# Genetic Rescue of a Lethal "Null" Activity Allele of 6-Phosphogluconate Dehydrogenase in *Drosophila melanogaster*

Abstract. *While a null activity mutant allele of the structural gene for 6-phosphogluconate dehydrogenase in* Drosophila melanogaster *is lethal, a similar mutation for glucose-6-phosphate dehydrogenase is not. Double mutant combinations lacking both enzyme activities, obtained either by recombination or by mutagen treatment of a chromosome bearing the lethal allele, result in a restoration of viability. The indispensability of the pentose phosphate shunt in* Drosophila *appears to depend upon the specific position of the block within the pathway.*

The existence of induced X-linked lethal mutations associated with the absence of 6-phosphogluconate dehydrogenase (6PGD; E.C. 1.1.1.43) activity has been reported in *Drosophila* (*1*, *2*). Because 6PGD is one of the enzymes of the pentose phosphate shunt, the lethality of these mutants was taken to indicate that proper functioning of the pathway is necessary for survival in this organism (*1*). This contention is now challenged by our recovery of an ethyl methanesulfonate (EMS)–induced viable fertile mutant exhibiting no measurable activity of glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49), the first enzyme of the shunt (*3*). This mutation seems to be an allele of the sex-linked structural gene *Zw* since it fails to complement with a leaky mutant $Zw^{lo}$ (Table 1) which yields a G6PD with altered kinetic properties and heat stability. We are designating this new mutation $Zw^{n1}$.

Apparently, a block of the pentose phosphate shunt at the level of the first enzyme does not affect viability, while a block at a later step is lethal. We therefore attempted to rescue individuals carrying the 6PGD null, lethal mutation $l(1)Pgd\text{-}A^n$ [see (*1*)] by introducing $Zw^{n1}$ into their genome. To this end we crossed $w^a\ l(1)Pgd\text{-}A^n/M\text{-}5$ females to $cv\ v\ Zw^{n1}$/Y males. The $F_1$ females [$w^a\ l(1)Pgd\text{-}A^n/cv\ v\ Zw^{n1}$] were then crossed to *FM7*/Y males. [*M*-5 and *FM7* denote multiply inverted, balancer X chromosomes; for a description of genetic symbols see Lindsley and Grell (*4*).] White-apricot ($w^a$) crossveinless (*cv*) vermilion (*v*) male zygotes, produced by recombination between the two maternal chromosomes, would be expected to carry the $Pgd\text{-}A^n$ and $Zw^{n1}$ alleles. A large proportion of recovered male progeny were, in fact, $w^a\ cv\ v$; two of these were bred to appropriate females and lines were established. Enzyme measurements on both strains failed to detect any significant 6PGD or G6PD activity, indicating that the recombinant genotype was $w^a\ l(1)Pgd\text{-}A^n\ cv\ v\ Zw^{n1}$. Thus, the addition of $Zw^{n1}$ to the $l(1)Pgd\text{-}A^n$ mutation suppresses the lethal character of the latter. Enzyme measurements performed on one of the two recombinant strains are presented in Table 2. We successfully reconstructed the $l(1)Pgd\text{-}A^n\text{-}Zw^+$ chromosome by crossing $w^a\ l(1)Pgd\text{-}A^n\ cv\ v\ Zw^{n1}/y\ cv\ v\ f$ females to males bearing $w^+ \cdot$ Y, a Y chromosome with an X fragment including $Pgd^+$. Forty-two nonyellow ($y^+$), forked (*f*) males produced by this mating were progeny tested: 39 produced no live males and were presumed to be $w^a\ l(1)Pgd\text{-}A^n\ cv\ v\ f/w^+ \cdot$ Y. Three males produced live sons and were presumed to be $w^a\ l(1)Pgd\text{-}A^n\ cv\ v\ f\ Zw^{n1}/w^+ \cdot$ Y. The presence of $Zw^{n1}$ was confirmed biochemically.

Employing the genetic procedures just described, we constructed a second double mutant using the $Zw^{lo}$ allele. Again, the double mutant males, $w^a\ l(1)Pgd\text{-}A^n\ f\ B\ Zw^{lo}$, exhibiting no detectable 6PGD and the expected low level of G6PD activity (Table 2), were viable and fertile. In order to determine the maximum level of G6PD which will mediate rescue of the $l(1)Pgd\text{-}A^n$ mutation, females heterozygous for $Zw^+$ and $Zw^n$ and, therefore, wih 50 percent G6PD activity, were generated and their relative viability was determined. While such females can be obtained in substantial numbers, they are only half as viable as lethal-bearing females with no G6PD activity. Furthermore, while the latter are fertile, the former are sterile.

