

Sena, Elissa and R. J. MacIntyre. Cornell University. Adenosine triphosphatase activity of 3rd larval instar alkaline phosphatase (Aph).

Several non-specific enzymes have been described in *Drosophila melanogaster*. Among these is an alkaline phosphatase reported by Beckman and Johnson (1964). It has been demonstrated that larval alkaline phosphatase bands separated by starch gel electrophoresis and stained by the technique of Beckman and Johnson (1964) all show adenosine triphosphatase activity (ATP-ase activity). The latter activity is observed using a modified lead conversion method (Allen, 1963). The identity between alkaline phosphatase and ATP-ase banding in larva can also be shown with the alizarin red S method (see Sandler and Bourne, 1961).

Attempts at identification of the non-specific alkaline phosphatase as a true ATP-ase have made use of the known specific inhibitors of ATP-ase activity, N-Ethyl maleimide (NEM) and p-chloromercuribenzoic acid (PCMB) reported by Padykula and Herman (1955 a and b). These inhibitors were tested in the following manner: After electrophoresis of the larval enzymes, the starch gels were sliced into thicknesses of 2 mm. One strip was placed into barbital buffer at pH 9.0 containing the inhibitor in question. Concentrations of NEM tested were: 5×10^{-4} M, 5×10^{-3} M, and 1×10^{-3} M. The PCMB concentrations used were 5×10^{-3} M, 2.5×10^{-3} M, 2×10^{-2} M and 1×10^{-2} M. These gels were incubated at room temperature for 1/2 to 1 hour, then rinsed in two changes of 2% CaCl₂ for ten minutes and in distilled water. Finally, the gels were stained for ATP-ase activity by the lead conversion method (Allen, 1963). The intensity and patterns of banding were compared with controls, i.e. 2 mm strips from the same initial gel, which had been subjected to the same treatments except that the inhibitor was absent from the barbital buffer. In this way qualitative effects of the inhibitors could be detected, but substantial inhibition was not observed with either inhibitor at any concentration. In order to re-name this non-specific alkaline phosphatase as an ATP-ase, specific and reversible inhibition of this enzyme should first be demonstrated. At this point, the adenosine triphosphatase activity of the 3rd larval instar alkaline phosphatase must be regarded as spurious.

References

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Falke, Ernest V. and R. J. MacIntyre. Cornell University. The genetic localization of a non-specific leucine amino-peptidase in *Drosophila melanogaster*.

exist as two forms which differed in electrophoretic mobility. Genetic tests revealed that the two forms of the enzyme are controlled by codominant alleles on Chromosome III (Beckman and Johnson, 1964).

Stocks homozygous for the alleles controlling either the "fast" enzyme or the "slow" enzyme were obtained from Dr. T. R. F. Wright and used to more precisely map the locus of the gene. The third chromosome marker stocks used were ru h th st cu sr e^s ca (rucuca) and st sbd e^s ro ca (steroca). Both stocks were found to be monomorphic for the "fast" band and, thus, presumably homozygous for the Lap D^F allele.

The approximate position of the gene was determined with the rucuca stock and a stock which was homozygous for the allele controlling the "slow" band (Lap DS). The final analysis was done with the steroca stock.

Since enzyme activity is strong in single pupae but not in single adults, the test-cross adult progeny could be classified by morphological phenotype only. It was therefore necessary to cross single males carrying recombinant chromosomes, whose morphological phenotype was known, to homozygous Lap D^F/Lap D^F virgin females, and analyze single pupal squashes from these crosses. This procedure then completed the phenotypic analysis of the testcross progeny.

Leucine aminopeptidase (Lap) activity has been demonstrated in starch gel zymograms of *Drosophila melanogaster* pupae. One of the zones of activity, the D zone, or Lap D, was found to

The analysis of the pupae was carried out using the methods outlined by Wright (1963) for starch gel electrophoresis. The trays were run for four hours at 5-8 v/cm and stained using L-leucyl-beta-naphthylamide as the substrate and Black K salt as the dye (Beckman and Johnson, 1964). Crosses with the rucuca stock indicated that the Lap D gene is located between ro and ca. The relative frequency with which the Lap D alleles assorted with the outside markers ro and ca of the steroca chromosome, in crossovers in the ro-ca region, was used to determine the exact position of the Lap D gene. Twelve pupae were tested individually from the mating of each testcross male carrying a recombinant chromosome. If the male's genotype was Lap D^F/Lap D^S, two bands were observed in the D zone of approximately half the pupae so analysed. If the male's genotype was Lap D^F/Lap D^F, only one band was observed in the D zone of all the pupae. In this way, sixty-five recombinant chromosomes were tested. From the data obtained, the locus of the Lap D gene was found to be 98.3±0.5 on Chromosome III.

DL-alanyl-beta-naphthylamide-HCl (AAP) was substituted for the Lap substrate (L-leucyl-beta-naphthylamide-HCl, or LAP). When the top half of a gel was stained with the AAP substrate and the bottom half with the LAP substrate, the banding pattern was the same for both. If the gels were placed one on top of the other, the bands were superimposable. Although the enzymes produced better bands with the LAP substrate, they also showed an observable amount of activity with the AAP substrate.

Although squashes of single adults gave little, if any, activity in starch gel, 10 lambda of a concentrated homogenate of a large number of adult flies when inserted into starch gel gave a heavy banding pattern of aminopeptidases, some of which are apparently different from those observed in the pupae.

References

Beckman, L. and F. M. Johnson, 1964, Hereditas 51: 221.; Wright, T. R. F., 1963, Genetics 48: 787.

Rowan, Sister M. Joan. University of Rochester. Is Plum^K homologous to the mutants of the brown locus?

A mutant, Pm^K, which phenotypically resembles previously known Plum mutants, has been reported by Krivshenko (DIS 38:75). This mutant is associated with an inversion in 2R (proximal break at the begin-

ning of 41 and distal break in 57 E-F). Slatis (Genetics 40:5, 1955) has described about 30 variegated position effects of the brown locus; all are due to chromosomal rearrangements involving 59D to 59F and the chromocentral heterochromatin of Y, 2, 3, or 4. However, the breakage point in the case of Pm^K is definitely in the region 57C - 57F. It was thus necessary to determine whether Pm^K is indeed homologous to the mutants of the brown locus or whether it is a separate mutant associated with the 57 region itself.

Initially it was found that the lethals associated with Pm^K and Pm (laboratory stock) are not homologous since Pm^K and Pm were viable in the homozygous condition as if they were associated with separate loci and were both present in the heterozygous condition.

If Pm^K is homologous to the bw locus, it should be possible to separate it from its associated inversion. (However, the fact that the presence of the inversion may or may not be necessary for the expressivity of the Plum character must also be considered). Slatis was not able to carry out such analyses because of the close proximity of the breakage points of his variegated bw mutants to the bw locus itself.

For genetical analysis, the Pasadena stock: px slt sp (#278, DIS, 1964) was used. The location of these markers on 2R is as follows; the bw locus is also indicated as well as the distal end of the inversion associated with Pm^K.

distal break	px	bw	slt	sp
99-100	100.5	104.5	106.3	107.0

Females Pm^K/px slt sp were test-crossed to px slt sp males, and among 3,548 progeny were found two plexus flies and one Pm^K slt sp fly (this latter female was crossed to