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Genomics of protein folding in the endoplasmic reticulum, secretion stress and glycosylation in the aspergilli

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ABSTRACT

We review aspects of the process of protein secretion by *Aspergillus niger* and *Aspergillus nidulans*, based on analysis of their genome sequences. Where useful, we have taken a comparative approach with the yeasts *Saccharomyces cerevisiae* and *Pichia pastoris*. The four fungi discussed in the review include two species which comprise a well-developed model yeast (*S. cerevisiae*) and filamentous fungus (*A. nidulans*) and two species which are exploited for their capabilities in the secretion of proteins (*P. pastoris* and *A. niger*). It has not been possible to cover all aspects of protein secretion and we have therefore restricted discussion to events which are largely, though not exclusively, located within the lumen of the endoplasmic reticulum.

1. Introduction

The secretion of proteins is an essential component of the lifestyles of most microorganisms and the saprophytic filamentous fungi have become adapted for the secretion of enzymes that degrade polymeric organic matter. In addition, the secretory process affords the ability to extend hyphae and elaborate new cell membrane and wall components. The secretion of proteins by filamentous fungi supports their lifestyle and, in many cases, is a component of their pathogenic mechanisms. Several fungal species are exploited commercially for their native enzymes and, increasingly, as hosts for secreted production of heterologous proteins. *Aspergillus niger* and *Aspergillus. oryzae* are important industrial fungal species for enzyme production and both have published genome sequences (Pel et al., 2007; Machida et al., 2005) which provide an important opportunity to investigate the genetic basis of protein secretion. In addition, an increasing number of other aspergilli, including different strains of a given species (e.g. *A. niger*), as well as other species of filamentous fungi, have sequenced genomes. Thus, comparative genomics analysis can be undertaken at various levels and for a range of purposes. It is our objective here to focus on the genomics of two key aspects of protein secretion in *A. niger* and to relate that to *Aspergillus nidulans* (Galagan et al., 2005) where appropriate and to *Saccharomyces cerevisiae* which has served as the model fungus for many cellular functions including secretion. Comparison with the yeast *Pichia pastoris* is also mentioned in some instances, making use of the proprietory genome sequence (Integrated Genomics Inc.). *P. pastoris* is a yeast that is often used as a cell factory for protein expression and genomic-based analyses with this organism are increasing (Gasser et al., 2007). It is not possible to cover all aspects of protein secretion so we focus on core elements of endoplasmic reticulum (ER)[1] lumenal functionality: protein folding, protein glycosylation and quality control. ER functions do not occur in isolation so we extend beyond the ER where relevant and include the Golgi where relevant to glycosylation. Note that the accession numbers of genes referred to in the text are listed in Table 1.

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[1] *Abbreviations used:* Dol, dolichol; Dol-P, dolichol phosphate; Dol-PP, dolichol pyrophosphate; ER, endoplasmic reticulum; ERAD, ER-associated degradation; Gal, galactose; Gal*f*, galactofuranose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Man, mannose; NMR, nuclear magnetic resonance; ORF, open reading frame; OST, oligosaccharyl transferase; PDI, protein disulphide isomerase; SPR, surface plasmon resonance; UGGT; UDPGlc:glycoprotein glucosyltransferase; UPR, unfolded protein response.

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Table 1
Summary of the genes involved in protein-folding, UPR and glycosylation

Protein name/description	*S. cerevisiae* gene locus	*A. niger* ORF number	*A. nidulans* ORF number	Cloned *Aspergillus* orthologue
Synthesis and transport of sugar nucleotide donors				
Glucose (hexose) kinase	*GLK1*/YCL040W	An12g08610	AN8689.3	*glkA* Panneman et al. (1996)
	HXK1/YFR053C	An15g05940 An16g01620 An02g14380 An06g00380 An13g00510	AN2180.3 AN7459.3 AN2638.3 AN4255.3	
Phosphoglucomutase	*PGM1*/YKL127W *PGM2*/YMR105C	An02g07650 (An07g06780)	AN2867.3 (AN4591.3)	*pgmB* Hoffmann et al. (2000)
UDP-glucose pyrophosphorylase	*UGP1*/YKL035W	An12g00820	AN9148.3	
Glucose-6-isomerase	*PGI1*/YBR196C	An16g05420	AN6037.3	
Glutamine:fructose-6-phosphate transaminase	*GFA1*/YKL104C	An18g06820 An03g05940	AN10709.3	
Glucosamine-6-phosphate *N*-acetyltransferase	*GNA1*/YFL017C	An12g07840	AN8706.3	
Phosphoacetylglucosamine mutase	*PCM1*/YEL058W	An18g05160 An18g05170	AN4234.3	
UDP-*N*-acetylglucosamine diphosphorylase	*UAP1*/YDL103C	An12g00480	AN9094.3	
Mannose-6-phosphate isomerase	*PMI40*/YER003C	An04g03200 An08g06350	AN1715.3 AN0667.3	
Phosphomannomutase	*ALG4*/YFL045C	An18g06500 (An07g06780)	AN10710.3 (AN4591.3)	
Mannose-1-phosphate guanylyltransferase	*MPG1*/YDL055C	An04g04990 An11g02380	AN5586.3 AN1911.3	
Galactokinase	*GAL1*/YBR020W	An16g04160	AN4957.3	
UTP:hexose-1-phosphate uridylyltransferase	*GAL7*/YBR018C	An02g03590	AN6182.3	
UDP-glucose-4-epimerase	*GAL10*/YBR019C	An12g10410 An14g03820 An02g11320	AN2951.3 AN4727.3	
→Mutarotase part of *S. cerevisiae* Gal10p		An11g10890 An02g09090	AN3432.3	
UDP-Galp mutase	n.a.	An02g08660 An16g02380	AN3112.3	*glfA* Bakker et al. (2005)
GDP-mannose transporter	*VRG4*/YGL225W	An17g02140 (An11g02020)	AN8848.3 AN9298.3	
UDP-*N*-acetyl-glucosamine transporter	*YEA4*/YEL004W	An03g06940	AN8875.3	
UDP-galactose transporter	*HUT1*/YPL244C	An18g04260 (An08g10400)	AN4068.3	
Guanosine diphosphatase	*GDA1*/YEL042W	An08g04450	AN1082.3	
Apyrase	*YND1*/YER005W	An14g02370	AN0210.3	
Synthesis of ER-precursor structure for N-glycosylation				
UDP-*N*-acetyl-glucosamine-1-P transferase	*ALG7*/YBR243C	An02g03240	AN5888.3	
N-Acetylglucosaminyldiphosphodolichol *N*-acetylglucosaminyltransferase: catalytic part	*ALG13*/YGL047W	An01g09110		
N-Acetylglucosaminyldiphosphodolichol *N*-acetylglucosaminyltransferase: transmembrane part	*ALG14*/YBR070C		AN5736.3	
Chitobiosyldiphosphodolichol β-mannosyltransferase	*ALG1*/YBR110W	An06g01100	AN5346.3	
Glycolipid 3,6-α-mannosyltransferase activity	*ALG2*/YGL065C	An14g05910	AN6874.3	
GDP-mannose:Man(3/4)GlcNAc(2) α-1,2-mannosyltransferase	*ALG11*/YNL048W	An18g05910	AN5725.3	
Flippase	*RFT1*/YBL020W	An02g14940	AN7425.3	
Dol-P-mannose:Man(5)GlcNAc(2)-PP-dolichyl α-1,3-mannosyltransferase	*ALG3*/YBL082C	An18g02360	AN0104.3	
Dol-P-mannose:Man(6/8)GlcNAc(2)-PP-dolichyl α-1,2-mannosyltransferase	*ALG9*/YNL219C	An08g07020	AN10118.3	
Dol-P-mannose:Man(7)GlcNAc(2)-PP-dolichyl α-1,6-mannosyltransferase	*ALG12*/YNR030W	An01g08460	AN3588.3	
Dol-P-glucose:Man(9)GlcNAc(2)-PP-dolichyl α-1,3-glucosyltransferase	*ALG6*/YOR002W	An02g12630	AN4864.3	
Dol-P-glucose:Glc(1)Man(9)GlcNAc(2)-PP-dolichyl α-1,3-glucosyltransferase	*ALG8*/YOR067C	An04g08820	AN7301.3	
Dol-P-glucose:Glc(2)Man(9)GlcNAc(2)-PP-dolichyl α-1,2-glucosyltransferase	*ALG10*/YGR227W	An02g02980	AN5902.3	
Dolichyl-phosphate β-D-mannosyltransferase (polypeptide 1)	*DPM1*/YPR183W	An16g04330	AN4947.3	
Dolichyl-phosphate β-D-mannosyltransferase polypeptide 2 (DPM2)		An01g05200	AN11234.3	
Dolichyl-phosphate β-D-mannosyltransferase polypeptide 3 (DPM3)		An14g00270	AN6992.3	
Dolichyl-phosphate glucosyltransferase	*ALG5*/YPL227C	An03g04410	AN7715.3	
"Mannose-P-dolichol utilization defect 1" (Lec35/MPDU1)		An04g03130	AN1721.3	
The oligosaccharyl transferase (OST)				
Alpha subunit (essential) of OST—ribosphorin I	*OST1*/YJL002C	An02g14560	AN7472.3	
Epsilon (essential) subunit of OST	*OST2*/YOR103C	An18g03920	AN4031.3	
Essential subunit of OST	*STT3*/YGL022W	An16g08570	AN1455.3	
Beta (essential) subunit of OST	*WBP1*/YEL002C	An07g04190	AN4683.3	

Delta (essential) subunit of OST—Ribophorin II	*SWP1*/YMR149W	An04g03495	AN1683.3	
Gamma subunit of OST	*OST3*/YOR085W	An02g14930	AN7426.3	
Subunit of OST	*OST4*/YDL232W	An08g07485		
Zeta subunit of OST	*OST5*/YGL226C-A			
Subunit of OST	*OST6*/YML019W	An02g14930	AN7426.3	
ER-processing of N-glycans and protein quality control				
Glucosidase I	*CWH41*/YGL027C	An15g01420	AN6606.3	
Glucosidase II—alpha subunit	*ROT2*/YBR229C	An09g05880	AN11054.3	*gls2* α Geysens et al. (in preparation)
→Other glycosyl hydrolase family 31 members		An18g05620 An01g10930 An04g06920 An01g04880 An09g03300	AN3504.3 AN0941.3 AN2017.3 AN0280.3 AN7345.3 AN8953.3 AN7505.3 AN10935.3 AN7120.3	
Glucosidase II—beta subunit	*GTB1*/YDR221W	An13g00620	AN10702.3	
Calnexin	*CNE1*/YAL058W	An01g08420	AN3592.3	*clxA* Wang et al. (2003)
UDP-glucose:glycoprotein glucosyltransferase	(*KRE5*/YOR336W)	An07g06430	AN4623.3	
ER degradation enhancer, α-1,2-mannosidase-like protein (EDEM)	*MNL1*/*HTM1*/YHR204W	An12g00340	AN11163.3	
ER-mannosidase I (and Man9-mannosidase) → other glycosyl hydrolase family 47 members	*MNT1*/YDR483W	An18g06220	AN5748.3 AN0551.3	
		An06g01510 An01g12550 An04g06990	AN3733.3 AN0787.3 AN3566.3 AN2045.3	*mds1A* Eades and Hintz (2000) *mds1B* (as above) *mds1C* (as above)
ER-mannosidase II	(*AMS1*/YGL156W)	An02g11720	AN2936.3	*mds2* Eades et al. (1998)
ER quality control lectin belonging to the OS-9 protein family	*YOS9*/YDR057W	An16g08470	AN1416.3	
Golgi processing of N-glycans				
Initiating α-1,6-mannosyltransferase	*OCH1*/YGL038C	An03g01090 An05g01750 An05g02320 An07g04940 An11g07490 An12g07020 An14g07140	AN4716.3 AN8986.3 AN7534.3 AN1620.3 AN2626.3	
Subunit of Golgi mannosyltransferase complexes M-Pol I and M-Pol II	*MNN9*/YPL050C	An03g05010 An04g01260 An15g06230	AN7672.3 AN4395.3 AN10265.3	
Subunit of Golgi mannosyltransferase complex M-Pol I	*VAN1*/YML115C	Same as for MNN9	Same as for *MNN9*	
Subunit of Golgi mannosyltransferase complex M-Pol II	*ANP1*/YEL036C	same as for MNN9	same as for *MNN9*	
Subunit of Golgi mannosyltransferase complex M-Pol II	*MNN10*/YDR245W	An15g03330	AN7562.3	
Subunit of Golgi mannosyltransferase complex M-Pol II	MNN11/YJL183W	An04g05940	AN1969.3	
Subunit of Golgi mannosyltransferase complex M-Pol II	*HOC1*/YJR075W	same as for OCH1	same as for *OCH1*	
Golgi α-1,2-mannosyltransferase	MNN2/YBR015C	An04g06730 An14g06060 An15g00920	AN6571.3 AN6857.3	
Golgi α-1,2-mannosyltransferase	*MNN5*/YJL186W	Same as for MNN2	Same as for *MNN2*	
Golgi α-1,3-mannosyltransferase → other member of this protein family (MNT2–4)	*MNN1*/YER001W	An15g04810		
Putative positive regulator of the mannosylphosphate transferase	*MNN4*/YKL201C	An18g03940 An08g05380	AN10496.3 AN1048.3	
N-Acetylglucosaminyltransferase	*GNT1*/*YOR320C*	An03g02990 An11g10260	AN11141.3 AN7983.3	
Protein-folding in the ER				
Protein disulphide isomerase*	*PDI1*/*YCL043C*	An02g14800	AN7436.3	*pdiA* Ngiam et al. (1997)
Member of the protein disulphide isomerase family (membrane-bound)*	*EPS1*/*YIL005W*	An12g04950	AN5970.3	
Member of the protein disulphide isomerase family*	*EUG1*/*YDR518W*			
Member of the protein disulphide isomerase family*	*MPD1*/*YOR288C*			
Member of the protein disulphide isomerase family*	*MPD2*/*YOL088C*			
Member of the protein disulphide isomerase family*		An01g04600	AN0248.3	*tigA* Jeenes et al. (1997)
Member of the protein disulphide isomerase family*		An18g02020	AN0075.3	*prpA* Wang et al. (2000)
Calnexin	*CNE1*/*YAL058W*	An01g08420	AN3592.3	*clxA* Wang et al. (2003)
Peptidyl-prolyl *cis–trans* isomerase (cyclophilin), ER-resident	*CPR5*/*YDR304C*	An00g07689	AN4467.3	*cypB* Derkx and Madrid (2001)
Peptidyl-prolyl *cis–trans* isomerase (cyclophilin), ER-resident**	*FPR2*/*YDR519W*	An01g06670	AN8343.3	
Membrane protein with a J domain required for the folding capacity of the endoplasmic reticulum	*ERJ5*/*YFR041C*	An07g07340	AN5770.3	

(*continued on next page*)

Table 1 (*continued*)

Protein name/description	*S. cerevisiae* gene locus	*A. niger* ORF number	*A. nidulans* ORF number	Cloned *Aspergillus* orthologue
Thiol oxidase required for oxidative protein-folding***	*ERO1/YML130C*	An16g07620	AN1510.3	*eroA* Harvey et al. (unpublished)
Flavin-linked sulfhydryl oxidase	*ERV2/YPR037C*	An13g01225	AN3759.3	
Flavin adenine dinucleotide (FAD) synthetase	*FAD1/YDL045C*	An08g07810	AN0591.3	
Flavin-containing monooxygenase; catalyzes oxidation of biological thiols	*FMO1/YHR176W*	An12g06490	AN8206.3	
Protein with a role in UDP-galactose transport to the Golgi lumen	*HUT1/YPL244C*	An18g04260	AN4068.3	
Ubiquitin-like protein	*RUB1/YDR139C*	An08g06370	AN0670.3	
One of several homologues of bacterial chaperone DnaJ	*SCJ1/YMR214W*	An05g00880	AN6170.3	
Hsp70 molecular chaperone, ER-resident	*KAR2/YJL034W*	An11g04180	AN2062.3	*bipA* van Gemeren et al. (1997)
Hsp70 molecular chaperone, ER-resident	*LHS1/YKL073W*	An01g13220	AN0847.3	*lhsA* Barnes et al. (unpublished)
Hsp70 molecular chaperone, ER-resident		An12g10590		
UPR				
Basic leucine zipper (bZIP) transcriptional activator of amino acid biosynthetic genes	*GCN4/YEL009C*	An01g07900	AN3675.3	
Basic leucine zipper (bZIP) transcription factor regulates the unfolded protein response	*HAC1/YFL031W*	An01g00160	AN9397.3	
Serine-threonine kinase and endoribonuclease; regulates Hac1p synthesis	*IRE1/YHR079C*	An01g06550	AN0235.3	
Evolutionarily conserved protein, provides resistance to agents that induce the unfolded protein response	*ORM1/YGR038W*	An01g08980	AN1933.3	
Evolutionarily conserved protein, provides resistance to agents that induce the unfolded protein response	*ORM2/YLR350W*	An01g08980	AN1933.3	
Type 2C protein phosphatase	*PTC2/YER089C*	An08g00830	AN1358.3	
tRNA ligase, required for tRNA splicing	*TRL1/YJL087C*	An08g01480	AN1296.3	

Aspergillus gene numbers between brackets indicate genes with low sequence similarity. *S. cerevisiae* locus names between brackets encode gene products with homology towards the described protein, but having a different functionality (Kre5p) or localization (Ams1p).

* See Fig. 3.

** This protein is membrane-bound in *S. cerevisiae* but has a C-terminal KDEL ER-retention signal in the aspergilli. The yeast and *Aspergillus* proteins have sequence similarity (e−24).

