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DISSERTATION

Nuclear transport and cellulase production in *Trichoderma reesei*

ausgeführt zum Zwecke der Erlangung des akademischen Grades eines Doktors der Naturwissenschaften unter der Leitung von

Privatdoz. Mag. Dr. rer. nat. Bernhard Seiboth

und

Univ. Prof. Dr. Christian P. Kubicek

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von

Mag. rer. nat. Sara Ghassemi

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CONTENT

KURZFASSUNG

Unter den Mikroorganismen, die (Hemi-)Zellulose abbauen können, ist der Pilz *Trichoderma reesei* derzeit der wichtigste industrielle Produzent von cellulolytischen und hemicellulolytischen Enzymen, die für Produktion von Biokraftstoffen und Bioraffinerieprodukten aus Lignocellulose eingesetzt werden. Das Xylanaseregulatorprotein 1 (XYR1), ein Transkriptionsfaktor des Zinc binuclearen Cluster typs ist der Hauptaktivator der Zellulase und Hemizellulase Genexpression.

Um die Gene zu aktivieren muss XYR1 aus dem Zytoplasma in den Zellkern transportiert werden. Der Mechanismus dieses nuklearen Imports ist noch unbekannt. Transport von Proteinen über 30 kDa in den Kern geschieht mit Hilfe von spezifischen nuklearen Transportern, sogenannten Karyopherinen. In meiner Dissertation konnte ich zeigen, dass das Genom von *T. reesei* zehn Karyopheringene (ein α-Importin und neun β-Importine) aufweist. Um jene β-Importine, die am Kernimport von XYR1 beteiligt sind zu identifizieren, habe ich Deletionsmutanten von 9 der 10 Karyopherine hergestellt und deren Rolle beim Transport von XYR1 in den Zellkern untersucht. Ich konnte zeigen, dass das ß-Importin KAP8, ein Ortholog von *Saccharomyces cerevisiae* Pse1 / Kap121 und *Aspergillus nidulans* KapI, essentiell für den nuklearen Import von XYR1 und die Induktion von Zellulasen und Hemizellulasen durch Laktose und Sophorose ist. Darüber hinaus konnte ich zeigen, dass KAP8 eine wichtige Rolle bei der Ausbildung asexueller Sporen sowie der Toleranz gegenüber verschiedenen Arten von Stress spielt. Die Ergebnisse weisen damit auf eine neue, wichtige Ebene der Zellulasebildung hin, welche für die Verbesserung industrieller Stämme genutzt werden kann.

SUMMARY

Among the microorganisms capable of (hemi-) cellulose degradation, the filamentous fungus *Trichoderma reesei* is the currently most important industrial producer of cellulolytic and hemicellulolytic enzymes which are used for 2nd generation biofuels and biorefinery production from lignocellulosic biomass. Aiming to achieve higher enzyme yields, proteins involved in transcriptional regulation of (hemi-) cellulase gene expression have been intensively studied in the last decade.The xylanase regulator 1 (XYR1), a zinc binuclear cluster transcription factor, is the main activator of cellulase and hemicellulase gene expression. The mechanism of nuclear import, however, has not yet been studied in *T. reesei*. It was thus unknown how XYR1 crosses the nuclear envelope. Macromolecule transport demands participation of specific nuclear carriers of the karyopherin-ß superfamily. The genome of *T. reesei* encodes 10 karyopherins (one α-importin and nine β-importins) acting as nuclear importers. XYR1 contains a classical, three-partite nuclear localisation signal, and thus theoretically requires interaction with the α-importin and at least one of the ten β-importins. To identify the β-importin(s) responsible for nuclear import of XYR1. I have generated gene deletion strains of 9 of the 10 karyopherins of *T. reesei*, and tested their importance for XYR1 transport. I could demonstrate that the *T. reesei* ß-importin KAP8, an orthologue of *Saccharomyces cerevisiae* Pse1/Kap121 and *Aspergillus nidulans* KapI, is essential for nuclear import of XYR1, and furthermore show that KAP8 plays a major role in asexual sporulation and stress resistance in *T. reesei*.

Acknowledgements

First of all I would like to thank Christian P. Kubicek for giving me the opportunity to perform my Ph.D. thesis at the Technical University of Vienna and for his contributions to all aspects of my work on this thesis.

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Finally I want thank my parents. Words cannot express my gratitude for your unconditional love , care and everything that you have done.I would also like to thank my beloved brother,Amir for always supporting me and always being there for me.

INTRODUCTION

1.1 Cellulose and hemicellulose polymers

1.1.1 Cellulose

The β-(1,4)-linked glucose polymer cellulose is a principal component of plant biomass, and produced by utilisation of solar energy and carbon dioxide. Cellulose is usually combined with hemicelluloses and has an about annual production of 7.2 ×1010 tones. Hydrogen bounds link hydroxyl groups of the glucose monomers to oxygen molecules on the same or neighboring chains and hold the chains together side by side and build microfibrils with a high strength. This strength is the most important source of rigidity for the plant cell wall (Kubicek, 2012).

1.1.2 Hemicellulose

Hemicelluloses are complex heterogenous polysaccharides which besides cellulose represent about 20-35% of lignocellulose or plant dry matter. Unlike cellulose, hemicelluloses are chemically heterogenous and composed of pentoses, hexoses and sugar acids. Xylan is the most abundant of these complex polymers and characterized by a backbone chains of β-1, 4-linked D-xylose units. Xylans are the major hemicelluloses hardwood while softwood hemicelluloses contain only glucomannans.(Kubicek, 2012).

1.2 Cellulases and hemicellulases of *T. reesei*

Trichoderma reesei is an ascomycete with an outstanding ability to produce high amounts of a wide range of carbohydrate active enzymes (CAZy) capable of decomposing a wide range of complex polysaccharides, including cellulose and hemicelluloses(Seiboth *et al.,* 2012).

T. reesei was firstly isolated from cotton canvas of US army tents on the Solomon Islands during World War II (Reese, 1976). This wild-type isolate QM6a, (Quarter Master 6a) is the basis for all strain used in industry for the production of enzymes which were produced within the last decades mainly by random mutagenesis and selection (Rubini *et al*., 2010).

Two broad categories of cellulases have been identified: cellobiohydrolases which cleave off cellobiose residues from ends of the cellulose chains, and endoglucanases that attack the β-glycosidic bonds within the cellulose chain (Amore *et al*., 2013).The cellulase activity produced by *T.reesei* is composed of a complement of endoglucanases (EGI/Cel7B, EGII/Cel5A, EGIII/ Cel12A, EGIV/Cel61A, and EGV/Cel45A) and the cellobiohydrolases (CBHI/Cel7A, and CBHII/Cel6A) that act synergistically to cleave cellulose to cellobiose (glycosyl-1,4-glucose) and two-glucosidases

(BGLI/Cel3A and BGLII/Cel1A) that are implicated in hydrolyzing of cellobiose to glucose which serves as an easily utilizable carbon source for fungi (Kubicek *et al*., 2009). The glycoside hydrolase 61 (GH61) family is another group of proteins expressed in *T. reesei* and enhanced the performance of cellulases in lignocellulose hydrolysis (Langston & Shaghasi, 2011).

These enzymes are in fact lytic polysaccharide mono-oxygenases (LPMO) and are summarized in the new CAZy class auxiliary activities. These enzymes are copper-dependent and cleave the β-(1→4) glycosidic bonds in these substrates, leaving the C1 or the C4 carbon oxidized.

In addition to classical cellulases, swollenin protein (encoded by the gene swo1) with sequence similarity to plant expansins was also isolated from *T. reesei* (Saloheimo *et al*., 2002) The production of these enzymes is an energy demanding process and the regulation of expression of these enzymes in a carbon source depended manner ensures that the enzymes are only produced, when the fungus needs to use plant polymers as sole carbon source. Therefore these degrading enzymes are only synthesized in presences of an inducer, such as celluloses and their transcripts are not formed in the presence of glucose, fructose, or glycerol (Aro *et al*., 2005, Kubicek *et al.*, 2009) .It has been reported that major cellulase genes are expressed under inducing conditions and required the obligatory presence of an inducer suggesting the regulation of cellulase gene expression in a consistent way, although the promoter titration effects in higher producer mutants affected relative ratio of their cellulase gene expression(Foreman *et al*., 2003).

1.2.1 **Regulation of (hemi-) cellulase gene expression in *T. reesei***

Several transcription factors, involved in the expression of cellulase genes have been identified, including four positive transcriptional activators (XYR1, ACE2, ACE3 and the HAP2/3/5 complex) as well as two repressors (ACE1, and the carbon catabolite repressor CRE1)(Aro *et al.*, 2005).

The presence of easily metabolizable carbon sources in the enviroment like glucose causes repression of genes necessary for utilization of other alternatively carbon sources, which is called carbon catabolite repression.

In *T.reesei* and other ascomycetous fungi, the key player for CCR is the Cys2His2 type transcription factor CRE1/CreA. CRE1 is the major negative regulator of cellulase and hemicellulase gene expression and also represses expression of the regulator gene xyr1 (Mach-Aigner *et al*., 2008, Seiboth *et al*., 2012). XYR1 on the other side, the xylanase regulator 1, is considered to be the main activator of cellulase and hemicellulase gene expression and belongs to the zinc binuclear cluster proteins (Stricker *et al*., 2006). ACE1 (activator of cellulases 1) is a second negative regulator of

cellulases, and has been shown to repress the expression of the cellulase genes cbh1, cbh2, egl1 and egl2 under otherwise inducing conditions(Takanori *et al*., 2009).

The cellulase activator ACE2 belongs to the class of zinc binuclear cluster proteins which is found exclusively in fungi. ACE2 binds to the same promoter elements as XYR1, and acts as a co-activator of transcription. The deletion of ACE2 causes reduced expression of all of major cellulase genes upon induction by cellulose but did not affect induction by sophorose(Aro *et al.*, 2001).Recently ACE3 was discovered and found to be detrimental for cellulase production and for the expression of several cellulase genes and its deletion significantly reduced xylanase activity and expression of xylan-degrading enzyme genes.(Häkkinen *et al*., 2014),

The HAP2/3/5 complex binds to the CCAAT motif, and is assumed to play a role in opening up the chromatin structure required for transcription (Zeilinger *et al*., 2003) . XYR1, the xylanase regulator 1, is considered to be the main activator of cellulase and hemicellulase gene expression and belongs to the zinc binuclear cluster proteins (Stricker *et al*., 2006).

1.3 **The zinc-finger transcription factor XYR1**

Zinc-binding proteins are one of the largest families of transcriptional regulators in eukaryotes and based on a specific structural DNA-binding motif are also termed „zinc finger" proteins.

CysX2CysX6CysX5–12CysX2CysX6–8Cys is a fungal-specific zinc-binding motif in these proteins (MacPherson *et al.*, 2006). The first- and best-characterized zinc cluster protein is Gal4p, a transcriptional activator of genes involved in regulation of the catabolism of galactose in the budding yeast *Saccharomyces cerevisiae*. Zinc-finger proteins are divided in three major classes: Class I zinc-finger TFs encompass the Cys2His2 (C2H2) protein, the most common type of transcription factor; Class II contains the Cys4 (C4) zinc-finger TFs which bind to a DNA sequence in the regulatory region of their target genes as homodimers or heterodimers (homodimers recognized invert repeats within the target nucleic acid sequence, whereas heterodimers bind to direct repeats (Laity *et al*., 2001)); and Class III zinc finger proteins contain a DNA-binding domain (DBD) that consists of six cysteine residues bound to two zinc atoms and is also referred to a zinc-binuclear-cluster, or Zn(II)2Cys6 (Zn2C6) TF. The proteins of this class contain only one zinc finger unit that binds to two zinc atoms. They seem to interact with DNA as monomers, homodimers, or heterodimers(MacPherson *et al*., 2006).

Zinc cluster proteins can be furthermore seperated into three functional domains: cysteine-rich DNA-binding domain (DBD), regulatory domain and the acidic region.

These regions play an important role in DNA-binding specificity, protein-DNA and protein-protein interactions(MacPherson *et al*., 2006).The DBD contains three subregions: the zinc finger itself, a linker and dimerization domain. The linker region is located C-terminally to the zinc cluster motif and it can appear in different forms .These linkers are not highly conserved, and thought to mediate sequence-specific DNA binding (MacPherson *et al*., 2006).

The dimerization region made up of heptad repeats is located C-terminally to the linker (Schjerling & Holmberg, 1996). The heptad repeats of the dimerization region are most likely responsible for dimerization and protein-protein interactions (MacPherson *et al*., 2006).

The regulatory domain contains an important region termed the middle homology region, which separates the DBD from the C-terminal acidic region and spans about 80 amino acids. This region plays a role in regulating the transcriptional activity. The C-terminally located, acidic domain acts as the major activation domain and deletion of about 10 C-terminal amino acids lead to the inactivation of the transcription factor (MacPherson *et al*., 2006).

XYR1 binds to a 5´-GGC(A/T)-3´ motif in the cellulose and hemicellulose promoters (Takanori *et al*., 2009), and its deletion results in elimination of cellulase induction by all known inducers (Stricker *et al*., 2006).

The consensus sequences for XYR1 have been found in all inducible T.reesei cellulase promoters (Takanori *et al*., 2009). XYR1 of *T. reesei* is orthologous to XlnR from *Aspergillus niger*, the first isolated transcriptional activator which regulates the expression of genes encoding xylanolytic and some cellulolytic enzymes *in Aspergillus* (Hasper *et al*., 2004). Protein alignment of both transcription factors showed a high degree of amino acid sequence identity. Figure 1 shows a simplified overview over the annotated key domains of XYR1. Functional regions of XYR1 consist of the N- terminal (Zn2C6) DNA-binding domain followed by an Arg-Arg-Arg-Leu-Trp amino acid motif, a marker for a fungal specific transcription-factor domain and a putative coiled- coil domain (Lupas *et al.,* 1991).The second coil-coiled is found in the C-terminal coiled-coil nuclear import domain together with a mono-partite NLS (nuclear localization signal) which might be involved in nuclear import dynamics(Hasper *et al*., 2004). The N-terminal DNA-binding domain is orthologous to the highly conserved transcription regulator Gal4 from *S. cerevisiae* (Schjerling & Holmberg, 1996).

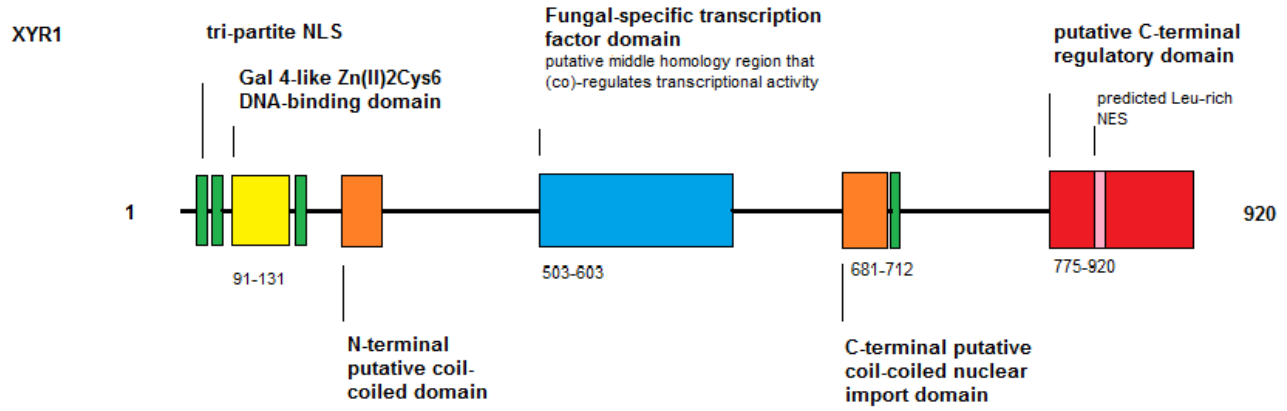

Figure 1. Domain architecture of XYR1. the Zinc finger DNA-binding domain (yellow) with a flanking tripartite NLS (green) is the first part of the domain followed by an N-terminal putative coil-coiled domain (orange) A fungal-specific transcription factor domain (blue) the C-terminal coiled-coil nuclear import domain with a mono-partite NLS (orange followed by green).and a putative C-terminal regulatory domain (red).A predicted NES sequence within this regulatory domain is predicted to be involved in nuclear export.

A tripartite NLS located in the *Gal4*-like binding domain appears to be required for nuclear import of XYR1, probably functioning as a second putative coiled-coil domain involved in nucleoplasmatic shuttling. In *A. niger*, however, this putative nuclear import domain has been reported to be non-functional. (Hasper *et al*., 2004)

Interestingly, NLS, NES (nuclear export signal)and DNA-binding domains of the Zinc finger cluster show partially overlap (Fernandes- Martinez *et al.*, 2003, Xiao *et al.*, 2003), which is a common finding among zinc finger proteins (Ware *et al*., 2006, Fernandes- Martinez *et al*., 2003). Difficulties in differentiating import and export motif from DNA-binding domains was also indicated in previous studies (Ware *et al*., 2006, LaCasse & Lefebvre, 1995). It has been proposed that the co-evolution of NLS and the DNA-binding domain occurred in a way that active nuclear entry and DNA binding can occur independently (LaCasse & Lefebvre, 1995),. The C-terminal putative coiled-coil region of XYR1 which has been found in other transcriptional activators likely play a role in dimerization(Hasper *et al.,* 2004). The putative C-terminal regulatory domain with a predicted nuclear export signal (NES) motif is the last domain region of XYR1 that is conserved within the Zn2Cys6 transcription factor family (Fornerod *et al*., 1997).

Several studies in mutants of *A. niger* lacking about 120 amino acids of the putative regulatory C-terminal domain showed that a putative C-terminal coiled-coil region is involved in nuclear import of XlnR. After deletion of the coiled-coil region, XlnR was found in the cytoplasm, while deletion of the C-terminus downstream of the coiled-coil region resulted in nuclear import of XlnR (Hasper *et*

al., 2004). This suggests that the orthologous region in *T. reesei* XYR1 probably is involved in nuclear import as well.

1.4 Nuclear import through nuclear pore complexes is facilitated by karyopherins

The nucleus is surrounded by a double membrane, called the nuclear envelope (NE). The nucleoplasm and cytoplasm communicate through nuclear pores, which are multiprotein complexes inserted into the NE. Figure2 shows a schematic model of nuclear pore complexes (NPCs) embedded in the NE.

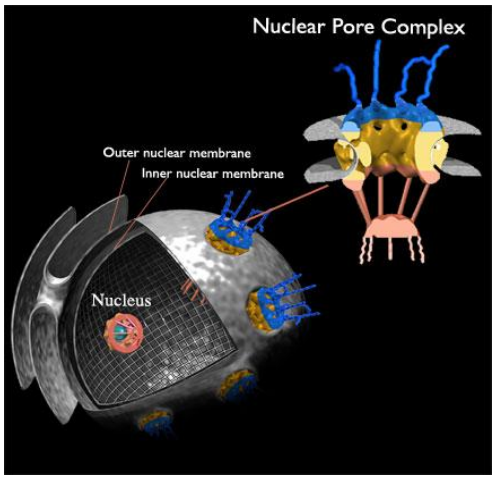

Figure 2. Schematic representation of the nuclear pore complex. NPCs are pores in the nuclear envelope and are a part of a transport channels which participate in the bidirectional transport of macromolecules between the cytoplasm and nucleus. (Image Credit: D. Stoffler & U. Aebi M.E. Mueller-Institute for Structural Biology, Biozentrum University of Basel, Switzerland)

Molecules smaller than 30 kDa are able to passively diffuse through nuclear pores (Görlich & Kutay, 1999), whereas larger molecules require active transport with the help of specific soluble nuclear carriers which are called Karyopherin (Kap) α and β.

The majority of these transporters belong to the karyopherin-β superfamily classified into importins and exportins, depending on their main transport direction (Mosammaparast & Pemberton, 2004). Both serve as transient adaptor proteins for the import and/or export of diverse cargo molecules, such as proteins but also tRNAs, through the NPC (Görlich & Kutay, 1999).Importins are divided in to importin α and ß. Fifteen importin-β1–like proteins have been identified in yeast and 22 in mammals (Mosammaparast & Pemberton, 2004, Ström & Weis, 2001).

1.4.1 **Importin α and ß can function synergistically in cargo transport**

Importin α is composed of a small N-terminal importin ß-binding (IBB) domain which consist of a basic stretch of about 40 highly conserved residues and a large NLS binding domain made up of ten tandem armadillo (ARM) repeats. Each arm is composed of three helices which form a cNLS-binding groove. This is followed by a C-terminal region, composed of the tenth ARM repeat which is thought to be important for binding to the export receptor CAS/Cse1(Cingolani *et al*., 1999).

On the other hand importin ß showed NPC- , IBB and Ran- binding activities. The interaction with Ran domain has been mapped to the N terminal region while the C terminal region has IBB binding activities. Macromolecules enter or leave the nucleus via its specific nuclear localization signals (NLS), or nuclear export signals (NES), respectively.

Most karyopherins, including importin ß, interact directly with nuclear localization signal (NLS)- and nuclear export signal (NES)-containing cargos. Importin ß can also interact indirectly with hundreds of different NLSs through importin α which recognize the NLS and serves as adaptor (Lange *et al*., 2007). Figure 3 shows a schematic overview on importin α/β domain structure and heterocomplex formation. Different functional domains and binding partners are indicated. The bottom panel shows the assembled structure of importin α in a trimeric complex with a bipartite nucleoplasmin classical nuclear localization signal peptide and importin β of *S. cerevisiae*(Goldfarb *et al*., 2004).

