Methods for Investigating the UPR in Filamentous Fungi

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Résumé en anglais	Filamentous fungi have a high-capacity secretory system and are therefore widely exploited for the industrial production of native and heterologous proteins. However, in most cases, the yields of nonfungal proteins are significantly lower than those obtained for fungal proteins. One well-studied bottleneck appears to be the result of slow or aberrant folding of heterologous proteins in the ER during the early stages of secretion within the endoplasmic reticulum, leading to stress responses in the host, including the unfolded protein response (UPR). Most of the key elements constituting the signal transduction pathway of the UPR in Saccharomyces cerevisiae have been identified in filamentous fungi, including the central activation mechanism of the pathway, that is, the stress-induced splicing of an unconventional (nonspliceosomal) intron in orthologs of the HAC1 mRNA. This splicing event relieves a translational block in the HAC1 mRNA, allowing for the translation of the bZIP transcription factor Hac1p that regulates the expression of UPR target genes. The UPR is involved in regulating the folding, yield, and delivery of secretory proteins and that has consequences for fungal lifestyles, including virulence and biotechnology. The recent releases of genome sequences of several species of filamentous fungi and the availability of DNA arrays, GeneChips, and deep sequencing methodologies have provided an unprecedented resource for exploring expression profiles in response to secretion stresses. Furthermore, genome-wide investigation of translation profiles through polysome analyses is possible, and here, we outline methods for the use of such techniques with filamentous fungi and, principally, Aspergillus niger. We also describe methods for the batch and controlled cultivation of A. niger and for the replacement and study of its hacA gene, which provides either a UPR-deficient strain or a constitutively activated UPR strain for comparative analysis with its wild type. Although we focus on A. niger, the utility of the hacA-deletion strategy is also described for use in investigating the virulence of the plant pathogen Alternaria brassicicola.
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