The Drosophila Stock Centers and their implications for developmental biology

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ABSTRACT Mutations are central to functional analyses of genes and their products. In vertebrates, gene clones may be readily available, but there is often a lack of mutations. In *Drosophila melanogaster*, which has been used in genetic research for almost a decade, mutations defining thousands of genes have been isolated. Much of the basic genetic knowledge available today has been obtained from fundamental experiments done on the fruitfly. This year's Nobel laureates in physiology and medicine, E.B. Lewis, C. Nüsslein-Volhard and E. Wieschaus, were rewarded for their important contributions to our understanding of the genetic control of early embryonic development where they used *Drosophila melanogaster* as model system. Such experiments often result in huge numbers of mutant strains that should be maintained to aid in the localization and functional analyses of new genes in the future. For this reason Drosophila Stock Centers have been established in Europe and North America. Japan is also planning to build an Asian Drosophila Stock Center. The objectives of Drosophila Stock Centers are to maintain strains with well characterized mutations, check their constitution and distribute strains together with information about their genetic defects to research groups around the world. The European Commission has recently acknowledged the importance of stock centers as part of the biological research infrastructure by supporting the European Drosophila Stock Centre in Umeå.

KEY WORDS: *Drosophila melanogaster, fruitfly, embryonic development, stock centers, mutant strains*

The fruitfly, *Drosophila melanogaster*, is one of the reference species in the HUGO project (Merriam *et al.*, 1991) and plays an important role for understanding the function of the hundreds of thousands of open reading frame sequences that will be found in the human, and other genomes. The *Drosophila* genome is estimated to contain between 5,000 and 15,000 genes (based on the numbers of lethal mutations and of transcription units, respectively), which should be compared to man's 100,000. In vertebrates it is difficult to trace the specific function of the genes that are important for early development due to the lack of mutations. On the other hand, several hundreds of the sequenced genes in *Drosophila* have counterparts in vertebrate species. This conformity makes it possible to perform mutational analyses in *Drosophila* which, to some extent, will indicate in what direction to look for function in higher organisms.

The comparably cheap handling of fruitflies, their short generation time and large number of offspring makes them ideal for genetic studies. A small genome in combination with its highly differentiated structures also makes them ideal for studies of functionality. *Drosophila* is as complex as many vertebrates with respect to metabolism, motion, sensory perception and behavior and they even have the capacity to learn and memorize. Several huge mutation screens have been made by different labs, in order to saturate the genome with lethal, visible or behavioral mutations. The ultimate goal is to isolate at least one mutant per locus, providing markers for each gene in the genome and thus making possible the genetic dissection of the developmental processes.

Several types of mutation screens have been performed in *Drosophila* (e.g. Reuter and Wolff, 1981). The classical tools for inducing mutations have been ionizing radiation or chemical mutagens. Nowadays, P-element mediated transformation or P-element hybrid dysgenesis screens are often used methods. In the latter, a P-element is induced to transpose within the germ line of flies carrying a stable transposase source, thus creating mutations at a high frequency from transposon insertions (Cooley *et al.*, 1988). This procedure has great advantages compared to ordinary chemical or X-ray induced mutagenesis, since the P-element can be used as a tag to clone the DNA surrounding the insertion site and thereby facilitate the cloning of mutated genes. The P-element can also be remobilized by simple genetic crosses in order to create reversions or short deletions around the insertion site. The enhancer trap technique makes use of DNA constructs which have been introduced into the genome by P-element mediated transformation. It is based on a minimal promoter driving a bacterial ß-galactosidase *(lacZ)* gene, which can be activated in specific patterns during development in transgenic flies (reviewed in Bellen *et al.*, 1990). This

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can be used to identify genes that are active in a certain tissue or during certain developmental stages. Some examples of *Drosophila* embryonal expression patterns in enhancer trap lines are shown in Figure 1. Enhancer trap detection screens have been done to search for genes specifically active in embryos (Bellen *et al.,* 1989), in ovaries (Grossniklaus *et al.,* 1989), in the brain and imaginal discs (Gibson and Gehring, 1991) and recently in the tracheal system (Samakovlis *et al.,* 1994) of *Drosophila*. The enhancer trap method has also been used on transgenic mice (Allen *et al.,* 1988).