*** Ero1p is membrane-associated in *S. cerevisiae* but has a C-terminal retention sequence in the *Aspergillus* spp.

2. Assisted protein folding in *Aspergillus*

Proteins destined for secretion are assisted in their folding within the lumen of the ER, may be glycosylated and progress towards secretion provided they pass the quality control checkpoints that monitor folding. Proteins that are incompletely folded are retained within the ER but, if folding continues to be incomplete, then they may be dislocated from the ER for degradation by the proteasome. Accumulation of unfolded proteins within the lumen of the ER causes ER stress and stimulates a homeostatic response called the unfolded protein response (UPR). The molecular mechanisms underlying each of these events have some commonality across the eukaryotes although the detail varies and those differences may be functionally important. These topics have been reviewed extensively and repeatedly in higher and lower eukaryotes. A recent review from Schröder (2008a) summarizes the molecular basis of ER stress in higher eukaryotes and other reviews focus on *S. cerevisiae* (Schröder and Kaufman, 2005) or the filamentous fungi (Archer and Turner, 2006). The UPR has been implicated in several cellular processes, including the regulation of expression of the ER lumenal environment (e.g. levels of foldases and the chaperone activity), endoplasmic reticulum-associated protein degradation (ERAD), autophagy, apoptosis, nitrogen-sensing and meiosis, depending on the organism but the diversity of the UPR's involvement has yet to be explored in detail in the filamentous fungi. The expression of heterologous proteins may result in ER stress and,

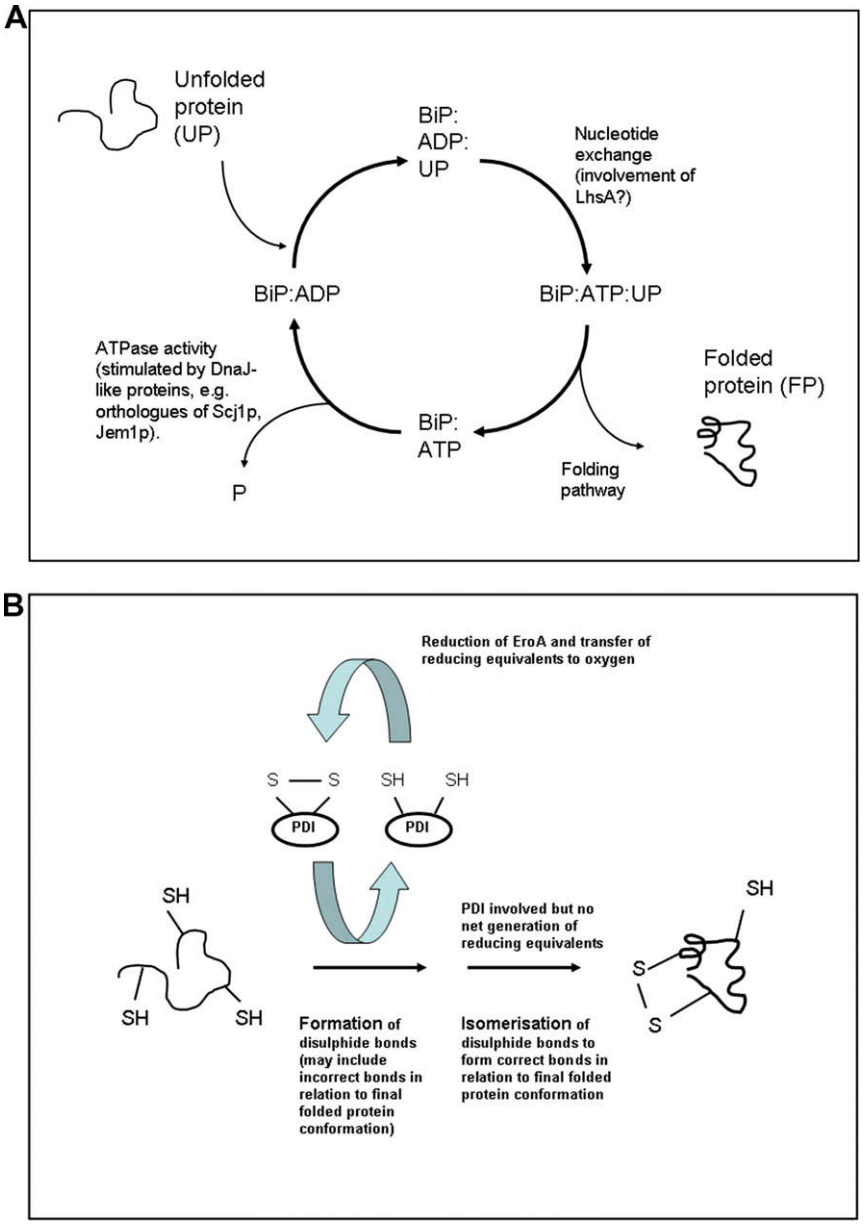

Fig. 1. (A and B) The assisted conformational folding (A) and formation of disulphide bonds (B) occurs in the lumen of the endoplasmic reticulum. These events may occur contemporaneously. The major chaperone (BiP) and foldase (PDI) are shown for simplicity although, as discussed in the text, their functionalities may also reside in other proteins. The chaperones hold the conformation of the secretory protein and may therefore be referred to as holdases. Note that PDI, a foldase, also has chaperone activity. Partially folded glycoproteins may leave the chaperone cycle and enter the calnexin cycle as described later. Nucleotide exchange in *Aspergillus* may not involve an orthologue of yeast Sil1p and that activity is therefore predicted to reside in LhsA. The formation of disulphide bonds is expected to occur when cysteines are suitably positioned during the folding process. The disulphide bonds may be incorrect in relation to the conformation of the correctly folded protein and, therefore, reduced PDI functions in their reduction (with reduced glutathione transferring reducing equivalents to regenerate reduced PDI) or in their isomerisation. These activities are discussed in the text in relation to the PDI family proteins. For more detailed review of the chaperone cycle and the foldase activity, refer to Schröder (2008a,b).

therefore, the UPR has often been viewed as an area that could be targeted for manipulation in order to provide improved strains for production purposes (Schröder, 2008b; Archer and Turner, 2006). *S. cerevisiae* can be used as the organism against which the aspergilli should be compared at the genomic level because of its status as a well-studied model fungus. It appears at first sight to be a useful comparison in relation to protein secretion, especially as filamentous fungi such as *A. niger* and *A. oryzae*, are such prodigious secretors of proteins in comparison to *S. cerevisiae*. Some other yeast species appear to be better protein secretors than *S. cerevisiae* and that has led, for example, to analysis of ER stress and the UPR in *P. pastoris* (Gasser et al., 2007; Graf et al., 2008). Even so, it should be remembered that *S. cerevisiae* can be made to be productive and is exploited commercially as a host for secreted heterologous protein production indicating it has certain advantages over other species for some applications. Some species have clearly evolved to have effective secretion of enzymes that support their natural lifestyles and, through comparative genomics, it is therefore feasible to ask what differences there are between species, e.g. between *A. niger* and *S. cerevisiae*.

3. The chaperone cycle in *Aspergillus*

The putative chaperone-encoding genes have been described in *A. niger* (Pel et al., 2007), *Aspergillus fumigatus* (Nierman et al., 2005), *A. oryzae* (Machida et al., 2005) and *A. nidulans* (Galagan et al., 2005). The chaperone cycle is displayed in a simple form in Fig. 1A and we focus here solely on the ER-resident Hsp70s, BipA and LhsA. Calnexin, which is a chaperone that binds to partially folded proteins, is discussed below in the context of quality control functionality in relation to the folding and secretion of glycoproteins. The ER-resident peptidyl-prolyl isomerases are not discussed further here: for further information on fungal PPIs including those resident in the ER, we refer to Pemberton (2006), Derkx and Madrid (2001) and Tremmel and Tropschug (2007). While the ER provides the environment necessary for assistance in the complexities of folding of glycoproteins and the formation and isomerisation of disulphide bonds, it also houses the detection system for recognition of problems in folding and secretion that might, if left alone, cause harm to the organism. Thus, the ER is the site of recognition of problems in folding and the initiation of homeostatic mechanisms to counteract problems.

BiP:ATP binds to unfolded proteins largely through exposed hydrophobic regions on the unfolded proteins and this binding holds the proteins in an unaggregated state that affords a helpful folding environment. Binding of DnaJ-like co-chaperones stimulates ATPase activity, and the BiP:ADP:protein complex is secured. ADP:ATP exchange is stimulated in *S. cerevisiae* by Sil1p (Sls1p is a synonym) and reduces affinity of BiP:ADP for protein substrates and releases bound protein (summarized by Schröder and Kaufman, 2005). The DnaJ protein Scj1p has a C-terminal ER-retention sequence whereas Jem1p, another DnaJ protein), is retained in the ER by association with the membrane. Both Sil1p and Lhs1p are likely to be retained in the ER by C-terminal retention se-

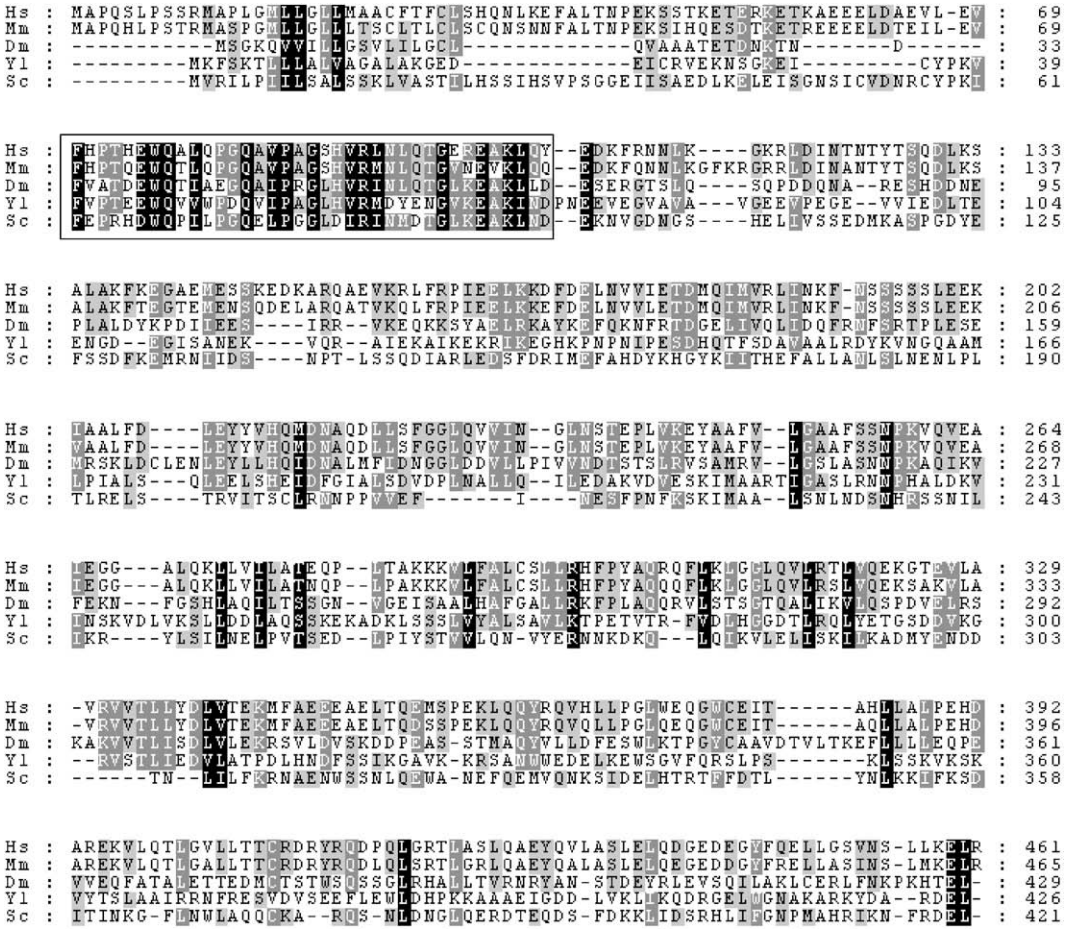

Fig. 2. Alignment of the amino acid sequences of Sil1p from *S. cerevisiae* with a selection of orthologues: human (Hs), mouse (Mm), *Drosophila* (Dm), *Yarrowia lipolytica* (Yl) and *Saccharomyces cerevisiae* (Sc). The Figure was provided by Colin Stirling, University of Manchester. Note the similarity within the boxed region but lack of similarity elsewhere. *Aspergillus* spp. lack an obvious homologue although other filamentous fungi may have one, e.g. *Neurospora crassa*.

quences and both have nucleotide exchange activity (Tyson and Stirling, 2000; Steel et al., 2004) and, although neither *SIL1* nor *LHS1* is essential, a double deletion is lethal. The aspergilli contain orthologues of DnaJ proteins but appear to lack an orthologue of *SIL1* and repeated attempts at deleting *lhsA* from *A. niger* have been unsuccessful (S. Barnes and D. Archer, unpublished). Although viable heterokaryons were obtained during the deletion protocol, all conidia that developed into mycelium contained *lhsA*. Thus, it appears that the aspergilli differ from *S. cerevisiae* in the functionality of the chaperone cycle. That does not imply that all filamentous fungi differ from *S. cerevisiae* in that respect because putative Sil1p orthologues are present in other species, e.g. *Neurospora crassa* and *Trichoderma reesei*. Although the expect values for the two putative Sil proteins in *N. crassa* and *T. reesei* compared to Sil1p in *S. cerevisiae* are 2e−10 and 2e−8, respectively, the similarities are high within the domain that characterizes Sil proteins across the eukaryotes (Fig. 2).

The ER-resident Hsp70 chaperone proteins Lhs1p and Kar2p (BiP) have been identified in *S. cerevisiae* and orthologues are found in all other fungi investigated so far. Sequence alignments of BiP show that it is a highly conserved protein in *A. niger*, *A. nidulans*, *S. cerevisiae* and *P. pastoris* with 61.1% identity across those species. Analysis of the BiP protein sequences from all 4 organisms using Interpro, NCBI and Pfam demonstrated that the domain architecture was well conserved across the species including the N-terminal ATPase and DnaK domains and C-terminal substrate binding domain (Bukau et al., 2006). Sequence alignment of Lhs proteins revealed less sequence conservation. The amino acid identity between the two *Aspergillus* spp. was 70% but was 21–25% between the *A. niger* LhsAp protein and the orthologues in *S. cerevisiae* and *P. pastoris*. The amino acid identity of Lhs proteins across all four species was poor at only 11.2%. Lhs proteins are about 30% longer than the BiP proteins. *S. cerevisiae* Lhs1p has been shown to function as a nucleotide exchange factor (Steel et al., 2004) and that functionality may reside in the long C-terminal region. A third Hsp70 protein that is presumptively resident in the ER lumen, due it having a C-terminal retention sequence, is found in *A. niger* (An12g10590) but apparently not in *A. nidulans* or *S. cerevisiae*. No functionality studies have been reported for these proteins.

Many of the genes that encode chaperone cycle proteins are transcriptionally up-regulated by the unfolded protein response (see below) in both *S. cerevisiae* (Travers et al., 2000) and *A. niger* (Guillemette et al, 2007). In *A. niger*, calnexin, *bipA*, *lhsA*, and *scjA* were all described as being regulated by the UPR. In contrast, the gene encoding the ER-resident PPI (*cypB*) was not up-regulated by either dithiothreitol (DTT) or tunicamycin (but was up-regu-

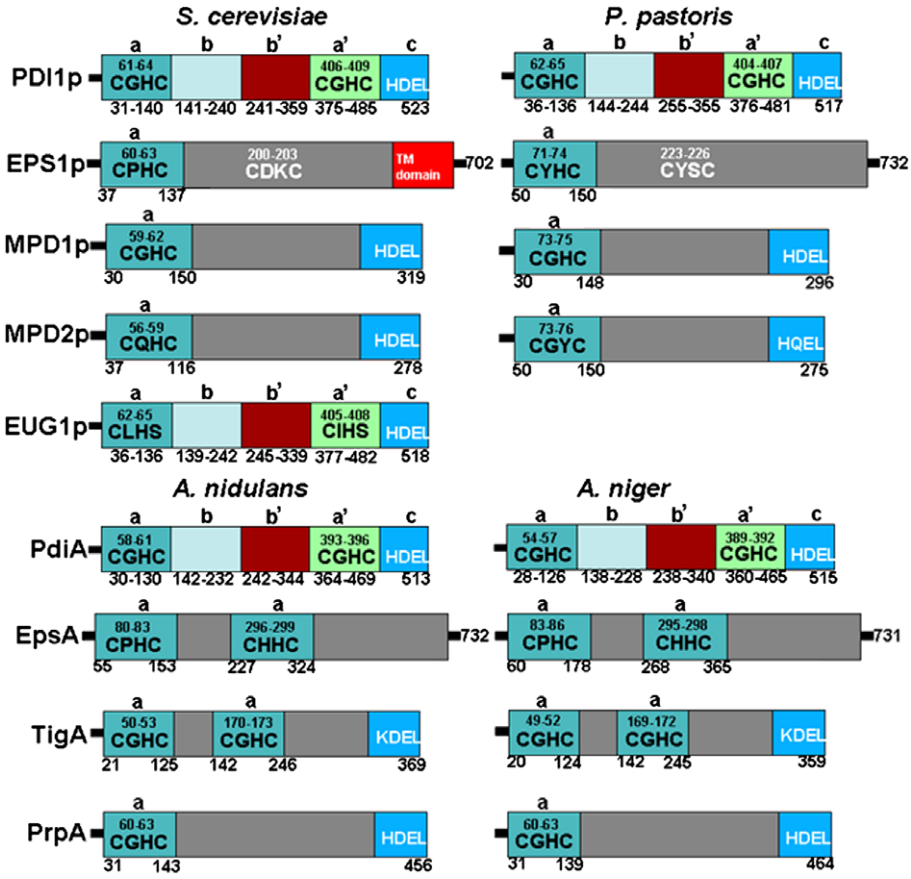

Fig. 3. Schematic representations of PDI and the PDI-like proteins from *S. cerevisiae*, *P. pastoris*, *A. niger* and *A. nidulans*. Domains were assigned in accordance with the *S. cerevisiae* Pdi1p domain nomenclature as described in Tian et al. (2006). The architecture and domain arrangements of all proteins were determined by cross referencing the analysis of the protein sequences from three separate analytical sources: NCBI, Interpro and Pfam. Each of these sequence analysis programmes operates different methods for identifying conserved protein domains. Pfam uses multiple sequence alignments and hidden Markov models, Interpro uses eleven databases and combines hidden Markov models, multiple sequence alignments, experimental data and PSI-blast searches, whereas NCBI uses a variant of PSI-blast called RPS-Blast to search a collection of 4 external databases as well as NCBI's own database. Each of the three programmes only recognizes a protein as containing a conserved domain if it scores lower than its stringency filter (*E*-value). This value varies depending on the methods used by the programmes. To ensure that the sequence analysis is not missing any domains that are present but have fallen just outside of the stringency parameters of the programmes the *E*-value for Pfam (the only programme with an adjustable *E*-value) was also increased from 1 to 10. In addition, the *E*-value was decreased from 1 to 0.1 to confirm the presence of the predicted domains. Thus, the domains shown in Fig. 2 have confidence limits as defined by the predictive programmes and, the case of Pfam, our deliberate testing of the criteria.

lated by expression of a heterologous protein) and An12g10590 (the additional ER-resident Hsp70 in *A. niger*) was up-regulated by DTT but not by tunicamycin or expression of a heterologous protein.

4. Oxidative folding in *Aspergillus*

Protein disulphide isomerases (PDIs) are multi-functional enzymes that can catalyze the formation, reduction and isomerisation of disulphide bonds as well as having molecular chaperone activity for proteins with and without disulphide bonds (Gruber et al., 2006; Tian et al., 2006). The role of PDI in oxidative folding and formation of disulphide bonds is shown in Fig. 1B. The expression of *PDI1* in yeast and *pdiA* in the aspergilli is up-regulated by the unfolded protein response and the level of their gene product may enhance the secreted levels of at least some heterologous proteins expressed in fungi (Song et al., 2002; Ngiam et al., 2000; Moralejo et al., 2001; Xu et al., 2005). The domain architecture of Pdi1p from *S. cerevisiae* was revealed by limited proteolysis to give the structure, a, b, b′, a′, c (Fig. 3) (Gruber et al., 2006). The domains a and a′ adopt a thioredoxin fold which each contain one active CGHC catalytic motif (Tian et al., 2006). These catalytic motifs are oxidized in the *S. cerevisiae* ER by the membrane-bound flavoprotein Ero1p using molecular oxygen (Pagani et al., 2001; Gruber et al., 2006). Retention of Ero1p in *P. pastoris* appears also to be probably achieved by a membrane-associating region, although the *A. niger* (HEEL) and *A. nidulans* (RDEL) orthologues, EroA, have putative C-terminal ER-retention sequences, indicating a divergence from yeasts which may have functional significance. The oxidized version of PDI can then oxidize and isomerise partially folded proteins to form disulphide bonds. The b and b′ domains, despite adopting a thioredoxin fold are redox inactive with the c domain containing the ER retrieval/retention signal (Gruber et al., 2006; Tian et al., 2006). Since the discovery of *PDI1*, numerous additional homologues have been identified and studied. In *S. cerevisiae* there are four such homologues, whereas in *P. pastoris*, *A. niger* and *A. nidulans* only three have been identified (Fig. 3).

Pdi1p is expressed in higher quantities than any of the PDI-homologues suggesting it is the predominant disulphide isomerase (Nørgaard et al., 2001). This is corroborated by experimental data that demonstrates that it has reductase, oxidative re-folding and isomerase activities which are significantly higher than any of the homologues (Nørgaard et al., 2001; Kimura et al., 2005). Kulp et al. (2006) showed that the domain architecture of Pdi1p is essential as it creates asymmetry in the rate of oxidation by Ero1p for the a and a′ domains. This asymmetry was shown to be responsible for the separate isomerase (a) and oxidase (a′) activities of Pdi1p (Kulp et al., 2006; Xiao et al, 2004). Furthermore, using surface plasmon resonance (SPR) it has been demonstrated that Pdi1p binds to the ER lumenal Hsp70 chaperone Kar2p (BiP) (Kimura et al, 2005). This interaction was demonstrated to be necessary for the chaperone activity of Pdi1p but had no affect on the protein's other activities (Kimura et al., 2005).

