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Drosophila melanogaster as an Experimental Organism

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The fruit fly *Drosophila melanogaster* has been used as an experimental organism in studies of genetics since the early 1900s. It is now widely used not only in classical and molecular genetics but also, with many new biochemical, cell biological, and physiological techniques, to research problems requiring a multidisciplinary approach, such as those of developmental biology and neurobiology.

MANY PROBLEMS IN EUKARYOTIC CELL BIOLOGY CAN BE most easily studied in unicellular organisms, such as yeast, or in cell cultures derived from multicellular organisms. Other problems, however, currently can be studied meaningfully only in intact animals. This may be because we do not know how to mimic crucial aspects of the organismal environment in vitro, because cell-cell interactions play an important role, or because the process under study involves a behavior that is not currently understood in terms of the properties of individual cells. Examples include pattern formation in the embryo and the development and function of organ systems, such as the nervous system. *Drosophila's* intermediate level of complexity, in combination with its sophisticated genetics, makes it particularly well suited for the study of basic problems in metazoan biology.

Drosophila melanogaster, the Most Genetically Manipulable Metazoan

The fruit fly has a small size and a short life cycle—features that make feasible the raising of large numbers of individuals for the many generations required for genetic analysis (Fig. 1). It also has a small genome, 1/20 the size of a typical mammalian genome, which facilitates molecular genetic analysis (*1*). Other organisms share these features, however, and they are not the primary reasons for *Drosophila*'s place in modern genetics. For this, credit must go to the hundreds of skillful and creative geneticists who have developed the wide range of tools available for use with this organism. Genetic studies with *Drosophila* began in T. H. Morgan's laboratory at Columbia University in 1909 (*2*). A year later, Morgan described the isolation of the first *Drosophila* mutant, *white eye*, and the observation that its inheritance was sex-linked (*3*). In 1915, Morgan, Sturtevant, Muller, and Bridges published their classic book *Mechanisms of Mendelian Inheritance* (*4*), establishing the relation between genes and chromosomes. Over the past 80 years, much of what we know about recombination, mutation, chromosome rearrangements, and other genetic phenomena has been discovered through the use of *Drosophila* as an experimental organism. It is important to remember that most of the techniques now used in genetic work with *Drosophila* were developed in the course of these efforts.

Use of Mutations to Dissect Complex Processes into Discrete Steps

In *Drosophila* it is possible to screen systematically for all genes that can mutate to produce a given phenotype. This phenotype can be any characteristic for which an assay can be devised, and a wide variety of such screens have been, and are being, carried out. Most common are screens for mutations affecting either viability or aspects of adult or embryonic morphology (*5*). Morphological analysis has advanced to the level of individual cells by the use of antibodies that allow the scoring of specific cells or cell types (*6*). In addition, screens for mutations affecting oogenesis (*7*), sex determi-

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nation (*8*), sensory perception (*9*), and learning (*10*) have been performed. Such genetic screens can provide an unbiased way to identify genes that function in forming a particular structure or in carrying out a particular process. No prior information about the biochemical nature of the structure or process is required. Thus, even processes that might be extremely complex, such as pattern formation in the developing embryo (*11*) or circadian rhythms (*12*), can be genetically dissected into identifiable components that can be individually approached. Mutation of many important genes is lethal to the organism. However, it is easy to maintain stocks of recessive mutations whose homozygosity would result in either death or sterility (*13–15*).

Mutations in over 3000 genes have been described and analyzed in *Drosophila* (*16*), and this number is growing rapidly. Sufficient numbers of individual flies can be examined to allow isolation of mutations in nearly all genes that contribute to the phenotype of interest. However, the number of flies that can be handled conveniently is usually not sufficient to look for phenotypes that cannot be generated by inactivation of a gene, but require a much rarer event such as a particular nucleotide change or amino acid change in the gene product, as is the case in certain types of second-site suppressor mutations (*17*).