Systematic screens for mutations in genes affecting early embryogenesis have been performed by Nüsslein-Volhard and Wieschaus (1980; Jürgens *et al.,* 1984; Nüsslein-Volhard *et al.,* 1984; Wieschaus *et al.,* 1984) at the European Molecular Biology Laboratories in Heidelberg. The maternal-effect mutants found in such screens imply that the maternal contribution is of a rather general and global nature. Some 20 genes have been identified that establish the initial anterior-posterior and dorsal-ventral pattern during oogenesis. During the cellular blastoderm stage the main features of a segmental pattern are determined by the actions of gap genes, pair-rule genes and segment polarity genes. These genes subdivide the embryo into smaller and smaller domains, distinguished by different patterns of gene expression. It has been possible, with genetic analysis and cell biological methods, to order these genes and their products in a hierarchy which is temporally and spatially determined. The homeotic selector genes within the *Antennapedia* and *Bithorax* complexes (ANT-C and BX-C) become active in the blastoderm. The definitive expression pattern is not complete until after gastrulation, however, when it helps to establish the identification and specialization of the segments.

There are several examples where *Drosophila* has shed light on mammalian development. The homeobox sequences encode an approximately 60 amino acid long DNA-binding domain, the homeodomain, composed of 3 alpha helical regions similar in structure to a class of bacterial DNA-binding proteins. These DNA sequences were first described and isolated in the *Antennapedia* complex members, *fushi tarazu* and *Antennapedia*, but they are also found in other key members of the set of about 50 control genes that define the anteroposterior pattern in *Drosophila*. About 40 homeobox containing genes, the Hox genes, have been isolated in mouse thanks to sequence homologies. Another example of conserved DNA sequences, with a presumed specific function, is the paired-type homeobox, which has been found within the Pax gene family in mammals. This regulatory domain was first identified in the *Drosophila* pair-rule segmentation gene *paired*. Several Hox and Pax genes have also been isolated in *Xenopus* and humans by means of sequence homologies (reviewed by Kessel and Gruss, 1990). The homeotic complex in *Drosophila* consists of 8 genes clustered in 2 groups (ANT-C and BX-C), but the splitting is not essential. Intriguingly, the relative order of the genes within the homeotic clusters reflects the expression along the body axis of the *Drosophila* embryo. Analysis of the structure and expression of the Hox genes have also shown this remarkable constancy in chromosomal organization and in spatial expression patterns. The 4 vertebrate Hox clusters are supposed to have arisen by 2 duplications followed by loss of individual genes. Bachiller and coworkers (1994) have looked at the effects of mouse Hox genes under heat shock control on *Drosophila* larval patterns in transgenic strains. The results suggest a general conservation of the functional hierarchy of homeotic genes that correlates with the order of genes and expression patterns.

Another example of comparative analysis between *Drosophila* and vertebrates is the *Wnt-1* gene. It was first identified as a proto-oncogene causing mouse mammary tumors (Nusse and Varmus, 1982) and it encodes one member of a family of putative signaling molecules. The *Wnt-1* gene is expressed in the developing nervous system of the mouse in a highly organized manner, with distinct anteroposterior and dorsoventral restrictions. Later on, the *Drosophila* orthologue, the segment polarity gene *wingless*, was cloned (Baker, 1987). This has stimulated a powerful molecular, genetic and cellular investigation of *wingless* signaling in patterning of body segments (reviewed by McMahon, 1992; Nusse and Varmus, 1992). In the trunk of the early *Drosophila* embryo *wingless* is expressed in 1 row of cells, just anterior to each parasegment boundary. The protein adheres to the surface of producing cells or associates with the extracellular matrix, and thus behaves as its mammalian counterpart. The *wingless* protein positively regulates expression of the *engrailed* gene in adjacent cells through complicated interactions involving products of several other segment polarity genes. *engrailed* encodes a transcription factor and is expressed in a row of cells just posterior to those cells that express *wingless*. The wingless receptor, a very central ingredient in the pathway for signaling, is however, still unknown.

