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Imaginal disc regeneration takes flight

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Drosophila imaginal discs, the larval precursors of adult structures such as the wing and leg, are capable of regenerating after damage. During the course of regeneration, discs can sometimes generate structures that are appropriate for a different type of disc, a phenomenon termed transdetermination. Until recently, these phenomena were studied by physically fragmenting discs and then transplanting them into the abdomens of adult female flies. This field has experienced a renaissance following the development of genetic ablation systems that can damage precisely defined regions of the disc without the need for surgery. Together with more traditional approaches, these newer methods have generated many novel insights into wound healing, the mechanisms that drive regenerative growth, plasticity during regeneration and systemic effects of tissue damage and regeneration.

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Introduction

Regeneration is the process by which tissues, organs or organisms restore missing or damaged parts. Regeneration is widespread in nature and observed in diverse taxa (reviewed by Refs. [1,2]) including the cnidarian *Hydra*, flatworms, urodele amphibians (*e.g.*, salamanders), and zebrafish. Approaches mostly derived from experimental embryology, such as amputation of limbs and transplantation of tissues, have been applied in these organisms and have provided important insights into cellular aspects of regeneration. While it is now possible, at least in principle, to modify genes in each of these organisms using genome-editing technologies, it is still difficult to carry out large-scale forward genetic screens.

In contrast, *Drosophila melanogaster* is an organism that has been studied by geneticists for over a hundred years. As a result, a number of sophisticated genetic tools are available to study biological processes. These include tools to manipulate gene expression in a tissue-specific manner as well as the efficient generation of genetic mosaics. Additionally, it is possible to conduct genetic screens in a variety of ways (*e.g.*, chemical mutagenesis, RNAi screens) that can be used to deconstruct a complex process such as regeneration that occurs in a living organism.

In this review we focus on studies of regeneration in *Drosophila* imaginal discs which are the larval primordia of adult structures such as the wing and eye. Experiments on imaginal disc regeneration were pioneered by the group of Ernst Hadorn ([3–6] and reviewed in Refs [7,8]). Although these studies revealed many fascinating aspects of regeneration including the propensity of tissues to change fate during regeneration (transdetermination) [9], the technical difficulties associated with these experiments curtailed the development of this field, and until recently, relatively few laboratories studied regeneration in imaginal discs. The development of genetic approaches to ablate tissues in a predictable and spatially defined way without needing surgery [10,11] has resulted in a renaissance of the study of imaginal disc regeneration. In this article, we first summarize the classical literature on regeneration and then discuss recent work derived from genetic approaches.

Discovery of the regenerative capacity of *Drosophila* imaginal discs

In pioneering studies that began in the 1940s, imaginal discs were cut into fragments that were subsequently implanted and cultured in adult female abdomens where regeneration occurred [3–6]. In the absence of molecular techniques, the only way true regeneration could be distinguished from other forms of growth was to demonstrate that the new tissue acquired the developmental potential of portions of the disc that had been ablated. This was assessed by re-implanting the cultured fragments into a larva where they differentiated during metamorphosis. Morphological markers were used to assess the extent of regeneration. Implanted fragments generated additional tissue by localized cell proliferation (Figure 1A), reminiscent of a regeneration blastema observed following limb amputation in salamanders. Additionally, the isolated blastemas alone were shown capable of regenerating the lost structures [12], and

confrontation of blastemas from distant parts induced regrowth of missing structures between those parts (intercalary growth) [13].

These studies also provided two unexpected results. First, certain types of disc fragments, instead of regenerating the missing portion, generated mirror-image duplications [6,14]. Second, after long periods of culture, structures that normally derive from other discs were sometimes derived from the proliferating blastema, a phenomenon known as transdetermination [15,16].