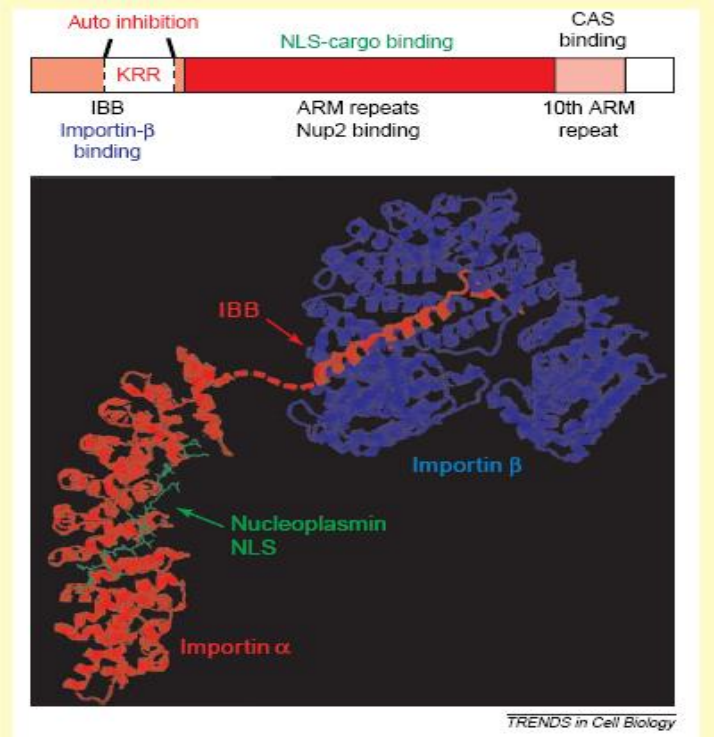

Figure 3. Importin α/β domain structure. (A) Schematic representation of the key domains of importin α**. (B)** 3D helical model of the importin α/β complex. (Goldfarb *et al.*, 2004)

Generally NLS recognition is mediated by either the α subunit of the α/β heterodimer or by importin β alone. In either case, the β subunit mediates docking of the complex at the NPC, followed by translocation to the nucleoplasm (Wagstaff & Jans, 2009). Those NLSs exclusively bound by an importin α are called ‘‘classical’’ NLS (C-NLC) C-NLS s are divided in monopartite and bipartiete NLS. A monopartite NLS is composed of single cluster of amino acids PKKKRKV; The virus 40 (SV40) T antigen is the best characterized examples for this class. A bipartite NLS was first identified in the non-nucleic acid binding protein, nucleoplasmin. The nucleoplasmin NLS consists of two seperate basic clusters of amino acids KR[PAATKKAGQA]KKKK (LaCasse & Lefebvre, 1995). NLS-cargo proteins which bypass the requirement for an adaptor protein and bind directly to importin ß are called non-classical NLS. The binding of the import substrate to the importin α/β heterodimer (Görlich *et al*., 1995a) is the initial cytoplasmatic event in NLS dependent nuclear import. Subsequently dissociation of nuclear protein cargo is triggered by binding of the small GTPase RanGTP to the importin ß, which releases the cargo into the nucleoplasm (Görlich & Kutay, 1999).A high concentration of RanGTP in the nucleus is generated by RanGEF, which converts RanGDP to RanGTP. Karyopherin-β and RanGTP are recycled back though the pore into the cytoplasm for additional rounds of cargo import and the hydrolysation of RanGTP to RanGDP (Gilchrist *et al*., 2002).The nuclear export receptor CAS mediates the export of karyopherin-α from nucleus to cytoplasm (Kau *et al*., 2004).This export involves complex formation with RanGTP, and then CAS is transported back into the nucleus through the pore. Cargoes that contain a nuclear export signal (NES) usually form a complex with CRM1 and RanGTP before they are exported out of the nucleus. In the cytoplasm, hydrolysis of RanGTP to RanGDP by RanGAP promotes complex dissociation. CRM1 is then transported back into the nucleus, where it can reassociate with a NES-containing protein and RanGTP to start the process over(Kau *et al.*, 2004). Figure 4 summarizes these events schematically.

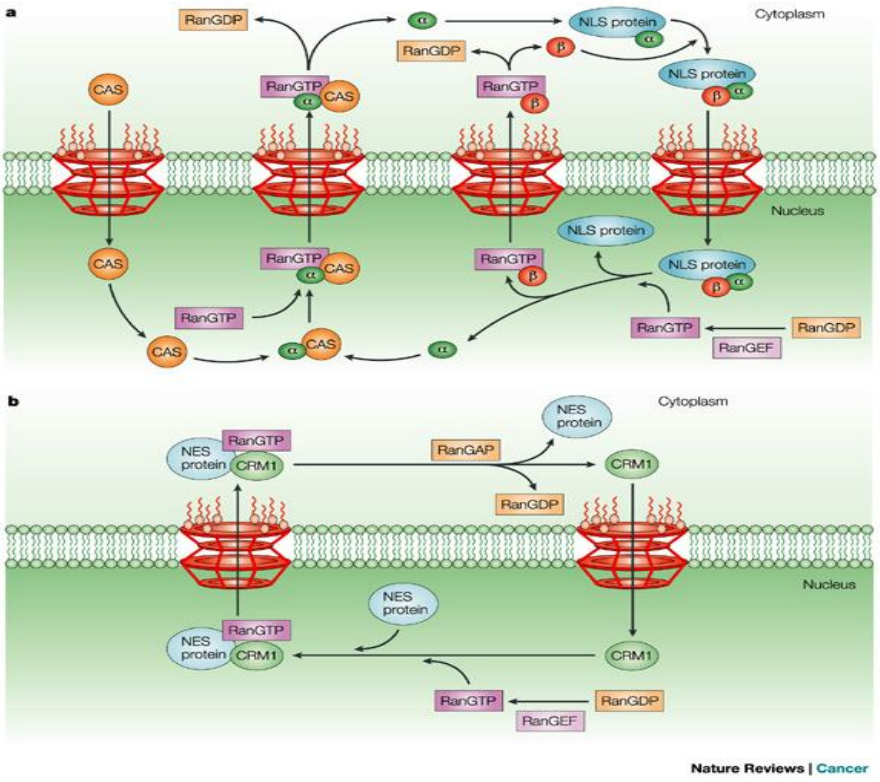

Figure4: Nucleoplasmatic schuttling through the nuclear pore complex. a) heterodimer of importin α and importin β accomplishes the nuclear import of proteins carrying classical nuclear localization signals (NLS),RanGTPase and RanGAP regulate nuclear/cytoplasmic release of the cargo and allow nuclear import against the concentration gradient of the protein.b) Cargoes containing nuclear export signal (NES) are exported out of the nucleus through binding to CRM1 and RanGTP(Kau *et al.*, 2004).

1.5 Karyopherins in *A. nidulans*

In filamentous fungi, karyopherins have so far only been identified and functionally studied in *A. nidulans* (Markina-Iñarrairaegui *et al*., 2011) and Neurospora crassa (Takeda *et al*., 2013). Yet information as to their involvement in nuclear transport of Zn(2)Cys(6) cluster transcription factors is still sparse: the regulator of the *A. nidulans* ethanol regulon, AlcR, for instance requires three importin-related proteins, Kap104, Sxm1, and Nmd5 (Nikolaev *et al*., 2003), whereas the rate-limiting step of nitrate regulation in the same fungus has been shown to be KapK-dependent (= CRM1/exportin 1) export of the pathway-specific regulator NirA (Bernreiter *et al*., 2007). BLAST searches and in silico analyses identified 17 loci coding for nuclear transporters in *A. nidulans*. The systematic characterizations of the nuclear transport determine the cell cycle–specific KAP distribution map and four essential nuclear transport pathways in *A. nidulans.*

Aim of the Thesis

T. reesei is an important industrial producer of cellulolytic and hemicellulolytic enzymes, which are used for biofuel production from lignocellulosic biomass. Induction of cellulases must involve nuclear import of the main transcriptional activator xylanase regulator 1 (XYR1) from the cytoplasm and nuclear import may thus constitute a pace-making step in the efficacy of cellulase gene expression. This limitation might be overcome once by understanding the mechanism of XYR1 nuclear import.

As this mechanism has so far been virtually unknown for *T. reesei*, I want to provide new insights into karyopherin function, which were assumed to mediate XYR1 nucleo-cytoplasmic shuttling under (hemi-) cellulase inducing conditions. New insights in XYR1 nuclear import are likely to offer novel means to improve industrial cellulase yields, and might further improve the impact of *T. reesei* for the biofuel and biorefinery industry.

Contribution of the authors

CHAPTER1

Identification of the karyopherin-ß superfamily of *Trichoderma reesei*

Sara Ghassemi performed the experiments. Christian P. Kubicek, Verena Seidl-Seiboth, Alexander Lichius and Bernhard Seiboth contributed to the planning of the experiments and helped in the interpretation of the results.

CHAPTER 2

Functional Characterization of the ß-importin KAP8 (Pse1/Kap121) in *Trichoderma reesei*.

Sara Ghassemi performed all experimental work of this study except, live cell imaging experiment which was performed by Alexander Lichius and Silvia Herold performed growth tests of A. nidulans MAD2666 and mutant strains at the Department of Cellular and Molecular Biology, Madrid, Spain. in the lab of Dr. Eduardo A. Espeso.The deep RNA-seq related part of the work was performed by Fréderique Bidard at IFP Energies Nouvelles, Department Biotechnology, 92852 Rueil Malmaison Cedex, Paris F-75005, France. Christian P. Kubicek and Bernhard Seiboth participated in the interpretation of the results.

2 Materials and Methods

2.1 Strains and culture conditions

The *Trichoderma reesei* strain QM9414 (ATCC 26921) and recombinant mutants derived from it were used throughout this work. Strain propagation, transformant selection and purification were performed on potato dextrose agar (PDA). For experimentation, strains were grown in Mandels-Andreotti (MA) medium (Mandels & Andreotti, 1978), using glucose, lactose, or cellulose (in the form of carboxymethylcellulose, CMC) as sole carbon sources at final concentrations of up to 1% (w/v) as indicated. Induction by sophorose was performed by replacing fungal biomass from 24 hrs 1 % (w/v) glycerol pre-cultures into fresh medium containing 1.4 mM sophorose as only carbon source as described by (Sternberg & Mandels, 1979). All strains are maintained as 50% (v/v) glycerol stocks at -80°C at TUCIM (http://www.vt.tuwien.ac.at/tucim/). Table 1 lists all *T. reesei* strains used and produced in this study.

Table1: *Trichoderma reesei* strains used in this work

Strain	Additional genotype	Mating type	Reference
T. reesei QM9414	*None*	*mat1-2*	*Mandels and Andreotti 1978*
T. reesei CBS 999.79	*None*	*mat1-2*	*Seidl et al. 2008*
T. reesei TU-6	*pyr4-*	*mat1-2*	*Gruber et al. 1990*
T. reesei Δtku70	*Δtku70 /pyr4-*	*mat1-2*	*Guangtao t al. 2009*
T. reesei gfp-xyr1	*nat1*	*mat1-2*	*Lichius et al. 2014*
T. reesei Δkap8	*Δtku70*	*mat1-2*	*this study*
T. reesei Δkap8 RT	*kap8+, nat1, Δtku70*	*mat1-2*	*this study*
T. reesei Δkap8 gfp-xyr1	*nat1, Δtku70*	*mat1-2*	*this study*
T. reesei Δkap8 RT xyr1-gfp	*kap8+, nat1, Δtku70*	*mat1-2*	*this study*
T. reesei Δkap1	*Δtku70, pyr4+*	*mat1-2*	*this study*
T. reesei Δkap2	*Δtku70, pyr4+*	*mat1-2*	*this study*
T. reesei Δkap4	*Δtku70, pyr4+*	*mat1-2*	this study
T. reesei Δkap5	*Δtku70, pyr4+*	*mat1-2*	this study
T. reesei Δkap6	*Δtku70, pyr4+*	*mat1-2*	this study
T. reesei Δkap7	*Δtku70, pyr4+*	*mat1-2*	this study
T. reesei Δkap9	*Δtku70, pyr4+*	*mat1-2*	this study
T. reesei Δkap10	*Δtku70, pyr4+*	*mat1-2*	this study

The *Aspergillus nidulans* strains used in this study are given in Table 2. The *pyrG89* mutant MAD1425 served as parental strain for the importin knock-out mutants. All *A. nidulans* mutants were produced in the lab of Eduardo Espeso, CIB, Madrid, Spain (Markina-Iñarrairaegui *et al.*, 2011), and propagated on complete medium plates at 37°C. The parental and the mutant strains were transferred to minimal medium (MM) plates containing either 1% glucose or 1% CMC as carbon source. Biotin, ammonium tartrate and pyridoxine were added to the medium.

Table 2: *Aspergillus nidulans* strains used in this work

Strain	Number		Genotype	Reference
Parental strain	TN02A3		*pyrG89; pyroA4, ΔnkuA::argB (argB2)*	Ayak et al. 2006
	MAD2666	*pyrGAf*	*pyrG89; pyroA4, ΔnkuA::argB (argB2), pyrG*	E.A. Espeso
ΔkapC	MAD2157	*ΔkapC::pyrGAf*	*pyrG89; pyroA4, ΔnkuA::argB (argB2);*	A. Markina-Iñarrairaegui
ΔkapD	MAD2324	*ΔkapD::pyrGAf*	*pyrG89, pyroA4, ΔnkuA::argB (argB2)*	A. Markina-Iñarrairaegui
ΔkapG	MAD2158	*ΔkapG::pyrGAf*	pyrG89; pyroA4, ΔnkuA::argB (*argB2*);	A. Markina-Iñarrairaegui
ΔkapH	MAD2174	*ΔkapH::pyrGAf*	pyrG89; pyroA4, ΔnkuA::argB (*argB2*);	A. Markina-Iñarrairaegui
ΔkapI	MAD2159	*ΔkapI::pyrGAf*	pyrG89; pyroA4, ΔnkuA::argB (argB2);	A. Markina-Iñarrairaegui
ΔkapJ	MAD2326	*ΔkapJ::pyrGAf*	pyrG89; pyroA4, ΔnkuA::argB (*argB2*);	A. Markina-Iñarrairaegui
ΔkapL	MAD2160	*ΔkapL::pyrGAf*	pyrG89; pyroA4, ΔnkuA::argB (*argB2);*	A. Markina-Iñarrairaegui
ΔkapM	MAD1837	*ΔkapM::pyrGAf*	pyrG89; pyroA4, ΔnkuA::argB (*argB2);*	A. Markina-Iñarrairaegui
ΔkapN	MAD2329	*ΔkapN::pyrGAf*	pyrG89; pyroA4, ΔnkuA::argB (*argB2)*;	A. Markina-Iñarrairaegui
ΔmexA	MAD3017	*ΔmexA::pyrGAf*	pyrG89; pyroA4, ΔnkuA::argB (*argB2);*	A. Markina-Iñarrairaegui
ΔnxtA	MAD2332	*ΔnxtA::pyrGAf*	pyrG89; pyroA4, ΔnkuA::argB (*argB2*);	A. Markina-Iñarrairaegui

Escherichia coli strains JM109 (Promega, Madison, Wisconsin), or Stellar® (#636763,Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France) were used for plasmid construction and amplification using standard molecular cloning techniques(Sambrook & Russell, 2001).

2.2 Media and Solutions

2.2.1 Potato Dextrose Agar (PDA)

39g/l PDA (Difco) were dissolved in tap water, autoclaved and tempered to 50°C prior to pouring into Petri dishes.

2.2.2 Luria Bertani Medium (LB)

LB medium with the following composition was used for *E. coli* cultivations.

Peptone	10g/l
Yeast Extract	5g/l
NaCl	10g/l

For plates 1.5% (w/v) agar- agar was added.

Mandels Andreotti Medium (MA)

For cultivation of fungal strains in liquid culture, minimal medium with the following composition was used:

KH_2PO_4	2g/l
(NH4)SO4	1.4g/l
CaCl2*2H2O	0.3g/l
MgSO4*7H2O	0.3g/l
100x Trace Element Stock	10ml/l

100x Trace Element Stock:

FeSO4*7H2O	0.5g/l
ZnSO4*7H2O	0.2g/l
MnSO4*7H2O	0.2g/l PH=5.5

Minimal Liquid Medium was autoclaved and tempered to 50°C.

2.3 Medium for single spore isolation

To obtain purified colonies from single spores, transformants were transferred to PDA plates containing 0.1% (v/v) Triton- X 100.

2.4 Growth tests of fungal strains on MA plates

In order to analyse the growth characteristic of *T. reesei* strains and the transformant generated in this study (see Table), 5µl spore solution of each of the respective strains were placed in the center of MA plates supplemented with different carbon sources and incubated at 28°C. Measurement of radial growth was started after 24 hours and carried out at 48 hours and 72 hour after incubation. Pictures were taken after 72 hours of incubation.

Table 3: Used carbon sources and concentrations.

Carbon source	*Concentration [w/v]*	*Concentration [mM]*
Glucose	1%	50
Lactose	1%	20
Xylose	1%	65
Cellulose in its carboxymethyl form	1%	-

2.5 Harvesting of fungal spores

Carbon source free MA plates were inoculated with 10 µl of spore suspension. After 4-5 days incubation at 28°C in the dark sufficient conidiation usually covered the surface of the plate. Spores were collected by rinsing the whole plate with a solution containing 0.9% NaCl and 0.05% Tween, and filtering the spore solution through a sterile miracloth-filter tip to remove hyphal fragments.

2.6 Determination of the spore concentration

The crude spore suspension was then filtered through glass wool to remove mycelial framents, and conidia were sedimented by centrifugation (5000xg, 10 min). Finally, the spore pellet was resuspended in 2.5 g/l phytagel (Phytagel^{TM,} SIGMA, Steinheim, Germany), well mixed and the transmission was measured at 590 nm in a Biolog standard turbidimeter. The number of total conidia was calculated using a calibration curve previously prepared with *T. reesei* QM9414 conidia.

10 ml of a Biolog inoculation solution (0.25% Phytagel, 0.03% Tween 80) were added to a Biolog tube. The tube was put in the machine and turned until the transmission was on zero. Afterwards 10-50µl of a *T. reesei* spore solution, harvested as described above, was added and the transmission measured until a transmission of 75% was achieved . The added spore solution contained a concentration of 4x107 spores. From this value the volume of the spore stock solutions was calculated that was needed to inoculate liquid cultures.

2.7 Determination of fungal biomass

Liquid cultures were prepared in 500ml Erlenmeyer flasks with 250ml of MA medium. The medium was inoculated with $1x10^6$spores/ml and incubated at 28°C and 200rpm. After the desired incubation time, the shake flasks were emptied by filtering through glass microfiber filter (Whatmanglass microfiber filters) whose empty dry weight were individually determined before. Filtered biomass was washed with tap water and then dried at 80°C over night. The next day biomass dry mass was weighed again and original biomass concentrations were calculated.

2.8 Molecular biology standard techniques

2.8.1 Construction of *T. reesei* recombinant strains

To study the function of importins, we constructed *T. reesei* strains in which all encoded importin genes, apart from *kap3*, were individually deleted. *T. reesei* strain *Δtku70*(Guangtao *et al.*, 2009) was used as a recipient for the targeted gene deletion cassettes. For selection, the coding region of the individual importin genes was replaced by the *T. reesei pyr4* (orotidine-5'-phosphate decarboxylase-encoding) gene (Gruber *et al.*, 1990). This was performed by amplifying 1.2 kb of the up- and downstream non-coding regions of the respective importins from genomic DNA of *T. reesei Δtku70* with the primer pair series 5f+5r and 3f+3r. The 5' and 3' ends of 5r and 3r bear sequences complementary to the *pyr4* gene, whereas the primer series 5f and 3r have 5' and 3' ends complementary to the pRS426 yeast vector. The nucleotide sequences of all primers used for construction of recombinant strains are given in Table 4. The two resulting PCR fragments and the 2.7 kb fragment of the *pyr4* gene were subsequently recombined into a XhoI/EcoRI linearized vector backbone of pRS426 using the endogenous yeast homologous recombination system, and the resulting gene replacement cassette was transformed by electroporation into *T. reesei* (Schuster *et al.*, 2012).

Genetic complementation of the *T. reesei* Δ*kap8* mutant strain was performed as follows: briefly, pAL10-Lifeact vector(Lichius, 2010) was first amplified with primers listed in Table 5, and the

resulting 3.5 kb vector backbone was purified by gel extraction with a commercial Gel and PCR clean-up system kit (#28704,QIAGEN, Hilden,Germany). Subsequently, the 4.1kb PCR fragment including promoter, coding and terminator region of the *kap8* gene were amplified from genomic *T. reesei* DNA and merged with the vector backbone by InFusion® recombinational PCR cloning (#639649, Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France). The resulting vector pSG002 was verified by DNA sequencing.

Table 4: Oligonucleotides used for the construction of knock-out cassettes

Primer name		5'-3 Sequence
Kap1	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGTAGAGCACTATCACGTCACC
Kap1	reverse	CGACGATATCAGCTTCCATATTCCGACTATATTACAGGGCAGACAGAGG
Kap1	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTACAAGTGTTTGTGCATCTCTC
Kap1	reverse	GCGGATAACAATTTCACACAGGAAACAGCTCGTCGTCTGC
Kap2	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGGTCTCTCTCGTTCCAAAAGC
Kap2	reverse	CGACGATATCAGCTTCCATATTCCGACTAATACACGTAGCTGAGTCTCG
Kap2	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTATTCGAGTGGTTACAGTCTCC
Kap2	reverse	GCGGATAACAATTTCACACAGGAAACAGCCCTAAGGTAAGGTATGCAGG
Kap3	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGGACAAACTCCATCAGTCTC
Kap3	reverse	CGACGATATCAGCTTCCATATTCCGACTAGATGCACAGTAAGAGTCAGG
Kap3	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTAGAATTCTGACGAAGCTGACG
Kap3	reverse	GCGGATAACAATTTCACACAGGAAACAGCACTGGAAAGAGTTGAGGTCC
Kap4	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGCTACTAGATCCTGCTTCTGG
Kap4	reverse	CGACGATATCAGCTTCCATATTCCGACTAGGAGGAGGGTAGAAGAAAGG
Kap4	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTAGGAGGAAAACCAGGAAGAGG
Kap4	reverse	GCGGATAACAATTTCACACAGGAAACAGCTAAGGCAGGTAGGTAGTTGG
Kap5	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGTATTGGAGTAGAGCAGGTCG
Kap5	reverse	CGACGATATCAGCTTCCATATTCCGACTATGCCTGTGTGTACAGGTAGC
Kap5	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTAAGTATGTAGGTAGGGCAAGC
Kap5	reverse	GCGGATAACAATTTCACACAGGAAACAGCGCTTACTGGTAGTCCAGACC

Kap6	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGGCATGGTAAGACTAAGGTGG
Kap6	reverse	CGACGATATCAGCTTCCATATTCCGACTATGAGAGACAGGAGACACAGG
Kap6	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTAGGGCATACTAGCTGTCTTGG
Kap6	reverse	GCGGATAACAATTTCACACAGGAAACAGCGTGGATGATGATGTCAGACG
Kap7	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGCAGCGACTTCTTGTAGATGG
Kap7	reverse	CGACGATATCAGCTTCCATATTCCGACTAGTTGGTGGTGGTGTAGATGC
Kap7	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTAACTCCTCATCCGTATACTCG
Kap7	reverse	GCGGATAACAATTTCACACAGGAAACAGCCCTTTCCAAACCTTCAGAGC
Kap8	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGGCTTGAGATAGGTTCACACC
Kap8	reverse	CGACGATATCAGCTTCCATATTCCGACTAGAGGTACCTGGTCATGTAGG
Kap8	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTACCCATACTGGGATAAGTACC
Kap8	reverse	GCGGATAACAATTTCACACAGGAAACAGCAGCGTCTCCATGTACTCACG
Kap9	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGGAACTCAATGCGTAACCTCC
Kap9	reverse	CGACGATATCAGCTTCCATATTCCGACTACTGTCTGTCTACAGGTGTGC
Kap9	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTACAGGATACACTCCCATATCC
Kap9	reverse	GCGGATAACAATTTCACACAGGAAACAGCTACCCTCCTCCTGTCTAACC
Kap10	forward	GTAACGCCAGGGTTTTCCCAGTCACGACGGGTTCGAAGAACACAGAAGC
Kap10	reverse	CGACGATATCAGCTTCCATATTCCGACTAGCCGTAGTAGAGAGAGTTCC
Kap10	forward	AGAAAAGCACAAAGAAGAGGCTCCAACTATACAGCGAAAGAGTACGAGG
Kap10	reverse	GCGGATAACAATTTCACACAGGAAACAGCCTATGAGGGACTCTGTATGC

Table 5: Primers used for vector construction

Primers used for construction of pSG003 vector (**Retransformation of the *T. reesei* Δkap8**)

Primer name	**5'-3' Sequence**
BamHI-Kap8-ORF-F	CAAAGATATCGGATCCATGTCCCTCCTCCTGTCG
ClaI-kap8-Ter-R	AGGTCGACGGTATCGATCTAGCTGAATATCCCAT

Table 6: Primers used for vector construction

Primers used for construction of pSG001 vector (**N-terminal GFP-fusions to XYR1**)

Primer name	5'-3' Sequence
pTX-nat-F	ATGCATGTGCTGTGTTCCTCA
pTX-nat-R	TCTAGATTTGTATCTGCGAATT
TX-nat-IF-F	AGATACAAATCTAGAATGGCCACCCTCGACG
TX-nat-IF-R	ACACAGCACATGCATTCAGGGGCAGGGCATG

2.8.2 **Fungal transformation**

Gene replacements cassettes were amplified from the respective plasmids by PCR and transformed into *T. reesei Δtku70* as linear DNA fragments using electroporation (Schuster *et al.*, 2012). All primary transformant strains were purified twice for mitotic stability, and the genotype of the purified strains was verified by PCR analysis of isolated genomic DNA. For each locus, primers were designed that bind (a) outside of the knock-out cassette but inside the flanking region of the respective gene, (b) inside the selection marker gene *pyr4,* and (c) inside the ORF of the wild type gene. With this set of primers it was verified that the gene replacement occurred at the intended locus. An additional primer pair amplifies 500 bp of the target ORF was used to confirm absence of the original locus from the whole genome, i.e. verify the homokaryotic status of the gene deletion strain. All primers used for PCR-genotyping are listed in Table 7.