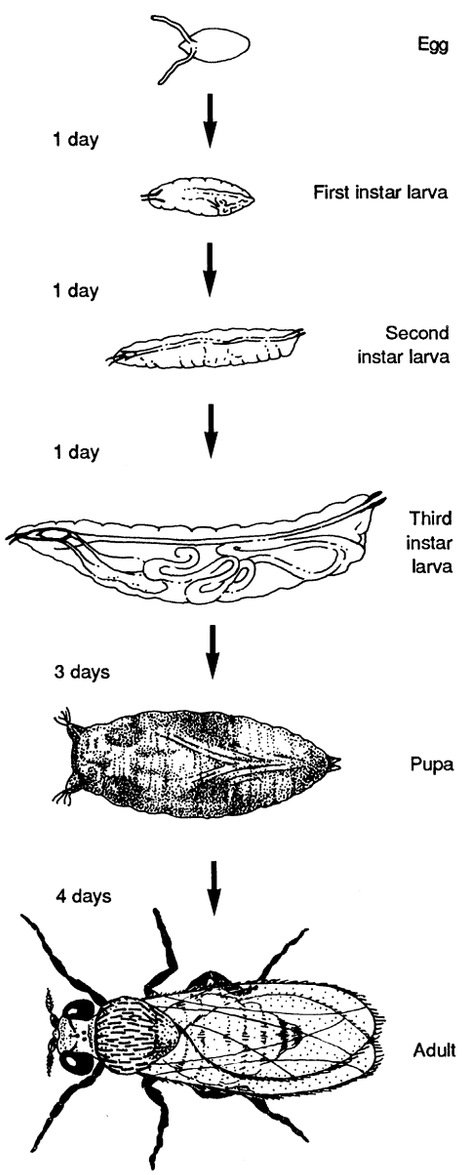

Fig. 1. Diagram of the *Drosophila* life cycle. The approximate time span of each developmental stage at 25°C is indicated.

Use of Genetic Tools to Analyze Gene Function, Cell Lineage, and Cell Interaction

Once mutations that affect the process of interest have been identified, they can be studied by standard genetic methods. Genetic complementation tests can be used to estimate the number of different genes involved in the process (*11*). The phenotypes of individuals carrying mutations in more than one of the genes can be studied to infer hierarchies of gene function. Measuring the rates of meiotic recombination between a mutation and other genes can be used to map the relative chromosomal position of the mutated gene. In addition to these general methods, a set of extremely useful genetic tools, many unique to *Drosophila*, can be applied to the analysis of mutations. These same tools provide noninvasive approaches for studying many aspects of the development and function of normal individuals which are not easily addressed by classical manipulative techniques.

These approaches rely largely on the production of mosaics, individuals that bear clones of genetically altered somatic cells. These clones can be produced either by chromosome loss (*18*) or by induced mitotic recombination (*19*) and can be recognized by the presence of cell-autonomous genetic markers (*20*). A large number of cell-autonomous genetic markers have been identified, allowing analysis of nearly all parts of the animal. By using mosaic animals, it has been possible to construct genetic "fate maps," which plot the relative positions in the early embryo of precursor cells for structures that arise later in development (*18*). Use of this method has made it possible to address questions, such as "What parts of an animal have to be male for it to behave as a male in mating?" (*21*). In addition, analysis of mosaic individuals can be used to determine the cell-autonomy of gene action (*19*), founder cell numbers (*22*), growth patterns (*23*), and restrictions of cell fate (*24*).

A few general examples may illustrate some of these applications. Suppose a gene is known to be essential for the development of a particular cell type, "X." This gene might be required in cell type X itself, in neighboring cells that send an important inductive signal, or in distant cells that provide a required hormone or metabolite. These alternatives can be distinguished by asking what cells, in a mosaic animal, must have a wild-type copy of the gene in order to permit normal development of cell type X. Mosaics are also used in the study of genes that when mutated result in the death of the animal. Such a gene could be required in every cell in the body or in just a particular cell type or organ. Clones of marked cells that are homozygous for the mutant gene can be induced, by mitotic recombination or chromosome loss, in a background of heterozygous cells. If the gene is a "housekeeping" gene required by each cell for its own viability, no marked cells will survive and no clones will be observed. Conversely, if marked clones are observed, then expression of the gene in the cells of the clone cannot be essential for the viability of either these cells or the organism. By examining a large number of clones, one can deduce which cells require the gene for the whole organisms to survive.

