The wing imaginal disc

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Abstract

The Drosophila wing imaginal disc is a tissue of undifferentiated cells that are precursors of the wing and most of the notum of the adult fly. The wing disc first forms during embryogenesis from a cluster of ~30 cells located in the second thoracic segment, which invaginate to form a sac-like structure. They undergo extensive proliferation during larval stages to form a mature larval wing disc of ~35,000 cells. During this time, distinct cell fates are assigned to different regions, and the wing disc develops a complex morphology. Finally, during pupal stages the wing disc undergoes morphogenetic processes and then differentiates to form the adult wing and notum. While the bulk of the wing disc comprises epithelial cells, it also includes neurons and glia, and is associated with tracheal cells and muscle precursor cells. The relative simplicity and accessibility of the wing disc, combined with the wealth of genetic tools available in Drosophila, have combined to make it a premier system for identifying genes and deciphering systems that play crucial roles in animal development. Studies in wing imaginal discs have made key contributions to many areas of biology, including tissue patterning, signal transduction, growth control, regeneration, planar cell polarity, morphogenesis, and tissue mechanics.

Keywords: wing; Drosophila; imaginal disc; FlyBook

Introduction

In holometabolous insects like Drosophila, which undergo complete metamorphosis, the precursors of most adult structures of the head, thorax, and genitalia are maintained during larval stages as distinct clusters of undifferentiated cells called imaginal discs. The name refers to the final, adult stage of insect development, which is classically known as the imaginal disc. The wing imaginal disc (henceforth, wing disc) gives rise to the wing and wing hinge, and also the dorsal half of the body wall in the second thoracic segment (T2, also known as the mesothorax), which in Drosophila comprises the bulk of the thorax (Fig. 1a and b). This includes the back of the thorax, the notum, and part of the lateral sides of the thorax, the pleura.

The imaginal discs are easily recognizable within the body cavity of the larva (Fig. 1c), which facilitated classical approaches involving transplantation, as well as more recent approaches incorporating both analyses of dissected fixed discs and live imaging. The relatively flat morphology of the wing disc, with most cells in a single epithelial layer, has also facilitated imaging-dependent approaches and contributed to their popularity as an experimental model. The imaginal discs undergo extensive growth during the larval stages of Drosophila, with the wing imaginal disc increasing in size over 1,000-times. In contrast to many larval tissues, which become polyploid, the imaginal disc cells remain diploid, and discs grow by increasing cell numbers. The extensive growth of the wing disc has contributed to its utility as a model for studies of organ size control, and to the identification in wing discs of genes that play key, conserved roles in controlling growth of animal tissues. The growth of wing discs has also facilitated methods for creating genetic mosaics through induction of recombination and growth of mitotic clones, thus enabling analysis of requirements for genes that are also required at earlier stages of development, and distinguishing autonomous from nonautonomous effects.

During wing disc development, distinct cell fates are assigned to different regions, and the wing disc transitions from a simple sac of cuboidal epithelial cells to an organ with complex epithelial morphology that includes regional differences in cell shape and a pattern of local folding around the future wing hinge. Patterning of the wing discs is mediated by several highly conserved signaling pathways, and many of the components of these pathways were first identified and characterized based on their roles in wing discs. Classical genetic studies identified and characterized many mutations that affect wing size, shape, and posture (Waddington 1940 and Lindsley and Grell 1968), in part because flies do not need their wings to survive or reproduce in laboratory culture. These mutations have contributed to the identification of genes that play important roles in wing discs. Indeed, some of the genes that are crucial for normal wing development were first identified over 100 years ago (Morgan and Bridges 1916, Bridges and Morgan 1919). Even today, investigators studying the effects of genetic alterations on wing discs often examine adult wings as the final outcome of processes that occurred during wing disc development. Studies in wing discs take advantage of the many sophisticated genetic techniques
Embryonic origin of the wing disc

The wing disc is thought of as a larval structure, but the initial specification of wing discs occurs during embryogenesis. The 2 wing discs of each developing fly (left and right) arise from cells in the lateral epidermis of T2 around embryonic stages 11–13 (Bate and Arias 1991; Cohen et al. 1993; Requena et al. 2017). The imaginal disc primordia can be identified in the embryo by their distinct cellular morphology (Madhavan and Schneiderman 1977; Bate and Arias 1991), but analysis of their specification has been greatly aided by the identification of genes that are specifically expressed in these cells, together with the cis-regulatory modules that drive expression in disc primordia (Williams et al. 1991; Cohen et al. 1993; Fuse et al. 1996; Requena et al. 2017). Examination of molecular markers, together with lineage analysis, has revealed that 2 adjacent populations of cells together give rise to wing discs (Requena et al. 2017) (Fig. 2). Around embryonic stage 11 thoracic imaginal disc primordia (TP) are specified, recognizable by expression of the transcription factors Snail (Sna) and Vestigial (Vg) (Williams et al. 1991; Cohen et al. 1993; Fuse et al. 1996; Requena et al. 2017) (Fig. 2). The wing primordia appear on the dorsal sides of Dll-expressing TP cells, and include both cells from the TP and cells just dorsal to the TP. The close association of the wing and leg imaginal disc primordia is consistent with lineage studies showing that individual marked clones created at early embryonic stages can contribute to tissue from both leg and wing discs (Wieschaus and Gehring 1976; Lawrence and Morata 1977). Around embryonic stage 14, the T2 imaginal disc primordia become physically separated into distinct leg and wing primordia, as the wing primordia cells migrate dorsally (Fig. 2). A similar process in T3 separates leg and haltere disc primordia.

The dual origin of wing disc cells has implications for the evolutionary origin of the insect wing. Two main hypotheses have been suggested (Clark-Hachtel and Tomoyasu 2016). The gill-exite hypothesis proposes that wings evolved from an outgrowth that at the base of the leg that functioned as a gill in aquatic insects, whereas the paranotal hypothesis proposes that wings evolved from an extension of the notum. The observation that the wing disc primordium includes populations of cells that are both shared and distinct from the leg disc primordium has been interpreted as supporting a unified hypothesis that combines the paranotal and gill-exite hypotheses (Niwa et al. 2010; Clark-Hachtel and Tomoyasu 2016; Requena et al. 2017; Linz and Tomoyasu 2018).

Several different approaches have been used to estimate the number of cells in an embryonic wing disc primordium. By direct examination of the morphologically identifiable wing disc primordium in the embryo after their separation from the leg primordia, Bate and Arias (1991) estimated that a primordium has 24 cells, whereas counting of Vg-expressing cells around this stage led (Cohen et al. 1993) to estimate a primordium contains around 30 cells. A similar analysis in the first instar larva yielded an
estimate of 38 cells (Madhavan and Schneiderman 1977), but this could have included associated tracheal, nerve, or adepithelial cells. Martín et al. (2009) inferred a founder size of 55 cells from measurements of clone sizes and the number of cells in late third instar wing discs, however, they induced their clones around the time when Madhavan and Schneiderman (1977) estimated that wing disc cells begin dividing, which may have skewed the estimate. By estimating the frequency of mosaicism in clonal analysis experiments using a single marker (Lawrence and Morata 1977) estimated a wing primordium has \( \approx 20 \) cells. By using a method (Tie-Dye) that generates multiple clone labels and examining frequencies of labeled clones (Worley et al. 2013) estimated with 73% confidence that it derives from 25 to 31 cells. Thus, a variety of approaches suggest that the initial number of wing disc primordium cells is likely around 25–30. Around embryonic stage 14, the wing primordia invaginate to form small epithelial sacs that remain connected to the embryonic epidermis. They undergo little or no cell division until near the end of the first larval instar (Madhavan and Schneiderman 1977; Bate and Arias 1991).

The specification of the wing disc primordium requires signals that inform cells of their segmental identity, and their anterior–posterior and dorsal–vental location within the segment. Segmental identity is provided by Hox genes, and formation of the thoracic and wing primordia is repressed in more anterior segments by Sex combs reduced and in more posterior segments by Ultrabithorax, abdominal A, and Abdominal B (Vachon et al. 1992; Carroll et al. 1995; Gebelein et al. 2002; Requena et al. 2017). The Hox gene expressed in T2, Antennapedia, is not required for formation of TP but does enhance Dll expression in the TP (Uhl et al. 2016). The TP is specified around the anterior–posterior (A–P) compartment boundary, such that it includes both anterior and posterior cells from its inception. This can be explained by the role of the Drosophila Wnt protein Wingless (Wg), which is expressed along the anterior side of the A–P boundary in the embryo. Wg is required for specification of the thoracic imaginal discs, as revealed by the requirement for Wg for expression of Dll in the TP (Cohen 1990; Cohen et al. 1993) (Fig. 2a). The lateral location of the TP is set by repression in more dorsal cells from Decapentaplegic (Dpp) signaling, and repression in more ventral cells from epidermal growth factor receptor (EGFR) signaling (Goto and Hayashi 1997).

As the TP splits into leg and wing primordia, distinct effects of Wg, Dpp, and EGFR signaling are observed, which act in concert to specify distinct thoracic imaginal disc primordia (Fig. 2b). Dpp becomes expressed in a lateral stripe just dorsal to the TP, and promotes wing primordium fate (Goto and Hayashi 1997; Hamaguchi et al. 2004; Requena et al. 2017), as well as, at lower levels, proximal leg fate (Goto and Hayashi 1997). Conversely, EGFR signaling from more ventral cells represses wing primordium fate, while promoting leg fate (Kubota et al. 2000; Requena et al. 2017). Dpp signaling also leads to local repression of Wg expression through upregulation of Dorsocross (Doc) transcription.
factors. This results in lower levels of Wg signaling, which favors wing disc fate over leg disc fate (Kubota et al. 2003; Requena et al. 2017). Thus, dynamic integration of positional cues results in specification of 2 bilaterally symmetric clusters of ~25–30 embryonic cells as wing disc primordia.

**Cell biology of the wing disc**

Although the wing disc is sometimes treated as a simple sheet of epithelial cells, it has a complex morphology, including heterogeneities in cell shape, type, and organization.

**Wing disc epithelial cells**

The early wing disc is a flat sac of cuboidal epithelial cells, with the apical sides toward the lumen. As the wing disc begins to grow, differences in cellular morphology appear. Cells on one side flatten, forming a thin squamous epithelium called the peripodial membrane or peripodial epithelium (PE) (Auerbach 1936; McClure and Schubiger 2005) (Fig. 3a). The peripodial epithelium ultimately constitute a small fraction of wing disc cells, roughly 5% at the end of larval development (McClure and Schubiger 2005), and contribute correspondingly little to the cuticle of the adult fly (Milner et al. 1984). Nonetheless, they may interact with other cells (Gibson and Schubiger 2000; Pallavicini and Shashidhara 2005), and they play essential roles in the morphogenetic transformation of the disc that occurs during metamorphosis (Milner et al. 1984; Pastor-Pareja et al. 2004; Alzada et al. 2013). Cells on the other side elongate apico-basally as their density increases, forming a columnar epithelium (Fig. 3, a and b). Most of the growth, and ultimately most of the cells, of the wing disc are in the columnar epithelium, also referred to as the disc proper (DP), and consequently most studies effectively treat the wing disc as an epithelial monolayer. Near the edge of the wing disc there is a transition zone with roughly cuboidal cells (McClure and Schubiger 2005; Alzada et al. 2010) (Fig. 3, a and b). By late third instar columnar cells become densely packed and increasingly tall, particularly in the central region of the disc that will give rise to the wing, where cells can be ~40 μm tall and ~2 μm wide (Fig. 3, b and d). These columnar cells become pseudostratified (the nuclei are not all in the same plane), a physical necessity as the widths of the cells become less than the width of the nucleus (Fig. 3, b, c, and d). Division of these pseudostratified cells involves a process of the cells becoming less than the width of the nucleus (Fig. 3, b, c, and d). This process is disturbed, BM formation is impaired, or BM proteins are degraded, columnar cells flatten toward a cuboidal shape (Domínguez-Giménez et al. 2007; Pastor-Pareja and Xu 2011; Ma et al. 2017). The BM also influences the distribution of extracellular signaling molecules (Ma et al. 2017).

**Nonepithelial cells in the wing disc**

In addition to the epithelial cells that make up the bulk of the wing disc, the wing disc also contains smaller numbers of other cell types. Neurons and associated glia are embedded within the wing disc epithelium. They form from sensory organ precursor cells that are selected through a lateral inhibition process mediated by Notch signaling, and they will form sensory bristles in the notum and along the anterior edge of the wing in adult flies (Huang et al. 1991; Gómez-Skarmeta et al. 2003). These neurons send axons between the epithelium and basal lamina of the wing disc.

Adult muscle precursor cells (AMP, originally described as dendritosphial cells) underlie the notum and are located between the columnar disc epithelium and the BM (Madhavan and Schneiderman 1977) (Fig. 3, a and b). These cells are mesodermal in origin, and they will form the flight muscles of the adult fly (Bate et al. 1991; Fernandes et al. 1991). Their location, proliferation and patterning depend upon signals from wing disc epithelial cells, including Wg, Notch, fibroblast growth factor and hedgehog (Hh) (Gunage et al. 2014; Hatori and Kornberg 2020; Everetts et al. 2021).

The wing disc is also tightly associated with tracheal cells (Inoue and Hayashi 2007), which provide oxygen to wing disc cells and form a primordium for the air sac that will deliver oxygen to
flight muscles in the adult (Fig. 3a). Wing disc tracheal patterning also depends upon signaling from disc cells, mediated at least in part through cytonemes (Roy et al. 2014; Du et al. 2018; Hatori and Kornberg 2020). Thus, although the wing disc is often thought of as a simple epithelial organ, the tight association and exchange of signals with mesodermal and tracheal cells emphasize that

Fig. 3. Cell biology of the wing disc. a) Schematics of a horizontal view of the wing disc (top left) and sections across the length and width of the disc to illustrate different cell types, including the squamous PE (gray) columnar cells of the wing (olive), hinge (brown) and notum (orange), and AMP (pink) and tracheal (blue) cells underlying the notum. Red arrows point to the hinge–notum (top), hinge–hinge (middle), and hinge–pouch (bottom) folds. b) Confocal micrographs of late third instar wing disc. Top left panel displays a maximum projection of E-cad staining (green). Panels at right and bottom show slices across the length and width of the disc, and include staining for DNA and F-actin as well as E-cad. c) Schematic section of columnar wing disc epithelia, illustrating pseudostratification, with nuclei in blue, and relative locations of marginal zone (green), adherens junctions (red), and septate junctions (dark blue) indicated. BM is indicated in orange at bottom. d) Extracted surface of confocal micrograph of apical surface of the wing region of the wing disc, with cells outlined by E-cadherin staining. Note the variations in apical cell size. Red arrows highlight the fold at the edge of the wing pouch, yellow arrows highlight a few examples of mitotic cells, which are transiently enlarged as they round up. e) Confocal micrographs of vertical sections through the wing disc, showing DNA (blue), F-actin (red), and at left collagen (encoded by viking, green) and at right E-cad (green).
the wing disc, like more complex vertebrate organs, includes a diversity of cell types that are organized by signaling interactions between epithelia and neighboring nonepithelial cells.

**Patterning of the wing disc**

Intensive studies of how cells in different regions of the wing disc acquire distinct fates have yielded fundamental insights into conserved mechanisms of tissue patterning and the signaling networks responsible for establishing it.

Cleavage of dissected third instar wing discs and transplantation of disc fragments back into larval hosts about to undergo metamorphosis was used to create the first detailed fate maps of the wing disc (Bryant 1975a) (Fig. 1a). This was possible because by the end of the third instar, the characteristic shape of the wing disc makes distinct regions readily identifiable, and disc fragments corresponding to morphologically identifiable regions differentiate into consistent adult structures. In broad terms, the 4 main regions of the late third instar wing disc are the wing pouch, a roughly oval-shaped region that gives rise to the wing blade; the proximal wing and wing hinge, a folded region that gives rise to structures at the base of the wing; the roughly triangular notal region, which gives rise to most of the back of the fly in the thorax, and the PE, which gives rise to some of the pleura. The asymmetry of the wing disc also makes it possible to distinguish anterior from posterior sides.

Expression profiling approaches, including microarrays, enhancer mapping, and single cell RNA sequencing, have revealed intricate and complex patterning of gene expression in wing disc cells by the end of the third larval instar (Butler et al. 2003; Jory et al. 2012; Bageritz et al. 2019; Deng et al. 2019; Everetts et al. 2021). The distinct fates of different regions of the wing disc are specified through a process of progressive refinement as disc development proceeds. Beginning with an initial subdivision of the wing disc into broad regions, the disc then becomes further subdivided as a series of transcription factors, signaling molecules, and their targets become expressed in distinct patterns.

**Establishment of wing disc patterning**

The wing disc contains regionally distinct cell types from its inception in the embryo, as it forms straddling the A-P compartment boundary, and thus includes both anterior and posterior cells (Fig. 2, a and b). Posterior cells are defined by expression of the transcription factor Engrailed (En) (Morata and Lawrence 1975; Kornberg 1981) (Fig. 4a). Although some wing disc cells originate as part of an early, initially tll-expressing TP that also gives rise to the leg imaginal disc and others originate from a slightly more dorsal primordium (Fig. 2), lineage analysis shows that cells from either primordia can contribute to each of the main regions of the wing disc (Requena et al. 2017).

During the first and second larval instars, the wing disc becomes subdivided into distinct appendage (wing) vs body wall regions. This subdivision is largely dependent upon the regionalized expression of 3 secreted signaling molecules: Vein (Vn), Wg, and Dpp (Fig. 4b). Vn, a ligand for the EGFR, is expressed in more dorsal cells of the early wing disc, whereas Wg is expressed in more ventral cells of the early wing disc (Couso et al. 1993; Williams et al. 1993; Ng et al. 1996; Simcox et al. 1996). Vn and Wg signaling antagonize each other, helping to maintain distinct dorsal and ventral territories (Baonza et al. 2000; Wang et al. 2000). Jak-Stat signaling, which is elevated in the ventral half of the wing disc, is required to maintain restriction of EGFR signaling to more dorsal cells at late second and early third instar (Recasens-Alvarez et al. 2017). The origin of the dorsal vs ventral differences in Vein and Wg expression in the early wing disc is not entirely clear. It has been reported that Vn expression is induced de novo in the first instar wing disc by Dpp signaling across the lumen from the overlying, future peripodial cells, and then maintained by a positive feedback loop through EGFR signaling (Wang et al. 2000; Paul et al. 2013), although this then raises the question as to what localizes Dpp expression to these overlying cells.

Notum fate is promoted by expression of the 3 homeodomain transcription factors of the Iroquois complex (Iro-C), Araucan, Caupolican, and Mirror (Diez del Corral et al. 1999). Loss of the
Iro-C transforms notum cells into wing hinge cells. Vn is required to promote Iro-C expression in dorsal cells of the wing disc (Wang et al. 2000; Zecca and Struhl 2002a) (Fig. 4b). Conversely, Wg contributes to the transcriptional program that defines the future wing, which is first distinguished by elevated expression of Vg and reduced expression of Teashirt (Tsh) (Williams et al. 1993; Cousso et al. 1995; Wu and Cohen 2002) (Fig. 4b). This is soon followed by repression of Homothorax (Hth) expression, and activation of Nubbin (Nub) expression (Ng et al. 1996; Azpiazu and Morata 2000; Zirin and Mann 2004). Dpp signaling has a complex role in this initial subdivision of the wing disc. After the initial contribution of Dpp in peripodial cells to activating Vn expression, Dpp, which becomes more highly expressed in the ventral half of the DP, plays a key role in repressing notal fates by repression expression of Iro-C genes (Cavodeassi et al. 2002).

Around the same time that the wing disc is subdivided into wing vs body wall regions, it also becomes subdivided into peripodial vs DP regions. This subdivision also depends upon Wg and EGFR signaling, but in this case both of these pathways suppress peripodial fate (Fig. 4d) (Baena-López et al. 2003). Thus, it appears that different combinations of the same signals differentiate wing, notum, and PE, with EGFR promoting notum, Wg promoting wing, and absence of both signals resulting in PE. It has also been reported that formation of PE requires nonautonomous contributions from Hh and Dpp signaling (McClure and Schübiger 2005). The requirement for Dpp might now potentially be explained by its role in promoting Vn expression (Paul et al. 2013).

