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Drosophila Imaginal Discs as a Playground for Genetic Analysis: Concepts, Techniques and Expectations for Biomedical Research

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Abstract

Drosophila imaginal discs are epithelial tissues perfectly suited to use them as a playground to define the functional contribution of genes to epithelial development and organ morphogenesis. The more we know about the discs and the mechanisms directing their development, the best prepared we are to assign specific “functions” to individual genes based on phenotypic observations. Conversely, and thinking from the perspective of the gene, the more we know about its function, the best inferences we could make about the mechanisms underlying imaginal disc *development*. This reciprocal relationship, coupled to the arsenal of possible experimental approaches available in *Drosophila* genetics, genomics and cellular biology, makes these tissues excellent systems to address biological problems with biomedical relevance. In this review, an overview of three interconnected aspects related to the use of *Drosophila* imaginal discs as an experimental system to analyze gene function is given: (i) imaginal discs biology, with a focus in the genetic mechanisms involved in pattern formation; (ii) concepts and available experimental tools for the analyses of gene function and (iii) uses of *Drosophila* and the imaginal discs for addressing biomedical problems.

Keywords: *Drosophila*, genetic analysis, growth control, pattern formation, imaginal disc

1. Introduction

Imaginal discs are epithelial tissues that grow within the larva of holometabolous insects and differentiate most of the external parts of the adult during metamorphosis [1]. They are named after the adult structures they form, for example, the wing imaginal disc makes the

wing and the thorax, while the leg discs develop the leg appendages and the pleura. Each disc has a characteristic size, shape, histology and fate map, and they are connected to the larval epidermis and to the tracheal system of the larvae [1]. Imaginal discs are a favorite subject of study for developmental and cell biologists, and the analysis of their characteristics has shaped key concepts in developmental biology, including the notions of cell determination, cell autonomy and positional information [2]. The study of imaginal discs is also contributing to identify and characterize the cellular and biochemical mechanisms underlying these concepts.

A key peculiarity that in part account for the success of the imaginal discs as experimental model systems is that they retain a considerable developmental plasticity during larval development. Thus, when let unperturbed, each imaginal disc will undergo with a clock-like precision cell divisions, growth and territorial specification, forming a fixed inventory of cuticular structures during differentiation. Simultaneously, the discs remain extremely plastic and reactive to experimental manipulations during most of their development. This developmental plasticity is particularly manifested when the discs are cut and transplanted into adult host, where disc fragments can reconstitute the missing parts (“regeneration”) and even alter their identity (“transdetermination”) [2, 3]. The ability to regenerate has been more recently observed *in situ* in experiments in which disc fragments are mechanically removed or where particular regions are eliminated through the induction of cell death [3–5].

Imaginal discs are also extremely reactive to genetic manipulations, and altering the expression of genes encoding a wide variety of proteins related to epithelial development in imaginal cells results in precise adult phenotypes [6]. The responsive nature of imaginal discs to genetic and other experimental manipulations is one of the reasons explaining why imaginal discs have been repetitively used in developmental biology. In fact, they have traditionally been either drivers or early adopters of novel experimental approaches directed to unravel the genetic and cellular basis of epithelial biology and organ morphogenesis [2]. In this manner, imaginal discs not only played a key role in the transition from experimental embryology to developmental genetics but also in the posterior move from formal mechanistic interpretations to increasingly detailed molecular and cellular descriptions.

Another aspect that explains the success of imaginal discs as experimental tools at different historical periods is the richness of biological processes participating in their development and differentiation. Thus, most common developmental operations, including cell proliferation and death, cell growth and differentiation, pattern formation and tissue mechanics and morphogenesis, as well as their underlying molecular mechanisms, can be analyzed in the discs. Because these processes are common to the development of all multicellular organisms and also regulated by conserved genes and molecular interactions, the discs are a most convenient system to dissect genetically complex developmental mechanisms. In this review, some key aspects of imaginal disc biology are summarized, and the experimental tools available to analyze the contribution of genes to the development of imaginal disc development are described. We will also summarize how to capitalize on the knowledge we have about the discs to address problems with biomedical impact.

