# Summary

In **Chapter 1**, a general introduction is given related to enzyme and metabolite production using filamentous fungi, with special attention to some possible mechanisms to explain why *A. niger* is such a good protein secretor.

**Chapter 2** describes the systematic investigation of the function of all members of the Rho family GTPases present in *A. niger*. Based on loss-of-function studies, we have showed that six Rho GTPases (RacA, CftA, RhoA, RhoB, RhoC, RhoD) exert distinct and overlapping functions during the life cycle of *A. niger*. Overall, our data show that individual Rho GTPases contribute differently to growth and morphogenesis within fungi. RacA and CftA collectively ensure polarity maintenance, whereby the main protagonist in *A. niger* is RacA. Interestingly, in *A. nidulans* deletion of the *cftA* resulted in a more pronounced effect on morphology compared to the *racA* deletion. The comparison between *A. niger* and *A. nidulans* indicates that the partitioning of the roles between CftA and RacA varies even among closely related filamentous fungi.

In **Chapter 3**, the *racA* mutant was subjected to more detailed studies to elucidate the impact of the altered morphology on the protein production yields as well as on the transcriptome. Surprisingly, physiological profiles including maximum specific growth rates and specific protein production rates were nearly identical despite the significant difference in their morphology. By following exocytotic (SncA-GFP) and endocytotic (SlaB-YFP, AbpA-CFP) markers together with protein yield determination, it was shown that the increase in hyphal tips did not result in an increase of protein production yields. The transcriptomic analysis of three morphological mutants (Δ*racA*, *ramosa-1* apical branching mutants; PglaA-RacAG18V, an apolar growing mutant, in which RacA is trapped in its 'on-state' by mutating the predicted GTP binding and hydrolysis domain) revealed that several signaling and metabolic pathways were altered in these morphological mutants involved in the polar tip growth. With regard to an increase of protein secretion, it would be interesting to challenge the Δ*racA* strain to overexpress a certain protein of interest to see the effect of hyperbranching on the protein secretion.

In **Chapter 4**, we performed transcriptome analyses by comparing an *A. niger* wild-type strain to a glucoamylase overexpressing strain under the same growth rate. Using GO term enrichment analysis, four higher-order categories were identified in the up-regulated gene set: i) ER membrane translocation, ii) protein glycosylation, iii) vesicle transport and iv) ion homeostasis. Among these, about 130 genes had predicted functions for the passage of

proteins through the ER and those genes included target genes of the HacA transcription factor that mediates the unfolded protein response (UPR), e.g. *bipA*, *clxA*, *prpA*, *tigA* and *pdiA*. Comparison of this dataset to other datasets in which *A. niger* was triggered to induce an unfolded protein response, a core set of 40 genes was identified which are key for the intensified traffic of proteins through the secretory pathway. The consistent up-regulation of a gene encoding the predicted acetyl-coenzyme A (CoA) transporter suggests a possible role for transient acetylation to ensure correct folding of secreted proteins

In **Chapter 5**, we established a GFP-v-SNARE reporter strain in which the trafficking and dynamics of secretory vesicles can be followed *in vivo* to study the process of protein secretion in *A. niger*. The biological role of seven secretion-specific genes, known to function in key aspects of the protein secretion machinery in *S. cerevisiae*, was analyzed using the GFP-v-SNARE reporter strain. This study revealed that the orchestration of exocyst-mediated vesicle transport is only partially conserved in *S. cerevisiae* and *A. niger* which serves as a basis to understand differences in secretion mechanisms between the species.

In **Chapter 6**, the major findings from the research described in the thesis are summarized, highlighted and interesting topics for future research are discussed.