A separate confirmation of the interactions just discussed was obtained in a search for EMS-induced revertants of $l(1)Pgd\text{-}A^n$. The criteria for reversion were the ability of adult sons of EMS-treated $w^a\ l(1)Pgd\text{-}A^n/w^+ \cdot$ Y males to survive and reproduce without the $Pgd^+$ bearing duplication $w^+ \cdot$ Y. Of three reversions obtained, two were autosomal traits, and one was X-linked. The latter (*R121*) still lacked any 6PGD activity while exhibiting greatly reduced G6PD levels (Table 2). Complementation tests with $Zw^{n1}$ indicate that the R121 chromosome bears a newly induced mutant at the *Zw* locus (Table 1). The presence of the apparently unaltered $l(1)Pgd\text{-}A^n$ allele renders this chromosome analogous to the double mutants generated by re-

Table 1. Enzyme activities in adult females expressed as mean micromoles of coenzyme reduced per minute per milligram of protein ± 95 percent confidence interval. The enzyme assay conditions used were those of Lucchesi and Rawls (*6*).

| Genetic constitution | *Zw* alleles | G6PD activity | Determinations (No.) |
|---|---|---|---|
| *FM7/FM7* | $Zw^A/Zw^A$ | 0.041 ± .002 | 7 |
| $FM7/cv\ v\ Zw^{n1}$ | $Zw^A/Zw^{n1}$ | 0.022 ± .002 | 4 |
| $FM7/Zw^{lo}$ | $Zw^A/Zw^{lo}$ | 0.028 ± .002 | 5 |
| $cv\ v\ Zw^{n1}/Zw^{lo}$ | $Zw^{n1}/Zw^{lo}$ | 0.007 ± .002 | 4 |
| *FM7/R121* | $Zw^A$/*R121* | 0.023 ± .001 | 7 |
| $R121/cv\ v\ Zw^{n1}$ | $Zw^{n1}$/*R121* | 0.003 ± .001 | 6 |

Table 2. Enzyme activities in adult males expressed as mean micromoles of coenzyme reduced per minute per milligram of protein ± 95 percent confidence interval. Enzyme assay conditions used are those of Lucchesi and Rawls (*6*). Zero activity indicates no detectable change in optical density for 5 minutes.

| Genetic constitution | *Pgd* allele | 6PGD | | *Zw* allele | G6PD | |
|---|---|---|---|---|---|---|
| | | Activity | Determinations (No.) | | Activity | Determinations (No.) |
| Wild type (Samarkand) | *Pgd-B* | 0.081 ± .004 | 7 | $Zw^A$ | 0.092 ± .005 | 6 |
| $y\ w^a$ | *Pgd-A* | 0.051 ± .003 | 13 | $Zw^A$ | 0.074 ± .003 | 15 |
| $w^a\ l(1)Pgd\text{-}A^n\ cv\ v\ Zw^{n1}$ | $l(1)Pgd\text{-}A^n$ | <0.001 | 7 | $Zw^{n1}$ | 0.000 | 6 |
| $w^a\ l(1)Pgd\text{-}A^n\ f\ B\ Zw^{lo}$ | $l(1)Pgd\text{-}A^n$ | <0.001 | 4 | $Zw^{lo}$ | 0.016 ± .005 | 7 |
| *R121* | $l(1)Pgd\text{-}A^n$ | <0.001 | 12 | *R121* | 0.008 ± .001 | 12 |

combination. This analogy has recently been extended to include three "revertants" of another lethal *Pgd* allele which represent mutations to reduced G6PD levels (*5*).

Although fairly common among prokaryotes and lower eukaryotes, the rescue of lethal mutations by genetic means is relatively undocumented in higher forms. The pentose phosphate shunt pathway of *Drosophila* provides an excellent model system with which to study this type of genetic interaction.

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**References and Notes**

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15 September 1976; revised 28 December 1976

## Serum Complement-Like Opsonic Activities in Human, Animal, Vegetable, and Proprietary Milks

Abstract. *Human, animal, proprietary, and soy milks are comparable to human serum C5 in opsonization of baker's yeast. Bovine milk and human serum opsonically reconstitute C5-deficient mouse serum. Such reconstitution is selectively inhibited by antiserum to human C5. Further characterization suggests that bovine milk contains material structurally and functionally similar, but not identical, to human C5.*

The serum complement system is a major participant in the human inflammatory response (*1*). In the course of studies involving one of the complement proteins, that is, the fifth component, or C5, we have observed functionally analagous opsonic activities to serum C5 in human, animal, proprietary, and vegetable milks. In this report we review the steps leading to our findings and compare a number of functional properties of human serum C5 with those of bovine milk.

