Characterization of a multicopper oxidase gene cluster in *Phanerochaete chrysosporium* and evidence of altered splicing of the *mco* transcripts

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Abstract

A cluster of multicopper oxidase genes (*mco1*, *mco2*, *mco3*, *mco4*) from the lignin-degrading basidiomycete *Phanerochaete chrysosporium* is described. The four genes share the same transcriptional orientation within a 25 kb region. *mco1*, *mco2* and *mco*3 are tightly grouped, with intergenic regions of 2·3 and 0·8 kb, respectively, whereas *mco4* is located 11 kb upstream of *mco*1. All are transcriptionally active, as shown by RT-PCR. Comparison of cDNAs and the corresponding genomic sequences identified 14–19 introns within each gene. Based on homology and intron composition, two subfamilies of *mco* sequences could be identified. The sequences have copper-binding motifs similar to ferroxidase proteins, but different from fungal laccases. Thus, these sequences constitute a novel branch of the multicopper oxidase family. Analysis of several cDNA clones obtained from poly(A) RNA revealed the presence of transcripts of various lengths. Splice variants from *mco2*, *mco3* and *mco4*were characterized. They generally exhibited the presence of one to five introns, whereas other transcripts lacked some exons. In all cases, the presence of introns leads to frame shifts that give rise to premature stop codons. In aggregate, these investigations show that *P. chrysosporium* possesses a novel family of multicopper oxidases which also feature clustering and incomplete processing of some of their transcripts, a phenomenon referred to in this paper as ‘altered splicing’.