

DROSOPHILA MELANOGASTER - THE MODEL ORGANISM OF CHOICE FOR THE COMPLEX BIOLOGY OF MULTI-CELLULAR ORGANISMS

Kathleen M. Beckingham^{1*}, J. Douglas Armstrong², Michael J. Texada¹, Ravi Munjaal¹, Dean A. Baker²

¹ Department of Biochemistry and Cell Biology, MS-140, Rice University, Houston, Texas 77251.

² School of Informatics, Institute for Adaptive and Neural Computation Edinburgh, EH1 2QL, UK.

ABSTRACT

Drosophila melanogaster has been intensely studied for almost 100 years. The sophisticated array of genetic and molecular tools that have evolved for analysis of gene function in this organism are unique. Further, *Drosophila* is a complex multi-cellular organism in which many aspects of development and behavior parallel those in human beings. These combined advantages have permitted research in *Drosophila* to make seminal contributions to the understanding of fundamental biological processes and ensure that *Drosophila* will continue to provide unique insights in the genomic era. An overview of the genetic methodologies available in *Drosophila* is given here, together with examples of outstanding recent contributions of *Drosophila* to our understanding of cell and organismal biology. The growing contribution of *Drosophila* to our knowledge of gravity-related responses is addressed.

INTRODUCTION

All biological processes are based on the use of a selected set of proteins to perform the many steps that underlie the final coherent set of events. It is an implicit assumption of modern biology that every activity of every organism can be viewed in this way. Given this recognition, two fundamentally different approaches to identifying these proteins can be applied. The biochemical approach takes the route of actually isolating the proteins themselves and characterizing them, first *in vitro* and more recently, with the advent of recombinant DNA technology, by reintroducing versions of the gene encoding a given protein back into the cell to test the roles hypothesized for the protein. In contrast, the genetic approach begins at the level of the organism with the proposition that the proteins involved in a particular process can be identified by first identifying their genes. The genes are identified by their ability to disrupt the process in question when mutated. In other words, if a mutation to a particular gene disrupts a particular process, then the protein encoded by that gene must play a role in that process. With this approach, much activity is spent in determining what type of protein is encoded by each mutated gene.

Although the route from mutation to sequenced gene may be long and hard, genetics is an extremely powerful approach because it can be applied to much more complex biological processes than biochemistry. Clearly for fundamental, universal processes that can be studied in cell-free extracts, the biochemical approach is highly effective. But for complex processes, such as developmental events or behavioral responses, in which many components of the whole organism are in play, genetics offers perhaps the only viable route to dissecting out the protein components.

The genetic approach has been used to probe gene/protein function in many organisms. However, the extent to which genetic methods and tools have been developed for *Drosophila melanogaster* far exceeds that for any other complex multi-cellular organism. As a result, *Drosophila* stands alone in terms of its ability to provide insight into complex biological processes. Many of the dramatic advances in understanding the genetic basis of development and behavior have come, and will continue to come, from *Drosophila*.

In this review we will first delineate the historical and practical reasons that have led to *Drosophila* becoming such a pre-eminent model organism and then discuss some of the unique advantages and molecular methodologies that it now offers for studying gene/protein function. We will then provide examples of biological processes where genetic work in *Drosophila* has opened up molecular understanding with relevance across all of evolution and has permitted insights into our own molecular makeup. In particular, recent work that uses *Drosophila* to address gravity-related phenomena will be discussed.

THE DEVELOPMENT OF DROSOPHILA AS A GENETIC SYSTEM

Mendel's genetic studies of the garden pea were the serendipitous consequence of his duties in the monastery garden. In contrast, *Drosophila* came to be a central organism in genetics as a result of careful consideration by T. H. Morgan, who, in the early 1900's, was looking for a suitable species in which to perform studies of heredity. *Drosophila* met all his criteria; a small species with a short life cycle that could be easily reared to produce large numbers of progeny. The intense studies of *Drosophila* that Morgan and his highly talented students (C. B. Bridges, H. J. Muller and A. H. Sturtevant) went on to perform gave rise to many of the concepts that are

* Correspondence to: Kathleen M. Beckingham
Dept. Biochemistry and Cell Biology
Houston, PO Box 1892, TX 77251
Email: kate@bioc.rice.edu
Phone: 713-348-4016; Fax: 713-348-5154

fundamental to genetics today. The recognition of genes as linear arrays on the chromosomes grew directly from their research, as did the demonstration of recombination between homologous chromosomes and the chromosomal basis of sex determination (Sturtevant, 1965).

But beyond these theoretical contributions, the genetic tools evolved from these studies have placed *Drosophila* in its unparalleled position in terms of understanding gene/protein function. In particular, two types of special chromosomes were developed as result of their work. Balancer chromosomes were first created by Muller (18). These chromosomes with multiple re-arrangements cannot recombine with a homologous chromosome partner, and therefore their use allows particular arrangements of mutations on a given chromosome to be maintained through many generations. Balancer chromosomes are available for all three of the major *Drosophila* chromosomes and their use is an essential component of most genetic manipulations in the organism. Deficiency chromosomes, each lacking a defined chromosome region, were another offshoot of their work. These chromosomes are invaluable in genetic mapping since a recessive mutation will show a phenotype when paired with a deficiency chromosome that is lacking the region containing the mutated gene. At this point in time, sets of deficiency chromosomes are available from the *Drosophila* stock centers that cover the entire genome and allow straight forward mapping of a mutation to a unique small chromosome region. These sophisticated tools are not available for any other organism.

In addition to these "man-made" genetic tools, the organism itself has conferred further advantages for genetic studies. The rich array of external appendages (multiple bristle types, wings, eyes, antennae and so on) that can be mutated without producing lethality has allowed for the isolation of many marker mutations that are the work-horse tools of most genetic schemes. In addition, several unusual aspects of *Drosophila* biology have proved extremely valuable. These biological eccentricities were not known, or anticipated, at the time of Morgan's decision to work on *Drosophila*. Along with insects from several other evolutionary groups, members of the Genus *Drosophila* do not show meiotic recombination in the male. This provides another useful mechanism in genetic schemes for avoiding rearrangements in arrays of mutations assembled on particular chromosomes. In addition, *Drosophila* belongs to a group of organisms that makes extensive use of an unusual growth mechanism in certain tissues: DNA replication proceeds without nuclear division or cytokinesis such that polyploid nuclei, often with thousands of copies of the chromosomes, are produced in huge cells. In some tissues the chromosome copies are retained together in perfect register such that giant, so-called polytene, chromosomes with intricate, reproducible patterns of bands are produced. The polytene chromosomes of the *Drosophila* larval salivary glands are particularly fine and have been intensely studied (Figure

1). The maps created for these chromosomes by Bridges (1935) are still in use and the positions of many of the deficiencies (and duplications) in the chromosomes used today in genetic mapping were identified by the changed banding patterns seen in the polytene chromosomes. A method for hybridizing DNA fragments encoding particular genes to the polytene chromosomes was developed by M.-L. Pardue (Pardue and Gall, 1969; Pardue et al., 1970) and with the advent of recombinant DNA this provided an even more rapid route to positioning a gene within the genome - by hybridizing its DNA to the polytene chromosome set.