It seems equally important to have the correct genes active in a certain segment as it is to have these genes downregulated in other segments. A group of *Drosophila* genes called the *Polycomb* group *(Pc-G)* are involved in the clonal transmission of the repressed state of homeotic regulatory genes through development (reviewed by Paro, 1990). Two modes of regulation have been proposed, either *Pc-G* gene products maintain an inactive chromatin structure by promoting DNA packaging, or they act as negative transcription factors. So far, 12 *Pc-G* genes have been identified in *Drosophila*, and as many as 40 might exist (Jürgens, 1985). *Polycomb*, which has given the name to the group, is primarily zygotically expressed and acts as a down-regulator of many homeotic loci. In heterozygous mutant males this can be seen as additional sex comb teeth on the second and third legs. Sex combs are structures normally only found on the first pair of legs on *Drosophila* males. Embryos lacking *Polycomb* function show strong posterior segmental transformation, which is of course lethal (Lawrence *et al.,* 1983). Interestingly, the *Polycomb* gene contains a domain (the chromo-domain) coding for a protein motif present also in a heterochromatin-associated protein encoded by the *Suppressor-205-of-variegation* gene (Paro and Hogness, 1991). This finding supports the idea that an inactive chromatin structure is achieved by the gene products. The chromo-domain is conserved in mammals and across the animal and plant kingdoms (Pearce *et al.,* 1992, and references herein). Another *Pc-G* gene, the *Posterior sex combs (Psc)* has been found to share a 600 nucleotide domain with the murine *bmi-1* oncogene (Brunk *et al.,* 1991). The *Drosophila* protein is nuclear and binds at about 45 loci on polytene chromosomes (Martin and Adler, 1993). The gene products of *Polycomb* (Zink and Paro, 1989) and *polyhomeotic*, another *Pc-G* gene (Franke *et al.,* 1992),