A key transcription factor in the initial specification of PE is Bowl (Fig. 4d) (Nusinow et al. 2008). Bowl protein is detected specifically in peripodial cells, and not in DP cells, due to the action of Lines, which is active in DP cells and promotes degradation of Bowl. Broad and early loss of Bowl can convert FE into DP cells, and complementarily, loss of Lines can convert DP cells into PE cells. However, later maintenance of distinct FE and DP fates does not depend upon Bowl. The mechanism that establishes the differential activity of Lines has not been described but would seem likely to be downstream of Wg and EGFR signaling. Elongation of columnar cells in the future wing is promoted by Dpp and Wg signaling (Widmann and Dahmann 2009a,b). Consistent with this, misexpression of Spalt (Sal) complex genes, which are targets of Dpp signaling, can alter the morphology of PE cells toward that of columnar cells (Tang et al. 2016). Moreover, misexpression of Lines in PE cells allows expression of Sal, and this contributes to a peripodial to columnar cell transformation, because the transformation is suppressed if Sal expression is knocked down (Tang et al. 2016).

Compartmentalization of the wing disc

Development of the wing disc is critically dependent upon its subdivision into orthogonal A–P and dorsal–ventral (D–V) regions called compartments, as communication between cells in these compartments establishes signaling centers that direct further wing patterning and growth. A compartment boundary forms when a mechanism for separating cells is coupled to heritable control of gene expression that defines positional identity. This creates distinct populations of nonintermixing cells. Conversely, noncompartmental subdivisions form when the maintenance of spatially distinct gene expression profiles depend upon cells’ position rather than their lineage. For example, in the wing disc, the A–P and D–V subdivisions are compartmental, but the subdivisions into notum vs wing, or peripodial vs columnar epithelium, are not.

Compartmentalization was first discovered in the Drosophila wing but subsequently identified in a variety of invertebrate and vertebrate tissues (Irvine and Rauskolb 2001; Dahmann et al. 2011). The discovery of compartments was made possible by the development of techniques for genetically marking individual cells and their descendants through induction of mitotic recombination. This led to the observation that the spatial distributions of clones of cells respect a boundary along the middle of the wing (Garcia-Bellido et al. 1973). For example, individual clones are always composed of only anterior or only posterior cells. Moreover, in most of the wing clone boundaries are irregular, but along the middle of the wing they form a straight line that demarcates the A–P compartment boundary. Remarkably, this boundary does not correspond to any distinct morphological features that can explain how cells are separated. Moreover, the boundary is maintained even when cells in 1 compartment are given a growth advantage compared with cells in the other compartment by using dominant slow-growth mutations called Minutes (Garcia-Bellido et al. 1976). The anterior–posterior compartmental subdivision of the wing begins during embryogenesis even before the disc primordia form (Garcia-Bellido et al. 1973, 1976; Wieschaus and Gehring 1976). A–P compartmentalization is dependent upon the posterior-specific expression of En (Morata and Lawrence 1975; Kornberg 1981), which is established in the embryo and then maintained by stable inheritance of the chromatin state (Moazed and O’Farrell 1992; Brenn et al. 1995; DeVido et al. 2008).

Around the beginning of the second larval instar, the wing disc is further subdivided into distinct dorsal and ventral compartments (Garcia-Bellido et al. 1976), specified by dorsal expression of the transcription factor Apterous (Ap) (Cohen et al. 1992; Díaz-Benjumea and Cohen 1993; Blair et al. 1994) (Fig. 4c). The dorsal-specific expression of Ap is promoted during the second instar by EGFR signaling (Wang et al. 2000; Zecca and Struhl 2002b). Ap expression initially overlaps Iro-C expression, but during second instar Ap becomes expressed in a broader domain that encompasses the dorsal half of the wing primordia as well as the notal region (Wang et al. 2000; Zecca and Struhl 2002a). This is thought to occur as a consequence of ap transcription being only transiently dependent upon EGFR signaling, and then heritably maintained independent of EGFR through autoregulation and maintenance of the chromatin state (Zecca and Struhl 2002b; Oktaba et al. 2008; Bieli et al. 2015). As the wing disc grows, this results in Ap being expressed in a broader domain than Iro-C, which continues to require EGFR signaling (Zecca and Struhl 2002b). The separation of the Ap domain from the Iro-C domain is essential for allowing the formation and growth of the future wing (Zecca and Struhl 2002a; Rafel and Milan 2008).

Signaling between compartments

Along the A–P compartment boundary, P cells signal to A cells through the Hh pathway. Hh is expressed specifically by posterior wing disc cells and can only productively signal to anterior wing disc cells because En represses expression of the Hh pathway transcription factor Cubitus interruptus (Ci) in posterior cells (Eaton and Kornberg 1990; Zecca et al. 1995) (Fig. 4a). Repression of Ci by En also plays a key role in establishing the posterior-specific expression of Hh, as Ci represses Hh in anterior cells both directly, and indirectly, through regulation of scribbler (sbb, also known as mtv) (Methot and Basler 1999; Apidianakis et al. 2001; Bejarano et al. 2007; Bejarano and Milán 2009). The complementary expression of Hh and Ci result in Hh pathway activation in a stripe of cells along the anterior side of the A–P compartment border, with the width of this stripe corresponding to the distance...
over which Hh can productively spread. Hh signaling is best known in the wing disc for inducing expression of the BMP family member Dpp (Basler and Struhl 1994). Dpp has profound effects on wing patterning and growth, consequently manipulations of Hh signaling can have similarly dramatic effects (Basler and Struhl 1994; Tabata and Kornberg 1994).

Signaling across the D–V boundary is mediated by the Notch pathway, which is activated along both sides of the boundary (Fig. 4c). Notch is activated in dorsal boundary cells by signaling from the Notch ligand Delta (Dl) and in ventral boundary cells by signaling from the Notch ligand Serrate (Ser) (Diaz-Benjumea and Cohen 1995; de Celis et al. 1996; Doherty et al. 1996). The differential signaling of Dl and Ser is regulated by Fringe (Fng) (Irvine and Wieschaus 1994; Kim et al. 1995; Fleming et al. 1997; Panin et al. 1997; Klein and Arias 1998b), which inhibits Ser binding to Notch and enhances Dl binding to Notch by glycosylating the Notch extracellular domain (Bruckner et al. 2000; Moloney et al. 2000; Xu et al. 2007). The dorsal-specific expression of Ser and Fng, established by Apl, combine to limit Ser to signaling to ventral wing cells, while the presence of Fng and the cis-inhibition of Notch ligands leads Dl to preferentially signal to dorsal, Fng-expressing cells (de Celis and Bray 1997; Panin et al. 1997; LeBon et al. 2014). Targets of Notch activation at the D–V boundary, including Wg and Vg (Kim et al. 1995; Rulifson and Blair 1995; Kim et al. 1996), play key roles in wing patterning and growth, and consequently interactions between dorsal and ventral cells are essential for wing development (Diaz-Benjumea and Cohen 1993; Irvine and Wieschaus 1994).

Separating cells into distinct compartments

Compartmentalization requires a mechanism for separating cells, and early hypotheses suggested that transcription factors that specify compartmental identity might also regulate cell affinity, and thereby sort cells into distinct populations. However, the hypothesized cell affinity molecules proved elusive. A breakthrough in understanding compartmentalization then came with the realization that signaling between compartments plays a key role in separating them. For example, anterior cells that cannot receive the Hh signal can cross the A–P compartment boundary (Blair and Ralston 1997, Rodriguez and Basler 1997). The mechanism by which Hh signaling maintains the boundary remains only partially understood. One key factor appears to be the levels of Interference hedgehog (Ihog) and Brother of ihog (Boi) proteins, which are downregulated by Hh signaling. These 2 proteins act redundantly as Hh coreceptors, but can also mediate cell adhesion and contribute to A–P cell segregation in wing discs independently of their function as Hh receptor components (Hsia et al. 2017). Nonetheless, Ihog/Boi cannot completely explain the segregation of cells to A and P compartments, and there is also evidence for a contribution of Dpp signaling (Shen and Dahmann 2005), and a role for actomyosin-mediated tension (Landsberg et al. 2009; Rudolf et al. 2015).

Multiple mechanisms also contribute to separation of dorsal and ventral cells. The LRR proteins Capricious (Caps) and Tartan (Tmn) are expressed specifically by dorsal cells during second and early third instar, and their expression contributes to segregation of dorsal and ventral cells, presumably by mediating cell adhesion (Millán et al. 2001). Intercellular signaling is also essential to maintenance of the D–V compartment boundary (Michelli and Blair 1999; Rauskolb and Irvine 1999). Notch signaling across the D–V boundary establishes a line of elevated cytoskeletal tension, including elevated levels of F-actin and myosin (Major and Irvine 2005, 2006; Allee et al. 2012). This upregulation of cytoskeleton tension keeps cells separated and maintains the straightness of the boundary. Cytoskeletal regulation by Notch cannot be explained by the canonical Notch transcriptional pathway, and the mechanism by which Notch signaling regulates cytoskeleton tension at the D–V boundary remains unknown. Differential expression of Caps and Tmn can also contribute to elevated tension along the D–V boundary (Michel et al. 2016). At late third instar additional mechanisms, including a zone of nonproliferating cells (ZNC) established downstream of Notch signaling, also contribute to maintenance of the D–V boundary (O’Brochta and Bryant 1985; Becam et al. 2011).

Wing disc morphogens

Compartment boundaries play a fundamental role in wing disc patterning by acting as sites of production for Dpp and Wg, which spread from compartment boundary cells to direct the expression patterns of genes throughout the developing wing disc. Since the shape of the boundary can affect the distribution of these signals, it has been suggested that the relatively straight and smooth compartment boundaries are important in part because they provide a reproducible morphogen distribution (Dahnmann and Basler 1999). The concept of a morphogen was first proposed by Turing (1952), who suggested that specification of different cell types in different places could be explained by molecules that would exhibit spatial differences in concentration and specify different fates according to their concentration. A simple way to produce a concentration gradient is to have a localized source, such as the stripes of Wg or Dpp expression along compartment boundaries, together with a “sink” that removes molecules and thus prevents them from accumulating to uniformly high levels throughout the tissue (Crick 1970). This mechanism also correlates the concentration of the morphogen to distance from the source, and so provides a means for specifying position within a tissue, a concept which has become central to our understanding of morphogens (Wolpert 1969; Sharpe 2019). Some of the first compelling tests arguing for the existence of morphogens in a cellular system were performed on Wg and Dpp within the wing disc. The importance of Dpp and Wg signaling to wing development, combined with the tractability of the wing disc as a model, has stimulated decades of study centered on their roles in wing patterning, including whether they act as morphogens, how they spread through tissues, and how they promote growth. In parallel, numerous investigations have taken advantage of the wing disc to identify and characterize components of these and other signaling pathways that play key roles in wing disc patterning.

The Dpp morphogen gradient

Dpp regulates the expression of genes that are activated or repressed in broad domains surrounding the stripe of Dpp transcription, including optomotor-blind (omb) and the Sal complex genes spalt major (salm) and spalt-related (sarl) (Fig. 5a). The long-range action of Dpp on downstream target genes was demonstrated by contrasting the nonautonomous effects of Dpp to the cell-autonomous effects of loss or activation of the Dpp receptor Thickveins (Tkv) (Nellen et al. 1996; Lecuit and Cohen 1998). Moreover, a gradient of Dpp protein decreasing away from the A–P boundary has been visualized using Dpp:GFP transgenes (Entchev et al. 2000; Teleman and Cohen 2000). A gradient of Dpp pathway activity can be visualized using an antibody against phosphorylated Mad, a key transcription factor of the Dpp pathway, although this gradient differs in shape from the Dpp protein gradient due to modulation of Dpp receptor levels (Tanimoto
The argument that Dpp acts as a morphogen in the wing disc was further supported by observations that lower levels of Dpp pathway activity are needed to promote expression of Omb, whereas higher levels of pathway activity are needed to promote expression of Sal, which can explain why Omb is normally expressed in a broader domain than Sal (Nellen et al. 1996). Regulation of Omb and Sal by Dpp is mediated through creation of an inverse gradient of the transcriptional repressor Brinker (Brk) (Fig. 5a), which is repressed by Dpp signaling (Campbell and Tomlinson 1999; Jaźwińska et al. 1999; Minami et al. 1999; Müller et al. 2003).

**How does Dpp spread through the wing disc?**

The wing disc has provided an outstanding system for investigating how long-range secreted signals spread through tissues. Models that have been proposed for how Dpp spreads from the A–P boundary to more lateral regions of the wing disc include transcytosis, transport through cytonemes, and diffusion.
Transcytosis was suggested by observations that endocytosis is required for formation of the Dpp gradient (Entchev et al. 2000). However, others have argued that the effects of endocytosis could be explained by altered levels of cell surface receptors (Lander et al. 2002), and direct analysis did not reveal requirements for endocytosis or Tkv in the spread of Dpp (Belenkaya et al. 2004; Schwank et al. 2011a). It has also been proposed that long-range Dpp signaling could be mediated through cytonemes (Ramírez-Weber and Kornberg 1999; Hsiung et al. 2005; Roy et al. 2011, 2014). This is suggested by observations that cytonemes from lateral cells orient toward and contact Dpp-expressing cells, and that Tkv can be observed moving along cytonemes. However, the contribution of cytonemes to Dpp gradient formation and signaling in wing disc epithelial cells remains unclear, as experimental tests of the consequences of cytoneme disruption (Roy et al. 2014) rely on manipulations that could also affect other processes. The most popular explanation for how Dpp spreads through the wing disc is that of extracellular diffusion, although there remains disagreement over whether Dpp gradient formation is best explained by free diffusion or by diffusion that is restricted through binding to receptors and glypicans. Glypicans are heparan sulfate proteoglycans attached to the cell-surface through glycosylphosphatidylinositol (GPI) anchors. They regulate developmental signaling pathways by binding secreted signaling molecules, and they have been implicated at various steps of signaling including control of movement, stability, signaling, and intracellular trafficking (Yan and Lin 2009). It is clear that both levels of Tkv (Lecuit and Cohen 1998; Tanimoto et al. 2000), and levels of the 2 Drosophila glypicans, Daily and Daily-like (Dlp) (Fujise et al. 2003; Belenkaya et al. 2004; Akiyama et al. 2008), influence the shape of the Dpp gradient, although it has also been argued that this could reflect effects on steps other than diffusion, and that the best explanation for how Dpp spreads through tissue is simply free diffusion (Zhou et al. 2012).

An elegant recent test of parameters that influence the shape of morphogen gradients used GFP and synthetic GFP-binding proteins based on nanobodies to mimic the Dpp morphogen gradient in the wing disc (Stapornwongkul et al. 2020). These experiments revealed that free diffusion could generate a gradient in the wing disc, however, a gradient approximating the shape of the normal Dpp gradient required a combination of high affinity, signaling receptors, and low affinity, nonsignaling, GPI-anchored binding proteins, presumably mimicking the contribution of glypicans. The low affinity binding proteins limit leakage of free GFP outside of the wing disc and may also contribute to gradient formation by diffusing within and between cells. Thus, observations of synthetic GFP gradient formation suggest that restricted diffusion plays a key role in Dpp gradient formation.

**Dpp gradient scaling**

The Dpp gradient is maintained over days during larval development, during which its relative size adapts to the increasing size of the wing disc. This raises the question of how the size and shape of the gradient is adjusted to match the altered dimensions of a growing disc, a process referred to as scaling. Three key factors have been identified that influence the shape of the Dpp gradient and are regulated by Dpp signaling, thus providing potential mechanisms for scaling: Dpp receptors, glypicans, and a secreted, feedback regulator of Dpp signaling, Pentagone (Pent). Pent, which is repressed by Dpp signaling, interacts with and promotes endocytosis of glypicans to broaden Dpp distribution in the wing disc, and has been suggested to play an essential role in gradient scaling (Vuilleumier et al. 2010; Ben-Zvi et al. 2011; Hamaratoglu et al. 2011; Norman et al. 2016). However, a more recent study reported that Pent could not explain scaling throughout the entire wing disc and proposed instead that feedback regulation of Dpp receptors and glypicans is also required to account for gradient scaling (Zhu et al. 2020).

**The Wg signaling gradient**

Although Wg is initially broadly expressed in the ventral region of the second instar wing disc, by early third instar this broad expression disappears and Wg transcription in the distal wing becomes concentrated along the cells straddling the D–V compartment boundary, where Notch is active (Cousso et al. 1993; Williams et al. 1993; Díaz-Benjumea and Cohen 1995; Rulifson and Blair 1995) (Fig. 5b). Expression of downstream targets of Wg signaling like Vg and Dll can be detected up to 15–20 cells away from the D–V boundary in late third instar wing discs. A Wg protein gradient declining away from the boundary can be directly visualized by antibody staining (Neumann and Cohen 1997; Strigini and Cohen 2000), and higher levels of Wg expression are needed to induce expression of genes or reporter constructs that are normally expressed closer to the D–V boundary, consistent with a concentration-dependent response to Wg (Zecca et al. 1996; Neumann and Cohen 1997). Direct, long-range effects of Wg were demonstrated by comparing the nonautonomous effects of Wg expression to the cell autonomous effects of transgenes that activate or block the Wg signaling pathway (Zecca et al. 1996; Neumann and Cohen 1997). In addition, although Wg is normally a secreted protein, an active, membrane-tethered form (Nrt-Wg) could be created, which rather than acting at long range only activates Wg signaling in neighboring cells (Zecca et al. 1996). While these observations implied that Wg acts as a morphogen in the wing disc, subsequent studies called this into question. Most strikingly, it was found that replacing endogenous Wg with the membrane tethered Nrt-Wg could still support development of nearly normal (though smaller) wings, apparently arguing against the importance of long-range diffusion or formation of spatial gradients for wing development (Alexandre et al. 2014). However, more recently studies have revealed that Nrt-Wg can actually be detected several cells away from its site of synthesis on the D–V boundary (Chaudhary et al. 2019). In addition, a feedback loop with the Wg receptor Frizzled 2 (Fz2), which is downregulated by Wg signaling, contributes to long-range signaling even in the absence of detectable Wg (Chaudhary et al. 2019), and the authors’ experiments implied that Wg could directly signal up to 11 cells away from the D–V boundary, but longer range effects were dependent upon Fz2. However, it is not yet clear if the effects of Fz2 on cells apparently outside the range of Wg secreted from D–V boundary cells reflect true ligand-independent signaling, a persistence of response to earlier exposure to Wg, or a heightened response to undetectably low levels of Wg.

Investigations into how Wg spreads through the wing disc have paralleled investigations of how Dpp spreads, including suggestions of spread by transcytosis, cytonemes, free diffusion, or restricted diffusion. An added complication for Wg is that due to a lipid modification that is essential for its activity (Willert et al. 2003), it is not very soluble as a free protein, and associates with lipid binding proteins (Panáková et al. 2005). As for Dpp, levels of both signaling receptors and glypicans modify the Wg gradient (Baeg et al. 2001; Franch-Marro et al. 2005; Schilling et al. 2014). However, the 2 Drosophila glypicans have distinct roles in Wg signaling, with Dally contributing to active signaling as a coreceptor and Dlp required for spread of Wg through the wing disc (Lin and...
Proximal-distal wing patterning

In addition to A–P and D–V patterning, appendages like the wing also have a proximal–distal (P–D) axis, with distinct cell fates specified at different distances from the body. Wg and Dpp act combinatorially in the wing disc to regulate P–D patterning of the developing wing by promoting expression of distally expressed genes and repressing expression of proximally expressed genes (Williams et al. 1993; Ng et al. 1995; Kim et al. 1996; Klein and Arias 1998a; Azpiazu and Morata 2000; Wu and Cohen 2002; Weihe et al. 2004; Zirin and Mann 2004). Indeed, the intersection of the A–P and D–V compartment boundaries defines the center of the developing wing pouch, and ultimately, the distal tip of the adult wing (Fig. 6a). The main subdivision of the wing field is between the distal wing and proximal wing regions. The distal wing forms the wing pouch in the larval disc and the wing blade in the adult. The proximal wing and wing hinge form structures at the base of the wing. The terms proximal wing and wing hinge are often used interchangeably, although formally they are distinct, with the proximal wing cells in between the distal wing and the wing hinge (Bryant 1975a; Díez del Corral et al. 1999). The distal wing is characterized by expression of Vg and Scalloped (Sd) (Campbell et al. 1992; Williams et al. 1993), whereas the proximal wing is characterized by expression of Teashirt (Tsh), Hth, and Zn finger homeodomain 2 (Zfh2) (Azpiazu and Morata 2000; Casares and Mann 2000; Wu and Cohen 2002; Whitworth and Russell 2003). The expression patterns of these genes are partially overlapping, and additional genes including round (rn), nab, nab, elbow B (elb), no ocelli (noc), and defective proventriculus (dve) have been identified that are expressed in distinct proximal–distal domains, establishing different subregions of the developing wing (Ng et al. 1995; St Pierre et al. 2002; Weihe et al. 2004; Terriente Félix et al. 2007) (Fig. 6a). Proximal–distal patterning of the wing also depends upon mutually repressive interactions between distally and proximally expressed genes (Azpiazu and Morata 2000; Casares and Mann 2000; Wu and Cohen 2002; Whitworth and Russell 2003; Weihe et al. 2004).