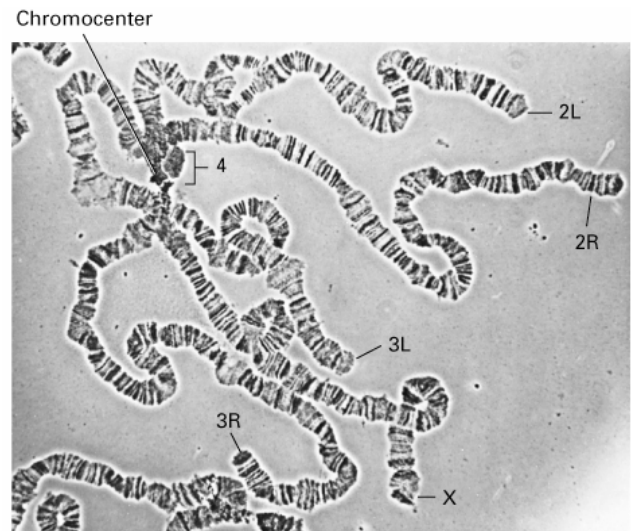


Figure 1. The polytene chromosome set of the *Drosophila* larval salivary glands. *The *Drosophila* chromosome complement consists of two large metacentric chromosomes (chromosomes 2 and 3) and the single telocentric X (first) chromosome. Although seen here, the tiny fourth chromosome is rarely visible. The tips of the left and right arms of chromosomes 2 and 3 are labeled, as is the single arm of the X. The chromosomes are fused at their centromeres at a structure which is called the chromocenter. The banding patterns seen here are highly reproducible. Genes can be mapped to individual bands by hybridization of their cloned DNA to preparations of the chromosomes.*

THE TRANSPOSON BASED TOOL KIT FOR IN-DEPTH MOLECULAR GENETIC ANALYSIS IN *DROSOPHILA*

The revolution initiated by the invention of DNA cloning technology has enhanced gene function analysis not just in *Drosophila* but in all organisms under study. One special consequence in *Drosophila*, however, was that this methodology permitted the molecular basis of an unusual genetic phenomenon termed hybrid dysgenesis, identified and studied by M. Kidwell, to be explained. Kidwell (Kidwell et al., 1977) discovered that high levels of mutagenesis occurred when certain strains (termed P and M strains) of *Drosophila* were crossed to one another, but only if the P strain was used to provide the male parent, not the female. O'Hare and Rubin (1983) established that the behavior of a class of transposable DNA elements,

present only in P strains and hence termed P elements, is the underlying basis of hybrid dysgenesis. Movement of P elements is repressed in the P strains but in crosses to M strains, which lack the elements, repression is released. Transposition of the elements to new locations in the genome is thus allowed, and as a result, new mutations are generated.

Since their discovery, the biology of P element transposons has been exploited to generate an impressive variety of molecular tools for *in vivo* analysis of gene function in *Drosophila*. In initial ground-breaking work, Spradling and Rubin (1982; Rubin and Spradling, 1982) made modified versions of P elements and demonstrated that these could be used to reintroduce cloned genes back into the organism. The benefits of this so-called transformation technique are multiple. First, it permits genetic rescue of mutations - that is, reversion of a mutant phenotype to the normal wild type state by re-introduction of a "good" version of the gene. But more importantly, when combined with the extensive mutant collections available in *Drosophila*, it permits a detailed analysis of gene function not possible in other organisms. Thus, for many genes, a completely non-functional mutation (null mutation) of the gene has been isolated previously. By

introducing different modified versions of a gene into its null genetic background through P element transformation, the ways in which the modified gene can support the complete set of functions of the normal gene can be addressed. For example, by this approach an investigator could address the function of particular regions of the protein coding sequence *in vivo*, by determining the ability of appropriately modified gene constructs to restore the mutant phenotype to normalcy.

But P elements have been put to even more sophisticated genetic uses. Three powerful P-based methodologies deserve special mention. One technique addresses the central quest in genetics - that of determining, once a mutant phenotype of interest has been identified, which gene is affected by the mutation. Most of *Drosophila* genetics to date has relied upon chemically induced mutations. Such mutagens typically produce single base changes in DNA. Given that the genome of *Drosophila* is $\sim 1.65 \times 10^8$ bases in total, identifying the location of that single base change has some of the quality of finding a needle in a hay stack. Without the sophisticated genetic/molecular methods developed in *Drosophila*, this task would be impossible, but even with these tools, it can take years of effort to identify the genes affected by such

The Gal4 system

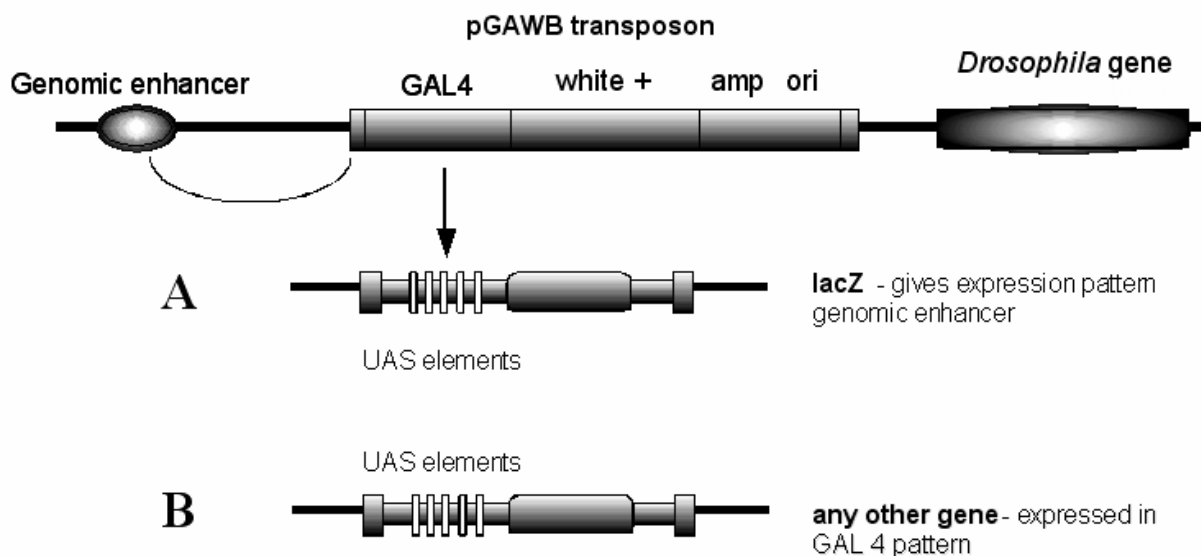


Figure 2. The Gal4 system for directed gene expression. The upper diagram shows a Gal4 P element transposon (pGawB - see Brand and Perrimon, 1993) inserted into the *Drosophila* genome in a typical position close to an enhancer element that would normally regulate an adjacent gene (shown here to the right of the transposon). The Gal4 coding sequence within the transposon will be expressed in the pattern dictated by the enhancer element. In addition to the Gal4 gene, pGawB contains the white⁺ gene (a marker used to identify successful integration of the transposon into the genome) and bacterial plasmid sequences (amp^r ori) to allow cloning of the DNA adjacent to the insertion site. In order to identify the Gal4 expression pattern produced by the enhancer, flies carrying the pGawB insertion are crossed to flies carrying a second P-based insertion that has the bacterial β galactosidase (lacZ) gene linked to a promoter region carrying five Gal4 binding sites (UAS sequences) (see A in figure). The progeny of this cross will carry both constructs in their genomes and thus will express lacZ in the pattern of the Gal4. Given that lacZ is very easily detected histochemically, this allows simple determination of the Gal4 expression pattern. Once the pattern is known, it can be used to express any gene of interest (see B in figure) by a similar procedure.

mutations. P elements, by inserting themselves into the genomic DNA, naturally act as mutagens. In order to be able to determine very rapidly which gene is affected by a particular P element insertion, versions of P elements that permit the rapid and simple cloning of the DNA adjacent to their insertion point have been generated (Cooley et al., 1988). Given that the *Drosophila* genome is completely sequenced and at an advanced level of annotation, in most cases, sequencing of the P-element adjacent DNA permits immediate and unambiguous positioning of the P insertion within the genome. This ability to make "tagged" mutations that readily lead to the affected gene is a powerful advantage that is now being used throughout the *Drosophila* community.

