GENETIC CHARACTERISATION AND ISOLATION OF THE *XPRF* GENE OF *ASPERGILLUS NIDULANS*

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ABSTRACT

Aspergillus nidulans provides a convenient model for studying gene regulation mechanisms in eukaryotes. In this study the regulation and secretion of extracellular proteases was the subject of some investigations around the regulation of gene expression in this organism.

Extracellular proteases are secreted to the growth medium to break the polypeptide chains and facilitate their uptake. Amino acid residues in proteins can act as nitrogen, carbon and sulphur sources. Proteins do not appear as favourite nutrients and when preferred nitrogen, carbon and sulphur sources are present, extracellular protease enzymes are produced at low levels.

There are major regulatory circuits in *A. nidulans* which control the metabolism of different nutrients. Several regulatory genes have appeared to be involved in the control of extracellular protease production. These include *areA*, mediating nitrogen metabolite repression (Arst and Cove, 1973), *creB* and *creC*, affecting carbon catabolite repression (Hynes and Kelly, 1977) and *xprE*, a new putative regulatory locus involved in production of extracellular proteases production in response to carbon and nitrogen limitation (M.E. Katz, unpublished data).

A new mutation was isolated which, in addition, seemed to affect the extracellular protease production. This study was focused on genetic characterisation of this mutated gene, designated *xprF1*, and molecular isolation of the wild-type *xprF*$^{+}$. *xprF1* caused elevated levels of extracellular protease activities in response to carbon and/or nitrogen limitation. *xprF1* did not express

any effect on sulphur regulation. Different genetic tests showed that *xprF1* segregates as a single gene located on the chromosome VII, 30.1 map units left to the *amdA* gene. *xprF1* also expressed pleiotropic effects on the utilisation of secondary nitrogen sources. In respect to the effect on the extracellular protease production, *xprF1* is recessive to its wild-type allele whereas for the effect on the utilisation of nitrogen sources, it is semi-dominant to *xprF*$^+$. Genetic data suggest that the *xprF* gene may have a regulatory role in production of extracellular proteases and utilisation of secondary nitrogen sources.

Co-transformation experiments using chromosome-specific cosmid clones from a wild-type genomic library (Brody *et al.*, 1991) for transformation of an *xprF1* strain resulted in isolation of a clone, L32F12, which carries the *xprF*$^+$. Transformed colonies express wild-type milk clearing activities with varied morphologies on hypoxanthine (as a secondary nitrogen source). Genetic studies on several transformed strains showed no evidence for gene replacement events. In fact, L32F12 appeared to integrate fairly stable at non-homologous sites.

According to results from this study several possibilities for the function of the *xprF* gene are discussed at the end. In addition, a model for the putative regulatory pathway in which the *xprF* is involved is speculated.

Contents

List of Figures

N.B. All photographs showing milk clearing activities on milk plates were prepared after 3 days. Since the standard scoring is carried out after 2 days, there could be some slight differences between scores and what are seen in photographs.

List of Tables