Comparative analysis of regulation of methionine-cysteine biosynthesis in ascomycetes

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Fungal methionine-cysteine (Met-Cys) biosynthesis was extensively researched in *Neurospora crassa*, and only about ten years ago this metabolic pathway began being studied in *Aspergillus nidulans*. Many enzymatic activities in this pathway has not been proven by cloned genes in *N.crassa*, and only some genes have been cloned in *A.nidulans*. Biosynthesis of Met-Cys is controlled by cascade of several regulators. On the level of transcription this pathway is regulated by Cys3p in *N.crassa*. In *A.nidulans*, the transcriptional regulator is MetRp. These regulators are not closely related, but swapping of bZIP domain from Cys3 into MetR complemented the MetR deletion, indicating that the bZIP domains of these proteins are functionally interchangeable [1]. The literature data argues that only sulfate uptake pathways are regulated (including sulfate uptake and subsequent reduction of sulfate to sulfide, uptake of exogenous proteins, choline-O-sulfate, tyrosine-O-sulfate and methionine), whereas the core enzymes of this metabolic pathway seem to be expressed constitutively. Binding sites for Cys3p upstream of two genes of *N.crassa* have been published [2].

The aim of this study was to analyze regulation of the Met-Cys genes in other ascomycete genomes. At the first step, I identified reliable orthologs for all known fungal Met-Cys genes in both genomes, resulting in 10 genes from *N.crassa* and 9 genes from *A.nidulans* (*S.cerevisiae* and *N.crassa* have two sulfate permeases, whereas the only one candidate gene was identified in *A.nidulans*). Among potentially regulated genes I could not identify reliable orthologs for methionine permease, choline-O-sulfate permease and choline sulfatase.

Attempts to identify potential binding sites for the transcriptional regulators in *N.crassa* and *A.nidulans* proceeded in the following directions:

1. Four published binding sites of *N.crassa* were used to construct a positional weight matrix and to search for new potential sites in the genomes of *N.crassa* and *A.nidulans*. High-scring sites were observed upstream of a third of genes. This showed that the information content of experimentally verified binding was insufficient to construct a specific recognition rule.

2. Published binding sites for Cys3 suggested that true sites might be preferably located close to each other with a spacer of about 20 nt. Adding this requirement resulted in selection of only two initial genes in *N.crassa*, whereas relaxing the recognition threshold lead to finding

sites not only upstream the known regulated genes, but also upstream of about 30% of all *N.crassa* genes. Again, the rule proved to be insufficiently specific.

3. Binding sites for transcriptional regulators tend to be conserved in related genomes. I used the genome of *Aspergillus fumigatus* (TIGR, Unfinished Microbial Genomes) to search for conserved sites of *Aspergillus* spp. Alignments of upstream regions of orthologous regulated genes of *A.nidulans* and *A.fumigatus* contained multiple highly conserved regions of 20-30 nt that could reflect “phylogenetic footprints” of regulatory signals. However, these conserved regions die not overlap with the predicted sites even with very low, non-specific cutoff.

These observations argue against the hypothesis of conservation of the *N.crassa* recognition signal in the *Aspergillus* genomes. As more genomes of ascomycetes become available, the taxonomic range of conservation of the *Neurospora* signal may be determined and the *Aspergillus*-specific signal may be identified.

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References

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