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Review

Molecular and cellular mechanisms involved in leg joint morphogenesis

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ABSTRACT

In summary, the patterning of the presumptive leg depends on gradients of Dpp and Wg morphogens, which lead to the establishment of the proximo-distal axis marked by the expression of Hth, Dac and Dll in broad domains along the leg. Then, EGFR signaling specifies the tarsal region by regulating the expression of tarsal gap genes in different tarsal segments. This patterning is closely linked to the formation of rings of Notch activation in the distal part of each leg segment. These rings of Notch activation are further regulated by different mechanisms: (1) the maintenance of a sharp border of Dl expression, (2) the inhibition of N activation in cells located proximally to the ligands, thus restricting N activity specifically to the distal part of cells. This localised activation of Notch induces the expression of Dysfusion which controls the expression of both pro-apoptotic genes and RhoGTPase regulators. Finally, apoptotic cells appear within the pro-apoptotic domain, and while dying, generate a transient pulling force. This force constitutes a mechanical signal that propagates to the rest of the tissue and triggers cytoskeleton reorganisation specifically in the presumptive fold, where RhoGTPase regulators are expressed. Altogether, this complex array of patterning and signaling leads to precise cellular mapping of the developing leg to correctly position local cell shape modifications, inducing tissue folding.

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Morphogenesis, or the shaping of tissues, relies on the combination of number of processes including cell rearrangements, cell–cell and cell-matrix adhesion dynamics, cell division, cell death, cell shape changes and cell intercalation [1,2].

During development, presumptive tissues require patterning before any morphogenesis can take place. This includes the establishment of tissue coordinates, which depend on axis positioning. Then, a complex network of cell signaling defines different domains within a tissue, at which stage well orchestrated cell shape remodeling can occur. Among the different types of cell–cell signaling, mechanical signals were recently found to play an important role in tissue morphogenesis.

Here, I summarize the information we have on leg joint formation in Drosophila, from patterning of the presumptive leg to the morphological consequences arising at the cellular level, focusing on the very first steps of joint morphogenesis which result in the generation of folds in the developing leg.

1. Determination of presumptive joint domains

The Drosophila leg is a segmented appendage composed of nine different segments articulated by joints. Joints consist of multicellular patterned features present between each segment [3]. The segmentation pattern of the leg is first visible in the developing leg that progressively divides into concentric segments bounded by constrictions. These constrictions, or folds, appear during third instar larvae and prepupal stages and are driven by the sequential activation of the Notch pathway in restricted domains along the proximo-distal axis of the leg [4]. This first wave of cell rearrangement is followed by a more complex cellular reorganisation occurring later during pupal stage and leading to the formation of the adult structure [5]. Adult joints differ from segments in that they contain intersegmental membrane and are devoid of bristles. Joint morphology varies from joint to joint with true joints (proximal joints from the coxa to the tibia–tarsus joint, plus the tarsus-pretarsus joint) clearly distinguishable from more distal joints (the tarsal joints). Tarsal joints are identical to one another and are composed of a "socket" in the proximal part and an interlocking "ball" in the distal part. Although the regulatory networks and resulting adult structures are different, Notch (N) is involved in the segmentation of the whole leg. Indeed, in N mutant contexts, all types of leg joints are absent. Reciprocally, ectopic activation of N leads to the formation of ectopic joint-like structures [6–8]. As such, the N pathway represents a crucial regulator of joint formation.

More generally, the N pathway appears recurrently used to establish boundaries. In the fly, besides its involvement in leg segmentation, N establishes the wing margin and wing veins; a role that is conserved during evolution since N is also known to establish somite borders in vertebrates [9]. Interestingly, it has been proposed that differential levels of Notch activity could be responsible for the distinction in joint morphology among species [10].

Historically, the huge number of works aimed at characterising the process of segmentation of the developing leg were focused on the role of N in leg joint differentiation and the molecular mechanisms involved in the regulation of N signaling. It is only recently that the complex network involved in the cellular rearrangement occurring during leg folding has been tackled, a first step that seems essential for the correct segmentation of the adult leg.