Mitotic recombination generally occurs at very low rates but can be increased greatly by x-irradiation. Thus the investigator can control the time at which marked clones are induced. By observing the marked cells later in development, one can learn much about patterns of cell growth and restrictions of cell fate. By generating such clones in flies carrying particular mutations, one can study the role of other genes in controlling these parameters. For example, cell clones induced after blastoderm formation in the posterior region of the wing never contribute to the anterior region, indicating that a lineage restriction has been established (*25*). However, when clones are similarly induced in animals carrying a mutation in the *engrailed* gene, no such lineage restriction is observed, indicating that *en-*

grailed gene function is required to establish or maintain this posterior-anterior lineage restriction (*25*).

High Resolution Cytogenetic Analysis Made Possible by Polytene Chromosomes

Cytogenetic analysis in *Drosophila* is greatly facilitated by the giant polytene chromosomes present in the salivary glands of third instar larvae (*26*). These interphase chromosomes contain more than 1000 strands of chromatin precisely aligned to produce a characteristic and highly reproducible banding pattern (Fig. 2). Polytene chromosomes are useful in correlating cytological and genetic maps. The positions of chromosomal inversions, deletions, and other rearrangements in particular mutant strains can be determined by analysis of polytene chromosomes by use of light microscopy. Moreover, genetic and molecular maps can be aligned using the technique of in situ hybridization (*27*) to place cloned DNA segments on the polytene chromosome map. Some of the bands that can be seen in the light microscope correspond to less than 10 kb of DNA, with the average of the approximately 5000 visible bands being about 25 kb. Thus, the resolution of cytogenetic analysis in *Drosophila* is orders of magnitude greater than in other animals and there is no large gap between what can be seen in the light microscope and what can be cloned in a single recombinant DNA molecule.

Many of the roles traditionally played by cytological maps of polytene chromosomes may be fulfilled instead by ordered molecular maps of cloned DNA segments now being constructed for many organisms (*28*), including *Drosophila* (*29*). However, such molecular maps are made from a particular strain and cannot be used readily when the analysis of different strains is required, as in the mapping of chromosome rearrangements or the positions of transposable elements in different populations.

From Genetic Function to DNA Clone

Many genetic loci in *Drosophila* have been identified by their phenotypic effects on development, physiology, or behavior. For a large fraction of these, the gene product is unknown and methods that depend only on knowledge of the phenotype must be used to molecularly clone the gene. Two approaches have been taken: one relies on cytogenetic and molecular mapping, the other on transposon mutagenesis. In the first approach, classical genetic mapping is used initially to determine the position of the gene relative to known genes and to the breakpoints of chromosomal deletions or other rearrangements. A large number of such rearrangements exist (*16*), and it is generally feasible to produce more for a chromosomal location of interest. Then the region of the genome containing the desired gene is isolated by chromosome walking (*30*) or chromosome microdissection (*31*). In chromosome walking overlapping segments of DNA are isolated, starting from the nearest known cloned DNA segment, by sequentially screening a genomic library, so that one extends the cloned region in an ordered way toward the gene of interest. In cloning by microdissection, the chromosomal region containing the desired gene is physically dissected from the polytene chromosomes of the salivary glands and used as a source of DNA for constructing a library in a bacteriophage λ vector. Both of these methods yield DNA sequences from the region containing the gene of interest. The problem is then reduced to identifying the DNA sequences comprising the gene within the larger cloned region. In some cases, the region defined by the positions of chromosome rearrangements or restriction site polymorphisms may encompass only the gene of interest. In other cases, looking for DNA sequences that have a transcript accumulation pattern that matches the expected pattern of expression of the desired gene has permitted the localization of a gene in a chromosome walk. The most rigorous test, however, is to find whether a cloned DNA segment can complement the mutant defect when introduced into the fly genome by DNA transformation methods.

In the second approach, transposon tagging, transposable elements are used to "tag" a gene biochemically to aid in its cloning (*32*). Transposable elements are segments of DNA that move as discrete units from place to place in the genome (*33*). The insertion of a transposable element into a new genomic site often inactivates a gene located at that site. Indeed, a large fraction of spontaneous mutations in *Drosophila* appears to be due to transposable element insertions. P transposable elements have proven useful to molecular geneticists studying *Drosophila* as tools for transposon tagging because their mobility is under genetic control and can be manipulated experimentally (*34*).

