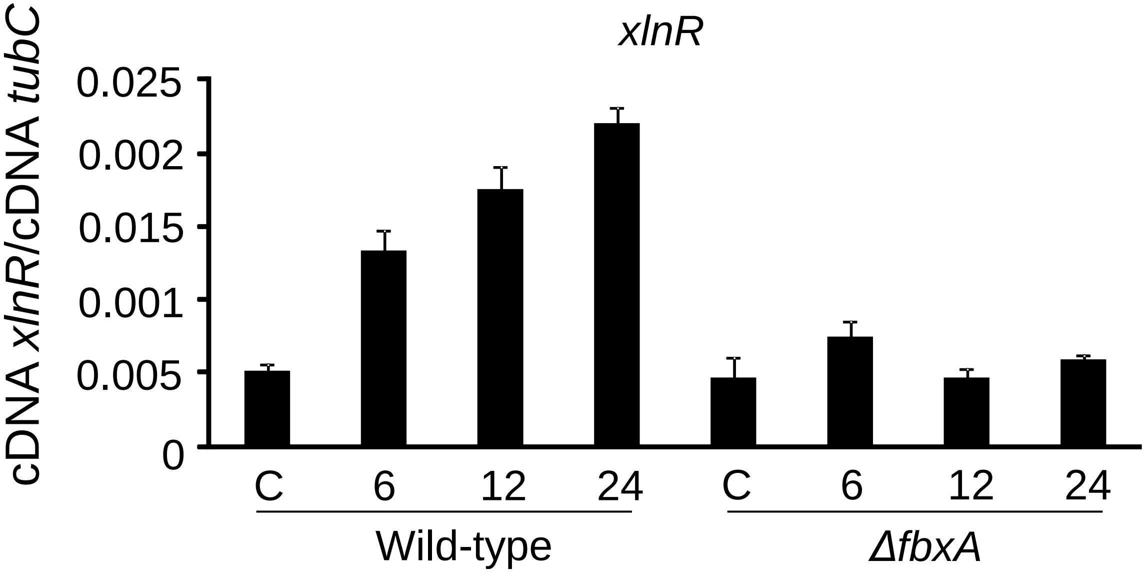


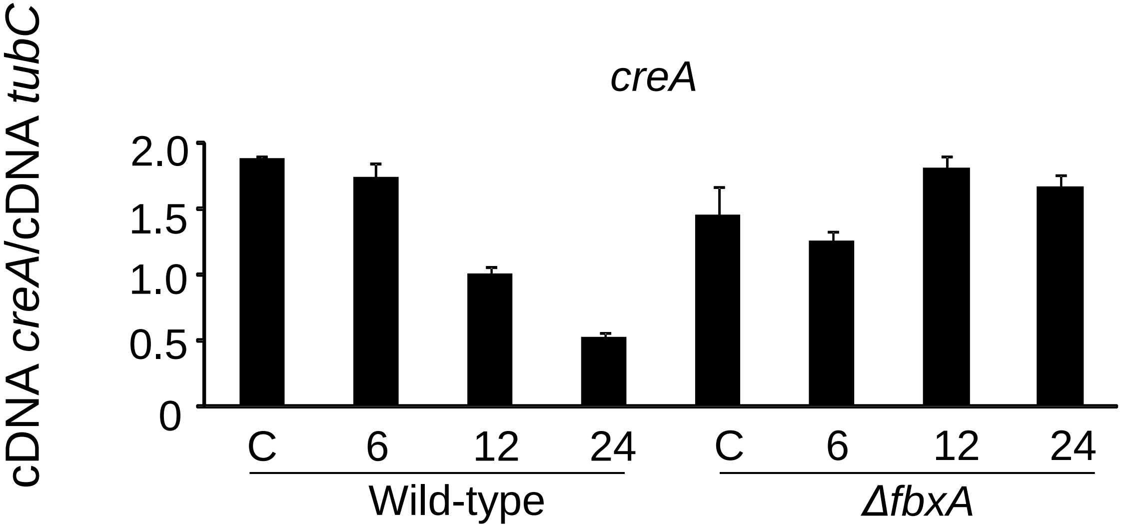
The *xlnA* and *xlnD* mRNA accumulation in *A. nidulans* strains

xlnA	Control	Xylose 1 h	Xylose 4 h
Wild-type	0.04 ± 0.00	0.92 ± 0.15	1.14 ± 0.06
ΔfbxA	0.007 ± 0.00	0.31 ± 0.08	1.10 ± 0.09