Vg plays a key role in linking signaling from compartment boundaries to wing development. Vg is a transcriptional coactivator that partners with the DNA-binding protein Sd to specify future wing blade cells (Williams et al. 1991; Campbell et al. 1992; Williams et al. 1993; Halder et al. 1998; Paumard-Rigal et al. 1998; Simmonds et al. 1998). Vg and Sd are required for survival of wing pouch cells, and misexpression of Vg can transform cells in other imaginal discs toward wing blade fate (Kim et al. 1996; Liu et al. 2000). Regulation of Vg expression involves input from compartment boundary signals, including Dpp, Wg, and Notch (Couso et al. 1995; Kim et al. 1995, 1996; Neumann and Cohen 1996b; Kim et al. 1997; Klein and Arias 1998a), which act through 2 distinct enhancers: a boundary enhancer that responds to Notch activation, and a quadrant enhancer that requires Dpp and Wg signaling (Kim et al. 1996, 1997) (Fig. 6b). Activation of Vg from either enhancer also requires auto-regulation from Vg-Sd (Campbell et al. 1992; Williams et al. 1993; Halder et al. 1998; Paumard-Rigal et al. 1998; Simmonds et al. 1998; Klein and Arias 1998a; Zecca and Struhl 2007a), although at the quadrant enhancer Yorker (Yki) can substitute for Vg (Zecca and Struhl 2010).

During early wing disc development, proximal wing fate is promoted by Wg (Klein and Arias 1998a; Casares and Mann 2000; Whitworth and Russell 2003). Cells that receive Wg rather than Vg, and fail to receive Notch activation, form proximal wing. While early specification of proximal wing fate depends upon the broad ventral expression of Wg, after this fades Wg becomes expressed in 2 concentric circles in the proximal wing: an inner ring induced during early third instar, and an outer ring induced during mid-third instar (Couso et al. 1993; Williams et al. 1993) (Fig. 6a). The inner ring is established by signaling from Vg-expressing distal wing cells (Ng et al. 1995; Liu et al. 2000; del Alamo Rodríguez et al. 2002) (Fig. 6b). This signaling is mediated by the Ds-Fat pathway and is regulated by the differential expression of Four-jointed (Fj) and Dachsous (Ds), which are activated and repressed, respectively, downstream of Vg (Cho and Irvine 2004; Zecca and Struhl 2010). These rings of Wg expression in the proximal wing are maintained through a positive regulatory loop with Hth (Azpiazu and Morata 2000; Casares and Mann 2000; del Alamo Rodríguez et al. 2002), and contribute to proximal wing and hinge patterning and growth. Hinge patterning also involves local activation of the Jak-Stat pathway, mediated by localized expression of the Jak-Stat pathway Unpaired (Upd) ligands (Ayala-Carnago et al. 2013; Johnstone et al. 2013). Dorsal hinge cells also express Drop (Dr, also known as Msh), which contributes to repression of Iro-C complex genes, thereby maintaining separation of wing hinge from notum (Villa-Cuesta and ModeLL 2005).

Although different proximal–distal domains are not separated by strict lineage restrictions, cells in different regions tend not to intermix. This is evident when the expression of transcription factors that define distinct regions is altered. Thus, for example, clones of cells that are mutant for Iro-C genes within the notum tend to sort out from neighboring, Iro-C expressing cells (Villa-Cuesta et al. 2007), and clones of cells forced to express Vg in the proximal wing will sort out from neighboring cells that lack, or express only low levels of, Vg (Liu et al. 2000).

Patterning of the late third instar wing disc

The patterning of the wing disc established by Wg, Dpp and other signaling molecules is ultimately manifest in the placement of distinct structures at precise locations in the adult wing and notum. The cellular-level resolution needed to achieve this begins to appear around the end of larval development.

Wing margin

By late third instar, gene expression patterns characteristic of distinct cell types that will form along the edge of the wing, the wing margin, have been established. The margin is maintained by continued Notch activation along the D–V boundary, but at this stage Notch is activated by a feedback loop between D–V boundary cells, which express Wg in response to Notch activation, and flanking cells, which express Notch ligands in response to Wg signaling (de Celis and Bray 1997; Micchelli et al. 1997). Wing margin hairs and bristles are formed by these flanking cells. Anterior wing margin cells form mechanosensory and chemosensory bristles, while posterior wing margin
cells form long, noninnervated, hairs (Fig. 5c). Proneural genes like achaete (ac) and senseless (sen) are upregulated by Wg signaling in cells adjacent to the Wg stripe on the D–V boundary (Fig. 5b) (Phillips and Whittle 1993; Couso et al. 1994; Rulifson and Blair 1995; Jafar-Nejad et al. 2006). These stripes of proneural gene expression then resolve into clusters of cells that give rise to the sensory organ precursor cells that will later form wing margin bristles.

Wing veins

The wing blade is formed from 2 main cell types—vein and intervein (Fig. 5c). The wing veins provide rigidity to the adult wing,
and tubes for tracheae, nerves and hemolymph (Blair 2007). Vein cells are more densely packed than intervein cells, and secrete thicker, more darkly pigmented cuticle. The Drosophila wing has 5 main longitudinal veins (L1–L5), which run along the length of the wing, and 2 main cross-veins (ACV and PCV), which run perpendicular to the longitudinal veins and connect L3 to L4 (ACV) and L4 to L5 (PCV). The longitudinal veins are specified in the late third instar wing disc, while the cross-veins are specified in the pupal wing disc. Vein formation is promoted by EGFR signaling, and expression of Rhomboid, which promotes activation of the EGFR ligand Spitz, is one of the earliest markers of longitudinal veins in the wing disc (Sturtevant et al. 1993). Intervein cells are defined by expression of Blistered (Bs), which promotes intervein fate and suppresses vein fate (Fristrom et al. 1994; Montagne et al. 1996).

The position of most longitudinal veins is specified downstream of the anterior–posterior patterning established by Hh and Dpp signaling. Distinct networks of genes regulate the positioning of each longitudinal vein, but they follow a common logic wherein veins are specified along the borders of genes expressed in different A–P domains. The most central veins, L3 and L4, which form straddling the A–P boundary, are positioned primarily by Hh signaling, which acts through regulation of Collier (Col). Cells receiving high levels of Hh signaling express Col and become intervein cells, whereas cells bordering Col expression form L3 and L4. (Mullor et al. 1997; Strigini and Cohen 1997; Biels et al. 1998; Vervoort et al. 1999; Mohler et al. 2000). The more peripheral veins, L2 and L5, are positioned by Dpp signaling, which acts through regulation of several genes expressed in broad domains, including the Aristotle, Brk, Omph, Optix, Salm, and Salr transcription factors, which then define along their borders stripes of gene expression that will become L2 and L5 (Gómez-Skarmeta and Modolell 1996; Sturtevant et al. 1997; Lunde et al. 1998; de Celis and Barrio 2000; Cook et al. 2004; Sugimori et al. 2016; Martin et al. 2017a). Each of the proven stripes express distinct genes, including knirps and knirps-related in L2, abrupt in L5, and iro-C genes in L3 and L5. The L1 vein forms along the anterior wing margin.

The veins are restricted to narrow stripes of cells by a negative feedback loop with Notch signaling, which promotes intervein fate and inhibits vein fate (de Celis and Garcia-Bellido 1994; Sturtevant and Bier 1995; de Celis and Garcia-Bellido 1994; in L3 and L5. The L1 vein forms along the anterior wing margin. EGFR signaling upregulates expression of Notch ligands in vein cells, which then signal to neighboring cells to repress vein fate. During pupal development, Dpp becomes upregulated along vein territories EGFR, Notch, and Dpp signaling positions the wing veins. The expression patterns of both proneural genes of the achaete-scute complex (Haenlin et al. 1997; García-García et al. 1999; Calleja et al. 2000), together with genes that antagonize achaete-scute complex gene activity (Usui et al. 2008). The number and positioning of macrochaete is sufficiently precise that each has a unique name (Figs. 1 and 5d) (Stern 1955). Small mechanosensory bristles (microchaetae), which form in rows along the notum (Fig. 5d) arise from stripes of proneural gene expression that are patterned by Notch signaling during pupal development (Corson et al. 2017; Couturier et al. 2019). For both macro- and microchaete, a single sensory precursor (SOP) cell is selected from a cluster of cells expressing proneural genes; the SOP then undergoes stereotyped divisions to form the mechanosensory organ. Patterning of the notum downstream of Wg and Dpp also determines sites of attachment for flight muscles by regulating the expression pattern of the stripe gene (de Celis et al. 1999; Ghazi et al. 2003).

**Planar cell polarity of wing disc cells**

Wing disc epithelial cells, like many other tissues, exhibit planar cell polarity (PCP). PCP is the polarization of cells within the plane of the tissue, perpendicular to apical–basal polarity, and is readily visible in derivatives of the wing disc through the orientation of hairs and bristles in the adult wing and notum (Fig. 7) (Mlodzik 2020). Indeed, the Drosophila wing has long been one of the primary models for discovery of PCP components and analysis of their functions (Gubb and Garcia-Bellido 1982). Each epithelial cell makes a single hair, the location and orientation of which is controlled by PCP pathways (Wong and Adler 1993). Insect hairs are nonsensory actin-rich cellular extensions; it has been proposed that the wing hairs serve an aerodynamic role by guiding air flow over the surface of the wing (Wootton 1992). Insect bristles are multicellular mechanosensory and chemosensory organs; their orientation is also controlled by PCP pathways.

Two main PCP pathways have been described, the canonical, or Fz-dependent, PCP pathway and the De-Fat PCP pathway (Strutt and Strutt 2021) (Fig. 7, a and b). Components of each pathway localize near apical junctions, and their localization becomes polarized in conjunction with establishment of PCP. In the wing region of the wing disc, polarization occurs along the proximal–distal axis, and some components of each pathway localize to the proximal sides of cells, whereas other components localize to the distal sides. Proximally localized transmembrane proteins in 1 cell physically interact with distally localized proteins in the neighboring cells, which helps to establish, maintain, and propagate PCP.

Overall orientation of polarity in the De-Fat system is governed by expression gradients of 2 of the components: Ds, which is a large cadherin family protein that binds to Fat, and Fj, which is a Golgi-localized kinase that modulates binding between Fat
and Ds by phosphorylating their extracellular domains (Matakatsu and Blair 2004; Ishikawa et al. 2008; Brittle et al. 2010; Simon et al. 2010). The Ds gradient, from proximal to distal in the wing, and the Fj gradient, from distal to proximal, combined with binding between Fat and Ds, result in cellular polarization of Ds and Fat with Ds accumulating distally and Fat accumulating proximally (Clark et al. 1995; Villano and Katz 1995; Ma et al. 2003; Cho and Irvine 2004; Matakatsu and Blair 2004; Strutt et al. 2004; Ambegaonkar et al. 2012; Brittle et al. 2012). Polarization of Ds and Fat leads to polarization of the Dach protein, which is removed from apical membranes by Fat (Mao et al. 2006). The Ds and Fj gradients in the wing are established downstream of the Wg and Dpp gradients, at least in part through Vg. Ds and Fj expression is graded across the distal wing at early third instar, but flattens by the end of third instar, except near the edge of the wing pouch (Cho and Irvine 2004). Nonetheless, polarization is maintained, likely due to an ability to maintain polarization through cell division, combined with the propagation of polarization from expression boundaries at the edge of the wing pouch (Ambegaonkar et al. 2012; Wortman et al. 2017).

The mechanisms that direct overall orientation of polarity in the Fz system remain unclear (Sagner et al. 2012). Since 2 of the key components, Fz and Dsh, also participate in Wnt-β-catenin signaling, it had been thought that Wg or other Wnt proteins, which are expressed at the D–V boundary of the wing, might play a role in orienting Fz-PCP. Evidence for this was reported (Wu et al. 2013), but more recent studies have ruled out the possibility of Wnts contributing to orientation of Fz-PCP in the Drosophila wing disc (Ewen-Campen et al. 2020; Yu et al. 2020).

Fig. 7. PCP in the wing disc. a) Schematics illustrating polarization of proteins of the Ds-Fat PCP pathway in the wing disc. Left panel shows schematic cross-section through cells, right panel shows 3D perspective. At pupal stages, wing hairs (brown triangles), pointing distally, will form near the distal vertices of each cell. b) Schematics illustrating polarization of proteins of the Fz PCP pathway in the wing disc. Left panel shows schematic cross-section through cells, right panel shows 3D perspective. The Stan (also known as Flamingo) protein accumulates on both distal and proximal cell membranes. c) Example of PCP in the adult wing, illustrated by distally pointing hairs in wild-type, and misoriented hairs in a prickle mutant. Portions of this figure reproduced from Ambegaonkar and Irvine (2015).
The 2 PCP pathways can act independently, but also cross-talk with each other (Adler et al. 1998; Strutt and Strutt 2002; Ma et al. 2003; Merkel et al. 2014; Olofsson et al. 2014). They are linked in some contexts by the expression of a particular isoform of the prickle-spiny legs (pk-sple) locus, which is one of the key components of the Fz-PCP pathway (Gubb et al. 1999). The Sple isoform can bind to Dachs and Ds, and when this form predominates the Ds-Fat pathway can direct the orientation of the Fz pathway (Ayukawa et al. 2014; Merkel et al. 2014; Ambegaonkar and Irvine 2015). Recognition of the genetic interaction between these pathways had led to the suggestion that Ds-Fat could be responsible for orienting Fz-PCP (Adler et al. 1998; Strutt and Strutt 2002; Ma et al. 2003; Matakatsu and Blair 2004), however, subsequent studies have revealed that this normally only occurs under conditions where Sple is the predominant isoform (Ayukawa et al. 2014; Merkel et al. 2014; Ambegaonkar and Irvine 2015). In the wing, for example, Pk is the predominant isoform (Olofsson et al. 2014), so the Fz pathway must be oriented by other, as yet unidentified, cues.

Although hair polarity is typically visualized in the adult, and hairs first form during pupal development, imaging, and conditional knock down experiments have revealed that PCP is actually established in the wing disc by the middle of the third instar, as revealed by the polarization of PCP proteins (Sagner et al. 2011). In the wing, there is a remarkable reorientation of polarity that occurs during pupal development. In the larval wing disc, PCP is largely oriented toward the D–V boundary, but in the adult wing it is largely oriented toward the distal tip of the wing. This reorientation is associated with oriented cell division, and cell rearrangement that occur during puation as a result of contraction of the wing hinge, which generates an anisotropic tension that realigns PCP along the proximal-distal axis (Aigouy et al. 2010). PCP also orients ridges that form between cells in the adult wing (Doyle et al. 2008; Hogan et al. 2011). In the posterior wing, these ridges to not realign during puation, and consequently there is a discordance between the orientation of PCP for wing hairs and wing ridges. In the notum, PCP is oriented along the anterior–posterior axis of the fly, and there are again roles for both Fz and Ds-Fat PCP pathways in orienting hairs and bristles (Gho and Schweisguth 1998; Lu et al. 1999; Adler 2012).

**Growth of the wing disc**

The wing disc undergoes extensive growth during larval development (Fig. 8), and studies in wing discs have yielded fundamental insights into diverse factors that control cell proliferation and organ size, including components of key growth-regulating pathways, and the roles of tissue patterning, metabolism, and mechanics on regulation of organ growth.

**Parameters of wing disc growth**

The wing disc normally grows through increases in cell number, rather than increases in cell size, and imaginal disc cells remain diploid. Notably, however, the size of the wing disc can be uncoupled from cell number through experimental manipulations of cell cycle regulators, which yield a relatively normally size wing disc or compartment even with altered cell sizes (Weigmann et al. 1997; Neufeld et al. 1998). There is relatively little apoptosis during wild-type wing disc development (Milán et al. 1997), so size regulation is achieved primarily through controlling cell proliferation rather than cell death.

Direct counts of labeled nuclei in the DP led to an estimate of 30,350±1,400 cells at end of third instar (Martín et al. 2009), although this did not include the peripodial cells. McClure and Schubiger (2005) estimated that at late third instar the DP has 39,200±1,170 cells, and the PE had 2,099±236 cells. Some cell division continues during pupal development (Milán et al. 1996a, 1996b), and by counting hairs in the adult derivatives of the wing and notum, Garcia-Bellido and Merriam (1971) estimated that the adult derivatives of the wing disc comprise ~52,000 cells, although this is a rough estimate as wing hinge cells were not counted. Considering that the wing disc starts from a population of ~25–30 cells in the embryo, we can infer that it undergoes a more than 1,000-times increase in cell number during larval development, which corresponds to roughly 10 cell divisions.

Both temporal and spatial variations in growth rates occur over these 10 cell divisions. Growth rates gradually decline as the
wing disc ages, with a doubling time of ~6 h during second instar increasing to ~30 h by the end of third instar (García-Bellido and Merriam 1971; Bryant and Levinson 1985; Bittig et al. 2009; Martín et al. 2009). Small scale local variations in growth of clones or DNA replication can be detected, although for most of wing development growth rates are similar across different regions of the wing disc (González-Gaitán et al. 1994; Milán et al. 1996a, 1996b). However, there are exceptions to this. At late third instar, the ZNC occurs near the dorsal–ventral compartment border (O’Brochta and Bryant 1985), established downstream of Notch and Wg signaling (Johnston and Edgar 1998; Duman-Scheel et al. 2004; Herranz et al. 2008). Careful analysis of growth patterns at different stages of development led to realization that cells in the center of developing wing transiently grow at a faster rate than more proximal cells during early phases of wing disc growth (Mao et al. 2015). Quantitation of EdU labeling or clones sizes at different time points throughout wing disc development further revealed that this reverses in older wing discs, with proliferation rates slightly lower in the more distal parts of the wing disc when compared with more proximal regions (Johnston and Sanders 2003; Pan et al. 2018). Growth is also relatively lower in the hinge during early stages of larval development (Tozluoğlu et al. 2019). At the pupal stage, additional heterogeneities in cell proliferation appear, including between vein and intervein cells (Milán et al. 1996a, 1996b).

Transplantation experiments in which wing discs were dissected out of third instar larvae of different ages and then cultured in female abdomens for several days implied that wing discs grow to a preferred size regardless of the amount of time allowed for growth (García-Bellido 1965; Bryant and Levinson 1985). Similar conclusions have been reached without transplantation by genetically delaying pupariation (Parker and Struhl 2020; Strassburger et al. 2021). Thus, while the final size of an organ is a function of both the rate and duration of growth, there is a size control mechanism in wing discs that arrests growth when an appropriate size has been reached. Clonal analysis experiments have further implicated compartments as units of size control. Using Minutes, it is possible to create wing discs in which cells in the anterior or posterior compartment grow at a much different rate than cells in the complementary compartment. Remarkably, normal wings form, as the faster-growing compartment essentially stalls to let the slower growing compartment catch up near the end of larval development (García-Bellido et al. 1976; Martín and Morata 2006).