2. Genetic regulation of imaginal disc development

Imaginal discs are versatile, responsive and modular tissues for genetic experimentation. Internal regulatory processes not only determine their development but they also communicate and interact with other larval organs to influence growth and developmental timing [7–10]. In the discs, patterning and growth are interconnected aspects driven by conserved signaling pathways and complex transcriptional regulatory networks that coordinate gene expression along a field of epithelial cells. Although each imaginal disc has its own peculiarities, their modes of development share multiple common aspects, including regulated cell proliferation and the progressive generation of gene expression domains. Thus, a common feature of all discs is the existence of a continuous deployment and refinement of gene expression patterns that culminate in the allocation of cellular fates to individual cells or fields of cells. This process is linked to the position that each cell occupies in the epithelium, but it is mostly a consequence of each cell developmental history, as defined by the gene regulatory networks that were operating in its progenitors. The mechanistic links between gene activity and the patterned distribution of differentiated cells make possible to use phenotypic approaches to identify and characterize the function of individual genes through genetic analysis.

The origin of imaginal cells is the embryonic ectoderm. It is in this epithelial layer where a set of gene regulatory events defines the position of groups of cells as imaginal precursors [1, 11] (**Figure 1**). The specification of each imaginal primordium follows the same logic of gene regulatory events that will direct their subsequent development. Thus, the segmented embryonic ectoderm contains a Cartesian system of positional information along the antero-posterior (A/P) and dorso-ventral (D/V) axes defined by the expression of ligands

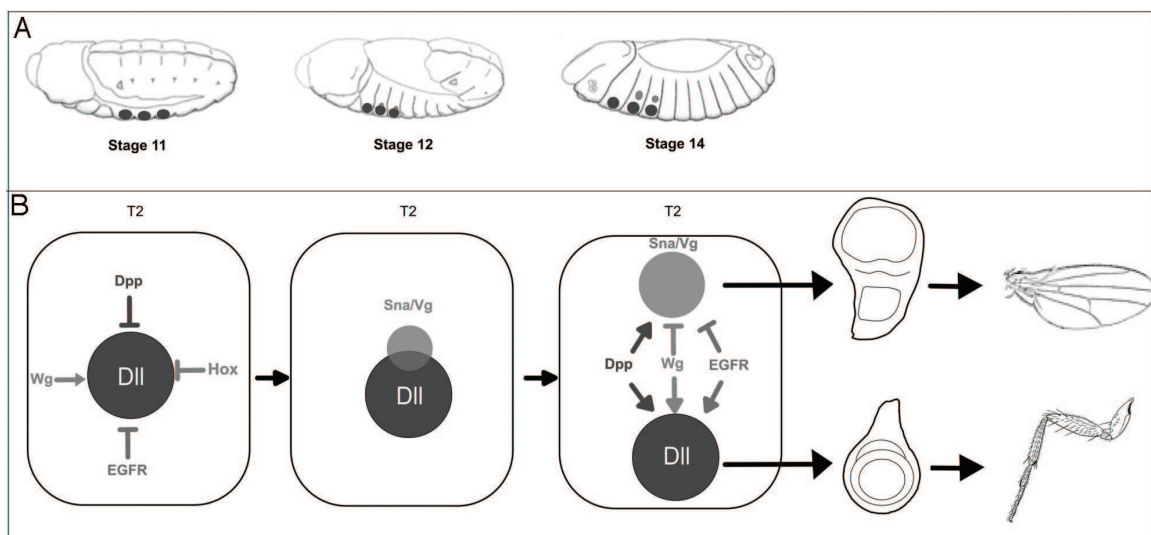


Figure 1. Specification of the thoracic appendage primordia. (A) Schematic representation of *Drosophila* embryogenesis indicating the position of the primordia for the thoracic discs in the T1, T2 and T3 segments. (B) Development of the disc primordium in the T2 segment. At stage 11, *Dll* expression (dark grey circle) is activated by *Wg* and repressed dorsally and ventrally by the *Dpp* and *EGFR* pathways, respectively. Some hours later, the wing primordium marked by the expression of *vg* and *sna* originates (light grey circle) and includes both *Dll*-expressing cells and *Dll* non-expressing cells. At stage 14, the wing and leg primordia are fully separated. The genetic inputs into *Dll* and *sna/vg* are schematized. Note that these genes integrate differentially the activity of the *Wg*, *Dpp* and *EGFR* pathways.

belonging to the Hedgehog (Hh), wingless (Wg), decapentaplegic (Dpp) and epidermal growth factor receptor (EGFR) pathways [1, 12–15] (**Figure 1**). The restricted expression of these ligands results in the generation of overlapping signaling domains in which pathway-specific transcription factors are expressed or active (**Figure 1**). These transcription factors act through *cis*-regulatory modules (CRM) present in a set of genes involved in the early determination of embryonic cells as imaginal precursors. Some of the best-characterized genes belonging to this class are *eyeless* (*eye*), *vestigial* (*vg*), *Distal-less* (*Dll*), *escargot* (*esg*), *buttonhead* (*btd*) and *Sp1* [11, 16–19]. The expression of these genes becomes restricted to groups of cells positioned with respect to parasegmental boundaries and located in precise A/P and D/V positions [20].

