assemia and sickle cell disease by the early second trimester (*19*), a time when intrauterine transfusion is technically feasible. Second, the optimal dose of donor stem cells remains to be defined. It is generally accepted that even partial reconstitution of normal hemoglobin production in patients with hemoglobinopathies may alleviate the clinical manifestations of these disorders. The donor hemoglobin levels of 14 to 29% achieved in this study are impressive when one considers that only a fraction of the 2×10^8 to 5×10^8 nucleated cells injected per kilogram of body weight were actually hematopoietic stem cells. Higher concentrations of stem cells in a similar volume of injected material should be well tolerated by the fetus, and may result in greater engraftment. Finally, our findings in this sheep model may not be applicable to the human fetus. Similar studies in a primate model are needed.

The successful achievement of hematopoietic chimerism in a large animal model by transplantation of fetal stem cells in utero suggests that this approach may provide an alternative to termination of pregnancy when, on prenatal diagnosis, a fetus is found to have a congenital hematopoietic disease.

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Amplification of an Esterase Gene Is Responsible for Insecticide Resistance in a California *Culex* Mosquito

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An esterase gene from the mosquito *Culex quinquefasciatus* that is responsible for resistance to a variety of organophosphorus (OP) insecticides was cloned in λgt11 phage. This gene was used to investigate the genetic mechanism of the high production of the esterase B1 it encodes in OP-resistant *Culex quinquefasciatus* Say (Tem-R strain) from California. Adults of the Tem-R strain were found to possess at least 250 times more copies of the gene than adults of a susceptible strain (S-Lab). The finding that selection by pesticides may result in the amplification of genes encoding detoxifying enzymes in whole, normally developed, reproducing insects emphasizes the biological importance of this mechanism and opens new areas of investigation in pesticide resistance management.

ALTHOUGH THE DEVELOPMENT OF resistance to chemical insecticides in arthropod pests constitutes a worldwide economic problem (*1*), there is virtually no information on the modifications that occur at the gene level in the process of acquiring resistance. Genes have been identified and sometimes mapped in studying the physiological or enzymological modifications in resistant insects. Hypotheses concerning genomic changes include structural changes as well as overproduction of either the target molecules or the detoxifying enzymes. For example, the modification of acetylcholinesterase encountered in some pests resistant to organophosphates (OP) and carbamates is believed to be a consequence of point mutation (*2*).

Here we show that overproduction of a detoxifying esterase is the result of gene amplification in *Culex quinquefasciatus* Say. In this mosquito, and in many other species of arthropods, OP resistance arises because one or several detoxifying esterases have become highly active and thus prevent the toxicant from reaching its target at lethal concentrations (*3*). Recently, one of these enzymes (esterase B1) was purified to homogeneity from the Californian strain Tem-R (*4*) and an antiserum raised against the single 67-kD structural polypeptide subunit it contains. The antiserum enabled us to estimate that esterase B1 is 500-fold more abundant in OP-resistant Tem-R than in susceptible (S-Lab strain) mosquitoes (*5*). In the present study, we have cloned a complementary DNA (cDNA) to messenger RNA (mRNA) of esterase B1 and we show that the corresponding genomic fragment is amplified at least 250-fold in resistant Tem-R.

We first verified that the in vitro translation products of total polyadenylated [poly(A)$^+$] RNA's isolated from larvae of Tem-R mosquitoes contain a 67-kD polypeptide comigrating with purified esterase B1 (Fig. 1A, lane 2) that could be specifically precipitated by its antiserum (Fig. 1B, lane 1). RNA's from Tem-R larvae were then utilized to prepare cDNA's. These cDNA's were introduced into the single Eco RI restriction site of the expression phage λgt11 and plated on an *Escherichia coli* Y1090 host. One recombinant phage, λgt-est, contained in the *lacZ* gene a cDNA insert coding for a 19-kD amino acid sequence immunoreactive with antiserum to esterase B1. This cDNA insert (700 bp) was purified and shown to be able to select by hybridization an mRNA that codes for esterase B1, as judged by in vitro translation (Fig. 1C) (*6*).