Table 7: Oligonucleotides used for PCR-genotyping

Primer name	5'-3' Sequence
Kap1 5p 200-F	CTATGAGCTGTCTTTCTGGA
Kap1 ORF-F	TGTGATGCTGTGCTTTGC
Kap1 1000-R	CAAGGAGCTCGACGAAGAT
Kap2 5p 200-F	TTGCCCATCTTCACCTATCT
Kap2 ORF-F	CACAGCCAGGGACTTTG
Kap2 1000-R	AGACGACCTGCATGATGTA
Kap3 5p 200-F	TAAGCCTTAGGTCACTGTC
Kap3 ORF-F	TCCTCGAAATGATTGGCA
Kap3 1000-R	GATCTTGTTGAGATACGGTC
Kap4 5p 200-F	ACCCAAGTTGAACTGACGCT
Kap4 ORF-F	GAAGATGATGGGAGGCAG

Kap4 1000-R	CATGCTTGTACGCCTTGAG
Kap5 5p 200-F	CTATCAGCTGTGGTCAAGGA
Kap5 ORF-F	AGATTTCTGGGAGCGTTG
Kap5 1000-R	GAGATGCTAGCAAGGTTGTC
Kap6 5p 200-F	GAAGGAGTTTGATCGATGAC
Kap6 ORF-F	ACGAGAGCCTTAGCGAG
Kap6 1000-R	ATGAGAAGGTCGAGGGTATA
Kap7 5p 200-F	TGTAGCTGTGCAGCTTCGAT
Kap7 ORF-F	GCAATCAGCATCATCTCC
Kap7 1000-R	GCAAAGTTTGAAATGAGGTC
Kap8 5p 200-F	TTGGCTCTACTCACTGCACT
Kap8 ORF-F	CCATACTAATTCGCCACT
Kap8 1000-R	TGTCTTGAACCACCGAAATG
Kap9 5p 200-F	CAATGTCGTAGATGGGAGTA
Kap9 ORF-F	CTCAACACCAGCGACAACG
Kap9 1000-R	AAACGGTCTGGATGAGGA
Kap10 5p 200-F	CATTTGCGAGTCTTTGCCAT
Kap10 ORF-F	ACGATACCGATGGACCAC
Kap10 1000-R	AGAAGCCCTTGACAATGCAT
Pyr4-F	ACCAGCAGACCAACGG
Pyr4-R	ACTGCATCCAAACCATCC

2.8.3 **Ethanol precipitation of DNA**

0.1 vol. of 3M sodium acetate (pH 5.2) was added to the DNA solution in an Eppendorf tube and mixed well. Two volumes of cold 90% EtOH were added and left at - 20 °C for 30 min. Subsequently the mix was centrifuged at 13000 rpm at 4 °C for 30 min, and the supernatant carefully decanted. The DNA pellet was washed in 1 vol. of 70 % ethanol and centrifuged for 5 min at 13000 rpm and at 4 °C. After discarding the supernatant the pellet was dried for 5-10 min at RT and resuspend in the appropriate volume of 10mM Tris-HCl buffer or water.

2.8.4 **Measuring DNA/RNA quantity and quality**

Quantity and quality of all DNA and RNA samples, including recovered pDNA, extracted gDNA, PCR products, DNA fragments after gel purification, or RNA and cDNA, were measured using the NanDrop ND-1000 spectrophotometer.

2.8.5 **Quick gDNA extraction protocol**

For the extraction of gDNA biomass was mechanically disrupted by means of glass beads and thorough vortexing. After incubating for 15 minutes and shaking at 200rpm at 65°C, 150µl of buffer P1 was added, followed by vortexing. Upon centrifugation for 1 minute at 10000 x g, the supernatant was transferred into a new Eppendorf tube containing 500µl of isopropanol. After another round of vortexing and centrifugation for 10 minutes at 10000 x g, the isopropanol was removed. The DNA pellet was washed with 75% ethanol. After that, the pellet was dried and resuspended in 30µl of nuclease free water.

Lysis Buffer:

Component	Final conc.	Quantity for 1L
Glucose	50 mM	10 ml
Tris	25 mM	8.2 g
EDTA	10 mM	1 ml

*EDTA pH 8, 0 with HCl

2.8.6 **Heat shock transformation and selection of E. coli**

Amplification of plasmid DNA was performed in *E. coli* after a standard heat shock transformation procedure. In brief, 50µl of competent bacteria cells from -80°C storage were thawed on wet ice and incubated with about 10ng of pDNA for 30 min. After a 45 sec heat shock at 42 °C in a water bath the cells were cool down on wet ice for 2 min, then recoverd with 500µl pre-warmed SOC and incubated for 30- 60 min at 37°C and 500 rpm in a heat block, After quick-pelleting the cells by centrifugation, the supernatant was discarded and the cell pellet resuspend in 200µl pre-warmed LB medium. 50µl and 150µl, respectively, were spread on pre-warmed LB-amp plates and incubated at 37°C o/n. Bacterial colonies cultivated and processed by mini or midi preparation using commercial kits.

Selection markers for *T.reesei* used in this study.

Selection marker	*Stock conc.*	*Solvent*	*Amount used*	*Final conc.*
Hygromycin B	200 mg/ml	dH_2O	100 µl/100 ml	200 µg/ml
Nourseothricin	5 mg/ml	dH_2O	100 µl/100 ml	50 µg/ml

2.9 Nucleic acid isolation and hybridization

Fungal biomass was harvested by vacuum filtration, washed with pre-cooled, distilled and sterile water, and shock frozen and ground in liquid nitrogen. For extraction of genomic DNA, plasmid DNA and RNA, purification kits (Wizard Genomic DNA Purification Kit, PureYield Plasmid Midiprep System and RNeasy plant kit, respectively, all from Promega) were used according to the manufacturer's specifications. Standard methods were used for electrophoresis, blotting and hybridization of nucleic acids (Sambrook & Russell, 2001).

2.10 Analysis of gene expression by quantitative RT-PCR

DNase treated (DNase I, RNase free; Fermentas) RNA (5 µg) was reverse transcribed with the RevertAid™ First Strand cDNA Kit (Fermentas) according to the manufacturer's protocol with a combination of oligo-dT and random hexamer primers. Gene specific oligonucleotides are listed in Table 8. All qPCR assays were performed on a Bio-Rad (Hercules, CA) iCycler IQ. For the reaction the IQ SYBR Green Supermix (Bio-Rad, Hercules, CA) was prepared for 25 µl assays with standard $MgCl_2$ concentration (3 mM) and a final primer concentration of 100 nM each. All assays were carried out in 96-well plates. The amplification protocol consisted of an initial denaturation step (3 min at 95°C) followed by 40 cycles of denaturation (15 sec at 95°C), annealing (20 sec; for exact temperatures see Supplementary Table S3) and elongation (10 sec at 72°C). Determination of primer efficiencies was performed beforehand using triplicate reactions from a dilution series of cDNA (1; 0.1; 0.01; 0.001). Amplification efficiency was then calculated from the given slopes in the IQ5 Optical system Software v2.0. Relative gene expression ratios were calculated using REST© Software (Pfaffl *et al.*, 2002). All samples were analyzed in at least two independent experiments with three replicates in each run.

Table 8: Primers used for RT-qPR.

Gene	Primer	5'-3' Seque	R^2	Efficiency
cbh1	qPCR-cbh1-F qPCR-cbh1-R	CCGAGCTTGGTAGTTACTCTG GGTAGCCTTCTTGAACTGAGT	0.990	98%
xyr1	qPCR-xyr1-F qPCR-xyr1-R	CCATCAACCTTCTAGACGAC AACCCTGCAGGAGATAGAC	0.987	100%
tef1	qPCR-tef1-F qPCR-tef1-R	CCACATTGCCTGCAAGTTCGC GTCGGTGAAAGCCTCAACGCA	0.995	95%

2.11 RNA-seq library preparation and analysis

Library preparation and Illumina sequencing were performed at the Ecole Normale Superieure Genomic Platform (Paris, France). Messenger (polyA+) RNAs were purified from 800 ng of total RNA using oligo (dT). Libraries were prepared using the stranded RNA-Seq library preparation TruSeq RNA Sample Prep Kits (Illumina). Libraries were multiplexed by four on three single flowcell lanes and subjected to 50 bp paired-end read sequencing on a HiSeq 2000 device. A mean of 47±6 million passing illumina quality filter reads was obtained for each of the 12 samples. RNA-Seq data analysis was done using the Eoulsan software version 1.2.2 (Jourdren *et al.*, 2012). Before mapping, poly N read tails were trimmed, reads ≤40 bases were removed, and reads with quality mean ≤30 were discarded. Reads were then aligned against the *T. reesei* reference genome (http://genome.jgi-psf.org/Trire2/Trire2.home.html) using Bowtie mapper (version 0.12.7) using the "-n 2 -l 34 -e 70 -k 2 --best" parameters. Only one alignment were kept in a given locus for each read, and reads alignments matching on more than one locus were removed. To compute gene expression, the *T. reesei* genome annotation was used. Gene expression was computed by counting all overlapping regions between alignments and referenced exons. To quantify the gene expression level, the relative transcript abundance was measured in reads per kilobase of exon per million mapped sequence reads (RPKM; (Mortazavi *et al.*, 2008)). The log2 ratios of the RPKM values were used to identify differentially expressed genes. To keep only the most differentially expressed genes, thresholds of four (sophorose vs. glycerol in the retransformant) and five (retransformant vs. *Δkap8* on sophorose) for log ratio were used. Read numbers <100 were considered as (almost) absence of transcription and not chosen for the evaluation. Genes were identified by the aid of a completely manually annotated *T. reesei* genome database proprietary to Christian P.Kubicek, Vienna University of Technology. The RNA-Seq gene expression data and raw fastq files are available at the GEO repository (www.ncbi.nlm.nih.gov/geo/).

2.12 Analysis of sexual and asexual development

To test the effect of importin gene deletion on sexual reproduction, *T. reesei MAT1-2* parental and mutant strains, were confronted with the compatible *MAT1-1* mating partner CBS999.79 as described previously in detail (Seidl *et al.*, 2009). All crosses, in which fruiting bodies were formed, were visually inspected until the maturation stage was achieved and ascospores were dispersed. Monoascosporic cultures were isolated by dispersing the solution with a cotton swab on multiple PDA plates. After overnight incubation several single germinated spores were selected by a stereomicroscope, transferred to a new PDA plate and cultivated at 28°C. To test for

photodependent conidiation, PDA plates were centrally inoculated with one 5 mm diameter mycelial plug taken from the edge of a 3-day-old colony and incubated for eight days at 28°C in either complete darkness or cycles of 12 h light/12 h darkness. Three biological replicates were prepared for each condition. Conidia from each plate were then harvested in equal volumes of physiological salt solution (0.1%, w/v, Tween 80 and 0.8% w/v NaCl) by gently rubbing the surface of the mycelium with a sterile Drygalski spatula. The crude spore suspension was then filtered through glass wool to remove mycelial fragments, and conidia were sedimented by centrifugation (5000xg, 10 min). Finally, the spore pellet was resuspended in 2.5 g/l phytagel (Phytagel^{TM,} SIGMA, Steinheim, Germany), well mixed and the transmission at 590 nm in a Biolog standard turbidimeter was measured. The number of total conidia was calculated using a calibration curve previously prepared with *T. reesei* QM9414 conidia.

2.13 Enzymatic assays and determination of fungal dry weight

Cellulase enzyme activities were determined using 1% (w/v) CMC and p-nitrophenyl-ß-D-lactobioside (Bailey & Tahtiharju, 2003). Total protein in the culture supernatant was determined by the method of Bradford (Bradford, 1976). Fungal dry weight was determined by filtering an aliquot of the culture through glass sinter funnels (porosity G1), washing with tap water and drying at 80 °C to constant weight.

2.14 Monitoring of XYR1 nuclear transport

Expression and subcellular localization of GFP-labeled fusion proteins was quantified using scanning confocal microscopy and image analysis as described recently in detail (Lichius *et al.*, 2014). Live-cell imaging was performed using a Nikon C1 confocal laser scanning unit mounted on a Nikon Eclipse TE2000-E inverted microscope base (Nikon GmbH, Vienna, Austria). GFP-labelled proteins were excited with the 488 nm laser line of an argon ion laser, and emitted fluorescence light separated by a Nikon MHX40500b/C100332 filter cube was detected with a photo-multiplier tube within the range of 500-530 nm. A Nikon Plan Apo VC 60×/1.2 water immersion objective lens was used, and laser intensity and laser dwell time during image acquisition were kept to a minimum to reduce photobleaching and phototoxic effects. Brightfield images were captured simultaneously with a Nikon C1-TD transmitted light detector mounted behind the condenser turret. Images were recorded with a maximum resolution of 1024x1024 pixels and saved as TIFF. HOECHST dye #34580 (*life* technologies, Invitrogen, cat# H21486) was used at a final concentration of 3.6µM to counterstain nuclei. HOECHST fluorescence was detected in the range

of 430-470 nm, after excitation of the dye with 405 nm of a HeNe laser and separation through a Nikon MHX40500a filter cube. This set up, unfortunately, was not capable of fully eliminating bleed-through of strong HOECHST signals into the GFP channel even when using sequential scanning.

3 Results

Chapter 1

3.1 Identification of the karyopherin-ß superfamily of *Trichoderma reesei*

We searched the *T. reesei* genome for potential orthologues of the 17 nuclear transporters previously identified in *A. nidulans* (Mans *et al.*, 2004, Markina-Iñarrairaegui *et al.*, 2011, Espeso & Osmani, 2008). Seventeen loci coding for nuclear transporters were found, of which 14 loci encoded proteins belonging to the karyopherin-β superfamily. Each of them showed a high similarity with only a single *A. nidulans* Kap protein (e-values between e-170 and 0). In agreement with Pyrenomycete nomenclature, these genes and proteins were designated as *kap/*KAP plus locus numbers from 1 to 14 (Table 9). They comprised four genuine exportins (KAP11 = *A. nidulans* KapK^{Crm1}, KAP13 = KapN, KAP5=KapE^{Cse1}, KAP12 = KapM^{Los1}), nine importins (KAP2=KapB^{Kap95}, KAP3=KapC^{Kap104}, KAP4=KapD^{Nmd5}, KAP6=KapG^{Kap114}, KAP7=KapH^{Kap120}, KAP8=KapI^{Pse1}, KAP9=KapJ^{Kap123}, KAP10=KapL and the mRNA transporter KAP14=KapF^{Mtr10}), and the importin-α homologue KAP1=KapA^{Srp1}. Additional *in silico* searches using Pfam domains related to this superfamily of proteins did not add more candidates to our predictions (data not shown).

Table: 9. Nuclear transporters in *T. reesei*

Trire2:	**Protein name**		**Protein type**	**Genefunction**
	T.reesei	***A.nidulans***		**conserved in *T. reesei***
21117	KAP1	KapA	Importin alpha	essential
74318	KAP2	KapB	Imortin beta	essential
80398	KAP3	KapC	Importin beta	non-essential
76859	KAP4	KapD	Importin beta	non-essential
73525	KAP5	KapE	Importin beta	essential
104161	KAP6	KapG	Importin beta	non-essential
64009	KAP7	KapH	Importin beta	non-essential
78158	KAP8	KapI	Importin beta	non-essential
23193	KAP9	KapJ	Importin beta	non-essential
77404	KAP10	KapL	Importin beta	non-essential
62120	KAP11	KapK	Exportin	non-essential
3892	KAP12	KapM	Exportin	non-essential
5991	KAP13	KapN	Exportin	non-essential
120152	KAP14	KapF	mRNA transporter Mtr10	essential

3.2 Phenotypic characterization of importin Knockout mutants of *T. reesei*

3.2.1 Growth test on cellulase -inducing and non-inducing carbon sources

Colony extension growth tests on cellulase-inducing and non-inducing carbon sources were conducted in order to identify importin mutants which grew poorly on different carbon sources (Figure 5). The transformant strains *Δkap*1, 2, 4, 6,7,8,9 and *Δkap10* as well as the parent strain *Δtku70* were compared.

Most importin gene knockout strains showed slower growth rates on 1% glucose and lactose plates compared to the parental strain. Only *Δkap4 and Δkap6* showed a strongly reduced growth on both glucose and lactose. On lactose a number of other Δ*kap* strains including Δ*kap5, 7, 9* and *10* showed a considerable reduction. Interestingly the *Δkap*8 showed similar growth rate on all tested carbon sources compared to the wild type, but significantly faster growth rates compared to other KAP mutants.

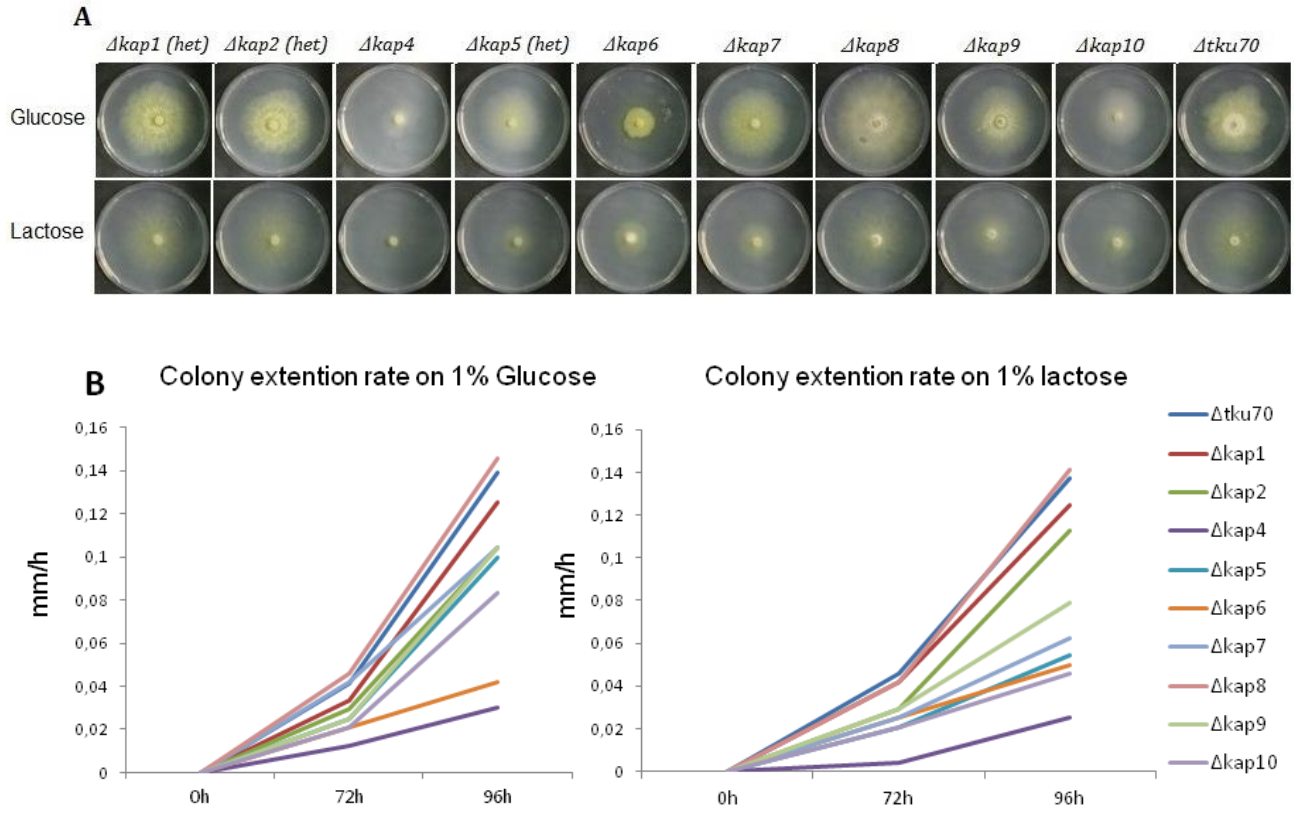

Figure5.Colony phenotypes of importin knock out in *T.reesei* and the reference strain *Δtku70*. (A) Macroscopic colony phenotypes of different *Δkap* strains. Pictures were taken after 72 h incubation at 28°C.**(B)** Colony extension rates on minimal medium plates containing 1% glucose and 1% lactose as carbon sources.

3.2.2 **Effect of importin knock outs on asexual sporulation**

We further investigated the effect of the importin knockouts on conidiogenesis in *T.reesei* .For this; strains were grown for five days on PDA plates (Figure 6). Additional spore counts showed that deletion of the *kap6* gene resulted in an aconidiate phenotype that correlated with upregulation of this gene during asexual differentiation (Metz *et al.*, 2011). (data not shown) Sporulation was also found to be reduced in the Δ*kap8* strain. It is important to note that the sporulation was affected in all *KAP* mutants. But the conidiation defect of Δ*kap8* seems to be an almost complete developmental block during conidiogenesis and not just a delay in sporulation as an extended incubation did not result in higher spore formation in this strain. The sporulation deffect of Δ*kap8* was fully rescued by ectopic introduction of *kap8* in this mutant.

