Cloning Allergens from *Aspergillus fumigatus*: The Filamentous Phage Approach

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During recent years, cDNA libraries and partial sequence knowledge of the primary structure of allergenic proteins have been used extensively to isolate allergens from different sources [1]. Although these techniques have proven very successful for determining the primary structure of allergens, they are a labour-intensive to approach cloning of several components from complex allergenic systems such as moulds which are known to produce a large number of different allergens [2], or pollen such as that from ragweed which is composed of at least 52 antigens of which 22 have been shown to bind to human serum IgE [3]. A rational cloning approach for heterogeneous protein mixtures sharing common properties, like allergens, requires a selective enrichment of clones expressing proteins able to bind human IgE antibodies. We have developed a new cloning system, termed pJuFo, designed to display cDNA products on the surface of filamentous phage M13 [4]. The physical linkage of cDNA gene products to the genetic information required for their production, achieved by exploiting the high-affinity interaction of the Jun and Fos leucine zippers (fig. 1), allows rapid and efficient screening of large libraries in semifluid systems [5]. We have applied the pJuFo cloning system to construct and screen a cDNA library from *Aspergillus fumigatus* displayed on the surface of filamentous phage M13 for gene products binding to human serum IgE. Phage displaying IgE-binding proteins were selectively enriched 105- to 106-fold over non-specific phage after six rounds of phage growth and selection on wells of a micro-titre plate coated with human IgE antibodies. Restriction enzyme analysis and preliminary sequence determination of 12 selected inserts revealed different open reading frames encoding proteins with a molecular mass ranging from 20 to 40 kD. The ability of the proteins to bind to human serum IgE was corroborated by enzyme-linked immunosorbent assay and by Western blot analysis [6]. Obvious advantages of the pJuFo cloning system compared to conventional solid-phase-based screening systems are the ability to access very large libraries with a powerful enrichment technique and the possibility to select for genes physically linked to their gene product as secreted and thus folded proteins. The demonstration that cDNA libraries can be displayed on the phage surface

and screened by product-ligand interaction has implications far beyond the isolation of allergenic proteins, and opens new perspectives in gene cloning.

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pelB

- Cys Jun Cys Gly Gly Gly Gly Ser gill (198-406) stop-''-stop

pelB

Cys Fos Cys cDNA stop

intracellular periplasm

COOH

extracellular

Fig. 1. a Essential elements of the pJuFo phagemid. The Jun leucine zipper flanked by Cys residues is expressed and secreted as the N-terminal fusion to a truncated gill gene product of the phagemid. The Fos leucine zipper flanked by Cys residues is used to produce Fos-cDNA A fusions secreted via the pelB leader and captured by the Jun leucine zipper. cDNA expression libraries can be constructed using the unique BglU, Xba\ and Kprñ restriction sites of the phagemid. The backbone of pJuFo is recruited from the previously reported pComb3 construct [6]. b Generation of phage surface display libraries. Jun/gill and Fos/cDNA heterodimers become incorporated into the phage surface together with wild-type gill product of the helper phage.

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