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The role of Dpp signaling in maintaining the *Drosophila* anteroposterior compartment boundary

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Abstract

The subdivision of the developing *Drosophila* wing into anterior (A) and posterior (P) compartments is important for its development. The activities of the selector genes *engrailed* and *invected* in posterior cells and the transduction of the Hedgehog signal in anterior cells are required for maintaining the A/P boundary. Based on a previous study, it has been proposed that the signaling molecule Decapentaplegic (Dpp) is also important for this function by signaling from anterior to posterior cells. However, it was not known whether and in which cells Dpp signal transduction was required for maintaining the A/P boundary. Here, we have investigated the role of the Dpp signal transduction pathway and the epistatic relationship of Dpp and Hedgehog signaling in maintaining the A/P boundary by clonal analysis. We show that a transcriptional response to Dpp involving the T-box protein Optomotor-blind is required to maintain the A/P boundary. Further, we find that Dpp signal transduction is required in anterior cells, but not in posterior cells, indicating that anterior to posterior signaling by Dpp is not important for maintaining the A/P boundary. Finally, we provide evidence that Dpp signaling acts downstream of or in parallel with Hedgehog signaling to maintain the A/P boundary. We propose that Dpp signaling is required for anterior cells to interpret the Hedgehog signal in order to specify segregation properties important for maintaining the A/P boundary.

Keywords: *Drosophila*; Imaginal disc; Compartment boundary; Cell segregation; Decapentaplegic; Optomotor-blind; T-box

Introduction

Cell adhesion is fundamental for the development of multicellular organisms. However, cells do not simply adhere to one another randomly. For example, when disaggregated frog embryos were allowed to reaggregate, cells segregated out and reestablished the layers to which they initially belonged (Townes and Holtfreter, 1955). This property of cells to selectively aggregate with some cells and to segregate out from others was termed cell affinity (Garcia-Bellido, 1966, 1972; Holtfreter, 1939). The underlying cell biological mechanisms of this cell behavior and the molecular nature of cell affinity remain poorly understood.

One system for studying the mechanisms underlying the segregation of cells during development is the formation of lineage boundaries that subdivide a number of vertebrate and insect tissues into groups of non-intermingling cells termed compartments (Blair, 2003; Dahmann and Basler, 1999; Irvine and Rauskolb, 2001; McNeill, 2000; Tepass et al., 2002; Vincent, 1998). Signaling across boundaries between adjacent compartments can lead to the local production of long-range signaling molecules that organize growth and patterning of the entire tissue (Lawrence and Struhl, 1996). The continuous segregation of cells at compartment boundaries is therefore important for the positioning and maintenance of such organizers and is crucial for the patterning of tissues.

The developing *Drosophila* wing is subdivided by two compartment boundaries. An early-arising compartment boundary separates anterior (A) and posterior (P) cells and a late-arising compartment boundary separates dorsal (D)

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and ventral (V) cells (Bryant, 1970; Garcia-Bellido and Merriam, 1971; Garcia-Bellido et al., 1973). It was originally proposed that the segregation of cells at these two compartment boundaries depends on compartment-wide cell affinities controlled by the activity of selector genes (Garcia-Bellido, 1975; Garcia-Bellido et al., 1973). However, more recently, it has become clear that signaling across compartment boundaries is at least equally important for segregating cells at these boundaries.