Fig. 2 shows the domain architecture of PDIs from *S. cerevisae*, *P. pastoris*, *A. nidulans* and *A. niger*. Each of the species encode a Pdi1p-like homo/orthologue containing the a, a′, b, b′, c domain architecture, with 2× CGHC catalytic domains per protein and a HDEL ER-retention/retrieval sequence. The proteins vary only slightly in size between the species with the lengths of the proteins ranging from 513 to 523 amino acids. Identity between the amino acid sequence of the proteins is significant, with Pdi1p from *S. cerevisiae* showing 43.9%, 35.5%, 35.7% identity to its homo/orthologues from *P. pastoris*, *A. niger* and *A. nidulans*, respectively, whereas the two *Aspergillus* proteins show 75.3% identity between them. It is likely that each of these proteins function in similar ways.

Deletion of the *EPS1* gene in *S. cerevisiae* is non-lethal and over-expression of *EPS1* in *PDI1* knockout strains is unable to completely compensate for loss of Pdi1p (Nørgaard et al., 2001). The architecture of *S. cerevisiae* Eps1p, a transmembrane protein that is a homologue of human transmembrane thioredoxin protein (TMX) (Gruber et al., 2006; Pel et al., 2007; Galagan et al., 2005) can be seen in Fig. 2. The thioredoxin catalytic sites of Eps1p are of a sub-optimal configuration (Pirneskoski et al., 2003) and recent studies have shown that Eps1p in *S. cerevisiae* has no oxidative re-folding activity and very little reductase activity but that it does have chaperone activity (Kimura et al., 2005). Using SPR it was shown that Eps1p was able to interact with Pdi1p, Eug1p, Mpd1p and Kar2p suggesting a significant role for this protein in the folding pathway (Kimura et al., 2005). These interactions were examined in more detail and Eps1p was shown to reduce the reductase activity of Mpd1p, abolish the chaperone activity of Pdi1p and increase the reductase activity of Eug1p, which has no activity on its own (Kimura et al., 2005). In addition, binding to Pdi1p, Kar2p or Mpd1p caused the abolition of the chaperone activity of Eps1p.

The architectures of EPS proteins from *S. cerevisiae*, *P. pastoris*, *A. niger* and *A. nidulans* are shown in Fig. 2. Sequence identity between EPS proteins from *S. cerevisiae* and *P. pastoris* is 24.7%, Although the EpsA sequence identities between the two *Aspergillus* species is 75.1%, the identities between the EPS proteins in the two yeasts and between the yeasts and *Aspergillus* spp. is low (20–30%). In all four species the overall architecture of EPS is similar with 2× sub-optimal CxxC catalytic sites and, in the two yeast species, one PDIa fold. Each of the two *Aspergillus* EPS proteins may have two PDIa folds although this apparent difference between the yeasts and aspergilli is an output of predictive programmes which may be overly discriminatory. None of the EPS proteins from *S. cerevisiae*, *P. pastoris*, *A. niger* or *A. nidulans* has a predicted C-terminal retention sequence. The protein has a transmembrane domain in *S. cerevisiae* (see above) and the orthologues in the other fungi each have a predicted transmembrane domain in some, but not all, domain analysis programmes.

Deletion of *MPD1* in *S. cerevisiae* is non-lethal and *MPD1* encodes the only PDI homologue where over-expression can compensate for loss of Pdi1p (Nørgaard et al., 2001). Mpd1p, has a weak reductase activity (and is the only PDI homologue to show any reductase activity), exhibits some oxidase activity but no chaperone activity (Kimura et al., 2005). Using SPR, Mpd1p was shown to interact with calnexin, leading to inhibition of its chaperone activity (Kimura et al., 2005). Interaction studies also showed that Mpd1p interacts with another yeast PDI homologue, Mpd2p and that this interaction has no affect on either protein's activity. Mpd2p in *P. pastoris* (Fig. 2) has only 17.2% amino acid identity with Mpd2p in *S. cerevisiae*. Both proteins contain one PDIa fold and a sub-optimal CxxC motif, CQHC in *S. cerevisiae* and CGYC in *P. pastoris*. In addition, each protein contains a C-terminal ER-retention/retrieval sequence, HDEL in *S. cerevisiae* and HQEL in *P. pastoris*.

The *tigA* gene was first identified in *A. niger* (Jeenes et al., 1997) and was subsequently identified in *A. nidulans* (Galagan et al., 2005). In both species, TigA is a 369 amino acid protein and the two proteins share 66.4% amino acid identity to one another. They both have 2× PDIa folds and 2× optimal CGHC catalytic domains in addition to a KDEL ER-retention sequence. Investigations into the function of TigA in *A. niger* have shown that it acts as an isomerase and chaperone (Liang et al., 2005). However, the chaperone activity appears to have some substrate specificity as it was unable to re-fold glyceraldehyde-3′-phosphate dehydrogenase but was able to re-fold prochymosin (Liang et al., 2005). In addition, the two CGHC motifs were shown to be unequal in activity with the N-terminal motif being more active.

The *prpA* genes in *A. niger* (Wang et al., 2000) and *A. nidulans* encode 456 and 464 amino acid proteins, respectively, which share 74.2% amino acid identity (Pel et al., 2007; Galagan et al., 2005; Zhou et al., 2004). Each protein contains one PDIa fold, one CGHC catalytic site and the HDEL ER-retention/retrieval sequence. In *A. niger* PrpA displayed 1% of the isomerase activity of PdiA and exhibited both chaperone and anti-chaperone activity (Zhou et al., 2004). PrpA may therefore function more as a chaperone than an oxidase *in vivo*.

In *S. cerevisiae*, Eug1p is a PDI-like protein that may contribute to protein folding (Nørgaard et al., 2001). Eug1p has 39% amino acid identity with Pdi1p and is similarly organized but has a mutated version of the CxxC motif, CxxS, which probably accounts for the diminished oxidizing capacity and lack of both reductase and isomerase activities (Nørgaard et al., 2001; Xiao et al., 2004; Kimura et al., 2005). Eug1p cannot restore viability to *PDI1* knockout strains (Nørgaard et al., 2001; Kimura et al., 2005). However, Eug1p did demonstrate significant chaperone activity and was shown to interact with Eps1p leading to enhanced reductase and chaperone activities (Kimura et al., 2005). This interaction with Eps1p increased the reductase and chaperone activities of this PDI-like protein (Kimura et al, 2005). There is no obvious direct homologue of Eug1p in *P. pastoris*, *A. niger* or *A. nidulans*.

Note that the expression of *pdiA*, *tigA*, *prpA*, *eroA*, but not *epsA*, were all up-regulated by ER stress in *A. niger* (Guillemette et al., 2007) although *tigA* appeared not to be affected by DTT.

5. The unfolded protein response (UPR)

As already outlined above, the UPR is a stress response initiated by detection of unfolded proteins in the ER that leads to the induction of homeostatic responses within the cell that include the transcriptional up-regulation of foldases and chaperones, as well as the up-regulation of ER-associated protein degradation (ERAD) by the proteasome. The UPR may be induced by several factors that include the expression of at least some heterologous proteins, especially at high yield, high level expression of some native proteins, or chemicals that adversely affect the folding environment within the ER. The transcriptional regulation of genes by unfolded secretory proteins has been described in *S. cerevisiae* (Travers et al., 2000), *A. niger* (Guillemette et al., 2007), *A. nidulans* (Sims et al., 2005), *P. pastoris* (Graf et al., 2008) and other fungal species, e.g. *T. reesei* (Arvas et al., 2006). Each of these studies has revealed that the UPR affects transcription of a larger number of genes than those directly involved in protein secretion. In addition to the transcriptional up-regulation of genes, the global analyses demonstrated transcriptional repression of some genes and, notably, those that encode some secretory proteins or wall/cell surface-associated proteins. The transcriptional down-regulation of genes encoding secretory proteins might also be considered homeostatic because the flux of proteins into the secretory system would then be reduced. That similar repression had been described in *T. reesei* (Pakula et al., 2003) and *A. niger* (Al-Sheikh et al., 2004) prior to the genome-wide analyses and has since been described in *S. cerevisiae* (Kimata et al, 2006). The sensing of unfolded proteins in the ER is the initial step in induction of the UPR. The transmembrane ribo-endonuclease Ire1p (in *S. cerevisiae*) must be activated by dimerisation (or the formation of higher order oligomers) and, then, autophosphorylation in response to unfolded proteins. The only known substrate for active Ire1p is the *HAC1*^{u} (*u*n-induced) mRNA which is cleaved at two positions, to remove an unconventional intron, before a ligase joins the two fragments to produce *HAC1*^{i} (*i*nduced) which encodes the transcription factor Hac1p. BiP (Kar2p) binds to the lumenal domain of Ire1p and may be titrated away from that association by binding to unfolded proteins (Bertolotti et al., 2000). That release of BiP from Ire1p leads to the aggregation of Ire1p (Kimata et al., 2007). Ire1p directly associates with unfolded proteins causing a change in shape leading to its activation (Credle et al., 2005; Kimata et al., 2007) and further factors may also be involved in the detection of unfolded proteins and subsequent signal initiation (Oikawa et al., 2007). The components of the UPR induction mechanism are conserved between yeasts and filamentous fungi although the intron in the *HAC1* mRNA is much larger in *S. cerevisiae* (252 bp) than it is in *hacA* mRNA from *A. niger*, *A. nidulans* and *T. reesei* (20 bp) (Mulder et al., 2004; Saloheimo et al., 2003). An intron that is removed before activation of the UPR in the *P. pastoris HAC1* mRNA has not been described. In contrast to the activation of *HAC1* mRNA in *S. cerevisiae*, *hacA* mRNA in both *A. niger* and *A. nidulans* is truncated in the 5′ UTR (Mulder et al., 2004; Saloheimo et al., 2003). The HAC transcription factors in each species recognize and bind to UPR elements (UPREs) in promoters of target genes and the sequences vary somewhat between species, target gene promoters and, where there is more than one UPRE in a promoter, between the UPREs (e.g. Mulder et al., 2006; Patil et al., 2004). Deliberate manipulation of levels of individual chaperones and foldases has led to improved secretion of some heterologous proteins (see above) and, similarly, up-regulation of the UPR by increased levels of expression of Ire or Hac^{i} has also improved the secretion of some target proteins in filamentous fungi (Valkonen et al., 2003, 2004; Nakajima et al., 2006). Genes regulated by the UPR are affected by several factors that include nutrient levels and growth rate (Pakula et al., 2005; Castrillo et al, 2007; Kuhn et al., 2001; Gasch et al., 2000; Schröder et al., 2000; Patil et al., 2004) and, if being expressed, the nature of any heterologous protein. From what we know to date the UPR, and its regulation and consequences, are similar in yeasts and the filamentous fungi

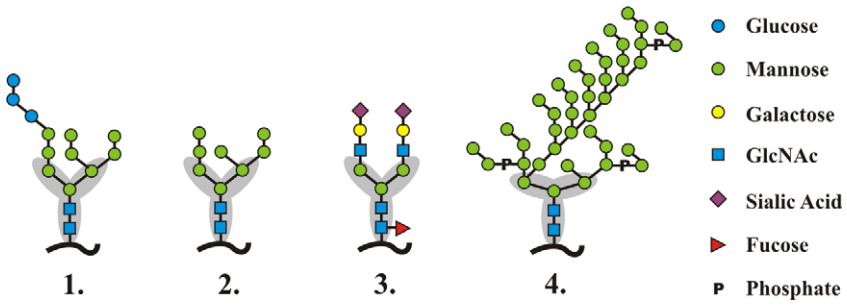

Fig. 4. Representation of different types of N-linked glycans: (1) The common precursor oligosaccharide $Glc_3Man_9GlcNAc_2$ that is transferred to the *N*-glycosylation site on a newly formed polypeptide chain in almost all eukaryotic organisms; (2) the high-mannose structure $Man_8GlcNAc_2$ (isomer B), a substrate for Golgi-located elongating mannosyltransferases in many yeast species as well as a structure that is also found on some secreted mammalian proteins; (3) a biantennary, core-fucosylated complex *N*-glycan, found on many mammalian proteins; (4) a hyperglycosyl structure, found on *S. cerevisiae* secreted proteins. The grey-shaded sugar residues within each *N*-glycan represent the common trimannosyl-chitobiose core ($Man_3GlcNAc_2$).

even if the fine detail can vary. Whether that level of detail contributes to differences in the capacity for protein secretion, remains to be seen.

6. Glycosylation of secreted proteins

Protein glycosylation is a major post-translational modification and plays essential roles in eukaryotic cells from fungi to humans (Kukuruzinska and Lennon-Hopkins, 1999). There are two main types of glycosylation, *N*- and *O*-glycosylation, in which oligosaccharides are attached either to the β-amide group of an asparagine residue or to the β-hydroxyl group of mainly serine and threonine residues.

In all eukaryotes, *N*-glycosylation predominantly occurs as a co-translational process where a pre-assembled, lipid-linked glycosyl structure—usually $Glc_3Man_9GlcNAc_2$ (Fig. 4)—is transferred to specific Asn-residues within the sequence Asn-X-Ser/Thr of a nascent polypeptide chain (Kornfeld and Kornfeld, 1985). After transfer to the protein, the original precursor oligosaccharide is further modified via the action of ER- and Golgi-resident glycosidases and glycosyl transferases. *N*-glycan processing within the ER is very conserved for all eukaryotes, mainly resulting in $Man_{8-9}GlcNAc_2$ structures in yeast (Jelinek-Kelly and Herscovics, 1988; Movsichoff et al., 2005; Ziegler et al., 1994) and $Man_{6-8}GlcNAc_2$ structures in mammalian cells (Weng and Spiro, 1993). However, once a glycoprotein is shuttled through the Golgi apparatus, the composition of the final *N*-glycan structure greatly depends on the specific eukaryotic organism in which it is produced. Although the final oligosaccharides all have the common trimannosyl-chitobiose ($Man_3GlcNAc_2$) core, originating from the lipid-linked precursor, *N*-glycans from yeasts and fungi tend to consist mainly of mannose residues (called high-mannose type) (Dean, 1999; Gemmill and Trimble, 1999; Maras et al., 1999) whereas the N-linked sugars synthesized by mammalian organisms are mainly of the complex type (Fig. 4), thus consisting of branching GlcNAc residues which are further modified with (sub)terminal galactose, fucose and sialic acid residues (Kornfeld and Kornfeld, 1985).

O-Glycosylation amongst the eukaryotes is diverse. The main pathway in higher eukaryotes is located in the Golgi and directly uses sugar nucleotides as monosaccharide donors for the sequential build-up of the *O*-glycosyl structure (Hanisch, 2001; Peter-Katalinic, 2005; Van den Steen et al., 1998). In yeasts and fungi however, *O*-glycosylation starts in the ER lumen where the initial reaction of sugar transfer to serine and threonine residues uses a dolicholphosphate-activated mannose residue as the monosaccharide donor (Tanner and Lehle, 1987). However, recent reports indicate that this type of *O*-mannosylation also occurs in mammalian cells where it is most probably also initiated in the ER (Endo, 2004; Willer et al., 2003). For this review, we will restrict ourselves to *N*-glycosylation within the aspergilli. For a comprehensive overview on structures, functions and genetics of *O*-glycosylation within fungi, the interested reader is referred to Goto (2007).

7. *N*-Glycan diversity in the aspergilli

In contrast to *S. cerevisiae*, where so-called hyperglycosyl structures consisting of more than 200 mannose residues (Fig. 4) can be added onto the glycoproteins (Dean, 1999; Herscovics and Orlean, 1993), *N*-glycans synthesized by *Aspergillus* species (Chiba et al., 1993; Colangelo et al., 1999a,b; Ftouhi-Paquin et al., 1997; Limongi et al., 1995; Molgaard and Larsen, 2002; Panchal and Wodzinski, 1998; Takayanagi et al., 1994, 1992; Takegawa et al., 1991; Wallis et al., 1999) are in many cases very similar to the high-mannose structures found on some mammalian proteins (Williams and Lennarz, 1984). In the latter case, *N*-glycans consist of a $Man_5GlcNAc_2$ backbone with 0–4 extra α-1,2-linked mannose residues.

Depending on the species and the protein under analysis, fungal-specific high-mannose *N*-glycans have been observed, where partial hydrolysis of α-1,2-linked mannose residues results in glycosyl structures onto which new mannose residues are added via α-1,3- (Takegawa et al., 1991) or α-1,6-linkages (Nakao et al., 1987). In some cases, elongation of the $Man_{8-9}GlcNAc_2$ ER-structure with a limited number of α-1,2-linked mannose residues can also occur, as seen for acid carboxy peptidase of *Aspergillus saitoi* (Chiba et al., 1993; Ichishima, 2000) and endopolygalacturonase I of *A. niger* (Colangelo et al., 1999a), carrying sugar chains-like $Man_{10-11}GlcNAc_2$ and $Man_{13}GlcNAc_2$, respectively. Reports on hyperglycosylation in filamentous fungi are rare and the range of mannose residues is rather limited: $Man_{18}GlcNAc_2$ has been observed on pectin methylesterase of *A. niger* (Warren et al., 2002) and $Hex_{7-26}GlcNAc_2$ on *A. niger* α-galactosidase A (Wallis et al., 2001).

In contrast, very small *N*-glycans ($Man_{3-4}GlcNAc_2$) have also been isolated, indicating extended hydrolysis of α-1,3- and α-1,6-linked mannose residues (Akao et al., 2006; Colangelo et al., 1999b; Limongi et al., 1995; Molgaard and Larsen, 2002; Woosley et al., 2006a,b) by putative Class 2 mannosidases. Indeed, apart from the characterization (Ichishima et al., 1981; Yamamoto et al., 1982; Yoshida et al., 2000) and cloning (Akao et al., 2006; Eades and Hintz, 2000; Inoue et al., 1995) of several Class 1 fungal α-1,2-mannosidases, a limited number of Class 2 α-mannosidases have also been purified (Amano and Kobata, 1986; Gaikwad et al., 1995) or cloned (Eades et al., 1998) from the aspergilli.

In some cases, the high-mannose *N*-glycan structures are decorated with extra glucose and galactose residues. NMR analysis clearly identified $GlcMan_9GlcNAc_2$ structures on α-D-galactosidase (Takayanagi et al., 1992) and α-D-glucosidase (Takayanagi et al., 1994) from *A. niger*. The presence of galactose residues was in many cases deduced from the results of a monosaccharide analysis (Ward et al., 2004) and could in some cases be further confirmed via NMR or immunodetection. In contrast to higher eukaryotes, the galactose residues on glycans of filamentous fungi are in the furanose (Gal*f*) and not in the pyranose form. Depending on the analyzed protein or the specific *Aspergillus* strain, Gal*f* residues were positioned in different ways onto the glycosyl structures, e.g. α-1,2-linked for *A. niger* α-D-galactosidase (Takayanagi et al., 1992) and α-D-glucosidase (Takayanagi et al., 1994) or β-1,5-linked on α-galactosidase A of *A. niger* N402 (Wallis et al., 2001). Finally, phosphate residues, either in a monoester or a diester linkage, were observed on both the high-mannose and galactomannan *N*-glycan chains from *A. oryzae* β-galactosidase (Nakao et al., 1987). The addition of phosphomannose to yeast *N*-glycans in a diester linkage is a well-described phenomenon (Jigami and Odani, 1999).

Prior to whole-genome sequencing, only a limited number of genes involved in *N*- (or *O*-)glycosylation of aspergilli had been cloned. In what follows, we aim to summarize the annotation results for protein *N*-glycosylation, obtained with the model organism *A. nidulans* and the related, but biotechnologically more relevant, species *A. niger*. In most cases, we refer to the locus names known for *S. cerevisiae*.