Complementary to the A/P and D/V coordinate systems, which are common to all embryonic segments, the ectoderm also bears a segment-specific code of homeotic genes resulting from the differential expression of the *Bithorax* (*Bx*) and *Antennapedia* (*Antp*) gene complexes [21]. The genes of the *Bx* complex prevent the development of imaginal primordia from the abdominal segments and confer different identities to the thoracic segments 2 and 3. Similarly, the genes of the *Antp* complex confer segmental identity to the imaginal precursors present in the cephalic segments [21]. Once the position, size and identity of each primordium are determined, its development implies cell proliferation and invagination from the embryonic surface [1]. For all subsequent stages, the discs will grow as hollow sacs within the interior of the larvae that remain connected through a stalk to the larval epidermis. From the primordia that were specified in the embryo to the mature third instar discs, there happens a considerable increase in size due to cell proliferation, and this increase is always accompanied by the generation of gene expression domains that progressively become coincident with the adult structures; each cell will give rise during metamorphosis (**Figure 1**). The same signaling pathways that participate in the specification of the primordia drive the generation of gene expression domains. The continuous interplay between signaling and transcription is a common aspect to the development of all discs. In this manner, at each time-point in the development of the discs, localized domains of signaling are converted into territories of gene expression, which in turn drive the generation of novel signaling domains directing further domains of gene expression (**Figure 2**).

The examples of the leg and wing discs illustrate how signaling-transcription networks coupled to the increase in the size of the epithelium have been adapted to generate diverse expression patterns associated to cell fate allocations. The leg and wing discs originate from the same primordium located in the ventral region of each hemisegment of the mesothorax (parasegment 5). These early primordia can first be recognized as a group of ~30 cells in each thoracic hemisegment that express the homeobox gene *Dll* [22]. *Dll* expression is activated by Wg and repressed dorsally and ventrally by the Dpp and EGFR pathways, respectively [23]. These *Dll*-expressing cells give rise to all regions of the adult thorax, including both the ventral (i.e., legs) and dorsal (i.e., wing and haltere) appendages [22, 24]. As the embryo develops, a group of cells of these early primordia moves dorsally to form the dorsal primordia (wing and haltere), lose *Dll* expression and activate the wing-promoting genes, *vestigial* (*vg*) and *snail* (*sna*) [16, 22, 25] (**Figure 1**). The remaining *Dll*-expressing cells in each thoracic hemisegment give rise to the leg discs [24, 26] (**Figure 1**). At the molecular level, the signals that govern the separation between the dorsal and ventral primordia are integrated by the CRMs of the *Dll* and *sna* genes. Dpp is required for the expression of *Dll* and *sna*, whereas Wnt and EGFR signalling repress wing fate and promote leg primordia formation [14, 15].