A second P element based technique allows the identification of the regulatory elements associated with particular genes. As a result of the molecular analysis of the insertion points of thousands of P elements, it has become clear that they preferentially insert into regions of genes that contain sequences regulating the expression of the genes. This is an intriguing finding and presumably reveals some aspect of chromatin function that has yet to be understood. Many of these regulatory sequences, termed enhancers, lie in the 5' flank of the transcribed region. Modified versions of P elements have been generated that will report the expression pattern dictated by an enhancer flanking a P insertion site (O'Kane and Gehring, 1987). These modified versions contain the coding sequence for a bacterial protein β galactosidase (*lacZ*) but with no associated transcription regulatory elements. As a result there is no expression of the *lacZ* in *Drosophila* unless its host P element is inserted so as to allow an adjacent enhancer to confer an expression pattern on *lacZ*. The pattern of *lacZ* expression has been examined in hundreds of these so-called "enhancer trap" lines and thus regulatory sequences that give gene expression in many different tissues have been identified.

Building on this approach, a further powerful P element based system (the GAL4-UAS system -see Figure 2) has been developed that allows patterns of enhancer-driven expression to be converted into the expression of any gene of interest (Brand and Perrimon, 1993; Duffy, 2002). This is achieved by using the gene for a yeast transcription factor, Gal4, in place of *lacZ*, in the "enhancer trap" P construct and then introducing into the genome, in a second P element, the gene of interest under the control of a promoter that will be activated by Gal4 binding. Thus the gene of interest will be expressed wherever Gal4 is expressed. The resulting ability to express particular genes in pre-selected tissues has many valuable applications. For example, although a gene of interest may be expressed in many tissues, the phenotype of a particular mutation to the gene may result from defects in only one of these tissues. This hypothesis can be tested by expressing the wild type, non-mutant, version of the gene in particular tissues of the mutant animal and determining in which tissues the mutant phenotype is rescued.

An example from the Beckingham lab. will illustrate this point (Wang et al., 2002). We identified a mutation to the universally expressed calcium signaling protein calmodulin that affected muscle behavior in the larval/pupal stages. We wished to determine whether these defects reflected problems in the musculature itself, in the nervous system innervating the muscles, or perhaps elsewhere in the animal. By expressing wild type calmodulin in either the muscles or the nervous system using this technique, we were able to show convincingly that the defects originated entirely within the muscles themselves.

Although the Gal4-UAS system allows directed gene expression in particular tissues it does not permit temporal control over when, in the development of that tissue, the gene will be expressed. However, systems are now evolving to address this need to control gene activation in terms of not just tissue but also time (reviewed in McGuire et al., 2004). Modification of the Gal4-UAS system so that Gal4 is activated only in response to feeding the hormone analog RU486 is one approach; use of a temperature sensitive version of the Gal4 repressor protein Gal80, which represses Gal4 at permissive temperatures but allows Gal4 expression upon shift to higher temperatures, is another.

Many proteins have vital roles in different tissues at different stages of development. A null mutation in such a gene will produce death at the first point in the life cycle of the organism at which the protein is essential. Thus, a classic null mutation cannot be used to address the roles of the protein beyond this point of death. For any recessive mutation, this problem can be overcome by so-called mosaic analysis. In this approach, the whole organism is heterozygous for the mutation and therefore phenotypically wild type. Then, some method is used to render the mutation homozygous in particular cells of the body so that the effects of the mutation can be addressed in those cells. Although classical *Drosophila* genetics provided some techniques for generating mosaic animals they were cumbersome and not generally applicable to any gene. Another advance that has grown out of the ability to readily transfer gene constructs into the genome with P element vectors is a generally applicable system for generating mosaic animals (Xu and Rubin, 1993). This system, termed the FLP-FRT system (Figure 3) uses a recombinase enzyme (FLP) from yeast that will induce sequence-specific recombination at a site termed FRT. FRT sequences are appropriately positioned on two homologous chromosomes one of which carries a mutation of interest. FLP activity is then used to induce an illegal recombination event that will result in a cell in which both homologous chromosomes carry the mutation. By using versions of the FLP enzyme that are under the control of either tissue-specific enhancers or a promoter induced by heat-shock, cell lineages homozygous for the mutation can be induced in either particular tissues, or throughout the animal at particular times.

It is important to recognize that, effective as these transposon-based methods are for gene analysis, any gene reintroduced into the organism in this way is not inserted so as to replace the version of the gene already present in the genome. Hence as alluded to above, to examine the function of the transgenic construct without interference from the endogenous chromosomal version of the gene requires the pre-existence of a null mutation of the gene in question. In contrast, in the mouse and in yeast, the

methods for re-introducing genes into the genome target their integration by homologous recombination so that they are substituted for the endogenous gene at the correct chromosomal locus (Thomas and Capecchi, 1987; Petes et al., 1991). Recently methods for this type of targeting of genes have also been developed in *Drosophila* and will be valuable for those genes for which a null mutation is not available (Rong and Golic, 2000).