8. Synthesis and transport of sugar nucleotides

In many cases, sugar nucleotides act as the activated monosaccharide donor substrates for the synthesis and elongation of oligosaccharides in the ER and Golgi. These activated sugar donors are generated within the cytoplasm/nucleus and need to be trans-

ported into the organelles of the secretion pathway, where the corresponding glycosyltransferases act. Based on the different monosaccharides found within *Aspergillus N*-glycans, we set out to identify the genes involved in the synthesis of UDP-Glc, UDP-GlcNAc, UDP-Gal*p*, UDP-Gal*f* and GDP-Man. For an extended overview on the metabolic pathway of the different sugar nucleotides, the interested reader is referred to Bulter and Elling (1999).

Cytosolic glucose is converted to glucose-6-P via a glucose kinase (Glk1p) (Walsh et al., 1983) and further processed to UDP-Glc by phosphoglucomutases (Pgm1p or Pgm2p) (Boles et al., 1994) and a uridylyltransferase (Ugp1p) (Daran et al., 1995). Via the isomerase Pgi1p (Dickinson et al., 1988), glucose-6-P is also partially converted to fructose-6-P. This fructose-6-P is further processed to GDP-Man via the successive action of the mannose-6-phosphate isomerase Pmi40p (Smith et al., 1992), the phosphomannomutase Alg4p/Sec53p (Kepes and Schekman, 1988) and the guanylyltransferase Mpg1p/Vig9p (Benton et al., 1996). Conversion to UDP-GlcNAc occurs by the consecutive actions of the transaminase Gfa1p (Watzele and Tanner, 1989), the *N*-acetyltransferase Gna1p (Mio et al., 1999), the mutase Pcm1p (Hofmann et al., 1994) and the UDP-*N*-acetylglucosamine diphosphorylase Uap1p (Mio et al., 1998). Orthologues were found in both *A. nidulans* and *A. niger* for all genes involved in the synthesis of these three sugar donors. Five and six genes with high sequence homology to glucose kinase (and other hexokinases) were found in *A. nidulans* and *A. niger*, respectively, with ORF An12g08610 from *A. niger* being identical to the previously cloned *glkA* gene (Panneman et al., 1996). Furthermore, whereas *S. cerevisiae* contains two phosphoglucomutase genes (*PGM1* and *PGM2*), only one orthologue could be identified in either *Aspergillus* sp., with the *A. nidulans* ORF AN2876.3 being almost identical to the previously cloned *pgmB* (Hoffmann et al., 2000). However, an extra gene showing equally low homology to phosphoglucomutase (Pgm1/2p) and phosphomannomutase (Alg4p) was identified in both aspergilli. Finally although the other activities involved in UDP-Glc, UDP-GlcNAc or GPD-Man synthesis are encoded by only one gene in *S. cerevisiae*, up to two orthologues can be found in either *A. niger* or *A. nidulans*.

Synthesis of UDP-Gal*p* from cytosolic galactose occurs via the successive action of the Gal1p galactokinase and a Gal7p uridylyltransferase (St. John and Davis, 1979) with both *GAL1* and *GAL7* having one orthologue in *A. niger* and *A. nidulans*. Interconversion between UDP-Glc and UDP-Gal is catalyzed by the Gal10p epimerase (Majumdar et al., 2004). In *S. cerevisiae*, *GAL10* encodes a protein with a N-terminal NAD^+ dependent epimerase and a C-terminal mutarotase domain. In *Aspergillus*, both domains are encoded by separate genes: orthologues were found in *A. nidulans* and *A. niger* for the epimerase part of yeast *GAL10* as well as for the mutarotase part. Conversion from UDP-Gal*p* into UDP-Gal*f* is catalyzed by the cytosolic UDP-Gal*p* mutase. Recently, the gene was cloned from *A. fumigatus* (*glfA*) (Bakker et al., 2005) and one or two orthologues were identified in *A. nidulans* and *A. niger*, respectively.

When using sugar nucleotides as monosaccharide donor substrates, the glycosyltransferases of the ER and Golgi generate nucleoside diphosphates (NDPs) as side products, which are inhibitory for the transferase activities. To prevent this inhibition, the generated NDPs need to be converted to nucleoside monophosphates (NMPs) via nucleoside diphosphatase (NDPase) activities (Hirschberg et al., 1998). Two such activities, a GDPase/UDPase (Gda1p) (Yanagisawa et al., 1990) and an apyrase (Ynd1p) (Gao et al., 1999), have been identified in *S. cerevisiae* and an orthologue for both of them was found in the two *Aspergillus* species. It is generally believed that the resulting GMP and UMP can act as substrates in an antiport mechanism where they are shuttled back to the cytosol while the corresponding sugar nucleotides simultaneously enter the lumen of the ER or Golgi apparatus (Hirschberg et al., 1998). One gene with high similarity to yeast *VRG4*, encoding such a GDP-Man transporter (Dean et al., 1997), was found for *A. niger*, while two orthologues were identified in *A. nidulans*. Furthermore, both fungal species also have one open reading frame showing high homology with *S. cerevisiae YEA4* and *HUT1*, the coding sequences for the UDP-GlcNAc and the UDP-Gal transporter, respectively (Nakanishi et al., 2001; Roy et al., 2000). Putative genes involved in the transport of UDP-Gal*f* across membranes have not been identified yet.

9. Synthesis of the dolicholphosphate-linked ER-precursor $Glc_3Man_9GlcNAc_2$

For the majority of eukaryotes studied to date, the synthesis of Asn-linked glycans starts with the 'en bloc' transfer of a tetradecasaccharide from the lipid-linked precursor structure Dol-PP-$GlcNAc_2Man_9Glc_3$ (Samuelson et al., 2005). Biosynthesis of this precursor structure occurs at the ER-membrane in a process known as the dolichol pathway (Burda and Aebi, 1999). The first reactions occur at the cytoplasmic face of the membrane and use sugar nucleotides as activated monosaccharide donors. Alg7p, an *N*-acetylglucosamine-phosphate transferase, initiates the synthesis process by converting Dol-P into Dol-PP-GlcNAc (Kukuruzinska and Robbins, 1987). The addition of a second GlcNAc residue in β-1,4-linkage is catalyzed by a heterodimer Alg13/14p, where Alg13p is the catalytic subunit that localizes at the ER-membrane via its interaction with the integral membrane protein Alg14p (Bickel et al., 2005; Chantret et al., 2005; Gao et al., 2005). Then, Alg1p adds a first mannose residue in a β-1,4 linkage (Couto et al., 1984). Finally, Alg2p (Jackson et al., 1993; O'Reilly et al., 2006) and Alg11p (Cipollo et al., 2001; O'Reilly et al., 2006), two α-mannosyltransferases known to interact with Alg1p, are responsible for the addition of four α-linked mannose residues and thus complete the build-up of the precursor structure at the cytosolic face of the ER-membrane. The resulting Dol-PP-$GlcNAc_2Man_5$ is now flipped to the lumenal face of the ER-membrane by the ATP-independent membrane-spanning flippase Rft1p (Helenius et al., 2002), the encoding gene being responsive to induction by DTT and the expression of a heterologous protein in *A. niger* (Guillemette et al., 2007). In both *A. niger* and *A. nidulans*, one orthologue could be identified for Alg7p, Alg1p, Alg2p, Alg11p and Rft1p. We were able to identify an orthologue to yeast Alg14p for *A. nidulans* but not for *A. niger*, although it could be found using Blast analysis in *Aspergillus clavatus* and *A. fumigatus*. Similarly, we found an orthologue for Alg13p in *A. niger*, but could not identify one in *A. nidulans*, although it is clearly present in *A. clavatus*, *A. fumigatus* and *A. oryzae*. This could be due to sequence gaps and/or low interspecies sequence homology of the genes.

Further elongation of Dol-PP-$GlcNAc_2Man_5$ to Dol-PP-$GlcNAc_2Man_9Glc_3$ occurs within the ER lumen via the action of the mannosyltransferases Alg3p, Alg9p and Alg12p (Aebi et al., 1996; Burda and Aebi, 1999; Burda et al., 1996; Frank and Aebi, 2005; Grubenmann et al., 2002; Huffaker and Robbins, 1983), two α-1,3-glucosyltransferases Alg6p (Reiss et al., 1996) and Alg8p (Stagljar et al., 1994), and the α-1,2-glucosyltransferase Alg10p (Burda and Aebi, 1998). One orthologue of the genes encoding each of these transferases was easily identified in both aspergilli.

The sugar donors used during the transfer reactions at the lumenal site of the ER-membrane are Dol-P-Man and Dol-P-Glc. They are generated at the cytosolic face via the transfer of mannose and glucose, from GDP-Man, respectively, UDP-Glc, onto Dol-P and then flipped across the ER-membrane. Dol-P-Glc is generated by Alg5p (Heesen et al., 1994) with an orthologue present in *A. niger* and *A. nidulans*. Dol-P-Man is synthesised by Dol-P-Man synthase,

encoded by yeast *DPM1* (Orlean et al., 1988). However, identification of other eukaryotic *DPM1* homologues indicated the existence of two classes of Dol-P-Man synthases (Colussi et al., 1997; Kruszewska et al., 2000; Mazhari-Tabrizi et al., 1996; Tomita et al., 1998; Zimmerman et al., 1996). One class consists of the synthases found, for example, in *S. cerevisiae* (Dpm1p), *Ustilago maydis* and *Trypanosoma brucei*, as they contain a typical hydrophobic C-terminal transmembrane region that is not present in the Dol-P-Man synthases of humans, mice, *T. reesei* and *Schizosacharomyces pombe*. Therefore, the latter class of *DPM1* gene products depend on Dpm2p and Dpm3p for stability and full synthase activity (Maeda et al., 2000; Maeda et al., 1998). Analogous to *T. reesei*, the *Aspergillus* orthologue of the *DPM1* gene products lack the C-terminal transmembrane region and, as a consequence, orthologues for the human Dpm2p and Dpm3p could be identified. Once synthesized, Dol-P-Man and Dol-P-Glc migrate across the ER-membrane. Although this process is not well understood yet, it seems to involve the *Lec35/MPDU1* gene (Anand et al., 2001; Kranz et al., 2001; Schenk et al., 2001), for which an orthologue could be identified in *A. niger* and *A. nidulans*, but not in *S. cerevisiae*.

10. Transfer of the ER-precursor structure to nascent polypeptide chains

Upon translocation of the nascent polypeptide chain across the ER-membrane via the translocon complex, the ER-precursor *N*-glycan structure can be co-translationally transferred from its dolichol pyrophosphate carrier onto specific Asn-residues within the sequence Asn-X-Ser/Thr. This transfer is catalyzed by a multisubunit membrane complex, called the oligosaccharyl transferase (OST) (Knauer and Lehle, 1999; Lennarz, 2007). Up to now, nine OST subunits have been identified in yeast with Ost1p, Ost2p, Stt3p, Wbp1p and Swp1p being essential for the activity of the complex (Dempski and Imperiali, 2004) and Ost3–6p being required for optimal sugar transfer. Recently, it has been proposed that two different OST isoforms exist in *S. cerevisiae*, depending on whether either Ost3p or the homologous Ost6p are present within the complex (Schwarz et al., 2005). Moreover, the Ost3p-containing OST was shown to specifically interact with the Sec61 translocon, whereas the Ost6p-complex specifically associates with the mechanistically different Ssh1 translocon (Yan and Lennarz, 2005). We were able to identify orthologues in both *A. niger* and *A. nidulans* for the five essential OST components. However, only one gene showing homology to both Ost3p and Ost6p was found, indicating that both *Aspergillus* species contain just one OST isoform. This could actually fit with the fact that the recent gene annotations could also identify only one type of translocon in both aspergilli (Pel et al., 2007). An ORF encoding a small polypeptide with low sequence homology towards Ost4p was identified for *A. niger* but not *A. nidulans*. Furthermore, no orthologues were found for the small Ost5p subunit. Although not essential for a basal functionality of the OST complex in yeast, the aspergilli sequences encoding these

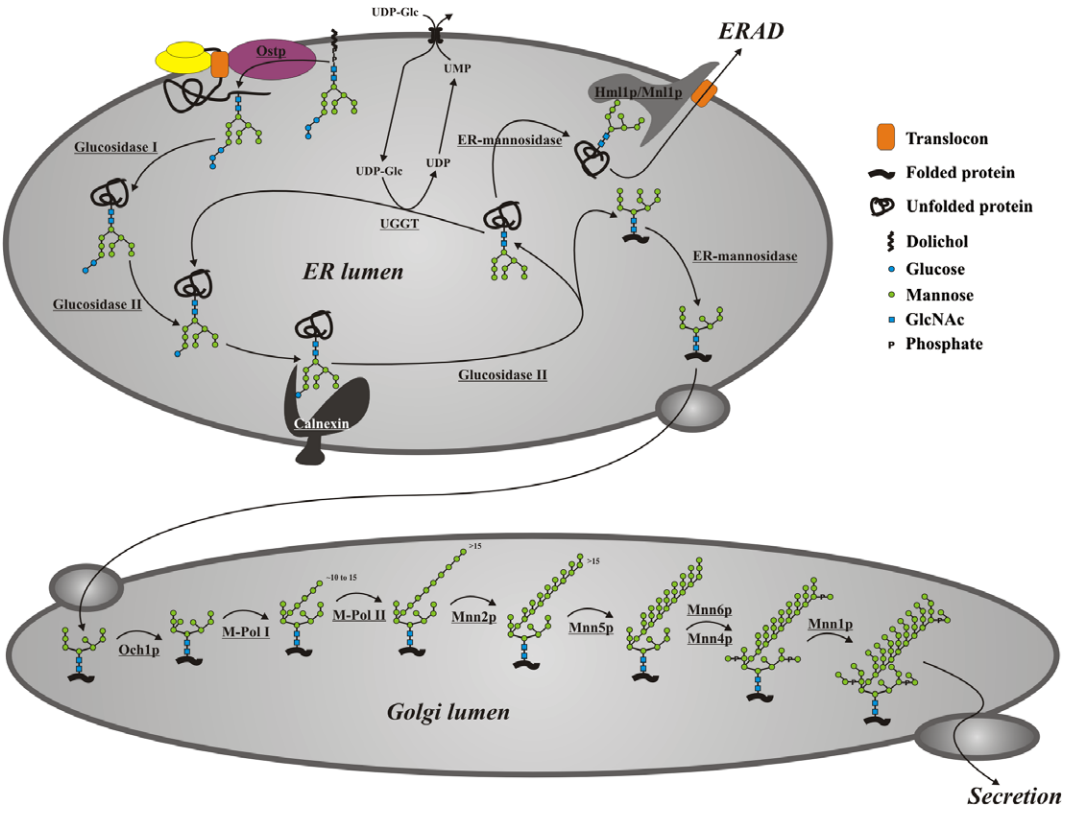

Fig. 5. Representation of *N*-glycosylation events in the ER (upper part) and the Golgi (lower part). The ER events also include a simplified version of the quality control cycle. After partial deglucosylation of protein-bound *N*-glycans by glucosidases I and II, the unfolded protein interacts with the membrane-bound lectin chaperone calnexin (there is no calreticulin in yeast and fungal systems) via its monoglucosylated structures. Upon final deglucosylation via glucosidase II, the glycoprotein is released from calnexin. If incompletely folded, its *N*-glycans are reglucosylated by UGGT so that the underlying glycoprotein can interact again with the lectin chaperone. Note that there is no UGGT in *S. cerevisiae*, but orthologues of the *S. pombe* protein were identified in the aspergilli. If the protein fails to fold after several rounds of de- and reglucosylation, its *N*-glycans will eventually be trimmed by ER-resident α-1,2-mannosidases (e.g. Mns1p in *S. cerevisiae* or its orthologues in the aspergilli). ER-localized lectin-like proteins recognizing these trimmed *N*-glycans (e.g. Hml1p/Mnl1p in *S. cerevisae* or its orthologues in the aspergilli) and target these unfolded proteins for ERAD. In contrast, when a glycoprotein is completely folded, it will not undergo reglucosylation by UGGT. After trimming of the high-mannose *N*-glycans by ER-resident α-1,2-mannosidases, the glycoprotein migrates to the Golgi apparatus via vesicular transport. In most yeast species, the *N*-glycans—upon entry into the Golgi—predominantly have the $Man_8GlcNAc_2$ (isomer B) structure. In *S. cerevisiae*, this structure can be further modified as represented in the lower part of the figure. Underlined are the names of the proteins involved in *N*-glycosylation and quality control.

small subunits might have been missed due to sequence gaps and/or low interspecies homology.

11. ER processing of *N*-glycans and protein quality control

Immediately after transfer of $Glc_3Man_9GlcNAc_2$ to the polypeptide chain, the α-1,2-glucose residue is removed by glucosidase I (Esmon et al., 1984; Romero et al., 1997). While glucosidase I is generally considered to be a type II membrane protein (Hettkamp et al., 1984; Kalz-Fuller et al., 1995; Kilker et al., 1981), its orthologue in both *A. niger* and *A. nidulans* lacked the transmembrane region, but contained a C-terminal HDEL-tag for ER localization. Hydrolysis of the two α-1,3-linked glucose residues is performed by glucosidase II, a heterodimer consisting of a catalytic alpha subunit and a beta subunit with a C-terminal HDEL-like tag (Arendt and Ostergaard, 1997; D'Alessio et al., 1999; Trombetta et al., 1996, 2001). Apart from being involved in ER-retention, the beta subunit also plays a role in correct folding of the alpha subunit (D'Alessio et al., 1999; Pelletier et al., 2000; Treml et al., 2000) and in its efficient positioning towards the glucosylated N-linked glycans (Deprez et al., 2005). Only recently, yeast *GTB1* was characterized as the *S. cerevisiae* orthologue of the glucosidase II beta subunit. It is significantly larger than the identified mammalian and *S. pombe* counterparts and was shown to be specifically involved in the trimming of the third, but not the second, glucose residue of the yeast ER-precursor (Wilkinson et al., 2006). Moreover, it does not contain a C-terminal HDEL-like tag or any transmembrane domains. Since the yeast alpha subunit, encoded by *ROT2* (Trombetta et al., 1996), also lacks any known localization signal, the ER-retention mechanism for the *S. cerevisiae* glucosidase II remains unknown. An orthologue of the glucosidase II beta subunit with a C-terminal KDEL-tag was found in *A. niger* and *A. nidulans* and was more of the mammalian/*S. pombe* type than of the *S. cerevisiae* type. Several sequences from both aspergilli, all encoding putative family 31 glycosyl hydrolases (Henrissat and Bairoch, 1993), showed high similarity towards the yeast Rot2p. However, in *A. niger*, only one of them (An09g05880, showing high similarity to the *A. nidulans* sequence AN11054.3) was almost completely identical, both at the amino acid as well as the nucleotide level, to the recently cloned functional glucosidase II alpha subunit from *A. niger* strain N402 (Geysens et al., in preparation).

Glucosidase II removes the two α-1,3-linked glucose residues in a sequential way (Hubbard and Robbins, 1979; Jakob et al., 1998), thereby first resulting in a monoglucosylated *N*-glycan intermediate that can interact with ER lectin chaperones such as the membrane-bound calnexin or, in higher eukaryotes, the soluble calreticulin (Helenius and Aebi, 2004; Ruddock and Molinari, 2006). Calnexin was previously cloned in *A. niger* (*clxA*) (Wang et al., 2003) and an orthologue was also identified in *A. nidulans*. However, both show a significantly higher sequence homology towards mammalian calnexin than towards the *S. cerevisiae* calnexin-like Cne1p (Parlati et al., 1995; Xu et al., 2004). There is no known calreticulin in fungi. The role of glucosidase, and the quality control cycle, is illustrated in Fig. 5.