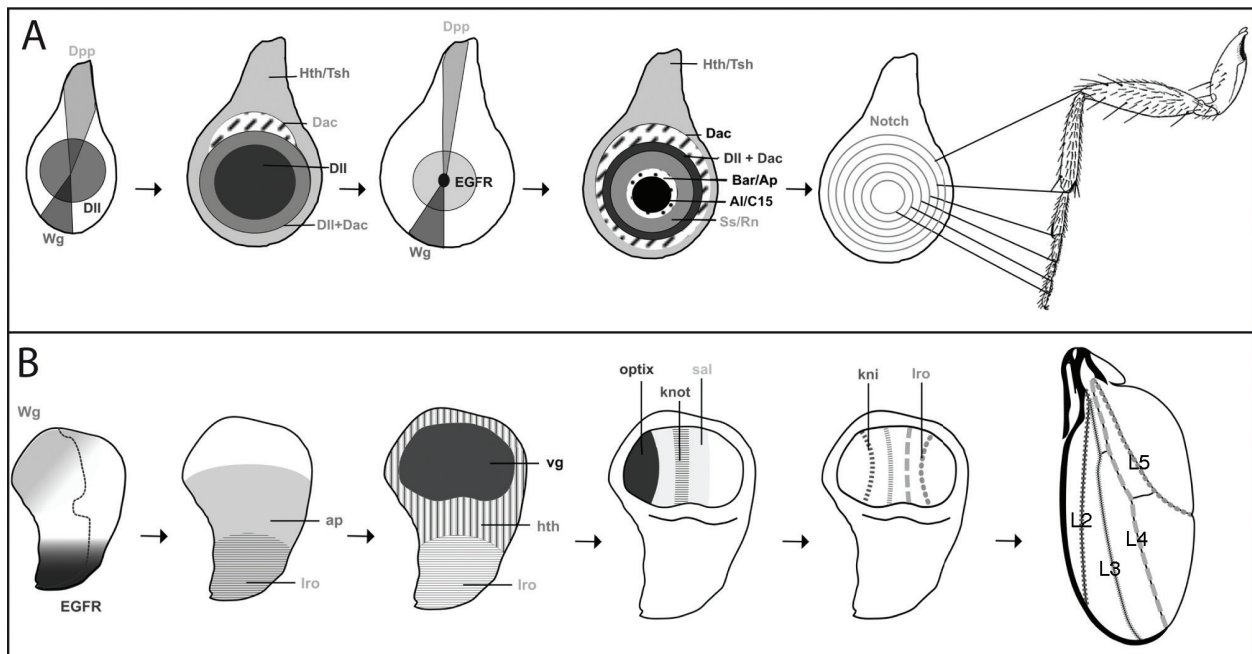


Figure 2. Overview of leg and wing imaginal disc patterning. (A) Schematic representation of the leg disc at different developmental stages, from early L2 (left) to late L3 (right). Initially, Wg (dark grey) and Dpp (light grey) activate *Dll* expression in the distal domain of the leg (grey circle) and repress *dac*. As the disc grows, *dac* expression (striped and grey circle) escapes the repression of Wg and Dpp, and the initial PD axis is established. Later on, the distal part of the leg is further subdivided in nested expression patterns by the activity of the EGFR pathway, which ligand is expressed in the distal-most tip of the leg disc. The combinatorial code of the PD axis patterning genes localized the activation of the Notch pathway (circles) in concentric rings that prefigure the joints of the adult leg. (B) Schematic representation of the wing disc at different developmental stages, from L2 (left) to late L3 (right). The L2 disc is subdivided into a proximal domain where the EGFR pathway is active (dark grey) and an anterior distal domain where the Wg pathway is active (light grey)—EGFR signaling activates the expression of the Iroquois genes (*Iro*) and apterous (*ap*). The confrontation of *ap* expressing and not expressing cells triggers Notch activation and vestigial (*vg*) expression in the primordium of the wing blade. Proximal cells express the homothorax gene (*hth*; striped) and will contribute to the hinge region of the wing. The wing blade is subdivided into expression domains due to the activity of the Hedgehog and Decapentaplegic signaling pathways. The expression of *optix* (black), *spalt* (*sal*; light grey) and *knot* (dark grey) is shown. The expression of *Knirps* (*kni*) and Iroquois (*Iro*) is then activated in provein territories (lines) from where later each longitudinal vein (L2-L5) differentiates.

From this point onwards, both groups of cells follow their development independently, using the information already present in the primordium to drive subsequent developmental steps. In the case of the wing disc, the first subdivision imposed over the antero-posterior compartment initial subdivision is between proximal and distal cells (Figure 2). Proximal cells express the EGFR ligand *vein* (*Vn*), whereas distal cells express the gene *wingless* (*wg*) [15, 27, 28]. Decreasing EGFR signalling levels from distal to proximal regions of the disc regulates the formation of nested expression domains of genes encoding transcription factors. In more proximal cells, the genes of the Iroquois (*Iro*) and *Spalt* (*sal*) complexes are expressed and contributed to the determination of the future thorax [27, 29–31]. In a more extended domain, the expression of *apterous* (*ap*) is also activated in response to the EGFR pathway [15, 32, 33], and the Notch signaling pathway is activated at the boundary between cells expressing and not expressing *ap* and drives the expression of *vestigial* (*vg*), a cofactor necessary for the specification of wing cells [25, 34–38]. From now onwards, the territories fated to become the thorax, hinge and blade will expand and further divide into smaller subdomains of gene expression related to pattern elements such as veins and sensory organs (Figure 2). In the expanding wing blade, for example,

most of the expression territories are established with respect to the A/P compartment boundary due to the activity of the Hh and Dpp signaling pathways [39]. In this manner, the central region of the wing is patterned by the differential response to Hh signaling of a battery of genes required for the positioning of the central wing veins (L3 and L4) and the central intervein (L3/4 intervein) [40]. Simultaneously, the lateral regions of the wing blade are patterned through a set of transcription factors which expression is regulated by the Dpp signaling pathway (**Figure 2**).