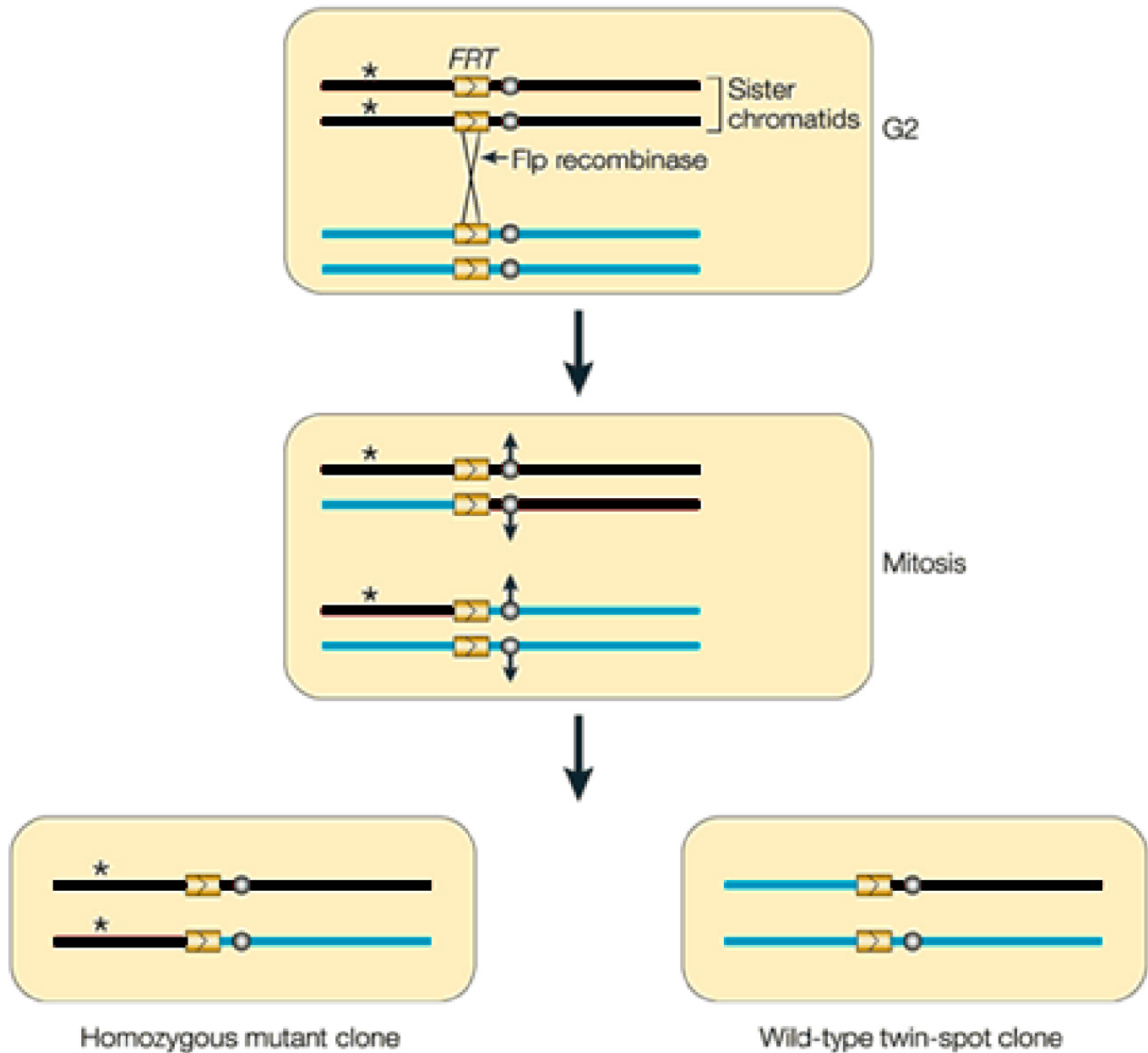


Figure 3. The FLP-FRT system for generation of mosaic tissues. The upper panel shows a pair of homologous chromosomes in a single cell of an animal that has been generated to allow mosaic analysis of a particular mutation (represented here by a star symbol on one of the chromosomes). The cell is heterozygous for the mutation and therefore phenotypically normal. The chromosomes are shown in G2 of the cell cycle, when sister chromatids for both chromosomes are present. FRT sequences are present at identical positions on the two chromosomes. Flp recombinase, expressed from another chromosome in the cell, causes an illegal recombination event between the FRT sequences on chromatids of the homologous pair. As a result, the two chromatids containing the mutation are now attached to different centromeres (see middle panel). After mitosis, when chromatids derived from each chromosome pair have segregated to the daughter cells (lower panel), one cell will be homozygous for the mutation and the other wild type. Reproduced with permission from *Nature Reviews in Genetics* 3, 176-188, 2002 (www.nature.com/reviews) copyright 2002, Macmillan Magazines Ltd.

THE INITIAL UNLEASHING OF THE POWER OF DROSOPHILA GENETIC SCREENS

The mutations initially studied by Morgan's group were naturally occurring ones. Indeed the discovery of the first white-eyed mutant fly by Morgan was a defining moment in Drosophila genetics. The critical advance of directly generating mutations was pioneered by Muller, when he initiated the use of ionizing radiation as a mutagen (Muller, 1927). Subsequently, more easily useable chemical mutagens - in particular DNA alkylating agents such as ethylmethane sulfonate - have been identified and used. The ability to generate mutants in a scattershot manner throughout the genome, led to the concept of the genetic screen; that is, a two-stage experimental protocol whereby mutants are generated and then those that affect a particular process of interest are isolated and studied. Although genetic screening of this type was established in microbial systems relatively early, it was not pursued in complex multi-cellular animals initially for two compelling reasons: first, in most species existing genetic analysis was too primitive to permit in-depth analysis of mutants, and second, the resources and time necessary for multi-generational analysis of hundreds or thousands of mutant lines made such screening completely impractical in most species.

In essence, Drosophila was the only complex animal species for which these two criteria could be met and in the 1970's directed screening efforts to identify mutants in particular processes were initiated. Three of these early screening efforts deserve particular mention for the far-reaching impact of their findings.

a) Mutants in visual processes

Pak (1979; 1995) used an electrophysiological assay to identify mutants which altered the electroretinogram of the fly and thus, by implication, to find genes with roles in Drosophila phototransduction and other vision-related events. Several of these mutations have proved to affect genes with general roles in intracellular signaling processes and thus they provide insights with broad significance into intracellular regulation. The *trp* mutations in particular have played an enormously important role in advancing understanding in neural signaling in mammalian systems. Originally named for the electrophysiological effect of the mutants (a transient receptor potential,) the *trp* gene proved to encode a novel kind of ion channel. Trp is now recognized as the founding member of a vast and varied family of related channels used throughout the nervous system across evolution and with roles in many sensory processes such as taste, touch, pain and temperature perception (Clapham, 2003; Huang, 2004).

b) Mutants in behavioral processes

Benzer, after his successful exploitation of bacteriophage genetics to address the nature of the genetic code, turned his attention to Drosophila genetics as a route to exploring

the biological phenomenon that interested him most - that of behavior. Although Benzer received considerable criticism for attempting to unravel complex phenomena one gene at a time, the genetic screening initiated in his lab. identified mutants that have played ground-breaking roles in understanding such universal phenomena as circadian rhythms, courtship and mating activity, and learning and memory. Mutants of the gene *period* were isolated by Konopka in Benzer's lab (Konopka and Benzer, 1971) and subsequent cloning of the *period* gene by the labs of Hall (a Benzer post-doc) and Rosbash (Reddy et al., 1984) has led to recognition of its pivotal role in regulating diurnal activity across all evolutionary orders. The first learning mutant, *dunce*, isolated in the Benzer lab by Quinn (Dudai et al., 1976) proved to encode an enzyme involved in cyclic nucleotide metabolism. Along with *rutabaga*, another Quinn mutation that affects adenylyl cyclase (Livingstone et al., 1984) these learning mutants have provided part of the now large body of evidence demonstrating the role of cyclic AMP in learning and memory in all biological systems.

c) Mutants in embryonic patterning

Nusslein-Volhard and Wieschaus as young new independent investigators at the EMBL labs in Heidelberg, undertook a large scale screen to identify genes that affect formation of the Drosophila embryonic body plan. A large number of mutations were produced, some with remarkable effects on the final form of the animal (Nusslein-Volhard and Wieschaus, 1980; Nusslein-Volhard et al., 1984; Jurgens et al., 1984). For example, the *even-skipped* and *odd-skipped* mutations generate larvae in which every other body segment is missing. The impact of this screen cannot be over-emphasized. Determining what kinds of genes were behind such unusual phenotypes was of great interest. Further, this work appeared just at the moment when cloning the gene affected by a given mutation was becoming feasible. The cloning and further study of genes identified by this screen is one of the seminal contributions of Drosophila to modern biology. Many of these genes are conserved in higher species and over the past 20 years we have seen the mammalian homologs identified and their fundamental roles in vertebrate cell function established. Indeed whole conserved pathways used across evolution to execute similar functions have been identified. The Hedgehog signaling pathway (Lum and Beachy, 2004) is a striking example of the impact of this screen: besides roles in embryonic development in all species, this pathway plays a critical part in regulation of tissue growth in adult vertebrates and is implicated in the transition to tumor growth in several cancers (Scott, 2003). Three key members of this pathway (Hedgehog, Patched and Smoothed) were identified by the Nusslein-Volhard/Wieschaus screen.

