Chapter 9. Imaginal disc growth, cell lineage restrictions and polarised cell affinities.

After hatching from the egg, most larval cells increase in size without further nuclear division and become increasingly polypoid. However, the adult body is constructed from diploid cells that are set aside in imaginal discs or histoblast nests within each embryonic segment. Separate A and P histoblast nests fuse to form the imaginal discs, which are attached to the trachea or mouth hooks during the larval stages [1]. The imaginal discs grow throughout larval development as flattened epithelial sacks, with a closely adherent peripodial membrane. Regulatory interactions take place across the thin, fluid-filled lumen between these two epithelial layers, but the peripodial cells do not contribute to adult structures (and their nuclei may become polyploid). During pupal metamorphosis disc eversion is driven by actomyosin contractions in the peripodial membrane, with extensive remodelling of the epithelial surface topography [2,3,4,5]. Towards the end of metamorphosis, the separate larval discs fuse to secrete the cuticular surface of the pharate adult, with the adult body being formed from a mosaic of separate clonal lineages [6].

In the anterior region, twin proboscal and clypeolabral discs are located on either side of the ventral midline. These discs contribute to the adult head capsule, with fused (L/R) tubular extensions: the proboscis and clypeolabrum. The proboscal discs lack a peripodial membrane, instead both opposed epithelial surfaces of the disc give rise to adult structures [7]. By contrast, the dorsal eye-antennal discs are formed by cell clusters coalescing from several embryonic segments, with no lineage restrictions between the adult eye, antenna, or head capsule. In the trunk region, separate ¨orthosegmental¨ (D and V) sets of discs give rise to the thorax; wing, haltere and leg structures, see [1]. The D prothoracic disc develops as a small ring surrounding an anterior tracheal tube, which contributes only a small humeral plate to the anterior notum, without a central limb outgrowth. As a result, the surface of the adult notum is formed almost entirely from the A compartment of the D mesothoracic disc. A narrow strip of cuticle below the scutellum is derived from the P mesothoracic compartment. The D metathoracic disc forms a compact, gyro-sensory, haltere in place of the metathoracic wing of other pterygote insects. By contrast, the abdominal tergites (D) and sternites (V) derive from histoblast nests that remain quiescent during larval growth. These abdominal nests proliferate, spread and fuse during the pupal stage, without forming distal appendages. The external genitalia derive from terminal genital discs, which can be transformed to homoeotic limb outgrowths [8,9]. Additional imaginal rings are anchored to the salivary glands and the mid- and hind-gut; which proliferate to provide the adult gut stem-cell lineages, see [1]. In general, the embryonic axial system is maintained throughout larval development, with bristles and hairs aligned with A > P axis of the adult body surface. However, this long axis is re-aligned along the Pr > Dist axis of adult limbs. An additional planar axis is not formed, instead the embryonic axial system is rotated in the centre of the thoracic imaginal discs. Epithelial surfaces remain 2D, whether wrapped around tubular outgrowths, or as flattened sheets.

The seminal study of Garcia-Bellido et al [10] found that the imaginal wing disc is sub-divided by lineage restrictions during growth. Fast-growing (*Minute+*) clones initiated at the beginning of the first larval instar define a straight antero-posterior (A/P) compartment boundary, which they do not cross. The dorsal and ventral wing cell populations later become separated by a D/V compartment boundary. The authors postulated that disc growth could be regulated by region-specific mitotic rates, preferred spindle orientations, mitotic waves, and differential cell recognition properties. Later work identified A/P compartmental boundaries in the leg, eye-antennal and genital discs, and between the abdominal histoblast nests. Surprisingly, the A/P boundary in the leg is not as straight as in the wing, with some bristles having either A or P provenance [11]. There is no D/V lineage restriction in the P leg, although

the A compartment may become sub-divided towards the end of larval development [11]. Meanwhile, the A/P lineage restriction in the antenna takes place during the middle of the second larval instar [12]. Taken together, these studies confirm that the compartmental boundaries delimit units of growth control, with differential expression of morphogenetic functions, reviewed in [1] [13].

