Aspergillus niger is a filamentous fungus that is ubiquitous and commonly found on decaying plant material. A. niger has a saprophytic lifestyle and plays an important role in the degradation and recycling of dead plant material. In order to break down the complex plant polymers into smaller molecules that can be taken up and serve as energy and nutrient sources, the fungus produces a variety of hydrolytic enzymes. Many of the secreted enzymes of A. niger, like amylases, proteases, pectinases, xylanases and lipases find applications in the baking, starch, textile, food and feed industry. In industrial fermentations these extracellular enzymes can be produced in large quantities, some up till 40g/l. Although the high capacity secretory pathway of A. niger is often successfully exploited for the production of homologous and other fungal enzymes, the expression of heterologous enzymes of non-fungal origin is often several orders of magnitude lower. It is generally accepted that protein folding in the endoplasmic reticulum (ER) is a major bottleneck in heterologous protein production. A better understanding of the secretory pathway and the processes involved is therefore of great importance for future optimisation of heterologous protein production in the fungus.

The ER constitutes a major part of the secretory pathway and it is here that protein folding takes place. A quality mechanism ensures that only correctly folded proteins are passed on to the downstream pathway. So when the folding rate of proteins in the ER is lower than the input of newly synthesised polypeptides, unfolded proteins start to accumulate within the lumen of the ER. This constitutes a threat to the cell, and the eukaryotic cell responds to it by the activation of a stress response pathway known as the Unfolded Protein Response (UPR). The UPR consists of a signal transduction cascade that transmits a signal from the lumen of the ER to the nucleus of the cell. The major result of the response is that the cell increases the production of ER localised chaperones and foldases, which are molecules that assist in protein folding.

In this thesis we focused on the properties and the regulation of the transcriptional activator of the A. niger UPR, HacA.

Chapter 1 of this thesis gives an overview of the processes and mechanisms within the secretory pathway, and describes the UPR in Saccharomyces cerevisiae and mammalian cells. Chapter 2 describes the cloning and characterisation of the A. niger hacA gene. The induced HacA1 protein shares 72% identical residues in its DNA binding domain with its yeast homologue, but exhibits only 25% over-all identity. Also the unconventional intron differs substantially. In yeast a 252 nt unconventional intron is removed from the HAC1* transcript upon secretion stress, whereas the unconventional intron splicing of A. niger
hacA\textsuperscript{u} only removes a 20 nt intron. As a consequence of the unconventional intron splicing, the reading frame downstream of the intron is changed. The reading frame downstream of the intron of hacA\textsuperscript{u} encodes a C-terminal domain of 222 amino whereas in hacA\textsuperscript{i} a smaller C-terminal domain of 128 amino acids is encoded. In addition to the unconventional intron splicing it was shown that the A. niger hacA transcript is truncated by 230 nt upon ER stress. GFP tagging of HacA\textsuperscript{i} proved its nuclear localisation, and over-expression of the spliced (and thus induced) hacA\textsuperscript{i} from the gpdA promoter resulted in constitutive upregulation of ER localised chaperones and foldases, and induced the transcription of the hacA gene itself as well. The hacA\textsuperscript{i} gene was also expressed in E. coli and protein was purified and used for DNA binding experiments. It was shown by Electrophoretic Mobility Shift Assays (EMSAs) that HacA\textsuperscript{i} was able to bind to promoter fragments of ER localised foldases and chaperones, and to its own promoter.

In Chapter 3 we focused more on the target sites of the transcriptional activator HacA. The sequence and characteristics of various Unfolded Protein Response Elements (UPREs) was unravelled. The promoters to which HacA\textsuperscript{i} binding was observed in Chapter 1 each contained a single UPRE, except the hacA promoter itself in which three UPREs were discovered. A comparison of the identified UPREs resulted in a consensus sequence for the A. niger UPRE corresponding to 5'-\textbf{CAN(G/A)NTGT/GCCT}-3'. The A. niger UPRE is a partially palindromic sequence (underlined) around a single spacer nucleotide (between brackets) followed by a conserved stretch of four nucleotides. By binding site selection (BSS) the optimal binding site for HacA was isolated which shares the characteristics of the natural UPREs, and only differs by the absence of the spacer nucleotide. The dissociation constants for the different UPREs were determined, and by reporter constructs it was shown that UPREs with different dissociation constants resulted in differential upregulation upon ER stress. In addition to the unconventional intron splicing, the A. niger hacA transcript is truncated at its 5'end by approximately 230 nt upon secretion stress. The mechanism behind this truncation has been unknown thus far, and has also not been reported for the UPR transcription factors of mammalian cells and yeast. In Chapter 4 we describe the mechanism behind the hacA mRNA truncation. The shorter hacA mRNA that appears during secretion stress is the result of transcription of the hacA gene from a new start site which lies closer to the ATG. This transcriptional switch is dependent on the UPRE2 in the hacA promoter and operated by the HacA\textsuperscript{i} protein itself. The shorter hacA transcript lacks the 5'-end half-site of a long inverted repeat that causes translational attenuation in the hacA\textsuperscript{u} transcript. Relieved from this translational block, the hacA\textsuperscript{i} mRNA can be efficiently translated and contribute to the pool of HacA\textsuperscript{i} protein, thereby strengthen the stress response. Based on our current knowledge a working model is presented at the end of the Chapter, showing the multistep activation of HacA. Finally, in Chapter 5 the most important results are discussed and a role for HacA\textsuperscript{u} as a negative regulator for the UPR is speculated upon.