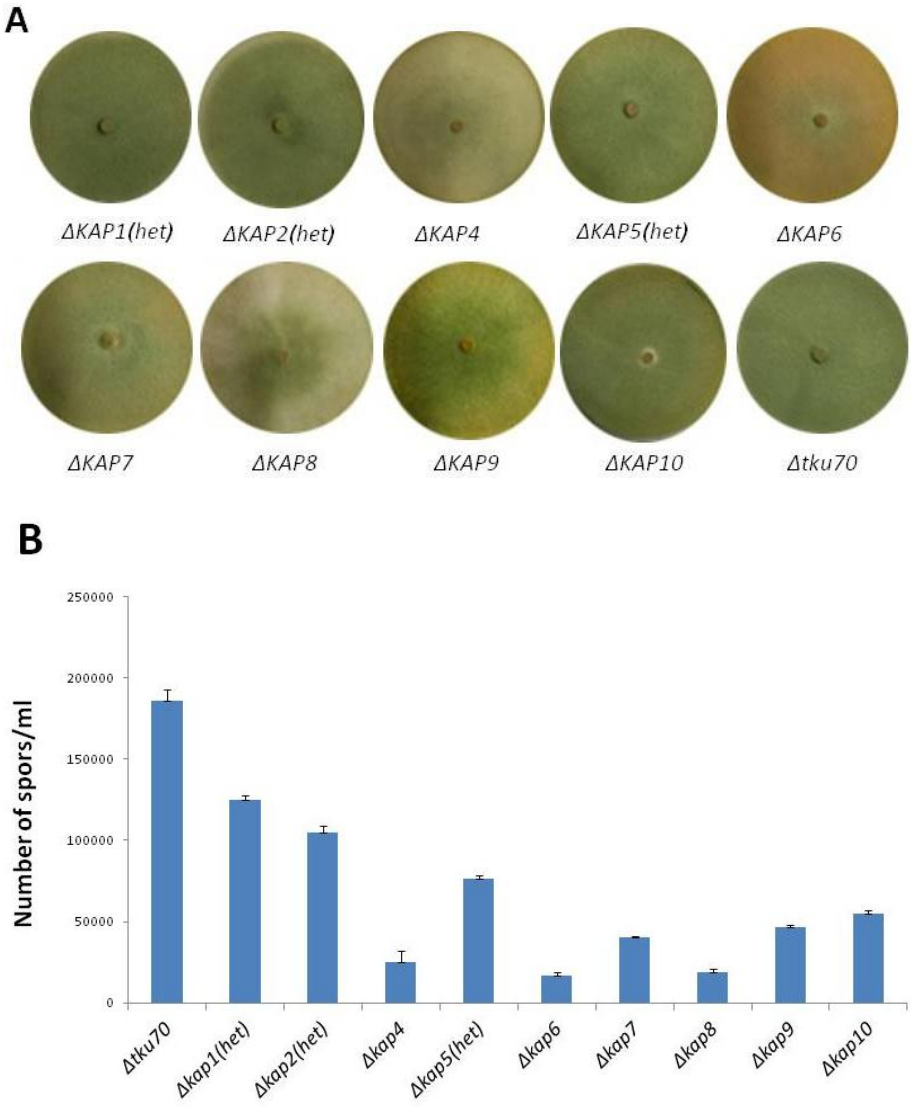

Figure 6. Effects of importin gene deletion on conidiogenesis in *T. reesei.* (A) Pictures were taken after 5 day incubation at 28°C. **(B)** Conidiogenesis was found to be significantly reduced in Δ*kap4, 6* and *8* in comparison to the parental QM9414 Δt*ku70* strain. Error bars indicate standard deviation.

3.2.3 **Biomass production of importin knock-out strains**

In addition to the tests of growth on solid medium also biomass formation of the *T. reesei* importin gene knockout strains in liquid cultivations was determined. Therefore MA medium containing 1 % glucose (w/v) as carbon source was used and samples were taken after 22h, 28h, 36h and 44h (Figure. 7) Δ*kap4* and the heterokaryotic Δ*kap5* showed a general slower growth in comparison to the reference strain Δ*tku70*. In contrast to the results obtained for colony extension on solid media, the levels of biomass accumulation during liquid cultivation for the Δ*kap6* were similar to the Δ*tku70* strain. Also the biomass accumulation for the Δ*kap8* was similar, despite its significantly faster colony extension on glucose plate, suggesting that either there is a difference in growth on plate and in shake flask or that the mycelia mate is just thinner and grows faster. No effect was found for the other Δ*kap* strains. Therefore, the results of the biomass formation analysis showed no significant difference between parental strain and gene knockout strains.

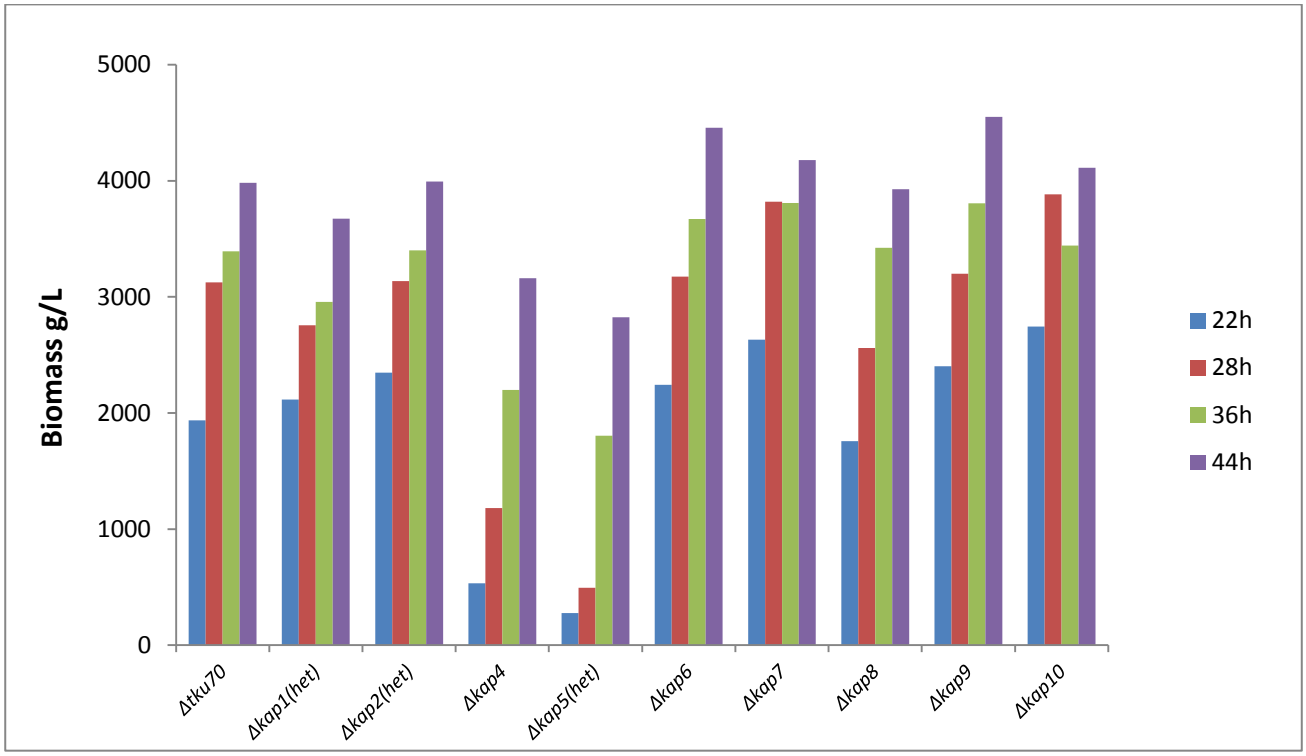

Figure 7. Biomass formation of different importin gene knockout strains. Biomass formation was measured during growth in shake flask in MA medium with 1 % w/v glucose. Samples were taken after 22h, 28h, 36h, and 44h. As reference strain the *Δtku70* was used

3.2.4 **Cellulase Gene Transcription Analysis**

To potentially identify the ß-importin of *T. reesei* that is reponsible for XYR1 import, we cultivated the knock-out strains on lactose and analyzed the formation of cellulase mRNA, the products of nuclear function of XYR1. Lactose, rather than cellulose was chosen for these experiments because lactose utilization is in contrast to cellulose independent of the action of secreted cellulases (Seiboth *et al.*, 2007). The gene encoding the major cellulase, *cel7A (= cbh1)*, was used as model for monitoring cellulase gene expression. The knock-out strains exhibited somewhat reduced *cel7A* expression, but most strains (including the heterokaryon strains) showed expression levels of >50% of the parental strain. Two exceptions were of note: Δ*kap10*, which showed less than 30% of *cel7A* expression, and the Students t-test determined this as significant (p = 0.00279 for 16 hrs, and P=0.00998 for 36 hrs). In addition, Δ*kap8* produced almost no *cel7A* transcript. Students t-test confirmed the significance of this result (p=0.0000023 and 0.00029 for 16 and 36 hrs, respectively) (Figure 8).

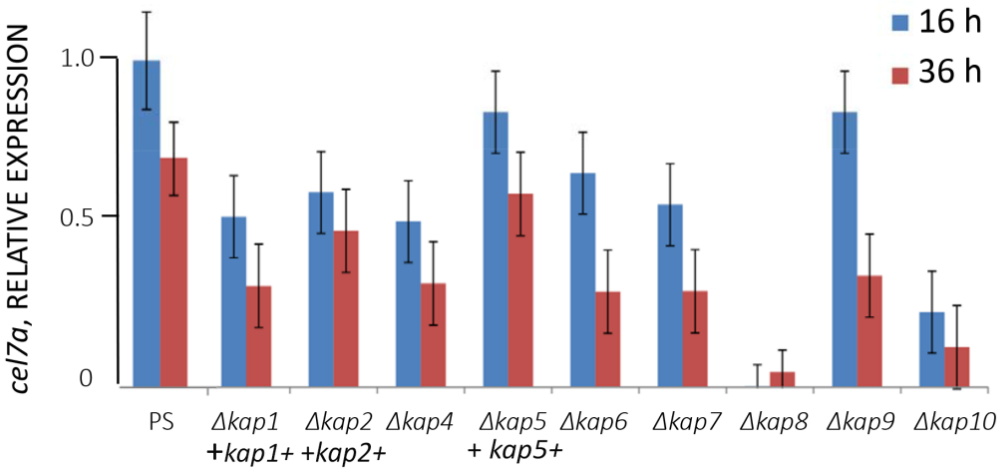

Figure 8. Expression of the *T. reesei* cellobiohydrolase gene during. growth on lactose for 16 hrs (blue bars) and 36 hrs (red bars) in nine ß-importin knock-out strains. Values are given as "relative gene expression", which is the ratio of expression of *cel7A* to that of the housekeeping gene tef1, normalized to the same ratio obtained with the parental strain *T. reesei* QM9414 after 16 hrs on lactose.

Conclusions Chapter 1

To investigate the function of nuclear transporters for XYR1 nuclear import upon cellulase induction we have generated gene deletion strains for nine of all 10 karyopherins in *T.reesei*. *Kap1, kap2* and *kap5* were found to be essential genes, in line with findings in *A. nidulans(Markina-Iñarrairaegui et al., 2011)* KAP1 is the only α-importin; hence, its cellular importance is not surprising; the roles of KAP2 and KAP5 in nuclear transport however, remain to be determined. The homologues of Kap2 from *Aspergillus nidulans*, *KapB/Kap95,* showed an accumulation at the nuclear periphery similar to that observed for nucleoporins(Osmani *et al.*, 2006), and KapB/Kap95 also accumulated in the proximity of the spindle pole body (SPB), the fungal centrosome. In *Aspergillus nidulans* KapECse1, the homologues of kap5 in *T. reesei* belongs to the group of genuine exportins which must be the specific importin-α exporter required for cytoplasmic recycling of this adapter (Markina-Iñarrairaegui *et al.*, 2011).

Of the remaining six non-essential β-importins only Δ*kap4*, Δ*kap6* and *Δkap8* showed recognizable growth defects on minimal medium independent of the used carbon source. The severe conidiogenesis defect of Δ*kap6* was the most pronounced mutant phenotype, in line with its significant up-regulation during conidiogenesis as reported earlier(Metz *et al.*, 2011). The growth defects of Δ*kap4* and Δ*kap6* were not connected to obvious changes in cellulase or xylanase gene expression, whereas cellulase gene expression was significantly decreased in Δ*kap8*, suggesting that KAP8 is involved in nuclear import of XYR1. Taken together, these data identified KAP8 as the prime candidate β-importin for shuttling XYR1 across the nuclear envelope in a α-importin-dependent way. Further experiments, featured in the following chapter 2, were carried out to verify this assumption.

Chapter 2

3.3 Functional characterization of the ß-importin KAP8 (Pse1/Kap121) in *Trichoderma reesei.*

3.3.1 Functional analysis of the *T. reesei* ß-importins reveals that kap8 is essential for cellulase gene expression *in T. reesei*

To verify whether KAP8 was indeed linked to XYR1-dependent cellulase transcription, the wild type *kap8* allele was reintroduced into *Δkap8* strain, and the expression of *cel7A* was followed by RT-qPCR analysis. As shown in Figure 9, expression of *cbh1* in the *Δkap8* strain was only 2 % of that in the complemented strain, in which *cbh1* transcript levels were fully restored. This finding confirmed that KAP8 is essential for cellulase gene expression and therefore is the most likely candidate for nuclear import of XYR1.

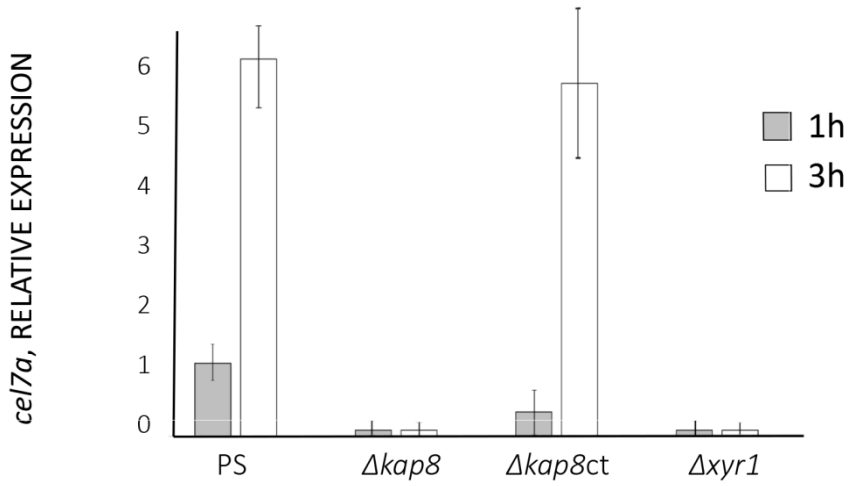

Figure 9. Expression of the cellobiohydrolase *cel7A* in *T. reesei* parental strain (PS), *Δkap8* and its complemented transformant Δ*kap8*ct, and Δ*xyr1* after 24 hrs pre-culture on 1 % (w/v) glycerol and subsequent transfer to 1.4 mM sophorose for 1h (grey bars) and 3 hrs (white bars), respectively. Relative expression levels are defined as above, but the ratio obtained for the retransformant at t=0 was used for normalization. Error bars indicate the standard deviation from n ≥ 3 biological replicates.

3.3.2 KAP8 is essential for nuclear import of XYR1 *in T. reesei*

In order to test whether KAP8 is indeed directly responsible for XYR1 uptake into the nucleus, a GFP-XYR1 fusion protein was expressed in *T. reesei Δkap8* and the complemented transformant and monitored its subcellular localization under inducing conditions. As reported previously (Lichius *et al*., 2014), nuclear import of XYR1 in response to a cellulase inducing signal is essential to activate xyr1 expression in an auto-regulatory manner and hence to produce sufficient amounts of XYR1 to elicit high-level cellulase and hemicellulase gene expression. As shown in Figure.10,

fluorescence microscopy demonstrate that the parent strain and the *kap8* complemented transformant imported XYR1 into the nuclei, whereas *Δkap8* did not. These data show that nuclear import of XYR1 is dependent on KAP8 function.

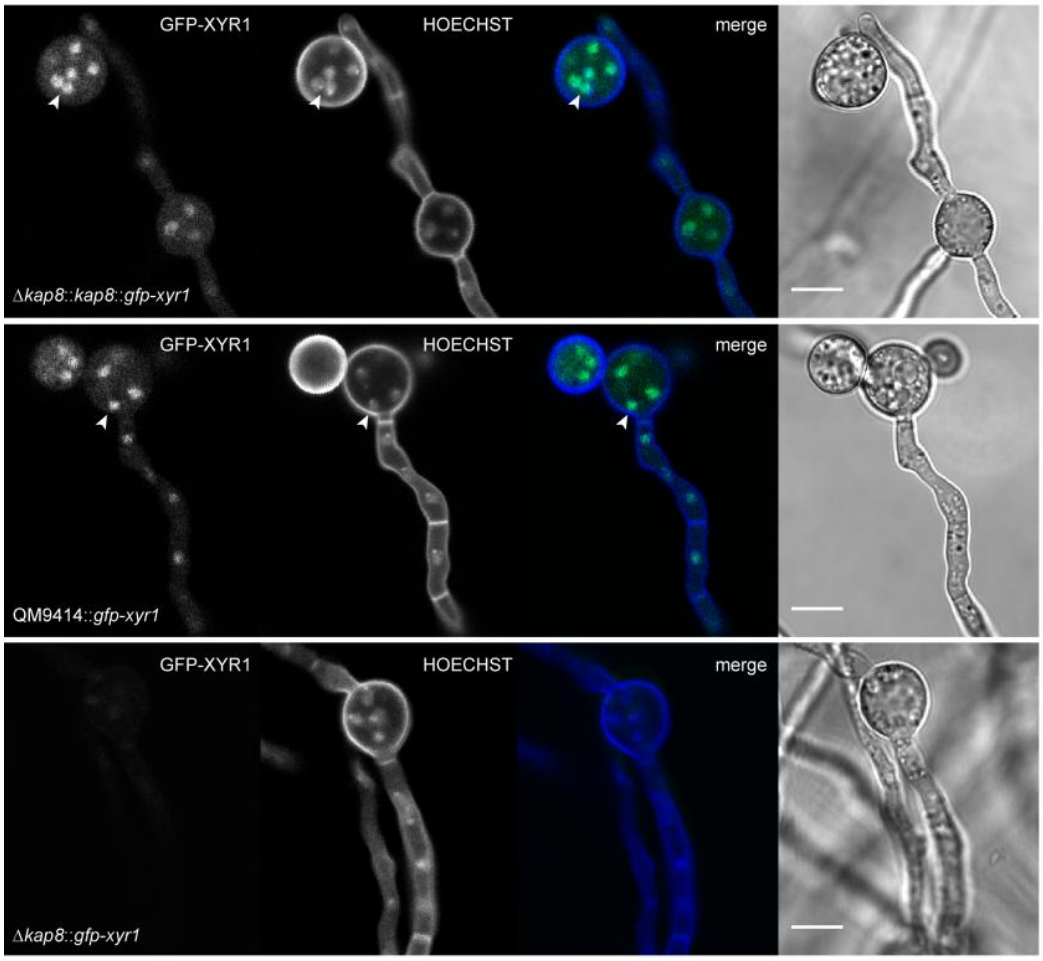

Figure 10. **Nuclear recruitment of GFP-XYR1 is dependent on KAP8 function**. In contrast to the Δ*kap8* strain, the genetically complemented strain *(Δkap8*RT/GFP-XYR1*)* is fully capable of importing GFP-XYR1 into the nucleus. In both cases, expression of *gfp-xyr1* was under control of the native *xyr1* promoter. Staining of nuclei with Hoechst dye confirmed nuclear recruitment of GFP-XYR1. Scale bars, 3 µm.

3.3.3 KAP8 is essential for the expression of the sophorose-induced plant cell wall degrading enzymes in *T. reesei*

The results described above provided the first evidence that KAP8 is essential for nuclear import of XYR1, and is indirectly important for the formation of the cellulase CEL7A. The expression of all cellulase genes of *T. reesei* is known to be coregulated by XYR1 (Kubicek, 2013). A similar effect of *kap8* on the expression of the other cellulase genes can be safely assumed. However, XYR1 also regulates the expression of several hemicellulases (Stricker *et al.*, 2008, Seiboth *et al.*, 2012, Amore *et al.*, 2013), and its full regulon in *T. reesei* has not yet been identified. We investigated the global change in gene transcription in the *Δkap8* strain and its rescued retransformant upon

induction with 1.4 mM sophorose after three hours. We detected 351 genes (including 57 CAZymes) that were at least 4-fold higher expressed on sophorose compared to the non-induced control (24 h glycerol pre-culture; Supplementary Table 3). 146 from them (including 44 CAZymes) were >10-fold induced. Apart of CAZymes, genes encoding unknown conserved proteins, metabolic enzymes and membrane proteins that function in solute uptake were most abundant (Figure 11). The rest of the genes identified (4-5 % of the transcriptome) consisted of genes encoding enzymes which react with molecular oxygen, genes encoding small secreted cysteine-rich proteins (SSCRPs) and transcription factors (two C2H2-type, each one of BZIP-, myb- and MYND/zinc finger type, and 11 Zn(2)Cys(6) zinc cluster proteins; Supplementary Table S4). The latter also included the already known regulatory genes *xyr1*, *clr2 (*Trire2:26163, which encodes the orthologue of the *N. crassa* and *A. nidulans* cellulase regulator CLR-2/ClrB; (Coradetti *et al.*, 2012, Häkkinen *et al.*, 2014), and *ace3* (Häkkinen *et al.*, 2014).

In the *Δkap8* strain, 195 of the 351 sophorose-induced genes were more than 5-fold downregulated compared with the parental control and exhibited almost no expression irrespective of the presence or absence of sophorose (Supplementary Table 4). They included 42 of the 57 CAZyme-encoding genes (73.7 %; Table 3), and comprised all cellulase and hemicellulase genes that were known or believed to be under control of XYR1, as well as many other genes for which XYR1 control has not yet been detected, such as α–D-galactosidases, α-D-fucosidases, α-D-mannosidases, AA9 polysaccharide monooxygenases and polygalacturonases. Besides CAZymes, also several members of other gene groups listed in Figure 11 exhibited significantly reduced expression in *Δkap8* strain, which was most, pronounced with genes encoding proteases and SSCRPs (Figure 11). The induction of *xyr1,* which itself is also induced by sophorose (*vide supra*), was only somewhat reduced (39 % after 3 hrs of induction, respectively; $p < 0.001$).

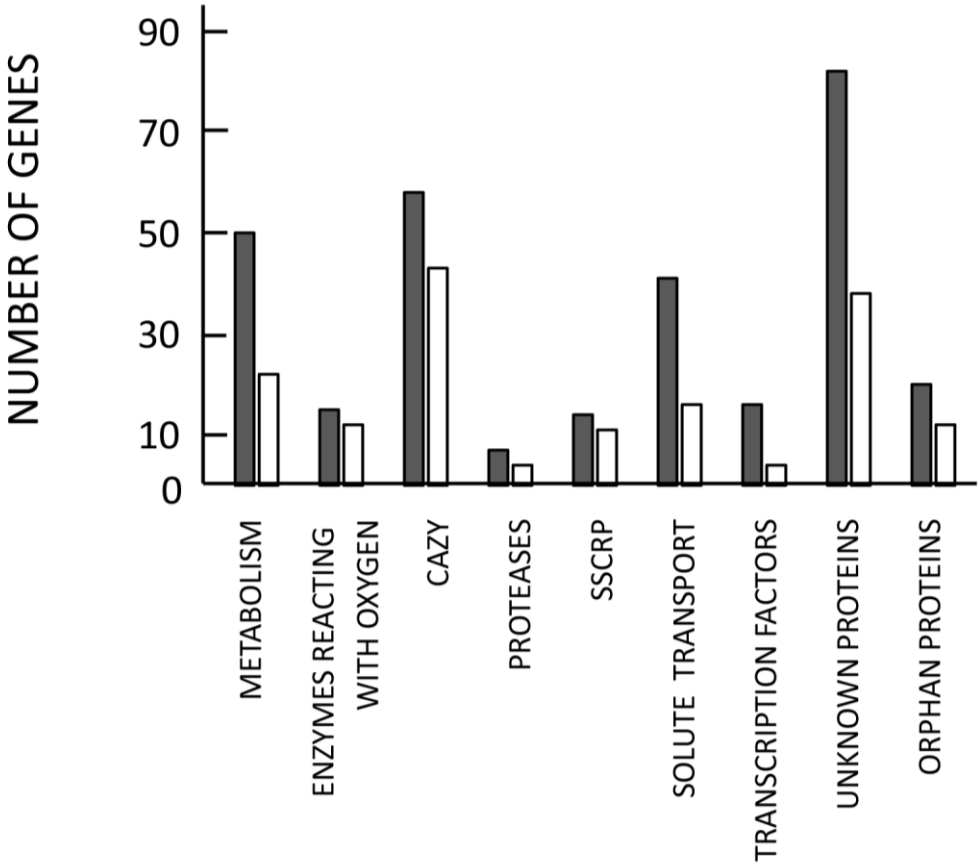

Figure 11. Number of genes, grouped according to functional similarity, that were induced by sophorose at least 4-fold (grey bars) and genes whose sophorose-induction was significantly lower (> 5-fold) in the *Δkap8* strain compared to the complemented transformant *Δkap8ct* (white bars). The grouping shown comprised 81.7 % of all sophorose-inducible genes of strain *Δkap8ct* as documented in Supplementary Table 3 Gene groups and abbreviations: METABOLISM (genes involved in intracellular catabolism, anabolism and secondary metabolism); CAZY (all extracellular glycosyl hydrolases, carbohydrate esterases and accessory enzymes); enzymes reacting with oxygen (oxidases; cytochrome P450- and FAD monooxygenases; dioxygenases); SSCRP (small, secreted, cysteine-rich proteins including hydrophobins and ceratoplatanins); SOLUTE transport (members of the major facilitator superfamily; amino acid transport; ion transport); unknown proteins (proteins that have orthologues in other species, but for which no function has as yet been described); and orphan proteins (proteins that have orthologues, if at all, only in other *Trichoderma* species, and for which also no function is as yet known).