Hippo signaling in wing discs

The Hippo signaling network contributes to the regulation of growth and cell fate throughout the metazoa (Misra and Irvine 2018; Zheng and Pan 2019). Many of the studies that first identified Hippo pathway components and deciphered their roles in the pathway were performed in wing discs, where mutations of pathway components can have dramatic effects on growth. Hippo signaling regulates the activity of the Yki transcriptional coactivator protein, which partners with Sd to activate target genes. Increased Yki activity in the wing disc stimulates growth, whereas loss of yki suppresses growth (Huang et al. 2005). Yki is inhibited by the upstream kinase Warts (Wts), which phosphorylates Yki to promote its cytoplasmic localization (Dong et al. 2007; Oh and Irvine 2008). Wts is activated by Hippo and related kinases, and Wts and Hippo are regulated by a wide variety of upstream cues. Indeed, a defining feature of the Hippo network is its sensitivity to diverse inputs, which enable it to integrate information about the physical environment, metabolism, and local patterning to modulate organ growth.

One key regulator of Hippo signaling in the wing disc is the Ds-Fat pathway. Regulation of the levels and localization of Dachs by gradients of Fj and Ds not only polarizes cells, it also, together with Dachs ligand with SH3s (Dlish, also known as Yamana), downregulates Wts and an additional upstream regulator of Yki, Expanded (Bennett and Harvey 2006; Cho et al. 2006; Silva et al. 2006; Willecke et al. 2006; Vrabioiu and Struhl 2015; Misra and Irvine 2016; Zhang et al. 2016). Consequently, manipulations of the Fj or Ds expression patterns can locally alter cell proliferation in the wing disc and influence wing size (Rogulja et al. 2008; Willecke et al. 2008). Complete loss of Dachs reduces the wing to less than half its normal size (Mao et al. 2006), whereas loss of Ds or Fat throughout the wing disc can increase its size (Bryant et al. 1988; Mahoney et al. 1991; Clark et al. 1995; Matakatsu and Blair 2006).

The Hippo pathway is also regulated in the wing disc by cytoskeletal tension. Even before a link to the Hippo pathway was made, theoretical considerations suggested that the crowding of cells in the center of the developing wing might inhibit growth, and act as a counter to growth-stimulating effects of wing disc morphogens (Aegerter-Wilmsen et al. 2007; Hufnagel et al. 2007). Artificial stretching of wing discs can stimulate cell proliferation (Schluck et al. 2013). Subsequent studies established that tension at AJ inhibited Wts by recruiting the Wts inhibitor Jub (Rauskolb et al. 2014; Sun et al. 2015). This recruitment is mediated through α-catenin, which can undergo a tension-dependent change in conformation (Yonemura et al. 2010; Rauskolb et al. 2014; Alégot et al. 2019; Sarpal et al. 2019). Experimental manipulations have also demonstrated suppression of Yki activity by growth-induced crowding (Pan et al. 2016), and correlated reductions in cytoskeletal tension that normally occur as cells become more crowded with decreasing Yki activity (Pan et al. 2018; Borreguero-Muñoz et al. 2019). In addition to changes in tension at AJ, it has also been proposed that cell shape could influence Hippo signaling by concentrating or diluting upstream regulators associated with cell–cell junctions, and consistent with this idea high levels of nuclear Yki have been observed in wing disc PE cells (Borreguero-Muñoz et al. 2019). Spectrins are also required for normal Yki activity in the wing disc (Deng et al. 2015; Fletcher et al. 2015), raising the possibility of additional modes of cytoskeletal regulation.

The Hippo pathway can also stimulate growth in wing discs in response to tissue damage or loss of cell polarity (Parsons et al. 2010; Grusche et al. 2011; Sun and Irvine 2011). These effects are mediated at least in part through activation of the Jnk pathway, which has also been proposed to contribute to normal wing disc growth through cross-regulation of Hippo signaling (Willsey et al. 2010; Grusche et al. 2011; Zheng and Pan 2021). Loss of cell polarity or cell–cell contact may also modulate Hippo signaling through transmembrane proteins that participate in homophilic binding and acts as upstream regulators of the Hippo pathway, including Echinoid and Crumbs (Chen et al. 2010; Grzeschik et al. 2010; Ling et al. 2010; Robinson et al. 2010; Yue et al. 2012).

Metabolic pathways also cross-talk with Hippo signaling in multiple ways (Ibar and Irvine 2020). In wing discs, Insulin signaling can regulate the Hippo pathway (Sträßer et al. 2012), Akt, which is a key downstream factor in Insulin and mTor pathways, can influence Yki activity through a mechanism that appears to involve a novel phosphorylation of Hippo (Borreguero-Muñoz et al. 2019), and mTOR has been reported to regulate Yki activity independently of Hippo signaling (Parker and Struhl 2015).
Influence of A–P and D–V patterning on growth of the wing disc

A link between the patterning of the wing disc and its growth was first suggested by regeneration experiments (Bryant 1975b; Haynie and Bryant 1976). If part of a wing disc is excised, and the remaining part is cultured in a female abdomen, the disc fragment can grow until the missing part is regenerated (or, in the case of smaller fragments, duplicated) (Fig. 9a). If 2 disc fragments are fused, growth will “fill in” the missing tissue. Together with similar studies of intercalary regeneration in other models, this suggested that developing appendages possess positional information that controls their growth (French et al. 1976; Bryant et al. 1981).

The link between patterning and growth was solidified by the discovery that genes that play key roles in the patterning of the wing disc also control its growth. When cells with different compartmental identities are juxtaposed, for example by misexpression of En in anterior cells or misexpression of Ap in ventral cells, growth is stimulated, and when these manipulations create a new intersection of A–P and D–V compartment boundary cells, this growth can be organized into partial wing duplications (Diaz-Benjumea and Cohen 1993; Zecca et al. 1995). Similar effects can be generated by misexpressing genes that play key roles in signaling between compartments, including Hh and Fng (Capdevila and Guerrero 1994; Irvine and Wieschaus 1994; Tabata and Kornberg 1994; Zecca et al. 1995). Genes that participate in intercompartmental signaling are also required for the normal growth of the wing, which reflects the essential roles that induction of Dpp, Wg and Vg along compartment boundaries plays in wing formation.

Fig. 9. Wing disc regeneration. a) When a portion of a wing disc is excised, the missing part can be regenerated through a process including healing of the epithelium, stimulation of cell proliferation near the cut edges, and tissue repatterning. Smaller fragments typically generate duplicated structures rather than regenerating. b) Wing disc regeneration can also occur after genetically induced ablation of cells in a defined region of the disc. c) The damage response is coordinated by Jnk, which is activated by ROS and triggers multiple responses that enable regeneration.
Growth control in the wing disc by Dpp

In addition to its central role in patterning the wing disc along the A–P axis, the morphogen Dpp is also required for normal wing disc growth and is able to induce overgrowth when misexpressed or over-expressed (Spencer et al. 1982; Capdevila and Guerrero 1994; Zecca et al. 1995). Experiments removing functional Dpp receptors, or deleting the expression or spread of Dpp from the A–P boundary, emphasize that Dpp signals directly to cells at a distance from the A–P boundary to promote growth (Burke and Basler 1996; Barrio and Milán 2017; Bosch et al. 2017; Matsuda and Affolter 2017). These observations raise a question that has been debated for over 30 years: how is the gradient of Dpp around the wing pouch formed, and how does it regulate growth, whereas in medial regions, where Dpp levels are normally very low, increasing Dpp pathway activity stimulates growth (Capdevila and Guerrero 1994; Zecca et al. 1995; Nellen et al. 1996). However, analysis of growth patterns reveals that cells in different regions respond differently to uniform increases in Dpp—in lateral regions, where Dpp levels are normally very low, increasing Dpp pathway activity stimulates growth, whereas in medial regions, where Dpp levels are normally high, uniformly increasing Dpp does not increase growth (Martín-Castellanos and Edgar 2002).

This leads to a second class of models, which suggest that the growth-promoting effects of Dpp are balanced by a growth inhibitor (Serrano and O’Farrell 1997). There is evidence for such inhibitors, although how each contributes remains to be clarified. One key factor is Brk. As for its effects on wing patterning, much of the influence of Dpp on wing growth is mediated through repression of Brk, which itself acts as a repressor of growth (Martin et al. 2004; Schwank et al. 2008). However, Brk cannot easily explain why the growth response to Dpp differs between medial and lateral cells, because Brk itself is a target of Dpp, and indeed it has been inferred based on examination of hypomorphic Dpp mutants that the distinct growth responses of medial and lateral cells are not established by Dpp (Schwank et al. 2008). Alternatives that have been suggested include differences in Fat activity or Vg expression (Martín-Castellanos and Edgar 2002; Schwank et al. 2011b). Differences in cytoskeletal tension are also a factor, as by mid-third instar cells in the center of the wing have lower levels of cytoskeletal tension at adherens junctions than more proximal cells (Aegerter-Wilmsen et al. 2012; Pan et al. 2018), and increasing cytoskeletal tension preferentially increases cell proliferation in the medial part of the disc where Dpp signaling is highest (Pan et al. 2016).

An alternative explanation that has been proposed is that cells need to experience continually increasing amounts of Dpp in order to continue growing. This was suggested by observations that cells across different positions of the Dpp gradient and at different stages of wing disc development experience an ~50% increase in levels of Dpp signaling per cell division (Wartlick et al. 2011). However, the significance of this has been questioned based on observations that cells can continue to grow in the absence of Dpp signaling if brk is mutant (Schwank et al. 2012), and that Dpp can be provided by heterologous, uniform expression from the tubulin promoter and wings still grow (Bosch et al. 2017).

Models for intercalary regeneration led to the suggestion that it could be the gradient, rather than the absolute level, of Dpp activity that promotes wing growth (Day and Lawrence 2000). This was supported by observations that juxtaposing cells with different levels of Dpp pathway activity transiently stimulates cell proliferation in the medial wing, whereas high uniform levels of activity inhibit growth in the medial wing (Rogulja and Irvine 2005). It has received further support from connections between Dpp signaling and the Ds-Fat pathway, which provides a mechanism for the Dpp expression gradient to regulate growth (Rogulja et al. 2008). However, the suppression of medial growth observed with high level uniform activation of Dpp appears to be an indirect consequence of high level proliferation in lateral cells (Schwank et al. 2008). Additionally, when the normal source of Dpp along the A–P boundary is eliminated and replaced with a moderate level of uniform Dpp expression, the wing disc can still grow (Bosch et al. 2017).

In weighing the evidence for or against various models, we emphasize that there appear to be multiple mechanisms through which Dpp can promote wing growth, and experimental support for 1 model does not necessarily exclude another. Thus, it seems clear that some of the contribution of Dpp to wing growth can be explained by a simple threshold model, but other mechanisms can also contribute, although how much remains subject to debate. In threshold models, the distance over which Dpp can spread and productively signal is a key factor in determining the size of the wing disc (Barrio and Milán 2020; Parker and Struhl 2020; Zecca and Struhl 2021), although one also has to consider differences in responsiveness to Dpp that exist between medial and lateral regions.

Growth control in the wing disc by Wg

Wg expressed by D–V boundary cells is required for growth of the distal wing (Couso et al. 1994; Neumann and Cohen 1997). However, expression of Wg cannot substitute for loss of Notch activation at the D–V boundary, because Wg is also an essential notch target there for promotion of wing growth (Kim et al. 1996; Klein et al. 1998; Klein and Arias 1998a). Wg signaling is normally graded, but low level uniform Wg expression can support wing growth (Baena-Lopez et al. 2009). Ectopic expression of Wg within the wing pouch has relatively little ability to promote growth, and high level Wg activity can actually inhibit growth in the distal wing (Neumann and Cohen 1996a; Klein and Arias 1998a; Johnston and Sanders 2003; Baena-Lopez et al. 2009). In contrast, elevated expression of Wg has strong mitogenic effects in the proximal wing, where the rings of Wg expression are also required for normal growth (Neumann and Cohen 1996a). Some reports have suggested that elevated Wg expression can increase growth in the proximal part of the wing pouch (Giraldez and Cohen 2003; Barrio and Milán 2020), but to the extent this has been reported it might be a consequence of the increased cell proliferation induced by Wg in the proximal wing, together with a shifting boundary between distal and proximal wing (Zecca and Struhl 2007b). Thus, most studies suggest that Wg has a potent ability to promote growth in the proximal wing, but in the distal wing its influence is permissive rather than instructive.

Control of wing growth by Vg

Vg, which together with its partner Sd is required for survival and growth of wing pouch cells (Kim et al. 1996; Liu et al. 2000), has 2
conceptually important roles in controlling wing growth. First, as a key target of Notch, Wg and Dpp. Vg integrates signaling from both A–P and D–V compartment boundaries. Second, Vg participates in a dynamic process that shifts cells from the proximal wing into the distal wing. These roles of Vg depend upon its regulation by its distinct boundary and quadrant enhancers, which maintain Vg expression after an initial transient phase of broad expression early in wing disc development.

Activation of Vg expression by Notch through the boundary enhancer generates a population of Vg-expressing cells in which Wg and Dpp can then activate the Vg quadrant enhancer, thereby maintaining Vg expression even in cells that are pushed away from the D–V boundary by cell proliferation (Kim et al. 1996). The maintenance of Vg expression is 1 mechanism by which threshold levels of Wg and Dpp contribute to wing growth, but Wg and Dpp are also required for wing disc growth independently of their role in promoting Vg (Zecca and Struhl 2007b; Barrio and Millán 2020; Parker and Struhl 2020, Zecca and Struhl 2021). Vg may also contribute to growth regulation by the Ds-Fat pathway, as it regulates Fj and Ds expression (Cho and Irvine 2004; Zecca and Struhl 2010). This can influence growth within the wing pouch, and also indirectly growth within the proximal wing, through induction of the inner ring of Wg expression (Cho and Irvine 2004; Zecca and Struhl 2010).

Analysis of Vg regulation led to the discovery that it could contribute to growth of the wing blade not only through autonomous effects on Vg-expressing cells, but also through a “feed-forward” mechanism that recruits neighboring, proximal cells into the future wing blade (Fig. 6b) (Zecca and Struhl 2007b, 2010, 2021). Three factors contribute to this recruitment (1) Vg expression is activated through its quadrant enhancer in the presence of Vg, Wg, and Dpp (Kim et al. 1996); (2) boundaries of Fj and Ds expression induce elevated Yki activity (Rogulja et al. 2008; Willecke et al. 2008); (3) Yki and Vg both partner with the same DNA binding protein, Sd, and while they have distinct activities, Yki can substitute for Vg in the activation of Vg expression through the quadrant enhancer (Zecca and Struhl 2010). This can result in a spread of Vg expression to neighboring cells. Because Vg regulates Fj and Ds expression (Cho and Irvine 2004; Zecca and Struhl 2010), this process can occur iteratively, expanding the developing wing blade. This mechanism has the capacity to spread over many cells (reflecting the range of Wg and Dpp signaling) under artificial conditions (Zecca and Struhl 2007b, 2010, 2021), although it remains unclear what fraction of normal wing size is dictated by this recruitment process, because Vg and Ds-Fat signaling are also required for normal growth within the wing pouch. We also note that on its own this process would not increase growth of the wing disc, rather it shifts cells from the proximal wing into the distal wing. It has been argued that it promotes disc growth due to the induction of Wg expression in the proximal wing, but Ds-Fat signaling, as revealed by the genetic requirement for dachs, is only required for induction of Wg in the proximal wing at early third instar (Cho and Irvine 2004). Additionally, a wg allele, spd,h, that eliminates Wg expression in the inner ring, strongly reduces the size of the proximal wing and hinge but has only mild effects on the size of the wing blade (Neumann and Cohen 1996a).

**Orientation of growth in the wing disc**

The shape of the wing disc, and ultimately the adult wing, depends not only on the pattern and amount of growth but also on the orientation of growth. During normal wing disc development, there is a bias in the orientation of growth along the proximal–distal axis of the distal wing, visible in the elongated shape of marked clones of cells (Bryant 1970; Resino et al. 2002). This orientation of growth is dependent upon the Ds-Fat pathway (Baena-Lopez et al. 2005; Mao et al. 2011). Ds-Fat signaling is also required for the orientation of cell divisions, which are normally biased along the proximal–distal axis, but the bias in cell division orientation is an insufficient explanation for how growth is oriented, because cell division orientation is randomized in mud mutant wing discs, yet mud does not significantly affect the orientation of growth in the wing disc or shape of the adult wing (Zhou et al. 2019). Quantitative analysis of cell behaviors in wing discs growing in ex vivo culture revealed that changes in shape of the developing wing pouch during growth reflect 3 main processes: oriented cell divisions, cell rearrangements, and cell shape changes (Dye et al. 2017). However, the relative contributions of these processes to growth orientation amongst individual discs can vary, and loss of division orientation in mud mutants could be compensated for by an increased contribution of cell rearrangements (Dye et al. 2017; Zhou et al. 2019).

In the proximal wing, growth is oriented circumferentially. In this region, growth orientation correlates with the circumferential orientation of mechanical stress. This stems in part from the initially faster growth of distal wing cells, which circumferentially stretches more proximal wing disc cells (Legoff et al. 2013; Mao et al. 2013). However, circumferentially oriented stress is maintained even after differential growth no longer occurs, and it has been proposed that it is instead driven largely by radially oriented cell rearrangements induced by a mechanosensitive feedback (Dye et al. 2021).

**Notum growth**

Growth of the notum has not been as intensively investigated as growth of the wing, although Dpp is required for normal notum growth, as the notum is much smaller in dpp mutant wing discs (Spencer et al. 1982). In addition to proliferation of cells initially fated to form notum, part of the growth of the notum comes from a shift of cells from the PE to the notum. This shift has been revealed by lineage tracing experiments (Pallavi and Shashidhara 2003; McClure and Schubiger 2005). It is also consistent with the genetic requirement of peripodial cells for growth of the notum, although part of this effect may reflect a contribution of PE to DP cell survival (Gibson and Schubiger 2000; Pallavi and Shashidhara 2003; Nusinow et al. 2008). By counting cells McClure and Schubiger (2005) estimated that at the beginning of the third instar there are 3 times as many cells in the DP as in the PE, but at late third instar the ratio is 20:1. As based on differences in growth rates one would have expected a final ratio of only 12:1, it appears that a substantial fraction of PE cells shift to the DP, and this is particularly important for growth of the notum. During pupal stages, live imaging has revealed that cell numbers in the notum are limited in part by a crowding-induced delamination (Marinari et al. 2012; Levayer et al. 2016).

**Integration of wing disc and organismal growth**

The influence of local factors like wing disc patterning must be integrated with systemic effects so that the growth of the wing disc is coordinated with the growth of the rest of the animal. The growth of the wing disc, like all parts of the fly, is influenced by metabolism and metabolic pathways (Mirth and Shingleton 2012; Okamoto and Yamanaka 2015). Starvation can lead to flies that are much smaller than normal, but with relatively normal proportions, although some variations in the effects of nutrition on different body parts, including the wing, have been observed.
The effects of starvation can be mimicked by blocking the Insulin or mTor signaling pathways (Chen et al. 1996; Böhni et al. 1999; Colombani et al. 2003). During larval development, the imaginal discs are bathed in hemolymph, which provides a mechanism for inter organ communication. A key focus of nutritional regulation during larval stages is the fat body. Activation of mTor signaling in the fat body promotes growth of other larval tissues by stimulating production of Drosophila insulin-like peptides (DilpPe) (Colombani et al. 2003; Géméniard et al. 2009). Studies in genetic mosaics have confirmed that Insulin and mTor signaling pathways are also autonomously required within imaginal disc cells for normal growth, and have revealed that these pathways affect cell size as well as cell number (Böhni et al. 1999; Weinkove et al. 1999; Oldham et al. 2000; Zhang et al. 2000; Broggiò et al. 2001).

A key external signal controlling the growth and development of the imaginal discs is the steroid hormone 20-hydroxyecdysone (which we will abbreviate as ecdysone). Ecdysone is synthesized by the prothoracic gland under the control of a wide range of developmental and environmental signals (Mirth and Shingleton 2012; Texada et al. 2020). Ecdysone is best known for its role in triggering molts between larval instars and the larval to pupal transition. However, small pulses of ecdysone also occur during the third instar (Warren et al. 2006), and a low level of ecdysone is required for growth of the larval wing disc (Herbosco et al. 2015). At least in part, this requirement for ecdysone reflects a role in regulating the expression of developmental patterning genes that are required for normal wing disc growth (Mirth et al. 2009; Dye et al. 2017; Parker and Struhl 2020). For reasons that are not yet understood, the dosage of ecdysone required for continued growth is proportional to disc size (Strassburger et al. 2021). The understanding that ecdysone is required for continued disc growth and normal gene expression has also contributed to success in growing wing discs in culture (Dye et al. 2017).