Upon removal of the last glucose residue by glucosidase II, the glycoprotein is released from the calnexin cycle when it has obtained its correct three-dimensional structure. In contrast, when the glycoprotein is incompletely folded, its *N*-glycans will be reglucosylated by UDP-Glc:glycoprotein glucosyltransferase (UGGT), which serves as a folding sensor within the ER quality control mechanism (Caramelo et al., 2004). In this way, the protein can re-enter the calnexin cycle for another round of folding assistance. Functional UGGT has been characterized in mammalian cells (Arnold and Kaufman, 2003; Tessier et al., 2000) and in *S. pombe* (*gpt1*) (Fanchiotti et al., 1998; Fernandez et al., 1996), but not in *S. cerevisiae* (Fernandez et al., 1994). Indeed, the homologous yeast *KRE5* gene does not seem to encode such a functional glucosyltransferase (Meaden et al., 1990). In both *A. niger* and *A. nidulans*, a single gene showing homology to both yeast *KRE5* and *S. pombe gpt1* was identified. However, both aspergilli gene products showed significantly more similarity to the *S. pombe* UGGT, especially in the C-terminal part of the protein where the more conserved transferase domain is located (Guerin and Parodi, 2003). Together with the presence of a more mammalian-like calnexin, this is an indication that the aspergilli, in contrast to *S. cerevisiae*, might possess a better-equipped ER quality control mechanism.

Before reaching the Golgi, *N*-glycans on correctly folded glycoproteins undergo a last trimming reaction within the ER lumen. In *S. cerevisiae*, the α-1,2-mannosidase Mns1p removes a single mannose residue resulting in $Man_8GlcNAc_2$ (isomer B) (Fig. 4) (Burke et al., 1996; Camirand et al., 1991; Jelinek-Kelly and Herscovics, 1988), whereas in mammalian cells the activities of ER-mannosidase I (Gonzalez et al., 1999; Tremblay and Herscovics, 1999), ER-mannosidase II (Bischoff et al., 1990; Weng and Spiro, 1993, 1996) and/or Man9-mannosidase (Bause et al., 1992, 1993) can result in the formation of $Man_{6-8}GlcNAc_2$ structures. Yeast Mns1p, the mammalian ER-mannosidase I and the mammalian Man9-mannosidase show significant sequence homology with one another (around 30% identity on protein level) and belong to glycosyl hydrolase family 47. Several sequences homologous to this family of mannosidases, were found in both *Aspergillus* species. Three of the *A. nidulans* orthologues show more than 98% identity (on amino acid level) with gene products from the previously cloned *mds1A* (AN3733.3), *mds1B* (AN0787.3) and *mds1C* (AN3566.3) genes (Eades and Hintz, 2000). Two *A. nidulans* sequences (AN5748.3 and AN0551.3) and one corresponding sequence of *A. niger* (An18g06220) showed highest homology towards both yeast Mns1p and the mammalian ER-mannosidase I, but it remains speculative whether these genes actually encode for the aspergilli counterparts of the ER-localized α-1,2-mannosidases. Because of the homology of these orthologues with the Golgi-residing mammalian α-1,2-mannosidases, and based on the truncated high-mannose *N*-glycans observed on some of the secreted *Aspergillus* glycoproteins, several of the aspergilli gene products may very well reside within the fungal Golgi apparatus.

One orthologue of the ER-mannosidase II was identified in both *A. niger* and *A. nidulans*, the gene product of the latter one showing 98% identity with a previously characterized Class 2 α-mannosidase (*msd2*) (Eades et al., 1998). Evidence suggests that the ER-mannosidase II enters the ER lumen via translocation from the cytosplasm (Weng and Spiro, 1996) and that it is intimately involved with the cytosolic degradation of free oligosaccharides in the mammalian cell (Suzuki et al., 2006). However, since the corresponding yeast protein Ams1p is thought to be a vacuolar α-mannosidase (Yoshihisa and Anraku, 1989; Yoshihisa and Anraku, 1990), the exact localization and function of the aspergilli orthologues remains speculative.

Finally, when a glycoprotein fails to reach its correct conformation within a reasonable time, it will be removed from the ER lumen and degraded in the cytosol via ER-associated degradation (ERAD) (McCracken and Brodsky, 2003). A timer mechanism, depending on the slow trimming rate of *N*-glycans by ER-resident α-1,2-mannosidases, seems to define the amount of time that an incompletely folded glycoprotein has to obtain its correct structure before being degraded (Helenius, 1994). Hence, in *S. cerevisiae*, incompletely folded ER-resident glycoproteins with a $Man_8GlcNAc_2$ structure are considered as ERAD substrates (Jakob et al., 1998). Sorting of these misfolded glycoproteins is at least partially dependent on lectin-like proteins, able to recognize the trimmed *N*-glycans. In yeast, two different ERAD-associated lectins have been identified within the ER. The first one, Htm1p/Mnl1p (Jakob

et al., 2001; Nakatsukasa et al., 2001), has significant homology towards the α-1,2-mannosidases of glycosyl hydrolase family 47. To date, several functional mammalian homologues of this protein, designated EDEM1 to 3 (Hirao et al., 2006; Hosokawa et al., 2001; Mast et al., 2005; Olivari et al., 2005), have been identified as well. Yos9p, the other yeast lectin involved in ERAD, might play a role as bridging protein between the machinery that extracts misfolded proteins from the ER lumen and the machinery involved in their retro-translocation across the ER-membrane (Bhamidipati et al., 2005; Kim et al., 2005; Szathmary et al., 2005). Indeed, Yos9p was shown to be associated to the retro-translocation complex and contains a mannose-binding domain similar to the one observed for the members of the mannose-6-P receptor family (Ismail and Ng, 2006), hence enabling interaction with misfolded proteins through their partially trimmed *N*-glycans. In both *A. niger* and *A. nidulans*, one orthologue was found for yeast Hml1p/Mnl1p which, on the protein level, shows somewhat higher sequence identity towards human EDEM1 and EDEM2. BlastP analysis using yeast Yos9p also resulted in one hit, albeit with a rather low overall sequence similarity, for each *Aspergillus* species. Whereas Yos9p and the putative *A. nidulans* orthologue contain a C-terminal HDEL-like tag, the *A. niger* variant does not.

12. Golgi processing of *N*-glycans

Upon entry of a correctly folded glycoprotein within the mammalian Golgi apparatus, the high-mannose structure is further trimmed to $Man_5GlcNAc_2$ by Golgi-localized α-1,2-mannosidases (Lal et al., 1998; Tremblay and Herscovics, 2000). In contrast, in *S. cerevisiae*, the ER-derived $Man_8GlcNAc_2$ structure becomes elongated with extra mannose residues, which can eventually result in hypermannosylation of glycoproteins (Dean, 1999; Herscovics and Orlean, 1993), as illustrated in Fig. 5.

As already mentioned, several N-linked glycans found on glycoproteins secreted by *Aspergillus* species, are the result of further hydrolysis of the ER $Man_8GlcNAc_2$ structure by α-1,2- and even α-1,3/6-mannosidase activities. With regard to the trimming of α-1,2-mannose residues, the identification and cloning of several orthologues for glycosyl hydrolase family 47 α-1,2-mannosidases (Eades and Hintz, 2000) might indicate that the aspergilli, unlike *S. cerevisiae*, have a Golgi enzymatic system similar to that of mammalian cells. In this regard, the characterization has been reported of an *A. oryzae* microsomal enzyme able to convert $Man_9GlcNAc_2$ into $Man_8GlcNAc_2$, and of an *A. oryzae* broad-specificity α-1,2-mannosidase (encoded by *fmanIB*) with a vesicular localization that differs from the ER (Akao et al., 2006; Yoshida et al., 2000). Finally, one cannot exclude that extracellular hydrolases, whose secretion into the medium might depend on the applied cultivation conditions, are responsible for certain post-secretional modifications on protein-bound *N*-glycans (Stals et al., 2004b). In this respect, also five open reading frames encoding putative mannosidases from glycosyl hydrolase family 92 were found for both aspergilli. As for the other mannosidase orthologues, further research will be necessary to establish whether these sequences actually encode a functional enzyme and whether these activities act, either intra- or extracellularly, on protein-linked *N*-glycans.

In *S. cerevisiae*, elongation of the $Man_8GlcNAc_2$ structure is initiated via the action of Och1p, the initiating α-1,6-mannosyltransferase. This enzyme catalyzes the addition of an α-1,6-mannose onto the α-1,3-linked mannose of the trimannosyl core (Nakayama et al., 1992). Further extension of this α-1,6-linked mannose residue to an α-1,6-mannose backbone of up to 50 residues occurs via the sequential activity of two mannosyltransferase complexes, M-Pol I and M-Pol II (Hashimoto and Yoda, 1997; Jungmann and Munro, 1998; Jungmann et al., 1999; Kojima et al., 1999). M-Pol I consists of two proteins, Mnn9p and Van1p, with Mnn9p presumably responsible for adding the second α-1,6-linked mannose residue of the backbone, as such generating a substrate that can be elongated by Van1p to up to 15 mannoses (Stolz and Munro, 2002). M-Pol II is a complex of Mnn9p, Mnn8p/Anp1p, Mnn10p/Bed1p, Mnn11p and Hoc1p and is responsible for finishing off the α-1,6-mannose backbone (Dean and Poster, 1996; Hashimoto and Yoda, 1997; Jungmann et al., 1999; Neiman et al., 1997). However, the precise functions of each of the individual components within this complex remain unclear. Furthermore, Och1p and Hoc1p share significant sequence homology with one another, and so do Anp1p, Van1p and Mnn9p. Via Blast analysis, several orthologues from the *S. cerevisiae* hyperglycosylation machinery have been identified in the two *Aspergillus* species. Five and seven coding sequences, showing varying homology to both *OCH1* and *HOC1*, could be established in *A. nidulans* and *A. niger*, respectively. Similarly, three orthologues for the homologous *ANP1/VAN1/MNN9* genes were found in both aspergilli. Finally, in both species we could also identify an orthologue for *MNN10* and for *MNN11*. Hence, since the addition of extra α-1,6-mannose residues on small high-mannose *N*-glycan structures as well as limited hypermannosylation have been reported in aspergilli, one or more of these orthologues might encode the necessary functional *Aspergillus* α-1,6-mannosyltransferases, either acting in a single way or in a coordinated manner via assembly into an enzyme complex. Alternatively, some of those genes might also be involved in *O*-glycosylation since, contrary to what is found in *S. cerevisiae*, α-1,6-linked mannoses are also regularly found on *Aspergillus O*-glycans (Goto, 2007).

During hyperglycosylation in *S. cerevisiae*, the α-1,6-mannose backbone is further modified by the addition of branches consisting of two α-1,2-linked mannose residues, added sequentially by Mnn2p and Mnn5p (Rayner and Munro, 1998), and a terminal α-1,3-linked mannose residue that is added by Mnn1p (Yip et al., 1994). The same two and three orthologues were identified in *A. nidulans* and *A. niger*, respectively, for both α-1,2-mannosyltransferases. In contrast, one gene showing homology to both *MNN1* and *MNT2* (which is a second member of the *MNN1* family) was established in *A. niger* but not in *A. nidulans*. With respect to some of the characterized *N*-glycans on *A. niger* glycoproteins (carrying extra α-1,2-mannoses on a $Man_{8-9}GlcNAc_2$ backbone or α-1,3-linked mannose residues on a $Man_5GlcNAc_2$ structure), several of the orthologues most probably encode a corresponding functional mannosyltransferase, although a few of them might be acting on a somewhat different acceptor substrate when compared to the process of *N*-glycan maturation in *S. cerevisiae*.

N-Glycan phosphomannosylation via the addition of a mannosephosphate residue in a diester linkage is well documented for *S. cerevisiae* (Ballou et al., 1990; Frevert and Ballou, 1982; Hernandez et al., 1989, 1992; Jigami and Odani, 1999) and the filamentous fungus *T. reesei* (De Bruyn et al., 1997; Garcia et al., 2001; Harrison et al., 2002; Hui et al., 2001; Maras et al., 1997a,b; Stals et al., 2004a), but was thus far only rarely observed in the aspergilli. Two genes from *S. cerevisiae* are known to be intimately involved in this glycan modification step, although their exact functions are still not completely understood. *MNN6* belongs to the *KRE2/MNT1* gene family (encoding several α-1,2-mannosyltransferases involved in *O*- and to lesser extent also *N*-glycosylation) and is assumed to code for a phosphomannosyltransferase (Wang et al., 1997), while *MNN4* has been described to encode a positive regulator for glycan phosphomannosylation (Odani et al., 1996, 1997). More recently, a gene called *PNO1*, specifically involved in phosphomannosylation of small high-mannose core structures (i.e. no hyperglycosyl structures) was described for *P. pastoris* (Miura et al., 2004). Although this open reading frame has some sequence similarity with *S. cerevisiae MNN4*, its gene

product is significantly smaller (777 versus 1178 amino acids) and lacks the C-terminal KE-rich repeat, typical for yeast Mnn4p. In both *A. niger* and *A. nidulans*, two orthologues were identified, sharing similarity towards the *MNN4* and the *PNO1* genes. Although the aspergilli sequences encode much smaller gene products (between 299 and 364 amino acids), significant conservation was observed in a 150 amino acid stretch containing the G[T/S]hhGhhxxxx hhxaxxDxD motif of the Fukutin protein family, suggested it to be involved in the transfer of sugarphosphate, and not only the monosaccharide itself, from a sugar nucleotide donor substrate (Aravind and Koonin, 1999).

Whereas termination of *N*-glycans with mannosephosphate or α-1,3-mannose residues is a common feature in *S. cerevisiae*, the oligosaccharides of some other fungal species are characterized by different capping residues such as α-1,2-GlcNAc in *Kluyveromyces lactis* (Raschke and Ballou, 1972), and α-1,2-linked Gal and β-1,3-linked pyruvylated Gal in *S. pombe* (Gemmill and Trimble, 1999). The gene encoding the *K. lactis* α-1,2-GlcNAc-transferase has been cloned (Guillen et al., 1999) and more recently a homologue (*GNT1*) was also identified in *S. cerevisiae* (Yoko-o et al., 2003). Within specific *N*-glycosylation mutants, Gnt1p was shown to be involved in the transfer of a GlcNAc residue onto the single *N*-glycan present on hen egg lysozyme (G49N). Deletion of *GNT1* resulted in loss of GlcNAc-transferase activity but had no obvious effects on growth or cell wall composition (Yoko-o et al., 2003). Two orthologues with low sequence homology were found for the yeast Gnt1p in both *A. niger* and *A. nidulans*. However, because of this low similarity and because of the fact that terminal α-linked GlcNAc residues have not yet been characterized on *Aspergillus N*- or *O*-glycans, it remains questionable whether they actually code for a functional transferase.

Recently, *pvg3* was shown to encode the *S. pombe* β-1,3-galactosyltransferase (Andreishcheva et al., 2004), but no *Aspergillus* orthologues could be identified so far. The genes known to encode α-1,2-galactosyltransferases in *S. pombe* belong to the *GMA12/MNN10* family (Chappell et al., 1994; Yoko-o et al., 1998). Hence, the two open reading frames of both *Aspergillus* species, previously identified as being homologous to *S. cerevisiae MNN10* and *MNN11*, were also found to be orthologues of the *S. pombe* galactosyltransferase genes. With an identity of ±40% at the amino acid level, the aspergilli sequences AN7562.3 and An15g03330 are clearly most related to yeast *MNN10*, whereas AN1969.3 and An04g05940 are approximately equally homologous to *MNN11* on the one hand and the *S. pombe* α-1,2-galactosyltransferase genes on the other. Since α-1,3-linked galactopyranose residues have been identified on *Aspergillus O*-glycans (Goto, 2007) (but not *N*-glycans), the possibility that these fungi encode for one ore more α-galactosyltransferases can certainly not be excluded.

In contrast to *O*-glycosylation, transfer of galactose residues to aspergilli *N*-glycans has only been observed for the furanose form. However, although several bacterial and trypanosomatid sequences have been deposited, no orthologues for these two different classes of galactofuranosyltransferases (Gal*f*T) could thus far be identified within filamentous fungi. This might indicate that the fungal Gal*f*Ts have yet another structure compared to both the bacterial and the trypanosomatid transferases.

Finally, the presence of terminal α-1,3-linked glucose was also established on *Aspergillus N*-glycans (Takayanagi et al., 1992, 1994). Rather than being the result of an extra glucosyltransferase activity, the glucosylated oligosaccharide—based on its structure and low abundancy in the total *N*-glycan pool—most probably reflects the fact that glucosidase II trimming was at its limit. This is in strong contrast with some *T. reesei* strains, where the predominant presence of monoglucosylated *N*-glycans is the result of a partially inactivating frame-shift mutation in the gene encoding the catalytic glucosidase II alpha subunit (Geysens et al., 2005).

13. Concluding remarks

The process of glycoprotein secretion has similarities in all eukaryotes. While the yeast *S. cerevisiae* has proven to be a useful model for investigations of the detail of protein secretion, closer inspection of parallels in other eukaryotes, and even among closely related fungal species, has revealed both similarities and differences. In higher eukaryotes, the secretion of proteins is important because defects may lead to a number of so-called protein-folding diseases (Luheshi et al., 2008; Lin et al., 2008). Even though protein secretion stress responses are more versatile in mammalian cells than they are in fungi, detailed analysis of fungal systems can serve as useful models for further exploration in higher eukaryotes. There are several reasons for studying protein secretion by *Aspergillus* spp. Firstly, the filamentous fungi are not budding yeasts and their biology merits study because of their importance in nature. They are more complex organisms than *S. cerevisiae*, the most obvious manifestation being the hypha, and exemplified by the sequenced *Aspergillus* spp. having about twice the number of predicted genes as found in *S. cerevisiae*. Some species are pathogenic (e.g. *A. fumigatus*) while others are used in biotechnology (e.g. *A. niger*, *A. oryzae* and *Aspergillus terreus*). The secretion of proteins is important for hyphal extension, degradation of plant polymers in natural ecosystems, pathogenicity and for biotechnological exploitation. Exploration of secretion processes that builds upon the genome sequences and post-genomic technologies is in its infancy but is rapidly advancing and offers promise of both greater understanding and improved applications.

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References

Aebi, M., Gassenhuber, J., Domdey, H., te Heesen, S., 1996. Cloning and characterization of the *ALG3* gene of *Saccharomyces cerevisiae*. Glycobiology 6, 439–444.

Akao, T., Yamaguchi, M., Yahara, A., Yoshiuchi, K., Fujita, H., Yamada, O., Akita, O., Ohmachi, T., Asada, Y., Yoshida, T., 2006. Cloning and expression of 1,2-alpha-mannosidase gene (*fmanIB*) from filamentous fungus *Aspergillus oryzae*: in vivo visualization of the FmanIBp-GFP fusion protein. Biosci. Biotechnol. Biochem. 70, 471–479.

Al-Sheikh, H., Watson, A.J.H., Lacey, G.A., Punt, P.J., MacKenzie, D.A., Jeenes, D.J., Pakula, T., Penttilä, M., Alcocer, M.J.C., Archer, D.B., 2004. Endoplasmic reticulum stress leads to the selective transcriptional downregulation of the glucoamylase gene in *Aspergillus niger*. Mol. Microbiol. 53, 1731–1742.

Amano, J., Kobata, A., 1986. Purification and characterization of a novel alpha-mannosidase from *Aspergillus saitoi*. J. Biochem. 99, 1645–1654.

Anand, M., Rush, J.S., Ray, S., Doucey, M.A., Weik, J., Ware, F.E., Hofsteenge, J., Waechter, C.J., Lehrman, M.A., 2001. Requirement of the *Lec35* gene for all known classes of monosaccharide-P-dolichol-dependent glycosyltransferase reactions in mammals. Mol. Biol. Cell 12, 487–501.

Andreishcheva, E.N., Kunkel, J.P., Gemmill, T.R., Trimble, R.B., 2004. Five genes involved in biosynthesis of the pyruvylated Galbeta1,3-epitope in *Schizosaccharomyces pombe* N-linked glycans. J. Biol. Chem. 279, 35644–35655.

Aravind, L., Koonin, E.V., 1999. The fukutin protein family-predicted enzymes modifying cell-surface molecules. Curr. Biol. 9, R836–R837.

Archer, D.B., Turner, G., 2006. Genomics of protein secretion and hyphal growth in *Aspergillus*. In: Brown, A. (Ed.), The Mycota XIII Fungal Genomics. Springer, pp. 75–96 (Chapter 5).