3.3.4 The *Aspergillus nidulans* KAP8 orthologue KapI is not involved in cellulase and xylanase formation

Our findings that KAP8 regulates XYR1 import and consequently cellulase and hemicellulase formation in *T. reesei* prompted us to investigate whether this mechanism is also conserved in other fungi. We chose to test this in *A. nidulans*, because its karyopherins have been investigated in detail and respective mutants are available (Markina-Iñarrairaegui *et al.*, 2011). We showed above that the *T. reesei* KAP8 orthologue in *A. nidulans* is KapI. In order to test, whether KapI is involved in the function of the *A. nidulans* XYR1 orthologue XlnR, we tested the growth of a Δ*kapI*

(and the other *kap* mutant strains) strain on D-glucose, D-xylose, birchwood xylan and carboxymethyl-cellulose. Induction of the enzymes required for catabolism of D-xylose, extracellular depolymerization of xylan and (in part) of cellulose have been shown to be dependent on XlnR function in *Aspergillus* spp. (Tsukagoshi *et al.*, 2001, Tamayo *et al.*, 2008). Consequently, we assumed that growth on these carbon sources would be reduced in the *A. nidulans* Δ*kapI* strain if KapI would perform the nuclear import of XlnR. However, the *A. nidulans* Δ*kapI* strain grew equally well as the wild type strain and other importin mutants on all tested carbon sources, thus illustrating that KapI either is not involved in XlnR nuclear import or that its function can be compensated by another karyopherin (Figure 12).

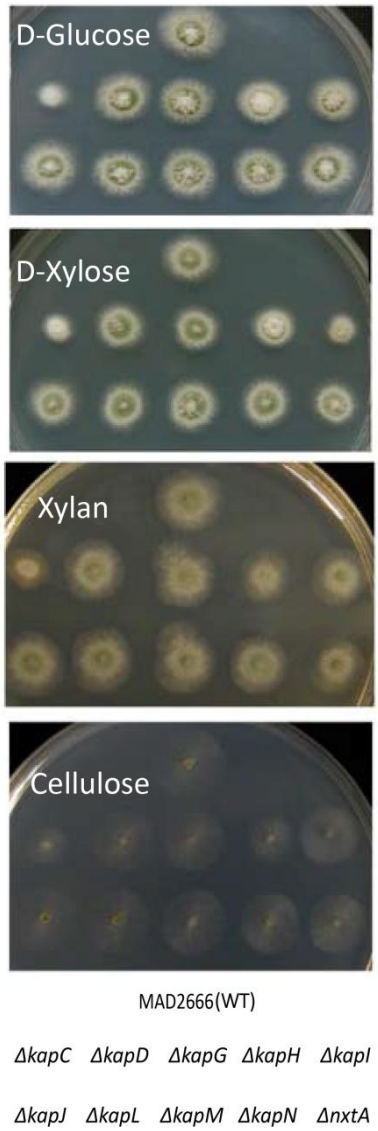

Figure 12. Growth of *A. nidulans* MAD2666 and mutant strains defective in selected *kap* genes (see Table 2) on D-glucose, the cellulose derivate CMC, xylan and D-xylose. Data from a single experiment are shown, but three replicates yielded consistent results.

3.3.5 *T. reesei* KAP8 function is required for asexual – but not sexual - development

In *A. nidulans*, conidiation of the Δ*kapI* strain is reduced by two orders of magnitude compared to the isogenic wild-type strain (Etxebeste *et al.*, 2009). In *T. reesei,* sporulation of the *Δkap8* strain was also reduced to <10 % of that of the parental strain (n=4; Figure 13A and 13B). In contrast to *A. nidulans*, however, we found no reduced hyphal extension rate in *T. reesei Δkap8* on D-glucose as a carbon source (0.14 vs. 0.11 cm/h; SD ± 0.02 cm/h). Also no significant differences were detected with respect to hyphal branching frequency, measured as the average length of hyphae between any two branches in germlings and at the periphery of mature colonies. In addition, the *Δkap8* strain and its complemented transformant grew at a similar colony extension rate and with a similar biomass yield in liquid medium on D-glucose as carbon source (data not shown).

In order to test whether KAP8 would be involved in the nuclear import of a transcription factor essential for sexual recombination, we mated *T. reesei Δkap8 MAT1-2* with a corresponding *T. reesei MAT1-1* partner (see Materials and Methods). As shown in Figure 13C, *T. reesei Δkap8* exhibited normal sexual development by producing fertile fruiting bodies in the same number and within the same time as the parent. We therefore conclude that KAP8 is not involved in nuclear transport of components essential for sexual development of *T. reesei*.

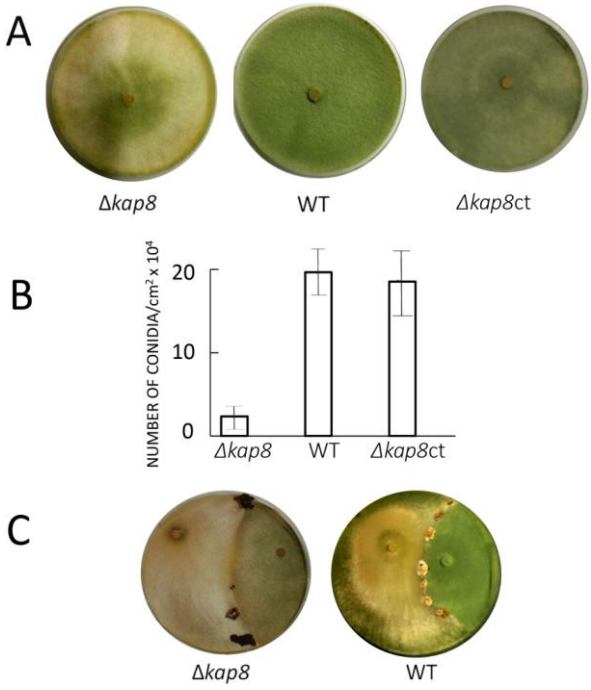

Figure 13. Effect of kap8 knock out on asexual and sexual development in *T. reesei*: (A) phenotype of *Δkap8*, the parental strain and the complemented transformant (*Δkap8ct*) on PDA plates; **(B)** quantification of conidia harvested from these cultures. Vertical bars indicate standard deviations (n ≥ 3); **(C)** fruiting body formation in confrontation with strain CBS999.79 (*MAT1-1*).

3.3.6 *T. reesei* KAP8 is involved in the general stress response

In *S. cerevisiae*, Kap121 is essential for the nuclear import of the oxidative stress regulator Yap1 (Isoyama *et al.*, 2001), and the antibiotic efflux regulator Pdr1 (Delahodde *et al.*, 2001 , Caudle *et al.*, 2011). We have therefore investigated whether the deletion of *T. reesei kap8* might have an effect on its response to stress. To this end, we cultivated *T. reesei Δkap8* and its rescued transformant under conditions known to elicit a stress response, such as high concentrations of sorbitol and KCl (osmotic and salt stress), extreme pH, growth-inhibiting temperature (37°C), fluconazole (azole toxicity), and H_2O_2 (oxidative stress). The results, shown in Figure 14, document that indeed the *Δkap8* strain exhibits significantly decreased growth under all these conditions, but the effect was most severe under osmotic stress, at low pH (pH2) and at elevated temperature (37°C). Interestingly, and in contrast to *A. nidulans* (Etxebeste *et al.*, 2009), none of these stress conditions rescued the sporulation deficiency of the *Δkap8* strain.

To potentially identify genes that could be causally related to this reduced stress resistance, we searched our transcriptomic data for genes whose expression on glycerol was either similar or even higher as that on sophorose, but strongly reduced under both conditions in the *Δkap8* strain. 163 genes fulfilled this criterion. Some of the major gene groups in this sample were the same as during induction with sophorose, i.e. genes encoding metabolic enzymes, MFS permeases and unknown proteins (Figure 15). Among the other genes, however, a few examples are noteworthy, e.g. the two-component histidine kinase Trire2:70943; the aquaglyceroporin Trir2:81082; three PTH11-type G-protein coupled receptors Trire2: 82041, 69904 and 122795; the translation initiation regulator GCN20 (Trire2: 22839); several putative cell wall proteins (Trire2: 123659,123475; and the *Trichoderma* cell-wall protein QI74 Trire2:74282;(Rey *et al.*, 1998); and five Zn(2)Cys(6) transcriptional activators of which the expression of two (Trire2: 112036, 112560) was absolutely dependent on KAP8 function.

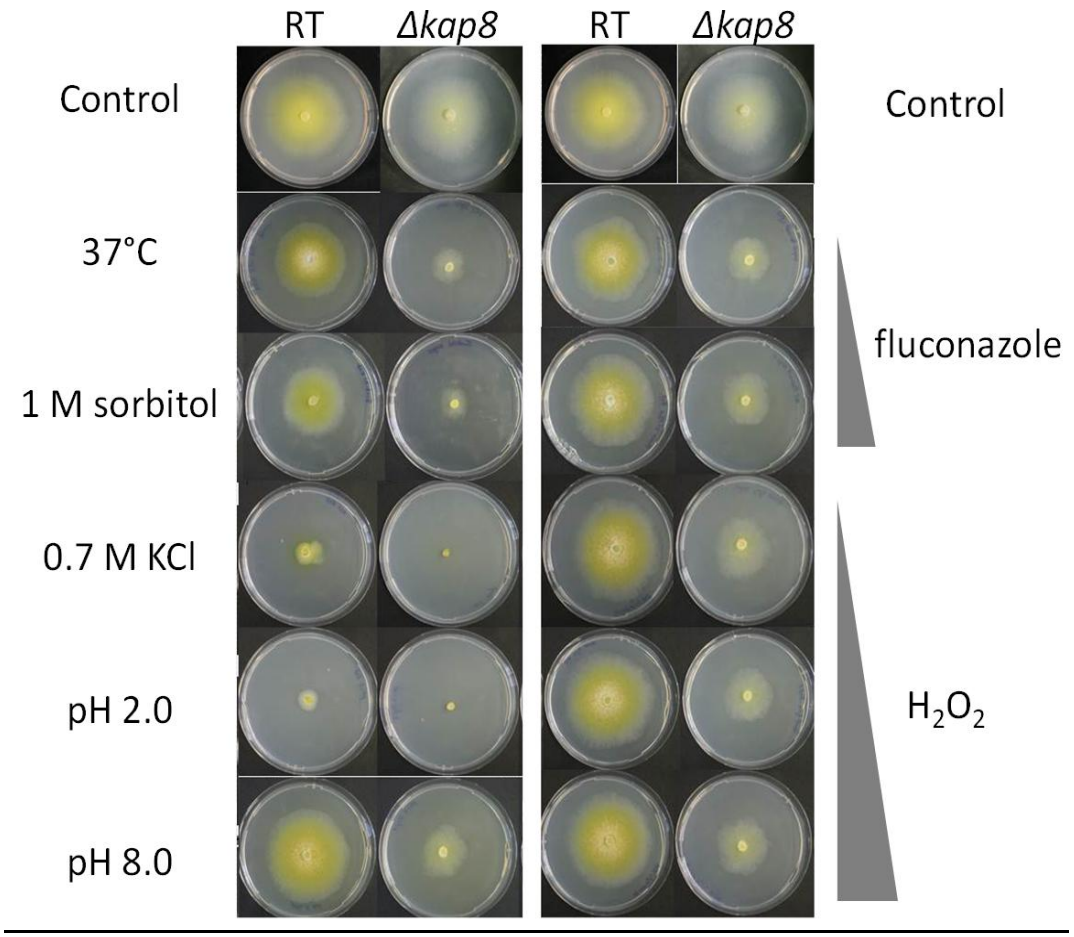

Figure 14. Growth of *T. reesei Δkap8* and its retransformant (*Δkap8*ct) on PDA in the presence of various stress-inducing agents. From top to bottom: 1.5, 10 and 20 mM H_2O_2; from top to bottom 0.5 and 2 µg/mL fluconazole. Other concentrations/conditions are directly indicated. The plates shown are from single experiments, but two further biological replicates yielded consistent results.

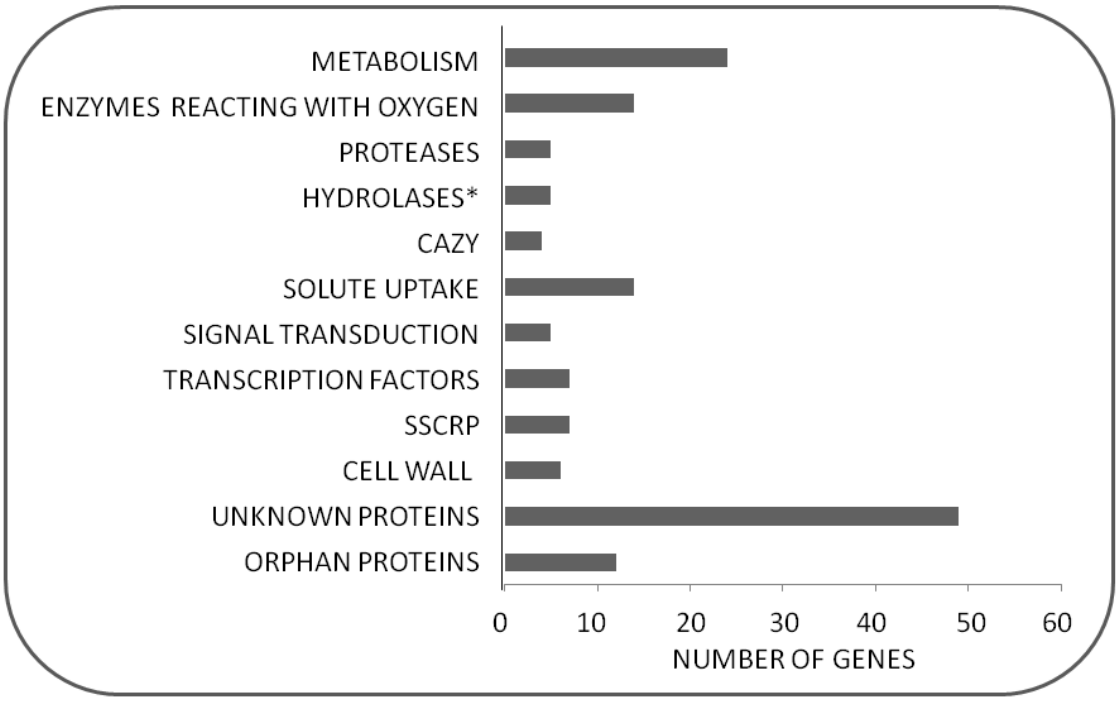

Figure 15. Inventory of gene groups that were not induced by sophorose but significantly downregulated in the *Δkap8* strain compared to the retransformant. The gene groups encounter 152 from 163 downregulated genes. Specification of gene groups is as described in the Legend to Figure 11. New groups include: HYDROLASES (extracellular lipases, esterases and amidases), SIGNAL TRANSDUCTION (involved in signal transduction pathways); and CELL WALL (proteins being components of the fungal cell wall, glycosyltransferases involved in their biosynthesis).

4 DISCUSSION

In this study, we provided the first evidence that the *T. reesei* ß-importin KAP8, an orthologue of *S. cerevisiae* Pse1/Kap121 and *A. nidulans* KapI, is essential for the nuclear import of the transcriptional regulator for cellulase and hemicellulase gene expression XYR1. Pse1 was first reported in yeast as an auxiliary import receptor of ribosomal protein L25, since the defective import of L25 observed in the *kap123*Δ strain was reversed by the overexpression of Pse1 (Schlenstedt *et al.*, 1997), and subsequently shown to be an essential protein that regulates multiple cellular transcription factors such as Pho4 (responding to phosphate stress; (Kaffman *et al.*, 1998), Yap1 (oxidative stress response; (Isoyama *et al.*, 2001), Pdr1 (membrane biogenesis; Delahodde *et al.*, 2001), the iron regulator Aft1 (Ueta *et al.*, 2003), Ste12 (mating response; (Leslie *et al.*, 2002) and a regulatory protein for sporulation (Chaves & Blobel, 2001). In filamentous fungi, the function of the *A. nidulans* KapI appears to be non-essential (Etxebeste *et al.*, 2009), and here we could confirm this also for *T. reesei* orthologue KAP8. Although its role in the control of cellular processes such as conidiation and hyphal branching has been demonstrated in *A. nidulans*, the transcriptional activators whose transport would be performed by KapI, and consequently impact the above processes, have not yet been identified (Etxebeste *et al.*, 2009). In the present paper, we demonstrate that KAP8 is essential for XYR1 transport and cellulase formation. Interestingly, cellulasse gene transcription occurs in *T. reesei* also during conidiation in the absence of an inducer, which would link XYR1 to sporulation. However, XYR1 has been shown to play no regulatory role in asexual sporulation (Metz *et al.*, 2011).

Transcriptomic analysis showed that a loss-of-function of KAP8 indeed led to the impairment of the induction of all cellulase and xylanase genes of *T. reesei*, and also of several other genes for which the regulation by XYR1 has not yet been demonstrated, most notably including α-D-galactosidases, α-D-mannosidases, and α-D-fucosidases. Their XYR1-dependent induction by the cellulase inducer sophorose implies that these enzymes belong to the standard repertoire of *T. reesei* when faced with a lignocellulosic substrate, and support previous interpretations (Ivanova *et al.*, 2013) that *T. reesei* is also strongly active on hemicellulose side chains. Häkkinen *et al.* 2012) have recently described the sophorose-inducible CAZome of *T. reesei*, and listed 56 genes. Although we detected almost the same number (55), only 30 and 31 genes, respectively, were identical between the two studies (Supplementary Table 2). We assume that our more stringent criteria for induction and the early time point (3 h), as well as the fact that RNAseq was used in the

present work whereas (Häkkinen *et al.*, 2012) used microarrays, may be responsible for this difference.

We also identified four transcription factors that were induced by sophorose and dependent on the function of KAP8. Interestingly, none of them has been found in a screening of *T. reesei* transcription factors that correlate with cellulose formation (Häkkinen *et al.*, 2014). Their relationship to cellulase and hemicellulase gene expression, if any, needs to be assessed, but this was beyond the scope of the present paper. The already known regulators of cellulose and hemicellulase gene expression in *T. reesei* (i.e. XYR1, ACE3, and CLR2) were all significantly induced by sophorose and their expression was reduced in the *Δkap8* strain. However, all of them exhibited still a significant residual level, indicating that their expression is at least partly independent of KAP8. Therefore, they all failed to pass our criteria for regulation by KAP8.

It is intriguing to note in this context, that KAP8 and also the nuclear transport factor 2 (Trire2:22294; an essential component for the small GTPase Ran which assist in the nuclear export of ß-importins), and ataxin-7 (Trire2:112346; which "gates" proteins to the nucleopore complex) have been shown to have become mutated during generation of the *T. reesei* cellulase hyperproducer mutant RUT C30 from its parent NG14 (Le Crom *et al.*, 2009). This suggested that changes in nuclear recruitment of transcription factors could contribute to RUT C30's enhanced cellulase formation (Le Crom *et al.*, 2009). The respective mutation in *kap8* is a single nucleotide exchange resulting in an A_{738}→T change within an Armadillo repeat in KAP8. However, despite of the importance of *kap8* for cellulase and hemicellulase formation as we have shown here, we consider it now less likely that this A_{738}→T mutation contributes to the increased cellulase production in strain RUT C30, because other mutations were meanwhile found that make up for most of the differences in productivity between RUT C30 and NG14, and thus the consequences of the KAP8 mutation, if any, can only be minor (C. Ivanova and B. Seiboth, unpublished data).

Having identified KAP8 as the importin that is responsible for nuclear import of XYR1 and consequently cellulase and hemicellulase gene expression in *T. reesei*, we wondered whether this function may also be conserved in other fungi. We chose *A. nidulans* for this purpose, because knock-out strains in all importins are available for this species, and because the role of XlnR in growth on xylan and xylanase gene expression has been documented (Tamayo *et al.*, 2008). However, we found that the *A. nidulans kapI* knock-out strain grew as well on xylan as its parent thus arguing against a role of KapI in XlnR function. There was also no difference between *ΔkapI* and its parent with respect to growth on cellulose, but we must note that a role of XlnR in

regulation of cellulase gene expression in *Aspergilli* is not clear (De Souza *et al.*, 2013). As noted above, regulation of cellulose gene expression in *A. nidulans* and *N. crassa* is mainly performed by CLR-2/ClrB (Coradetti *et al.*, 2012), and thus the role of XYR1 in cellulase gene transcription in *T. reesei* is so far unique among fungi. Owing to the above discussed ability of *ΔkapI* mutants to grow well on cellulose, it also appears rational to assume that KapI is either not or not exclusively involved in nuclear transport of ClrB in *A. nidulans*, and this fits also to our discussion of its *T. reesei* ortholog CLR2 (*vide supra*).

Interestingly, this defect in conidiation of the *A. nidulans ΔkapI* strain is suppressed under abiotic stress, whereas this was not the case in *T. reesei*. In accordance with *S. cerevisiae*, *T. reesei* KAP8 is necessary for a full stress response and the *Δkap8* strain is particularly sensitive against low pH and osmotic stress. Our transcriptomic data revealed two genes that were strongly downregulated in the *Δkap8* strain and that could therefore be involved in *T. reesei* osmotic stress i.e. the class VI histidine kinase SLN1 (Trir2:70943) and an aquaglyceroporin (Trir2:81082). Indeed, Sln1 is in *S. cerevisiae* involved in the high-osmolarity response by transmitting the osmolarity signal through the Sln1-Ypd1-Ssk1 two-component system and the Ssk2/Ssk22-Pbs2-Hog1 MAP kinase cascade (Hohmann, 2002). Whether Sln1-orthologues would function in the same way in Pezizomycota is unclear, however: In *Aspergillus spp.*, targeted deletion of the *Sln1* orthologue produced no stress-impaired phenotype (Furukawa *et al.*, 2008, Du *et al.*, 2006), whereas in the pyrenomycete *Magnaporthe oryzae*, which is evolutionary closer to *Trichoderma* (Wang *et al.*, 2009), deletion of the Sln1 orthologue MoSLN1 significantly affected fungal growth and morphology on different media, and resulted in impaired resistance to oxidative and osmotic stress (Zhang *et al.*, 2010). With regards to the aquaglyceroporin (Trir2:81082), *S. cerevisiae* maintains the osmotic equilibrium und osmotic stress by producing and retaining high concentrations of glycerol as a compatible solute, whose intracellular concentration is to a large extent determined by the regulated activity of aquaglyceroporins. A similar mechanism may occur in *T. reesei,* yet still requires testing.

