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HMG-CoA reductases of *Mucor circinelloides*

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Mucor circinelloides is a carotene producing zygomycete, which is used as a model organism in the study of carotenoid biosynthesis. Carotenoids and other important isoprenoids of the fungal cell (such as ergosterol and the prenyl group of certain proteins) are synthesized in the acetate-mevalonate pathway. The central step in the pathway is the conversion of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid catalysed by the HMG-CoA reductase enzyme.

The *M. circinelloides* genome contains three different HMG-CoA reductase genes (named as *hmgR1*, *hmgR2* and *hmgR3*), which were cloned using the sequence data available in the genome database of the fungus (http://genome.jgi-psf.org/Mucci2/Mucci2.home.html). We used the genes in gene expression studies to investigate their function. Relative transcription levels of the three genes during the life cycle and under different cultivation conditions (aerobic/anaerobic growth, different carbon sources, different temperature and salt stress) were analyzed by quantitative real-time PCR (qPCR). In these studies, *hmgR1* showed low relative transcription levels under all conditions, while *hmgR2* showed high transcription levels under all aerobic conditions. Under anaerobic condition, transcription of *hmgR3* increased significantly.

We built three different expression vectors (pNG1, pNG2 and pNG3 containing the genes *hmgR1*, *hmgR2* and *hmgR3*, respectively) and used PEG mediated protoplast transformation to elevate the copy number of the genes. The carotenoid production and the sensitivity of statins changed after elevating the copy number of the genes. Enhanced expression of *hmgR2* increased the amount of the ergosterol in the transformants. Elevated copy number of *hmgR3* affected the carotene production and the sensitivity of statins in the highest degree among the different types of transformants.

We used antisens RNA (asRNA) mediated gene silencing to investigate other function of each gene. Three different vectors (pAS1, pAS2 and pAS3) were built containing antisense DNA fragments of *hmgR1*, *hmgR2* and *hmgR3*, respectively between the promoter and terminal region of glyceraldehyde-3-phosphate dehydrogenase. After PEG mediated protoplast transformation, transformants were isolated (MS12-pAS1, MS12-pAS2 and MS12-pAS3). Macro- and micromorphology, carotene and ergosterol content and the growth rate were examined in the resulting transformants. Growth rate, germination of spores and ergosterol content decreased in the transformants MS12-pAS3. Moreover, transformants showed altered morphology with swollen, frequently branching hyphae indicating a possible role of *hmgR3* in the mycelial development. In the MS12-pAS2 transformants, ergosterol content also decreased, but the morphology did not change.

Our results suggest that *hmgR2* may play an important role in the general isoprenoid metabolism and highly expresses under aerobic conditions. According to the transformation and qPCR studies, *hmgR3* seems to have role in the mycelial development, carotene biosynthesis and may be necessary for the sensing of the oxygen concentration of the environment. Moreover *hmgR3* may necessary to the germination of sporangiospores and apoptotic processes.

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TAF10 proteins indicate structural and functional links between histone acetyltransferase and basal transcription factor complexes

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TATA-binding protein associated factors (TAFs) have been identified as subunits of the TFIID basal transcription factor complex required for RNA polymerase II initiation. More recent studies indicate that TAFs are also present in histone acetyl transferase complexes, which regulate transcription initiation and the organization of the chromatin structure. This observation raises the possibility of complex „trans-mutation" by which due to changes in subunit composition one type of multiprotein complex is converted to an other type as transcription initiation is progressing.

Of the two *Drosophila* GCN5 histone acetyltransferase (HAT)-containing complexes SAGA and ATAC, TAF10 subunits are present in the former while they are missing from the latter. Despite that we found that the gene expression alterations in *taf10* mutants are very similar to those observed in ATAC subunit (Ada2a, Ada3) mutants. First, we aimed to find out whether only *taf10* mutants have similar gene expression alterations to ATAC mutants or other TAFs mutants also show the ATAC specific gene expression patterns. For this we studied the gene expression pattern of *Drosophila* stocks in which *taf5*, *taf10* or *taf8* was downregulated by RNAi.