FURTHER DEVELOPMENT OF GENETIC SCREENING IN DROSOPHILA

Numerous *Drosophila* screens have been carried out since this period and genes with roles in processes as diverse as olfaction (Woodward et al., 1989), hearing (Eberl et al., 1997), sensitivity to ethanol (Guarnieri and Heberlein, 2003) and development of various organ systems have been identified. As proved true for the embryonic patterning screens, the extensive screening in the Rubin lab. focused on eye development (e.g. Therrien et al., 2000; Greenwald and Rubin, 1992) has uncovered genes and pathways that are universally conserved. The so-called Pax-Six-Eya-Dach signaling network is one such conserved pathway to emerge from this work (Ozaki et al., 2004). In addition, a "second order" class of screens has been developed that allows further proteins in a given pathway to be identified once one pathway member has been isolated through mutation. These so-called genetic interaction (or enhancer/suppressor) screens involve searching for second site mutations that suppress or enhance the phenotype of a given mutation in a starting gene of interest. The underlying assumption here is that only mutations that affect protein products that interact with one another, or at least have roles in the same process, could modify one another's phenotype in this way.

As discussed above, a major advance for genetic screening has involved the development of modified P transposons that can be used as insertional mutagens that then allowed rapid cloning of the adjacent DNA (Cooley et al., 1988). This approach is now widely in use, and our own studies of gravitaxis took advantage of this approach (see below). But in addition, genetic screening methods have been developed that take advantage of some of the more sophisticated P element based tools discussed above. One such technique allows the identification of genes with roles in particular processes by examining the effects of their over-expression or mis-expression in particular tissues (Rorth, 1996). The technique uses Gal4 expressed in a specific pattern to activate randomly inserted UAS-type promoter elements and thus to initiate expression of any endogenous gene adjacent to the insertion in the pattern determined by the Gal4 expression characteristics. For a fraction of the genes in *Drosophila*, even null mutations do not produce a phenotype, perhaps as a result of functional redundancy. This new method is valuable in that it permits functional analysis of such genes.

By combining aspects of the Gal4-UAS system, the FLP-FRT system and mutagenesis extremely sophisticated screens are now being developed and executed in *Drosophila*. One example will suffice. In order to identify genes that might contribute to the transition from a non-invasive to a metastatic tumor, Pagliarini and Xu (2003) devised a scheme in which FLP-FRT was used to generate mosaic cell lines in developing eye tissue. As a result of a single FLP-induced recombination event they could generate a clone of cells that simultaneously

expressed a fluorescent marker protein (GFP -for cell identification) and a gene that causes the cells to transition to non-invasive tumorous growth. In addition, this recombination event rendered the cells homozygous for one of a collection of random mutations being tested for their effects in a non-invasive tumor cell. Those mutations that caused a transition to metastatic growth could then be easily identified since fluorescent cells were no longer confined to the eye, but rather were spread throughout the organism. No other organism allows this kind of sophisticated, directed approach to identifying genes with roles in metastasis. The implications of this particular screen in terms of understanding human cancer are obvious.

DROSOPHILA IN THE GENOMIC ERA

With the sequencing of the genomes of several model organisms, a new so-called genomics era has arrived. Interestingly, the genomes of the vertebrate model organisms have proved to be "quasi polyploid" in that three or four highly related copies of many genes are present. Pleasingly this is not true of *Drosophila*; most *Drosophila* genes are unique and the complication of genetic redundancy is thus greatly diminished.

The new central approach of the genomics era is whole genome gene expression analysis using probes for all the genes in the organism displayed by micro-array technology. This approach compares expression patterns for two different experimental situations, such as a particular tissue before and after some experimental treatment, or at two different stages of development. Again, *Drosophila* brings particular advantages to this type of approach. Most notably, the ability to pursue array-based expression data by subsequent study of mutants and altered gene constructs adds a depth of analysis not possible in most organisms.

A wealth of *Drosophila* community resources has developed over the years including bioinformatic tools to handle the large data sets now accumulating from genomic studies. Several stock centers around the world maintain both classical and transgenic mutants. A variety of international consortia make datasets and molecular reagents available to the community at large. The FlyBase database (FlyBase@flybase.net) maintains a list of *Drosophila* researchers, references, genome and mutant information, and many user-contributed articles and tutorials. Of particular note is the Berkeley *Drosophila* Genome Project (BDGP: <http://www.fruitfly.org/>) which maintains developing genomic resources such as banks of cDNAs and stocks of newly generated gene disruption mutants. There is now also an initiative to maintain extensive raw datasets in a central, searchable database; FlyMine (<http://www.flymine.org>). FlyBase is a central repository for links to developing bioinformatics tools.

With the genome completely sequenced, the *Drosophila* community has begun development of tools for the

ultimate phase of genomic research - a full understanding of the function of each individual gene. Two useful tools are already available for this work. First, a large fraction of the proteins of the *Drosophila* proteome have been tagged with GFP by genomic insertion of an appropriate reporter construct (Kelso et al., 2004; <http://flytrap.med.yale.edu>). The GFP tag permits the expression pattern and subcellular location of the protein from a given gene to be easily investigated. Second, as a complement to standard mutations, libraries of DNA constructs that can be used to create mutant phenotypes by RNAi-mediated mRNA destruction have been generated (<http://www.hgmp.mrc.ac.uk/geneservice>). Currently, constructs for ~90% of the *Drosophila* genome are available. In addition, a large collection of new gene/small genomic deletions has been made by the company Exelixis, using more recently generated transposons such as piggybac, razorbac and warthog. These will aid studies of uncharacterized genes (<http://drosophila.med.harvard.edu/index.php?option=content&task=view&id=5&Itemid=27>).

DROSOPHILA AND HUMAN DISEASES

The promise of using the capacity for deep genetic analysis in *Drosophila* to advance molecular understanding of specific human diseases has recently begun to be realized. The major approach to date in modeling a human disease in *Drosophila* has been to express the protein product associated with a particular disease state in the fly and then to establish whether the changes associated with the disease are mimicked in this organism. For three major classes of neurodegenerative diseases, Alzheimer's, Parkinson's disease and the polyglutamine class of diseases (exemplified by Huntington's disease) this approach has been successful and the model disease state generated in the fly is being used for further analysis (Bonini and Fortini, 2003; Iijima et al., 2004). Candidate genes that might modify the severity of the disease-related phenotype (see suppressor and enhancer screens above) are being investigated. For both Parkinson's and the polyglutamine diseases, this approach has already established that chaperone proteins such as hsp70, which regulate protein folding, can ameliorate the disease phenotype (Bonini and Fortini, 2003). Current indications also suggest that general insights into the biological function of the disease protein (or its interaction partners) will come from this strategy of expressing in *Drosophila*. For example, over-expression in *Drosophila* of APP, the protein associated with Alzheimer's disease, produces a blistered wing phenotype (Fossgreen et al., 1998). This defect is associated with failed cell-cell adhesion in the wing epithelia and suggests that the processing enzyme that modifies APP (which is already present in *Drosophila*) might also play a role in processing critical cell adhesion molecules.

