Drosophila – a versatile model in biology & medicine

The fruit fly *Drosophila melanogaster* is a versatile model organism that has been used in biomedical research for over a century to study a broad range of phenomena. There are many technical advantages of using *Drosophila* over vertebrate models; they are easy and inexpensive to culture in laboratory conditions, have a much shorter life cycle, they produce large numbers of externally laid embryos and they can be genetically modified in numerous ways. Research using *Drosophila* has made key advances in our understanding of regenerative biology and will no doubt contribute to the future of regenerative medicine in many different ways.

Barbara H. Jennings

Transcriptional Regulation Group, UCL Cancer Institute, Paul O'Gorman Building, 72 Huntley St, London, WC1E 6BT, United Kingdom

E-mail: barbara.jennings@ucl.ac.uk

Many obvious practical and ethical obstacles severely limit the scope for experiments using humans in biomedical science, thus much of what we know about the underlying biology of cells and tissues comes from studies using model organisms such as mice, and the focus of this article, the fruit fly *Drosophila melanogaster* (Fig. 1a). *Drosophila* has been used productively as a model organism for over a century to study a diverse range of biological processes including genetics and inheritance, embryonic development, learning, behavior, and aging. Although humans and fruit flies may not look very similar, it has become well established that most of the fundamental biological mechanisms and pathways that control development and survival are conserved across evolution between these species.

The first documented use of *Drosophila* in the laboratory was by William Castle's group at Harvard in 1901, although the "father" of *Drosophila* research is undoubtedly Thomas Hunt Morgan[1]. Morgan greatly refined the theory of inheritance first proposed by Gregor Mendel, by using *Drosophila* to define genes and establish that they were found within chromosomes (long before it was even established that DNA is the genetic material). Morgan won the Nobel Prize in Physiology or Medicine in 1933 *"for his discoveries concerning the role played by the chromosome in heredity"*[2]. One of Morgan's protégés, Hermann Muller, won the Nobel Prize in Physiology or Medicine in 1946 *"for the discovery of the production of mutations by means of x-ray irradiation"*[3]. Using *Drosophila* in the 1920s, Muller discovered that x-rays caused a massive increase in the mutation rate of genes, and could actually break chromosomes[4]. Although irradiated flies looked normal, their offspring frequently showed the effects of mutation. This led to the realization that radiation causes harmful genetic defects in the offspring of exposed humans – a timely observation given that this was at the advent of man's attempts to harness and exploit nuclear fission.

