A BLOCK IN THE PENTOSE SHUNT AND IN A 
PURINE DEGRADATION PATHWAY INTERACT TO 
PRODUCE LETHALITY IN DROSOPHILA 
MELANOGASTER

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Abstract—Null activity alleles of the structural genes for glucose-6-phosphate dehydrogenase and xanthine dehydrogenase in Drosophila melanogaster have no or little measurable effect on the viability of mutant flies. In contrast, double mutant combinations lacking both enzyme activities result in lethality. This observation highlights a physiological interaction between metabolic pathways heretofore considered to be independent.

Key Word Index: glucose-6-phosphate dehydrogenase, hexose monophosphate shunt, xanthine dehydrogenase, purine catabolism, Drosophila melanogaster

INTRODUCTION

During the course of an investigation of the cis-acting sequences responsible for the dosage compensation of genes located on the X chromosome of Drosophila melanogaster (Lucchesi and Manning, 1987), we attempted to synthesize a stock suitable for transposon-mediated germline transformation (Rubin and Spradling, 1982). In order to allow the selection of transformants among the progeny of treated individuals, the transformation vector used carried the wild-type allele of a gene for which the recipient strain might, therefore, be mutant. The mutation in question, called ry (rosy), inactivates the autosomal structural gene for the enzyme xanthine dehydrogenase (EC 1.2.1.37) and produces a mutant eye color in homozygous individuals (Forrest et al., 1956). Furthermore, since the X-linked gene under study was Zw +, the structural gene for the enzyme glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49, Young et al., 1964), it was necessary for the recipient stock to lack this enzyme so that the activity of the newly introduced gene could be measured in transformants without the complication of an endogenous background. Starting with true-breeding lines homozygous for either Zw or ry null alleles, we have attempted to generate a stock of flies carrying both types of mutations. This resulted in the unexpected discovery of a highly specific synthetic lethal interaction between the two genes in question.

MATERIALS AND METHODS

Mutant strains and culture conditions

The Zw + gene is located on the X chromosome. Two mutant alleles of this gene were used: Zw +1, induced by ethyl methane sulfonate mutagenesis (Hughes and Lucchesi, 1977) and Zw +88 recovered from a hybrid dysgenesis cross (Nero, 1986); neither allele is able to produce significant levels of G6PD activity as determined by spectrophotometric assay. The mutant allele ry + 546, located on chromosome 3, consists of a deletion of coding sequences and produces no recognizable xanthine dehydrogenase (XDH) transcript. Throughout this report, the two null alleles of G6PD and the null XDH allele may be designated Zw − and ry −, respectively. In various crosses we made use of a balancer, i.e. a chromosome which prevents the recovery of crossover products and, thereby, maintains the integrity of the Zw −-bearing chromosome. The balancer bears a dominant mutation which allows the presence of this chromosome to be detected in heterozygotes. We used FM7a, a balancer described by Merriam (1968). Crosses were performed by placing 3–5 pairs of parents in standard Drosophila culture vials containing a cornmeal–molasses–Brewer’s yeast–agar medium seeded with live yeast. After the females oviposited for 6 days, the parents were removed. All crosses were maintained at 25°C.

Transformation experiments

These experiments were performed following the methods and procedures of Rubin and Spradling (1982). We introduced a DNA fragment with the transcribed region of the Zw + gene into the transformation vector Carnegie-20, supplied to us by Drs A. Spradling and G. Rubin. This plasmid vector contains a transposon (P element) capable of insertion into the Drosophila genome and the marker gene ry + (Fig. 1). Preblastoderm embryos from a ry + 546/ry − recipient strain were injected with the vector, reared to adulthood and mated to individuals from the same strain. Their progenies were examined for the presence of transformants detectable on the basis of their wildtype (ry +) eye color. Details of the construction of the transformation vectors and of the genetic, cytological and molecular characterization of the transformants will be published elsewhere.

Enzyme assays

Crude extracts were prepared by homogenizing adult males in 0.1 M Tris, 5 mM mercaptoethanol, 0.2 mM
Fig. 2. Crosses designed to generate a true-breeding line of flies simultaneously mutant for a null allele of Zw− and of ry+, the structural genes of G6PD and XDH. The X chromosome is represented by the open bars, the Y chromosome by the solid J-shaped symbol and chromosome 3 by the crosshatched bar. 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Fig. 3. In situ hybridization of a plasmid containing of a P element (p25.7 wc) to salivary gland chromosomes of a larva from a transformant line. The probe was labeled with a biotinylated deoxynucleotide and allowed to hybridize to its homologous sequences on the chromosomes; its presence was detected by the binding of a streptavidin-biotin-horse radish peroxidase complex (ENZO Biochem. Inc.) according to a method modified by E. Hafen (personal communication). The arrow indicates the position of the P-element sequences and, therefore, of the transduced Zw⁺; ry⁺ genes. In this particular example, the genes were inserted at 62E on the cytological map. The p25.7 wc plasmid happens to contain a DNA fragment homologous to sequences found at 17C on the cytological map (open arrow).

This provides a useful internal control for the effectiveness of the hybridization procedure.
genes is indicated by the small open box inserted in the symbols are described in the legend of Fig. 2, with the some of a transformant line with a Fig. 4. Crosses performed in order to replace the X chromosome. Note that the position of the transduced genes differs in different transformant lines. In addition to the progeny types indicated, the second cross produces balancer-bearing individuals which have been omitted from the figure.

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nucleic acid biosynthesis. The effects of a block in the committed step of the pathway are apparently easily counteracted by the dietary intake of intermediates such as 6-phosphogluconate, ribose-5-phosphate or 5-phosphoribosyl-1-pyrophosphate (PRPP). XDH catalyzes the oxidation of 2-amino-4-hydroxypteridine to isoaxanthopterin (Forest et al., 1956; Glassman, 1965). XDH also catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid. The expected effect of a ry- mutation is the accumulation of hypoxanthine. In the presence of a functioning hexose monophosphate shunt and a normal supply of PRPP, a rise in the level of hypoxanthine may be limited by the salvage pathway conversion of hypoxanthine to inosinate, a reaction requiring PRPP. In the absence of a functioning hexose monophosphate shunt, the supply of PRPP may be limited preventing the conversion of hypoxanthine to inosinate and resulting in a level of hypoxanthine accumulation deleterious to the organisms. This hypothesis could be tested by measuring levels of hypoxanthine in the pre-lethal stages of double mutant individuals, by determining the sensitivity of Z- individuals to an excess of dietary hypoxanthine, or by attempting to overcome the synthetic lethal effect by feeding intermediates of the pentose shunt.

**Table 1. G6PD and XDH activity in Zw- Y; ry-rY males carrying a Zw- and ry- transduced genes**

| Constitution | G6PD activity | XDH activity |
|--------------|---------------|--------------|
| Zw- Y; ry-ry | 0.3 ± 0.1 | --c |
| (control male escapers) | | |
| Zw- Y; ry+, Zw-ry- | 2.0 ± 0.3 | 1.8 ± 0.3 |
| (transformed males) | | |
| Zw- Y; ry+ry- | 3.5 ± 0.1 | 2.0 ± 0.3 |
| (wildtype control males) | | |

*aOne unit of G6PD activity is that necessary to reduce 1 μmol of NADP per mg of live wt/min.

*bActivity is expressed in arbitrary units of fluorescence per mg of protein/min. The reaction mixture from ry-ry- males exhibited no increase in fluorescence over time and was used to zero the fluorometer.

*cNot determined.