We therefore used λgt-est DNA as a probe to detect the esterase B1 gene in genomic DNA of OP-resistant (Tem-R) and -susceptible (S-Lab) adult mosquitoes. Equal quantities of genomic DNA from both types of mosquito were digested to completion with Eco RI and the fragments

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obtained were separated by agarose electrophoresis and blot-transferred onto nitrocellulose (*7*). A strong hybridization of labeled λgt-est DNA was observed with a single 2.1-kb fragment in Tem-R DNA (Fig. 2, A and B, lanes 1). On the contrary, λgt-est DNA hybridized only slightly with a 2.8-kb fragment of S-Lab DNA (Fig. 2, A and B, lanes 2). The difference in hybridization levels between Tem-R and S-Lab genomic DNA's with the probe was estimated [by a slot-blot assay (*8*)] to be at least 250-fold.

The 2.1-kb genomic fragment that was shown by the above experiments to contain at least part of the esterase B1 gene in Tem-R mosquitoes has the same size as one of the Eco RI bands that were previously detected (*9*) by an in-gel renaturation technique (*10*) as specifically amplified fragments in the genomic DNA of several OP-resistant strains of Californian *C. quinquefasciatus*. To confirm the identity of these two bands, the Eco RI fragments, after separation by agarose gel electrophoresis and before blot transfer, were subjected to two cycles of denaturation, renaturation, and in situ digestion with S1 nuclease (*10*). The purpose of this treatment was to select amplified fragments. Hybridization of the blot with labeled λgt-est DNA revealed the 2.1-kb band in Tem-R DNA (Fig. 2C, lane 1). No hybridization was detected with S-Lab DNA similarly treated (Fig. 2C, lane 2).

Thus, the λgt-est probe has revealed the presence of a larger number of copies of the esterase B1 gene in the genome of Tem-R than in the genome of S-Lab; our estimates of the amplification factor between the two strains is at least 250-fold. In addition, this probe has allowed us to show that one of the six Eco RI DNA fragments found earlier to be specifically amplified in the genome of the Tem-R strain (*9*) contains at least part of the esterase B1 gene. Specific genomic sequences have also been found amplified in strains of *Culex pipiens* and of houseflies (*9*) that are OP-resistant owing to the presence of highly active detoxifying enzymes (*11*). It is probable, therefore, that gene amplification is a widespread mechanism in the acquisition of resistance to pesticides.

Demonstration of gene amplification in a whole, normally developed, reproducing animal as a response to selection by pesticides in field conditions emphasizes the importance of a genetic phenomenon already widely studied in cultured cells, tumor cells, and during differentiation (*12*). Its discovery in a higher animal provides a model for the study of this phenomenon at the genetic and molecular levels and for evaluation of its evolutionary significance (*13*). One obvious question is: What could be the adaptative significance of such a phenomenon in the insect world, where chemical selection pressures as intense as those imposed by the use of pesticides were unknown? The existence of structural gene amplification in arthropods is likely to lead to strategy changes in resistance management, and more practical questions will have to be answered, such as how amplification is organized in the genome, how it is distributed in the different tissues of the insect, and how it is transmitted from generation to generation.