ISSN:1369 7021

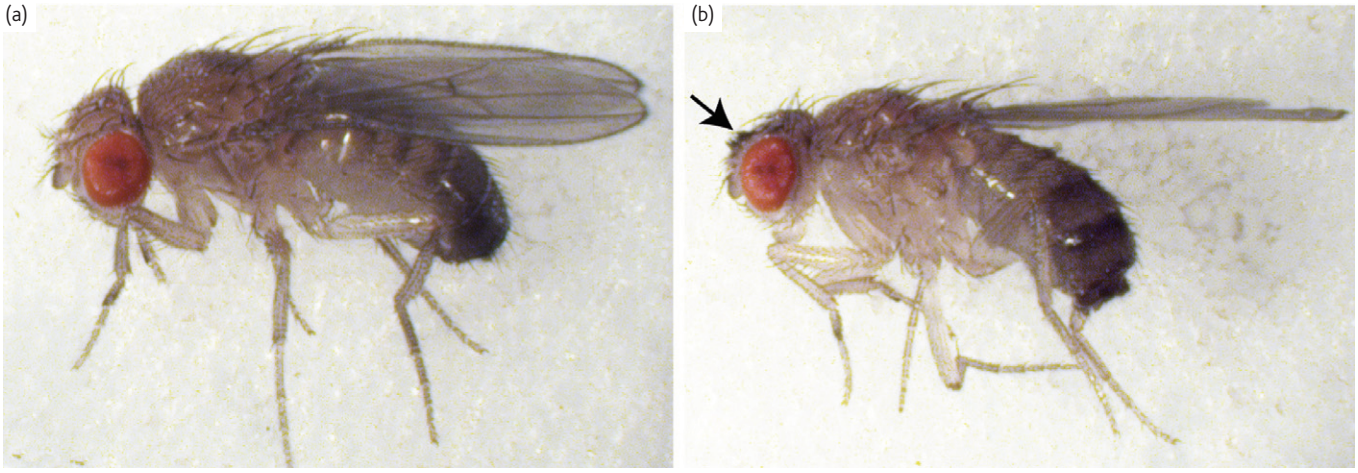

Fig. 1 (a) A wild type ("normal") male Drosophila melanogaster. (b) Drosophila carrying the groucho[1] mutation (named after Groucho Marx; an arrow points to the bushy "eyebrow").

Over the past four decades, *Drosophila* has become a predominant model used to understand how genes direct the development of an embryo from a single cell to a mature multicellular organism. In 1995, Christiane Nüsslein-Volhard, Eric Wieschaus, and Ed Lewis won the Nobel Prize in Physiology or Medicine "*for their discoveries concerning the genetic control of early embryonic development*"[5]. Many of the genes that they defined as being important for fly development have since been shown to be critical for all animal development, including humans. Although the final architecture of a fly and a human differs greatly, many of the underlying building blocks and engineering processes have been conserved through evolution and are strikingly similar.

In 1999, Craig Venter and colleagues used the *Drosophila* genome as a test bed to prove the practicality of the "shot-gun" approach for sequencing the human genome. This approach worked well and the first release of the sequence of the *Drosophila* genome was released in March 2000, just 11 months ahead of the human genome[6]. The sequence and annotation of the *Drosophila* genome is freely available to all and can be accessed via "Flybase", the outstanding online database dedicated to *Drosophila*[7]. Comparisons between the fully sequenced *Drosophila* and human genomes revealed that approximately 75 % of known human disease genes have a recognizable match in the genome of fruit flies consolidating its legitimacy as a model organism for medical research[8]. Currently, it is estimated that there are around 14 000 genes in *Drosophila*[6] and each of these has a dedicated page on Flybase that contains links to practically everything known about that gene including sequence, gene product sequence, known mutations, and related literature.

Drosophila – a genetic "work horse"

There has been a long history of using *Drosophila* genetics as a tool for understanding biology dating back to Morgan's experiments over 100 years ago. One convention in *Drosophila*, which Morgan initiated, is naming mutations to reflect the nature of their phenotype (the observable effects of the mutation). For example, one of the first mutations Morgan isolated is known as *white*; flies with this mutation have white eyes (instead of red)[9]. Genes are usually named after the first mutation isolated within them (e.g., *white*). This convention has lead to many genes with somewhat entertaining names. One example is *groucho*. Flies carrying the first mutant allele (variant) of *groucho* have extra bristles above their eyes resembling the bushy eyebrows sported by Groucho Marx (Fig. 1b), a famous film star and entertainer at the time the mutation was discovered[10]. Other genes include *tribbles* (cells divide ectopically in mutants; the name refers to an alien species in "Star Trek" that reproduced uncontrollably[11]), *wingless* (mutant flies lacked wings[12]), *Notch* (flies have a large notch taken out of the wing[4]) and *hedgehog* (larvae are round and have extra bristles[13]). Many genes first characterized in flies have subsequently been isolated and studied in mice and humans, and the fly names have been adopted or adapted. Examples of these include *Notch* (there are 4 Notch genes in mammals, *Notch1 – 4*), "*sonic hedgehog*" (related to *Drosophila hedgehog*[14]) and *Wnt* (*wingless* and *INT*-related[15]). However, if a related gene (orthologue) has been characterized in other model systems before mutant alleles are isolated in the fly, then the established name is adopted in *Drosophila* (e.g., *Spt5*[16,17]). All *Drosophila* genes were given a "CG number" during the annotation of the genome sequence, and there are still many uncharacterized genes with no known mutation or orthologues that are referred to by a CG number (e.g., CG10026)[7].