A typical protocol for transposon tagging using P elements would be as follows (*35*). Males from a strain containing P elements (a P strain) are crossed to females from a strain lacking P elements (an M strain). In such a cross P element transpositions are induced in the germline of the progeny. The progeny are bred and their offspring are screened or selected for new mutations in the gene of interest. In most cases, the new mutations will have resulted from P element insertions and the gene will be "tagged" with a P element. DNA corresponding to the mutant allele can then be retrieved from a genomic DNA library of the mutant strain by virtue of its sequence homology to the P element (*36*). Alternatively, strains containing one or only a few P elements can be constructed by P element–mediated transformation of M strains and used instead of naturally occurring P strains as the source of P elements in the initial cross (*37*). This approach has the advantage that P elements can be used that have been modified to contain genetic markers or other features to permit their easy identification and cloning.

The major advantage of transposon tagging as a cloning method

Table 1. Proteins that are highly similar in *Drosophila* and vertebrates.

Class	Protein	Reference
Cytoskeleton	Actin	(*76*)
	Myosin	(*77*)
	Tropomyosin	(*78*)
	Tubulin	(*79*)
	Spectrin	(*80*)
Neuronal function	Acetylcholinesterase	(*81*)
	Choline acetyl transferase	(*82*)
	Acetylcholine receptor	(*83*)
	Sodium channel	(*84*)
Transcription factors	Homeo box proteins	(*85*)
	Zinc finger proteins	(*86*)
Second messenger systems	Calmodulin	(*87*)
	Protein kinase C	(*88*)
	Protein kinase A	(*89*)
Oncogenes	Src	(*90*)
	Abl	(*91*)
	Myb	(*92*)
	Rel (dorsal)	(*93*)
	Int-1 (wingless)	(*94*)
Growth factors and receptor	TGF-β	(*95*)
	Insulin receptor	(*96*)
	EGF receptor	(*97*)
Cell and substrate adhesion	Fibronectin receptor-like	(*98*)
	Laminin	(*99*)
	NCAM*-like (amalgam)	(*100*)

*Neuronal cell and adhesion molecule.

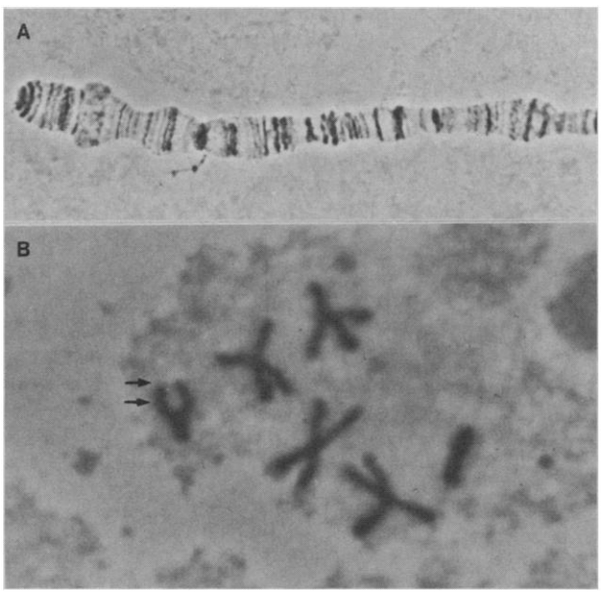

Fig. 2. (**A**) The distal third of the X chromosome as seen in the polytene chromosomes of third instar salivary glands. (**B**) A metaphase spread made from cells of the brain of a third instar male. The region of the X chromosome corresponding to that shown in the top panel is indicated by the arrows. This region contains approximately 8000 kb of DNA.

is that only the approximate cytogenetic location of the desired gene need be known. Thus, it can be readily applied to most of the *Drosophila* mutations already identified. Moreover, screens can be initiated for new mutations that have a particular phenotype with P element insertion used as the mutagen, thereby greatly facilitating the subsequent cloning of those genes (*37*). A major disadvantage in all transposon tagging methods, however, is that P element insertion is not random but shows target site preferences. Thus, not all genomic sites are mutated with equal frequency and perhaps only one-half of all genes will be mutated at rates high enough to make their isolation by this method practical (*38*).