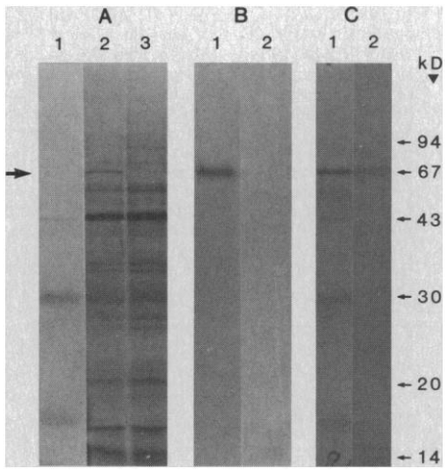

Fig. 1. Identification of a cDNA coding part of esterase B1. (A) Total poly(A)$^+$ mRNA was isolated from mosquito larvae (*14*). Each preparation was translated in vitro in a rabbit reticulocyte lysate system (Amersham) containing [^{35}S]methionine. The labeled products were identified by fluorography after electrophoresis on polyacrylamide gels (15%) in the presence of sodium dodecyl sulfate (SDS). The arrow on the left indicates the position to which purified esterase B1 polypeptide migrates. (Lane 1) Control without mRNA template; (lanes 2 and 3) products encoded by poly(A)$^+$ RNA's extracted from Tem-R and S-Lab larvae, respectively. (B) Immunoprecipitation of the products obtained from the translation of poly(A)$^+$ RNA's from Tem-R (lane 1) or S-Lab (lane 2) larvae by the antiserum to esterase B1. No polypeptide was immunoprecipitated when preimmune serum was used. (C) Hybrid selection of esterase B1 mRNA by the Eco RI–cDNA insert cloned into the λgt-est recombinant. Two micrograms of the insert were bound to a 4-mm square of nitrocellulose filter and allowed to hybridize with 50 μg of poly(A)$^+$ RNA from Tem-R larvae in 250 μl of buffer containing 50% formamide, 10 m*M* Hepes (*p*H 6.8), 5 m*M* EDTA, 0.3*M* NaCl, and 0.2% SDS during 4 hours at 37°C. After extensive washing with 1× standard saline citrate (SSC) and 0.5% SDS at 42°C, the annealed RNA was released into a solution containing 90% formamide, 10 m*M* Hepes, 1 m*M* EDTA, 0.1% SDS, and 25 μg/ml of calf liver transfer RNA at 47°C. Upon ethanol precipitation in the presence of 0.2*M* ammonium acetate (*p*H 5.5), the selected mRNA was translated in vitro and the product was separated by electrophoresis either directly (lane 1) or after immunoprecipitation with the antiserum to esterase B1 (lane 2).

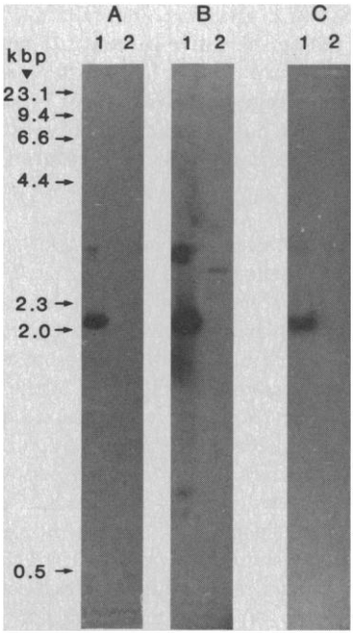

Fig. 2. Hybridization of λgt-est probe specific for the esterase B1 gene with Eco RI–digested genomic DNA of OP-susceptible or -resistant adult mosquitoes. (A and B) Chromosomal DNA (15 μg) isolated as described (*9*) was digested to completion with Eco RI and subjected to electrophoresis on an agarose gel (1%). The separated fragments were transferred to nitrocellulose and hybridized with the ^{32}P-labeled λgt-est probe (specific activity 2.1 × 10^8 cpm/μg) in a buffer containing 50% formamide, 0.1% SDS, 10% dextran sulfate, 20 m*M* sodium phosphate (*p*H 7.0), 5× SSC, and 1× Denhardt's buffer during 12 hours at 42°C. The final washes were performed at 68°C in 0.2× SSC. The same blot was autoradiographed during 18 hours (A) and 7 days (B), respectively. The arrows on the left indicate the migration of size markers on the same gel. (C) The separated Eco RI fragments of chromosomal DNA's were transferred to nitrocellulose after the gel was submitted to two cycles of denaturation, renaturation, and in situ S1 digestion, which allow the selection of amplified DNA fragments. The blot was hybridized as described above. (Lanes 1) Tem-R mosquitoes; (lanes 2) S-Lab mosquitoes.