Genetic analysis gives essential insight into the role played by individual factors in a given biological process. The range of genetic tools that have become available for *Drosophila* over the past century far surpass those for any other multicellular organism. The vast assortment of strains containing endogenous mutations is collated in Flybase, and these are usually available from the labs that generated them or from stock centers (for a review of *Drosophila* resources see[18]). It is now relatively simple to generate transgenic flies carrying DNA that activates or inhibits the expression of individual genes of interest,

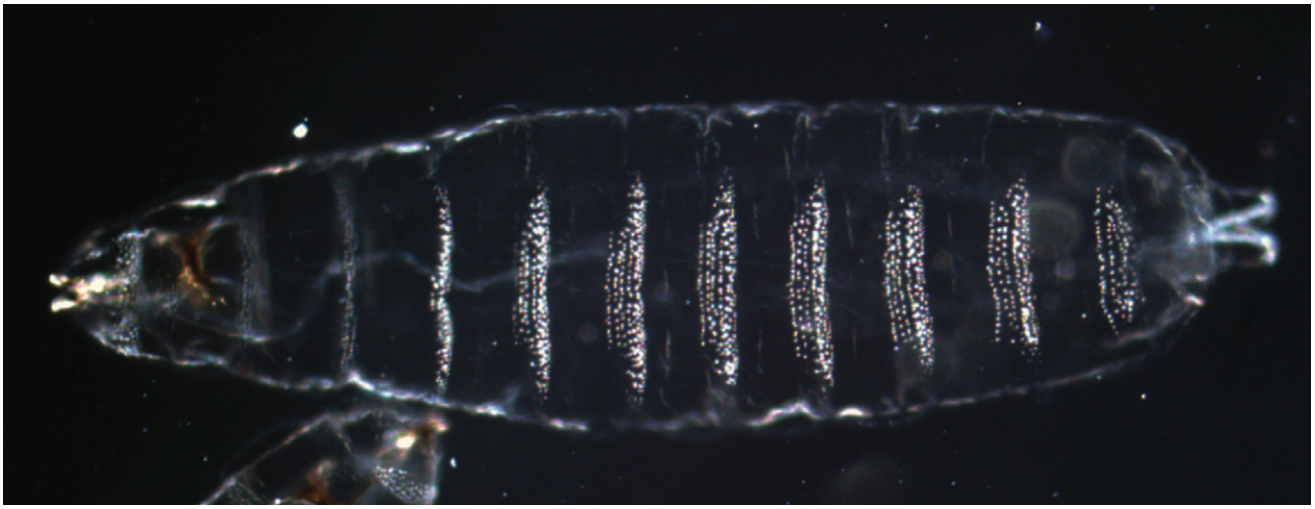

Fig. 2 Darkfield microscopy showing the cuticle (exoskeleton) of a young (1st instar) larva. The head is to the left of the image.

either throughout the organism or in defined tissues. Furthermore, the relatively short life cycle of the fly means that genetic experiments that would take months or even years in vertebrate models such as mouse or zebrafish can be completed in a matter of weeks.

It is possible to reduce expression of individual genes in *Drosophila* cells by expressing double-stranded RNA corresponding to that gene's sequence; this is known as RNA interference or RNAi[19]. The Vienna *Drosophila* RNAi Center houses a collection of transgenic fly lines, each carrying an inducible UAS-RNAi construct against a single protein coding gene[20]. Currently they accommodate over 22 000 different transgenic fly lines, which provide knockdowns for over 88 % of *Drosophila* genes. Expression of these transgenic RNAi constructs can be driven in a tissue specific manner using the "GAL4 system"[21,22] providing a simple, yet powerful strategy to study the role of individual genes in diverse biological processes. Other valuable resources include collections of transgenic lines that allow inducible overexpression of individual genes and transgenic lines expressing green fluorescent protein (GFP) or β-galactosidase (LacZ) in specific tissues or in the pattern of specific genes[7,18].