However, the great power of this *Drosophila* disease modeling approach lies in large scale genetic screening for mutations that enhance or suppress the disease

phenotype. By this route, completely novel genes involved in disease progression will be identified. In time, this will provide new potential targets for development of therapeutic drugs.

DROSOPHILA'S CONTRIBUTION TO RESEARCH INTO GRAVITY-BASED RESPONSES

a) Earth-based studies

Interestingly, geotaxis (now more correctly termed gravitaxis) was one of the first behaviors investigated in *Drosophila* at the beginning of the last century (Carpenter, 1905). However, further work was not undertaken again until the late 1950's/ early 1960's when Hirsch began extensive studies of the phenomenon. At that time, the issue of inheritance of behavioral traits was of intense interest because of its far-reaching social implications in terms of human behavior. Hirsch's primary goal was thus to identify defined behavioral responses and then to address their heritability. He designed vertical multi-choice mazes to examine gravitactic behavior in walking, as opposed to flying, flies (Hirsch, 1959). By selecting flies from a wild type population that emerged either at the high or the low maze exits he was able to demonstrate stable heritance of the maze behavioral traits over many generations with maximal behavioral separation between high and low lines occurring after 48 generations (Hirsch and Erlenmeyer-Kimling, 1961). Today, after more than 700 generations, these lines still breed true in terms of their maze behavior.

Erlenmeyer-Kimling and Hirsch (1961; Hirsch and Erlenmeyer-Kimling, 1962) established that the determinants for these traits were dispersed throughout the chromosome set, but prevailing methodology precluded actually identifying any of the loci involved. More recently however, Toma et al. (2002) have used the genomics approach described above to identify these loci. They compared patterns of gene expression in the high and low strains by DNA microarray technology and identified several genes for which expression levels differed significantly between the two strains. For three genes, they were able to use previously identified mutants of the loci to demonstrate that the genes had roles in gravitaxis. Thus mutants at these genes showed altered behavior in the maze.

We have taken an alternative approach to identify genes with roles in gravitaxis (Armstrong, Texada, Munjaal, Baker and Beckingham, manuscript submitted). We have used the gravitactic maze as the central assay of a genetic screen to identify mutants with altered behavior in this gravitactic response. By examining mutations caused by a Gal4 expressing "enhancer trap" type P transposon (see above), we have been able to quickly move from the mutants we have identified to the affected genes. The actual sense organs that detect gravitational force are not known in *Drosophila*. However, recognizing that Gal4 will be expressed in those tissues that are the likely

origins of the mutant phenotype, we have been able to identify candidate peripheral sense organs that might mediate graviperception. In particular, a set of sensory structures on the *Drosophila* head show strong expression of Gal4 in many of our mutant lines (Figure 4).

Bhattacharya and collaborators are addressing another behavioral response to gravity in *Drosophila* (Sanchez, Stowers, Shenasa, Fahlen and Bhattacharya, manuscript in preparation). They have established that upon subjecting flies to hypergravitational force (1.8g-8g) a highly stereotypic locomotor response is initiated, the features of which suggest that increased g force stimulates an adaptive program of gene expression. Whole genome micro-array studies and candidate gene approaches are being used to identify genes important for this locomotor response. The mushroom body in the *Drosophila* brain, which is important for olfactory learning and memory and for locomotion, has been shown to be involved in regulating this behavioral response to hypergravity. Our *de novo* forward genetic screen studies have identified several novel genes with roles in gravitaxis. But a striking aspect of all three of these approaches is that many of the gravity-related genes identified have clear homologs in human beings. As shown in Table 1, several of these genes have predicted roles in neural signaling or morphogenesis. Interestingly, gravity-related genes with roles in regulating circadian activity have been identified

by both Toma et al. and Bhattacharya and colleagues (Table 1). The Bhattacharya lab has also identified a role for conserved genes of the cAMP pathway (*dunce*, *rutabaga*) known to be involved in learning and memory in addition to genes affecting longevity and stress (*methuselah* and *I'm not dead yet*). The roles of these various genes are thus much broader than the original studies of them might have indicated. Further, it is hard to imagine any route by which the roles of these genes in gravity-based responses could have been identified in any mammalian system, given the limited methodology currently available in these organisms. But with the characterization of these genes in *Drosophila*, the possibility for mammalian studies is now open. Hopefully future studies will address the roles of these proteins in gravitaxic responses in mammals.

b) Micro-gravity experiments

The features of *Drosophila* that make it ideal for genetic studies of a multi-cellular organism also make it ideal for experiments in the micro-gravity environment. On the International Space Station (ISS), where mass and volume are critical issues, the small size and fecundity of *Drosophila* make it possible to use large numbers of individuals for experiments - at least two orders of magnitude more than could be used with mammals. Further, the ease with which *Drosophila* can be reared in