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6. Complementary DNA's were prepared from poly(A)$^+$ RNA's of Tem-R larvae with an Amersham cDNA synthesis kit [V. Gubler and B. J. Hoffman, *Gene* **25**, 263 (1983)]. After addition of Eco RI linkers, cDNA's were introduced in phage λgt11 [R. A. Young and R. W. Davis, *Science* **222**, 778 (1983); *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1194 (1983)] and plated on *E. coli* λ1090 (Promega Biotec). Among the 5×10^4 recombinants initially screened, 14 displayed a strong positive signal after four plaque purifications with esterase B1 antiserum but not with a preimmune serum or a serum raised against *Culex* esterase A1 (*5*). One of the recombinant phages, λgt-est, was used to lysogenize *E. coli* Y1089. The proteins from the lysogens, after their separation by sodium dodecyl sulfate (SDS)–polyacrylamide electrophoresis and immunoblotting [H. Towbin *et al.*, in (*5*); R. Hawkes, E. Niday, J. Gordon, *Anal. Biochem.* **119**, 142 (1982)] were shown to include a β-galactosidase fusion protein of 135 kD that was immunoreactive with esterase B1 antiserum. The DNA of λgt-est was digested with Eco RI and used to hybrid-select the mRNA of esterase B1 from poly(A)$^+$ RNA's extracted from Tem-R larvae. This selected mRNA (Fig. 1C) produced a 67-kD polypeptide comigrating with purified esterase B1 and immunoprecipitated by its specific antibody. λgt-est cDNA is therefore specific for esterase B1.
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Occult *Drosophila* Calcium Channels and Twinning of Calcium and Voltage-Activated Potassium Channels

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In the membrane of the flight muscle cells of developing *Drosophila* a large calcium-sensitive potassium current, I_{Kc}, was found. It was present before the development of voltage-activated potassium channels and seems to be the first potassium current to develop in the membrane. Also present in these early cells were large numbers of occult (hidden) calcium channels, which remained inactive until the end of pupal development. These inactive calcium channels could be made to function by injecting adenosine triphosphate or ethyleneglycol tetraacetic acid into the early cells. I_{Kc} has kinetic properties resembling the later developing voltage-sensitive current I_{Kv}, and is distinct from the fast, transient calcium-dependent outward current I_{Ac}, which appears much later in development. I_{Ac} closely resembles the voltage-sensitive current I_{Av}, also present in these cells. Thus, both of the voltage-sensitive potassium channel types, I_{Av} and I_{Kv}, have similar calcium-sensitive counterparts, I_{Ac} and I_{Kc}, that are present in the same cells.

IN *Drosophila* DEVELOPMENT, THE LAST of three larval stages terminates with the formation of a puparium and the histolysis of larval muscles. In the following 4 days of pupal development, the adult flight muscles are made de novo. During this period these muscles are particularly accessible for the investigation of developing membrane electrical properties by voltage-clamp techniques (*1–3*). At the midpoint of pupal development a fast, transient voltage-dependent current appeared in the membrane and was mature at about 72 hours of pupal development (Fig. 1A). This current was termed the A current when first identified in molluscan neurons and is designated I_{Av} here. The maturation of I_{Av} is followed by the development of a slower, voltage-dependent K^+ current, I_{Kv}, which is mature at 96 hours of development (Fig. 1A). At the end of pupal development, when the adult is ready to eclose from the pupal case, a large calcium current, I_{Ca}, abruptly appears. There was evidence for the presence of a slow, Ca^{2+}-dependent K^+ current similar to I_{Kc} in other systems (*4*); we observed large, persistent currents in young adult cells after the addition of saline containing Ca^{2+} (6 m*M*) (Fig. 1B). These persistent currents (tails) had a reversal potential at the estimated K^+ equilibrium potential. The large, slow current tail present with Ca^{2+} (Fig. 1B, arrow 4) was absent when Ca^{2+} was removed (Fig. 1B, $-Ca^{2+}$); only a fast component due to I_{Kv} was evident. The current tail (arrow 4) is in the inward (downward) current direction because the K^+ reversal potential for this cell (approximately −60 mV) is more positive than the holding potential of −80 mV.

We investigated the developmental origin of this current with voltage clamping in pupal cells at the 72-hour stage (before I_{Kv} develops) (Fig. 1A). At this developmental stage, there is less net outward current. We determined the pattern of outward current in a 72-hour wild-type cell with no Ca^{2+} (Fig. 2, left) or 18 m*M* Ca^{2+} (Fig. 2, right) in the saline. The fast transient current, I_{Av}, was present in both conditions. However, in the right traces, I_{Kc} is also clearly present, as indicated by the rising outward current after the initial decline of I_{Av} and by the appearance of the large slow current tails after the termination of the voltage-clamp step pulse.

To eliminate I_{Av} from records at this stage

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