Life cycle of *Drosophila*

Like butterflies and moths, *Drosophila* undergo a four stage life cycle; egg, larva (Fig. 2), pupa, and fly. Once fertilized, the embryo develops in the egg for around one day (at 25 °C) before hatching as a larva. The larva eats and grows (and goes through three molts) over five days until it pupates and undergoes metamorphosis into the adult fly over the course of four days. During metamorphosis, most of the embryonic and larval tissue is destroyed. The adult tissues (e.g., wing, leg, eye) develop from groups of cells known as "imaginal discs" that have been set-aside since early embryonic development (Fig. 3).

Like humans, adult tissues generally do not regenerate in *Drosophila*; if you pull the wings off a fly, they will never grow back. However,

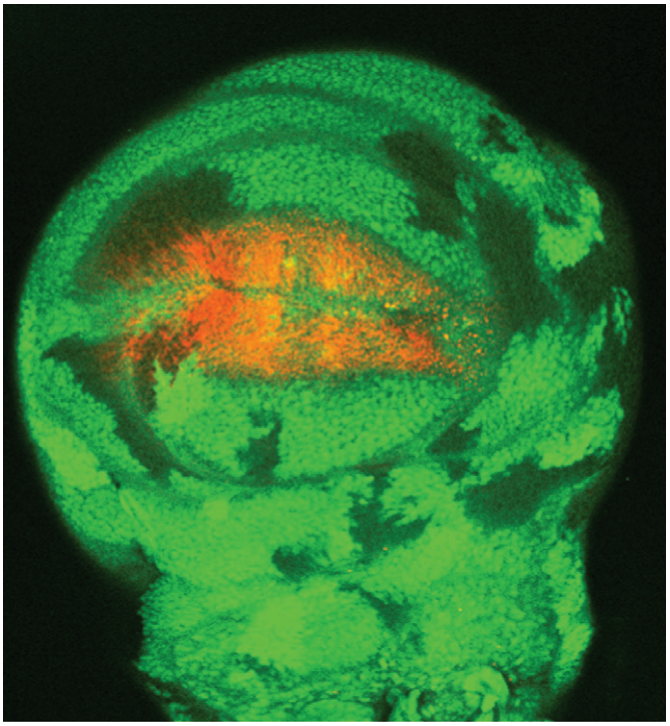

Fig. 3 Image of fluorescent immunohistochemical staining of an imaginal wing disc from Drosophila, taken using confocal microscopy.

imaginal discs do have the capacity to regenerate if damaged in certain conditions (see below), and over recent years have provided an invaluable model system to study the genetics of tissue regeneration[23].

Working with *Drosophila*

Drosophila are relatively inexpensive and easy to keep; indeed they are commonly used in high school biology classes as a teaching aid for demonstrating the basic principles of genetics and inheritance.

Furthermore, there are generally very few restrictions on their use in the laboratory as there are minimal ethical and safety issues (although some *Drosophila* strains are considered to be genetically modified organisms). Each female fly can lay up to ~100 eggs per day for up to 20 days. It takes approximately 10 days at 25 °C for an embryo to develop into a fertile adult fly[24]. Thus it is relatively easy to generate large numbers of embryos or flies for an experimental approach if required.

Although commonly referred to as a fruit fly and often found in the wild hanging around vineyards and orchards, *Drosophila melanogaster* actually eat the yeasts growing on the fruit rather than the fruit itself. Initially lab flies were kept in bottles containing rotting banana pulp[1], but these days it is most common to keep them in bottles containing a jelly-like food that is typically made from a mix of water, cornmeal, yeast, soy flour, malt extract, corn syrup, and agar[24] (Fig. 4). The jelly needs to be hard enough so that the flies don't get stuck in it, but soft enough for the larvae to crawl around and feed in. There are many variations on the basic recipe and pre-mixed formulations are available. Bottles and vials are plugged with foam or cotton wool to prevent the flies escaping and also to keep mites and other nasties out. *Drosophila* can be safely anaesthetized in carbon dioxide if it is necessary to manipulate individual flies (e.g., to set up a genetic cross). In most established fly labs, flies are placed on porous pads connected to a source of carbon dioxide and moved around with a fine tipped paintbrush while viewed with a stereomicroscope (Fig. 5). Ether can be used as an alternative to carbon dioxide, and a magnifying glass used in place of a stereomicroscope.