In the case of the leg, the restricted expression of Dpp and Wg in dorsal and ventral domains of the leg imaginal disc directs the formation of the proximo-distal (P/D) axis. Initially, high levels of Wg and Dpp activate *Dll* in the center of the leg disc and repress the expression of *dachshund* (*dac*). As the discs grow, *dac* escapes the repression of Wg and Dpp in the medial domain of the leg disc and is activated by *Dll* [23]. Once these genes are activated, their expression is locked by autoregulatory mechanisms. In the periphery of the disc, where combined low levels of Wg and Dpp are found, the expression of *homothorax* (*Hth*) and *teashirt* (*Tsh*) is activated. Later on, the activity of the EGFR pathway in the distal domain of the leg disc is required to activate a series of secondary P/D targets, including the tarsal restricted genes *aristalless* (*al*), *Bar* (*B*) and *rotund* (*rn*) [11, 23] (**Figure 2**). An important consequence of the P/D subdivision is the activation of the Notch pathway in concentric rings that subsequently correspond to the joints, movable structures separating adjacent leg segments [41]. Joint development is controlled by the action of subsidiary Notch target genes, such as the transcription factors encoded by *oddskipper* family genes (*odd*) and *dysfusion* (*dys*) in proximal and distal joints, respectively. In particular, *Dys* regulates the expression of several Rho-GTPase regulators and pro-apoptotic genes that together sculpt the tarsal joints [42, 43].

In summary, as the discs grow in size, its pattern is progressively established and prefigures as expression domains the position where different structures, such as veins, sensory organs and tarsal joints, that will differentiate during metamorphosis. This process relies upon regulatory mechanisms that link signaling with transcriptional regulation along the epithelium. Subsequently, each disc will initiate its differentiation and morphogenesis during pupal development, in a course that includes extensive morphogenetic movements and fusion between imaginal discs. The culmination of imaginal disc development is the generation of precise patterns of differentiation. As these patterns are under strict regulation of genes encoding transcription factors and signaling components, changes in the expression pattern or activity of these genes result in precise alterations of the pattern of cell differentiation and organ morphogenesis. These alterations, the mutant phenotypes, can be used as diagnostic criteria to define the requirements of the gene and to annotate its function in relation to the processes affected. Most of the impact of *Drosophila* in biological research is due to the availability of methods to analyze gene function *in vivo*. These methods rely on the power to generate and analyze mutations and to detect the timing and patterning of gene expression. It is through the analysis of the consequences of manipulating gene activity that specific functions could be assigned to particular genes.

3. Genetic analysis in *Drosophila*

When genetic analysis was first used to characterize the contribution of a gene to a particular process, the definition of “gene function” was abstract, referring more to the requirement of the gene than to the actual biochemical function of the protein it encoded, which was for the most

part unknown. Thus, genes encoding transcription factors or signaling molecules could be classified as “segmentation genes,” because they displayed mutant phenotypes affecting the segmentation of the embryo [44]. By looking at the particularities of the mutant phenotype, these genes could be further classified into discrete classes that later were shown to correspond to the different levels in the hierarchy of regulatory interactions driving segmentation [44]. During most of the 20th century, methods to analyze a gene were blind to a large extent. In this manner, mutations generated randomly were selected because they failed to complement a particular allele or gene deficiency [45, 46] or because they displayed or modified a phenotype in the tissue of interest [47–50]. Favorite methods to generate mutations were chemical (EMS), physical (ionizing radiations) and later through the mobilization of transposable elements [51, 52].

The availability of the *Drosophila* DNA sequence in 2000 [53], combined with the development of novel techniques to generate mutations in the following years, marked a shift in the way genes were studied. When the entire genetic map of the fly was open to scrutinize, it was necessary to develop new methods that allowed the generation of gene-specific mutations from the sequence (“reverse genetics”). In this manner, homologous recombination was adopted to fly genetics [54, 55], although the technical complexity and low frequency of these events precluded a generalized adoption by the fly community. More impact had the implementation of RNA interference (RNAi), which combined with the Gal4/UAS system, already extensively used to generate gain-of-function conditions [56] allowed a targeted reduction of mRNA levels of the gene of interest in specific tissues [57]. The generation of genome-wide collections of UAS-RNAi lines further allowed screening systematically the genome, either by searching for mutant phenotypes in the tissue of interest or for modifiers of a particular genetic condition [58, 59]. RNAi is being massively used as a first approach to identify the functional requirements of a gene or gene family of interest, but still has the problems of generating only hypomorphic conditions and the existence of off-target effects caused by sequence similarities.