1.1. Establishment of the proximo-distal axis

The first step identified so far in the process of joint formation is the localised expression of ligands Serate (Ser) and Delta (Dl), which leads to the activation of the transmembrane receptor N in a row of cells in the distal part of each segment.

The localised activation of N in the developing leg depends on the establishment of the proximo-distal axis of the leg that relies on the production of two ligands from the Wnt and transforming growth factor β signaling pathways, Wingless (Wg) and Decapentaplegic (dpp). These two morphogenes are expressed respectively in the most ventral and most dorsal sector of the disc. The diffusion of Wg and Dpp forms a concentric gradient with high levels of both proteins in the center and low levels at the periphery. This gradient of Wg and Dpp creates discrete ring-shape domains of expression of Hth, Dac and Dll along the newly formed proximo-distal axis. Leg development is dynamic and the expression profiles of these genes change as development proceeds [11–13]. At second instar, the leg disc is divided into two domains: a proximal domain defined by Hth expression and a distal domain defined by Dll expression. At early third instar (~72 h after egg-laying, AEL) a population of cells expressing Dac arise that lie at an intermediate position between the Dll- and Hth-expressing cells. At mid third instar (~96 h AEL) clear overlap between the Dll and Dac expression domains is visible. Finally, by late third instar (~120 h AEL), there is a thin band of cells expressing all three genes, corresponding to the future trochanter. These genes are considered as the leg gap genes [11] (see Fig. 1).

The subdivision of the leg in different territories along the proximo-distal axis is further implemented by the segmentation of the tarsus. The adult tarsus is divided into five segments (t1 to t5, from proximal to distal) at the end of which is the pretarsus that is characterised by a pair of claws. Several homeobox genes are expressed in distinct regions of the tarsus, including *aristaless* (*al*), *C15* and *dLim1* in the pretarsus, *Bar* (*B*) in t4 and t5, and *apterous* (*ap*) in t4, and finally *tarsalless* (*tal*), *spineless* (*ss*), *rotound* (*rn*) and *bric à brac* (*bab*) in the t1–t4 region. Patterning along the proximo-distal axis of the tarsus is controlled by a distal-to-proximal gradient of EGF-receptor (EGFR) signaling activity, established by a source of ligands in the center of the leg imaginal disc, corresponding to the presumptive tip of the adult appendage [14–16]. EGFR signaling is initiated in early third instar larvae by expression of the secreted ligand Vein (Vn), induced by Wg and Dpp in the central region of leg disc from 72 h to 96 h AEL [14,15,17]. Shortly after, the protease Rhomboid (Rho, required for processing and secretion of EGFR membrane-bound ligands) is expressed at low levels in the distal tip [17], suggesting that although Vn seems to be the essential player in EGFR activation, membrane bound ligands of the EGF signaling pathway are also involved in tarsus development. High levels of EGFR signaling are required to activate expression of the distal-most genes *al, C15* and *dLim1* which are required for the development of the claws and are expressed in the very center of the leg disc. In contrast, progressively lower levels are sufficient to activate more proximally expressed genes, such as *B, ap, ss, rn* and *bab* [14–17]. Regulatory interactions between these genes, together with EGFR signaling, define the pattern leading to the subdivision of the tarsus (see Fig. 1).

Thus, the proximo-distal axis is established by a combination of signals: first Dpp and Wg induce the regionalisation of the leg through the regulation of *Hth, Dac* and *Dll*, then EGFR specifies the tarsus region through the regulation of *ss, rn, bab, Bar, ap, Al, C15* and *dLim1*.

1.2. Formation of Notch activation rings

It has been suggested that distinct combinations of these proximo-distal patterning genes independently regulate each segmental ring of Notch ligand through different enhancers [18]. This regulation could explain that leg segmentation does not occur in a simple distal to proximal order, nor proximal to distal order, but rather shows a more complicated temporal sequence that could fit with the progressive modification of gap gene expression patterns. Indeed, the spatial and temporal expression of these different