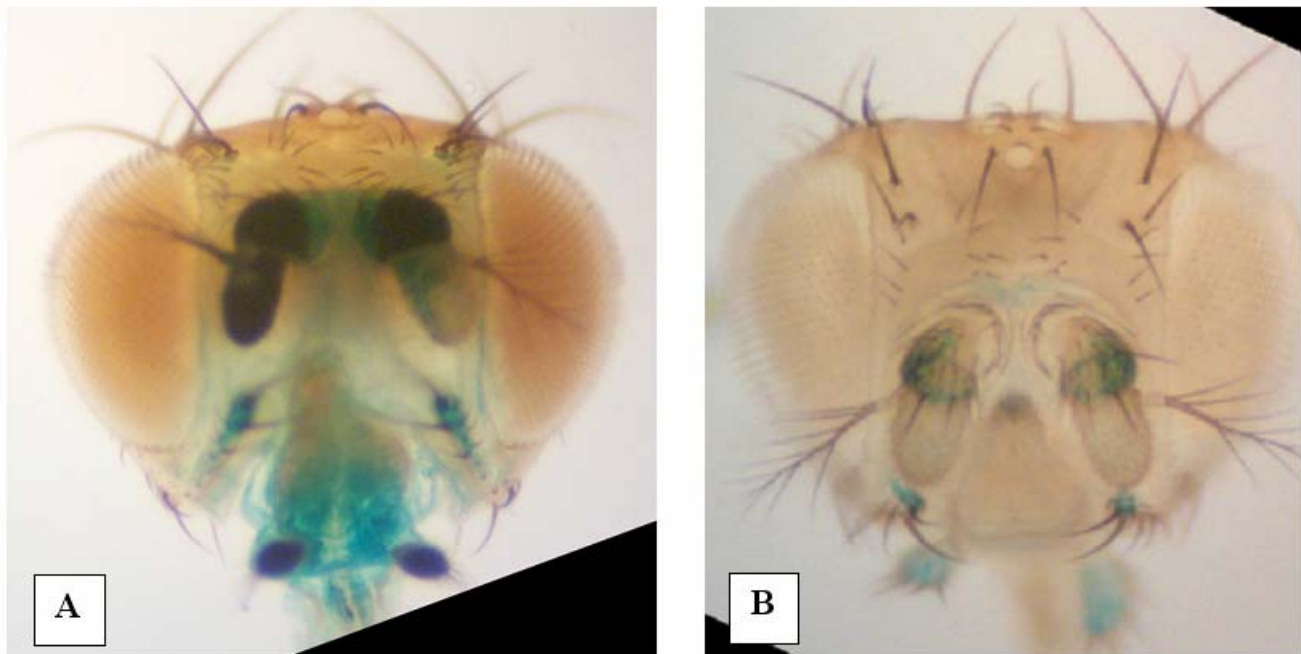


Figure 4. Gal4 expression patterns in facial sense organs of two *Drosophila* gravitaxic mutants. Heads from two different mutant fly lines with altered gravitaxic behavior in vertical mazes (see text) are shown. Each mutation is caused by a P{GawB}transposon insertion and shows Gal4 expression in the pattern of an adjacent enhancer sequence associated with the affected gene (see Figure 2). A. Head of a fly from the yuri gagarin mutant line. B. Head of a fly from the neil armstrong mutant line. The expression patterns for Gal4 were visualized by crossing each Gal4 transposon line to a UAS-lacZ line (see Figure 2) and detecting lacZ by a histochemical stain in the progeny. In each line, three pairs of sense organs in the head stain prominently for lacZ - these are the antennae (uppermost), the vibrissae (middle) and the maxillary palps (lower). Each of these structures contains mechano-sensory receptors that are therefore candidates for sensing gravity.

TABLE 1**Genes with Roles in Gravitaxic Behavior in *Drosophila* that Have Conserved Homologs in Humans**

<u>Gene</u>	<u>Protein Function</u>
^a Pendulin (Pen)	Nuclear importin
^a cryptochrome (cry)	Photopigment protein with role in circadian rhythms
^a Pigment-dispersing Factor (Pdf)	Neuropeptide that mediates circadian locomotor activity
^b broad (br)	Zinc-finger class transcription factor
^b off-track (otk)	Receptor tyrosine kinase with role in neural pathfinding (Human homologs are Trk neurotrophin receptors)
^b discslarge (dlg1)	MAGUK class protein with guanylate kinase, PDZ, SH3 and P-loop domains with a role in synapse structure (Human homolog is chapsyn-110)
^b escargot (esg)	Zinc-finger class transcription factor with role in peripheral nervous system development (Human homolog is SLUG)
^b Connector of kinase to AP-1 (Cka)	WD-40 domain protein, part of JNK signaling cascade (Human homologs are striatin, zinedin and cell cycle autoantigen SG2NA)
^c period (per)	Transcription factor that regulates circadian rhythm (Human homolog is PER3)
^c timeless (tim)	Transcription factor that regulates circadian rhythm (Human homolog is hTimeless)
^c dunce (dnc)	Phosphodiesterase that regulates cAMP levels (Human homolog is cAMP-specific 3', 5'-cyclic phosphodiesterase 4D)
^c rutabaga (rut)	Adenylate cyclase responsible for cAMP synthesis (Human homolog is brain adenylate cyclase 1)
^c I'm not dead yet (indy)	Sodium dicarboxylate cotransporter implicated in longevity (Human homolog is NADC3)
^c G-salpa60A	Component of transmembrane receptor signal transduction cascade involved in associative learning in mushroom body (Human homolog Guanine nucleotide-binding protein G-s-alpha 3)

^a = Toma et al. (2002); ^b = Armstrong et al., submitted (see text); ^c = Sanchez et al, in preparation (see text).

small, closed, containers allows design of experiments that need very little attention from the astronaut crew. *Drosophila* experiments in microgravity that take advantage of the powerful genetic methods available in this organism are planned in the near future. A collaborative experiment by Beckingham, Bhattacharya and Armstrong will investigate the behavior and gene expression changes evoked by microgravity in both wild type and mutant flies. An experiment by Thompson and Woodruff will use the genetic manipulations possible in *Drosophila* to gain information about the mutagenic effects of radiation levels on the ISS. Details of these experiments may be obtained from the NASA Task Book site (http://peer1.nasaprs.com/peer_review/index.cfm).

CONCLUSION

Based on appearance we might not readily conclude that *Drosophila melanogaster* and *Homo sapiens* have a great deal in common. But with the sequencing of both genomes it is now clear that more than half the genes in *Drosophila* have homologs in human beings. What's more, not just genes, but whole molecular pathways, originally delineated in *Drosophila* as a result of its sophisticated genetics, are now known to be conserved intact in mammalian systems. The value of using *Drosophila* to investigate fundamental biological processes with the distinct goal of uncovering insights directly relevant to human beings is thus evident. The major contributions of *Drosophila* to date have been in terms of molecular understanding of development and embryogenesis. But current research activities indicate that use of *Drosophila* to study human disease and behavioral phenomena is expanding and we may expect further substantial contributions in these areas. Despite its small size, *Drosophila* has a complex repertoire of behavioral responses including activities that relate readily to the human condition such as courtship and male-male aggression. The continually expanding array of resources in *Drosophila* will ensure that it will be a major source of insights into these more complex aspects of organismal biology. In terms of gravitational biology, *Drosophila* is already providing results that have implications for gravity-based responses in people. The use of *Drosophila* as a model organism to probe gene expression and behavior in micro-gravity fulfils two valuable goals in parallel. On one hand, data that can be interpreted in depth thanks to the wealth of genetic data available for the organism will be generated. Simultaneously, information pertinent to human responses in the microgravity environment will be produced.

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REFERENCES

- Bonini, N.M. and Fortini, M.E. 2003. Human neurodegenerative disease modeling using *Drosophila*. *Annual Reviews Neurosciences* 26: 627-656.
- Brand, A.H. and Perrimon, N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118: 401-415.
- Bridges, C.B. 1935. Salivary chromosome maps. *Journal of Heredity* 26: 60-64.
- Carpenter, F.W. 1905. The reactions of the pomace fly (*Drosophila ampelophila* Loew) to light, gravity and mechanical stimulation. *Contributions from the Zoological Laboratory of the Museum of Comparative Zoology at Harvard College* 162: 157-171.
- Clapham, D.E. 2003. TRP channels as cellular sensors. *Nature* 426:517-524.
- Cooley, L., Kelley, R. and Spradling, A. 1988. Insertional mutagenesis of the *Drosophila* genome with single P elements. *Science* 239: 1121-1128.
- Dudai, Y., Jan, Y.N., Byers, D., Quinn, W.G. and Benzer, S. 1976. *dunce*, a mutant of *Drosophila* deficient in learning. *Proceedings of the National Academy of Sciences USA* 73: 1684-1688.
- Duffy, J.B. 2002. Gal4 system in *Drosophila*: a fly geneticist's swiss army knife. *Genesis* 34: 1-15.
- Eberl, D.F., Duyk, G.M. and Perrimon, N. 1997. A genetic screen for mutations that disrupt an auditory response in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences USA* 94: 14837-14842.
- Erlenmeyer-Kimling, L. and Hirsch, J. 1961. Measurement of the relations between chromosomes and behavior. *Science* 134: 1068-1069.
- Fossgreen, A., Bruckner, B., Czech, C., Masters, C.L., Beyreuther, K. and Paro, R. 1998. Transgenic *Drosophila* expressing human amyloid precursor protein show -secretase activity and a blistered-wing phenotype. *Proceedings of the National Academy of Sciences USA* 95: 13703-13708.
- Greenwald, I. and Rubin, G.M. 1992. Making a difference: the role of cell-cell interactions in establishing separate identities for equivalent cells. *Cell* 68: 271-281.
- Guarnieri, D.J. and Heberlein, U. 2003. *Drosophila melanogaster*, a genetic model system for alcohol research. *International Reviews in Neurobiology* 54:199-228.
- Hirsch, J. 1959. Studies in experimental behavior genetics : II. Individual differences in geotaxis as a function of