In more recent years, the adaptation of the CRISPR/Cas9 technology to the fly is allowing an unprecedented level of precision and easiness with which a gene can be targeted [60–63]. This method is based on the use of the nuclease Cas9 guided by small RNAs (gRNAs) to generate double-strand breaks (DSB) at a target genomic locus, allowing its use as a highly efficient and specific system for gene edition. Using CRISPR/Cas9 allows targeted manipulation of a given gene in different manners. Thus, the CRISPR/Cas 9 system can be employed to generate sequence-specific DSB to disrupt the target locus when a single gRNA is used, resulting in the generation of small insertion or deletions (In/Dels) through the error-prone process of non-homologous end-joining (NHEJ) repair in the coding sequence. This approach can be used to disrupt coding genes, leading to an array of mutations ranging from hypomorphs to amorph alleles (**Figure 3**), caused by frameshifts in the reading frame, premature stop codons or triplet insertions or deletions [63–65]. The NHEJ repair system can also be directed by two gRNAs to delete a specific fragment flanking the targeted sequences [63, 66]. The resulting DNA change consists in a deletion of a longer sequence, which could include an entire open reading frame, an exon or also non-coding sequences such as candidate regulatory regions, being also an useful approach to analyze transcriptional regulation *in situ* (**Figure 3**).

A second application of the CRISPR-Cas9 system is to stimulate HDR (*homology-direct repair*) by using homologous DNA sequences as template for the DNA repair and two gRNAs, allowing precise

