

Proteomic approach to study the immune response of *Drosophila melanogaster*.

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Insects are devoid of an adaptive immune system and rely only on innate immune reactions for their defence. Genetic and molecular approaches have shown that *Drosophila* is a powerful model to study innate immunity, which seems to be remarkably conserved between flies and mammals [for review see Hoffmann & Reichhart, 2002]. To combat microbial infection, *Drosophila* activates multiple cellular and humoral responses, including proteolytic cascades and production of effector molecules, such as antimicrobial peptides. In order to investigate novel molecules that are regulated during the *Drosophila* humoral immune response, we developed differential display analysis of proteins contained in the blood (haemolymph) of immune-challenged versus unchallenged *Drosophila*. For molecules below 15 kDa, the analysis was performed with MALDI MS and μ -HPLC [Uttenweiler-Joseph *et al.*, 1998] leading to the characterisation of a large number of new peptides with, up to now, unknown function. For molecules larger than 15 kDa, we developed a proteomic approach using 2D electrophoresis and mass spectrometry. Conditions for the analysis of *Drosophila* haemolymph proteins were optimised (sample preparation, detergents, range of pI). Using silver stained gels, more than 300 proteins were detected from the haemolymph of 200 flies (figure 1). With these techniques (2D, MS), the data can be combined with the information available from total *Drosophila* genome sequence to accurately identify individual proteins.

Two distinct intracellular signalling cascades are involved in *Drosophila* immune response: the Toll and Imd pathways, which are activated by different microbial elicitors [Michel *et al.* 2001 ; Gottar *et al.* 2002]. Differential 2D analyses were performed on the haemolymph proteins after different immune stimuli (bacteria or fungi). After silver staining and scanning, the gels were analysed using PDQuest software. As compared to the control samples (uninfected *Drosophila*), more than 80 protein spots were found to be up or down regulated by at least a factor of five after immune challenge.

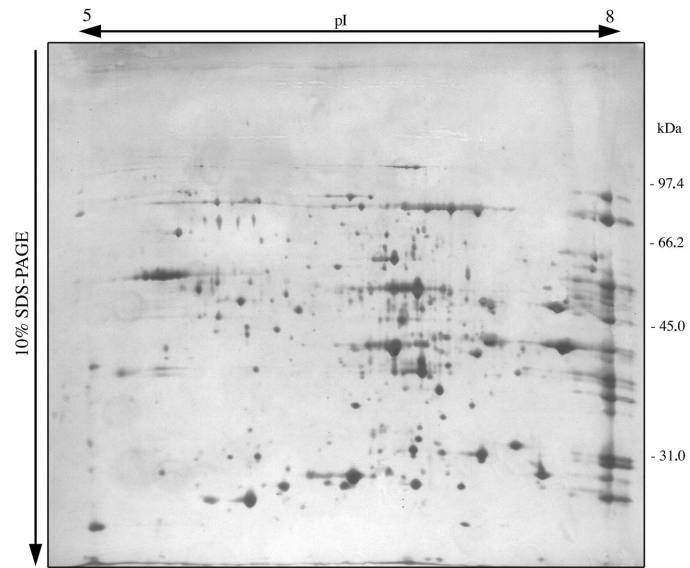


Figure 1: 2D gel electrophoresis of the haemolymph from 200 control flies.

The proteins making up these spots are under characterisation. In the case of fungal infection, we extended the analysis using narrow range immobilised pH gradient strips (5.5 – 6.7). Proteins present at a low concentration in the haemolymph, not detected using broad-range pI strips, could then be analysed (figure 2).

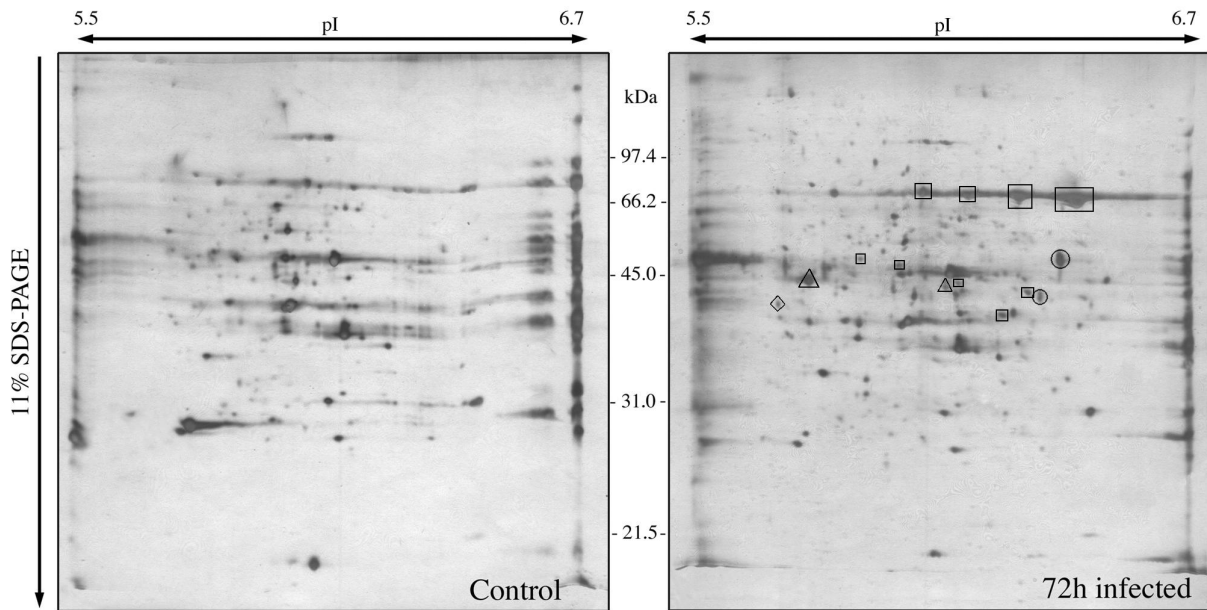


Figure 2: 2D gel electrophoresis on narrow range strips of the hemolymph of 500 control and 500 fungal infected *Drosophila*.

Among the differentially regulated proteins identified, several are of particular interest. Two of them were enzymes thought to be involved in the prophenoloxidase cascade (triangles). This cascade is part of the process of melanisation, an aspect of the humoral immune response. Two differentially regulated serine protease inhibitors (Serpins) were also identified (circles), one of which corresponds to the blood serpin Necrotic, involved in the Toll pathway [Levashina *et al.*, 1999]. We showed that the Necrotic protein was N-terminally cleaved following a fungal infection. The second serpin may represent a new candidate component of the antifungal response cascade. Transferrin (squares), a major transport protein for iron, was also upregulated following fungal infection. Interestingly, in infected *Drosophila* haemolymph, transferrin was found to be processed and several fragments were identified. The protease responsible for this cleavage could be part of the immune mechanism and should be further investigated. Another fungally regulated protein revealed by this proteomic approach was Peptidylglycine α -hydroxylating monooxygenase (Phm, diamond), which is known to be involved in the maturation of secretory peptides. Induction of this enzyme might be important for the production of active amidated immune effectors.

The precise function of all the proteins identified will have to be determined using complementary approaches, such as the analysis of *Drosophila* mutants. The characterisation of proteins specifically regulated by bacterial or fungal infection will also provide information on the mechanisms activating the immune response and will complement the microarray studies [Irving *et al.*, 2001].

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