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The role of autophagy during carbon starvation in *Aspergillus niger*
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**The role of autophagy during carbon starvation
in *Aspergillus niger***

Anne-Marie Burggraaf

The role of autophagy during carbon starvation in *Aspergillus niger*

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Outline

Filamentous fungi with a saprophytic lifestyle, like *Aspergillus niger*, commonly encounter limitations in the available carbon sources in their environment. During such conditions, very specific carbon starvation responses are induced aiming at survival and proliferation of the fungus. One of the processes that is highly activated by carbon starvation is autophagy. Autophagy is an intracellular degradation system which targets cytosolic components to lytic compartments for degradation and recycling of the building blocks of the cell. The process has been described in many species, most importantly in yeast and mammalian cells, and also including filamentous fungi. During autophagy in *A. niger*, cytoplasmic proteins and organelles are sequestered and delivered to vacuoles in double membrane vesicles, termed autophagosomes. Upon fusion of the outer membrane of the autophagosome with the vacuolar membrane, a single membrane vesicle is released into the hydrolytic environment of the vacuole. Following lysis of the autophagosomal membrane and hydrolytic degradation of the vesicular contents, breakdown products are transported back into the cytosol for reuse by the cell. The autophagy pathway is regulated by autophagy-related (Atg) proteins, which specifically support the different steps in the process. The process is highly induced by carbon starvation conditions, during which the recycling of nutrients is highly important for maintenance of the mycelium and to fuel asexual spore formation and cellular differentiation. This thesis aims at evaluating the autophagy process in the filamentous fungus *A. niger*, focusing on its role during carbon starvation, ER stress and unconventional protein secretion.

Chapter 1 reviews the specific responses to carbon starvation in *Aspergillus* species, giving insights into the most important cellular responses which include morphological responses such as asexual spore formation and recycling mechanisms such as the degradation and utilization of inner cell materials by autophagy and the degradation of cell wall constituents by extracellular hydrolytic enzymes.

In **chapter 2** phenotypical and cytological characterizations of *A. niger* autophagy deletion mutants in surface and submerged growth during carbon starvation are provided. The disruption of autophagy-related homologs in fluorescent reporter strains shows that both *atg1* and *atg8* are essential for autophagy, whereas *atg17* is not essential. By using automated image analysis it is demonstrated that cell death and outgrowth of cryptic hyphae is accelerated in autophagy deletion strains.

Chapter 3 describes the results of genome-wide transcriptional analysis comparing the *A. niger* $\Delta atg1$ mutant strain with the wild-type during submerged carbon starvation. Early

and late carbon starvation responses can be clearly distinguished, as genes related to cell division and DNA repair are higher expressed in the $\Delta atg1$ mutant at one day post carbon depletion whereas lower expression of metabolic processes is specific for the late phase of carbon starvation.

Chapter 4 shows the effects of applying ER stress in *A. niger* strains defective for functional autophagy and/or ER associated degradation (ERAD), by which the possible link between ER stress and autophagy is investigated. ER stress conditions are induced either by exposure to a chemical ER stressor or by expression of a disulfide bond mutated form of the glucoamylase protein. Fluorescent labeling of this misfolded protein visualizes higher accumulating protein levels in the ER in ERAD-defective background strains as compared to wild-type and autophagy single deletion mutants.

In **chapter 5** the unconventionally secreted aspartic protease PepN is used as a target protein to describe the role of autophagy in unconventional protein secretion. Proteome and immunoblotting analyses demonstrate the presence of PepN in secretomes of wild-type, $\Delta atg1$ and $\Delta atg8$ strains, indicating that the secretion of PepN is independent of autophagy-essential Atg1 and Atg8.

