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Substrate specificity of esterases in D.pseudoobscura and D.melanogaster, with notes on the tissue localization of Esterase-5 in D.pseudoobscura.

Esterase-5 (Est-5) in D.pseudoobscura and Esterase-6 (Est-6) in D.melanogaster are apparently homologous loci (Abraham & Luchessi 1974). They reside on homologous chomosome segments and encode enzymes with similar catalytic properties. Est-5 maps to position 111.8, slightly distal to the visible mutant locus compressed (co) on the right arm of the X chromosome of D.pseudoobscura (Beckenbach 1981), and in D.melanogaster, Est-6 maps to III-36.8 (Wright 1963) close to the visible mutant locus gespleten (gs). The co and gs mutations produce similar phenotypic effects which further strengthens the case for homology. As part of a study of the determinants of esterase electrophoretic mobility, we have conducted tests to examine the substrate specificities and tissue localizations of these esterases. The results confirm that EST-5 and EST-6 resemble each other biochemically.

Homogenates (6 flies per 150 µl grinding solution: gel buffer with 5% sucrose and a dash of bromochlorophenol blue) were prepared from male and female D.melanogaster genotypically S/S, S/F and F/F for Est-5. For D.pseudoobscura samples were prepared from females with Est-6 genotypes of 100/100, 100/112 and 112/112 and from males with genotypes of 100/Y and 112/Y. Supernatants were run on polyacrylamide gels made by polymerizing 5% or 8% (w/v) solutions of acrylamide monomer and bisacrylamide cross linked (w/w ratio 19:1). The gel and electrode buffer was 87.7 mM Tris, 9.6 mM boric acid and 2.7 mM disodium EDTA pH 9.0 (at 25°C) after Keith (1983). All runs were carried out at 0°C.

A variety of chemical cocktails have been used in the past to detect the presence of enzymic gene products of Drosophila esterase loci. The enzymes (carboxyl esterases, E.C. 3.1.1.1) show a broad substrate specificity, but short chain aliphatic esters are usually found to make the best substrates. Both EST-5 and EST-6 hydrolyze β-napthyl esters more quickly than α-napthyl esters and short carbon chain aliphatic derivates (e.g., acetate) are preferred (see Narise & Hubby 1966, on EST-5 and Danford & Beardmore 1979, on Est-6). Nonetheless, EST-5 and EST-6 are routinely detected on gels by using α-napthyl acetate (α-NA) instead of or together with β-napthyl acetate (β-NA). Many different dyes which couple to the liberated napthol product have been used to visualize esterase activity on gels: Fast Red TR, Fast Garnet GBC, Fast Blue B, Fast Blue RR (these and all dyes and substrates used were provided by Sigma Chemical Co., St. Louis, MO, USA). Therefore, we designed a series of tests to examine the staining properties of these enzymes. After electrophoresis replicate gels of identical homogenates were stained in 0.1 M phosphate buffer pH 6.5 with 50 mg dye plus 20 mg of either only α-NA, only β-NA, or both α-NA and β-NA (substrates were added from 20 mg/ml solutions in acetone). These three substrate combinations were tested in the presence and absence of pronan-1-ol. The results are shown in Table 1.

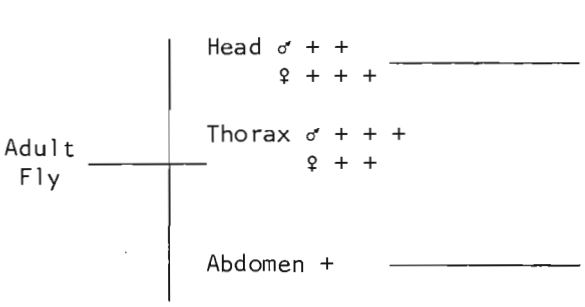
Table 1. Staining properties of the β-specific napthyl esterases EST-5 and EST-6.

Substrate	Propan-1-ol present?	Fast Red TR-napthol conjugate colour and intensity
α-NA	-	rust[1]
β-NA	-	orange[1]
α-NA + β-NA	-	orange
α-NA	+	rust; increased intensity
β-NA	+	orange; increased intensity
α-NA + β-NA	+	orange; increased intensity

1=with Fast Garnet GBC, the colours observed were brown and pink with α-NA and β-NA, resp.

No differences in staining properties were observed between sexes or genotype for either enzyme. The α-specific esterase Est-C from D.melanogaster, gives the same colour pattern except that the α-napthol-dye conjugate colour (rust or brown) predominates when α-NA + β-NA substrate mixtures are used.

Table 2. Tissue localization of Est-5 activity in D.pseudoobscura.



Eyes + +	Intensity of staining (visual estimate) is indicated by number of + symbols; ± indicates a trace of Est-5 activity. Sex-specific differences are indicated by ♂ and ♀ symbols.
Rest of head ±	
♂ Testes +	
♀ Ovaries +	
Gut ± (but + for a different esterase)	
Cuticle ±	
Malpigian tubules -	

In D.pseudoobscura samples run on 5% polyacrylamide gels, we observed a streak of esterase activity with slower mobility than EST-5. When 8% gels were used, the streaking was replaced by a concentrated band of activity. This locus stained rust coloured with α-NA plus β-NA substrate mixtures and Fast Red TR dye. Thus, we conclude that this represents an α-specific esterase (this zone did stain orange as expected with Fast Red TR and β-NA alone).

From these results we recommend the following: (i) For maximum detection of β-specific esterases, use β-NA plus Fast Red TR plus propan-1-ol. However, for ease of visual inspection and photography, rust or brown bands are more distinct than orange ones and thus α-NA plus Fast Red TR without propan-1-ol may be preferred. Depending on one's esthetic sense, other dyes such as Fast Garnet can be used, but the colour produced is somewhat faint.

(ii) To maximize information yield per gel, we recommend using both α-NA and β-NA plus Fast Red TR without propan-1-ol. This combination reveals all α- and β-specific esterases.

(iii) In order to pick out a favourite β-specific esterase from a background of α-specific esterases (e.g., EST-6 from EST-C in D.melanogaster) we suggest α-NA plus β-NA plus Fast Red TR (or Fast Garnet GBC) with propan-1-ol. The alcohol reduces the activity of the α-specific enzymes, and the presence of α-NA ensures that they come up a darker colour.

We studied the tissue distribution of Est-5 in D.pseudoobscura by dissecting out various organs from two adults carrying 112 allele only. Adult flies were divided into head, thorax, and abdomen. Organs within the abdomen were dissected and rinsed in saline. Eyes were excised from heads, and both eyes and eyeless heads were tested. Tissues were homogenized in 20 µl of grinding solution and 10 µl of supernatant loaded on gels. Whole flies used as controls were ground in 30 µl and 6 µl loaded on gels. The activity of EST-5 in the organ samples were judged visually by staining intensity (see Table 2).

Our results agree well with those of Lunday & Farmer (1983), especially with regard to the finding of high EST-5 activity within the eye of D.pseudoobscura. Our failure to find high EST-5 levels in saline-washed isolated abdominal tissues compared with levels in whole abdomens is also consistent with Lunday & Farmer's (1983) report that most EST-5 activity in D.pseudoobscura is present in the haemolymph. EST-6, on the other hand, has been shown to reside primarily in the anterior ejaculatory duct of male D.melanogaster (Sheehan et al. 1979).

Overall, the results of our substrate specificity experiment support the model of homology between Est-5 in D.pseudoobscura and Est-6 in D.melanogaster. The tissue localization of these enzymes, however, clearly demonstrates that these enzyme loci are now under different forms of developmental regulation.

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