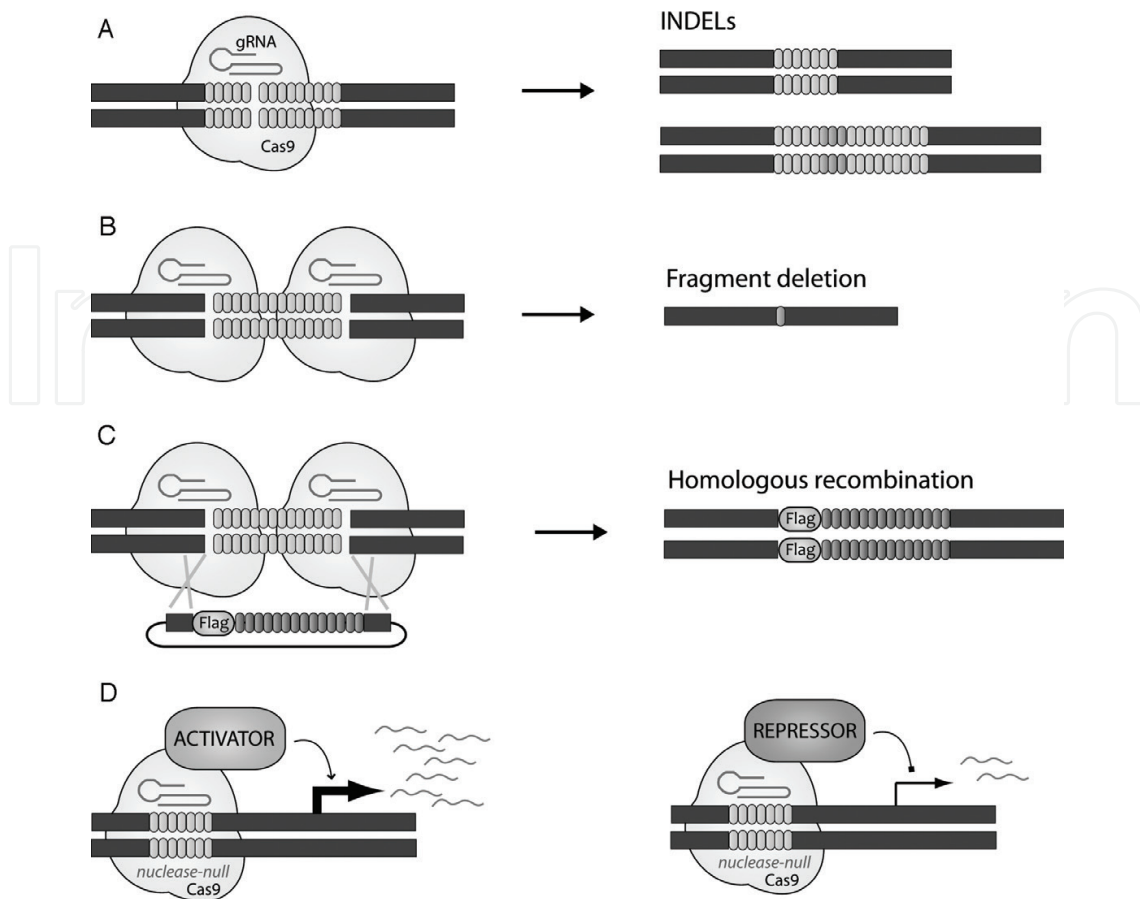


Figure 3. Applications of CRISPR/Cas9 for gene editing in *Drosophila*. Schematic representation of Cas9-DNA interactions leading to changes in the genomic sequence (A–C) or to changes in gene expression (D). The genomic DNA is represented as thick lines, Cas9 is represented by a grey shape, guide RNA (gRNA) are shown as grey lines and nucleotides targeted by gRNA as grey ovals. (A) Single gRNA leads to DNA double strand breaks (left) that are corrected by non-homologous end joining (right) leading to INDELS. (B) Double gRNAs lead to nearby double strand breaks (left) that when corrected result in a deletion of the DNA located between adjacent breaks (right). (C) The use of two gRNAs in the presence of sequences with homology to the target DNA (left) leads to the substitution of this DNA (knock-out) by the selected sequence (knock-in), in this case a tagged version of a coding region (Flag). (D) The targeting of inactive forms of Cas9 (nuclease-null Cas9) fused with activating (left) or repressor (right) domains leads to the corresponding changes in gene expression from adjacent promoters.

genome editing by generating sequence substitutions following a deletion (knock-out/knock-in). This method relies on supplying a homologous repair DNA (ssDNA or dsDNA) engineered with the desired modifications [62, 67–69]. This approach allows not only the induction of particular mutations in the genome but also the integration of ssDNA oligonucleotides for epitope TAGs into protein coding genes, allowing the tagging of endogenous proteins (**Figure 3**).

More interestingly, when stimulating HDR using longer homologous repair dsDNA (homology arms), the removed genomic region can be replaced with site-specific recombinase sequences such as attP sites [67], allowing subsequent integrations at this genomic position of modified versions of the gene or orthologous genes and sequences encoding for tagged proteins (**Figure 3**). Creation of a DSB dramatically increases the frequency of homologous recombination [70], allowing the expression *in situ* of protein variants expressed under the same regulatory DNA as the endogenous gene (**Figure 3**). In addition, *Flippase recognition target* (FRT) sites could be introduced flanking the region of interest to allow tissue-specific

or clonal deletion of specific sequences [67] (**Figure 3**). Engineered alleles can be subsequently interrogated by their impact on any particular part of the fly or used to follow the expression of the protein of interest due to the incorporation of different TAGs (**Figure 3**).

Beyond genome engineering, CRISPR/Cas9 has also been used to regulate endogenous gene expression in both cells and organisms without causing any mutation. For this approach, a nuclease-dead or inactive Cas9 is fused to a transcriptional activator or repressor domain and can be recruited to specific target DNA by its gRNAs, allowing activation through CRISPR or repression by CRISPR interference (**Figure 3**) [71–73]. In addition, an inactive Cas9 co-expressed with a gRNA can also be used for immunoprecipitation of specific DNA regions as a variant form of engineered DNA binding-mediated chromatin immunoprecipitation (enChIP), and associated proteins can be subsequently identified by mass spectrometry (enChIP-MS) [74]. In summary, CRISPR/Cas9 technology is providing a precise and efficient method for sequence-specific targeting of Cas9, resulting in genomic alterations, changes in gene expression and even the isolation of protein complexes bound to specific DNA regions. The application of this technology is triggering an unprecedented level of precision in genetic and genomic analysis in *Drosophila* research.

4. Uses of *Drosophila* and the imaginal discs to address biomedical problems

The precision by which the genome can be modified, combined with the knowledge we have about the cellular and molecular fundamentals of imaginal disc development, justify the use of these epithelial tissues as experimental models to address a variety of biological questions, including biomedical ones. According to the Homophila database, around 75% of human disease genes cataloged in OMIM (On-line Mendelian Inheritance in Man) database have close homologs in the fruit fly [75]. This high degree of evolutionary conservation makes even more compelling the use of *Drosophila melanogaster* to develop experimental models of human diseases [76, 77].