Concluding remarks

Summarizing, I have here identified a major player in the import of XYR1 and cellulose and hemicellulose gene expression. However, I have also shown that the expression of XYR1 despite being triggered by cellulase inducers is only partially impaired by loss-of-function of *kap8*. This implies that *xyr1* induction is only partially due to XYR1-dependent autoregulation (Lichius *et al.,* 2014) and thus requires other as yet unknown transcription factors whose nuclear recruitment is KAP8-independent. Identification of this factor may also open new avenues for improvement of cellulase production in *T. reesei*.

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Appendix

Supplementary Table 1 Glycosyl hydrolases (GH), carbohydrate esterases (CE) and accessory enzymes (AAs) of *T. reesei* that are induced by sophorose and impaired in their expression in *Δkap8*

		RPKM*			*Δkap8*ct/*Δkap8***
Trire2:	**Annotation**	***Δkap8***		***Δkap8*RT**	**[-fold]**
		soph***	**glyc**	**Soph**	
73643	AA9 polysaccharide monooxygenase CEL61a	548.6	9.5	76189.2	138.9
65215	CE4 imidase	200.5	20.1	3640.5	18.2
73632	CE5 acetyl xylan esterase AXE1	7.0	1.2	2846.3	408.8
73638	CIP1	108.6	2.4	83482.6	768.7
57179	GH105/GH88 glycosyl hydrolase	4.2	0.0	213.7	51.2
74223	GH11 endo-ß-1,4-xylanase XYN1	9.1	0.0	314.4	34.7
112392	GH11 endo-ß-1,4-xylanase XYN5	4.2	0.0	289.8	69.4
123232	GH12 endo-ß-1,4-glucanase	1.4	0.0	180.8	129.9
65162	GH18 endo-N-acetyl-ß-D-glucosaminidase Endo T	100.3	20.1	2195.8	21.9
5836	GH2 β-mannosidase	72.4	0.0	3192.5	44.1
102909	GH2 ß-glycosidase	53.6	66.4	294.9	5.5
55999	GH27 α-galactosidase	399.6	15.4	2259.5	5.7
72632	GH27 α-galactosidase AGL1	14.6	1.2	160.3	11.0
72704	GH27 α-galactosidase AGL3	192.9	17.8	4165.6	21.6
112140	GH28 exo-polygalacturonase PGX1	7.0	0.0	706.9	101.5
122780	GH28 exo-rhamnogalacturonase RGX1	669.1	39.1	50745.5	75.8
108671	GH3β-glucosidase/glucan1,4-β-glucosidase BGL3f	7.7	0.0	167.5	21.9
76672	GH3 β-glucosidase BGL1/CEL3a	2.8	0.0	132.6	47.6
46816	GH3 β-glucosidase CEL3d	146.9	26.1	1865.0	12.7
121127	GH3 β-xylosidase BXL1	271.5	1.2	23568.4	86.8
110894	GH30 endo-β-1 6-galactanase	104.4	58.1	534.3	5.1
69276	GH30 endo-β-1,4-xylanase	2.1	0.0	1762.2	843.7
111849	GH30 endo-β-1,4-xylanase XYN4	142.7	13.0	8683.6	60.8
69944	GH31 α-xylosidase/α-glucosidase	23.7	0.0	415.1	17.5
60085	GH31 α-glucosidase	43.9	8.3	427.5	9.7
79960	GH47 α-1,2-mannosidase	271.5	3.6	5769.6	21.2
120312	GH5 endo-β-1,4-glucanase EGL2/CEL5a	9.7	0.0	29758.3	3053.1
49976	GH5 endo-β-1,4-glucanase EGL5/CEL45a	158.7	3.6	25111.8	158.2
56996	GH5 β-mannanase MAN1	0.7	0.0	226.1	324.7
55319	GH54 α-L-arabinofuranosidase ABF3	118.4	4.7	10592.8	89.5
70845	GH55 β-1,3-glucanase	237.4	60.4	2030.4	8.6
72567	GH6 cellobiohydrolase CBH2/CEL6a	245.1	7.1	372237.7	1518.9
76210	GH62 α-L-arabinofuranosidase ABF2	0.0	0.0	115.1	ND
72526	GH67 α-Glucuronidase GLR1	337.7	13.0	7793.8	23.1
123989	GH7 cellobiohydrolase CBH1/CEL7a	985.1	7.1	1255793.1	1274.7
122081	GH7 endo-β-1,4-glucanase EGL1/CEL7b	142.7	1.2	180819.3	1266.9

49081	GH74 xyloglucanase CEL74a	394.8	360.3	8088.7	20.5
74198	GH92 α-1,2-mannosidase	44.6	24.9	897.0	20.1
79921	GH92 α-1,2-mannosidase	503.4	88.9	3199.7	6.4
58802	GH95 α-L-fucosidase	37.6	8.3	494.2	13.1
5807	GH95 α-L-fucosidase	32.7	5.9	319.6	9.8
123992	Swollenin	595.3	55.7	34626.7	58.2

* RPKM reads per kilobase of exon per million mapped sequence reads
** Ratio of induction by sophorose in the retransformant strain Δkap8 RT over *Δkap8*
*** Soph, sophorose; glyc, glycerol.

Supplementary Table 2: comparison of CAZymes induced by sophorose in this study and by Häkkinnen et al. (2012) and reduced by KAP8 loss-of-function

Cazy group	number	number of genes *		
		*Δkap8*RT	*Δkap8*	Häkkinen et al (2012)
glycosyl hydrolase family	1	**2**		1
	2	2	2	2
	3	8	4	8
	5	**3**	3	2
	6	1	1	1
	7	2	2	2
	10	1		1
	11	2	2	**3**
	12	1	1	1
	13	**2**		
	15			**1**
	16			**2**
	18	1	1	**3**
	27	**4**	3	3
	28	**2**	2	1
	30	3	3	3
	31	**3**	2	2
	35			**1**
	43	1		1
	45			**1**
	47	**2**	1	1
	54	1	1	1
	55	**1**	1	
	62	1	1	1
	64			**1**
	65	**1**		
	67	1	1	1
	71			
	74	**1**	1	
	75			
	76	1		1

	79			1
	92	**2**	2	1
	95	2	2	2
	105	1	1	1
	115			**1**
accessory enzymes family	9	1	1	**2**
carbohydrate esterase family	1			**1**
	3			**1**
	4	**1**	1	
	5	1	1	1
Total CAZys		**55**	**40**	**56**

***** Red and bold numbers highlighten those gene numbers that are higher in on of the two studies

Supplementary Table 3: Genes induced >4-fold by the presence of 1.8 mM sophorose in the T. *reesei* Δkap8 retransformant

Trire2:	Annotation	Soph		glyc		RPKM		induction
		log2FC	p-value	log2FC	p-value	soph	glyc	[-fold]
50593	5' nucleotidase	2,68	0,00	0,92	0,00	637,07	79,43	8,02
73643	AA9 polysaccharide monooxygenase CEL61a	13,91	0,00	4,56	0,14	76189,19	1,17	65226,50
74187	AAA+-type ATPase	2,58	0,00	1,23	0,13	208,59	26,87	7,76
103041	Acetamidase	4,25	0,01	4,26	0,01	629,88	18,69	33,70
77093	acid sphingomyelin phosphodiesterase (probably vacuolar)	2,87	0,00	2,64	0,00	870,32	98,12	8,87
106885	acyl-CoA dehydrogenase	2,42	0,04	1,76	0,13	1352,23	179,88	7,52
54667	acyl-CoA synthetase	3,00	0,00	5,65	0,00	4107,03	453,21	9,06
69753	ADA, Adenosine deaminase	2,17	0,00	2,15	0,00	1592,67	357,43	4,46
66689	Adenine deaminase/adenosine deaminase	1,42	0,07	5,82	0,00	125,36	25,70	4,88
2038	Alcohol dehydrogenase, class IV	3,31	0,00	2,03	0,04	257,14	25,90	9,93
68590	Alcohol dehydrogenase, class V	2,46	0,02	4,19	0,00	899,09	154,19	5,83
80659	alcohol oxidase AOX1	7,10	0,09	0,45	0,96	516,85	3,50	147,49
70860	allantoate permease, 10 TM domains	2,25	0,00	1,52	0,00	4087,51	807,14	5,06
62576	alpha/beta hydrolase	2,55	0,00	-4,03	0,00	1466,28	296,69	4,94
5889	Amidase	2,44	0,00	2,85	0,00	791,20	123,82	6,39
62172	amino acid permease (PotE?)	2,66	0,00	2,60	0,00	247,63	49,06	5,05
66819	Amino acid transporter LysP	1,53	0,00	3,58	0,00	123,30	28,03	4,40
61114	Amino acid transporter LysP	1,75	0,00	1,26	0,01	590,83	102,79	5,75
59952	Amino acid transporter PotE	2,34	0,00	2,14	0,00	131,52	32,71	4,02
59843	AMP-dependent synthetase and ligase, acetoacetyl-CoA synthase-like	3,07	0,19	3,20	0,16	166,46	30,37	5,48
123673	Ankyrin	1,93	0,01	4,25	0,00	477,80	105,13	4,55
63653	Ankyrin	4,97	0,01	4,87	0,02	705,91	57,24	12,33
70907	Ankyrin	2,78	0,00	0,89	0,11	124,33	24,53	5,07
82619	arginosuccinate synthetase	1,68	0,26	-Inf	0,08	12857,47	2725,11	4,72
104073	aspartate racemase	2,82	0,00	2,11	0,02	325,73	36,21	9,00
109538	BZIP transcriptional regulator	3,25	0,00	3,16	0,01	187,01	31,54	5,93

35552	C2H2 transcription factor	1,72	0,00	3,24	0,00	598,02	102,79	5,82
108357	C2H2 transcriptional regulator	3,39	0,00	2,79	0,00	30388,13	3760,02	8,08
62362	calcium transporting ATPase, ion pump	2,27	0,00	1,78	0,00	6486,79	1226,47	5,29
67761	calpain-like protease	2,44	0,00	3,94	0,00	2659,24	483,58	5,50
22459	carboxypeptidase A	4,57	0,00	0,94	0,00	1736,52	85,27	20,37
81430	Cation transporting ATPase	1,98	0,00	4,10	0,00	9649,52	1836,21	5,26
65215	CE4 imidase	2,91	0,00	3,80	0,00	3640,53	388,97	9,36
73632	CE5 acetyl xylan esterase AXE1	Inf	0,00	2,31	0,50	2846,25	0,00	ND
104277	cell wall protein, distantly related to A. niger CwpA.	2,75	0,00	5,33	0,00	4230,34	812,98	5,20
64397	Ceramidase family protein, associated toCellulase signal transduction (PMID: 15288024)	2,93	0,00	2,73	0,00	17849,20	1978,71	9,02
60374	choline oxidase	3,43	0,00	3,81	0,00	322,64	16,35	19,73
73638	CIP1	16,27	0,00	3,91	0,21	83482,59	0,00	ND
123029	Cu2+/Zn2+ superoxide dismutase SOD1	2,14	0,00	0,78	0,00	12918,09	3117,58	4,14
109811	Cytochrome P450	2,24	0,00	3,82	0,00	304,15	65,41	4,65
69648	Cytochrome P450 CYP11/CYP12/CYP24/CYP27 subfamilies	4,07	0,03	1,17	0,57	129,47	4,67	27,71
70842	Cytochrome P450 CYP2 subfamily	4,74	0,00	3,04	0,08	391,49	11,68	33,52
57555	cytochrome P450 monooxygenase	2,56	0,03	3,91	0,01	164,40	25,70	6,40
65141	cytochrome P450 monooxygenase	3,34	0,00	3,24	0,02	544,59	37,38	14,57
4999	cytochrome P450 monooxygenase	3,60	0,09	5,19	0,03	732,03	60,21	12,16
59338	D-Alanine aminotransferase	2,20	0,00	3,04	0,00	302,09	51,40	5,88
47077	Dioxygenase	2,55	0,01	2,99	0,00	1753,99	328,23	5,34
61293	dipeptidyl peptidase 5	2,01	0,00	5,03	0,00	228,11	35,04	6,51
2044	dipeptidyl peptidase 5	2,12	0,00	1,85	0,00	187,72	43,04	4,36
123955	Epl1/Sm1	3,65	0,12	3,05	0,27	333,95	18,69	17,87
22637	ERCC8 (CSA, CKN1) involved in transcription-coupled nucleotideExcision repair	1,68	0,23	1,56	0,24	1979,02	422,84	4,68
106120	esterase/lipase	3,53	0,00	3,57	0,00	3290,15	293,19	11,22
65677	Esterase/lipase/thioesterase	2,17	0,01	0,81	0,35	669,95	151,85	4,41
5345	FAD-containing oxidoreductase	4,52	0,00	1,24	0,00	3078,48	116,81	26,36
110666	ferric reductase	Inf	0,00	4,69	0,07	317,51	0,00	ND
76696	Flavin-containing monooxygenase	3,54	0,00	5,66	0,00	13062,97	954,31	13,69

23083	Flavoprotein monooxygenase	4,60	0,04	2,39	0,27	7850,32	530,30	14,80
81511	Formamidase	1,86	0,00	1,69	0,00	658,65	141,34	4,66
108482	fumarylacetoacetate hydrolase	2,88	0,00	0,06	0,96	2782,55	272,16	10,22
106798	GCN5-N-acetyltransferase	1,77	0,04	1,03	0,25	4104,98	958,99	4,28
103537	GCN5-N-acetyltransferase	3,11	0,01	-0,13	0,96	185,98	19,86	9,37
69868	GDP-mannose a-mannosyltransferases;Distant relative	2,96	0,11	1,45	0,46	428,48	39,71	10,79
120749	GH1 ß-glucosidase BGL2/CEL1a	10,33	0,00	9,00	0,00	200233,47	142,50	1405,10
22197	GH1 ß-glucosidase CEL1b	6,84	0,00	5,67	0,00	31816,40	239,45	132,87
120229	GH10 endo-ß-1,4-xylanase XYN3	Inf	0,02	Inf	0,88	94,53	0,00	ND
57179	GH105/GH88 glycosyl hydrolase	8,67	0,00	Inf	0,10	213,73	1,17	182,97
74223	GH11 endo-ß-1,4-xylanase XYN1	7,15	0,00	Inf	0,01	314,42	0,00	ND
112392	GH11 endo-ß-1,4-xylanase XYN5	9,22	0,00	Inf	0,12	289,76	0,00	ND
123232	GH12 endo-ß-1,4-glucanase	Inf	0,04	Inf	0,77	180,85	0,00	ND
59578	GH13 α-glucosidase	2,10	0,00	0,82	0,20	123,30	22,19	5,56
108477	GH13 α-glucosidase/oligo α-glucosidase	1,96	0,00	2,60	0,00	2756,86	520,96	5,29
65162	GH18 endo-N-acetyl-ß-D-glucosaminidase Endo T	5,09	0,00	2,51	0,04	2195,83	85,27	25,75
5836	GH2 β-mannosidase	5,07	0,00	5,34	0,00	3192,53	53,73	59,42
102909	GH2 protein	2,21	0,00	0,14	0,81	294,90	54,90	5,37
65986	GH27 α-galactosidase	2,16	0,00	5,59	0,00	707,97	106,29	6,66
55999	GH27 α-galactosidase	4,97	0,00	4,08	0,01	2259,54	66,58	33,94
72632	GH27 α-galactosidase AGL1	3,44	0,04	3,99	0,04	160,29	10,51	15,25
72704	GH27 α-galactosidase AGL3	3,67	0,05	3,19	0,08	4165,60	226,61	18,38
112140	GH28 exo-polygalacturonase PGX1	Inf	0,00	4,60	0,17	706,94	0,00	ND
122780	GH28 exo-rhamnogalacturonase RGX1	4,09	0,02	4,68	0,01	50745,53	2187,80	23,19
108671	GH3 β-glucosidase/glucan 1,4-β-glucosidase BGL3f	8,21	0,00	Inf	0,05	167,49	0,00	ND
76672	GH3 β-glucosidase BGL1/CEL3a	Inf	0,01	Inf	0,34	132,55	0,00	ND
104797	GH3 β-glucosidase BGL3j	2,44	0,00	2,21	0,00	1974,91	276,83	7,13
121735	GH3 β-glucosidase CEL3b	4,00	0,00	7,71	0,00	106538,28	10025,56	10,63
82227	GH3 β-glucosidase CEL3c	6,32	0,00	4,30	0,00	57016,53	487,09	117,06
46816	GH3 β-glucosidase CEL3d	4,13	0,01	2,18	0,18	1864,96	81,76	22,81

76227	GH3 β-glucosidase CEL3e	2,48	0,00	2,94	0,00	4039,21	561,84	7,19
121127	GH3 β-xylosidase BXL1	10,20	0,00	5,74	0,01	23568,42	16,35	1441,23
110894	GH30 endo-β-1 6-galactanase	3,52	0,00	1,32	0,23	534,31	29,20	18,30
69276	GH30 endo-β-1,4-xylanase	11,48	0,01	Inf	0,47	1762,21	0,00	ND
111849	GH30 endo-β-1,4-xylanase XYN4	8,12	0,00	3,59	0,13	8683,64	17,52	495,61
69944	GH31 α-xylosidase/α-glucosidase	6,26	0,02	5,78	0,05	415,12	3,50	118,46
82235	GH31 α-glucosidase	3,61	0,00	4,65	0,00	33896,11	3446,98	9,83
60085	GH31 α-glucosidase	3,71	0,00	2,84	0,00	427,45	30,37	14,07
3739	GH43 β-xylosidase/α-L-arabinofuranosidase	3,65	0,00	5,12	0,00	1391,01	110,61	12,58
79960	GH47 α-1,2-mannosidase	5,01	0,00	5,25	0,00	5769,57	165,87	34,78
65380	GH47 α-1,2-mannosidase	1,94	0,00	-0,32	0,57	6853,62	1485,79	4,61
120312	GH5 endo-β-1,4-glucanase EGL2/CEL5a	Inf	0,00	0,57	0,91	29758,26	0,00	ND
49976	GH5 endo-β-1,4-glucanase EGL5/CEL45a	9,75	0,01	4,51	0,13	25111,77	15,18	1653,73
56996	GH5 β-Mannanase MAN1	8,63	0,02	1,41	0,89	226,06	0,00	ND
55319	GH54 α-L-arabinofuranosidase ABF3	3,38	0,00	3,87	0,00	10592,79	1239,32	8,55
70845	GH55 β-1,3-glucanase	2,87	0,00	2,07	0,00	2030,40	197,40	10,29
72567	GH6 Cellobiohydrolase CEL6A/CBH2	13,62	0,00	2,83	0,37	372237,65	18,69	19917,32
76210	GH62 α-L-arabinofuranosidase ABF2	Inf	0,02	Inf	1,00	115,08	0,00	ND
123456	GH65 α,α-trehalase	2,63	0,00	5,35	0,00	35674,77	5922,12	6,02
72526	GH67 α-Glucuronidase GLR1	6,82	0,00	5,43	0,01	7793,80	30,37	256,63
123989	GH7 Cellobiohydrolase CBH1/CEL7a	13,71	0,00	3,24	0,30	1255793,06	54,90	22874,46
122081	GH7 Endo-β-1,4-glucanase EGL1/CEL7b	15,95	0,00	2,88	0,36	180819,35	0,00	ND
49081	GH74 Xyloglucanase CEL74a	4,34	0,07	0,16	0,98	8088,71	276,83	29,22
55802	GH76 α-1,6-mannanase	3,83	0,00	1,17	0,00	3847,07	254,64	15,11
79921	GH92 α-1,2-mannosidase	2,87	0,00	2,60	0,00	3199,72	402,98	7,94
74198	GH92 α-1,2-mannosidase	3,61	0,10	1,75	0,43	897,03	56,07	16,00
58802	GH95 α-L-fucosidase	4,32	0,00	3,46	0,02	494,24	17,52	28,21
5807	GH95 α-L-fucosidase	3,60	0,01	2,75	0,05	319,56	18,69	17,10
22915	glucose oxidase	1,86	0,00	3,50	0,00	505,54	119,14	4,24
49946	Glutathione S-transferase	2,05	0,00	1,69	0,02	701,80	150,68	4,66

64172	Glutathione-dependent formaldehyde-activating,GFA	1,89	0,00	1,80	0,00	1016,23	245,29	4,14
123978	GMC methanol oxidase	4,12	0,46	4,82	0,39	375,05	80,60	4,65
37525	GPCR , contains RGS domain	1,97	0,00	3,48	0,00	1773,51	406,49	4,36
70096	Gß-WD40 domain protein	2,14	0,00	1,10	0,11	407,93	58,40	6,98
80340	GT α-1,6-mannosyltransferase	2,59	0,02	2,48	0,02	2291,39	471,90	4,86
82626	half-sized ABC transporter	3,18	0,07	4,49	0,01	307,23	66,58	4,61
106171	HET protein	4,06	0,00	2,35	0,00	7249,21	422,84	17,14
41699	HET protein	2,56	0,00	1,72	0,00	382,24	47,89	7,98
122220	HET protein	2,02	0,00	0,30	0,54	151,05	22,19	6,81
63240	HET-E-1, putative	1,40	0,00	1,64	0,00	881,62	213,76	4,12
119989	HFB2	2,54	0,00	6,06	0,00	559639,35	112966,50	4,95
123967	HFB3	2,88	0,00	7,56	0,00	156672,42	26809,57	5,84
106538	HFB4	4,03	0,05	1,50	0,53	133,58	3,50	38,12
71094	Intradiol ring-cleavage dioxygenase	3,03	0,04	0,84	0,67	103,78	10,51	9,87
49753	L-arabinitol 4-dehydrogenase	2,50	0,00	2,10	0,00	851,82	124,98	6,82
121418	lipase G-D-S-L	9,15	0,00	6,62	0,02	12934,53	21,03	615,19
66662	Mandelate racemase/muconate lactonizing protein	4,64	0,00	2,05	0,12	224,00	1,17	191,77
3049	methionine aminopeptidase	2,07	0,00	0,83	0,00	3603,44	858,54	4,20
112126	Methylmalonate semialdehyde dehydrogenase	2,12	0,00	3,43	0,00	3485,38	735,88	4,74
77517	MFS hexose transporter	2,51	0,00	1,36	0,00	7136,19	1001,04	7,13
46819	MFS hexose transporter	3,71	0,00	2,81	0,01	378,13	19,86	19,04
55077	MFS maltose permease	2,00	0,00	0,47	0,11	621,65	144,84	4,29
65915	MFS permease	2,12	0,00	3,99	0,00	2372,56	520,96	4,55
121482	MFS permease	4,27	0,00	3,17	0,00	8399,02	363,27	23,12
111888	MFS permease	2,53	0,00	5,32	0,00	856,96	134,33	6,38
71059	MFS permease	2,49	0,00	7,21	0,00	246,61	32,71	7,54
67752	MFS permease	2,58	0,00	2,99	0,00	3177,12	446,20	7,12
53475	MFS permease	1,79	0,00	2,38	0,00	656,59	147,18	4,46
106330	MFS permease	2,74	0,00	3,71	0,00	6051,12	656,46	9,22
76897	MFS permease	1,76	0,00	2,65	0,00	1304,96	303,70	4,30