The homeobox transcription factors encoded by *engrailed* (*en*) and *invected* (*inv*) are expressed in P cells and act as selector genes for the P compartment (Brower, 1986; Coleman et al., 1987; Kornberg et al., 1985; Lawrence and Morata, 1976; Morata and Lawrence, 1975; Poole et al., 1985). Clonal analysis has shown that P cells lacking En and Inv activity often no longer segregate at the A/P boundary with P cells, but instead intermingle with A cells (Blair and Ralston, 1997; Hidalgo, 1994). Conversely, A cells ectopically expressing En, if in contact with P cells, segregate into the P territory (Dahmann and Basler, 2000). En regulates cell segregation mainly by controlling the signaling of the secreted molecule Hedgehog (Hh). In P cells, En both facilitates the expression of Hh and represses the transcription of the Zn-finger transcription factor Cubitus interruptus (Ci), an essential component of the Hh signal transduction pathway (Dominguez et al., 1996; Eaton and Kornberg, 1990; Tabata et al., 1992). Thus, P cells produce Hh but cannot respond to it. In contrast, A cells express Ci and can respond to Hh secreted from P cells. One response to this unidirectional signaling of Hh from P to A cells is the specification of an A cell affinity required to maintain the segregation of cells at the A/P boundary. Anterior cells lacking the function of the seven-pass transmembrane protein Smoothened (Smo), and hence the ability to transduce the Hh signal (Alcedo et al., 1996; van den Heuvel and Ingham, 1996), no longer segregate with A cells but instead segregate into P territory (Blair and Ralston, 1997; Rodriguez and Basler, 1997). This control of cell segregation by Hh signaling requires the transcription factor Ci, indicating that Hh controls A/P cell segregation by regulating the transcription of target genes (Dahmann and Basler, 2000). Hh signaling is not only necessary, but also sufficient to control cell segregation. P cells ectopically expressing Ci, and thus activating the Hh pathway, segregate into the A territory (Dahmann and Basler, 2000). Recently, two subunits of the *Drosophila* mediator complex, Skuld (Skd) and Kohtalo (Kto), have been shown to be required for the normal segregation of cells at the A/P boundary (Janody et al., 2003). It has been proposed that Skd and Kto assist Ci to regulate some of its target genes, including those involved in cell segregation. Despite several efforts (e.g., Vegh and Basler, 2003), Hh target genes required for the segregation of cells at the A/P compartment boundary have not been identified.

Signaling across the A/P boundary is also bidirectional. In response to Hh, a narrow stripe of cells along the A side of the A/P boundary produces the long-range signaling molecule Decapentaplegic (Dpp), a member of the TGFβ superfamily (Masucci et al., 1990; Padgett et al., 1987). Dpp acts as a morphogen by specifying cell fates in both compartments along the A/P axis in a concentration-dependent manner (Lecuit et al., 1996; Nellen et al., 1996). To direct precise patterning, the shape of the source of the Dpp morphogen must be stably maintained and the continuous segregation of cells at the A/P boundary may contribute to this.

Dpp signals through a Ser/Thr kinase receptor complex including the type I and II receptors Thickveins (Tkv) and Punt, respectively (Brummel et al., 1994; Letsou et al., 1995; Nellen et al., 1994; Penton et al., 1994; Ruberte et al., 1995). The binding of Dpp to its receptors induces Punt to phosphorylate Tkv which in turn phosphorylates the transcription factor Mothers against dpp (Mad) (Raftery and Sutherland, 1999; Tanimoto et al., 2000). Phosphorylated Mad enters the nucleus and, in concert with the Zn-finger protein Schnurri (Arora et al., 1995; Grieder et al., 1995), represses the transcriptional repressor Brinker (Brk) (Campbell and Tomlinson, 1999; Jazwinska et al., 1999; Minami et al., 1999; Muller et al., 2003). As a consequence, the extracellular Dpp gradient is converted into an inverse gradient of a transcriptional repressor. Brk, in a concentration-dependent manner, negatively controls the expression of Dpp target genes including *spalt-major* (*salm*), *spalt-related* (*salr*) (two neighboring and functionally related genes referred to in the following as *sal*), and *optomotor blind* (*omb*), which encodes a member of the T-box family of transcription factors (Campbell and Tomlinson, 1999; Jazwinska et al., 1999; Minami et al., 1999; Pflugfelder et al., 1992). As a consequence, *sal* and *omb* are expressed in nested regions centered around the Dpp expression domain with the *omb* expression domain being broader than the *sal* expression domain (Grimm and Pflugfelder, 1996; Sturtevant et al., 1997).

The current model presented above assumes that signals controlling A/P cell segregation are exclusively unidirectional from P to A cells. Based on the findings that A cells signal back to P cells via Dpp (Lecuit et al., 1996; Nellen et al., 1996) and that wings from flies hypomorphic for *dpp* have a distorted A/P boundary (Hidalgo, 1994), it has been proposed that A and P cells are specified for their segregation behavior by P to A and A to P signaling, respectively, and that Dpp might be the A to P signal involved (Blair and Ralston, 1997; Vincent, 1998). However, it was not known whether and in which cells Dpp signal transduction is required for maintaining the segregation of cells at the A/P boundary. Here, we have addressed these questions by analyzing the segregation of marked clones of cells unable to transduce the Dpp signal at the A/P boundary. We find that an Omb-mediated transcriptional response to Dpp is required in A cells but not in P cells to maintain the A/P boundary. Thus, our results do not support the proposal that Dpp signaling from A to P cells is required