We have recently shown that *Halloween* genes, which are expressed in the prothoracic gland and regulate ecdysone synthesis are

regulated by the ATAC HAT complex. As a result of ecdysone synthesis failure ATAC mutants arrest development at the larval-prepupal transition though they do not present any evident defect during larval development. We silenced *taf5*, *taf8*, *taf10* genes specifically in the ring gland where ATAC-regulated ecdysone synthesis occurs at late larva stage and observed developmental arrest before the prepupal transition, while there was no effect detectable on the other larval developmental stages. This phenotype is similar to that seen in ATAC mutants. 20-hydroxyecdysone feeding rescue TAF mutant L3 stage larvae and they reach pupa stage. These data indicate that TAFs influence the ecdysone synthesis similarly as it was observed in ATAC-specific *Ada2a* mutants. Furthermore, decreased expression of TAF proteins in the wing discs results in notched wing phenotype, which suggests that similarly to ATAC, TAF proteins also play role in apoptosis induction.

Our data suggest a functional interconnection between the ATAC HAT complex and the basal transcription factor TFIID. By further studies we aim to elucidate the details of the structural and functional interrelationship of the two complexes.

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The role of black *Aspergillus* species in food safety and human health as potential mycotoxin producers and opportunistic human pathogens

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Aspergillus is among the economically most important fungal genera. Species belonging to section *Nigri* (black Aspergilli) of this genus play an important role as human pathogens and mycotoxin producers, and in the food and biotechnological industries due to their ability to produce hydrolytic enzymes (lipases, amylases) as well as organic acids (citric acid, gluconic acid).

Aspergilli are one of the more difficult groups concerning classification and identification. New molecular approaches have shown that there is a high biodiversity, and the different species are difficult to be recognized based solely on their phenotypic characters. One of our aims was the production of a robust genus-wide phylogeny based on 6 gene sequences to get insight into the evolutionary relationships within this economically important genus. According to former studies, section *Nigri* was suggested to belong to subgenus *Circumdati;* however, our data indicate that sections *Cremei* and *Nigri* are unrelated to subgenus *Circumdati*, and possibly represent new subgenera.

Black Aspergilli are commonly found as soil organisms decomposing dead plant residues, as postharvest contaminants, and as pathogens of several crops including grapes, almond and onion. Black Aspergilli are of concern not only for their ability to destroy several agronomically important food crops, but also due to their ability to produce several mycotoxins including ochratoxins and fumonisins. In our work we isolated black Aspergilli from raisins, onions, figs and dates and identified the strains at the species level by comparing their partial calmodulin gene sequences. Various black *Aspergillus* were isolated from raisins and figs, while only *A. awamori* and *A. tubingensis* were identified on onions and dates, respectively. Fumonisin contamination of the samples was examined by HPLC-MS/MS technique. Fumonisins were detected in all products in varying quantities. Several fumonisin isomers have been identified for the first time in black Aspergilli.

Nowadays *Aspergillus* species cause human infections more frequently. 92 clinical isolates from The Netherlands were assigned at the species level using sequence analysis of part of the calmodulin gene. 55 A. *tubingensis*, 21 *A. acidus*, 14 *A. niger* and 2 *A. awamori* isolates were detected. Antifungal susceptibility tests of the isolates are in progress. *Aspergillus* is also considered to be the predominant causative organism of otomycosis, with *Aspergillus niger* as the most frequently described species. We analysed black Aspergilli isolated from otomycosis cases in Iran and Hungary. The results indicate that *A. niger* is not the only black *Aspergillus* species involved in otomycosis cases: *A. awamori* and *A. tubingensis* are also able to cause ear infections. Antifungal susceptibility tests revealed that all isolates were highly susceptible to terbinafine, while exhibited moderate susceptibilities against amphotericin B and ketoconazole. *A. niger* and *A. awamori* were found to have higher MICs for ketoconazole than *A. tubingensis*.

Regarding the population structure of black Aspergilli, only limited data are available. We started to examine the structure of various black *Aspergillus* populations using molecular methods. Regarding the distribution of the mating type genes in different species, close to 1:1 ratios were observed in *A. niger* and *A. tubingensis* populations regardless of the origin of the isolates. However, most *A. awamori* isolates were found to carry the MAT1 idiomorph. Analysis of the genetic variability of the isolates by UP-PCR analysis is in progress.

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