The collections of fly strains, each carrying an insertion of a genetically marked transposable element, that are generated by transposon mutagenesis and by gene transfer experiments are proving very useful for a variety of purposes. For example, with only 1000 such transposable element insertions any site in the genome would be expected to be within 100 kb of an element. Any gene of interest could then be mapped by recombination relative to the markers carried by the transposable element, localized to between two such elements, and isolated by a chromosomal walk between these elements.

From DNA Clone to Genetic Function

Often a segment of DNA will be isolated first, not on the basis of its corresponding to a particular genetic locus, but rather because it displays a desired biochemical property: the DNA might hybridize to RNA present in one tissue or in one developmental stage, it may be similar to another cloned gene from the same or a different organism, or it may encode an antigen that reacts with an antibody of interest. Each of these properties has been exploited with great success in *Drosophila* and other organisms to isolate genes that have a pattern of expression or DNA sequence that indicates they are performing a function of interest. For example, many *Drosophila* genes have been cloned because they contain a homeo box domain (*39*), or are homologous to a particular mammalian oncogene (*40*) or receptor (*41*). Other genes have been selected because they are expressed in the eye (*42*) or at the blastoderm stage of development (*43*), or because they encode an antigen that reacts with a monoclonal antibody that stains the surfaces of a subset of neurons (*44*).

The demonstration that a gene's product is present at the right time and place or has similarity to genes of known or suggestive biochemical function does not prove, however, that it plays a role in the process under study. To get beyond such "guilt by association" arguments one needs to know how the process is affected when the gene product is removed. Mutation of the gene offers a general method for removing its product. Once a gene has been cloned, and its position on the genetic map has been determined by in situ hybridization to polytene chromosomes, a variety of genetic methods (*15*) can be used to isolate mutations that map in the general area of the gene. Then, assuming that a mutation of the gene of interest produces a detectable phenotype, such a mutation can be identified from among all the mutations in the area by its ability to be complemented by the cloned gene (*45*), or by its producing an alteration either in the DNA sequence of the gene (*46*) or in the abundance of the gene product (*47*). If mutation of the gene is lethal to the whole organism, it is nonetheless possible to ask what role, if any, it plays in individual cells by making clones of cells that are homozygous for the mutant gene in a background of heterozygous cells.

Introduction of Normal and Altered Genes Back into the Genome

The experimental utility of gene transfer methods is evident at two levels. The most straightforward and routine application is to determine whether a cloned segment of DNA encodes a product that is missing or altered in a particular mutant (that is, genetic complementation). A more sophisticated application of gene transfer involves the systematic manipulation of a gene and the determination of the biological consequences of the changes introduced. In *Drosophila*, stable gene transfer into the germline can be achieved by using P transposable elements as vectors (*48*).

The strategy for using P elements as vectors for gene transfer is based on mimicking the events that take place during a cross between P and M strains. In such a cross, P elements on paternally contributed chromosomes enter the M strain egg and are induced to transpose at high rates. An analogous situation occurs if DNA containing an autonomous 3-kb P element is microinjected into an M cytotype embryo shortly after fertilization. This element can transpose from the injected DNA to the germline chromosomes of the host embryo in a reaction that is catalyzed by a "transposase" protein encoded by the P element. Smaller P elements that lack the DNA sequences encoding this protein can also transpose if co-injected with the 3-kb element. Other DNA segments of interest can be transferred into the germline if they are inserted within such internally deleted P elements and then co-injected with the 3-kb element. Figure 3 shows a typical protocol for such a gene transfer experiment.

Because genes transferred by means of P element vectors are incorporated into the germlines of their hosts, their function can be assayed in all cell types and developmental stages in subsequent generations. Although the transferred genes are not inserted at their normal chromosomal locations, they appear to be regulated properly and, in the majority of cases, exhibit correct tissue and temporal specificity of expression (*49*).

A cloned gene can be altered by mutagenesis in vitro and then put back into the genome where its function can be assessed. Specific changes in the amino acid sequence of the encoded protein can be

engineered and the effects of these changes determined in the organisms's normal environment, where behavioral or developmental phenotypes caused by the altered gene can be monitored. In addition, mutations outside the protein-coding region of the gene can be made and assayed in vivo to determine which cis-acting DNA sequences control the tissue specificity and the developmental timing of gene expression.

