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Wing tips: The wing disc as a platform for studying Hedgehog signaling

Tom A. Hartl, Matthew P. Scott *

Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305, USA
Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA
Department of Bioengineering, Stanford University School of Medicine, Stanford, CA 94305, USA

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ABSTRACT

Hedgehog (Hh) signal transduction is necessary for the development of most mammalian tissues and can go awry and cause birth defects or cancer. Hh signaling was initially described in *Drosophila*, and much of what we know today about mammalian Hh signaling was directly guided by discoveries in the fly. Indeed, Hh signaling is a wonderful example of the use of non-vertebrate model organisms to make basic discoveries that lead to new disease treatment. The first pharmaceutical to treat hyperactive Hh signaling in Basal Cell Carcinoma was released in 2012, approximately 30 years after the isolation of Hh mutants in *Drosophila*. The study of Hh signaling has been greatly facilitated by the imaginal wing disc, a tissue with terrific experimental advantages. Studies using the wing disc have led to an understanding of Hh ligand processing, packaging into particles for transmission, secretion, reception, signal transduction, target gene activation, and tissue patterning. Here we describe the imaginal wing disc, how Hh patterns this tissue, and provide methods to use wing discs to study Hh signaling in *Drosophila*. The tools and approaches we highlight form the cornerstone of research efforts in many laboratories that use *Drosophila* to study Hh signaling, and are essential for ongoing discoveries.

1. Introduction

Initial analyses of Hh-mediated developmental events were conducted in the embryo, where Hh ligands are necessary for larval segment polarity. Larvae with null *hh* mutations lose naked cuticle tissue. What remains is a shrunken body covered with disorganized denticles, which gave rise to the name "hedgehog" [1,2]. This same phenotype occurs in embryos lacking function of *wingless* (*wg*) [2]. In fact, a primary function of Hh signaling in the embryo is to promote the expression of *wingless* (*wg*) in adjacent cells [3–5]. Wg is necessary to specify ectodermal cells that secrete larval cuticle [6]. Wg also feeds back to induce expression of *engrailed* (*en*), which drives expression of *hh*. This forms a positive feedback circuit that sustains *wg* and *hh* expression in neighboring cells [4,7–10].

Understanding steps in Hh signal transduction began with studies of embryos that implicated another segment polarity gene "*patched*" (*ptch*). Embryos with diminished Ptch expanded *wg* expression [7,8]. This indicated that Ptch represses *wg*, yet paradoxically, Ptch and Wg were found to be transcribed in the same row of cells [11]. Thus, Ptch could only be a repressor of *wg* expression if it were functionally inactivated at a post-transcriptional level. It was hypothesized that Ptch, a transmembrane protein [12,13], might be a receptor that is deactivated through the binding of an unknown ligand. The inhibition of the receptor would lead to *wg* induction. The hypothetical ligand was theorized correctly to be Hh [11]. That idea was based on knowledge that Hh is produced by cells adjacent to those that co-transcribe *ptch* and *wg* [9,14], and that *wg* expression is dependent upon *hh* [4,7–10]. Consistent with the idea that Hh blocks Ptch's repression of *wg* expression, *hh*; *ptch* double mutant embryos had the *ptch* phenotype of *wg* overexpression – proving that *ptch* is epistatic to and thus downstream of *hh* [11]. This model was proven through the subsequent demonstration that Hh is a secreted molecule [15] that can bind Ptch [16,17], be sequestered by Ptch [18], and negatively influence Ptch function [19].

The study of Hh signaling in the embryo established a blueprint of genetic interactions that has been built upon and refined over the last two decades. The imaginal wing disc has often facilitated these studies. The wing disc is made up of about 50,000 cells that develop into the adult wing and into body wall structures to which the wing is attached (Fig. 1A and B). The ventral and dorsal most regions develop into the pleura and notum structures of the dorsal thoracic body wall where the wings are attached. More internal

* Corresponding author. Address: Clark Center, West Wing W252, 318 Campus Drive, Stanford University School of Medicine, Stanford, CA 94305, USA. Fax: +1 (650) 725 2952.
E-mail address: mscott@stanford.edu (M.P. Scott).

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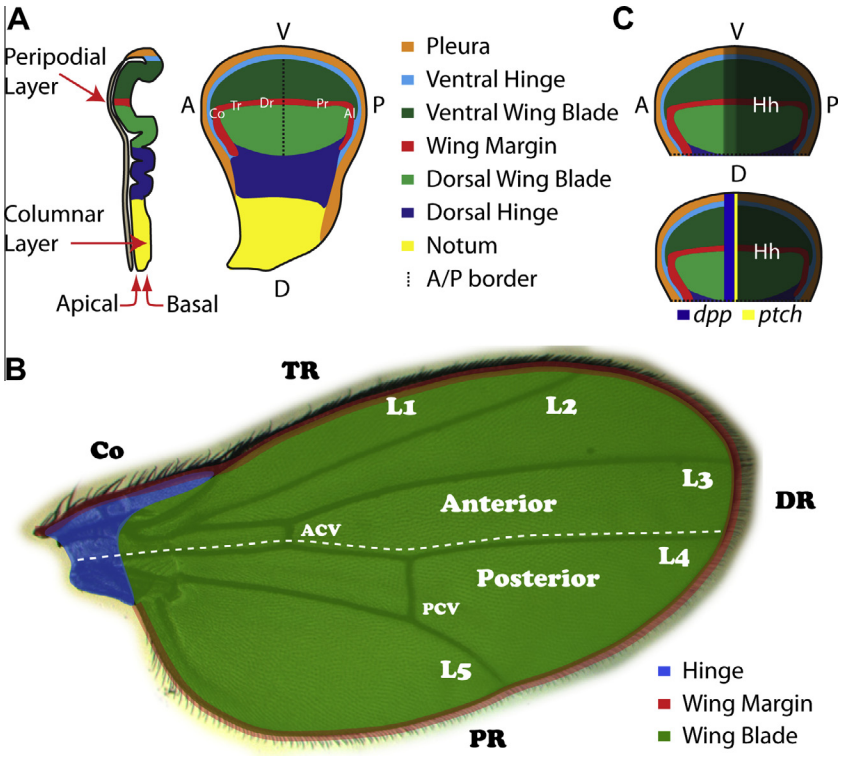