60945	MFS permease	1,55	0,02	4,88	0,00	3316,86	711,36	4,66
69026	MFS permease	3,89	0,00	5,32	0,00	4744,10	290,85	16,31
105260	MFS permease	2,92	0,00	2,69	0,00	306,20	31,54	9,71
77785	MFS permease	1,59	0,01	2,86	0,00	3214,11	768,59	4,18
54632	MFS permease	7,97	0,00	6,27	0,00	25106,63	100,45	249,93
69611	MFS permease	2,43	0,01	3,92	0,00	1447,79	169,37	8,55
28409	MFS permease	2,54	0,04	5,50	0,00	721,33	113,30	6,37
60329	MFS permease	4,31	0,00	3,72	0,00	473,69	22,19	21,34
4774	MFS permease	2,77	0,02	3,98	0,00	340,82	49,81	6,84
69651	MFS permease	2,17	0,00	2,00	0,00	168,51	30,37	5,55
123473	MFS permease	2,11	0,01	2,67	0,00	3022,99	485,92	6,22
59388	MFS permease	1,66	0,00	1,53	0,00	99,67	19,86	5,02
70108	MFS permease	2,34	0,02	2,87	0,01	106,86	12,85	8,32
67541	MFS permease	7,46	0,00	Inf	0,01	285,65	1,17	244,55
80058	MFS permease	2,47	0,18	2,18	0,24	267,16	24,53	10,89
56684	MFS permease	5,67	0,02	1,70	0,46	886,76	9,34	94,90
50894	MFS permease	6,60	0,00	3,21	0,00	7094,06	63,08	112,47
78833	MFS permease (fucose permease)	4,08	0,00	4,73	0,00	7066,31	353,93	19,97
65191	MFS permease (maltose permease)	2,50	0,19	4,10	0,03	7755,79	842,18	9,21
69834	MFS permease,	2,82	0,09	3,91	0,01	29280,46	3139,78	9,33
79202	MFS permease, associated with cellulose signalling	10,47	0,00	10,67	0,00	58434,52	36,21	1613,76
69081	mitochondrial cytochrome b2, putative	2,73	0,00	4,12	0,00	2259,54	309,54	7,30
47930	Mitochondrial oxoglutarate/malate carrier proteins	2,03	0,00	4,30	0,00	24274,34	5634,78	4,31
69771	Monocarboxylate transporter	2,88	0,00	1,94	0,03	1720,08	318,88	5,39
106248	monosaccharide transporter (galactose permease ?)	3,32	0,00	0,12	0,80	2270,84	231,28	9,82
122374	MRSP1/expansin-like	4,13	0,00	4,52	0,00	17941,68	874,89	20,51
3267	MRSP1/expansin-like	3,09	0,00	3,30	0,00	2196,09	257,69	8,52
3405	MSF cellulose response transporter CTR1	10,94	0,00	10,26	0,00	721340,49	366,02	1970,77
107848	multicopper oxidase type 1, secreted	3,78	0,02	3,79	0,01	2395,17	134,33	17,83
4124	myb transcriptional regulator	3,58	0,00	1,11	0,00	108,48	9,07	11,96

67971	MYND-type Zn-finger protein	2,73	0,00	4,02	0,00	2584,23	306,03	8,44
119790	myo-inositol oxygenase ,	6,07	0,00	7,94	0,00	2594,51	31,54	82,27
111236	N-acetyltransferase of bacterial origin	1,81	0,00	-0,08	0,93	184,96	33,87	5,46
56499	N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D, putative	2,15	0,00	0,05	1,00	182,90	37,38	4,89
47432	NAD(P) transhydrogenase beta subunit	1,97	0,00	3,84	0,00	24006,15	4004,15	6,00
76204	NADH:flavin oxidoreductase/12-oxophytodienoate reductase	2,31	0,05	2,30	0,05	110,97	12,85	8,64
66766	Nitrilase	2,53	0,23	2,79	0,17	281,54	30,37	9,27
123786	NRPS	1,72	0,24	1,24	0,38	6907,05	1180,92	5,85
69946	NRPS, siderophore synthase	1,69	0,23	-0,08	0,99	5099,62	791,95	6,44
50323	OOC1	5,66	0,04	Inf	0,59	103,78	1,17	88,85
80980	peptidyl arginine deiminase	3,54	0,00	6,24	0,00	27145,25	2044,12	13,28
60988	phosphate transporter	4,22	0,03	3,66	0,05	1223,79	56,07	21,83
80003	Phosphoglycerate dehydrogenase and related dehydrogenases	2,52	0,00	4,97	0,00	14505,62	1683,19	8,62
71092	Phytase	4,25	0,00	1,69	0,24	17691,99	700,84	25,24
59482	PKS	2,24	0,12	2,91	0,03	5102,71	651,78	7,83
54260	potential indoleamine 2,3-dioxygenase	2,34	0,00	-3,49	0,00	303,12	42,05	7,21
61304	Prolidase and Aminopeptidase P	2,67	0,00	2,76	0,00	1038,83	160,03	6,49
122415	Protein disulfide isomerase 1	2,37	0,00	-0,61	0,37	84690,96	17827,10	4,75
106939	protein kinase	3,41	0,00	0,49	0,56	103,78	8,18	12,69
27992	PTH11 GPCR	3,26	0,00	1,43	0,08	1586,50	136,66	11,61
121308	PutA delta-1-pyrroline-5-carboxylate dehydrogenase	4,27	0,00	5,69	0,00	7096,11	324,72	21,85
111932	PutA delta-1-pyrroline-5-carboxylate dehydrogenase	4,09	0,00	4,87	0,00	612,41	33,87	18,08
80091	quinate permease	2,83	0,00	4,89	0,00	1203,24	123,82	9,72
69692	Quinoprotein amine dehydrogenase beta chain-like protein	6,67	0,03	3,39	0,25	465,47	1,17	398,49
4213	ribonuclease T2	4,14	0,00	2,98	0,00	5491,99	312,24	17,59
104393	SAM-dependent methyltransferase	2,25	0,00	3,09	0,00	291,82	67,75	4,31
6108	SAM-dependent methyltransferase	2,38	0,00	1,71	0,03	3395,98	537,31	6,32
106315	serine protease	3,58	0,00	3,55	0,00	799,42	38,55	20,74
78639	Serine/threonine protein kinase	2,12	0,00	1,61	0,00	8433,95	2032,44	4,15
65869	Shikimate kinase	2,58	0,00	5,08	0,00	1381,00	193,90	7,12

106164	short chain dehydrogenase/reductase	8,19	0,00	8,95	0,00	101,73	0,00	ND
70334	short chain dehydrogenase/reductase	2,54	0,00	4,73	0,00	802,50	140,17	5,73
60517	short chain dehydrogenase/reductase	3,71	0,00	3,14	0,00	913,47	74,76	12,22
67938	short chain dehydrogenase/reductase	2,43	0,03	4,01	0,00	229,14	43,22	5,30
30668	short chain dehydrogenase/reductase	2,61	0,00	2,79	0,00	1875,24	247,63	7,57
53331	short chain dehydrogenase/reductase	1,74	0,17	3,69	0,00	965,88	231,28	4,18
70520	short chain dehydrogenase/reductase	2,05	0,19	4,22	0,00	461,36	60,74	7,60
123079	short chain dehydrogenase/reductase	2,93	0,11	4,06	0,02	8931,28	866,71	10,30
65067	short chain dehydrogenase/reductase	1,84	0,22	1,29	0,56	144,88	21,03	6,89
2570	short chain dehydrogenase/reductase		NA	-0,25	1,00	0,00	0,00	ND
105970	short chain dehydrogenase/reductase	1,99	0,00	1,06	0,13	1483,75	283,84	5,23
54086	short chain dehydrognease/reductase	2,74	0,02	2,45	0,03	2736,31	293,19	9,33
80019	short-chain dehydrogenase/reductase	2,72	0,00	3,94	0,00	12161,83	2166,77	5,61
122556	Short-chain dehydrogenase/reductase	8,04	0,00	Inf	0,06	608,30	2,34	260,39
63568	SNF2-like helicase, ATPase domain	1,77	0,00	2,76	0,00	846,68	191,56	4,42
102908	SSCRP	2,99	0,00	1,04	0,00	2879,14	530,30	5,43
122422	SSCRP	2,87	0,00	2,39	0,00	5552,76	752,24	7,38
108663	SSCRP	9,85	0,00	6,41	0,00	6313,14	1,17	5404,75
120697	SSCRP	3,92	0,02	4,09	0,02	2597,59	94,61	27,45
123199	SSCRP	5,77	0,00	2,65	0,03	635,01	12,85	49,42
121739	SSCRP	2,56	0,09	1,86	0,21	3382,62	606,23	5,58
104227	SSCRP	5,18	0,02	0,93	0,76	132,55	5,84	22,70
111915	SSCRP	4,12	0,00	1,63	0,80	473,69	21,03	22,53
102851	SSCRP	2,47	0,22	-0,54	0,86	136,66	32,71	4,18
104050	SSCRP	5,41	0,13	0,21	1,00	481,91	14,02	34,38
123992	Swollenin	6,87	0,02	3,79	0,15	34626,69	203,24	170,37
2211	Transketolase	5,11	0,00	2,95	0,03	677,39	19,58	34,59
104322	unique protein	3,27	0,00	4,14	0,00	4055,66	484,75	8,37
108833	unique protein	2,58	0,00	1,83	0,00	4878,71	944,97	5,16
109562	unique protein	3,77	0,00	5,21	0,00	812,78	36,21	22,45

107076	unique protein	2,94	0,00	4,27	0,00	127,41	17,52	7,27
106340	unique protein	1,68	0,00	1,25	0,00	221,95	39,71	5,59
112520	unique protein	3,01	0,00	3,34	0,00	459,31	84,10	5,46
104867	unique protein	1,42	0,06	1,86	0,01	107,89	22,19	4,86
122007	unique protein	3,65	0,01	3,06	0,02	3612,79	505,77	7,14
42752	unique protein	1,96	0,01	1,76	0,02	517,87	110,97	4,67
108143	unique protein	3,91	0,00	1,98	0,05	552,81	52,56	10,52
120504	unique protein	6,20	0,00	2,48	0,07	16947,03	193,90	87,40
108340	unique protein	6,19	0,02	4,84	0,09	719,27	3,50	205,26
105515	unique protein	2,33	0,01	1,46	0,10	386,35	47,89	8,07
48266	unique protein	1,55	0,02	-0,88	0,18	387551,94	50063,53	7,74
123095	unique protein	3,52	0,09	2,55	0,21	308,26	57,24	5,39
109944	unique protein	5,17	0,00	-1,45	0,27	837,44	42,05	19,91
122629	unique protein	2,40	0,19	0,50	0,83	1071,71	117,98	9,08
111762	unique protein, 1TM	1,74	0,04	0,44	0,68	174,68	30,37	5,75
121883	unique secreted protein	5,01	0,00	3,09	0,01	108,92	0,00	ND
105860	unknown protein	2,61	0,00	1,72	0,00	9087,46	1233,48	7,37
22590	unknown protein	1,90	0,00	4,04	0,00	214,75	46,72	4,60
5614	unknown protein	1,93	0,00	3,10	0,00	1368,67	317,72	4,31
103064	unknown protein	2,57	0,00	3,48	0,00	626,79	82,93	7,56
78448	unknown protein	2,77	0,00	2,86	0,00	1037,80	147,18	7,05
122499	unknown protein	2,69	0,00	2,78	0,00	457,25	63,08	7,25
111990	unknown protein	1,92	0,00	3,86	0,00	540,48	114,47	4,72
122582	unknown protein	2,65	0,00	2,47	0,00	1447,79	242,96	5,96
120031	unknown protein	2,67	0,00	5,62	0,00	33959,82	5244,64	6,48
123261	unknown protein	2,41	0,00	2,65	0,00	2268,78	379,62	5,98
62285	unknown protein	1,97	0,00	2,98	0,00	674,06	124,98	5,39
5347	unknown protein	2,58	0,00	4,21	0,00	287,71	46,72	6,16
108674	unknown protein	2,04	0,00	2,15	0,00	541,51	122,65	4,42
74745	unknown protein	3,46	0,00	4,25	0,00	4347,47	434,52	10,01

69963	unknown protein	1,96	0,00	2,05	0,00	575,42	131,99	4,36
57383	unknown protein	1,71	0,00	1,96	0,00	200,37	49,06	4,08
103205	unknown protein	5,34	0,00	3,81	0,00	1226,87	23,36	52,52
109339	unknown protein	1,77	0,00	1,81	0,00	1104,59	275,66	4,01
106623	unknown protein	1,80	0,00	2,96	0,00	226,06	44,39	5,09
22284	unknown protein	4,28	0,00	4,85	0,00	65468,99	2378,19	27,53
122087	unknown protein	3,52	0,00	1,77	0,00	484,99	56,07	8,65
32716	unknown protein	1,81	0,01	3,91	0,00	144,88	31,54	4,59
111095	unknown protein	3,15	0,00	3,18	0,00	521,98	66,58	7,84
104174	unknown protein	1,90	0,00	2,15	0,00	784,00	171,71	4,57
102966	unknown protein	6,41	0,00	6,44	0,00	17494,70	395,98	44,18
69375	unknown protein	1,95	0,02	3,41	0,00	631,93	106,29	5,95
64656	unknown protein	2,23	0,00	2,68	0,00	147,96	30,37	4,87
69316	unknown protein	2,03	0,01	3,18	0,00	480,88	78,26	6,14
103455	unknown protein	1,75	0,00	1,23	0,00	497,32	115,64	4,30
120176	unknown protein	2,77	0,01	-3,96	0,00	164,40	36,21	4,54
111778	unknown protein	2,25	0,00	2,20	0,00	837,44	209,08	4,01
65735	unknown protein	2,29	0,00	2,73	0,00	11424,06	1500,97	7,61
56211	unknown protein	2,32	0,00	1,02	0,00	584,66	113,30	5,16
110220	unknown protein	2,89	0,00	2,45	0,00	314,42	30,37	10,35
105330	unknown protein	2,49	0,00	3,06	0,00	146,94	11,68	12,58
79726	unknown protein	3,58	0,00	2,94	0,00	131,52	10,51	12,51
75380	unknown protein	1,94	0,17	Inf	0,00	102,75	25,70	4,00
40830	unknown protein	2,16	0,02	2,75	0,00	1006,98	169,37	5,95
71123	unknown protein	2,76	0,01	3,04	0,00	366,83	26,87	13,65
66865	unknown protein	1,73	0,00	1,64	0,00	1001,84	228,94	4,38
60482	unknown protein	1,70	0,08	2,72	0,00	1333,73	329,40	4,05
75450	unknown protein	2,03	0,00	1,25	0,00	5733,61	999,87	5,73
59801	unknown protein	2,70	0,00	2,40	0,00	856,96	85,27	10,05
108586	unknown protein	4,07	0,02	Inf	0,00	971,01	52,56	18,47

107775	unknown protein	2,48	0,00	2,07	0,01	692,55	89,94	7,70
72581	unknown protein	2,34	0,01	2,54	0,01	596,99	124,98	4,78
109317	unknown protein	2,11	0,00	1,92	0,01	3163,76	538,48	5,88
124007	unknown protein	2,64	0,00	2,16	0,01	302,09	19,86	15,21
106556	unknown protein	3,61	0,00	0,76	0,01	694,61	43,22	16,07
76075	unknown protein	2,74	0,00	2,34	0,01	2499,98	365,61	6,84
4952	unknown protein	2,72	0,00	2,25	0,02	223,12	33,92	6,58
30084	unknown protein	2,80	0,00	2,08	0,04	154,13	25,70	6,00
52476	unknown protein	2,95	0,03	2,58	0,07	1614,25	140,17	11,52
56840	unknown protein	7,08	0,03	5,40	0,08	10624,65	70,08	151,60
44640	unknown protein	2,87	0,00	1,66	0,09	16047,94	1800,00	8,92
35202	unknown protein	4,00	0,00	2,12	0,09	248,66	11,68	21,29
104295	unknown protein	6,99	0,01	3,97	0,10	1564,93	7,01	223,29
104333	unknown protein	1,99	0,00	0,83	0,14	462,39	100,45	4,60
111758	unknown protein	4,44	0,00	1,99	0,16	1365,59	43,22	31,60
102904	unknown protein	3,27	0,02	1,87	0,20	1646,10	156,52	10,52
69777	unknown protein	2,10	0,02	1,10	0,23	130,50	30,37	4,30
54226	unknown protein	3,28	0,01	-1,50	0,28	216,81	16,35	13,26
104422	unknown protein	Inf	0,02	Inf	0,50	139,74	0,00	ND
104556	unknown protein	4,34	0,00	-0,92	0,66	215,78	11,68	18,47
111875	unknown protein	2,02	0,01	-0,15	0,90	6143,59	1095,65	5,61
123911	unknown protein	2,42	0,00	-0,10	0,91	219,89	36,21	6,07
23228	unknown protein Duf1479	3,54	0,05	5,53	0,00	17570,74	1080,47	16,26
70639	unknown protein with WD repeats	1,60	0,00	2,97	0,00	213,73	45,55	4,69
112387	unknown protein with WD repeats	2,18	0,03	0,98	0,39	246,61	45,55	5,41
3363	unknown protein, 9 TM	3,11	0,36	1,83	0,59	1444,83	167,81	8,61
120837	unknown protein, 1 TM domain	2,80	0,02	2,09	0,08	1094,32	249,97	4,38
56546	unknown protein, 3TM	3,14	0,00	4,95	0,00	848,74	45,55	18,63
122579	unknown protein, 4TM	4,26	0,00	1,82	0,47	556,92	23,36	23,84
123120	unknown protein, 8 TM	2,02	0,00	3,00	0,00	3066,15	754,57	4,06

59368	unknown protein, Duf341	4,89	0,06	4,59	0,08	5340,07	134,33	39,75
5849	unknown protein, Duf967	2,71	0,00	4,07	0,00	2587,32	431,02	6,00
106043	unknown protein, GFA-domain	3,03	0,00	2,24	0,00	2851,39	505,77	5,64
106129	unknown protein, only present in ascomycota	2,13	0,00	0,42	0,34	127,41	21,03	6,06
119576	unknown protein, only present in ascomycota and Streptomyces	2,22	0,00	4,19	0,00	4102,92	683,32	6,00
111887	unknown protein, only present in fungi	3,00	0,00	5,64	0,00	199,34	17,52	11,38
55887	unknown protein, secreted	7,32	0,00	3,75	0,00	7422,87	53,73	138,15
111138	unknown protein, secreted	2,13	0,12	3,04	0,03	192,15	36,21	5,31
70991	unknown protein, ThiJ/PfpI domain	3,47	0,01	2,06	0,18	202,42	9,34	21,66
68997	unknown TPR domain protein, unknown	2,63	0,00	2,27	0,01	203,45	38,55	5,28
66103	WbbJ Acetyltransferase (isoleucine patch superfamily)	1,79	0,00	2,06	0,00	2124,93	502,27	4,23
107776	xylose reductase	3,43	0,00	1,88	0,00	18493,46	1367,81	13,52
104072	xylose transporter	6,37	0,00	3,43	0,00	1319,35	9,34	141,19
120357	Zinc-binding oxidoreductase	2,03	0,07	6,42	0,00	15373,88	2651,52	5,80
65854	Zn2Cys6 transcriptional regulator	2,53	0,00	5,74	0,00	959,71	144,84	6,63
70351	Zn2Cys6 transcriptional regulator	2,82	0,00	3,42	0,00	2370,51	296,69	7,99
105269	Zn2Cys6 transcriptional regulator	2,49	0,00	3,90	0,00	343,19	52,56	6,53
112134	Zn2Cys6 transcriptional regulator	3,22	0,00	1,80	0,00	939,16	74,76	12,56
102499	Zn2Cys6 transcriptional regulator	2,17	0,18	2,35	0,11	213,73	30,37	7,04
121121	Zn2Cys6 transcriptional regulator	3,17	0,00	1,29	0,16	1977,99	157,69	12,54
121415	Zn2Cys6 transcriptional regulator	6,81	0,00	1,51	0,43	7826,69	66,58	117,55
122208	Zn2Cys6 transcriptional regulator XYR1	3,87	0,00	3,17	0,00	70560,39	4012,32	17,59
77513	Zn2Cys6 transcriptional regulator ACE3	2,98	0,00	2,82	0,00	21363,35	2402,72	8,89
55105	Zn2Cys6 transcriptional regulator AmyR, amylase regulator	1,94	0,00	2,73	0,00	583,64	119,14	4,90
26163	Zn2Cys6 transcriptional regulator CLR-2	6,24	0,00	6,60	0,00	1210,43	8,18	148,04

Supplementary Table 4: Genes whose expression in the presence of 1.8 mM sophorose decreased >5-fold in *T. reesei Δkap8* compared to the *Δkap8* retransformant

Trire2:	annotation	Soph		glyc		RPKM		reduction
		log2FC	p-value	log2FC	p-value	*Δkap8*	*Δkap8*RT	[-fold]
123989	GH7 Cellobiohydrolase CBH1/CEL7a	-1,4227	0,654704022	13,7129	0,000793722	985	1255793	1274,734
103041	Acetamidase	2,53045	0,138853728	4,25025	0,010330684	81	630	7,799276
77093	acid sphingomyelin phosphodiesterase (probably vacuolar)	2,34988	1,45506E-12	2,86678	3,65769E-24	120	870	7,225853
54667	acyl-CoA synthetase	7,42957	3,92395E-10	3,00243	0,002437445	127	4107	32,41265
66689	Adenine deaminase/adenosine deaminase	6,49384	2,91638E-08	1,42356	0,074205882	18	125	6,925295
2038	Alcohol dehydrogenase, class IV	1,16089	0,273291102	3,31128	0,000651618	47	257	5,417361
68590	Alcohol dehydrogenase, class V	3,50721	0,000957252	2,45739	0,021232969	159	899	5,664023
80659	alcohol oxidase AOX1	-0,8478	0,862919703	7,09731	0,090410054	12	517	43,66876
59843	AMP-dependent synthetase and ligase, acetoacetyl-CoA synthase-like	2,71745	0,215014313	3,06681	0,194250376	42	166	3,919557
63653	Ankyrin	5,17819	0,014684235	4,9726	0,009985238	24	706	28,96947
70907	Ankyrin	0,86661	0,146726066	2,78449	2,18828E-09	16	124	7,764426
82619	arginosuccinate synthetase	10,3234	2,71107E-09	1,67989	0,257319496	0	12857	#DIV/0!
104073	aspartate racemase	1,69542	0,053436519	2,81552	0,000789816	34	326	9,548056
109538	BZIP transcriptional regulator	4,89477	2,59648E-06	3,2482	5,40481E-06	3	187	67,15266
108357	C2H2 transcriptional regulator	2,52939	2,91384E-06	3,38687	1,43932E-09	3612	30388	8,413213
66766	Carbon-nitrogen hydrolase	2,70783	0,157279497	2,53065	0,225000106	36	282	7,929251
22459	carboxypeptidase A	0,28006	0,441400936	4,56567	1,81128E-62	107	1737	16,19637
65215	CE4 imidase	4,58398	2,79005E-08	2,9119	0,000229943	201	3641	18,15643
73632	CE5 acetyl xylan esterase AXE1	-Inf	0,470784611	Inf	0,000410429	7	2846	408,8195
104277	cell wall protein, distantly related to A. niger CwpA.	5,21012	7,68396E-14	2,75409	1,59178E-05	572	4230	7,391981
60374	choline oxidase	2,31652	4,30056E-06	3,43274	3,5441E-21	55	323	5,866166
73638	CIP1	-3,6191	0,306538176	16,2667	0,000198276	109	83483	768,651
69648	Cytochrome P450 CYP11/CYP12/CYP24/CYP27 subfamilies	-0,3007	0,935691941	4,07433	0,025777707	12	129	10,93889
70842	Cytochrome P450 CYP2 subfamily	3,9213	0,011385944	4,74381	7,84497E-05	2	391	187,4371
65141	cytochrome P450 monooxygenase	4,72673	0,000342088	3,34498	0,004026536	19	545	27,93635