Arendt, C.W., Ostergaard, H.L., 1997. Identification of the CD45-associated 116-kDa and 80-kDa proteins as the alpha- and beta-subunits of alpha-glucosidase II. J. Biol. Chem. 272, 13117–13125.

Arnold, S.M., Kaufman, R.J., 2003. The noncatalytic portion of human UDP-glucose: glycoprotein glucosyltransferase I confers UDP-glucose binding and transferase function to the catalytic domain. J. Biol. Chem. 278, 43320–43328.

Arvas, M., Pakula, T., Lanthaler, K., Saloheimo, M., Valkonen, M., et al., 2006. Common features and interesting differences in transcriptional responses to secretion stress in the fungi *Trichoderma reesei* and *Saccharomyces cerevisiae*. BMC Genomics 7, 32.

Bakker, H., Kleczka, B., Gerardy-Schahn, R., Routier, F.H., 2005. Identification and partial characterization of two eukaryotic UDP-galactopyranose mutases. Biol. Chem. 386, 657–661.

Ballou, L., Hernandez, L.M., Alvarado, E., Ballou, C.E., 1990. Revision of the oligosaccharide structures of yeast carboxypeptidase Y. Proc. Natl. Acad. Sci. USA 87, 3368–3372.

Bause, E., Bieberich, E., Rolfs, A., Volker, C., Schmidt, B., 1993. Molecular cloning and primary structure of Man9-mannosidase from human kidney. Eur. J. Biochem. 217, 535–540.

Bause, E., Breuer, W., Schweden, J., Roeser, R., Geyer, R., 1992. Effect of substrate structure on the activity of Man9-mannosidase from pig liver involved in N-linked oligosaccharide processing. Eur. J. Biochem. 208, 451–457.

Benton, B.K., Plump, S.D., Roos, J., Lennarz, W.J., Cross, F.R., 1996. Over-expression of *S. cerevisiae* G1 cyclins restores the viability of *alg1 N*-glycosylation mutants. Curr. Genet. 29, 106–113.

Bertolotti, A., Zhang, Y.H., Hendershot, L.M., Harding, H.P., Ron, D., 2000. Dynamic interaction of BiP and ER stress transducers in the unfolded protein response. Nat. Cell Biol. 2, 326–332.

Bhamidipati, A., Denic, V., Quan, E.M., Weissman, J.S., 2005. Exploration of the topological requirements of ERAD identifies Yos9p as a lectin sensor of misfolded glycoproteins in the ER lumen. Mol. Cell 19, 741–751.

Bickel, T., Lehle, L., Schwarz, M., Aebi, M., Jakob, C.A., 2005. Biosynthesis of lipid-linked oligosaccharides in *Saccharomyces cerevisiae*: Alg13p and Alg14p form a complex required for the formation of GlcNAc(2)-PP-dolichol. J. Biol. Chem. 280, 34500–34506.

Bischoff, J., Moremen, K., Lodish, H.F., 1990. Isolation, characterization, and expression of cDNA encoding a rat liver endoplasmic reticulum alpha-mannosidase. J. Biol. Chem. 265, 17110–17117.

Boles, E., Liebetrau, W., Hofmann, M., Zimmermann, F.K., 1994. A family of hexosephosphate mutases in *Saccharomyces cerevisiae*. Eur. J. Biochem. 220, 83–96.

Bukau, B., Weissman, J., Horwich, A., 2006. Molecular chaperones and protein quality control. Cell 125, 443–451.

Bulter, T., Elling, L., 1999. Enzymatic synthesis of nucleotide sugars. Glycoconj. J. 16, 147–159.

Burda, P., Aebi, M., 1998. The *ALG10* locus of *Saccharomyces cerevisiae* encodes the alpha-1,2 glucosyltransferase of the endoplasmic reticulum: the terminal glucose of the lipid-linked oligosaccharide is required for efficient N-linked glycosylation. Glycobiology 8, 455–462.

Burda, P., Aebi, M., 1999. The dolichol pathway of N-linked glycosylation. Biochim. Biophys. Acta 1426, 239–257.

Burda, P., te Heesen, S., Brachat, A., Wach, A., Dusterhoft, A., Aebi, M., 1996. Stepwise assembly of the lipid-linked oligosaccharide in the endoplasmic reticulum of *Saccharomyces cerevisiae*: identification of the *ALG9* gene encoding a putative mannosyl transferase. Proc. Natl. Acad. Sci. USA 93, 7160–7165.

Burke, J., Lipari, F., Igdoura, S., Herscovics, A., 1996. The *Saccharomyces cerevisiae* processing alpha 1,2-mannosidase is localized in the endoplasmic reticulum, independently of known retrieval motifs. Eur. J. Cell Biol. 70, 298–305.

Camirand, A., Heysen, A., Grondin, B., Herscovics, A., 1991. Glycoprotein biosynthesis in *Saccharomyces cerevisiae*. Isolation and characterization of the gene encoding a specific processing alpha-mannosidase. J. Biol. Chem. 266, 15120–15127.

Caramelo, J.J., Castro, O.A., de Prat-Gay, G., Parodi, A.J., 2004. The endoplasmic reticulum glucosyltransferase recognizes nearly native glycoprotein folding intermediates. J. Biol. Chem. 279, 46280–46285.

Castrillo, J.I., Zeef, L.A., Hoyle, D.C., Zhang, N., Hayes, A., et al., 2007. Growth control of the eukaryotic cell: a systems biology study in yeast. J. Biol. 6, 4.

Chantret, I., Dancourt, J., Barbat, A., Moore, S.E., 2005. Two proteins homologous to the N- and C-terminal domains of the bacterial glycosyltransferase Murg are required for the second step of dolichyl-linked oligosaccharide synthesis in *Saccharomyces cerevisiae*. J. Biol. Chem. 280, 9236–9242.

Chappell, T.G., Hajibagheri, M.A., Ayscough, K., Pierce, M., Warren, G., 1994. Localization of an alpha 1,2 galactosyltransferase activity to the Golgi apparatus of *Schizosaccharomyces pombe*. Mol. Biol. Cell 5, 519–528.

Chiba, Y., Yamagata, Y., Iijima, S., Nakajima, T., Ichishima, E., 1993. The carbohydrate moiety of the acid carboxypeptidase from *Aspergillus saitoi*. Curr. Microbiol. 27, 281–288.

Cipollo, J.F., Trimble, R.B., Chi, J.H., Yan, Q., Dean, N., 2001. The yeast *ALG11* gene specifies addition of the terminal alpha 1,2-Man to the Man5GlcNAc2-PP-dolichol *N*-glycosylation intermediate formed on the cytosolic side of the endoplasmic reticulum. J. Biol. Chem. 276, 21828–21840.

Colangelo, J., Licon, V., Benen, J., Visser, J., Bergmann, C., Orlando, R., 1999a. Characterization of the glycosylation of recombinant endopolygalacturonase I from *Aspergillus niger*. Rapid Commun. Mass Spectrom. 13, 1448–1453.

Colangelo, J., Licon, V., Benen, J., Visser, J., Bergmann, C., Orlando, R., 1999b. Characterization of the N-linked glycosylation site of recombinant pectate lyase. Rapid Commun. Mass Spectrom. 13, 2382–2387.

Colussi, P.A., Taron, C.H., Mack, J.C., Orlean, P., 1997. Human and *Saccharomyces cerevisiae* dolichol phosphate mannose synthases represent two classes of the enzyme, but both function in *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. USA 94, 7873–7878.

Couto, J.R., Huffaker, T.C., Robbins, P.W., 1984. Cloning and expression in *Escherichia coli* of a yeast mannosyltransferase from the asparagine-linked glycosylation pathway. J. Biol. Chem. 259, 378–382.

Credle, J.J., Finer-Moore, J.S., Papa, F.R., Stroud, R.M., Walter, P., 2005. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA 102, 18773–18784.

D'Alessio, C., Fernandez, F., Trombetta, E.S., Parodi, A.J., 1999. Genetic evidence for the heterodimeric structure of glucosidase II. The effect of disrupting the subunit-encoding genes on glycoprotein folding. J. Biol. Chem. 274, 25899–25905.

Daran, J.M., Dallies, N., Thines-Sempoux, D., Paquet, V., Francois, J., 1995. Genetic and biochemical characterization of the *UGP1* gene encoding the UDP-glucose pyrophosphorylase from *Saccharomyces cerevisiae*. Eur. J. Biochem. 233, 520–530.

De Bruyn, A., Maras, M., Schraml, J., Herdewijn, P., Contreras, R., 1997. NMR evidence for a novel asparagine-linked oligosaccharide on cellobiohydrolase I from *Trichoderma reesei* RUTC 30. FEBS Lett. 405, 111–113.

Dean, N., 1999. Asparagine-linked glycosylation in the yeast Golgi. Biochim. Biophys. Acta 1426, 309–322.

Dean, N., Poster, J.B., 1996. Molecular and phenotypic analysis of the *S. cerevisiae* MNN10 gene identifies a family of related glycosyltransferases. Glycobiology 6, 73–81.

Dean, N., Zhang, Y.B., Poster, J.B., 1997. The *VRG4* gene is required for GDP-mannose transport into the lumen of the Golgi in the yeast, *Saccharomyces cerevisiae*. J. Biol. Chem. 272, 31908–31914.

Dempski Jr., R.E., Imperiali, B., 2004. Heterologous expression and biophysical characterization of soluble oligosaccharyl transferase subunits. Arch. Biochem. Biophys. 431, 63–70.

Deprez, P., Gautschi, M., Helenius, A., 2005. More than one glycan is needed for ER glucosidase II to allow entry of glycoproteins into the calnexin/calreticulin cycle. Mol. Cell 19, 183–195.

Derkx, P.M.F., Madrid, S.M., 2001. The foldase CYPB is a component of the secretory pathway of *Aspergillus niger* and contains the endoplasmic retention signal HEEL. Mol. Genet. Genomics 266, 537–545.

Dickinson, J.R., Smith, M.E., Swanson, T.R., Williams, A.S., Wingfield, J.M., 1988. The *cdc30* mutation in *Saccharomyces cerevisiae* affects phosphoglucose isomerase, the cell cycle and sporulation. J. Gen. Microbiol. 134, 2475–2480.

Eades, C.J., Gilbert, A.M., Goodman, C.D., Hintz, W.E., 1998. Identification and analysis of a class 2 alpha-mannosidase from *Aspergillus nidulans*. Glycobiology 8, 17–33.

Eades, C.J., Hintz, W.E., 2000. Characterization of the class I alpha-mannosidase gene family in the filamentous fungus *Aspergillus nidulans*. Gene 255, 25–34.

Endo, T., 2004. Structure, function and pathology of *O*-mannosyl glycans. Glycoconj. J. 21, 3–7.

Esmon, B., Esmon, P.C., Schekman, R., 1984. Early steps in processing of yeast glycoproteins. J. Biol. Chem. 259, 10322–10327.

Fanchiotti, S., Fernandez, F., D'Alessio, C., Parodi, A.J., 1998. The UDP-Glc:glycoprotein glucosyltransferase is essential for *Schizosaccharomyces pombe* viability under conditions of extreme endoplasmic reticulum stress. J. Cell Biol. 143, 625–635.

Fernandez, F., Jannatipour, M., Hellman, U., Rokeach, L.A., Parodi, A.J., 1996. A new stress protein: synthesis of *Schizosaccharomyces pombe* UDP-Glc:glycoprotein glucosyltransferase mRNA is induced by stress conditions but the enzyme is not essential for cell viability. EMBO J. 15, 705–713.

Fernandez, F.S., Trombetta, S.E., Hellman, U., Parodi, A.J., 1994. Purification to homogeneity of UDP-glucose:glycoprotein glucosyltransferase from *Schizosaccharomyces pombe* and apparent absence of the enzyme from *Saccharomyces cerevisiae*. J. Biol. Chem. 269, 30701–30706.

Frank, C.G., Aebi, M., 2005. *ALG9* mannosyltransferase is involved in two different steps of lipid-linked oligosaccharide biosynthesis. Glycobiology 15, 1156–1163.

Frevert, J., Ballou, C.E., 1982. Yeast invertase polymorphism is correlated with variable states of oligosaccharide chain phosphorylation. Proc. Natl. Acad. Sci. USA 79, 6147–6150.

Ftouhi-Paquin, N., Hauer, C.R., Stack, R.F., Tarentino, A.L., Plummer Jr., T.H., 1997. Molecular cloning, primary structure, and properties of a new glycoamidase from the fungus *Aspergillus tubigensis*. J. Biol. Chem. 272, 22960–22965.

Gaikwad, S.M., Keskar, S.S., Khan, M.I., 1995. Purification and characterization of alpha-D-mannosidase from *Aspergillus* sp.. Biochim. Biophys. Acta 1250, 144–148.

Galagan, J.E., Calvo, S.E., Cuomo, C., Ma, L.-J., Wortman, J.R., et al., 2005. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature 438, 1105–1115.

Gao, X.D., Kaigorodov, V., Jigami, Y., 1999. *YND1*, a homologue of *GDA1*, encodes membrane-bound apyrase required for Golgi *N*- and *O*-glycosylation in *Saccharomyces cerevisiae*. J. Biol. Chem. 274, 21450–21456.

Gao, X.D., Tachikawa, H., Sato, T., Jigami, Y., Dean, N., 2005. Alg14 recruits Alg13 to the cytoplasmic face of the endoplasmic reticulum to form a novel bipartite UDP-*N*-acetylglucosamine transferase required for the second step of N-linked glycosylation. J. Biol. Chem. 280, 36254–36262.

Garcia, R., Cremata, J.A., Quintero, O., Montesino, R., Benkestock, K., Stahlberg, J., 2001. Characterization of protein glycoforms with N-linked neutral and phosphorylated oligosaccharides: studies on the glycosylation of endoglucanase 1 (Cel7B) from *Trichoderma reesei*. Biotechnol. Appl. Biochem. 33, 141–152.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., et al., 2000. Genomic expression programs in the response of yeast cells to environmental changes. Mol. Biol. Cell 11, 4241–4257.

Gasser, B., Maurer, M., Rautio, J., Sauer, M., Bhattacharyya, A., et al., 2007. Monitoring of transcriptional regulation in *Pichia pastoris* under protein production conditions. BMC Genomics 8, 179.

Gemmill, T.R., Trimble, R.B., 1999. Overview of N- and O-linked oligosaccharide structures found in various yeast species. Biochim. Biophys. Acta 1426, 227–237.

Geysens, S., Pakula, T., Uusitalo, J., Dewerte, I., Penttila, M., Contreras, R., 2005. Cloning and characterization of the glucosidase II alpha subunit gene of *Trichoderma reesei*: a frameshift mutation results in the aberrant glycosylation profile of the hypercellulolytic strain Rut-C30. Appl. Environ. Microbiol. 71, 2910–2924.

Gonzalez, D.S., Karaveg, K., Vandersall-Nairn, A.S., Lal, A., Moremen, K.W., 1999. Identification, expression, and characterization of a cDNA encoding human endoplasmic reticulum mannosidase I, the enzyme that catalyzes the first mannose trimming step in mammalian Asn-linked oligosaccharide biosynthesis. J. Biol. Chem. 274, 21375–21386.

Goto, M., 2007. Protein *O*-glycosylation in fungi: diverse structures and multiple functions. Biosci. Biotechnol. Biochem. 71, 1415–1427.

Graf, A., Gasser, B., Dragosits, M., Sauer, M., Leparc, G., et al., 2008. Novel insights into the unfolded protein response using *Pichia pastoris* specific DNA microarrays. BMC Genomics 9:390.

Grubenmann, C.E., Frank, C.G., Kjaergaard, S., Berger, E.G., Aebi, M., Hennet, T., 2002. *ALG12* mannosyltransferase defect in congenital disorder of glycosylation type lg. Hum. Mol. Genet. 11, 2331–2339.

Gruber, C.W., Cemazar, M., Heras, B., Martin, J.L., Craik, D.J., 2006. Protein disulphide isomerase: the structure of oxidative folding. Trends Biochem. Sci. 31, 455–464.

Guerin, M., Parodi, A.J., 2003. The UDP-glucose:glycoprotein glucosyltransferase is organized in at least two tightly bound domains from yeast to mammals. J. Biol. Chem. 278, 20540–20546.

Guillemette, T., van Peij, N.N.M.E., Goosen, T., Lanthaler, K., Robson, G.D., van den Hondel, C.A.M.J.J., Stam, H., Archer, D.B., 2007. Genomic analysis of the secretion stress response in the enzyme-producing cell factory *Aspergillus niger*. BMC Genomics 8, 158.

Guillen, E., Abeijon, C., Hirschberg, C.B., 1999. The genes for the Golgi apparatus *N*-acetylglucosaminyltransferase and the UDP-*N*-acetylglucosamine transporter are contiguous in *Kluyveromyces lactis*. J. Biol. Chem. 274, 6641–6646.

Hanisch, F.G., 2001. *O*-Glycosylation of the mucin type. Biol. Chem. 382, 143–149.

Harrison, M.J., Wathugala, I.M., Tenkanen, M., Packer, N.H., Nevalainen, K.M., 2002. Glycosylation of acetylxylan esterase from *Trichoderma reesei*. Glycobiology 12, 291–298.

Hashimoto, H., Yoda, K., 1997. Novel membrane protein complexes for protein glycosylation in the yeast Golgi apparatus. Biochem. Biophys. Res. Commun. 241, 682–686.

Heesen, S., Lehle, L., Weissmann, A., Aebi, M., 1994. Isolation of the *ALG5* locus encoding the UDP-glucose:dolichyl-phosphate glucosyltransferase from *Saccharomyces cerevisiae*. Eur. J. Biochem. 224, 71–79.

Helenius, A., 1994. How N-linked oligosaccharides affect glycoprotein folding in the endoplasmic reticulum. Mol. Biol. Cell 5, 253–265.

Helenius, A., Aebi, M., 2004. Roles of N-linked glycans in the endoplasmic reticulum. Annu. Rev. Biochem. 73, 1019–1049.

Helenius, J., Ng, D.T., Marolda, C.L., Walter, P., Valvano, M.A., Aebi, M., 2002. Translocation of lipid-linked oligosaccharides across the ER membrane requires Rft1 protein. Nature 415, 447–450.

Henrissat, B., Bairoch, A., 1993. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. 293, 781–788.

Hernandez, L.M., Ballou, L., Alvarado, E., Tsai, P.K., Ballou, C.E., 1989. Structure of the phosphorylated N-linked oligosaccharides from the *mnn9* and *mnn10* mutants of *Saccharomyces cerevisiae*. J. Biol. Chem. 264, 13648–13659.

Hernandez, L.M., Olivero, I., Alvarado, E., Larriba, G., 1992. Oligosaccharide structures of the major exoglucanase secreted by *Saccharomyces cerevisiae*. Biochemistry 31, 9823–9831.

Herscovics, A., Orlean, P., 1993. Glycoprotein biosynthesis in yeast. FASEB J. 7, 540–550.

Hettkamp, H., Legler, G., Bause, E., 1984. Purification by affinity chromatography of glucosidase I, an endoplasmic reticulum hydrolase involved in the processing of asparagine-linked oligosaccharides. Eur. J. Biochem. 142, 85–90.

Hirao, K., Natsuka, Y., Tamura, T., Wada, I., Morito, D., Natsuka, S., Romero, P., Sleno, B., Tremblay, L.O., Herscovics, A., Nagata, K., Hosokawa, N., 2006. EDEM3, a soluble EDEM homolog, enhances glycoprotein endoplasmic reticulum-associated degradation and mannose trimming. J. Biol. Chem. 281, 9650–9658.

Hirschberg, C.B., Robbins, P.W., Abeijon, C., 1998. Transporters of nucleotide sugars, ATP, and nucleotide sulfate in the endoplasmic reticulum and Golgi apparatus. Annu. Rev. Biochem. 67, 49–69.

Hoffmann, B., LaPaglia, S.K., Kubler, E., Andermann, M., Eckert, S.E., Braus, G.H., 2000. Developmental and metabolic regulation of the phosphoglucomutase-encoding gene, *pgmB*, of *Aspergillus nidulans*. Mol. Gen. Genet. 262, 1001–1011.