- chromosome variations in synthesized *Drosophila* populations. *Journal of Comparative and Physiological Psychology* 52: 304-308.
- Hirsch, J. and Erlenmeyer-Kimling, L. 1961. Sign of taxis as a property of the genotype. *Science* 134: 835-836.
- Hirsch, J. and Erlenmeyer-Kimling, L. 1962 Studies in experimental behavior genetics: IV. Chromosome analyses for geotaxis. *Journal of Comparative and Physiological Psychology* 55: 732-739.
- Huang, C.L. 2004. The transient receptor potential superfamily of ion channels. *Journal of the American Society of Nephrology* 15: 1690-1699.
- Iijima, K., Liu, H.-P., Chiang, A.-S., Hearn, S.A., Konsolaki, M. and Zhong, Y. 2004. Dissecting the pathological effect of human A 40 and A 42 in *Drosophila*: a potential model for Alzheimer's disease. *Proceedings of the National Academy of Sciences USA* 101: 6623-6628.
- Jurgens, G., Wieschaus, E., Nusslein-Volhard, C. and Kluding, H. 1984. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* II. Zygotic loci on the third chromosome. *Roux's Archives of Developmental Biology* 193: 283-295.
- Kelso, R.J., Buszczak, M., Quinones, A.T., Castiblanco, C., Mazalupo, S. and Cooley, L. 2004. Flytrap, a database documenting a GFP protein-trap insertion screen in *Drosophila melanogaster*. *Nucleic Acids Research* 32: D418-D420.
- Kidwell, M.G., Kidwell, J.F. and Sved, J.A. 1977. Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. *Genetics* 86: 813-833.
- Konopka, R.J. and Benzer, S. 1971. Clock mutants of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences USA* 68: 2112- 2116.
- Livingstone, M.S., Sziber, P.P. and Quinn, W.G. 1984. Loss of calcium/calmodulin responsiveness in adenylate cyclase of rutabaga, a *Drosophila* learning mutant. *Cell* 37: 205-215.
- Lum, L. and Beachy, P.A. 2004. The Hedgehog response network: sensors, switches, and routers. *Science* 304: 1755-1759.
- McGuire, S.E., Roman, G. and Davis, R.L. 2004. Gene expression systems in *Drosophila*: a synthesis of time and space. *Trends in Genetics* 20: 384-391.
- Muller, H.J. 1918. Genetic variability, twin hybrids and constant hybrids, in a case of balanced lethal factors. *Genetics* 3: 422-499.
- Muller, H.J. 1927. Artificial transmutation of the gene. *Science* 46: 84-87.
- Nusslein-Volhard, C. and Wieschaus, E. 1980. Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287: 795-801.
- Nusslein-Volhard, C., Wieschaus, E. and Kluding, H. 1984. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* I. Zygotic loci on the second chromosome. *Roux's Archives of Developmental Biology* 193: 267-282.
- O'Hare, K. and Rubin, G.M. 1983. Structure of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* 34: 25-35.
- O'Kane, C.J. and Gehring, W.J. 1987. Detection in situ of genomic regulatory elements in *Drosophila*. *Proceedings of the National Academy of Sciences USA* 84: 9123-9127.
- Ozaki, H., Nakamura, K., Funahashi, J., Ikeda, K., Yamada, G., Tokano, H., Okamura, H.O., Kitamura, K., Muto, S., Kotaki, H., Sudo, K., Horai, R., Iwakura, Y. and Kawakami, K. 2004. Six1 controls patterning of the mouse otic vesicle. *Development* 131: 551-562.
- Pagliarini, R.A. and Xu, T. 2003. A genetic screen in *Drosophila* for metastatic behavior. *Science* 302: 1227-1234.
- Pardue, M.L. and Gall, J.G. 1969. Molecular hybridization of radioactive DNA to the DNA of cytological preparations. *Proceedings of the National Academy of Sciences USA* 64: 600-604.
- Pak, W.L. 1979. Study of photoreceptor function using *Drosophila* mutants. In: *Neurogenetics: Genetic approaches to the nervous system*. (Breakfield, X. Ed.) New York: Elsevier-North Holland, pp. 67-99.
- Pak, W.L. 1995. *Drosophila* in vision research. The Friedenwald Lecture. *Investigative Ophthalmology and Visual Science* 36: 2340-2357.
- Pardue, M.L., Gerbi, S., Eckardt, R.A. and Gall, J.G. 1970. Cytological localization of DNA complementary to ribosomal RNA in polytene chromosomes of Diptera. *Chromosoma* 29: 268-290.
- Petes, T.D., Malone, R.E. and Symington, L.S. 1991. In: *Molecular and cellular biology of the yeast Saccharomyces*. (Broach, J.R., Pringle, J.R. and Jones E. W., Eds.) Plainview, New York: Cold Spring Harbor Laboratory Press, pp. 407-521.
- Reddy P, Zehring, W.A., Wheeler, D.A., Pirrotta, V., Hadfield, C., Hall, J.C. and Rosbash, M. 1984. Molecular analysis of the *period* locus in *Drosophila melanogaster*

and identification of a transcript involved in biological rhythms. *Cell* 38: 701-10.

Rong, Y.S. and Golic, K.G. 2001. Gene targeting by homologous recombination in *Drosophila*. *Science* 288: 2013-2018.

Rorth, P. 1996. A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proceedings of the National Academy of Sciences USA* 93: 12418-12422.

Rubin, G.M. and Spradling, A.C. 1982. Genetic transformation of *Drosophila* with transposable element vectors. *Science* 218: 348-353.

Scott, M.P. 2003. Cancer: a twist in a hedgehog's tale. *Nature*: 425: 780-782.

Spradling, A.C. and Rubin, G.M. 1982. Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* 218: 341-347.

Sturtevant, A.H. 1965. *A history of genetics*. Harper and Rowe, New York, pp.1-165.

Therrien, M., Morrison, D.K., Wong, A.M. and Rubin, G.M. 2000. A genetic screen for modifiers of a kinase suppressor of Ras-dependent rough eye phenotype in *Drosophila*. *Genetics* 156: 1231-1242.

Thomas, A.R. and Capecchi, M.R. 1987. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell* 51: 503-512.

Toma, D.P., White, K.P., Hirsch, J. and Greenspan, R.J. 2002. Identification of genes involved in *Drosophila melanogaster* geotaxis, a complex behavioral trait. *Nature Genetics* 31: 349-353.

Wang, B., Bolduc, C. and Beckingham, K. 2002. Calmodulin UAS-constructs and the in vivo roles of calmodulin: analysis of a muscle-specific phenotype. *Genesis* 34: 86-90.

Woodard, C., Huang, T., Sun, H., Helfand, S.L. and Carlson, J. 1989. Genetic analysis of olfactory behavior in *Drosophila*: a new screen yields the ota mutants. *Genetics* 123: 315-326.

Xu, T. and Rubin, G.M. 1993. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* 117: 1223-1237.