Fig. 1. Anatomy and fate map of the imaginal wing disc. The richness of pattern of the wing facilitates analyzing the effects of perturbed developmental signals. (**A**, **B**) The primordia of the wing disc are color coded to match the adult wing structures they develop into in **B** (pleura, notum, and alula are not shown). During metamorphosis, the wing disc is restructured and differentiates so that the wing margin is everted and the dorsal and ventral wing hinges and blades slide over one another to form the dorsal and ventral epithelial sheets of the adult wing. Cells of the wing pouch's margin will differentiate into unique classes of bristles of the anterior (Costal, Co; Triple Row, Tr; Double Row, Dr). The posterior margin carries hairs (Posterior Row, PR and Alula Row, Ar (Ar not shown in B). The pleura and notum differentiate into the thoracic body structure attached to the wing hinge and blade. The anterior wing blade carries three longitudinal veins (L1–L3) and the Anterior Crossvein (ACV), while posterior carries longitudinal veins L4 and L5, and the Posterior Crossvein (PCV). Veins L2 and L4 are associated with the ventral surface, Veins L3 and L5 are dorsal. The anterior has campaniform sensilla (not shown), which are mechanosensory organs with stereotyped locations on the wing. Four campaniform sensilla reside along L3, two at the anterior proximal wing margin, and one along the ACV [150,151]. (**C**) Hh is transcribed in the posterior compartment and is secreted anteriorly where it contacts ~12 rows of cells abutted against the A/P border [28]. There it induces target genes like *ptch* at high concentrations next to the A/P border. The high level of Ptch at the A/P border leads to Hh sequestration, and thus limits Hh spreading to a total of ~12 cellular rows. Hh can also induce *dpp* at lower doses. This figure is adapted from recommended literature [21,23,113,152,153].

disc regions will become the ventral and dorsal wing hinge, and the centrally located wing pouch differentiates into the adult wing blade.

The wing disc is subdivided into anterior and posterior compartments that are separated by an anatomical boundary called the A/P border. These compartments are defined by lineage restrictions; clones of cells will expand to fill parts of a single compartment, but normally do not cross the A/P border [20]. Anterior structures such as wing veins 1–3, the anterior crossvein (ACV), and Costal (Co), triple row (Tr), and double row (Dr) bristles along the wing margin are established by patterning events in the anterior compartment of the wing disc. Posterior structures such as the posterior row (Pr) and alula hairs (Al), wing veins 4 and 5, and the posterior crossvein (PCV) are patterned in the posterior compartment of the wing disc [21–23] (Fig. 1A and B).

The wing disc consists of two juxtaposed layers of epithelial cells referred to as the "columnar layer" cells and the "peripodial layer" cells. The columnar and peripodial layers are separated by a lumen. The two cell layers are situated such that their apical surfaces are oriented toward the lumen and their basolateral surfaces are oriented away from the lumen [22] (Fig. 1A).

Hh patterns the entire wing through cellular events it induces in the imaginal wing disc [15,24,25]. Wing development is orchestrated by many molecular events, starting with the expression of *hh* exclusively in the posterior compartment in response to the Engrailed and Invected transcription factors (Fig. 1C) [9,15,26,27]. Hh ligands are secreted directionally from the posterior compartment and reach ~12 cell rows near the A/P border of the anterior compartment (Fig. 1C) [15,24,28]. Hh secretion depends upon a balance of Dispatched, Dlp, and Dally, which promote Hh release and flow through the tissue, and Ihog, and Boi, which restrain Hh release [28–30]. Mature Hh protein carries N-terminal palmitic acid and C-terminal cholesterol adducts [31–33] that can impact its movement through tissue and its strength as a signaling molecule [34]. Lipid modifications generally tether proteins to the cell membrane, so the flow of dually lipidated Hh protein through tissues may be facilitated by packaging into multimers [35–38], exovesicles [39], and lipoproteins [40–42]. An exciting new model for how Hh moves through tissues came from the recent discovery that Hh navigates wing disc epithelia along the exterior of cellular extensions of the basolateral membrane called cytonemes that can span up to 70 μm in length [29,30,43,44].

In most cases, Hh signaling controls tissue development by regulating post-translational modifications of the Cubitus Interruptus (Ci) transcription factor and, by extension, the genes Ci regulates [45,46] (Fig. 2). In the absence of Hh ligands, the kinases Protein Kinase A (PKA), Casein Kinase 1 (CK1), and Glycogen Synthase Kinase 3 (GSK3) phosphorylate the C-terminus of full-length (155 kDa) Ci, which is termed Ci^F. The multi-site phosphorylation upon Ci^F create a site primed for ubiquitination by SCF^{Slimb}, leading to partial proteosome processing and removal of Ci's C-terminal *trans*-activation domain [47–56]. The resulting 75 kDa Ci, with its N-terminal zinc-finger DNA binding domain intact, behaves as a transcriptional repressor termed Ci^R [56]. Ci^F is complexed