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Lucchesi, JC
Manning, JE

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

JOHN C. LUCCHESI¹* and JERRY E. MANNING²

¹Department of Biology, University of North Carolina, Chapel Hill, NC 27514, U.S.A.

²Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717, U.S.A.

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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 must, 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*ⁿ¹, induced by ethyl

methane sulfonate mutagenesis (Hughes and Lucchesi, 1977) and *Zw*^{H7a} 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*⁵⁰⁶, 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*^{506/ry}⁵⁰⁶ 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

*To whom all correspondence should be addressed.

Fig. 1. Diagram of the transformation vector used in the experiments described in the text. The wildtype G6PD structural gene (Zw^+) contained within a 6 kilobase fragment was inserted in the Carnegie-20 transformation vector of A. Spradling and G. M. Rubin. Open bars, plasmid sequences; solid bars, P-element sequences; crosshatched bar, DNA fragment containing the Zw^+ sequences; stippled bar, ry^+ gene. The vector was injected into recipient embryos in its circular form.

EDTA, 0.1 mM NADP buffer (pH 8.0) at a concentration of 10 mg of wet wt/ml. G6PD activity was measured as the increase in absorbance at 340 nm resulting from the reduction of NADP (Lucchesi and Rawls, 1973). XDH assays were carried out by following the change in fluorescence when 2-amino-4-hydroxy pteridine is oxidized to isoxanthopterin as described by Forrest *et al.* (1956) and modified by Spradling and Rubin (1983).

RESULTS AND DISCUSSION

For the sake of brevity, only the first generation of crosses necessary to generate a line, simultaneously mutant for one of the G6PD null alleles and for the XDH null allele, is presented in Fig. 2. This cross produced a total of 980 $Bal/Zw^{n1}; ry^{506}/ry^{506}$ females, 621 $Bal/Bal; ry^{506}/ry^{506}$ females, 459 $Bal/Y; ry^{506}/ry^{506}$ males, and 11 $Zw^{n1}/Y; ry^{506}/ry^{506}$ males. Similar results were obtained with the Zw^{H7a} allele (data not shown). The two classes of females are expected to occur in equal frequencies; the discrepancy between this expectation and the observed values can be ascribed to the reduced viability resulting from homozygosity for the balancer chromosome and its dominant marker. The $Zw^{n1}/Y; ry^{506}/ry^{506}$ males should be as frequent as the most frequent female class (980) or, at least, as frequent as their Bal -bearing brothers (459). The occurrence of only 11

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 bars. Bal is a balancer chromosome; we used FM7a, described by Merriam (1968). Zw^- represents either Zw^{n1} or Zw^{H7a} ; ry^- null allele used is ry^{506} . The parentheses indicate the lethality or semi-lethality of the double mutant genotype.

such males is significant. Furthermore, these few individuals lived for only a few days and appeared to be sterile. The recovery of poorly viable and sterile $Zw^-; ry^-$ individuals prevented the performance of the next crosses and, therefore, impeded the synthesis of the desired recipient line. Balanced heterozygous stocks were established, nevertheless, by crossing $Zw^-/Bal; ry^-/ry^-$ females with their Bal -carrying brothers.

Using the transformation vector described in Fig. 1, transformations were obtained anyway with the ry^{506}/ry^{506} stock instead of a $Zw^-; ry^-$ stock as a source of recipient embryos and a number of separate lines were established by backcrossing individual transformants to flies of the appropriate sex from the recipient stock. To insure that each transformant line represented a separate insertional event, only one transformant produced by a given recipient was retained. By monitoring the transmission of the ry^+ phenotype in these lines, sex chromosome versus autosomal linkage of the newly inserted (transduced) genes could be determined. The precise cytological mapping of these inserts was performed by *in situ* hybridization to larval salivary gland polytene chromosomes of labeled probes homologous to the P-element sequences in the transformation vector (Fig. 3).

Crosses were performed to replace the X chromosome of transformant lines with an X containing a Zw^- allele (Fig. 4). In the progeny of these crosses, $Zw^-; ry^-$ flies of either sex were, once again, very rare (in a typical cross, a total of 9/546 individuals were obtained). In contrast, flies carrying the transduced Zw^+ and ry^+ genes in their genome were more viable and, therefore, relatively abundant (162/546 individuals). In order to establish that the transduced genes are active, G6PD and XDH activity levels were measured in rescued individuals (Table 1).

The observation of rescue by transformation is important in that it rules out the possibility that the lethality of double mutant individuals may be due to some unidentified effect of the genetic background of the stocks used in the crosses. Transformed males and females carrying the transduced gene differ from their semilethal sibs only by the presence of the Zw^+ and ry^+ alleles. In fact, since the two stocks used to recover transformants and to replace their X chromosomes with a Zw^- allele (Fig. 4) were the same as those used to attempt to synthesize the double mutant recipient strains, the transformants have the same genetic background as the semi-lethal double mutant individuals generated by the scheme in Fig. 2. These results establish the specificity of the lethal interaction between the Zw^- and ry^- alleles. This is supported by the fact that the two Zw^- alleles used in these experiments are of different origin and are present on X chromosome with different histories.

Although no specific biochemical basis for the lethal interaction can be described at this time, a possible correlation which could lead to or result from synergistic interferences with nucleotide metabolism should be considered. G6PD is the first enzyme in the pentose monophosphate pathway. The primary metabolic function of this pathway is the production of pentose whose ribo- and deoxyribonucleotide derivatives are used for coenzyme and especially for

Fig. 3. *In situ* hybridization of a plasmid containing of a P element (p π 25.7 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 p π 25.7 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.

Fig. 4. Crosses performed in order to replace the X chromosome of a transformant line with a Zw^- -bearing X. The symbols are described in the legend of Fig. 2, with the addition that the presence of the transduced Zw^+ and ry^+ genes is indicated by the small open box inserted in the crosshatched 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.

Table 1. G6PD and XDH activity in $Zw^-/Y; ry^-/ry^-$ males carrying a Zw^+ and ry^+ transduced genes

Constitution	G6PD activity ^a	XDH activity ^b
$Zw^-/Y; ry^-/ry^-$ (control male escapers)	0.3 ± 0.1	— ^c
$Zw^-/Y; [ry^+, Zw^+]/ry^-$ (transformed males)	2.0 ± 0.5	1.8 ± 0.3
$Zw^+/Y; ry^+/ry^-$ (wildtype control males)	3.5 ± 0.1	2.0 ± 0.3

^aOne unit of G6PD activity is that necessary to reduce 1 μ mol 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.

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 isoxanthopterin (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 Zw^- individuals to an excess of dietary hypoxanthine, or by attempting to overcome the synthetic lethal effect by feeding intermediates of the pentose shunt.

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