Hofmann, M., Boles, E., Zimmermann, F.K., 1994. Characterization of the essential yeast gene encoding *N*-acetylglucosamine-phosphate mutase. Eur. J. Biochem. 221, 741–747.

Hosokawa, N., Wada, I., Hasegawa, K., Yorihuzi, T., Tremblay, L.O., Herscovics, A., Nagata, K., 2001. A novel ER alpha-mannosidase-like protein accelerates ER-associated degradation. EMBO Rep. 2, 415–422.

Hubbard, S.C., Robbins, P.W., 1979. Synthesis and processing of protein-linked oligosaccharides in vivo. J. Biol. Chem. 254, 4568–4576.

Huffaker, T.C., Robbins, P.W., 1983. Yeast mutants deficient in protein glycosylation. Proc. Natl. Acad. Sci. USA 80, 7466–7470.

Hui, J.P., Lanthier, P., White, T.C., McHugh, S.G., Yaguchi, M., Roy, R., Thibault, P., 2001. Characterization of cellobiohydrolase I (Cel7A) glycoforms from extracts of *Trichoderma reesei* using capillary isoelectric focusing and electrospray mass spectrometry. J. Chromatogr. B 752, 349–368.

Ichishima, E., 2000. Unique catalytic and molecular properties of hydrolases from *Aspergillus* used in Japanese bioindustries. Biosci. Biotechnol. Biochem. 64, 675–688.

Ichishima, E., Arai, M., Shigematsu, Y., Kumagai, H., Sumida-Tanaka, R., 1981. Purification of an acidic alpha-D-mannosidase from *Aspergillus saitoi* and specific cleavage of 1,2-alpha-D-mannosidic linkage in yeast mannan. Biochim. Biophys. Acta 658, 45–53.

Inoue, T., Yoshida, T., Ichishima, E., 1995. Molecular cloning and nucleotide sequence of the 1,2-alpha-D-mannosidase gene, *msdS*, from *Aspergillus saitoi* and expression of the gene in yeast cells. Biochim. Biophys. Acta 1253, 141–145.

Ismail, N., Ng, D.T., 2006. Have you HRD? Understanding ERAD is DOAble! Cell 126, 237–239.

Jackson, B.J., Kukuruzinska, M.A., Robbins, P., 1993. Biosynthesis of asparagine-linked oligosaccharides in *Saccharomyces cerevisiae*: the *alg2* mutation. Glycobiology 3, 357–364.

Jakob, C.A., Bodmer, D., Spirig, U., Battig, P., Marcil, A., Dignard, D., Bergeron, J.J., Thomas, D.Y., Aebi, M., 2001. Htm1p, a mannosidase-like protein, is involved in glycoprotein degradation in yeast. EMBO Rep. 2, 423–430.

Jakob, C.A., Burda, P., Roth, J., Aebi, M., 1998. Degradation of misfolded endoplasmic reticulum glycoproteins in *Saccharomyces cerevisiae* is determined by a specific oligosaccharide structure. J. Cell Biol. 142, 1223–1233.

Jeenes, D.J., Pfaller, R., Archer, D.B., 1997. Isolation and characterisation of a novel stress-inducible PDI-family gene from *Aspergillus niger*. Gene 193, 151–156.

Jelinek-Kelly, S., Herscovics, A., 1988. Glycoprotein biosynthesis in *Saccharomyces cerevisiae*. Purification of the alpha-mannosidase which removes one specific mannose residue from Man9GlcNAc. J. Biol. Chem. 263, 14757–14763.

Jigami, Y., Odani, T., 1999. Mannosylphosphate transfer to yeast mannan. Biochim. Biophys. Acta 1426, 335–345.

Jungmann, J., Munro, S., 1998. Multi-protein complexes in the *cis* Golgi of *Saccharomyces cerevisiae* with alpha-1,6-mannosyltransferase activity. EMBO J. 17, 423–434.

Jungmann, J., Rayner, J.C., Munro, S., 1999. The *Saccharomyces cerevisiae* protein Mnn10p/Bed1p is a subunit of a Golgi mannosyltransferase complex. J. Biol. Chem. 274, 6579–6585.

Kalz-Fuller, B., Bieberich, E., Bause, E., 1995. Cloning and expression of glucosidase I from human hippocampus. Eur. J. Biochem. 231, 344–351.

Kepes, F., Schekman, R., 1988. The yeast *SEC53* gene encodes phosphomannomutase. J. Biol. Chem. 263, 9155–9161.

Kilker Jr., R.D., Saunier, B., Tkacz, J.S., Herscovics, A., 1981. Partial purification from *Saccharomyces cerevisiae* of a soluble glucosidase which removes the terminal glucose from the oligosaccharide Glc3Man9GlcNAc2. J. Biol. Chem. 256, 5299–5603.

Kim, W., Spear, E.D., Ng, D.T., 2005. Yos9p detects and targets misfolded glycoproteins for ER-associated degradation. Mol. Cell 19, 753–764.

Kimata, Y., Ishiwata-Kimata, Y., Yamada, S., Kohno, K., 2006. Yeast unfolded protein response pathway regulates expression of genes for antioxidative stress and for cell surface proteins. Genes Cells 11, 59–69.

Kimata, Y., Ishiwata-Kimata, Y., Ito, T., Hirata, A., Suzuki, T., Oikawa, D., Takeuchi, M., Kohno, K., 2007. Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins. J. Cell Biol. 179, 75–86.

Kimura, T., Hosoda, Y., Sato, Y., Kitamura, Y., Ikeda, T., Horibe, T., Kikuchi, M., 2005. Interactions among yeast protein disulphide isomerase proteins and endoplasmic reticulum chaperone proteins influence their activities. J. Biol. Chem. 280, 31438–31441.

Knauer, R., Lehle, L., 1999. The oligosaccharyltransferase complex from yeast. Biochim. Biophys. Acta 1426, 259–273.

Kojima, H., Hashimoto, H., Yoda, K., 1999. Interaction among the subunits of Golgi membrane mannosyltransferase complexes of the yeast *Saccharomyces cerevisiae*. Biosci. Biotechnol. Biochem. 63, 1970–1976.

Kornfeld, R., Kornfeld, S., 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54, 631–664.

Kranz, C., Denecke, J., Lehrman, M.A., Ray, S., Kienz, P., Kreissel, G., Sagi, D., Peter-Katalinic, J., Freeze, H.H., Schmid, T., Jackowski-Dohrmann, S., Harms, E., Marquardt, T., 2001. A mutation in the human *MPDU1* gene causes congenital disorder of glycosylation type If (CDG-If). J. Clin. Invest. 108, 1613–1619.

Kruszewska, J.S., Saloheimo, M., Migdalski, A., Orlean, P., Penttila, M., Palamarczyk, G., 2000. Dolichol phosphate mannose synthase from the filamentous fungus *Trichoderma reesei* belongs to the human and *Schizosaccharomyces pombe* class of the enzyme. Glycobiology 10, 983–991.

Kuhn, K.M., DeRisi, J.L., Brown, P.O., Sarnow, P., 2001. Global and specific translational regulation in the genomic response of *Saccharomyces cerevisiae* to a rapid transfer from a fermentable to a nonfermentable carbon source. Mol. Cell. Biol. 21, 916–927.

Kukuruzinska, M.A., Lennon-Hopkins, K., 1999. *ALG* gene expression and cell cycle progression. Biochim. Biophys. Acta 1426, 359–372.

Kukuruzinska, M.A., Robbins, P.W., 1987. Protein glycosylation in yeast: transcript heterogeneity of the *ALG7* gene. Proc. Natl. Acad. Sci. USA 84, 2145–2149.

Kulp, M.S., Frickel, E.M., Ellgaard, L., Weissman, J.S., 2005. Domain architecture of protein disulphide isomerase facilitates its dual role as an oxidase and isomerase in Ero1p-mediated disulphide formation. J. Biol. Chem. 281, 876–884.

Lal, A., Pang, P., Kalelkar, S., Romero, P.A., Herscovics, A., Moremen, K.W., 1998. Substrate specificities of recombinant murine Golgi alpha1, 2-mannosidases IA and IB and comparison with endoplasmic reticulum and Golgi processing alpha1,2-mannosidases. Glycobiology 8, 981–995.

Lennarz, W.J., 2007. Studies on oligosaccharyl transferase in yeast. Acta Biochim. Pol. 54, 673–677.

Liang, Y., Li, W., Ma, Q., Zhang, Y., 2005. Functional analysis of tunicamycin-inducible gene A polypeptide from *Aspergillus niger*. Biochem. Cell Biol. 83, 654–658.

Limongi, P., Kjalke, M., Vind, J., Tams, J.W., Johansson, T., Welinder, K.G., 1995. Disulfide bonds and glycosylation in fungal peroxidases. Eur. J. Biochem. 227, 270–276.

Lin, J.H., Walter, P., Yen, T.S.B., 2008. Endoplasmic reticulum stress in disease pathogenesis. Annu. Rev. Pathol. 3, 399–425.

Luheshi, L.M., Crowther, D.C., Dobson, C.M., 2008. Protein misfolding and disease: from the test tube to the organism. Curr. Opin. Chem. Biol. 12, 25–31.

Machida, M., Asai, K., Sano, M., Tanaka, T., Kumagai, T., et al., 2005. Genome sequencing and analysis of *Aspergillus oryzae*. Nature 438, 1157–1161.

Maeda, Y., Tanaka, S., Hino, J., Kangawa, K., Kinoshita, T., 2000. Human dolichol-phosphate-mannose synthase consists of three subunits, DPM1, DPM2 and DPM3. EMBO J. 19, 2475–2482.

Maeda, Y., Tomita, S., Watanabe, R., Ohishi, K., Kinoshita, T., 1998. DPM2 regulates biosynthesis of dolichol phosphate-mannose in mammalian cells: correct subcellular localization and stabilization of DPM1, and binding of dolichol phosphate. EMBO J. 17, 4920–4929.

Majumdar, S., Ghatak, J., Mukherji, S., Bhattacharjee, H., Bhaduri, A., 2004. UDPgalactose 4-epimerase from *Saccharomyces cerevisiae*. A bifunctional enzyme with aldose 1-epimerase activity. Eur. J. Biochem. 271, 753–759.

Maras, M., De Bruyn, A., Schraml, J., Herdewijn, P., Claeyssens, M., Fiers, W., Contreras, R., 1997a. Structural characterization of N-linked oligosaccharides from cellobiohydrolase I secreted by the filamentous fungus *Trichoderma reesei* RUTC 30. Eur. J. Biochem. 245, 617–625.

Maras, M., Saelens, X., Laroy, W., Piens, K., Claeyssens, M., Fiers, W., Contreras, R., 1997b. In vitro conversion of the carbohydrate moiety of fungal glycoproteins to mammalian-type oligosaccharides—evidence for *N*-acetylglucosaminyltransferase-I-accepting glycans from *Trichoderma reesei*. Eur. J. Biochem. 249, 701–707.

Maras, M., van Die, I., Contreras, R., van den Hondel, C.A., 1999. Filamentous fungi as production organisms for glycoproteins of bio-medical interest. Glycoconj. J. 16, 99–107.

Mast, S.W., Diekman, K., Karaveg, K., Davis, A., Sifers, R.N., Moremen, K.W., 2005. Human EDEM2, a novel homolog of family 47 glycosidases, is involved in ER-associated degradation of glycoproteins. Glycobiology 15, 421–436.

Mazhari-Tabrizi, R., Eckert, V., Blank, M., Muller, R., Mumberg, D., Funk, M., Schwarz, R.T., 1996. Cloning and functional expression of glycosyltransferases from parasitic protozoans by heterologous complementation in yeast: the dolichol phosphate mannose synthase from *Trypanosoma brucei brucei*. Biochem. J. 316, 853–858.

McCracken, A.A., Brodsky, J.L., 2003. Evolving questions and paradigm shifts in endoplasmic-reticulum-associated degradation (ERAD). Bioessays 25, 868–877.

Meaden, P., Hill, K., Wagner, J., Slipetz, D., Sommer, S.S., Bussey, H., 1990. The yeast *KRE5* gene encodes a probable endoplasmic reticulum protein required for (1–6)-beta-D-glucan synthesis and normal cell growth. Mol. Cell. Biol. 10, 3013–3019.

Mio, T., Yabe, T., Arisawa, M., Yamada-Okabe, H., 1998. The eukaryotic UDP-*N*-acetylglucosamine pyrophosphorylases. Gene cloning, protein expression, and catalytic mechanism. J. Biol. Chem. 273, 14392–14397.

Mio, T., Yamada-Okabe, T., Arisawa, M., Yamada-Okabe, H., 1999. *Saccharomyces cerevisiae GNA1*, an essential gene encoding a novel acetyltransferase involved in UDP-*N*-acetylglucosamine synthesis. J. Biol. Chem. 274, 424–429.

Miura, M., Hirose, M., Miwa, T., Kuwae, S., Ohi, H., 2004. Cloning and characterization in *Pichia pastoris* of *PNO1* gene required for phosphomannosylation of N-linked oligosaccharides. Gene 324, 129–137.

Molgaard, A., Larsen, S., 2002. A branched N-linked glycan at atomic resolution in the 1.12 A structure of rhamnogalacturonan acetylesterase. Acta Crystallogr. D Biol. Crystallogr. 58, 111–119.

Moralejo, F.J., Watson, A.J., Jeenes, D.J., Archer, D.B., Martin, J.-F., 2001. A defined level of protein disulfide isomerase expression is required for optimal secretion of thaumatin by *Aspergillus awamori*. Mol. Genet. Genomics 266, 246–253.

Movsichoff, F., Castro, O.A., Parodi, A.J., 2005. Characterization of *Schizosaccharomyces pombe* ER alpha-mannosidase: a reevaluation of the role of the enzyme on ER-associated degradation. Mol. Biol. Cell 16, 4714–4724.

Mulder, H.J., Saloheimo, M., Penttilä, M., Madrid, S., 2004. The transcription factor HACA mediates the unfolded protein response in *Aspergillus niger*, and up-regulates its own transcription. Mol. Genet. Genomics 271, 130–140.

Mulder, H.J., Nikolaev, I., Madrid, S.M., 2006. HACA, the transcriptional activator of the unfolded protein response (UPR) in *Aspergillus niger*, binds to partly palindromic UPR elements of the consensus sequence 5′-CAN(G/A)NTGT/GCCT-3′. Fungal Genet. Biol. 43, 560–572.

Nakajima, K., Asakura, T., Maruyama, J., Morita, Y., Oike, H., et al., 2006. Extracellular production of neoculin, a sweet-tasting heterodimeric protein with taste-modifying activity, by *Aspergillus oryzae*. Appl. Environ. Microbiol. 72, 3716–3723.

Nakanishi, H., Nakayama, K., Yokota, A., Tachikawa, H., Takahashi, N., Jigami, Y., 2001. Hut1 proteins identified in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are functional homologues involved in the protein-folding process at the endoplasmic reticulum. Yeast 18, 543–554.

Nakao, Y., Kozutsumi, Y., Funakoshi, I., Kawasaki, T., Yamashina, I., Mutsaers, J.H., Van Halbeek, H., Vliegenthart, J.F., 1987. Structures of oligosaccharides on beta-galactosidase from *Aspergillus oryzae*. J. Biochem. (Tokyo) 102, 171–179.

Nakatsukasa, K., Nishikawa, S., Hosokawa, N., Nagata, K., Endo, T., 2001. Mnl1p, an alpha-mannosidase-like protein in yeast *Saccharomyces cerevisiae*, is required for endoplasmic reticulum-associated degradation of glycoproteins. J. Biol. Chem. 276, 8635–8638.

Nakayama, K., Nagasu, T., Shimma, Y., Kuromitsu, J., Jigami, Y., 1992. *OCH1* encodes a novel membrane bound mannosyltransferase: outer chain elongation of asparagine-linked oligosaccharides. EMBO J. 11, 2511–2519.

Neiman, A.M., Mhaiskar, V., Manus, V., Galibert, F., Dean, N., 1997. *Saccharomyces cerevisiae HOC1*, a suppressor of pkc1, encodes a putative glycosyltransferase. Genetics 145, 637–645.

Ngiam, C., Jeenes, D.J., Archer, D.B., 1997. Isolation and characterisation of a gene encoding protein disulphide isomerase, *pdiA*, from *Aspergillus niger*. Curr. Genet. 31, 133–138.

Ngiam, C., Jeenes, D.J., Punt, P.J., van den Hondel, C.A., Archer, D.B., 2000. Characterisation of a foldase, protein disulphide isomerase A, in the protein secretory pathway of *Aspergillus niger*. Appl. Environ. Microbiol. 66, 775–782.

Nierman, W., Pain, A., Anderson, M.J., Wortman, J.R., Kim, S., et al., 2005. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. Nature 438, 1151–1156.

Nørgaard, P., Westphal, V., Tachibana, C., Alsøe, L., Holst, B., Winther, J.R., 2001. Functional differences in yeast protein disulphide isomerases. J. Cell Biol. 152, 553–562.

Odani, T., Shimma, Y., Tanaka, A., Jigami, Y., 1996. Cloning and analysis of the *MNN4* gene required for phosphorylation of N-linked oligosaccharides in *Saccharomyces cerevisiae*. Glycobiology 6, 805–810.

Odani, T., Shimma, Y., Wang, X.H., Jigami, Y., 1997. Mannosylphosphate transfer to cell wall mannan is regulated by the transcriptional level of the *MNN4* gene in *Saccharomyces cerevisiae*. FEBS Lett. 420, 186–190.

Oikawa, D., Kimata, Y., Kohno, K., 2007. Self-association and BiP dissociation are not sufficient for activation of the ER stress sensor Irel. J. Cell Sci. 120, 1681–1688.

Olivari, S., Galli, C., Alanen, H., Ruddock, L., Molinari, M., 2005. A novel stress-induced EDEM variant regulating endoplasmic reticulum-associated glycoprotein degradation. J. Biol. Chem. 280, 2424–2428.

O'Reilly, M.K., Zhang, G., Imperiali, B., 2006. In vitro evidence for the dual function of Alg2 and Alg11: essential mannosyltransferases in N-linked glycoprotein biosynthesis. Biochemistry 45, 9593–9603.

Orlean, P., Albright, C., Robbins, P.W., 1988. Cloning and sequencing of the yeast gene for dolichol phosphate mannose synthase, an essential protein. J. Biol. Chem. 263, 17499–17507.

Pagani, M., Pilati, S., Bertoli, G., Valsasina, B., Sitia, R., 2001. The C-terminal domain of yeast Ero1p mediates membrane localization and is essential for function. FEBS Lett. 508, 117–120.

Pakula, T.M., Laxell, M., Huuskonen, A., Uusitalo, J., Saloheimo, M., Penttilä, M., 2003. The effects of drugs inhibiting protein secretion in the filamentous fungus *Trichoderma reesei*: evidence for down-regulation of genes that encode secreted proteins in the stressed cells. J. Biol. Chem. 278, 45011–45020.

Pakula, T.M., Salonen, K., Uusitalo, J., Penttliä, M., 2006. The effect of specific growth rate on protein synthesis and secretion in the filamentous fungus *Trichoderma reesei*. Microbiology 151, 135–143.

Panchal, T., Wodzinski, R.J., 1998. Comparison of glycosylation patterns of phytase from *Aspergillus niger* (*A. ficuum*) NRRL 3135 and recombinant phytase. Prep. Biochem. Biotechnol. 28, 201–217.

Panneman, H., Ruijter, G.J., van den Broeck, H.C., Driever, E.T., Visser, J., 1996. Cloning and biochemical characterisation of an *Aspergillus niger* glucokinase. Evidence for the presence of separate glucokinase and hexokinase enzymes. Eur. J. Biochem. 240, 518–525.

Parlati, F., Dominguez, M., Bergeron, J.J., Thomas, D.Y., 1995. *Saccharomyces cerevisiae CNE1* encodes an endoplasmic reticulum (ER) membrane protein with sequence similarity to calnexin and calreticulin and functions as a constituent of the ER quality control apparatus. J. Biol. Chem. 270, 244–253.