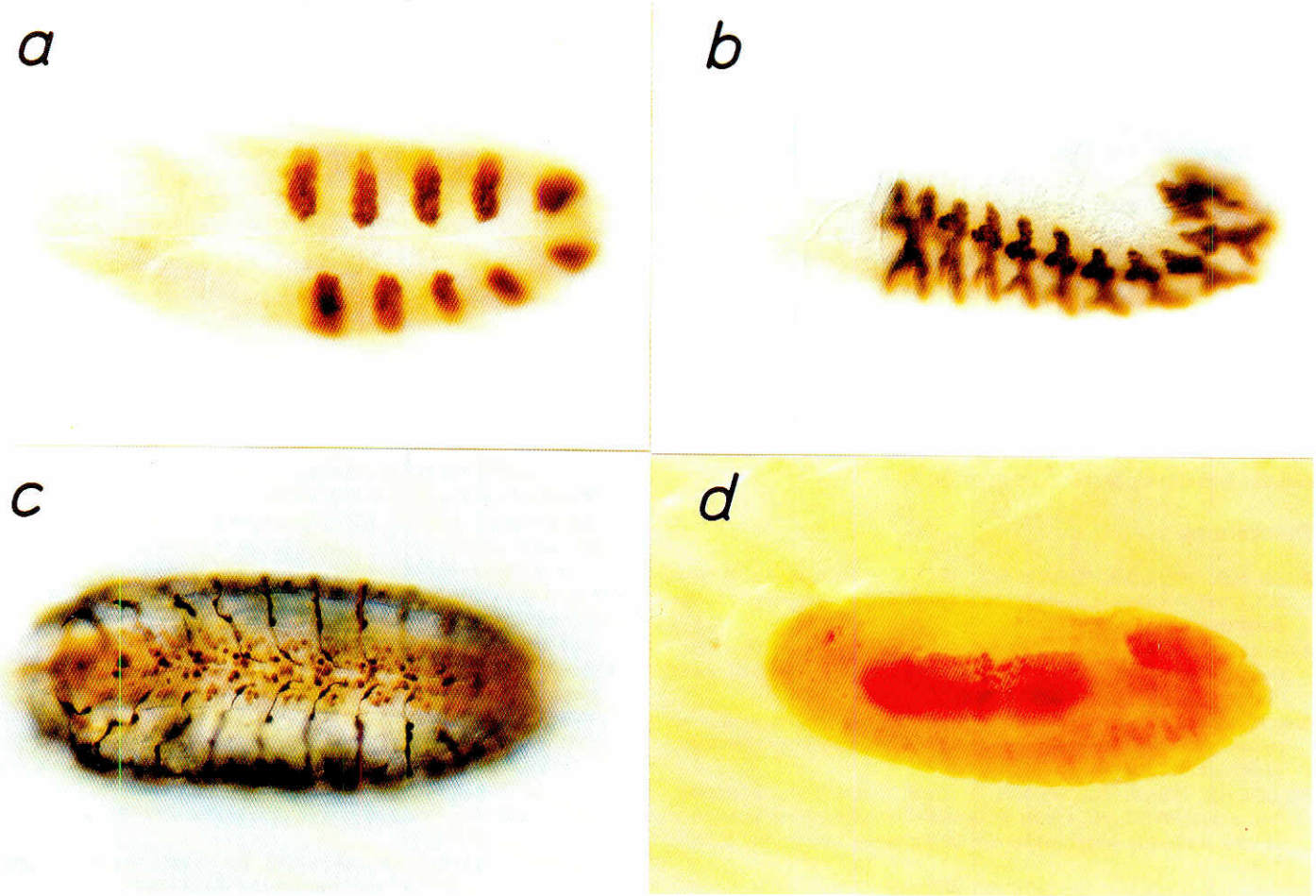

Fig. 1. Examples of embryonal expression patterns in enhancer trap lines of *Drosophila melanogaster*. **(a)** *An early embryo (stage 11) that expresses the ß-galactosidase reporter gene in cells that will become part of the tracheal system. The reporter gene construct has happened to be inserted close to an enhancer that specifically regulates a nearby gene important for the development of tracheal cells. The produced ß-galactosidase is made visible by the use of an antibody that specifically binds to the ß-galactosidase enzyme. The antibody is coupled to horse-radish peroxidase, which drives a reaction giving a brown colored compound.* ***(b)*** *The same line as in* ***(a)*** *but stained later in development (stage 12).* ***(c)*** *A ventral view of another enhancer trap line which expresses the ß-galactosidase gene in both tracheal cells and cells that will develop into the central nervous system.* ***(d)*** *An embryo from yet another enhancer trap line, where the reporter gene is inserted close to a gene that is specifically activated in cells that will give rise to the midgut.* ***(a,b,c)*** *Anterior is to the left;* ***(a,b,d)*** *dorsal is at the top. Photographs kindly supplied by Dr. Christos Samakovlis.*

have been found to bind to almost all of the chromosomal locations to which the *Psc* protein bind. However, the *Pc* protein does not bind directly to DNA, but probably achieves its specificity by interacting with more segment- and tissue-specific factors.

The strongest advantages with *Drosophila* are the large amounts of knowledge gathered over the 90 years this organism has been used in genetic studies, and also the vast numbers of mutants and strains that have been constructed and propagated in various labs around the world. In attempts to simplify the stock keeping labour for drosophilists, the *Drosophila* Stock Centers were started. The European *Drosophila* Stock Center is located at the Dept. of Genetics, Umeå University, Sweden, and the two centers established for the American Drosophila community are located at the Dept. of Biology, Indiana University, Bloomington, Indiana 47405, and Dept. of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403. The *Drosophila* Stock Center in Umeå started in 1981, initially as a joint project supported by the European Science Foundation, ESF, but since 1989 it has been financed entirely by the Swedish Natural Science Research Council (NFR) and Umeå University.

Many *Drosophila* embryonic lethal mutants and enhancer trap lines isolated in the large screens mentioned above are kept in the *Drosophila* Stock Centers. In addition to the lethal strains, there are also a variety of sterile mutants, behavioral mutants, enhancer and suppressor mutants, which are mapped genetically or cytologically, but whose functions still await illumination. Mutations in over 4000 genes and over twice as many rearrangements in *Drosophila* have been described and analyzed (Lindsley and Zimm, 1992). The *Drosophila* Stock Centers naturally also keep thousands of specified deficiencies, easily scorable markers and balancer chromosome strains to aid in the mapping, identification and genetic analysis of *Drosophila*

genes. Stock lists are available electronically on the FlyBase Server at Bloomington, Indiana (gopher ftp.bio.indiana.edu or ftp ftp.bio.indiana.edu), and appear in printed versions in the *Drosophila Information Service* on a regular basis. The European *Drosophila* Stock Center in Umeå distributes well over 2000 strains per year, filling requests from all over the world. The larger Bloomington Stock Center distributed over 12000 stocks in 1992, of which 28% were sent outside the USA.

Discussions are currently underway about establishing a stock center in Europe for the maintenance of transgenic mouse stocks, which will be of major importance for facilitating the research on mammalian development. However, while many of the tools for functional analysis are specific for *Drosophila*, such as polytene chromosomes, and while some of the results obtained may be specific for insects, many of the results from the cloning and mapping of the *Drosophila* genome will continue to be of profound value for all biology because of the universality of cellular and developmental mechanisms.

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