Differential A and P cell affinities have been confirmed by many subsequent studies including [14] [15]. Differential affinities could generate irregular, curved boundaries between separate cell populations. However, to maintain a straight boundary must require an additional stabilising component. By analogy, the unstable interface between oil and water forms dispersed droplets of oil-in-water and water-in-oil. A straight oil/water interface requires that the two liquid phases have different densities, within a uniform gravitational field. By comparison, the differential A and P cell affinities in the wing disc are regulated by Hh signalling, with Hh expression restricted to P cells. Clones of *cubitus interuptus* (*ci*) that express ectopic Hh in the anterior wing form circular, curved interfaces with surrounding A cells; with the extrusion of some clumps of epithelial cells [16]. The simplest mechanism to maintain a straight boundary might be if the A and P cells induce the expression of heterotypic, trans-membrane signalling receptors, restricted to lateral cellular interfaces that are in direct contact. The measured bond-tension between wing disc cells is increased at both the A/P and the D/V boundaries [17] [18] [19]. Increasingly sophisticated methods for inducing marked clones have confirmed the classical studies using the *Minute* technique [10] [14] [20] [21]. In addition, small twin-clones induced late in development may intercalate with surrounding cells [21]. By implication, imaginal disc cells may adjust their position with respect to their neighbours via movements like those driving germ band extension during gastrulation. In this context, the polarised distribution of E-cadherin regulates cell shape (and movement) at embryonic segmental boundaries [22]. Furthermore, several of the core PCP mutants have roles in convergent-extension, in *Drosophila* and vertebrate systems [23] [24] [25] (Wang et al. 2005). Notably, the D/V margin of the wing disc includes a zone of non-proliferating cells, which are qualitatively distinct from the A/P boundary cells [27]. The domineering non-autonomy around *frz* and *pk* clones may cross the A/P boundary, but does not cross the D/V wing margin [28] [29], and unpublished observations. As the pupal wing disc everts, its epithelial surface stretches along the P/D axis of the limb and folds around the D/V margin, as the two surfaces of the adult wing blade come together to form a double-sided sandwich. Taken together, these studies establish that the growth of imaginal discs is regulated within compartmental fields, with extensive remodelling during pupal metamorphosis [1] [2] [30]. The growth of the larval wing disc is regulated via re-deployment of key embryonic functions: including *dpp*, along the A/P boundary; *wingless* (*wg*), around the D/V margin, and *hedgehog* (*hh*), in the posterior compartment [31] [32]. The stages at which A/P boundaries becomes straight and D/V boundaries become smoothly curved, may vary between the different discs; and is delayed until the pupal stage, following the migration of abdominal histoblasts. Compartmental growth constraints may be particularly critical in the wing disc, to ensure an exact match between top and bottom surfaces of the wing, and its precise aerodynamic shape. Notably, the A/P boundary defines the line along which the wing folds during rapid climbing [34], and is the centre of gravity of the flying insect. More generally, morphogenetic twin-fields may be regulated around a D/V axis of mirror symmetry (AMS), with an orthogonal set of A/P AMSs in segmented organisms.

Summary:

The proliferative growth of imaginal discs is regulated within discrete compartments, with undifferentiated epithelial cells maintained separately from the increasingly polytene larval tissues. A narrow lumen separates the convoluted disc surface from a

closely opposed "peripodial" membrane. Discrete embryonic cell clusters fuse to form the eye-antennal, thoracic and genital discs, while the abdominal histoblast nests remain separate until pupal metamorphosis. The cell-lineage discontinuities at compartment boundaries may represent a limiting case, with heterotypic cell-adhesion molecules localised to the interface between separate cell populations. The straight (parasegmental) boundary in the wing disc is qualitatively distinct from the curved (segmental) boundary, which acts as a barrier to PCP signalling between the D and V surfaces. Epithelial cells may intercalate during pupal metamorphosis, before imaginal disc eversion.

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