Multiple signals converge to regulate ecdysone production during larval development. Nutritional status, through insulin signaling, appears to influence ecdysone production by regulating the growth of the prothoracic gland (Mirth et al. 2005). Importantly, ecdysone production is also regulated by signaling from the wing and other imaginal discs. It has long been known that certain classes of tumor-inducing mutations, or tissue damage that triggers regeneration, can delay pupariation. A key molecular mechanism for this delay was provided by the discovery that it is in part mediated by induction of the relaxin-like signaling peptide, Dilp8, in damaged or tumorous wing discs (Colombani et al. 2012; Garelli et al. 2012). Dilp8 signals to its receptor Lgr3 in larval brain and prothoracic gland to downregulate ecdysone production, slowing growth and delaying metamorphosis (Colombani et al. 2015; Jaszczak et al. 2016). Dilp8 also coordinates the relative growth of wing discs and other organs during development. This function is visible, for example, by the variability in wing size (referred to as fluctuating asymmetry) in flies lacking Dilp8 signaling (Colombani et al. 2012; Garelli et al. 2012). This coordination reflects the fact that in addition to activation by damage signals, Dilp8 is also regulated by inputs that report the growth status of imaginal discs. Dilp8 is upregulated by Yki (Boone et al. 2016); Yki activity normally declines in wing discs as they approach their mature size (Pan et al. 2018; Borreguero-Muñoz et al. 2019), and deletion of the enhancer that mediates Yki regulation of Dilp8 results in fluctuating asymmetry similar to that of loss of Dilp8 (Boone et al. 2016). Dilp8 regulation also contributes to a mechanism through which an individual slow-growing imaginal disc can slow growth of other organs; the transcription factor Xrp1 is upregulated in slow growing imaginal disc cells and upregulates Dilp8 expression (Boulan et al. 2019). Dilp8 thus provides a mechanism for sensing, through multiple inputs, when imaginal disc growth is completed and metamorphosis can ensue. While regulation of Dilp8 can occur in other imaginal discs, most studies of Dilp8 have focused on wing discs, which presumably have a key role in Dilp8 regulation as the largest of the imaginal discs. Other signals secreted by imaginal discs, including Dpp and Upd3, can also influence ecdysone production (Setiawan et al. 2018; Cao et al. 2021; Romão et al. 2021). Dpp pathway activation in the prothoracic gland declines in older larvae, suggesting that, as for Dilp8, levels of Dpp released by imaginal discs may help coordinate growth and developmental transitions (Setiawan et al. 2018).

Signaling from muscles also influences the growth of wing and other imaginal discs. This signaling is mediated by the activin family member Myoglianin (Myo) (Upadhyay et al. 2020), which is expressed by larval muscles. Wing discs in Myo mutants are ~40% smaller than in wild-type larvae. This signaling may help coordinate the size of appendages and cuticular body parts with the size of the larval body and the musculature that will be attached to them.

**Regeneration of the wing disc**

**Wing discs as a model for regeneration**

The wing disc has long been recognized as for its regenerative capacity, which provided early insights into the relationship between patterning and growth, and more recently has enabled studies investigating how tissues respond to and repair damage (Worley and Hariharan 2021). Regeneration is a process of reformation of damaged or excised tissue. It typically requires recognition of the damage, followed by regrowth and repatterning of the missing tissue. While adult wings of Drosophila cannot regenerate, wing discs can recover from damage or removal of tissue by regenerating the missing cells. Early regeneration studies relied on the fact that transplantation of discs into larval hosts led to differentiation on schedule with metamorphosis of the host, but discs could alternatively be transplanted into female abdomens, where they could grow without differentiating (Hadorn and Buck 1962; Bryant 1971, 1975a, 1975b). During this growth phase, damaged wing discs regenerate missing tissue (Fig. 9a). The success of regeneration was assayed by retransplantation into larval hosts, or examination of cell proliferation and molecular markers of disc cell fates (O’Brochta and Bryant 1987; Mattila et al. 2005).

More recently, techniques were developed to excise tissue from imaginal discs without removing them from the larva (Pastor-Pareja et al. 2008; Diáz-García and Baonza 2013). Additionally, genetic approaches have been developed to kill cells in defined regions of the disc during larval development and then examine the ability of the disc to regenerate this tissue within the larva (Smith-Bolton et al. 2009; Bergantinos et al. 2010; Herrera et al. 2013) (Fig. 9b). These genetic approaches are amenable to doing genetic and genomic analysis and screens, and have yielded insights into processes, molecules and pathways that contribute to regeneration, as well as how regeneration is coordinated with the development of the rest of the organism.

**Jnk activation coordinates wing disc regeneration**

Activation of Jnk signaling is essential for regeneration in wing discs and acts through multiple downstream effectors to coordinate elimination of dying cells, tissue remodeling, stimulation of proliferation, modulation of cell fate, and systemic responses
that facilitate effective regeneration (Fig. 9c) (Bosch et al. 2005; Mattila et al. 2005; Bertoglinos et al. 2010; Grusche et al. 2011; Sun and Irvine 2011; Colombani et al. 2012; Garelli et al. 2012). Reactive oxygen species (ROS), which are generated by dying cells, are key inducers of Jnk activation, as well as of p38 MAPK activation, which also contributes to regeneration (Santabárbara-Ruiz et al. 2015; Fogarty et al. 2016). Efficient regeneration requires an appropriate level and duration of Jnk signaling, and genes that modulate ROS levels are required for appropriate Jnk activation and consequently, wing disc regeneration (Brock et al. 2017; Khan et al. 2017). Jnk activation also feeds back on ROS production to modulate the duration of the response (Khan et al. 2017).

Regenerative growth in wing discs

Jnk activation is required for early steps in regeneration including cellular processes that close wounds, modify cell fates and stimulate cell proliferation (Bosch et al. 2005; Mattila et al. 2005; Bertoglinos et al. 2010). In wing discs, as in many other contexts, a population of cells near the wound site, often referred to as a blastema, are stimulated to undergo increased proliferation (O’Brochta and Bryant 1987). The majority of blastema cells in wing disc regeneration experiments arise from cells in which Jnk activation was induced (Bosch et al. 2008).

The stimulation of proliferation during regeneration shares features with apoptosis-induced compensatory cell proliferation (Hu et al. 2004; Pérez-Garíjo et al. 2004; Ryoo et al. 2004; Morata et al. 2011), a phenomena in which induction of cell death by irradiation or expression of proapoptotic genes stimulates proliferation of neighboring cells in a Jnk-dependent process. Jnk signaling promotes cell proliferation through cross-talk with other signaling pathways, including Wg, Hippo, and Jak-Stat. Dying cells can also release Dpp, but Dpp is not actually required for the induction of proliferation by dying cells (Pérez-Garíjo et al. 2009). It has also been reported that Wg is not required for compensatory cell proliferation (Pérez-Garíjo et al. 2009), however, Wg is required for normal regeneration after genetically induced tissue damage, during which Wg expression is induced near wound edges in a Jnk-dependent process (Smith-Bolton et al. 2009).

Jnk activation in regenerating wing discs also leads to activation of Yki, and Yki is required for regenerative growth after tissue damage (Grusche et al. 2011; Sun and Irvine 2011). Indeed, regenerative growth in the wing disc is even more sensitive to Yki levels than normal developmental wing growth (Grusche et al. 2011; Sun and Irvine 2011; Repiso et al. 2013). One mechanism through which Jnk pathway activation increases Yki activity is direct phosphorylation of the Wts inhibitor Jub by Jnk, which promotes binding of Jub to Wts (Sun and Irvine 2013). The Ds-Fat pathway also contributes to Yki regulation during regeneration (Grusche et al. 2011; Repiso et al. 2013). The Ds-Fat pathway also influences the orientation of growth during regeneration and replacement of dying cells (Li et al. 2009; Repiso et al. 2013). Growth can be oriented toward dying cells in a Fat-dependent process; this presumably makes regenerative growth more efficient at closing wounds.

The Upd family of Jak-Stat pathway ligands are upregulated downstream of Jnk during wing disc regeneration, and Jak-Stat signaling contributes to promotion of cell proliferation as well as loss of cell fate specification (Pastor-Pareja et al. 2008; Katsuyama et al. 2015; Santabárbara-Ruiz et al. 2015; La Fortezza et al. 2016; Ahmed-de-Prado et al. 2018). During regeneration of the intestine Upd is upregulated downstream of Yki (Karpowicz et al. 2010; Ren et al. 2010; Shaw et al. 2010; Staley and Irvine 2010), so it is possible that Yki also mediates the upregulation of Upd during wing disc regeneration, although to our knowledge this has not been directly tested in wing discs.

Cancer has been described as a wound that does not heal, and there are notable similarities between the mechanisms that stimulate growth in regenerating wing discs and wing discs with mutations in neoplastic tumor suppressor genes (Mirzoyan et al. 2019; Gong et al. 2021). Many neoplastic tumor suppressor mutations disrupt epithelial cell polarity, which triggers activation of responses that parallel those that occur during regeneration, including activation of Jnk, activation of Yki, and activation of Jak-Stat signaling. These responses can be modulated by local disc patterning, as for example tumors in wing discs form preferentially in the proximal wing and hing where Jak-Stat signaling is normally active (Tamori et al. 2016).

Repatterning during wing disc regeneration

Wing disc patterning has to be re-established after tissue loss or damage, as even the fundamental separation of wing disc cells into A–P and D–V compartments can be disrupted (Díaz-García and Baonza 2013; Herrera and Morata 2014). Repatterning of regenerating tissue in wing discs is thought to follow a similar process as during normal development, including re-establishment of compartment boundaries (Smith-Bolton et al. 2009; Bertoglinos et al. 2010; Herrera and Morata 2014), and expression of Dpp and Wg along these boundaries (Mattila et al. 2004; Díaz-García and Baonza 2013). However, the ability to repattern is limited, as in some cases damaged wing discs are unable to regenerate missing structures. In classic disc fragmentation experiments, it was observed that larger fragments of a wing disc could regenerate the missing parts, but smaller fragments generated duplicated structures rather than regenerating (Bryant 1975a, 1975b) (Fig. 9a). In more recent experiments employing localized induction of cell death, it was observed that the wing has a greater capacity for regeneration than the notum, and that fragments that are exclusively notum or wing are unable to regenerate the complementary region (Martin et al. 2017b).

Regulation of chromatin modifiers is important for modifications of cell fate downstream of Jnk, and components of both repressive and activating complexes, as well as chromosome remodeling complexes, are regulated by tissue damage and influence regeneration (Klebes et al. 2005; Lee et al. 2005; Blanco et al. 2010; Skinner et al. 2015; Tian and Smith-Bolton 2021). The regulation of chromatin modifiers during regeneration may also explain why regeneration is sometimes associated with transdifferentiation (Klebes et al. 2005; Lee et al. 2005), in which cells in a regenerating disc sometimes switch fate toward that of a different imaginal disc.

Modulation of developmental timing during wing disc regeneration

Wing disc damage can delay pupariation, and this delay contributes to efficient regeneration by providing time for regenerative growth and repatterning to occur in the damaged disc (Hackney and Cherbas 2014). A key factor mediating this delay is Dilp8, which is secreted by regenerating imaginal disc cells (Colombani et al. 2012; Garelli et al. 2012). In the absence of Dilp8, damage-induced developmental delay does not occur and regeneration is compromised. Dilp8 secreted from damaged wing disc cells signals to its receptor, Lgr3, in the brain and prothoracic gland to delay pupariation by inhibiting synthesis of ecdysone (Colombani et al. 2015; Garelli et al. 2015). Expression of Dilp8 is promoted by multiple pathways upregulated during regeneration, including Jnk, Yki, and Jak-Stat (Colombani et al. 2012; Katsuyama et al.

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2015; Boone et al. 2016). In addition to regulating Dilp8, Upd3 cytokines secreted by regenerating or tumorous larval tissues can also act directly on the prothoracic gland to suppress edcsyne production (Cao et al. 2021; Româo et al. 2021).

The regenerative capacity of wing discs decreases over the course of the third larval instar (Smith-Bolton et al. 2009), and multiple factors have been identified as contributing to this decline. A damage-responsive enhancer of wg is subject to epigenetic silencing as wing discs age, reducing the ability of the wing disc to regenerate (Harris et al. 2016). Increases in edcsyne levels also suppress regenerative capacity through upregulation of broad and concomitant downregulation of chimino (Narbonne-Reveau and Maurange 2019). Increases in edcsyne during the latter part of the third instar also impair regeneration by regulating the localization of the septate junction protein coracle, and consequently decreasing the permeability of the wing disc epithelium (DaCrema et al. 2021). This suppresses the ability of Dilp8, which is secreted into the wing disc lumen (Colombani et al. 2012), to reach its receptor Lgr3 in the brain and prothoracic gland, and likely also reduces secretion of other signals from imaginal discs, like Dpp and Upd3, that can influence edcsyne production.

Morphogenesis of the wing disc

During larval stages, the wing disc is a sac-like epithelial monolayer that nonetheless has a complex morphology, formed through variations in cell shape and epithelial folding. Then, during metamorphosis the relative flat epithelium of the wing disc undergoes a remarkable transformation to generate the 3D morphology of the adult wing and notum.

In recent years, genetic and imaging approaches have been combined with physical modeling to provide insights into how this transformation occurs.

Formation of folds in the larval wing disc

The epithelium of the DP is initially flat, but as it grows folds begin to appear at precise locations, such that by mid-third instar the wing imaginal disc exhibits a reproducible pattern of folds separating the wing pouch region of the disc from the notal region (Fig. 3, a and b). Three distinct folds, referred to as the hinge–notum, hinge–hinge, and hinge–pouch folds. These folds are positioned by genes that pattern the wing disc.

The hinge–notum fold forms at the border of Iro-C gene expression, and genetic manipulations of Iro-C expression show that folds can be induced at ectopic Iro-C borders, with the non-expressing cells undergoing apical–basal shortening and invagination (Villa-Cuesta et al. 2007). Omm, which is expressed in the wing pouch and hinge but not the notum, also contributes to hinge–notum fold formation (Wang et al. 2016). The Jak-Stat pathway Upd ligands become expressed in cells that will form the hinge, and Jak-Stat signaling is also required for normal formation of the hinge–notum fold (Johnstone et al. 2013). The hinge–pouch fold forms in cells expressing Doc proteins, whose expression is delimited by repression of Vg in more distal cells and repression by Hth in more proximal cells (Sui et al. 2012). Loss- and gain-of-function experiments established that Doc genes contribute to fold formation. The hinge–hinge fold is positioned in part by Wg expression, which is expressed just distal to this fold and represses fold formation (Sui and Dahmann 2020).

Recent studies have identified cellular mechanisms associated with fold formation. All 3 folds are characterized by an apical–basal shortening of cells, and redistribution of microtubules from predominantly apical to predominantly basal (Sui et al. 2012; Wang et al. 2016). Local degradation of basal ECM has been described at the hinge–pouch (Sui et al. 2012) and hinge–hinge folds (Sui et al. 2018). There are also intriguing differences between the folds, however, as the hinge–hinge fold is characterized by decreased basal tension, whereas the hinge–pouch fold is characterized by increased lateral tension (Sui et al. 2018). The formation of folds by alterations in basal or lateral tension can be reproduced in computational simulations using 3D vertex models (Sui et al. 2018). Computational and experimental analyses suggest that differential growth between different regions of the wing disc also contribute to fold formation (Tozluoğlu et al. 2019).

Morphogenesis of the pupal wing disc

During metamorphosis, the wing disc undergoes a complex morphogenesis, triggered by edcsyne, to form the adult wing, hinge, and notum. These processes were first described in classic studies decades ago (Auerbach 1936; Waddington 1939; Fristrom and Fristrom 1975), but new insights have been revealed through application of modern genetic techniques, live imaging, and modeling.

Wing disc eversion

The first step in converting the larval wing disc into the pupal wing is eversion, during which the imaginal discs effectively turn inside out, such that appendages extend out of the body cavity and the apical surfaces are now on the surface of the developing animal rather than facing a central lumen (Fig. 10). At the beginning of this process, the developing wing pouch begins to elongate and flattens along the D–V compartment boundary to generate the bilayered shape of the adult wing (Fristrom and Fristrom 1975; Aldaz et al. 2010). The dorsal and ventral surfaces of the wing blade become attached through integrin binding to laminin (Brower and Jaffe 1989; Henchcliffe et al. 1993; Brabant et al. 1996; Martin et al. 1999; Sun et al. 2021), but undergo phases of flattening, inflation, and reattachment during pupal development (Waddington 1939; Fristrom and Fristrom 1975; Fristrom et al. 1993). Myosin-generated forces in the peripheral cells contribute to the folding of the wing and the eversion of the wing disc (Aldaz et al. 2013). Around this time, stalk cells that maintain connection of the disc to the larval epithelium invade the larval epidermis and become migratory, expanding the space through which the disc will evert (Pastor-Pareja et al. 2004). The PE then ruptures, and the disc everts through the opening formed by ruptured PE and stalk cells.

Morphogenesis of the wing

After eversion the wing continues to elongate and expand. Elongation of the wing during early pupal development (4–5 h after puparium formation) is accompanied by oriented cell intercalation and cell shape changes (Díaz-de-la-Loza et al. 2018). These appear to be directed by anisotropic stresses, visible through the polarization of myosin accumulation along cell–cell junctions. Expansion of the surface area of the developing wing is accomplished by cell flattening, as cells transition from columnar to cuboidal (Fristrom and Fristrom 1975; Díaz-de-la-Loza et al. 2018). This flattening is accompanied by degradation of the ECM.

Later in pupal development, the developing wing is shaped by a contraction of the wing hinge region, in concordance with attachment of the distal wing to the pupal cuticle (Turner and Adler 1995; Ettouney et al. 2015; Ray et al. 2015). The attachment of the distal wing is mediated through apical ECM proteins,
including Dumpy. The anchoring of the distal wing, in conjunction with the contraction of the hinge, generates an anisotropic tension that contributes to further wing elongation. This late reshaping of the wing is accompanied by a combination of cell shape changes, cell rearrangements, and cell divisions, all oriented by the anisotropic tension, and reproducible in computational modeling (Etournay et al. 2015; Guirao et al. 2015; Ray et al. 2015). Cells in the late pupal wing blade become more isometric, adopting a relatively even hexagonal packing in a process that depends upon PCP proteins and the physical properties of wing cells (Classen et al. 2005; Farhadifar et al. 2007). Wing cells secrete cuticle during the middle of pupal development, and then after eclosion undergo an epithelial–mesenchymal transition and apoptosis, such that the mature adult wing is mostly composed of cuticle without underlying cells (Johnson and Milner 1987; Kiger et al. 2007; Link et al. 2007; Sobala and Adler 2016).

**Notum fusion**

The notal region of the disc also undergoes morphogenetic changes to spread and replace the larval epidermis, adopt the adult notal shape, and fuse with notal cells from the contralateral wing disc. The fusion of the notal region of the 2 wing discs at the midline of the adult fly is mediated by a subset of peripodial cells at the edge of the notum and requires Jnk pathway activity in these cells (Agnès et al. 1999; Zeitlinger and Bohmann 1999; Martin-Blanco et al. 2000; Tripura et al. 2011).