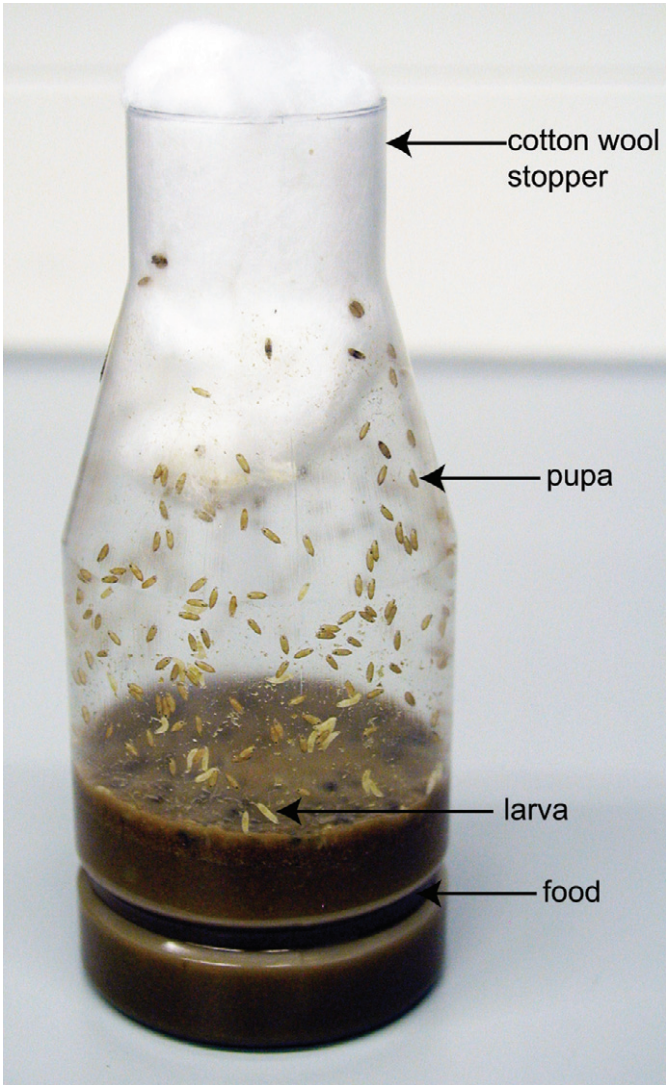

Fig. 4 A bottle containing Drosophila larvae and pupae.

To date, it has not been possible to find an efficient way to freeze down *Drosophila* gametes or embryos, thus it is necessary to keep fly strains as living stocks. Typically stocks of flies are kept at 18 °C as this slows the life cycle down to approximately 28 days. This means that each fly stock needs to be turned over on to fresh food just once every month, leaving enough time between feeds for a decent vacation.

Drosophila as a model for regenerative biology and medicine

Most of our understanding of the biology underlying tissue regeneration comes from experiments using model organisms, including *Drosophila*. In order to either stimulate tissue regeneration at the site of injury or to generate tissues anew, it is necessary to understand which gene products are involved and how they interact with each other. Not surprisingly, the pathways and processes that we know to be activated during tissue regeneration are first used during embryonic development. In both contexts, cells must divide to increase in number, and then stop dividing and differentiate into specific cell types in specific locations. These events must be highly regulated to give rise to the complicated organs and tissues found in animals and to also avoid uncontrolled cell division and improper differentiation, i.e., cancer.

The regenerative capacity of *Drosophila* imaginal discs has been studied for over 40 years[23]. Fragments of imaginal discs transplanted into the abdomens of adult female flies can survive for a number of days. The cells in these discs do proliferate, but do not differentiate. In contrast, fragments of imaginal discs transplanted into larvae (just before pupation) do differentiate into the tissue for which they were originally destined. However, discs that have been cultured repetitively in different fly abdomens (while cells proliferate), can differentiate into an alternative tissue demonstrating "transdetermination". Thus, it was deduced that although the future identity of the discs is determined early in embryonic development, it is not fixed until the initiation of differentiation. Another interesting observation is that imaginal discs that have been cultured in adult hosts before being allowed to differentiate in larvae will either regenerate or duplicate the tissue depending on the precise origin of the disc fragment [23]. These classic observations were made long before any understanding of the underlying molecular patterning pathways that have since been deduced to some extent from genetic analysis.