4999	cytochrome P450 monooxygenase	6,36455	0,007429257	3,60378	0,090922373	27	732	27,38949
57555	cytochrome P450 monooxygenase	3,77842	0,005710292	2,55907	0,034096374	19	164	8,433614
59338	D-Alanine aminotransferase	3,26981	1,91823E-12	2,1989	6,05528E-09	54	302	5,562942
61293	dipeptidyl peptidase 5	3,87411	2,23151E-07	2,01164	0,001543703	75	228	3,033759
123955	Epl1/Sm1	4,83828	0,052665988	3,64603	0,12106907	7	334	47,96618
106120	esterase/lipase	3,63402	2,81973E-29	3,53192	3,99605E-34	290	3290	11,3328
65677	Esterase/lipase/thioesterase	0,81756	0,308568929	2,17372	0,007277701	113	670	5,939972
110666	ferric reductase	-Inf	1	Inf	0,00028806	21	318	15,20159
76696	Flavin-containing monooxygenase	3,97856	7,02355E-06	3,53862	8,51628E-05	2465	13063	5,300253
23083	Flavoprotein monooxygenase	0,94064	0,680276149	4,59783	0,043328859	1559	7850	5,036062
103537	GCN5-N-acetyltransferase	-0,6759	0,592385931	3,10695	0,005429163	26	186	7,219858
69868	GDP-mannose a-mannosyltransferases;Distant relative	1,3245	0,467902797	2,95526	0,109014634	31	428	13,67651
57179	GH105/GH88 glycosyl hydrolase	Inf	1	8,6726	0,000988072	4	214	51,16393
74223	GH11 endo-ß-1,4-xylanase XYN1	Inf	0,277413138	7,15314	0,000578942	9	314	34,74
112392	GH11 endo-ß-1,4-xylanase XYN5	Inf	1	9,21617	0,00393478	4	290	69,36648
123232	GH12 endo-ß-1,4-glucanase	NA	NA	Inf	0,037349852	1	181	129,8777
65162	GH18 endo-N-acetyl-ß-D-glucosaminidase Endo T	2,51246	0,034442003	5,09226	8,30763E-05	100	2196	21,90251
5836	GH2 β-mannosidase	5,11146	0,004780266	5,06894	0,00416825	72	3193	44,09199
102909	GH2 protein	0,01157	1	2,20702	1,71319E-11	54	295	5,501017
55999	GH27 α-galactosidase	1,37396	0,396619075	4,97125	0,003147257	400	2260	5,654121
72632	GH27 α-galactosidase AGL1	3,34844	0,076659782	3,43623	0,041416964	15	160	10,9637
72704	GH27 α-galactosidase AGL3	3,35679	0,057012195	3,66826	0,052169187	193	4166	21,6001
112140	GH28 exo-polygalacturonase PGX1	-Inf	1	Inf	0,000816452	7	707	101,5407
122780	GH28 exo-rhamnogalacturonase RGX1	6,12716	0,000439673	4,08567	0,017833788	669	50746	75,84593
108671	GH3 β-glucosidase/glucan 1,4-β-glucosidase BGL3f	Inf	1	8,21182	0,001865261	8	167	21,8699
76672	GH3 β-glucosidase BGL1/CEL3a	NA	NA	Inf	0,013195586	3	133	47,59721
46816	GH3 β-glucosidase CEL3d	1,23656	0,450879981	4,13005	0,012288909	147	1865	12,69539
121127	GH3 β-xylosidase BXL1	0,82311	0,752888743	10,1996	8,66437E-05	272	23568	86,80081
110894	GH30 endo-β-1 6-galactanase	-0,3142	0,842107395	3,51981	0,001200109	104	534	5,116393
69276	GH30 endo-β-1,4-xylanase	Inf	1	11,4769	0,006480315	2	1762	843,7128

111849	GH30 endo-β-1,4-xylanase XYN4	0,71054	0,802206688	8,12173	0,002797447	143	8684	60,84236
69944	GH31 α-xylosidase/α-glucosidase	3,05484	0,376012893	6,26118	0,01968778	24	415	17,53696
60085	GH31 α-glucosidase	1,87023	0,005100991	3,70875	4,52149E-11	44	427	9,74551
79960	GH47 α-1,2-mannosidase	4,19926	3,12786E-06	5,01229	2,58703E-08	272	5770	21,24892
120312	GH5 endo-β-1,4-glucanase EGL2/CEL5a	-Inf	0,232997791	Inf	0,00070162	10	29758	3053,074
49976	GH5 endo-β-1,4-glucanase EGL5/CEL45a	1,70394	0,57479853	9,75103	0,005181157	159	25112	158,1978
56996	GH5 β-Mannanase MAN1	-0,0986	1	8,62692	0,024938968	1	226	324,6942
55319	GH54 α-L-arabinofuranosidase ABF3	7,46436	1,86418E-16	3,38175	1,2153E-05	118	10593	89,49925
70845	GH55 β-1,3-glucanase	2,33746	1,63302E-12	2,86525	1,90256E-19	237	2030	8,552328
72567	GH6 Cellobiohydrolase CEL6A/CBH2	-1,3289	0,686730107	13,6239	0,000824627	245	372238	1518,922
73643	GH61 polysaccharide monooxygenase CEL61a	-2,6842	0,394062935	13,9148	0,000575411	549	76189	138,8753
76210	GH62 α-L-arabinofuranosidase ABF2	NA	NA	Inf	0,023707429	0	115	#DIV/0!
72526	GH67 α-Glucuronidase GLR1	2,40798	0,18309676	6,82236	0,001039746	338	7794	23,08159
122081	GH7 Endo-β-1,4-glucanase EGL1/CEL7b	-3,6508	0,247538025	15,9517	0,000207489	143	180819	1266,919
49081	GH74 Xyloglucanase CEL74a	-0,3901	0,893328128	4,33681	0,067281975	395	8089	20,49056
74198	GH92 α-1,2-mannosidase	1,5302	0,464652999	3,61246	0,104568745	45	897	20,13196
79921	GH92 α-1,2-mannosidase	2,30473	0,000374467	2,87366	1,61637E-05	503	3200	6,356706
58802	GH95 α-L-fucosidase	2,39195	0,107984717	4,31731	0,00333378	38	494	13,14629
5807	GH95 α-L-fucosidase	2,07648	0,141457533	3,59996	0,00842978	33	320	9,765946
123978	GMC methanol oxidase	6,31764	0,236231694	4,12323	0,46231616	17	375	21,54789
37525	GPCR , contains RGS domain	5,80709	8,85324E-65	1,96933	1,29187E-15	97	1774	18,32643
80340	GT α-1,6-mannosyltransferase	3,07098	0,004714538	2,58613	0,023707429	438	2291	5,232461
106171	HET protein	0,27271	0,304801333	4,05992	1,39643E-62	1432	7249	5,061911
41699	HET protein	0,71992	0,266989594	2,56098	1,68652E-05	81	382	4,733003
119989	HFB2	7,96967	1,94169E-50	2,5428	6,77058E-09	24007	559639	23,31168
123967	HFB3	9,16131	2,4562E-99	2,87665	3,05889E-18	7345	156672	21,33035
106538	HFB4	2,05196	0,347898532	4,03069	0,047917088	6	134	23,98309
71094	Intradiol ring-cleavage dioxygenase	1,98952	0,172513165	3,0313	0,038882462	6	104	18,63302
121418	lipase G-D-S-L	2,9122	0,291060101	9,14552	0,002472971	153	12935	84,44732
66662	Mandelate racemase/muconate lactonizing protein	-0,3606	0,856936212	4,64169	0,000477273	42	224	5,274465

3049	methionine aminopeptidase	4,18115	2,73999E-55	2,06941	1,28098E-18	84	3603	42,96027
46819	MFS hexose transporter	0,61679	0,628570434	3,71064	0,000925289	88	378	4,276573
121482	MFS permease	3,51205	4,01864E-38	4,26977	1,01664E-64	266	8399	31,5808
56684	MFS permease	0,1062	0,99820451	5,6725	0,016906611	34	887	25,9936
67541	MFS permease	Inf	0,538107462	7,46397	0,004458723	13	286	21,59449
69651	MFS permease	2,43687	1,82695E-05	2,17464	1,45889E-06	12	169	14,23793
54632	MFS permease	1,81947	0,12369925	7,96527	9,71591E-09	1949	25107	12,88378
111888	MFS permease	5,82439	4,27372E-37	2,53224	1,17177E-19	81	857	10,61109
60329	MFS permease	2,2806	0,030290451	4,30586	4,96103E-06	46	474	10,30882
80058	MFS permease	2,0228	0,255433221	2,46819	0,184814738	33	267	7,994364
70108	MFS permease	2,57245	0,022225172	2,34293	0,022340354	18	107	5,90353
69611	MFS permease	3,4262	7,8486E-05	2,43498	0,005675668	253	1448	5,728701
105260	MFS permease	1,30854	0,002165483	2,92028	6,08528E-17	81	306	3,759085
50894	MFS permease	0,76863	0,010039276	6,59649	1,3054E-122	377	7094	18,7998
65191	MFS permease (maltose permease)	5,44572	0,003467589	2,50369	0,192850417	299	7756	25,96728
69834	MFS permease,	4,56443	0,004050968	2,8152	0,088256025	1536	29280	19,06471
69771	Monocarboxylate transporter	3,5099	4,16531E-05	2,87567	0,00085049	100	1720	17,15713
106248	monosaccharide transporter (galactose permease ?)	0,58262	0,053508254	3,3189	3,39272E-32	127	2271	17,92143
122374	MRSP1/expansin-like	5,84196	1,59822E-87	4,13496	1,03506E-66	332	17942	54,02596
107848	multicopper oxidase type 1, secreted	2,3206	0,112391209	3,7821	0,017415345	421	2395	5,695831
111236	N-acetyltransferase of bacterial origin	-0,1739	0,791708981	1,81336	2,65402E-06	43	185	4,28482
56499	N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D, putative	2,35944	3,03288E-06	2,14653	1,64865E-07	10	183	17,51381
76204	NADH:flavin oxidoreductase/12-oxophytodienoate reductase	1,8357	0,128513839	2,31489	0,047385427	26	111	4,194614
123786	NRPS	3,22289	0,012134068	1,7176	0,235086671	346	6907	19,96153
69946	NRPS, siderophore synthase	0,81808	0,548609723	1,69019	0,230726856	490	5100	10,40455
50323	OOC1	Inf	0,315819719	5,66143	0,043593869	1	104	149,0641
80980	peptidyl arginine deiminase	7,91756	1,86157E-15	3,53878	3,01355E-05	521	27145	52,05589
60988	phosphate transporter	3,20564	0,078804699	4,21954	0,025498593	83	1224	14,77123
71092	Phytase	0,93454	0,518171247	4,24567	0,00346452	1236	17692	14,31648
59482	PKS	4,02585	0,002919741	2,23555	0,121131296	292	5103	17,45055

61304	Prolidase and Aminopeptidase P	2,38466	7,48581E-07	2,66788	7,77034E-09	187	1039	5,546904
122415	Protein disulfide isomerase 1	-0,4517	0,500388071	2,36849	0,000120964	15309	84691	5,532093
106939	protein kinase	-1,2621	0,116134718	3,40835	3,43797E-06	26	104	4,02876
27992	PTH11 GPCR	1,80772	0,021697339	3,26328	6,42419E-05	118	1587	13,4838
121308	PutA delta-1-pyrroline-5-carboxylate dehydrogenase	5,84417	2,88777E-25	4,26949	9,99069E-20	242	7096	29,37304
69692	Quinoprotein amine dehydrogenase beta chain-like protein	0,17506	1	6,66952	0,030730189	19	465	23,87767
106315	serine protease	2,72117	0,009993979	3,58393	0,000586589	86	799	9,259973
78639	Serine/threonine protein kinase	1,91856	9,48381E-06	2,12263	2,14043E-06	1577	8434	5,348364
65067	short chain dehydrogenase/reductase	3,89443	0,011041608	1,83932	0,221796438	4	145	34,68324
123079	short chain dehydrogenase/reductase	5,40028	0,002323353	2,92656	0,10546813	340	8931	26,28765
53331	short chain dehydrogenase/reductase	4,08252	0,000674592	1,74436	0,170471093	164	966	5,90353
67938	short chain dehydrogenase/reductase	3,00289	0,00983781	2,42813	0,026824434	72	229	3,195357
122556	Short-chain dehydrogenase/reductase	Inf	0,279458684	8,03837	0,000162401	3	608	174,7445
80019	short-chain dehydrogenase/reductase	5,89786	1,05506E-22	2,71901	4,27067E-07	525	12162	23,16783
111915	SSCRP	6,09267	0,000246678	4,12121	0,000963482	0	474	#DIV/0!
104050	SSCRP	2,11998	0,550359644	5,41403	0,130592425	1	482	692,1889
108663	SSCRP	0,95435	0,698799929	9,85304	8,31621E-06	168	6313	37,62582
123199	SSCRP	1,77643	0,157339034	5,76938	4,76124E-07	18	635	35,08059
104227	SSCRP	0,91376	0,783074316	5,18078	0,01874066	4	133	31,73147
122422	SSCRP	4,02114	1,5251E-08	2,86552	3,87051E-05	200	5553	27,78979
120697	SSCRP	4,81264	0,004673617	3,92025	0,022094808	96	2598	27,03646
121739	SSCRP	3,6293	0,010380726	2,56264	0,086539584	169	3383	19,99426
102851	SSCRP	1,30297	0,524962792	2,47163	0,224453515	8	137	17,84476
102908	SSCRP	1,97226	2,89602E-15	2,98614	1,9264E-35	162	2879	17,8251
123992	Swollenin	2,08372	0,412644948	6,86906	0,018595944	595	34627	58,17049
2211	Transketolase	0,24037	0,921469287	5,11243	0,000349165	128	677	5,301621
109944	unique protein	0,97286	0,457689228	5,16836	2,76E-05	3	837	300,7111
108340	unique protein	2,95608	0,33127712	6,18852	0,024593083	24	719	29,51765
120504	unique protein	0,35865	0,839503269	6,19945	4,07138E-05	735	16947	23,05088
108143	unique protein	2,66325	0,005990715	3,91467	4,33033E-05	33	553	16,89414

122629	unique protein	1,20848	0,490761719	2,39581	0,192850417	66	1072	16,20364
112520	unique protein	3,24487	0,002600898	3,0057	0,003601912	56	459	8,144685
107076	unique protein	3,92711	7,12984E-06	2,93757	5,70566E-08	17	127	7,320377
104322	unique protein	3,1748	2,89233E-24	3,26884	1,64888E-28	574	4056	7,069549
105515	unique protein	1,36008	0,103454446	2,33204	0,005617243	60	386	6,452696
123095	unique protein	1,82707	0,357685649	3,51818	0,091460504	72	308	4,257353
111762	unique protein, 1TM	0,82619	0,322721114	1,739	0,041077362	33	175	5,227084
121883	unique secreted protein	1,98498	0,243574547	5,00713	1,79567E-11	3	109	31,28871
105860	unknown protein	1,7159	0,000359683	2,61036	1,62263E-07	1333	9087	6,816034
104422	unknown protein	NA	NA	Inf	0,020738965	1	140	200,72
108586	unknown protein	Inf	0,000555283	4,06776	0,022677257	13	971	77,48383
52476	unknown protein	5,49676	0,000110802	2,95399	0,026911101	23	1614	70,26095
56840	unknown protein	3,65328	0,209908832	7,07896	0,034305142	208	10625	51,03888
104556	unknown protein	0,63427	0,677555891	4,34381	0,001277192	6	216	34,43726
103205	unknown protein	3,20494	3,96466E-06	5,33518	4,15294E-20	38	1227	32,04007
104295	unknown protein	0,63851	0,864182234	6,99475	0,008484224	82	1565	19,04889
102904	unknown protein	2,27032	0,099630998	3,27327	0,02204827	93	1646	17,64451
35202	unknown protein	1,1592	0,376012893	4,00122	0,00063404	17	249	14,88182
111875	unknown protein	1,06906	0,13794877	2,0167	0,006531305	453	6144	13,55499
54226	unknown protein	-1,642	0,19216321	3,28198	0,01068151	22	217	10,04552
79726	unknown protein	2,68079	0,005264792	3,58365	5,83661E-08	13	132	9,942788
120031	unknown protein	5,73149	1,35754E-15	2,67307	5,86501E-05	3807	33960	8,920614
106623	unknown protein	3,62883	3,568E-12	1,80282	4,04581E-06	30	226	7,551027
111778	unknown protein	3,1079	1,42409E-07	2,24663	9,98787E-05	113	837	7,424965
111095	unknown protein	3,13699	3,3785E-07	3,15229	1,30768E-08	72	522	7,209119
44640	unknown protein	1,02484	0,287665582	2,87202	0,003251767	2349	16048	6,831753
5347	unknown protein	4,2179	1,60478E-12	2,58167	3,9656E-10	43	288	6,665276
69963	unknown protein	2,19374	7,00503E-12	1,96068	8,37296E-12	97	575	5,946002
122087	unknown protein	0,75006	0,046069634	3,51922	6,66403E-30	86	485	5,663549
74745	unknown protein	3,31361	7,43168E-08	3,46072	2,30257E-08	774	4347	5,615521

59801	unknown protein	1,61311	0,052862547	2,70093	0,001503038	159	857	5,375048
111758	unknown protein	-0,7035	0,655868045	4,44115	0,002371175	260	1366	5,258573
69777	unknown protein	1,05133	0,245179944	2,10427	0,016669515	26	130	5,065867
105330	unknown protein	2,51542	0,007328719	2,49192	0,003450977	33	147	4,3969
30084	unknown protein	1,23375	0,234747122	2,8034	0,004162986	43	154	3,570684
64656	unknown protein	1,75079	0,008245245	2,22566	0,000391971	54	148	2,724706
23228	unknown protein Duf1479	3,93195	0,022557689	3,54467	0,054159767	3363	17571	5,225174
112387	unknown protein with WD repeats	0,00319	1	2,18225	0,03224879	73	247	3,373446
56546	unknown protein, 3TM	4,38321	5,85006E-06	3,13738	0,000449529	102	849	8,293054
122579	unknown protein, 4TM	5,37195	0,000962131	4,26355	0,00157294	3	557	199,9821
59368	unknown protein, Duf341	5,26306	0,038289151	4,88525	0,064315366	69	5340	77,47638
106043	unknown protein, GFA-domain	4,63089	5,74446E-10	3,03	1,72569E-05	79	2851	36,24402
111887	unknown protein, only present in fungi	5,73239	4,59257E-08	2,99653	1,8516E-07	18	199	11,01235
55887	unknown protein, secreted	2,24026	5,63196E-06	7,32433	2,53862E-56	123	7423	60,23602
111138	unknown protein, secreted	3,08338	0,020954308	2,12964	0,117526133	24	192	7,88543
70991	unknown protein, ThiJ/PfpI domain	2,43319	0,079985653	3,46606	0,007725281	10	202	20,76778
68997	unknown TPR domain protein, unknown	1,5782	0,072774546	2,63251	0,002646961	65	203	3,142202
104072	xylose transporter	1,46624	0,257351708	6,37282	7,08007E-07	47	1319	27,86813
121415	Zn2Cys6 transcriptional regulator	1,96309	0,276031049	6,81328	0,000685566	28	7827	281,0449
105269	Zn2Cys6 transcriptional regulator	3,41645	0,000159613	2,49371	0,003267332	54	343	6,319805

Curriculum Vitae

Mag. rer. nat. Sara Ghassemi

Dresdner Strasse 42/41
1200 Vienna, Austria
Tel.: +43-69916033660
E-Mail: sara_ghassemi@yahoo.com

PERSONAL INFORMATION

Date of birth: 18th Mai 1983
Place of birth: Tehran, Iran
Citizenship: Iranian (Permanent residency)

ACADEMIC CAREER

Since 10/2011	Ph.D. studies in Molecular Biotechnology Institute of Chemical Engineering , Research Division Biotechnology and Microbiology, Vienna University of Technology
10/2003 – 07/2011	Diploma studies in Genetics and Molecular Pathology Institute of Molecular Biology University of Vienna

ACADEMIC CAREER DETAILS

POSTGRADUATE STUDIES

09/2011 – 09/2014	PhD Student and research assistant, Institute of Chemical Engineering, Vienna University of Technology PhD project: Nuclear transport and cellulase production in *Trichoderma reesei* Supervisor: Prof. Christian P. Kubicek, Dr. Bernhard Seiboth

UNDERGRADUATE STUDIES

10/2003 – 07/2011	Molecular Biology Student at the University of Vienna

02/2010 – 07/2011 Diploma thesis at cancer research institute of medical university of Vienna
Diploma thesis title: Over-expression of fibroblast growth factor 5 (FGF5) in melanoma
Supervisor: Assoc. Prof. Dr. Michael Grusch

PUBLICATIONS

Mol Microbiol. 2015 Jan 27. doi: 10.1111/mmi.12944.
The ß-importin KAP8 (Pse1/Kap121) is required for nuclear import of the cellulase transcriptional regulator XYR1, asexual sporulation and stress resistance in *Trichoderma reesei*
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Giessrigl B, Yazici G, Teichmann M, Kopf S, Ghassemi S, Atanasov A, Dirsch V, Grusch M, Jäger W, Ozmen A, Krupitza G.

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Fibroblast growth factor receptors as therapeutic targets in human melanoma: synergism with BRAF inhibition.
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POSTER

Ghassemi S., Lichius A., Kubicek C. P., Seidl-Seiboth V. Nuclear transport and cellulase production in *Trichoderma reesei* poster at 4th ÖGMBT Annual Meeting Graz, 17. - 19. September 2012

Ghassemi S., Lichius A., Seiboth B., Seidl-Seiboth V., Kubicek C.P.
Nuclear import and cellulase production in Trichoderma reesei Poster at the
12th EUROPEAN CONFERENCE ON FUNGAL GENETICS Seville, March 23-27, 2014

Ghassemi S., Lichius A., Seiboth B., Kubicek C.P.
Nuclear import and cellulase production in Trichoderma reesei Poster at the 6 th ÖGMBT Annual Meeting Vienna, 15.-18. September 2014

LANGUAGES

Persian: Native speaker
German: Fluently spoken and written
English: Fluently spoken and written