In 1968, we described a familial opsonic defect (*2*). Clinical improvement of the proband resulted upon the administration of fresh plasma, while stored plasma was ineffective. The plasma factor responsible for the patient's improvement was identified through the use of an assay in vitro of the quantitation of uptake by human polymorphonuclear leukocytes (PMN's) of baker's yeast particles opsonized by human serum. Serums from the proband, her mother, and a number of other family members were deficient in their enhancement of yeast particle uptake by normal human PMN's.

When opsonically deficient serum from the patient was mixed with serum from mice genetically deficient in C5, no improvement in yeast opsonization was observed. However, full reconstitution of yeast opsonization occurred after addition to the patient's serum of either low concentrations of normal mouse serum (not C5 deficient) or normal human serums. Further, full reconstitution of either the patient's serum or C5-deficient mouse serum resulted from the addition of physiologic doses of highly purified human C5. In contrast to the mouse serum, which completely lacks the C5 protein, however, the opsonically abnormal human serums were found to contain normal levels of C5 by immunochemical and hemolytic measurements. Thus, it was hypothesized that the abnormality in the C5 molecule was functional rather than quantitative in the abnormal subjects (*3*).

Table 1. Effects of various antiserums on yeast opsonic activities of human serum and bovine milk. Results are expressed as average numbers of yeast particles ingested per PMN. Each number listed for an individual antiserum is based upon at least four separate measurements. Average values are shown, as no appreciable variation was found among individual measurements for any antiserum.

| Antiserum against | Normal human serum | Bovine milk |
|---|---|---|
| | 3.5 ± 0.4* | 3.4 |
| C3 | 2.7† | 3.3 |
| C4 | 3.4 | 3.4 |
| C5 | 2.0† | 1.9† |
| IgG | 3.3 | 3.6 |
| IgM | 3.4 | 3.3 |
| IgA | 3.3 | 3.3 |
| Whole human serum | 1.8† | 3.5 |
| $\alpha_2$-Macroglobulin | 3.1 | 3.4 |
| Ceruloplasmin | 3.2 | 3.2 |
| $\alpha_1$-Antitrypsin | 3.0 | 3.0 |
| Albumin | 3.5 | 3.5 |
| Fibrinogen | 3.4 | 3.5 |
| Transferrin | 3.1 | 3.0 |

*The mean value for normal human serum is based upon determination of opsonic activity of 121 individual normal human serums. †The depression of opsonic activity was statistically significant ($P < .001$). For normal human serum, ± 0.4 equals two standard deviations.

The hypothesized defect was verified through the demonstration of functional abnormalities in the C5 isolated from the opsonically deficient human serum (*4*). Studies of the isolated C5 from deficient serum revealed a restricted, primary functional defect analagous to the one suggested in whole serum from the patient. Yeast opsonic activity was absent, but hemolytic function was normal.

As other patients with the disorder were observed, clinical similarities were noted to a syndrome described by Leiner in 1908 (*5*). Of relevance was Leiner's observation that the illness was limited almost exclusively to breast-fed infants (41 of 43 cases). He observed clinical improvement upon placing the infant on bottled (cow) milk feedings or, occasionally, upon changing the wet nurse.

Based upon these observations, we compared yeast opsonic activities in a variety of milks with that of normal human serum.

Suspensions of baker's yeast [$1 \times 10^9$ yeast particles per milliliter of Earle's balanced salt solution (EBSS)] were incubated with one of the milks or with human serum for 30 minutes at 37°C. The yeast suspensions were then washed three times, resuspended in EBSS and incubated with a suspension of human PMN's ($5 \times 10^6$ PMN's per milliliter of EBSS). After phagocytosis had occurred, the average numbers of yeast particles ingested per PMN were determined by microscopic examination.

The results of these experiments are shown in Fig. 1. Human milk, bovine milk, goat milk, and proprietary formulas such as Enfamil and Isomil (which contain no animal protein), were comparable to human serum in yeast opsonic activity (*6*). The addition of antiserum to C5 significantly decreased the opsonic activities of the milks and serum. Each of these experiments was performed with three different preparations of antiserum to C5 (*7*).

In order to better assess the specificity of the reaction with antiserum to C5, we conducted similar experiments with a number of other antiserums against a variety of antigens, including C3, C4, IgG, IgM, IgA, whole human serum, $\alpha_2$-