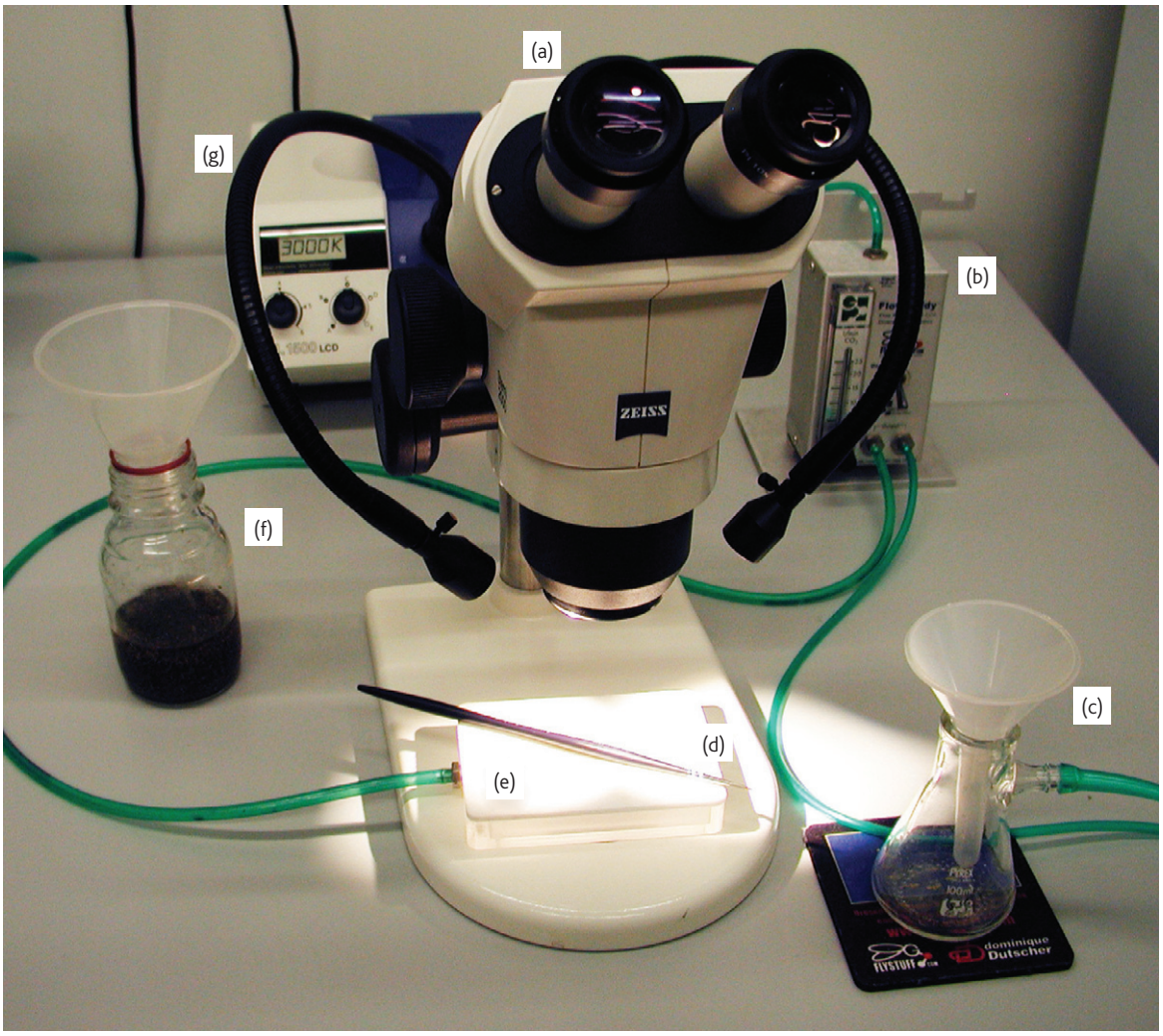

Fig 5. A typical bench set up for work with Drosophila: (a) stereomicroscope, (b) CO_2 regulator, (c) chamber for anesthesia, (d) paint brush, (e) porous pad connected to CO_2, (f) a fly morgue: a bottle containing methanol, and (g) a cold light source.