**Conclusion**

The Drosophila wing disc has been one of the most intensively studied organs in biology. This has resulted in an impressively detailed understanding of many aspects of its development. More broadly, discoveries elucidating fundamental aspects of wing disc development have established paradigms that have informed our understanding of organogenesis throughout the animal kingdom. To highlight just a few examples: Studies in wing discs have provided foundational insights into epithelial cell biology, including how cells divide in pseudostratified epithelia, how PCP is established and maintained, and how local cell shape changes combine to alter the shapes of epithelial tissues. The wing disc has been a particularly important model for understanding how tissues are patterned, beginning with the discovery of compartments, and the logic of developmental patterning that progresses from broad subdivisions to specification of discrete cell types at reproducible locations like bristles and veins. The discovery and characterization of signaling pathways that play key roles in establishing wing disc patterning, like the Notch, Wnt, Hh, and Dpp pathways, has identified mechanisms that play fundamental roles in tissue patterning across diverse animal species, and also established a model for identifying and characterizing components of these conserved pathways. As these signaling pathways are used reiteratively throughout the development of all animals, the identification and characterization of their components has had a particularly broad impact. Wing discs

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Fig. 10. Wing disc morphogenesis. a) Schematic illustrating steps in wing disc eversion, which occurs during the first half of prepupal development (the ~12-h period that serves as a transition between larval and pupal development). (i) The larval wing disc is attached to the cuticle by a stalk connected to the PE. (ii) Part of the PE attaches to the larval cuticle, and the wing pouch begins to elongate and flatten. (iii) The PE over the notum invades the larval cuticle and ruptures, generating an opening for the disc to evert through. The PE over the wing contracts (green arrows), in part through cells becoming columnar rather than squamous. (iv) The disc has everted through the hole created by invasion and rupture of the PE and larval cuticle. Eversion is driven by contraction of the remaining PE. b) Longitudinal sections of discs at (i) 0, (ii) 2, and (iv) 4 h after puparium formation, roughly corresponding to the same stages in the schematic. Green arrowheads point to the edges of the wing pouch area, which will form the distal wing. Images reproduced with permission from Domínguez-Giménez et al. (2007).
have also been essential to mechanistic investigations of fundamental developmental processes such as how cells are separated into compartments and how long-range signals spread through tissues.

The wing disc has also been a remarkable system for identifying factors that control organ growth and for characterizing their activity and how they are integrated with each other. For example, studies in wing discs, together with the eye disc, led to the discovery of the conserved Hippo signaling network, and laid the foundation of our understanding of its mechanism of action and its control of growth and cell fate. More recently, wing discs have aided investigations of interorgan communication and how systemic factors coordinate the growth and development of different organs within a developing organism. Although there remain gaps in our understanding of how wing disc growth is controlled, in no other organ is there a level of understanding approaching that which exists in the wing disc.

The accumulated foundation of knowledge, together with the many powerful tools available in Drosophila, ensure that the wing disc will continue to be fruitful terrain for elucidating fundamental aspects of biology for many years to come. New approaches, including improvements in disc culture that facilitate live imaging, ever more sophisticated genetic, molecular, and genomic techniques, and advances in image segmentation and quantitation, will aid investigations of interorgan communication and how systemic factors coordinate the growth and development of different organs within a developing organism. Although there remain gaps in our understanding of how wing disc growth is controlled, in no other organ is there a level of understanding approaching that which exists in the wing disc.

We thank the innumerable researchers who have collectively established the Drosophila wing disc as the most powerful system for experimental biology, and apologize to those researchers whose work could not be included. We thank Carlos Estrella for images of the embryonic primordia and Maria Dolores Martin Bermudo for images of wing disc eversion.

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Conflicts of interest

None declared.

Literature cited

Adler PN. The frizzled/stan pathway and planar cell polarity in the Drosophila wing. Curr Top Dev Biol. 2012;101:1–31.

Adler PN, Charlton J, Liu J. Mutations in the cadherin superfamily member gene dachsous cause a tissue polarity phenotype by altering frizzled signaling. Development. 1998;125(5):959–968.

Aegerter-Wilmsen T, Aegerter CM, Hafen E, Basler K. Model for the regulation of size in the wing imaginal disc of Drosophila. Mech Dev. 2007;124(4):318–326.

Aegerter-Wilmsen T, Heimlicher MB, Smith AC, de Reuille PB, Smith RS, Aegerter CM, Basler K. Integrating force-sensing and signaling pathways in a model for the regulation of wing imaginal disc size. Development. 2012;139(17):3221–3231.

Aigouy B, Farhadifar R, Staple DB, Sagner A, Röper J-C, Jülicher F, Eaton S. Cell flow reorients the axis of planar polarity in the wing epithelium of Drosophila. Cell. 2010;142(9):773–786.

Aikyama T, Kamimura K, Firkus C, Takeo S, Shimmii O, Nakato H. Daily regulates Dpp morphogen gradient formation by stabilizing Dpp on the cell surface. Dev Biol. 2008;313(1):408–419.

Aldaz S, Escudero LM, Freeman M. Live imaging of Drosophila imaginal disc development. Proc Natl Acad Sci U S A. 2010;107:14217–14222.

Aldaz S, Escudero LM, Freeman M. Dual role of myosin II during Drosophila imaginal disc metamorphosis. Nat Commun. 2013;4:1761.

Algéot H, Markosian C, Rauskolb C, Yang J, Kirichenko E, Wang, Y-C, Irvine, KD. Recruitment of JUB by alpha-catenin promotes Yki activity and Drosophila wing growth. J Cell Sci. 2019;132:jcs222018.

Alexandre C, Baena-Lopez A, Vincent JP. Patternning and growth control by membrane-tethered wingless. Nature. 2014;505(7482):180–185.

Alee M, Röper J-C, Landsberg KP, Pentzold TJ, Jülicher F, Dahmann C. Physical mechanisms shaping the Drosophila dorsoventral compartment boundary. Curr Biol. 2012;22(11):967–976.

Ambegaonkar AA, Irvine KD. Coordination of planar cell polarity pathways through Spiny legs. Elife. 2015;4:773.

Ambegaonkar AA, Pan G, Mani M, Feng Y, Irvine KD. Propagation of dachsous-fat planar cell polarity. Curr Biol. 2012;22(14):1302–1308.

Apidianakis Y, Grabiec D, Stifani S, Delidakis C. Groucho mediates a Cx-independent mechanism of hedgehog repression in the anterior wing pouch. Development. 2001;128(23):4361–4370.

Auerbach C. The development of the legs, wings and halteres in wild type and some mutant strains of Drosophila melanogaster. Proc R Soc Edinb B. 1936;58:787–815.

Ayala-Camargo A, Anderson AM, Amoyel M, Rodrigues AB, Flaherty MS, Bach EA. JAK/STAT signaling is required for hinge growth and patterning in the Drosophila wing disc. Dev Biol. 2013;382(2):413–426.

Ayukawa T, Akiyama M, Mummery-Widmer JL, Stoeger T, Sasaki J, Knoblich JA, Senoo H, Sasaki T, Yarnazaki M. Dachsous-dependent asymmetric localization of spiny-legs determines planar cell polarity orientation in Drosophila. Cell Rep. 2014;8(2):610–621.

Azpiazu N, Morata G. Function and regulation of homothorax in the wing imaginal disc of Drosophila. Development. 2000;127(12):2685–2693.

Baeg GH, Lin X, Khare N, Baumgartner S, Perrimon N. Heparan sulfate proteoglycans are critical for the organization of the extracellular distribution of wingless. Development. 2001;128(1):87–94.

Baena-Lopez LA, Baonza A, Garcia-Bellido A. The orientation of cell divisions determines the shape of Drosophila organs. Curr Biol. 2005;15(18):1640–1644.

Baena-Lopez LA, Franch-Marro X, Vincent JP. Wingless promotes proliferative growth in a gradient-independent manner. Sci Signal. 2009;2:ra60.

Baena-Lopez LA, Pastor-Pareja JC, Resino J. Wg and Egfr signalling antagonise the development of the peripodial epithelium in Drosophila wing discs. Development. 2003;130(26):6497–6506.
Cousse JP, Knust E, Arias AM. Serrate and wingless cooperate to induce vestigial gene expression and wing formation in Drosophila. Curr Biol. 1995;5(12):1437–1448.

Couturier L, Mazouni K, Corson F, Schweisguth F. Regulation of Notch output dynamics via specific E(spl)-HLH factors during bristle patterning in Drosophila. Nat Commun. 2019;10(1):3486.

Crick F. Diffusion in embryogenesis. Nature. 1970;225(5231):420–422.

DaCrema D, Bhandari R, Karanja F, Yano R, Halme A. Ecdysone regulates the Drosophila imaginal disc epithelial barrier, determining the length of regeneration checkpoint delay. Development. 2021;148:dev195057.

Dahmann C, Basler K. Compartment boundaries: at the edge of development. Trends Genet. 1999;15(8):320–326.

Dahmann C, Oster AC, Brand M. Boundary formation and maintenance in tissue development. Nat Rev Genet. 2011;12(3):43–55.

Dai J, Estrada B, Jacobs S, Sanzánchez-Sánchez BJ, Pastor-Parejo JC, Martín-Bermudo MD. Dissection of Nidogen function in Drosophila reveals tissue-specific mechanisms of basement membrane assembly. PLoS Genet. 2018;14(9):e1007483.

Day SJ, Lawrence PA. Measuring dimensions: the regulation of size and shape. Development. 2000;127(14):2977–2987.

de Celis JF, Barrio R. Function of the spalt/spalt-related gene complex and shape. Development. 2000;127(14):2977–2987.

De Ville Rodriguez A, Didiano D, Desplan C. Power tools for gene expression and clonal analysis in Drosophila. Development. 2019;146:dev179754.

Del Signore SJ, Hayashi T, Hatini V. odd-skipped genes and lines organise the length of regeneration checkpoint delay. Development. 2021;148:dev195057.

del Valle Rodriguez A, Didiano D, Desplan C. Power tools for gene expression and clonal analysis in Drosophila. Nat Methods. 2012;9(1):47–55.

Deng H, Wang W, Yu J, Zheng Y, Qing Y. Spectrin regulates Hippo signalling by modulating cortical actomyosin activity. Elife. 2015;4:e06567.

Deng M, Wang Y, Zhang L, Yang Y, Huang S, Wang, J, Ge, H, Ishibashi, T, Yan, Y. Single cell transcriptomic landscapes of pattern formation, proliferation and growth in Drosophila imaginal discs. Development. 2019;146:dev179754.

DeVido SK, Kwon D, Brown JL, Kassis JA. The role of polycomb-group response elements in regulation of engrailed transcription in Drosophila. Development. 2008;135(4):669–676.

Díaz-Beníuestra FJ, Cohen SM. Interaction between dorsal and ventral cells in the imaginal disc directs wing development in Drosophila. Cell. 1993;75(4):741–752.

Díaz-Beníuestra FJ, Cohen SM. Serrate signals through Notch to establish a Wingless-dependent organizer at the dorsal/ventral compartment boundary of the Drosophila wing. Development. 1995;121(12):4215–4225.

Díaz-de-la-Loza M-D-C, Ray RP, Ganguly B, Alt S, Davis JR, Hoppe A, Tapon N, Salbreux G, Thompson BJ. Apical and basal matrix remodeling control epithelial morphogenesis. Dev Cell. 2018;46(1):23–39.e5.

Díaz-García S, Baonza A. Pattern reorganization occurs independently of cell division during Drosophila wing disc regeneration in situ. Proc Natl Acad Sci U S A. 2013;110(32):13032–13037.

Diez del Corral R, Aroca P, M Gez-Skarmeta JL, Cavodeassi F, Modolell J. The Iroquois homeo domains are required to specify body wall identity in Drosophila. Genes Dev. 1999;13(13):1754–1761.

Doherty D, Feger G, Younger-Shepherd S, Jan LY, Jan YN. Delta is a ventral to dorsal signal complementary to Serrate, another Notch ligand, in Drosophila wing formation. Genes Dev. 1996;10(4):421–434.

Domínguez-Giménez P, Brown NH, Martín-Bermudo MD. Integrin-ECM interactions regulate the changes in cell shape driving the morphogenesis of the Drosophila wing epithelium. J Cell Sci. 2007;120(Pt 6):1061–1071.

Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, Gayyed MF, Anders RA, Maitra A, Pan D. Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell. 2007;130(6):1120–1133.

Doyle K, Hogan J, Lester M, Collier S. The Fz/PCD planar cell polarity signaling pathway controls Drosophila wing topography. Dev Biol. 2008;317(1):354–367.

Du L, Sohr A, Yan G, Roy S. Feedback regulation of cytoneme-mediated transport shapes a tissue-specific FGF morphogen gradient. Elife. 2018;7:e38137.

Duman-Scheel M, Johnston LA, Du W. Repression of dMyec expression by Wingless promotes Rbf-induced G1 arrest in the presumptive Drosophila wing margin. Proc Natl Acad Sci U S A. 2004;101(11):3857–3862.

Dye NA, Popović M, lyer KV, Fuhrmann JF, Fiscitello-Gómez R. Self-organized patterning of cell morphology via mechanosensitive feedback. Elife. 2021;10:e57964.

Dye NA, Popović M, Spannl S, Etournay R, Kainmüller D, Ghosh S, Myers EW, Jülicher F, Eaton S. Cell dynamics underlying oriented growth of the Drosophila wing imaginal disc. Development. 2017;144(3):4406–4421.

Eaton S, Auvinen P, Luo L, Jan YN, Simons K. CDC42 and Rac1 control different actin-dependent processes in the Drosophila wing. Development. 2017;144(3):4406–4421.

Eaton S, Wepf R, Simons K. Roles for Rac1 and Cdc42 in planar polarization by modulating cortical actomyosin activity. Elife. 2015;4:e07090.

Eaton S, Wepf R, Simons K. Roles for Rac1 and Cdc42 in planar polarization by modulating cortical actomyosin activity. Elife. 2015;4:e07090.
Everetts NJ, Worley MI, Yasutomi R, Yosef N, Haritharan IK. Single-cell transcriptomics of the Drosophila wing disc reveals instructive epithelium-to-myoblast interactions. Elife. 2021;10:e61276.

Ewen-Campen B, Comyn T, Vogt E, Perrimon N. No evidence that Wnt ligands are required for planar cell polarity in Drosophila. Cell Reports. 2020;32:108121.

Farhadifar R, Röper J-C, Aigouy B, Eaton S, Julicher F. The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. Curr Biol. 2007;17(24):2095–2104.

Fernandes J, Bate M, VijayRaghavan K. Development of the indirect flight muscles of Drosophila. Development. 1991;113(1):67–77.

Fleming RJ, Gu Y, Hukriede NA. Serrate-mediated activation of Notch is specifically blocked by the product of the gene fringe in the dorsal compartment of the Drosophila wing imaginal disc. Development. 1997;124(15):2973–2981.

Fletcher GC, Elbediwy A, Khanal I, Ribeiro PS, Tapon N, Thompson BJ. The Spectrin cytoskeleton regulates the Hippo signalling pathway. Embo J. 2015;34(7):940–954.

Fogarty CE, Diwanji N, Lindblad JL, Tare M, Amcheslavsky A, Makhijani K, Brückner K, Fan Y, Bergmann A. Extracellular reactive oxygen species drive apoptosis-induced proliferation via Drosophila macrophages. Curr Biol. 2016;26(5):575–584.

Franch-Marro X, Marchand O, Piddini E, Ricardo S, Alexandre C, Vincent J-P. Glypicans shunt the wingless signal between local signalling and further transport. Development. 2005;132(4):659–666.

French V, Bryant PJ, Bryant SV. Pattern regulation in epimorphic fields. Science. 1976;193(4257):969–981.

Fristrom D, Fristrom JW. The mechanism of evagination of imaginal discs of Drosophila melanogaster: I. General considerations. Dev Biol. 1975;43(1):1–23.

Fristrom D, Gotwals P, Eaton S, Kornberg TB, Sturtevant M, Bier E, Fristrom JW. Blistered: a gene required for vein/intervenin formation in wings of Drosophila Development. 1994;120(9):2661–2671.

Fristrom D, Wilcox M, Fristrom J. The distribution of PS integrins, laminin A and F-actin during key stages in Drosophila wing development. Development. 1993;117(2):509–523.

Fujise M, Takeo S, Kamimura K, Matsuo T, Aigaki T, Izumi S, Nakato H. Daily regulates Dpp morphogen gradient formation in the Drosophila wing. Development. 2005;130(8):1515–1522.

Fuse N, Hirose S, Hayashi S. Determination of wing cell fate by the escargot and snail genes in Drosophila. Development. 1996;122(4):1059–1067.

Garcia-Bellido A. Larval development of Drosophila melanogaster in the adult milieu. J Ins Physiol. 1965;11:1071–1078.

Garcia-Bellido A, Ripoll P, Morata G. Developmental compartmentalisation of the wing disc of Drosophila. Nat New Biol. 1973;245(147):251–253.

Garcia-Bellido A, Ripoll P, Morata G. Developmental compartmentalisation in the dorsal mesothoracic disc of Drosophila. Dev Biol. 1976;48(1):132–147.

Garcia-Bellido AB, Merriam JR. Parameters of the wing imaginal disc development of Drosophila melanogaster. Dev Biol. 1971;24(1):61–87.

Garcia-Carino MJ, Ramain P, Simpson P, Modolell J. Different contributions of pannier and wingless to the patterning of the dorsal mesothorax of Drosophila. Development. 1999;126(16):3523–3532.

Garelli A, Gontijo AM, Miguel V, Caparros E, Dominguez M. Imaginal discs secrete insulin-like peptide 8 to mediate plasticity of growth and maturation. Science. 2012;336(6081):579–582.

Garelli A, Heredia F, Casimiro AP, Macedo A, Nunes C, Garcez M, Dias ARM, Volonte YA, Uhlmann T, Caparros E, et al. Dilp8 requires the neuronal relaxin receptor Lgr3 to couple growth to developmental timing. Nat Commun. 2015;6:8732.

Gebelen B, Cuji L, Ryoo HD, Zhang W, Mann RS. Specificity of distal-less repression and limb primordia development by abdominal Hox proteins. Dev Cell. 2002;3(4):487–498.

Géninard C, Rulifson EJ, Léopol D. Remote control of insulin secretion by fat cells in Drosophila. Cell Metab. 2009;10(3):199–207.

Germani F, Bergantinos C, Johnston LA. Mosaic analysis in Drosophila. Genetics. 2018;208(2):473–490.

Ghazii A, Paul L, VijayRaghavan K. Prepattern genes and signaling molecules regulate stripe expression to specify Drosophila flight muscle attachment sites. Mech Dev. 2003;120(5):519–528.

Gho M, Schweisguth F. Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in Drosophila. Nature. 1998;393(6681):178–181.

Gibson MC, Schubiger G. Peripodial cells regulate proliferation and patterning of Drosophila imaginal discs. Cell. 2000;103(2):343–350.

Giraldez AJ, Cohen SM. Wingless and Notch signaling provide cell survival cues and control cell proliferation during wing development. Development. 2003;130(26):6533–6543.

Gómez-Skarmeta JL, Campuzano S, Modolell J. Half a century of neural prepatterning: the story of a few bristles and many genes. Nat Rev Neurosci. 2003;4(7):587–598.

Gómez-Skarmeta JL, Modolell J. Araucan and caupolican provide a link between compartment subdivisions and patterning of sensory organs and veins in the Drosophila wing. Genes Dev. 1996;10(22):2935–2945.

Gong S, Zhang Y, Tian A, Deng W-M. Tumor models in various Drosophila tissues. WIREs Mech. Dis. 2021;13:e1525.

González-Gaitán M, Capdevila M, García-Bellido A. Cell proliferation patterns in the wing imaginal disc of Drosophila. Mech Dev. 1994;46(3):183–200.

Goto S, Hayashi S. Specification of the embryonic limb primordium by graded activity of decapentaplegic. Development. 1997;124(1):125–132.

Grusche FA, Degoutin JL, Richardson HE, HarveyKF. The Salivary/Warts/Hippo pathway controls regenerative tissue growth in Drosophila melanogaster. Dev Biol. 2011;350(2):255–266.

Grzeschik NA, Parsons LM, Allott ML, Harvey KF, Richardson HE. Lgl, aPKC, and Crumbs regulate the Salvador/Warts/Hippo path- way through two distinct mechanisms. Curr Biol. 2010;20(7):573–581.

Gubb D, García-Bellido A. A genetic analysis of the determination of cuticular polarity during development in Drosophila melanogaster. J Embryol Exp Morphol. 1982;68:37–57.

Gubb D, Green C, Huen D, Coulson D, Johnson G, Tree D, Collier S, Roote J. The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in Drosophila imaginal discs. Genes Dev. 1999;13(17):2315–2327.

Guirao B, Rigaud SU, Bosveld F, Bailles A, López-Gay J, Ishiiha, S, Sugimura, K, Graner, F, Bellaiche, Y. Unified quantitative character- ization of epithelial tissue development. Elife. 2015;4:e08519.

Gunage RD, Reichert H, VijayRaghavan K. Identification of a new stem cell population that generates Drosophila flight muscles. Elife. 2014;3:e03126.

Hackney JF, Cherbas P. Injury response checkpoint and developmental timing in insects. Fly (Austin). 2014;8(4):226–231.

