Di Pasquale, Anna, Fara Misuraca, and Valentina Tesoro. Dipartimento Di Biologia Cellulare e Dello Sviluppo 'A. Monroy" - Sezione di Genetica, Università di Palermo, Parco D'orleans II, 90128 Palermo, Italy. Tel. 091-424459; Fax 091-420361. Immune response in the *tu-pb* melanotic tumor strain of *Drosophila melanogaster*: preliminary data.

It is well known that *Drosophila*, like other insects, resist bacterial infections through the induction of both cellular (Lackie, 1988) and humoral (Faye and Hultmark, 1993) responses; the cellular response involves the mobilization of hemocytes, which phagocytose or encapsulate microorganisms, whereas the humoral response utilizes antibacterial peptides, which effectively lyse bacteria or are bacteriostatic. Antibacterial peptides are synthesized in the fat bodies and secreted into the circulating hemolymph. Another component of the insect immunity is the activation of phenoloxidase and the clotting of the hemolymph. Phenoloxidase catalyzes the synthesis of melanin, resulting in the encapsulation of invading cells (Hultmark, 1993).

Several genes encoding anti-bacterial peptides have been cloned and characterized in *Drosophila melanogaster*. The induction of antibacterial peptides is controlled at the transcriptional regulation level (Faye and Hultmark, 1993).

The immune response has been shown to be activated not only in adults but also in pupae and third instar larvae. Recently it has been reported (Lemaitre *et al.*, 1995) that some genes controlling embryonic development [*dorsal (dl), Toll,* and *cactus*] are expressed in larval and adult fat bodies, where their RNA expression is enhanced upon injury. Mutants of these genes result in a melanotic tumour phenotype which is considered to be a spontaneous immune-like response. The Dorsal protein (Dl), normally localized in the cytoplasm of the fat body, is rapidly imported in the nucleus upon bacterial challenge. This nuclear uptake of Dl occurs spontaneously in mutants exhibiting melanotic tumours.

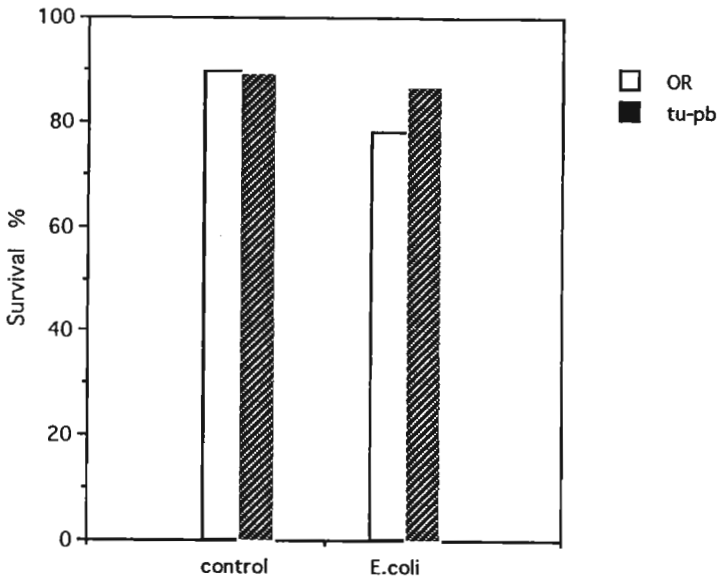

Figure 1. Survival of wild-type and mutant adults to bacterial infection.

In order to elucidate a possible relation between melanotic tumour manifestation and the immune response, we have started to investigate the response to the bacterial challenge in the melanotic tumour *tu-pb* strain of *D. melanogaster*.

Tu-pb is an atypical melanotic tumour mutant in which tumour manifestation is restricted to the head of the adult; penetrance is also incomplete and different in the sexes. Genetic analysis indicated that the *tu-pb* phenotype depends on at least two different loci, one recessive on the 3rd chromosome, the other, apparently semidominant, on the 2nd chromosome: only genotypes including both the two large *tu-pb* autosomes elicit tumour manifestation. *Tu-pb* larvae lack the precocious transformation of plasmocytes into lamellocytes which is typical of all melanotic tumour mutants, but retain most of the crystal cells in the lymph glands (Di Pasquale Paladino and Cavolina, 1983, 1984; Di Pasquale Paladino *et al.*, 1988).

To determine if *tu-pb* individuals have a different degree of susceptibility to bacterial infection, we analyzed the survival rates of Oregon-R wild-type flies and *tu-pb* mutants after bacterial challenge.