Recent studies have used *Drosophila* as a model for wound healing. It transpires that the molecular machinery and mechanisms driving wound healing resemble those found in tissue fusion events during animal development including a process called dorsal closure in the *Drosophila* embryo. Elegant studies using transgenic Green Fluorescent Protein (GFP) fusion genes (e.g., actin-GFP) in combination with time lapse imaging have revealed the precise sequence of cell shape changes and movements during dorsal closure and wound healing in *Drosophila* embryos[25]. Meanwhile, genetic analysis has identified many of the factors and pathways that direct and mediate these processes.

Although the tissue regeneration response after injury is highly complex, it generally involves activation of cell proliferation (to replace lost cells) followed by cell fate determination (patterning of the new tissue) and differentiation of cells to form the desired tissue[26]. The control of proliferation in mature organisms must be tightly controlled to prevent tumors; hence most adult tissues have very limited regenerative capacity. Stem cells in adult tissues are critical for regeneration as they have the ability to divide to renew themselves, and also produce daughter cells that differentiate in response to contextual cues. Typically, stem cells divide infrequently in adult tissues, however they can be stimulated to divide more rapidly in response to cell loss or wounding. Studies using *Drosophila* have made a significant contribution to our understanding of the molecular pathways that regulate stem cell activity in all animals[27]. For example, it was first shown in *Drosophila* that cells undergoing programmed cell death (apoptosis) in response to stress or damage produce molecular signals which activate stem cell proliferation to initiate tissue regeneration[26,28,29].

Drosophila as a tool in drug discovery

Drosophila is emerging as a valuable system for use in the clinical drug discovery process[30,31]. *Drosophila* can be used as a model to test the effects of novel drugs on the biochemical pathways conserved within

Instrument Citation

Leica MZ FLIII stereomicroscope
Zeiss Axioplan upright microscope
Zeiss LSM 510 confocal microscope

humans that control many key cellular activities for tissue regeneration such as cell division, differentiation, and movement. New drugs can be tested in *Drosophila* much faster than in mammalian models; indeed they may even be used for the initial high-throughput screening process as an alternative to cell culture. Screening in a whole organism promotes selection of compounds that have an enhanced safety profile for subsequent testing in expensive mammalian models. Furthermore, when using *Drosophila*, it may be relatively easy to manipulate the genetic background to mimic a diseased state to test for drug efficacy in that context.

Drosophila and bioengineering

Drosophila embryos have the potential to provide a valuable system for studying the biological safety of bioengineering technologies since they are relatively small (~500 μm) and can readily be produced in large numbers. Animal development is particularly sensitive to adverse environmental conditions that cause mechanical or genetic damage. Embryogenesis involves the precise coordination of numerous cellular processes including cell-cell communication, cell division, gene expression, cell death, cell movement, and cell shape changes. Disruption to any of these processes can have devastating consequences for the embryo including severe deformities, infertility, or death. Cultured mammalian cells, which are probably the most common system for analysis, provide a much less sophisticated model since they usually contain few, often only one, type of cell. Thus, it is difficult to evaluate potential damage to physical interactions and communication processes amongst the assortment of cells that are found in the very 3-dimensional environment of animal tissues.