Development of genetic ablation systems

In addition to the studies that demonstrated regeneration after physical fragmentation, diffuse damage to discs, such as with X-ray irradiation, was found to elicit additional cell divisions from the surviving cells [17]. Some authors use the term 'compensatory proliferation' to distinguish this type of response to injury from the more localized proliferation that results from physical damage. Both physical fragmentation and X-ray irradiation were, however, not capable of deleting defined portions of the disc with much precision. This problem was overcome by the development of genetic approaches to tissue ablation in imaginal discs [10,11] that took advantage of the Gal4/UAS system to target a pro-apoptotic gene (*e.g.*, *eiger*, *reaper*) to a defined region of the imaginal disc and the temperature-sensitive version of the Gal80 repressor ($Gal80^{ts}$) to restrict the ablation to a specific time window of imaginal disc development (Figure 1B). This offers the opportunity to compare the regenerative properties of imaginal discs of different maturity. Genetic ablation offers two advantages over the traditional fragmentation approach. First, since the discs are ablated *in situ*, they are able to generate the appropriate adult structures and thus the extent of regeneration can be assessed in a live fly; a transplanted disc develops within the abdomen of the host and needs to be excised from the abdomen of the recipient to be studied. Second, because it is far less laborious, genetic manipulations such as screens can be conducted in these flies thus enabling the discovery of novel regulators of regeneration. Other pro-apoptotic transgenes such as *debcl* (the pro-apoptotic *Drosophila* Bcl-2 ortholog) [18] and *hid* [19] have subsequently been used with this system. An analogous system was developed independently to study regeneration in the adult midgut [20]. Patches of tissue can also be ablated, albeit at random locations, by generating clones of tissue that are mutant for a temperature-sensitive cell-lethal mutation such as $sec5^{ts}$ [21].

As with fragmentation, genetic ablation usually results in localized regenerative growth characterized by an increase in the rate of proliferation of adjacent surviving cells [10,11]. However, clear differences are apparent in the response to ablation with different pro-apototic genes. These differences likely reflect the signaling pathways that are activated and the rate of cell killing by each pro-apoptotic gene leading to differences in the type of cell extrusion (apical versus basal), the extent to which proliferation is localized, and whether regeneration occurs concurrently with tissue loss [19].

Wound healing and early responses to tissue damage

The cut edges of fragmented discs heal very efficiently. Imaginal discs are epithelial sacs that consist of two layers of cells, the columnar epithelium (the 'disc proper') and the squamous peripodial epithelium. Transient heterotypic contacts between cut edges of the two layers appear during the first day of *in vivo* culture and involves contact mediated by filopodia and closure facilitated by an actin-rich cable [22–24] (Figure 1A). Within 48 hours in culture, the heterotypic interactions are resolved and continuity is re-established of both the disc proper and the peripodial eptihelium. Wound healing has been studied in other tissues in *Drosophila* including the epidermis of the embryo [25,26], the larva [27], the pupa [28] and the adult [29]; there are similarities and differences between those processes and wound healing in discs.

Morgan proposed that regeneration can occur either by local stimulation of cell proliferation (epimorphosis) or by re-patterning of existing tissue (morphollaxis) [1]. Most studies of imaginal disc regeneration demonstrate DNA synthesis or mitoses near the wound, that is a regeneration blastema [30–35] although a recent study suggests that morphollaxis could also occur to some extent [36]. Importantly, the first signs of cell proliferation precede the completion of wound healing [12,32,34,35,37] (Figure 1A). This argues strongly against the notion that cell proliferation is triggered by the juxtaposition of tissues with disparate positional identities and favor a mechanism where tissue damage directly stimulates cell proliferation. Fragmented discs respond to wounding by activation of the Jun N-terminal kinase (JNK), concentrated in regions near the edges of the wound. JNK is required for wound healing and also for cell proliferation in imaginal disc blastemas [24,35,37–39]. In addition to JNK, the p38 stress-activated protein kinase is also activated upon tissue damage and is required for regeneration [40••]. Reactive oxygen species (ROS) are produced rapidly after damage and act as chemoattractants for macrophages. This has been demonstrated in both the imaginal disc [41••] and the embryo [42] and ROS are required for activation of JNK and p38 [40••]. In embryonic wounds, the NADPH oxidase DUOX, which acts as a source of H_2O_2, is activated by calcium, and Ca^{2+} flashes have been observed following damage [43]. Intercellular Ca^{2+} waves, propagated via gap junctions are triggered by injury to imaginal discs [44,45].

Functional screens for genes that regulate the early stages of wound healing in *Drosophila* imaginal discs are only