Injury experiment (control) were performed by pricking third instar larvae or adults with a thin needle; for bacterial challenge, the needle was previously dipped into a concentrated bacterial culture of *E. coli*. Preliminary results give evidence that the *tu-pb* mutant exhibits slightly elevated survival rates as compared with the wild-type strain, after bacterial infection (Figure 1). Moreover, treatment of larvae sets in a melanization reaction which appears to persist in the adults and which can be induced by infection and even by a simple injury. *Tu-pb* flies exhibit a significantly higher level of this melanization reaction (Figure 2). To investigate about a possible correlation between resistance to infection,

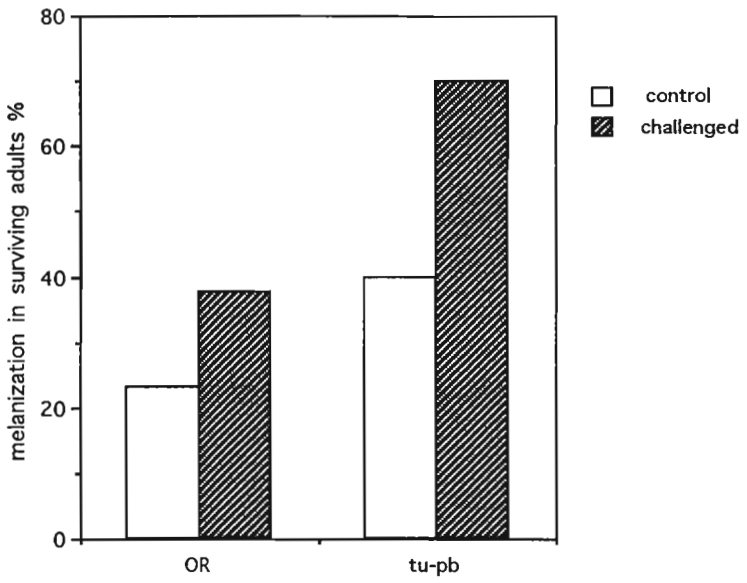

Figure 2. Percentage of melanization in Oregon-R and*tu-pb* adults survived to injury (control) or bacterial challenge as third instar larvae.

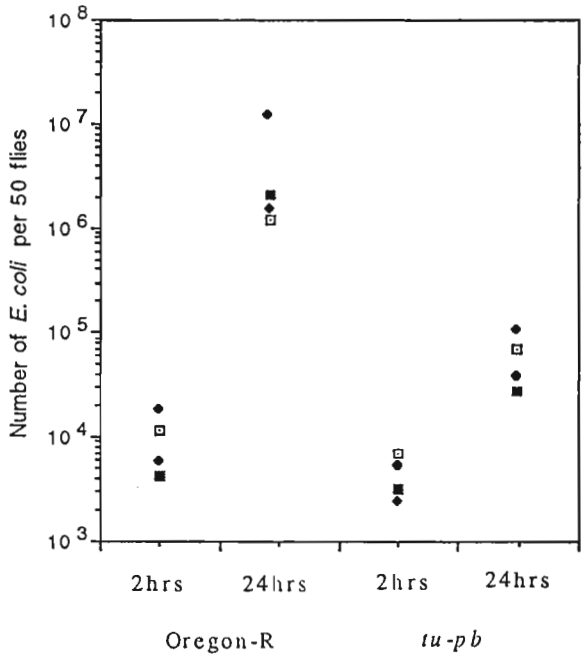

Figure 3. Bacterial growth in challenged Oregon-R and *tu-pb* adults infected with *E.coli* ampicillin resistent strain. Flies were homogenized either two or 24 h after infection, and lysates were cultured on plates containing ampicillin.

melanization and bacterial growth, bacterial proliferation assays were performed on challenged insects. The results obtained indicate that *tu-pb* mutants are able to inhibit bacterial growth more efficiently than wild type (Figure 3).

These preliminary observations could suggest that the *tu-pb* mutation probably enhances other immune mechanisms as proteolytic cascades and cellular reactions, essential for the host defense.

References: Di Pasquale Paladino, A., and P. Cavolina 1983, Dros. Inf. Serv. 59: 31; Di Pasquale Paladino, A., and P. Cavolina 1984, Dros. Inf. Serv. 60: 84; Di Pasquale Paladino, A., P. Cavolina, G. Romano, and R. Ribaudo 1988, Experientia 44: 777-779; Faye, I., and D. Hultmark 1993, In *Parasites and Pathogens of Insect*, vol. 2, Academic Press, pp. 25-53; Hultmark, D., 1993, TIG 9: 178-183; Lackie, A.M., 1988, Adv. Insect. Physiol. 21: 85-178; Lemaitre, B., M. Meister, S. Govind, P. Georgel, R. Steward, J.M. Reichart, and J.A. Hoffmann 1995, EMBO J. 14: 536-545; Sparrow, J.C.,1978, *The Genetics and Biology of* Drosophila, 2b (ed., M. Ashburner and T.R.F. Wright), pp. 277-313, Academic Press, N.Y.