Hadorn E, Buck D. On the differentiation of transplanted wing imaginal disc fragments of Drosophila melanogaster. Rev Suisse Zool. 1962;69:302–310.

Haenlin M, Cubadda Y, Blondeau F, Heitzler P, Lutz Y, Simpson P, Ramain P. Transcriptional activity of pannier is regulated
negatively by heterodimerization of the GATA DNA-binding domain with a cofactor encoded by the u-shaped gene of Drosophila. Genes Dev. 1997;11(22):3096–3108.

Halder G, Polaczyk P, Kraus ME, Hudson A, Kim J, Laughon A, Carroll S. The vestigial and scalloped proteins act together to directly regulate wing-specific gene expression in Drosophila. Genes Dev. 1998;12(24):3900–3909.

Hales KG, Korey CA, Larracuente AM, Roberts DM. Genetics on the fly: a primer on the Drosophila model system. Genetics. 2015;201(3):815–842.

Harnaguchi T, Yabe S, Uchiyama H, Murakami R. Drosophila Tbx6-related gene, Dorsocross, mediates high levels of Dpp and Scw signal required for the development of amnioserosa and wing disc primordium. Dev Biol. 2004;265(2):355–368.

Hassell G, de Lachapelle AM, Pyrowolakis G, Bergmann S, Hamaratoglu F, Iwaki DD, Kornberg TB. Hedgehog produced by the prothoracic gland. Genetics. 2016;204(2):703–709.

Hata R, Kornberg TB. Hedgehog produced by the Drosophila wing imaginal disc induces distinct responses in three target tissues. Development. 2020;147:dev195974.

Haynie JL, Bryant PJ. Intercalary regeneration in imaginal wing disc of Drosophila melanogaster. Nature. 1976;259(5545):659–662.

Henchcliffe C, García-Alonso L, Tang J, Goodman CS. Genetic analysis of the wingless gene reveals divergent functions during morphogenesis in Drosophila. Development. 1993;118(2):325–337.

Herboso T, Oliveira MM, Talamillo A, Pérez C, González M, Martín D, Sutherland JD, Shingleton AW, Mirth CK, Barrio R, et al. Ecdysone promotes growth of imaginal discs through the regulation of Thor in D. Sci Rep. 2015;5:e01831.

Herranz H, Peralta J, de Lachapelle AM, Pyrowolakis G, Bergmann S, Hamaratoglu F, Iwaki DD, Kornberg TB. Hedgehog produced by the Tbx6-related gene, Dorsocross, mediates high levels of Dpp and Scw signal required for the development of amnioserosa and wing disc primordium. Dev Biol. 2004;265(2):355–368.

Herreros T, de Lachapelle AM, Pyrowolakis G, Bergmann S, Affolter M. Dpp signaling activity requires Pentagone to scale with tissue size in the growing Drosophila wing imaginal disc. PLoS Biol. 2011;9(10):e1001188.

Hofma C, Yan D, Belenkaya TY, Lin X. Drosophila glypicans Daily and Dally-like shape the extracellular wingless morphogen gradient in the wing disc. Development. 2005;132(4):667–679.

Harris RE, Setiawan L, Saül J, Hariharan IK. Localized epigenetic silencing of a damage-activated Wnt enhancer limits regeneration in mature Drosophila imaginal discs. Elife. 2016;5:e11588.

Hatziiska A, Kirov N, Wieschaus E, Roth S, Rushlow C. The Drosophila gene brinker reveals a novel mechanism of Dpp target gene regulation. Cell. 1999;96(4):563–573.

Hayward K, Ghattas R, Comoglio F, Seimiya M, Cabuy E, Paro R. During development, the vestigial and scalloped proteins act together to directly regulate wing-specific gene expression in Drosophila. Genes Dev. 1997;11(22):3096–3108.

Hefner L, Oliveira MM, Talamillo A, Pan D. The Hippo signaling pathway coordinates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. Cell. 2005;122(3):421–434.

Hufnagel L, Telean AA, Rouault H, Cohen SM, Shraiman BI. On the mechanism of wing size determination in fly development. Proc Natl Acad Sci U S A. 2007;104(10):3835–3840.

Huh JR, Guo M, Hay BA. Compensatory proliferation induced by cell death in the Drosophila wing disc requires activity of the apical cell death caspase Dronc in a nonapoptotic role. Curr Biol. 2004;14(14):1262–1266.

Huppert SS, Jacobsen TL, Muskavitch MA. Feedback regulation is central to Delta-Notch signaling required for Drosophila wing vein morphogenesis. Development. 1997;124(17):3283–3291.

Hyman RO, Zhao Q. The evolution of cell adhesion. J Cell Biol. 2000;150(2):F89–96.

Ibar C, Irvine KD. Integration of Hippo-YAP signaling with metabolism. Dev Cell. 2020;54(2):256–267.

Ikma A, Netter S, Coen D. Prepatterning the Drosophila notum: the three genes of the Iroquois complex play intrinsically distinct roles. Dev Biol. 2008;317(2):634–648.

Inoue Y, Hayashi S. Tissue-specific laminin expression facilitates integrin-dependent association of the embryonic wing disc with the trachea in Drosophila. Dev Biol. 2007;304(3):90–101.

Irvine KD, Rauskolb C. Boundaries in development: formation and function. Annu Rev Cell Dev Biol. 2001;17:189–214.

Irvine KD, Wieschaus E. Fringe, a boundary-specific signaling molecule, mediates interactions between dorsal and ventral cells during Drosophila wing development. Cell. 1994;79(4):595–606.

Ishikawa HO, Takeuchi H, Haltiwanger RS, Irvine KD. Four-jointed is a Golgi kinase that phosphorylates a subset of cadherin domains. Science. 2008;321(5887):401–404.

Jafar-Nejad H, Tien AC, Acar M, Bellen HJ. Senseless and daughter-less confer neuronal identity to epithelial cells in the Drosophila wing margin. Development. 2006;133(9):1683–1692.

Jaszczak JS, Wolpe JB, Bhanderi R, Jaszczak RG, Halme A. Growth coordination during Drosophila melanogaster imaginal disc regeneration is mediated by signaling through the relaxin receptor 1Gr3 in the prothoracic gland. Genetics. 2016;204(2):703–709.

Jazwinka A, Kirov N, Wieschaus E, Roth S, Rushlow C. The Drosophila gene brinker reveals a novel mechanism of Dpp target gene regulation. Cell. 1999;96(4):563–573.

Johnson SA, Milner MJ. The final stages of wing development in Drosophila melanogaster. Tissue Cell. 1987;19(4):505–513.

Johnston LA, Edgar BA. Wingless and Notch regulate cell-cycle arrest in the developing Drosophila wing. Nature. 1998;394(6688):82–84.

Johnston LA, Sanders AL. Wingless promotes cell survival but constrains growth during Drosophila wing development. Nat Cell Biol. 2003;5(9):827–833.

Johnstone K, Wells RE, Strutt D, Zeidler MP. Localised JAK/STAT pathway activation is required for Drosophila wing hinge development. PLoS One. 2013;8(5):e65076.

Jory A, Estella C, Giorgianni MW, Slattery M, Laverty TR, Rubin GM, Mann RS. A survey of 6,300 genomic fragments for cis-regulatory activity in the imaginal discs of Drosophila melanogaster. Cell Rep. 2012;2(4):1014–1024.

Karpowicz P, Perez J, Perrimon N. The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. Development. 2010;137(24):4135–4145.

Katsuyama T, Comoglio F, Seimiya M, Cabuy E, Paro R. During Drosophila disc regeneration, JAK/STAT coordinates cell proliferation with Dilp8-mediated developmental delay. Proc Natl Acad Sci U S A. 2015;112(18):E2327–2336.

Khan SJ, Abidi SNF, Skinner A, Tian Y, Smith-Bolton RK. The Drosophila Duox maturation factor is a key component of a positive feedback loop that sustains regeneration signaling. PLoS Genet. 2017;13(7):e1006937.
Kiger JA, Natzele JE, Kimbrell DA, Paddy MR, Kleinbesselinke K, Green MM. Tissue remodeling during maturation of the Drosophila wing. Dev Biol. 2007;301(1):178–191.

Kim J, Irvine KD, Carroll SB. Cell recognition, signal induction, and symmetrical gene activation at the dorsal-ventral boundary of the developing Drosophila wing. Cell. 1995;82(5):795–802.

Kim J, Johnson K, Chen HJ, Carroll S, Laughon A. Drosophila Mad binds to DNA and directly mediates activation of vestigial by Decapentaplegic. Nature. 1997;388(6639):304–308.

Kim J, Sebring A, Esch JF, Krause ME, Vorwerk K, Magee J, Carroll SB. Integration of positional signals and regulation of wing formation and identity by Drosophila vestigial gene. Nature. 1996;382(6587):133–138.

Klebe A, Sustar A, Kechris K, Li H, Schubiger G, Kornberg TB. Regulation of cellular plasticity in Drosophila imaginal disc cells by the Polycomb group, trithorax group and lama genes. Development. 2005;132(16):3753–3765.

Klein T, Arias AM. Different spatial and temporal interactions between Notch, wingless, and vestigial specify proximal and distal pattern elements of the wing in Drosophila. Dev Biol. 1998a;194(2):196–212.

Klein T, Arias AM. Interactions among Delta, Serrate and Fringe modulate Notch activity during Drosophila wing development. Development. 1998b;125(15):2951–2962.

Klein T, Couso JP, Arias AM. Wing development and specification of dorsal cell fates in the absence of apertures in Drosophila. Curr Biol. 1998;8(7):417–420.

Kornberg T. Engrailed: a gene controlling compartment and segment formation in Drosophila. Proc Natl Acad Sci U S A. 1981;78(2):1095–1099.

Kubota K, Goto S, Eto K, Hayashi S. EGF receptor attenuates Dpp signaling and helps to distinguish the wing and leg cell fates in Drosophila. Development. 2000;127(17):3769–3776.

Kubota K, Goto S, Hayashi S. The role of Wg signaling in the patterning of embryonic leg primordium in Drosophila. Dev Biol. 2003;257(1):117–126.

La Fortezza M, Schenk M, Cosolo A, Kolybaba A, Grass I, Classen A-K, Link N, Chen P, Lu W-J, Pogue K, Chuong A, Mata M, Checketts J, Mao Y, Tournier AL, Hoppe A, Kester L, Thompson BJ, Tapon N. Histological analysis of the Drosophila wing imaginal discs. Wilhelm Roux Arch Dev Biol. 1977;183(4):269–305.

Lander AD, Nie Q, Wan FY. Do morphogen gradients arise by diffusion of Hh? Nature. 1999;400(6741):281–284.

Lin X, Perrimon N. Dolly cooperates with Drosophila Fz1 to transduce Wingless signalling. Nature. 1999;400(6741):281–284.

Lindley DL, Grell EH. Genetic Variations of Drosophila melanogaster. Washington, D.C.: Carnegie Institution of Washington; 1968.

Ling C, Zheng Y, Yin F, Yu J, Huang J, Hong Y, Wu S, Pan D. The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. Proc Natl Acad Sci U S A. 2010;107(23):10532–10537.

Link N, Chen F, Lu W-J, Fogue K, Chuong A, Mata M, Checketts J, Abrams JM. A collective form of cell death requires homeodomain interacting protein kinase. J Cell Biol. 2007;178(4):567–574.

Linz DM, Tomoyasu Y. Dual evolutionary origin of insect wings supported by an investigation of the abdominal wing serial homologs in Tribolium. Proc Natl Acad Sci U S A. 2018;115(4):E658–E667.

Liu X, Grammont M, Irvine KD. Roles for scaled and vestigial in regulating cell affinity and interactions between the wing blade and the wing hinge. Dev Biol. 2000;228(2):287–303.

Lu B, Usui T, Uemura T, Jan L, Jan YN. Flamingo controls the planar polarity of sensory bristles and asymmetric division of sensory organ precursors in Drosophila. Curr Biol. 1999;9(21):1247–1250.

Lunde K, Biels H, Nauber U, Bier E. The knirps and knirps-related genes organize development of the second wing vein in Drosophila. Development. 1998;125(1):4145–4154.

Ma D, Yang CH, McNeill H, Simon MA, Axelrod JD. Fidelity in planar cell polarity signalling. Nature. 2003;421(6922):543–547.

Ma M, Cao X, Dai J, Pastor-Pareja JC. Basement membrane manipulation in Drosophila wing disc affects Dpp retention but not growth mecanoregulation. Dev Cell. 2017;42(1):97–106.64.

Madhavan MM, Schneiderman HA. Histological analysis of the dynamics of growth of imaginal discs and histoblast nests during the larval development of Drosophila melanogaster. Wilhelm Roux Arch Dev Biol. 1977;183(4):269–305.

Mahoney PA, Weber U, Onofrechuk P, Biessmann H, Bryant PJ, Goodman CS. The fat tumor suppressor gene in Drosophila encodes a novel member of the cadherin gene superfamily. Cell. 1991; 67(5):853–868.

Major RJ, Irvine KD. Influence of Notch on dorsoventral compartmentalization and actin organization in the Drosophila wing. Development. 2005;132(17):3823–3833.

Major RJ, Irvine KD. Localization and requirement for Myosin II at the dorsal-ventral compartment boundary of the Drosophila wing. Dev Dyn. 2006;235(11):3051–3058.

Mao Y, Rauskolb C, Cho E, Hu W-L, Hayter H, Minihan G, Katz FN, Irvine KD. Dachs: an unconventional myosin that functions downstream of Fat to regulate growth, affinity and gene expression in Drosophila. Development. 2006;133(13):2539–2551.

Mao Y, Tournier AL, Bates PA, Gale JE, Tapon N, Thompson BJ. Planar polarization of the atypical myosin Dachs orients cell divisions in Drosophila. Genes Dev. 2011;25(2):131–136.

Mao Y, Tournier AL, Hoppe A, Kester L, Thompson BJ, Tapon N. Differential proliferation rates generate patterns of mechanical tension that orient tissue growth. EMBO J. 2013;32(21):2790–2803.

Marinat E, Menon I, Currans G, Gale J, Duke T, Baum B. Live-cell delamination counterbalances epithelial growth to limit tissue overcrowding. Nature. 2012;484(7395):542–545.
Martin D, Zusman S, Li X, Williams EJ, Khare N, DaRocha S, Chiquet-Ehrismann R, Baumgartner S. Wing blister, a new Drosophila laminin alpha chain required for cell adhesion and migration during embryonic and imaginal development. J Cell Biol. 1999;145(1):191–201.

Martín FA, Herrera SC, Morata G. Cell competition, growth and size control in the Drosophila wing imaginal disc. Development. 2009; 136(22):3747–3756.

Martín FA, Morata G. Compartments and the control of growth in the Drosophila wing imaginal disc. Development. 2006;133(22):4421–4426. (Cambridge, England)

Martín FA, Pérez-Garjio A, Moreno E, Morata G. The brinker gradient controls wing growth in Drosophila. Development. 2004;131(20):4921–4930.

Martin M, Ostale CM, de Celis JF. Patterning of the Drosophila L2 vein is driven by regulatory interactions between region-specific transcription factors expressed in response to Dpp signalling. Development. 2017a;144:3168–3176.

Martin R, Final N, Morata G. Distinct regenerative potential of trunk and appendages of Drosophila mediated by JNK signalling. Development. 2017b;144:3946–3956.

Martin-Blanco E, Pastor-Pareja JC, Garcia-Bellido A. JNK and decapentaplegic signaling control adhesiveness and cytoskeleton dynamics during thorax closure in Drosophila. Proc Natl Acad Sci U S A. 2000;97(14):7888–7893.

Martín-Castellanos C, Edgar BA. A characterization of the effects of Dpp signaling on cell growth and proliferation in the Drosophila wing. Development. 2002;129(4):1003–1013.

Matakatsu H, Blair SS. Interactions between fat and Dachsous and Tartan mediate cell interactions during DV boundary formation in the Drosophila wing. Cell. 2001;106(6):785–794.

Milner MJ, Bleasby AJ, Kelly SL. The role of the peripheral membrane of leg and wing imaginal discs of Drosophila melanogaster during evagination and differentiation in vitro. Wilehlm Roux Arch Dev Biol. 1984;193(3):180–186.

Minami M, Kinoshita N, Kamoshida Y, Tanimoto H, Tabata brinker is a target of Dpp in Drosophila that negatively regulates Dpp-dependent genes. Nature. 1999;398(6724):242–246.

Mirth C, Truman JW, Riddiford LM. The role of the prothoracic gland in determining critical weight for metamorphosis in Drosophila melanogaster. Curr Biol. 2005;15(20):1796–1807.

Mirth CK, Singleton AW. Integrating body and organ size in Drosophila: recent advances and outstanding problems. Front Endocrinol (Lausanne). 2012;3:49.

Mirth CK, Truman JW, Riddiford LM. The edcsyne receptor controls the post-critical weight switch to nutrition-independent differentiation in Drosophila wing imaginal discs. Development. 2009;136(14):2345–2353.

Misra JR, Irvine KD. The Hippo signaling network and its biological functions. Anr Rev Genet. 2018;52:65–87.

Mlodzik M. Planar cell polarity: moving from single cells to tissue-scale biology. Development (Cambridge, England). 2020;147(24):dev186346–186344.

Moloney DJ, Panin VM, Johnston SH, Chen J, Shao L, Wilson R, Wang Y, Stanley P, Irvine KD, Haltiwanger RS, et al. Fringe is a glycosyltransferase that modifies Notch. Nature. 2000;406(6794):369–375.

Montagne J, Groppe J, Guillemin K, Krasnow MA, Gehring WJ. Activation of knot leucine zipper in blistered. Development. 1996;122(9):2589–2597.

Micheilli CA, Blair SS. Dorsoventral lineage restriction in wing imaginal discs requires Notch. Nature. 1999;401(6752):473–476.

Micheilli CA, Rulifson EJ, Blair SS. The function and regulation of cut expression on the wing margin of Drosophila: Notch, wingless and a dominant negative role for Delta and Serrate. Development. 1997;124(8):1485–1495.

Micheilli CA, Rulifson EJ, Blair SS. The function and regulation of cut expression on the wing margin of Drosophila: Notch, wingless and a dominant negative role for Delta and Serrate. Development. 1997;124(8):1485–1495.

Michel M, Ailee M, Rudolf K, Bialas L, Jülicher F, Dahnmann C. The selector gene apterous and Notch are required to locally increase mechanical cell bond tension at the Drosophila dorsoventral compartment boundary. PLoS One. 2016;11(8):e0161668.

Milán M, Campuzano S, García-Bellido A. Cell cycling and patterned cell proliferation in the wing primordium of Drosophila. Prog Natl Acad Sci U S A. 1996a;93:640–645.

Milán M, Campuzano S, García-Bellido A. Developmental parameters of cell death in the wing disc of Drosophila. Prog Natl Acad Sci U S A. 1997;94:5691.

Milán M, Weihe U, Pérez L, Cohen SM. The LRR proteins capricious and Tartan mediate cell interactions during DV boundary formation in the Drosophila wing. Cell. 2001;106(6):785–794.