More complex mutations can also be constructed in vitro, including mutations that cause a gene to be expressed in a cell type where it is not normally active (*50*). For example, suppose a gene encoding a cell surface protein specific to cell type A has been isolated. The protein coding portion of this gene could be joined to the control region of a second gene whose expression is limited to another cell type, B, and the fusion gene introduced into the genome. Flies carrying the fusion gene should now express the protein on the surface of cell type B, directed by the fusion gene, as well as on the surface of cell type A (as a result of the unaltered copy of the gene present in the fly's genome). If this cell surface protein is involved in cell-cell-recognition, specific developmental abnormalities might be expected.

One problem with this type of approach is that the incorrect expression of many important genes will be lethal to the organism. Although genetic tools exist for handling recessive lethal mutations in *Drosophila*, mutations like the one described earlier are expected to be dominant. The classical way to overcome this difficulty is to make expression of the mutant gene conditional. For example, fusions can be made to transcriptional promoters that are inducible either by environmental factors, such as heat shock (*51*), or by an exogenous trans-acting factor that can be supplied only by mating to a specially engineered strain (*52*). An alternative solution would be to make fusions to promoters that are specific for nonessential cell types. For example, flies without eyes are viable under laboratory conditions and therefore fusions made to a promoter for a photoreceptor-specific gene (for example, rhodopsin) would be expected to be viable even if the fusion product resulted in death of the photoreceptor cells or otherwise disrupted eye development.

Other potential applications of gene transfer remain to be developed. For example, many genes have been cloned by virtue of their differential expression in certain cell types or developmental stages. However, these genes frequently do not correspond to known mutations, and thus the phenotype of an individual lacking the gene function cannot be discerned. Although with effort mutations in such a gene can be induced by classical means, a convenient and reliable method that utilizes the cloned copy of the gene to inactivate the corresponding chromosomal copy would be particularly useful. Methods based on homologous recombination, such as those used in yeast (*53*), are presently not available in *Drosophila*. It is possible that methods based on the production of an antisense RNA (*54*) can be adapted to *Drosophila* (*55*), although it is not clear that this method can completely inactivate a gene.

Embryological and Biochemical Methods Supplement Genetic Approaches

In addition to the genetic methods described above, it is possible to apply most of the techniques of experimental embryology to *Drosophila*. The embryo develops outside the mother and can be easily observed with a variety of techniques with varying degrees of resolution and invasiveness (*56*). Many gross morphological changes can be seen in living embryos by use of light microscopy, while at the other extreme fixed embryos can be readily stained, allowing one to see cellular details at high resolution. Methods for nuclear and cell transplantation (*57*), microsurgery (*58*), laser ablation (*59*), cell and organ culture (*60*), biochemical cell marking (*61*), immunocytology (*62*), and in situ hybridization (*63*) have been developed and widely applied.

Large quantities of *Drosophila* can be readily obtained at each stage of its life cycle. This facilitates biochemical analysis and has made it possible to obtain a wide variety of purified proteins. However, the difficulty in obtaining sufficient quantities of specific tissues or cell types for biochemical analysis is often a limiting factor. Extracts of embryos or cell cultures have been used for in vitro transcription (*64*) and RNA splicing studies (*65*). The ability to combine such biochemical studies with genetic analysis should be particularly useful in understanding how gene expression is controlled during development.

Use of *Drosophila* to Study the Development and Function of the Nervous Stystem

A number of scientists working with *Drosophila* are now using the embryological and genetic methods outlined here to investigate how the nervous system develops. Electrophysiological methods, including intracellular recording (*66*) and patch clamping (*67*), have been used in a variety of preparations. Given the small size of *Drosophila* neurons, however, it has been possible to map neuronal circuits in only a few cases (*68*). A large number of mutations affecting the electrical properties of nerve cells have been isolated (*69*), including