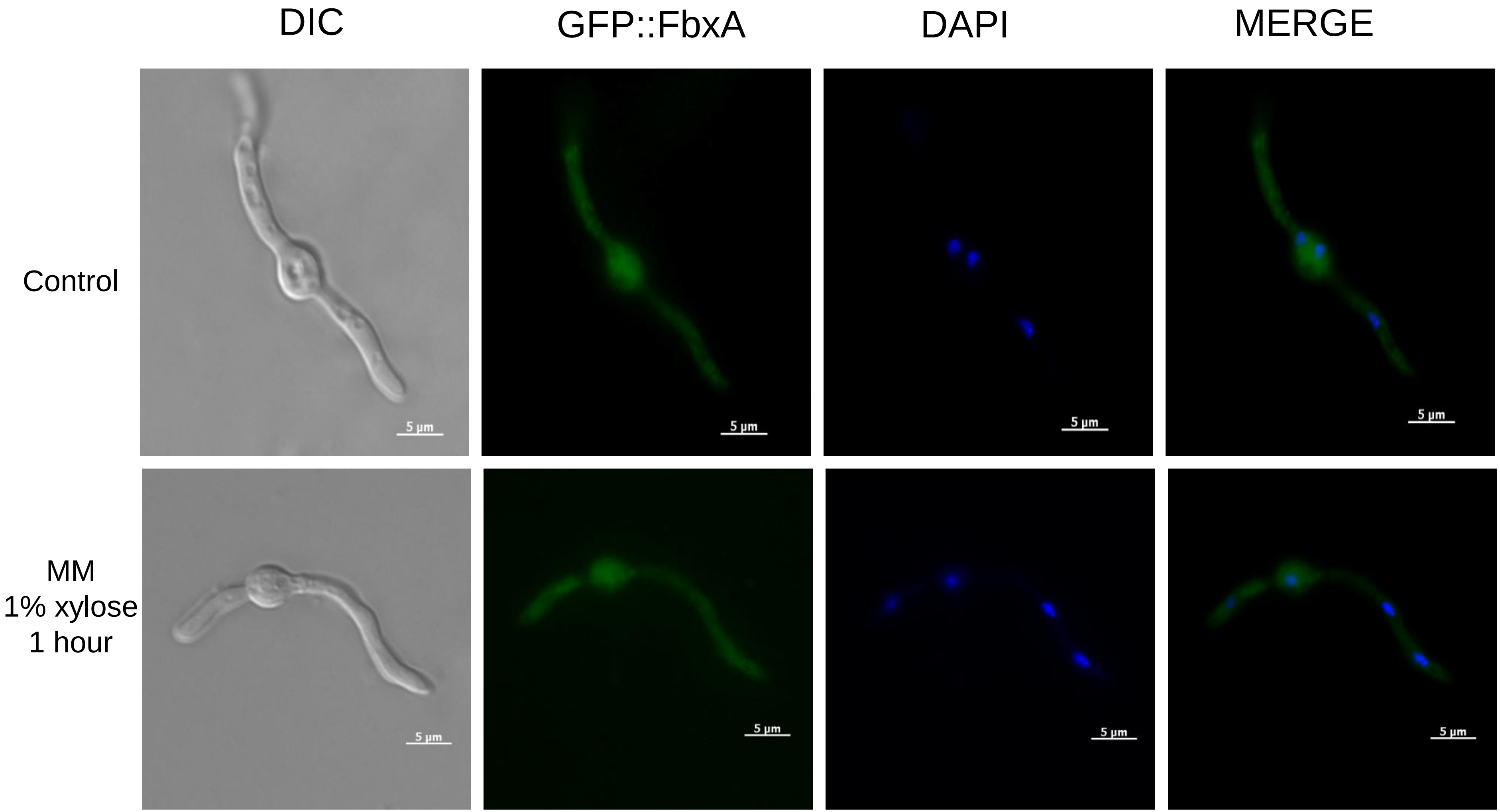
xlnD	Control	Xylose 1 h	Xylose 4 h
Wild-type	0.07 ± 0.007	7.00 ± 0.60	2.00 ± 0.06
ΔfbxA	0.12 ± 0.005	1.90 ± 0.10	0.60 ± 0.01

The *A. nidulans* xlnR and creA genes have decreased and increased, respectively, mRNA accumulation in the *ΔfbxA* mutant