Minami M, Kinoshita N, Kamoshida Y, Tanimoto H, Tabata brinker is a target of Dpp in Drosophila that negatively regulates Dpp-dependent genes. Nature. 1999;398(6724):242–246.
Morante G, Lawrence PA. Control of compartment development by the enrgailed gene in Drosophila. Nature. 1975;255(5510):614–617. Morante G, Shleevok E, Perez-Garajo A. Mitogenic signaling from apoptotic cells in Drosophila. Dev Growth Differ. 2011;53(2):168–176. Morgan TH, Bridges CB. Sex-linked inheritance in Drosophila. Publ Carnegie Inst. 1916;237:1–88. Müller B, Hartmann B, Pyrowolakis G, Basler K. Conversion of an extracellular Dpp/BMP morphogen gradient into an inverse transcriptional gradient. Cell. 2003;113(2):221–233. Mullor JL, Calleja M, Capdevila J, Guerrero I. Hedgehog activity, independent of decapentaplegic, participates in wing disc patterning. Development. 1997;124(6):1227–1237. Nakajima Y, Meyer E, Kroesen A, McKinney SA, Gibson MC. Epithelial junctions maintain tissue architecture by directing planar spindle orientation. Nature. 2013;500(7462):359–362. Narbonne-Reveau K, Maurange C. Developmental regulation of regenerative potential in Drosophila by ecdysone through a bistable loop of ZBTB transcription factors. PLoS Biol. 2019;17(2):e3000149. Nellen D, Burke R, Struhl G, Basler K. Direct and long-range action of a DPP morphogen gradient. Cell. 1996;85(3):357–368. Nematabakhsh A, Levis M, Kumar N, Chen W, Zartman JJ, Alber M. Epithelial organ shape is generated by patterned actomyosin contractility and maintained by the extracellular matrix. PLoS Comput Biol. 2020;16(8):e1008105. Neufeld TP, de la Cruz AF, Johnston LA, Edgar BA. Coordination of developmental modules. Evol Dev. 2010;12(2):168–176. Neumann CJ, Cohen SM. Long-range action of Wingless organizes the dorsal-ventral axis of Drosophila. Development. 1996b;122(11):3477–3485. Neumann CJ, Cohen SM. Distinct mitogenic and cell fate specification functions of wingless in different regions of the wing. Development. 1996a;122(6):1781–1789. Neumann CJ, Cohen SM. A hierarchy of cross-regulation involving Notch, wingless, vestigial and cut organizes the dorsal/ventral axis of the Drosophila wing. Development. 1996b;122(11):3477–3485. Neumann CJ, Cohen SM. Long-range action of Wingless organizes the dorsal-ventral axis of the Drosophila wing. Development. 1997;124(4):871–880. Ng M, Díaz-Benjumea FJ, Cohen SM. Nubbin encodes a POU-domain protein required for proximal-distal patterning in the Drosophila wing. Development. 1995;121(2):589–599. Ng M, Díaz-Benjumea FJ, Vincent JP, Wu J, Cohen SM. Specification of the wing by localized expression of wingless protein. Nature. 1996;381(6580):316–318. Niwa N, Akimoto-Kato A, Niimi T, Tojo K, Machida R, Hayashi S. Developmental regulation of recombination by ecdysone through a bistable loop of ZBTB transcription factors. PLoS Biol. 2019;17(2):e3000149. Oh H, Irvine KD. In vivo regulation of Yorkie phosphorylation and localization. Development. 2008;135(6):1081–1088. Okamoto N, Yamanaka N. Nutrition-dependent control of insect development by insulin-like peptides. Curr Opin Insect Sci. 2015;11:21–30. Oktaha K, Gutiérrez L, Gagneur J, Girardot C, Sengupta AK, Furlong EEM, Müller J. Dynamic regulation by polycomb group protein complexes controls pattern formation and the cell cycle in Drosophila. Dev Cell. 2008;15(6):877–889. Oldham S, Montagne J, Radimerski T, Thomas G, Hafen E. Genetic and biochemical characterization of dTOR, the Drosophila homolog of the target of rapamycin. Genes Dev. 2000;14(21):2689–2694. Olofsson J, Sharp KA, Matis M, Cho B, Axelrod JD. Prickle/spiny-legs isoforms control the polarity of the apical microtubule network in planar cell polarity. Development. 2014;141(14):2866–2874. Pallavi SK, Shashidhara LS. Egfr/Ras pathway mediates interactions between peripodial and disc proper cells in Drosophila wing discs. Development. 2005;130(20):4931–4941. Pallavi SK, Shashidhara LS. Signaling interactions between squamous and columnar epithelia of the Drosophila wing disc. J Cell Sci. 2005;118(Pt 15):3363–3370. Pan Y, Alégot H, Rauskolb C, Irvine KD. The dynamics of Hippo signaling during Drosophila wing development. Development. 2015;18:dev165712. Pan Y, Heemskerk I, Ibar C, Shraiman BI, Irvine KD. Differential growth triggers mechanical feedback that elevates Hippo signaling. Proc Natl Acad Sci U S A. 2016;113:E6974–E6983. Panáková D, Srong H, Marois E, Thiele C, Eaton S. Lipoprotein particles are required for hedgehog and wingless signalling. Nature. 2005;435(7038):58–65. Panin VM, Papayannopoulos V, Wilson R, Irvine KD. Fringe modulates Notch-ligand interactions. Nature. 1997;387(6636):908–912. Parker J, Struhl G. Scaling the Drosophila Wing: TOR-dependent target gene access by the hippo pathway transducer Yorkie. PLoS Biol. 2015;13(10):e1002274. Parker J, Struhl G. Control of Drosophila wing size by morphogen range and hormonal gating. Proc Natl Acad Sci U S A. 2020;117(50):31935–31944. Parsons LM, Grzeschik NA, Allott ML, Richardson HE. Lgl/aPKC and Crb regulate the Salvador/Warts/Hippo pathway. Fly (Austin). 2010;4(4):288–293. Pastor-Pareja JC, Grawe F, Martín-Blanco E, García-Bellido A. Invasive cell behavior during Drosophila imaginal disc eversion is mediated by the JNK signaling cascade. Dev Cell. 2004;7(3):387–399. Pastor-Pareja JC, Wu M, Xu T. An innate immune response of blood cells to tumors and tissue damage in Drosophila. Dis Model Mech. 2008;1(2–3):144–154; Discussion 153. Pastor-Pareja JC, Xu T. Shaping cells and organs in Drosophila by opposing roles of fat body-secreted collagen IV and perlec. Dev Cell. 2011;21(2):245–256. Paul L, Wang S-H, Manivannan SN, Bonanno L, Lewis S, Austin CL, Simcox A. Dpp-induced Egfr signaling triggers postembryonic wing development in Drosophila. Proc Natl Acad Sci U S A. 2013;110:5058. Paumard-Rigal S, Zider A, Vaudin P, Silber J. Specific interactions between Crb regulate the Salvador/Warts/Hippo pathway. Fly (Austin). 2010;4(4):288–293. Paul L, Wang S-H, Manivannan SN, Bonanno L, Lewis S, Austin CL, Simcox A. Dpp-induced Egfr signaling triggers postembryonic wing development in Drosophila. Proc Natl Acad Sci U S A. 2013;110:5058.
overgrowths caused by apoptotic cells in the Drosophila wing disc. Development. 2009;136(7):1169–1177.

Phillips RG, Whittle JR. wingless expression mediates determination of peripheral nervous system elements in late stages of Drosophila wing disc development. Development. 1993;118(2):427–438.

Rafel N, Milan M. Notch signalling coordinates tissue growth and wing fate specification in Drosophila. Development. 2008;135(24):3995–4001.

Ramírez-Weber FA, Kornberg TB. Cytonemes: cellular processes that project to the principal signaling center in Drosophila imaginal discs. Cell. 1999;97(5):599–607.

Ramos-Lewis W, Page-McCaw A. Basement membrane mechanics shape development: lessons from the fly. Matrix Biol. 2019;75–76:72–81.

Rauskolb C, Irvine KD. Notch-mediated segmentation and growth control of the Drosophila leg. Dev Biol. 1999;210(2):339–350.

Rauskolb C, Sun S, Sun G, Pan Y, Irvine KD. Cytoskeletal tension inhibits Hippo signaling through an Aju-Warts complex. Cell. 2014;158(1):143–156.

Ray RP, Matamoro-Vidal A, Ribeiro PS, Tapon N, Houle D, Salazar-Ciudad I, Thompson BJ. Patterned anchorage to the apical extracellular matrix defines tissue shape in the developing appendages of Drosophila. Dev Cell. 2015;34(3):310–322.

Recasens-Alvarez C, Ferreira A, Milán M. JAK/STAT controls organ size and fate specification by regulating morphogen production and signalling. Nat Commun. 2017;8:13815.

Ren F, Wang B, Yue T, Yun EY, Liu S, Kornberg TB. Specificity of decapentaplegic-dependent wingless expression during Drosophila regeneration. PLoS Genet. 2015;11(10):e1005595.

Santabarbara-Ruiz P, López-Santillán M, Martín-Rodríguez I, Binaquí-Casas A, Pérez L, Milán M, Corominas M, Serras F. ROS-induced JNK and p38 signaling is required for unpaired cytokine activation during Drosophila regeneration. PLoS Genet. 2019;15(11):e1008454.

Sato M, Saigo K. Involvement of pannier and u-shaped in regulation of decapentaplegic-dependent wingless expression in developing Drosophila notum. Mech Dev. 2000;93(1–2):127–138.

Schilling S, Steiner S, Zimmerli D, Basler K. A regulatory receptor network directs the range and output of the wingless signal. Development. 2014;141(12):2483–2493.

Schluck T, Nienhaus U, Aegerter-Wilmsen T, Aegerter CM. Mechanical control of organ size in the development of the Drosophila wing disc. PLoS One. 2013;8(10):e76171.

Schwank G, Dalessi S, Yang S-F, Yagi R, de Lachapelle AM, Affolter M, Bergmann S, Basler K. Formation of the long range Dpp morphogen gradient. PLoS Biol. 2011a;9(7):e1001111.

Schwank G, Restrepo S, Basler K. Growth regulation by Dpp: an essential role for Brinker and a non-essential role for graded signalling levels. Development. 2008;135(24):4003–4013.

Schwank G, Tauriello G, Yagi R, Kranz E, Koumoutsakos P, Basler K. Antagonistic growth regulation by Dpp and Fat drives uniform cell proliferation. Dev Cell. 2011b;20(1):123–130.

Schwank G, Yang SF, Restrepo S, Basler K. Comment on "Dynamics of dpp signaling and proliferation control". Science. 2012;335(6067):401; author reply 401.

Serrano O, O’Farrell PH. Limb morphogenesis: connections between patterning and growth. Curr Biol. 1997;7(3):R186–R195.

Setiawan L, Pan X, Woods AL, O’Connor MB, Hariharan IK. The BMP2/4 ortholog Dpp can function as an inter-organ signal that regulates developmental timing. Life Sci Alliance. 2018;1(6):e201800216.

Sharpe J. Wolpert’s French flag: what’s the problem? Development. 2019;146(24):dev185967.

Shaw RL, Kohlmaier A, Polesello C, Veelken C, Edgar BA, Tapon N. The Hippo pathway regulates intestinal stem cell proliferation during Drosophila adult midgut regeneration. Development. 2010;137(24):4147–4158.

Shen J, Dahmann C. The role of Dpp signaling in maintaining the Drosophila anteroposterior compartment boundary. Dev Biol. 2005;279(1):31–43.

Shingleton AW, Estep CM, Driscoll MV, Dworkin I. Many ways to be small: different environmental regulators of size generate distinct scaling relationships in Drosophila melanogaster. Proc Biol Sci. 2009;276:2625–2633.

Silva E, Tsatskis Y, Gardano L, Tapon N, McNeill H. The tumor-suppressor gene fat controls tissue growth upstream of expanded in the Hippo signaling pathway. Curr Biol. 2006;16(21):2081–2089.
Simcox AA, Grumbling G, Schnep B, Bennington-Mathias C, Hersperger E, Shearn A. Molecular, phenotypic, and expression analysis of vein, a gene required for growth of the *Drosophila* wing disc. Dev Biol. 1996;177(2):475–489.

Simmonds AJ, Liu X, Soanes KH, Krause HM, Irvine KD, Bell JB. Molecular interactions between vestigial and scalloped promote wing formation in *Drosophila*. Genes Dev. 1998;12(4):3815–3820.

Simon MA, Xu A, Ishikawa HO, Irvine KD. Modulation of fat: dachsous Glycoserine binding by the cadherin domain kinase four-jointed. Curr Biol. 2010;20(9):811–817.

Skinner A, Khan SJ, Smith-Bolton RK. Trichorax regulates systemic signaling during *Drosophila* imaginal disc regeneration. Development. 2015;142(20):3500–3511.

Smith-Bolton RK, Worley ML, Kanda H, Hariharan IK. Regenerative growth in *Drosophila* imaginal discs is regulated by wingless and Myc. Dev Cell. 2009;16(6):797–809.

Sobala LF, Adler PN. The gene expression program for the formation of wing cuticle in *Drosophila*. PLoS Genet. 2016;12(5):e1006100.

Sotillos S, De Celis JF. Interactions between the Notch, EGFR, and JAK/STAT receptors: An update. Mech Dev. 2005;123(3):738–752.

Spencer FA, Hoffmann FM, Gelbart WM. Decapentaplegic: a gene complex affecting morphogenesis in *Drosophila melanogaster*. Cell. 1982;28(3):451–461.

St Johnston D. The art and design of genetic screens: *Drosophila melanogaster*. Nat Rev Genet. 2002;3(3):176–188.

St Pierre SE, Galindo MI, Couso JP, Thor S. Control of *Drosophila* imaginal disc development by rotund and roughened eye: differentially expressed transcripts of the same gene encoding functionally distinct zinc finger proteins. Development. 2002;129(5):1273–1281.

Staley BK, Irvine KD. Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation. Curr Biol. 2010;20(17):1580–1587.

Stapornwongkul KS, de Gennes M, Cocconi L, Salbreux G, Vincent JP. Patterning and growth control in vivo by an engineered GFP gradient. Science. 2020;370(6514):321–327.

Stern C. Two or three bristles. Sci Prog (New Haven). 1955;Series 9:41–84.

Strassburger K, Lutz M, Müller S, Teleman AA. Ecdysone regulates cell differentiation by influencing stem cell proliferation. Curr Biol. 2010;20(17):89–102.

Tamori Y, Suzuki E, Deng WM. Epithelial tumors originate in tumor hotspots, a tissue-intrinsic microenvironment. PLoS Biol. 2016;14(9):e1002537.

Tang W, Wang D, Shen J. Asymmetric distribution of Spalt in *Drosophila* wing squamous and columnar epithelia ensures correct cell morphogenesis. Sci Rep. 2016;6:30236.

Teppas U, Tanentzapf G, Ward R, Fehon R. Epithelial cell polarity and the apical-basal polarity complex affecting morphogenesis in *Drosophila melanogaster*. Cell. 2007;131(10):1845–1852.

Teppas U, Cohen SM. Dpp gradient formation in the *Drosophila* wing imaginal disc. Development. 2000;127(1):19–28.

Tepass U. The apical polarity protein network in *Drosophila* imaginal discs. Cell. 1994;76(1):457–469.

Thompson BJ. Cell polarity: models and mechanisms from yeast, worms and flies. Development. 2013;140(1):13–21.

Tian Y, Smith-Bolton RK. Regulation of growth and cell fate during tissue regeneration by the two SWI/SNF chromatin-remodeling complexes of *Drosophila*. Genetics. 2021;217(1):1–16.

Tian Y, Smith-Bolton RK. Regulation of growth and cell fate during tissue regeneration by the two SWI/SNF chromatin-remodeling complexes of *Drosophila*. Genetics. 2021;217(1):1–16.

Tian Y, Smith-Bolton RK. Regulation of growth and cell fate during tissue regeneration by the two SWI/SNF chromatin-remodeling complexes of *Drosophila*. Genetics. 2021;217(1):1–16.

Tian Y, Smith-Bolton RK. Regulation of growth and cell fate during tissue regeneration by the two SWI/SNF chromatin-remodeling complexes of *Drosophila*. Genetics. 2021;217(1):1–16.

Tian Y, Smith-Bolton RK. Regulation of growth and cell fate during tissue regeneration by the two SWI/SNF chromatin-remodeling complexes of *Drosophila*. Genetics. 2021;217(1):1–16.
induction of pannier and u-shaped in Drosophila. Mech Dev. 2000;96(1):37–49.

Tozlouglu M, Duda M, Kirkland NJ, Barrientos R, Burden JJ, Muñoz JJ, Mao Y. Planar differential growth rates initiate precise fold positions in complex epithelia. Dev Cell. 2019;51(3):299–312.e4.

Tripura C, Chandrika NP, Susmitha VN, Noselli S, Shashidhara LS. Regulation and activity of JNK signaling in the wing disc peripodial membrane during adult morphogenesis in Drosophila. Int J Dev Biol. 2011;55(6):583–590.

Turing AM. The chemical basis of morphogenesis. Phil Trans Roy Soc Lond Ser B Biol Sci. 1952;237:37–72.

Turner CM, Adler PN. Morphogenesis of Drosophila pupal wings in vitro. Mech Dev. 1999;52(2–3):247–255.

Uhl JD, Zandvakili A, Gebelein B. A Hox transcription factor collective binds a highly conserved distal-less cis-regulatory module to generate robust transcriptional outcomes. PLoS Genet. 2016;12(4):e1005981.

Upadhyay A, Peterson AJ, Kim MJ, O’Connor MB. Muscle-derived myoglobin regulates Drosophila imaginal disc growth. Elife. 2020;9:e51710.

Urbano JM, Torgler CN, Molnar C, Tepass U, López-Varea A, Brown W. Pupal wings in Drosophila. Development. 2009;136(24):4165–4176.

Vervoort M, Crozatier M, Valle D, Vincent A. The COE transcription factor Collier is a mediator of short-range hedgehog-induced patterning in the abdomen of the Drosophila early embryo. Dev Biol. 2006;293(1):211–223.

Vuilleumier R, Springhorn A, Patterson L, Koidl S, Hammerschmidt W. Dorsal, something new. Cold Spring Harb Perspect Biol. 2021;13:e040733. doi: 10.1101/cshperspect.a040733.
Worley MI, Setiawan L, Hariharan IK. TIE-DYE: a combinatorial marking system to visualize and genetically manipulate clones during development in Drosophila melanogaster. Development. 2013;140(15):3275–3284.

Wortman JC, Nahmad M, Zhang PC, Lander AD, Yu CC. Expanding signaling-molecule wavefront model of cell polarization in the Drosophila wing primordium. PLoS Comput Biol. 2017;13(7):e1005610.

Wu J, Cohen SM. Repression of Teashirt marks the initiation of wing development. Development. 2002;129(10):2411–2418.

Wu J, Roman A-C, Carvajal-Gonzalez JM, Mlodzik M. Wg and Wnt4 provide long-range directional input to planar cell polarity orientation in Drosophila. Nat Cell Biol. 2013;15(9):1045–1055.

Xu A, Haines N, Dlugosz M, Rana NA, Takeuchi H, Haltiwanger RS, Irvine KD. In vitro reconstitution of the modulation of Drosophila Notch-ligand binding by Fringe. J Biol Chem. 2007;282(48):35153–35162.

Yan D, Lin X. Shaping morphogen gradients by proteoglycans. Cold Spring Harb Perspect Biol. 2009;1(3):a002493.

Yonemura S, Wada Y, Watanabe T, Nagafuchi A, Shibata M. Alpha-catenin as a tension transducer that induces adherens junction development. Nat Cell Biol. 2010;12(6):533–542.

Yu JJS, Maugarny-Cales A, Pelletier S, Alexandre C, Bellaiche Y, Vincent JP, McGough, IJ. Frizzled-dependent planar cell polarity without secreted Wnt ligands. Dev Cell. 2020;54(5):583–592.

Yue T, Tian A, Jiang J. The cell adhesion molecule echinoid functions as a tumor suppressor and upstream regulator of the Hippo signaling pathway. Dev Cell. 2012;22(2):255–267.

Zecca M, Basler K, Struhl G. Sequential organizing activities of engrailed, hedgehog and decapentaplegic in the Drosophila wing. Development. 1995;121(8):2265–2278.

Zecca M, Basler K, Struhl G. Direct and long-range action of a wingless morphogen gradient. Cell. 1996,87(5):833–844.

Zecca M, Struhl G. Control of growth and patterning of the Drosophila wing imaginal disc by EGFR-mediated signaling. Development. 2002a;129(6):1369–1376.

Zecca M, Struhl G. Subdivision of the Drosophila wing imaginal disc by EGFR-mediated signaling. Development. 2002b;129(6):1357–1368.

Zecca M, Struhl G. Control of Drosophila wing growth by the vestigial quadrant enhancer. Development. 2007a;134(16):3011–3020.

Zecca M, Struhl G. Recruitment of cells into the Drosophila wing primordium by a feed-forward circuit of vestigial autoregulation. Development. 2007b;134(16):3001–3010.

Zecca M, Struhl G. A feed-forward circuit linking wingless, fat-saceous signaling, and the warts-hippo pathway to Drosophila wing growth. PLoS Biol. 2010;8(6):e1000386.

Zecca M, Struhl G. A unified mechanism for the control of Drosophila wing growth by the morphogens decapentaplegic and Wingless. PLoS Biol. 2021;19(3):e3001111.