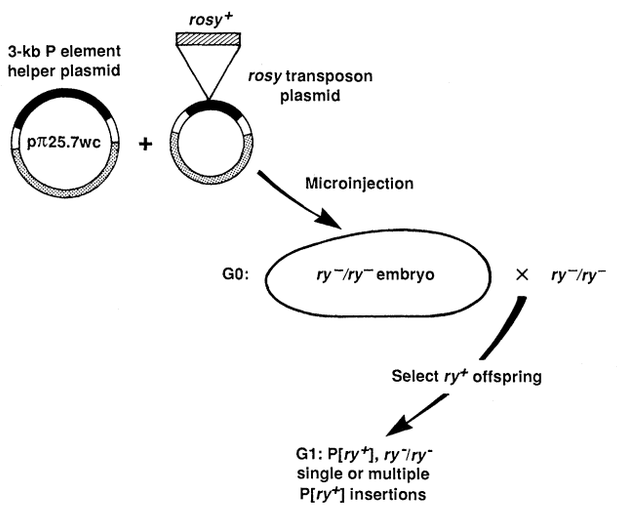

Fig. 3. Typical protocol for a gene transfer experiment. DNA of the plasmid pπ25.7wc, which carried a 3-kb P element, and DNA of the *rosy* transposon plasmid, which carried a wild-type *rosy* gene inserted into a small P element (to generate a *rosy* transposon), are co-injected into an embryo that is homozygous for a mutation in the *rosy* gene. The inclusion of the plasmid pπ25.7wc is necessary, since the *rosy* transposon can only transpose with the aid of protein factors encoded by the 3-kb "helper" P element. Approximately 10% of the injected embryos will survive to become fertile adults (G0 adults) and, in about one-third of these, transposition of the *rosy* transposon from the injected plasmid DNA to germline chromosomes will have occurred. Since transposition only occurs in the germline, the expression of the introduced *rosy* gene is not evident in the somatic tissues of the G0 adults, but if they are mated to ry^- individuals the expression of the *rosy* gene can be assayed in the next (G1) generation. In those offspring that show expression of the *rosy* gene (ry^+ offspring), single or multiple copies of the *rosy* transposon (P[ry^+]) are found inserted in the chromosomes and are stably inherited in future generations. The *rosy* gene (the structural gene for the enzyme xanthine dehydrogenase) affects eye color and thus is easily identified. Genes that have functions difficult to assay can be transferred by constructing a transposon that contains both the gene of interest and the *rosy* gene. Successful transfer of the entire transposon can be detected by identifying *rosy* gene function and then the ry^+ progeny can be assayed for the function of the second gene.

those in the structural genes for channels (*70*). Much has been learned from analysis of the electrophysiological effects produced by these mutations. Even lethal mutations have been amenable to study in nerve cells cultured from the homozygous embryos (*71*). Much information about the relationship of structure to function has been gained by studying genes encoding ion channels and receptors that have been expressed in heterologous systems, such as *Xenopus* oocytes (*72*). However, questions concerning the regulated expression, localization, and modification of such important molecules can only be fully answered if they are studied in their natural environment. *Drosophia* can be expected to play an important role in these studies. In addition, sensory transduction (*9*), neuronal pathfinding (*73*), various behaviors (*74*), biological rhythms (*12*), as well as learning and memory (*10*) are being analyzed in *Drosophila* by combined genetic and molecular approaches.

Concluding Remarks

When the techniques described here are used on an organism with the intermediate level of complexity of *Drosophila*, many challenging problems are accessible to experimental analysis. Will the answers obtained be relevant to mammalian organisms such as ourselves? The high degree of homology of a wide range of fundamental molecules suggests that many, perhaps nearly all, of the basic mechanisms used for the development and function of multicellular organisms were established before the evolutionary divergence of the progenitors of flies and humans some 500 million years ago. Representative examples of the high degree of primary sequence similarity observed between many *Drosophila* and vertebrate proteins are given in Table 1. This homology extends to functional properties. For example, the *Drosophila* insulin receptor binds to and is activated by bovine insulin (*75*). Mammalian organisms are more complex than *Drosophila*. However, there is no compelling reason not to believe, and much circumstantial evidence to support, the contention that most of this complexity is achieved by reiteration and adaptation of common, evolutionarily ancient processes.

Although other experimental organisms may equal or exceed *Drosophila* in the facility of a particular experimental area, only flies contain in one system the potential for the application of the tools of classical genetics, cytogenetics, molecular genetics, biochemistry, electrophysiology, cell cultures, and other cell biological techniques. *Drosophila*'s unique ability to support such a multidisciplinary approach, combined with its intermediate level of complexity, ensures that this animal will continue to play an important role in biological research for many years to come.

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