STRUCTURE FUNCTION ANALYSIS OF THE DEUBIQUITYLATING ENZYME FAM

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DECLARATION OF ORIGINALITY

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Poon-Yu Khut 04/10/06

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ABSTRACT

The ubiquitin pathway is a highly conserved post-translational modification system best characterised for its roles in protein degradation and intracellular trafficking and is involved in a diverse spectrum of cellular processes. Ubiquitylation is opposed by deubiquitylating enzymes (Dubs), and the ubiquitin specific peptidase (USP) class of Dubs remove ubiquitin from specific substrates, thereby affecting protein fate. USPs exhibit broad sequence diversity except over their catalytic cores and it has been suggested that this sequence variation constitutes their individual substrate-specific binding sites.

Fat Facets in Mouse (*Fam*) is a developmentally regulated USP whose function is crucial for mouse pre-implantation development. *Fam* is expressed in a complex fashion throughout development in a number of diverse tissue types and time points, well beyond its critical role in the early embryo. *Fam*'s orthologue in fly, *Fat Facets* (*faf*) is also developmentally regulated and is required for both drosophila eye and syncytial stage development.

Given the strengths of the zebrafish system as a developmental tool, the zebrafish orthologue of *Fam, usp9* was identified and found to be highly conserved. Analysis of its expression pattern found considerable overlap with the published mouse patterns. Given the similarities between the mouse and zebrafish systems, a series of cross-species experiments were conducted to determine whether exogenous expression of highly conserved regions of FAM, could cause dominant-negative phenotypes in developing zebrafish embryos.

Outside of the catalytic core, FAM's large N and C-terminal extensions consist of novel sequence bearing no similarity to any known domain. To delineate FAM domains, full-length FAM was expressed in insect cells and subjected to partial proteolysis. Combining this data with recent structural predictions and computer analyses of the FAM sequence, four FAM domains were characterised with the first domain containing three possible subdomains. The predominant helical nature of the N and C-terminal extensions of FAM were predicted to form scaffolding structures, well suited to protein binding.

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