Patil, C.K., Li, H., Walter, P., 2004. Gcn4p and novel upstream activating sequences regulate targets of the unfolded protein response. PLoS Biol. 2, E246.

Pel, H.J., de Winde, J.H., Archer, D.B., Dyer, P.S., Hofmann, G., et al., 2007. Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. Nat. Biotechnol. 25, 221–231.

Pelletier, M.F., Marcil, A., Sevigny, G., Jakob, C.A., Tessier, D.C., Chevet, E., Menard, R., Bergeron, J.J., Thomas, D.Y., 2000. The heterodimeric structure of glucosidase II is required for its activity, solubility, and localization in vivo. Glycobiology 10, 815–827.

Pemberton, T.J., 2006. Identification and comparative analysis of sixteen fungal peptidyl-prolyl *cis*/*trans* isomerase repertoires. BMC Genomics 7, 244.

Peter-Katalinic, J., 2005. Methods in enzymology: *O*-glycosylation of proteins. Methods Enzymol. 405, 139–171.

Pirneskoski, A., Klappa, P., Lobell, M., Williamson, R.A., Byrne, L., et al., 2004. Molecular characterization of the principal substrate binding site of the ubiquitous folding catalyst protein disulphide isomerase. J. Biol. Chem. 279, 10374–10381.

Raschke, W.C., Ballou, C.E., 1972. Characterization of a yeast mannan containing *N*-acetyl-D-glucosamine as an immunochemical determinant. Biochemistry 11, 3807–3816.

Rayner, J.C., Munro, S., 1998. Identification of the *MNN2* and *MNN5* mannosyltransferases required for forming and extending the mannose branches of the outer chain mannans of *Saccharomyces cerevisiae*. J. Biol. Chem. 273, 26836–26843.

Reiss, G., te Heesen, S., Zimmerman, J., Robbins, P.W., Aebi, M., 1996. Isolation of the *ALG6* locus of *Saccharomyces cerevisiae* required for glucosylation in the N-linked glycosylation pathway. Glycobiology 6, 493–498.

Romero, P.A., Dijkgraaf, G.J., Shahinian, S., Herscovics, A., Bussey, H., 1997. The yeast *CWH41* gene encodes glucosidase I. Glycobiology 7, 997–1004.

Roy, S.K., Chiba, Y., Takeuchi, M., Jigami, Y., 2000. Characterization of yeast Yea4p, a uridine diphosphate-*N*-acetylglucosamine transporter localized in the endoplasmic reticulum and required for chitin synthesis. J. Biol. Chem. 275, 13580–13587.

Ruddock, L.W., Molinari, M., 2006. *N*-Glycan processing in ER quality control. J. Cell Sci. 119, 4373–4380.

Saloheimo, M., Valkonen, M., Penttilä, M., 2003. Activation mechanisms of the HACI-mediated unfolded protein response in filamentous fungi. Mol. Microbiol. 47, 1149–1161.

Samuelson, J., Banerjee, S., Magnelli, P., Cui, J., Kelleher, D.J., Gilmore, R., Robbins, P.W., 2005. The diversity of dolichol-linked precursors to Asn-linked glycans likely results from secondary loss of sets of glycosyltransferases. Proc. Natl. Acad. Sci. USA 102, 1548–1553.

Schenk, B., Imbach, T., Frank, C.G., Grubenmann, C.E., Raymond, G.V., Hurvitz, H., Korn-Lubetzki, I., Revel-Vik, S., Raas-Rotschild, A., Luder, A.S., Jaeken, J., Berger, E.G., Matthijs, G., Hennet, T., Aebi, M., 2001. *MPDU1* mutations underlie a novel human congenital disorder of glycosylation, designated type If. J. Clin. Invest. 108, 1687–1695.

Schröder, M., Chang, J.S., Kaufman, R.J., 2000. The unfolded protein response represses nitrogen-starvation induced developmental differentiation in yeast. Genes Dev. 14, 2962–2975.

Schröder, M., 2008a. Engineering eukaryotic protein factories. Biotechnol. Lett. 30, 187–196.

Schröder, M., 2008b. Endoplasmic reticulum stress responses. Cell. Mol. Life Sci. 65, 862–894.

Schröder, M., Kaufman, R.J., 2005. ER stress and the unfolded protein response. Mut. Res. 569, 29–63.

Schwarz, M., Knauer, R., Lehle, L., 2005. Yeast oligosaccharyltransferase consists of two functionally distinct sub-complexes, specified by either the Ost3p or Ost6p subunit. FEBS Lett. 579, 6564–6568.

Sims, A.H., Robson, G.D., Hoyle, D.C., Oliver, S.G., Turner, G., et al., 2004. Use of expressed sequence tag analysis and cDNA microarrays of the filamentous fungus *Aspergillus nidulans*. Fungal Genet. Biol. 41, 199–212.

Smith, D.J., Proudfoot, A., Friedli, L., Klig, L.S., Paravicini, G., Payton, M.A., 1992. *PMI40*, an intron-containing gene required for early steps in yeast mannosylation. Mol. Cell. Biol. 12, 2924–2930.

Song, Y., Azakami, H., Shamima, B., He, J., Kato, A., 2002. Different effects of calnexin deletion in *Saccharomyces cerevisiae* on the secretion of two glycosylated amyloidogenic lysozymes. FEBS Lett. 512, 213–217.

St. John, T.P., Davis, R.W., 1979. Isolation of galactose-inducible DNA sequences from *Saccharomyces cerevisiae* by differential plaque filter hybridization. Cell 16, 443–452.

Stagljar, I., te Heesen, S., Aebi, M., 1994. New phenotype of mutations deficient in glucosylation of the lipid-linked oligosaccharide: cloning of the *ALG8* locus. Proc. Natl. Acad. Sci. USA 91, 5977–5981.

Stals, I., Sandra, K., Devreese, B., Van Beeumen, J., Claeyssens, M., 2004a. Factors influencing glycosylation of *Trichoderma reesei* cellulases. Part two: *N*-glycosylation of Cel7A core protein isolated from different strains. Glycobiology 14, 725–737.

Stals, I., Sandra, K., Geysens, S., Contreras, R., Van Beeumen, J., Claeyssens, M., 2004b. Factors influencing glycosylation of *Trichoderma reesei* cellulases. Part one: post-secretorial changes of the *O*- and *N*-glycosylation pattern of Cel7A. Glycobiology 14, 713–724.

Steel, G.J., Fullerton, D.M., Tyson, J.R., Stirling, C.J., 2004. Coordinated activation of Hsp70 chaperones. Science 303, 98–101.

Stolz, J., Munro, S., 2002. The components of the *Saccharomyces cerevisiae* mannosyltransferase complex M-Pol I have distinct functions in mannan synthesis. J. Biol. Chem. 277, 44801–44808.

Suzuki, T., Hara, I., Nakano, M., Shigeta, M., Nakagawa, T., Kondo, A., Funakoshi, Y., Taniguchi, N., 2006. Man2C1, an alpha-mannosidase, is involved in the trimming of free oligosaccharides in the cytosol. Biochem. J. 400, 33–41.

Szathmary, R., Bielmann, R., Nita-Lazar, M., Burda, P., Jakob, C.A., 2005. Yos9 protein is essential for degradation of misfolded glycoproteins and may function as lectin in ERAD. Mol. Cell 19, 765–775.

Takayanagi, T., Kimura, A., Chiba, S., Ajisaka, K., 1994. Novel structures of N-linked high-mannose type oligosaccharides containing alpha-D-galactofuranosyl linkages in *Aspergillus niger* alpha-D-glucosidase. Carbohydr. Res. 256, 149–158.

Takayanagi, T., Kushida, K., Idonuma, K., Ajisaka, K., 1992. Novel N-linked oligo-mannose type oligosaccharides containing an alpha-D-galactofuranosyl linkage found in alpha-D-galactosidase from *Aspergillus niger*. Glycoconj. J. 9, 229–234.

Takegawa, K., Kondo, A., Iwamoto, H., Fujiwara, K., Hosokawa, Y., Kato, I., Hiromi, K., Iwahara, S., 1991. Novel oligomannose-type sugar chains derived from glucose oxidase of *Aspergillus niger*. Biochem. Int. 25, 181–190.

Tanner, W., Lehle, L., 1987. Protein glycosylation in yeast. Biochim. Biophys. Acta 906, 81–99.

Tessier, D.C., Dignard, D., Zapun, A., Radominska-Pandya, A., Parodi, A.J., Bergeron, J.J., Thomas, D.Y., 2000. Cloning and characterization of mammalian UDP-glucose glycoprotein: glucosyltransferase and the development of a specific substrate for this enzyme. Glycobiology 10, 403–412.

Tian, G., Xiang, S., Noiva, R., Lennarz, W.J., Schindelin, H., 2006. The crystal structure of yeast protein disulphide isomerase suggests cooperativity between its active states. Cell 124, 61–73.

Tomita, S., Inoue, N., Maeda, Y., Ohishi, K., Takeda, J., Kinoshita, T., 1998. A homologue of *Saccharomyces cerevisiae* Dpm1p is not sufficient for synthesis of dolichol-phosphate-mannose in mammalian cells. J. Biol. Chem. 273, 9249–9254.

Travers, K.J., Patil, C.K., Wodicka, L., Lockhart, D.J., Weissman, J.S., Walter, P., 2000. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 101, 249–258.

Tremblay, L.O., Herscovics, A., 1999. Cloning and expression of a specific human alpha 1,2-mannosidase that trims Man9GlcNAc2 to Man8GlcNAc2 isomer B during *N*-glycan biosynthesis. Glycobiology 9, 1073–1078.

Tremblay, L.O., Herscovics, A., 2000. Characterization of a cDNA encoding a novel human Golgi alpha 1,2-mannosidase (IC) involved in *N*-glycan biosynthesis. J. Biol. Chem. 275, 31655–31660.

Tremmel, D., Tropschug, M., 2007. *Neurospora crassa* FKBP22 is a novel ER chaperone and functionally cooperates with BiP. J. Mol. Biol. 369, 55–68.

Treml, K., Meimaroglou, D., Hentges, A., Bause, E., 2000. The alpha- and beta-subunits are required for expression of catalytic activity in the hetero-dimeric glucosidase II complex from human liver. Glycobiology 10, 493–502.

Trombetta, E.S., Fleming, K.G., Helenius, A., 2001. Quaternary and domain structure of glycoprotein processing glucosidase II. Biochemistry 40, 10717–10722.

Trombetta, E.S., Simons, J.F., Helenius, A., 1996. Endoplasmic reticulum glucosidase II is composed of a catalytic subunit, conserved from yeast to mammals, and a tightly bound noncatalytic HDEL-containing subunit. J. Biol. Chem. 271, 27509–27516.

Tyson, J.R., Stirling, C.J., 2000. LHS1 and SIL1 provide a luminal function that is essential for protein translocation into the endoplasmic reticulum. EMBO J. 19, 6440–6452.

Valkonen, M., Ward, M., Wang, H., Penttilä, M., Saloheimo, M., 2003. Improvement of foreign protein production in *Aspergillus niger* var. *awamori* by constitutive induction of the unfolded protein response. Appl. Environ. Microbiol. 69, 6979–6986.

Valkonen, M., Penttilä, M., Saloheimo, M., 2004. The *ire1* and *ptc2* genes involved in the unfolded protein response pathway in the filamentous fungus *Trichoderma reesei*. Mol. Genet. Genomics 272, 443–451.

van den Steen, P., Rudd, P.M., Dwek, R.A., Opdenakker, G., 1998. Concepts and principles of O-linked glycosylation. Crit. Rev. Biochem. Mol. Biol. 33, 151–208.

van Gemeren, I.A., Punt, P.J., Drint-Kuyvenhoven, A., Broekhuijsen, M.P., van't Hoog, A., Beijersbergen, A., Verrips, C.T., van den Hondel, C.A., 1997. The ER chaperone-encoding *bipA* gene of black Aspergilli is induced by heat shock and unfolded proteins. Gene 198, 43–52.

Wallis, G.L., Easton, R.L., Jolly, K., Hemming, F.W., Peberdy, J.F., 2001. Galactofuranoic-oligomannose N-linked glycans of alpha-galactosidase A from *Aspergillus niger*. Eur. J. Biochem. 268, 4134–4143.

Wallis, G.L., Swift, R.J., Hemming, F.W., Trinci, A.P., Peberdy, J.F., 1999. Glucoamylase overexpression and secretion in *Aspergillus niger*: analysis of glycosylation. Biochim. Biophys. Acta 1472, 576–586.

Walsh, R.B., Kawasaki, G., Fraenkel, D.G., 1983. Cloning of genes that complement yeast hexokinase and glucokinase mutants. J. Bacteriol. 154, 1002–1004.

Wang, H., Entwistle, J., Morlon, E., Archer, D.B., Peberdy, J.F., Ward, M., Jeenes, D.J., 2003. Isolation and characterisation of a calnexin homologue, *clxA*, from *Aspergillus niger*. Mol. Genet. Genomics 268, 684–691.

Wang, H., Ward, M., 2000. Molecular characterisation of a PDI-related gene *prpA* in *Aspergillus niger* var. *awamori*. Curr. Genet. 37, 57–64.

Wang, X.H., Nakayama, K., Shimma, Y., Tanaka, A., Jigami, Y., 1997. *MNN6*, a member of the *KRE2*/*MNT1* family, is the gene for mannosylphosphate transfer in *Saccharomyces cerevisiae*. J. Biol. Chem. 272, 18117–18124.

Ward, M., Lin, C., Victoria, D.C., Fox, B.P., Fox, J.A., Wong, D.L., Meerman, H.J., Pucci, J.P., Fong, R.B., Heng, M.H., Tsurushita, N., Gieswein, C., Park, M., Wang, H., 2004. Production of antibodies and antibody fragments in *Apergillus niger*. Appl. Environ. Microbiol. 70, 2567–2576.

Warren, M.E., Kester, H., Benen, J., Colangelo, J., Visser, J., Bergmann, C., Orlando, R., 2002. Studies on the glycosylation of wild-type and mutant forms of *Aspergillus niger* pectin methylesterase. Carbohydr. Res. 337, 803–812.

Watzele, G., Tanner, W., 1989. Cloning of the glutamine:fructose-6-phosphate amidotransferase gene from yeast. Pheromonal regulation of its transcription. J. Biol. Chem. 264, 8753–8758.

Weng, S., Spiro, R.G., 1993. Demonstration that a kifunensine-resistant alpha-mannosidase with a unique processing action on N-linked oligosaccharides occurs in rat liver endoplasmic reticulum and various cultured cells. J. Biol. Chem. 268, 25656–25663.

Weng, S., Spiro, R.G., 1996. Endoplasmic reticulum kifunensine-resistant alpha-mannosidase is enzymatically and immunologically related to the cytosolic alpha-mannosidase. Arch. Biochem. Biophys. 325, 113–123.

Wilkinson, B.M., Purswani, J., Stirling, C.J., 2006. Yeast *GTB1* encodes a subunit of glucosidase II required for glycoprotein processing in the endoplasmic reticulum. J. Biol. Chem. 281, 6325–6333.

Willer, T., Valero, M.C., Tanner, W., Cruces, J., Strahl, S., 2003. *O*-Mannosyl glycans: from yeast to novel associations with human disease. Curr. Opin. Struct. Biol. 13, 621–630.

Williams, D.B., Lennarz, W.J., 1984. Control of asparagine-linked oligosaccharide chain processing: studies on bovine pancreatic ribonuclease B. An in vitro system for the processing of exogenous glycoproteins. J. Biol. Chem. 259, 5105–5114.

Woosley, B., Xie, M., Wells, L., Orlando, R., Garrison, D., King, D., Bergmann, C., 2006a. Comprehensive glycan analysis of recombinant *Aspergillus niger* endo-polygalacturonase C. Anal. Biochem. 354, 43–53.

Woosley, B.D., Kim, Y.H., Kumar Kolli, V.S., Wells, L., King, D., Poe, R., Orlando, R., Bergmann, C., 2006b. Glycan analysis of recombinant *Aspergillus niger* endo-polygalacturonase A. Carbohydr. Res. 341, 2370–2378.

Xiao, R., Wilkinson, B., Solovyov, A., Winther, J.R., Holmgren, A., Lundström-Ljung, J., Gilbert, H.F., 2004. The contribution of protein disulphide isomerase and its homologs to protein folding in the yeast endoplasmic reticulum. J. Biol. Chem. 279, 49780–49786.

Xu, X., Kanbara, K., Azakami, H., Kato, A., 2004. Expression and characterization of *Saccharomyces cerevisiae* Cne1p, a calnexin homologue. J. Biochem. 135, 615–618.

Xu, P., Raden, D., Doyle, F.J., Robinson, A.S., 2005. Analysis of unfolded protein response during single-chain antibody expression in *Saccharomyces cerevisiae* reveals different roles for BiP and PDI in folding. Metab. Eng. 7, 269–279.

Yamamoto, K., Hitomi, J., Kobatake, K., Yamaguchi, H., 1982. Purification and characterization of 1,2-alpha-mannosidase of *Aspergillus oryzae*. J. Biochem. 91, 1971–1979.

Yan, A., Lennarz, W.J., 2005. Two oligosaccharyl transferase complexes exist in yeast and associate with two different translocons. Glycobiology 15, 1407–1415.

Yanagisawa, K., Resnick, D., Abeijon, C., Robbins, P.W., Hirschberg, C.B., 1990. A guanosine diphosphatase enriched in Golgi vesicles of *Saccharomyces cerevisiae*. Purification and characterization. J. Biol. Chem. 265, 19351–19355.

Yip, C.L., Welch, S.K., Klebl, F., Gilbert, T., Seidel, P., Grant, F.J., O'Hara, P.J., MacKay, V.L., 1994. Cloning and analysis of the *Saccharomyces cerevisiae MNN9* and *MNN1* genes required for complex glycosylation of secreted proteins. Proc. Natl. Acad. Sci. USA 91, 2723–2727.

Yoko-o, T., Roy, S.K., Jigami, Y., 1998. Differences in in vivo acceptor specificity of two galactosyltransferases, the *gmh3*+ and *gma12*+ gene products from *Schizosaccharomyces pombe*. Eur. J. Biochem. 257, 630–637.

Yoko-o, T., Wiggins, C.A., Stolz, J., Peak-Chew, S.Y., Munro, S., 2003. An *N*-acetylglucosaminyltransferase of the Golgi apparatus of the yeast *Saccharomyces cerevisiae* that can modify N-linked glycans. Glycobiology 13, 581–589.

Yoshida, T., Kato, Y., Asada, Y., Nakajima, T., 2000. Filamentous fungus *Aspergillus oryzae* has two types of alpha-1,2-mannosidases, one of which is a microsomal enzyme that removes a single mannose residue from Man9GlcNAc2. Glycoconj. J. 17, 745–758.

Yoshihisa, T., Anraku, Y., 1989. Nucleotide sequence of *AMS1*, the structure gene of vacuolar alpha-mannosidase of *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 163, 908–915.

Yoshihisa, T., Anraku, Y., 1990. A novel pathway of import of alpha-mannosidase, a marker enzyme of vacuolar membrane, in *Saccharomyces cerevisiae*. J. Biol. Chem. 265, 22418–22425.

Zhou, H., Zhang, Y., Jia, C., Yang, K., 2004. Chaperone characteristics of PDI-related protein A from *Aspergillus niger*. Biochem. Biophys. Res. Commun. 321, 31–37.

Ziegler, F.D., Gemmill, T.R., Trimble, R.B., 1994. Glycoprotein synthesis in yeast. Early events in N-linked oligosaccharide processing in *Schizosaccharomyces pombe*. J. Biol. Chem. 269, 12527–12535.

Zimmerman, J.W., Specht, C.A., Cazares, B.X., Robbins, P.W., 1996. The isolation of a Dol-P-Man synthase from *Ustilago maydis* that functions in *Saccharomyces cerevisiae*. Yeast 12, 765–771.