The generation of *Drosophila* models for human diseases includes a variety of approaches, including blind and unbiased large-scale genetic screens, functional analysis of *Drosophila* genes orthologous for a known human disease gene, the expression of human genes in *Drosophila* tissues and the construction of genetic models that reproduce some characteristics of complex human syndromes such as cancer, kidney and metabolic diseases among many others [78–86]. These approaches are focused on either the gene or genes causing the disease or, complementary, the tissue where the disease is manifested. A successful example of the first approach, unbiased genetic screens, involved a mosaic screen of newly generated lethal mutations in the X chromosome that allowed the identification of 21 novel genes associated with human diseases for which no mutations were previously known [87]. The analysis of *Drosophila* genes with human counterparts contributing to human diseases has been mostly applied in the context of several neurodegenerative diseases, and some examples are the analysis of *parkin* and *Pink1*, genes related to Parkinson disease, and *sphingosine-1-P-lyase* for Charcôt-Marie-Tooth neuropathy [88]. In these cases, the *Drosophila* model allows a first approximation to study the functional relevance of the gene, including the consequences of its loss, its expression, the subcellular localization of the protein and its biochemical characteristics. Similarly, the expression of human proteins in flies has mostly targeted neurodegenerative diseases such

as Alzheimer disease, Tau-induced neurodegeneration (Tauopathy), polyglutamine diseases including Huntington disease and spinocerebellar ataxia, among others [89]. In this manner, it was shown that the overexpression of the human A β 42 protein in the central nervous system of *Drosophila* causes amyloid deposition, progressive learning defects, extensive neurodegeneration and shortened lifespan, all of the hallmarks of Alzheimer's disease [90].

Apart from offering a convenient experimental system to identify the function of a gene or the consequences of the expression of relevant protein variants, *Drosophila* has been instrumental in expanding the catalog of relevant genes contributing to the function and outcome of a particular genetic condition through the use of "genetic modifier screens". These experiments consist in the search for mutations that can either increase or suppress the phenotype caused by a genetic condition of interest, under the assumption that these modifying alleles might identify additional components relevant for the function of the protein of interest. Such approach has been applied to a variety of genetic backgrounds, either in blind genetic screens, i.e., through the generation of random mutations in a particular genetic background, or by introducing in these backgrounds the expression of RNAi directed against all genes or particular candidates. Using these approaches, it was found that mutations in Neprilysin 2 modify the progressive retinal degeneration concomitant with plaque formation caused by the overexpression of human A β 42 in the *Drosophila* eye [91].

Drosophila can also be effectively used for drug screens as well as in target discovery [77, 92]. Screening for novel drugs in flies enables for the selection of candidates with physiological characteristics that are difficult to analyze by cell culture or biochemical assays. In addition to high-throughput screening of potential therapeutics, *Drosophila* is a powerful tool for studying the molecular mechanism of a specific drug *in vivo*. The diminished effort and costs of analyzing the targets and possible "off-targets" in a *Drosophila* model make it useful as a first validation organism for establishing the efficacy and toxicity of a drug *in vivo*. In fact, some approved treatments, such as the kinase inhibitor vandetanib (ZD6474) for treating medullary thyroid carcinoma patients, were validated in *Drosophila* previous to clinical trials [93]. When using *Drosophila* for developing a treatment, it is important to consider the possible differences in pharmacokinetics of a drug in this organism and the differences in tissular distribution, which may affect the optimal doses of the compound. Also toxicity might be different, although a strong correlation has been shown [94]. Nowadays, there are several companies that have been using *Drosophila melanogaster* as human disease model for screening for therapeutic drugs such as Aktogen, En Vivo Pharmaceuticals, Genescent Corp and Medros Pharmaceuticals. Indeed, the ease of screening big samples of individuals has also been employed for drug discovery in *Drosophila* disease models. For example, a library of 2000 compounds was checked in a Fragile-X syndrome *Drosophila* model for pharmacological rescue [95], and 9 molecules were found to rescue lethality, among them three belonging to GABAergic inhibitory pathway. This and further studies led to the performance of human trials of GABAergic treatment (reviewed in [96]).

5. Conclusions

Several characteristics converge to sustain and reinforce the use of *Drosophila* in the post-genomic era as a motor for biomedical research. The availability of novel techniques to manipulate and scrutinize the genome with unprecedented sophistication and precision can

be used in experimental models, such as the imaginal discs, that are particularly well suited for genetic and molecular analyses. The convergence of technical improvements used in a favorable experimental system is sustained by the functional conservation of the relevant genes and the cellular processes they regulate and by the multiple adaptations that this system allows, including genetic, biochemical, cellular and pharmacological experimental approaches.

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