One goal of regenerative medicine is to be able to produce artificial tissues by precise manipulation of cells and growth factors. Various methods are currently under development to do this, including bio-electrosprays (reviewed in[32]). We used *Drosophila* embryos to show that this technique does not induce genetic or physical damage that significantly affects development or fertility, thus verifying that this procedure is safe for handling sensitive biological material[33]. In this context, one significant advantage that *Drosophila* has over vertebrate models (e.g., mouse, *Xenopus*, zebrafish) is that it is not subject to animal licensing laws. Thus we are free to allow the embryos to develop into adult flies and test fertility after the procedure without having to make lengthy applications for personal and project licenses.

Future directions

There is no doubt that research using *Drosophila* will lead to future breakthroughs in regenerative medicine. Many of these breakthroughs will stem from genetic studies teasing apart the fundamental molecular mechanisms of cell fate commitment and differentiation. However, flies have the potential to be used in many other novel and imaginative ways to address the central questions facing researchers in regenerative medicine. mt

Acknowledgements

I would like to thank Aamna Kaul for thoughtful reading of the manuscript. I am grateful for support from a MRC Career Development Award and a Wellcome Trust project grant.

REFERENCES

1 Kohler, R. E. *Lords of the fly : Drosophila genetics and the experimental life*. University of Chicago Press, Chicago (1994).
2 The Nobel Prize in Physiology or Medicine 1933. http://nobelprize.org/nobel_prizes/medicine/laureates/1933/.
3 The Nobel Prize in Physiology or Medicine 1946. http://nobelprize.org/nobel_prizes/medicine/laureates/1946/.
4 Muller, H. J. *Proc Natl Acad Sci USA* (1928) **14**, 714.
5 The Nobel Prize in Physiology or Medicine 1995. http://nobelprize.org/nobel_prizes/medicine/laureates/1995/.
6 Adams, M. D. *et al. Science* (2000) **287,** 2185.
7 Tweedie, S. *et al. Nucleic Acids Res* (2009) **37**, D555
8 Reiter, L. T., *et al., Genome Res* (2001) **11**, 1114.
9 Morgan, T. H. *Science* (1910) **32**, 120.
10 Lindsley, D. L. & Zimm, G. G., *The genome of Drosophila melanogaster*. Academic Press, San Diego (1992).
11 Seher, T. C. and Leptin, M., *Curr Biol* (2000) **10**, 623.
12 Sharma, R. P., *Drosophila Information Service* (1973) **50**, 134.
13 Jurgens, G., *et al., Roux Arch Devel Biol* (1984) **193**, 283.
14 Krauss, S., *et al., Cell* (1993) **75**, 1431.
15 Rijsewijk, F., *et al., Cell* (1987) **50**, 649.
16 Andrulis, E. D., *et al., Genes Dev* (2000) **14**, 2635.
17 Kaplan, C. D., *et al., Genes Dev* (2000) **14**, 2623.
18 Matthews, K. A., *et al., Nat Rev Genet* (2005) **6**, 179.
19 Carthew, R. W., *Curr Opin Cell Biol* (2001) **13**, 244.
20 Dietzl, G. *et al., Nature* (2007) **448**, 151.
21 Brand, A. H. and Perrimon, N., *Development* (1993) **118**, 401.
22 Elliott, D. A. and Brand, A. H., *Methods Mol Biol* (2008) **420**, 79.
23 Bergantinos, C., *et al. Bioessays* (2010) **32**, 207.
24 Stocker, H. and Gallant, P., *Methods Mol Biol* (2008) **420**, 27.
25 Martin, P. and Parkhurst, S. M., *Development* (2004) **131**, 3021.
26 Bergmann, A. and Steller, H., *Sci Signal* (2010) **3**(145), re8.
27 Pearson, J., *et al., Int J Dev Biol* (2009) **53**, 1329.
28 Ryoo, H. D., *et al., Dev Cell* (2004) **7**, 491.
29 Perez-Garijo, A., *et al., Development* (2004) **131**, 5591.
30 Bell, A. J., *et al., Fly* (2009) **3**, 39.
31 Giacomotto, J. and Segalat, L., (2010) *Br J Pharmacol* **160**, 204.
32 Jayasinghe, S. N., *Analyst* (2011) **136**, 878.
33 Joly, P., *et al., Biomicrofluidics* (2009) **3**, 44107.