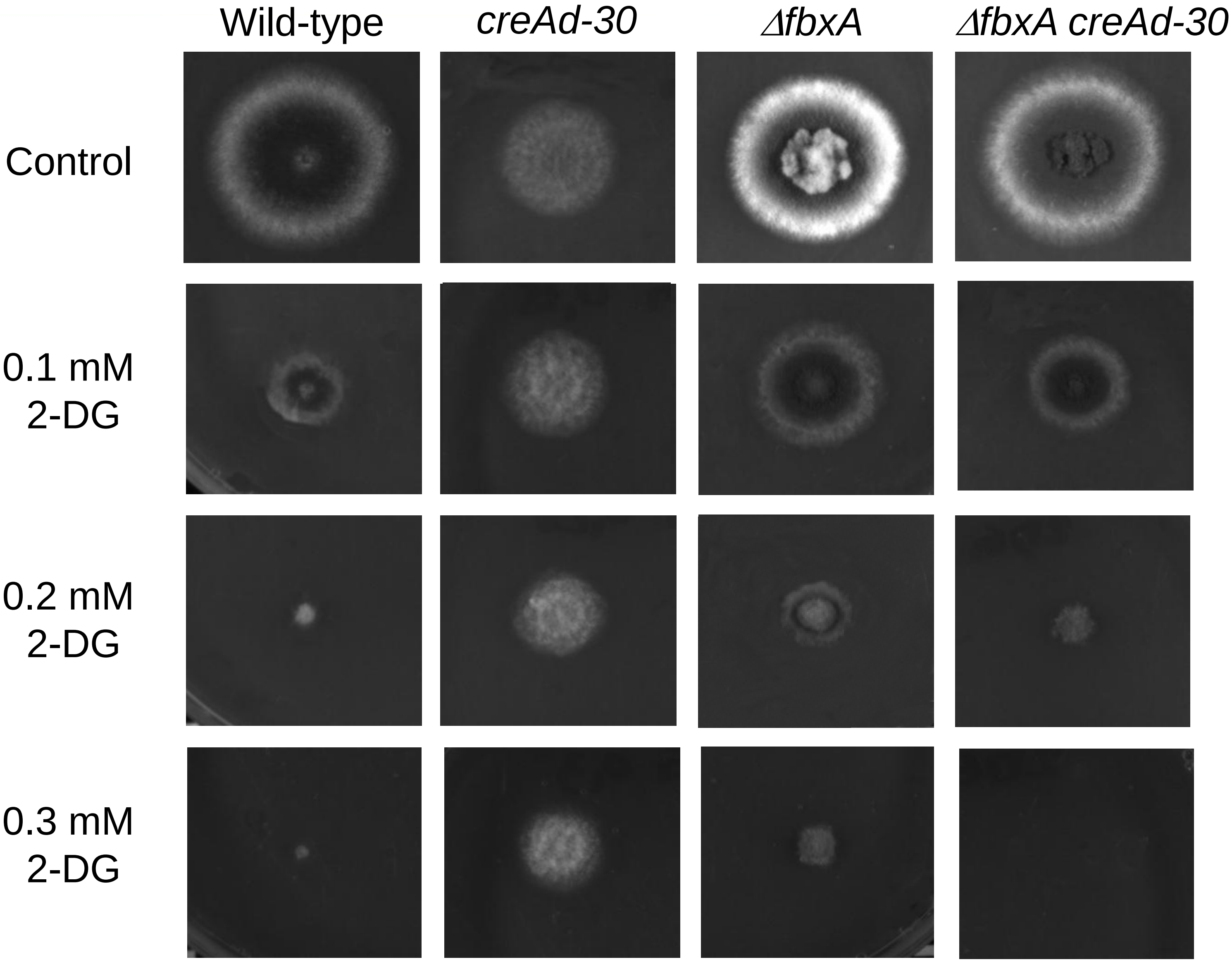


The *A. nidulans* GFP::FbxA is always located into the cytoplasm



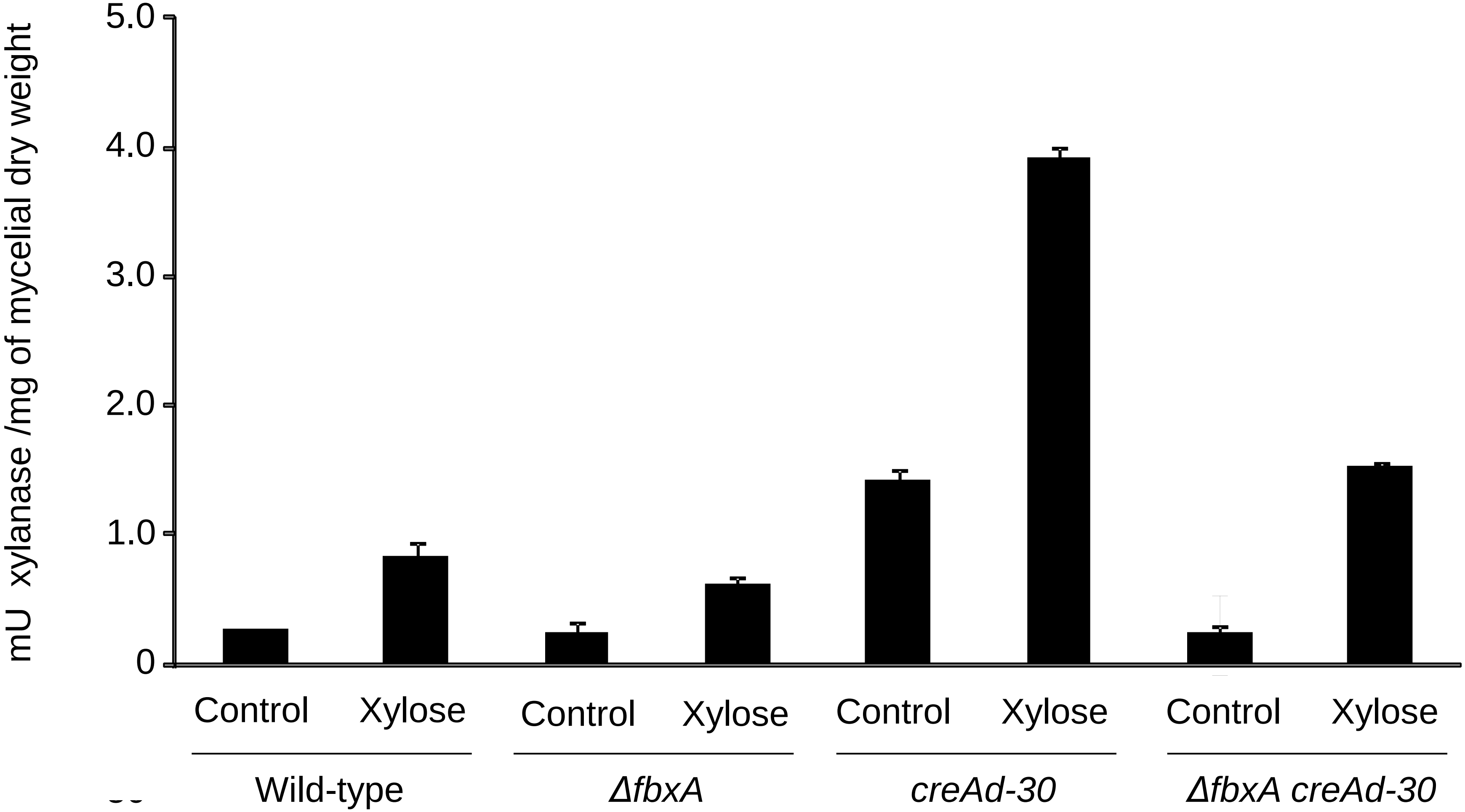
An explanation for the xylanase expression defect in the Δ*fbxA* mutant may be found in constitutive CCR

The *creAd-30* mutation suppresses the *ΔfbxA* 2-DG resistance



MM + xylose 1 %

The *creAd-30* mutation suppresses the lack of xylanase activity



Take home message

1) GlkA and SnfA are important for the CCR and xylanase expression.

2) We identified an F-box encoding gene that influence the xylanase expression.

3) Our work does not provide details of how FbxA influences *A. nidulans* xylanase induction and CCR. However, FbxA encodes a functional F-box protein that is together with CreA required for the expression of CCR-regulated genes.

4) It is possible FbxA ubiquitinates CreA and/or a (yet unidentifed) protein which is controlling xylan-degradation genes as well as glucose repression genes.

5) If FbxA deals with transcription factors in the nucleus, it deals either before they can enter the nucleus or it deals with transcription factors which have been exported from the nucleus.

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