

CHARACTERISATION OF A SELENOPHOSPHATE SYNTHETASE FROM A COLLECTION OF *P-lacW* INSERTION MUTANTS IN *Drosophila*

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We are interested in the regulation of cell proliferation and its role in development. As a model system we use the *Drosophila* imaginal discs development which shows extensive cell proliferation during larval stages. From a collection of *P-lacW* lethal insertion mutations in the second chromosome (Török et al. 1993) we have selected those with a late larval lethal phase and lacking or with reduced or abnormal imaginal discs. Our rationale is that mutations with such phenotypic effects would define genes somehow involved in cell proliferation. One of these mutations is the subject of the present study and has been characterised by a morphological analysis of the discs as well as by clonal analysis.

Disc morphology is characterised by a reduced and unorganised mass of cells. No folding or patterning can be detected in none of the imaginal discs of the larval stages.

Clonal analysis on wings has shown that homozygous clones, in a slow dividing (*Minute*) background as well as in twin clones, have the following features:

- 1.- clones are small in overall size.
- 2.- cells of the clone are small, as deduced from the size of the trichomes.
- 3.- borders of the clones are rounded.
- 4.- vein differentiation is reduced where the clone crosses a vein.
- 5.- ectopic veins may appear adjacent to clones.
- 6.- reduction of the wing regions can be detected when clones are in intervein regions.

We also have checked for the presence of apoptotic cells in the imaginal discs of the homozygous larvae. Both the reduction of the clone size and the associated reduction of the intervein regions could be explained by an increase of cell death. Therefore we labelled the 3' terminus of putative fragmented DNA by means of labelled nucleotides incorporated by a terminal deoxynucleotidyl transferase. The results show abundant apoptotic cells in the reduced homozygous imaginal discs.

After remobilization of the *P-lacW* insert in order to obtain viable revertants we have recovered genomic DNA fragments from both sides of the *P-lacW* insertion by plasmid rescue. DNA isolation, cloning and analysis have been performed according to standard protocols and one probe has been used to screen a cDNA library from adult flies. The gene we have cloned displays great similarity to the human selenophosphate synthetase, an essential component of selenoprotein synthesis. To our knowledge this is the first report of such an enzyme present in lower eukaryotes, since it was known only in prokaryotes and humans. Nevertheless, selenium is an essential trace element that has brought considerable interest owing to the recent identification of prokaryotic and eukaryotic proteins containing the amino acid selenocysteine. All of the selenoenzymes identified to date catalyse oxido-reduction reactions in which the selenocysteine is in the active site. It has been speculated that with the introduction of oxygen into the atmosphere, selenocysteine could have been counter selected as it is already oxidised. Thus, selenoprotein synthesis is now restricted to either anaerobic growth conditions or chemical environments that are well protected from oxygen. In fact, the function of one class of selenoenzymes, the glutathione peroxidases, is protection of cells from oxidative damage caused by the free radical by-products of hydrogen peroxides (Low and Berry, 1996). Further experiments will shed more light on the relationship between the selenoproteins and the activation of cell death mechanisms in those abnormal imaginal discs.

References

- Török, T., Tick, G., Alvarado, M., and Kiss, I. (1993). *P-lacW* insertional mutagenesis on the second chromosome of *Drosophila melanogaster*: isolation of lethals with different overgrowth phenotypes. *Genetics* 135, 71-80.
- Low, S.C. and Berry, M.J. (1996) Knowing when not to stop: selenocysteine incorporation in eukaryotes. *TIBS* 21, 203-208.