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Compartment boundaries: where, why and how?

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ABSTRACT In the 1960's, Garcia Bellido and colleagues uncovered the existence of developmental compartments in *Drosophila*. This observation has had a lasting impact on our understanding of developmental mechanisms in flies and vertebrates. Here, I review the work that demonstrated the existence of compartments. I then conjecture on why compartments exist and review the roles of various gene products in the maintenance of compartment boundaries.

KEY WORDS: *compartments, engrailed, clonal analysis,embryonic boundaries*

Introduction

A recent model for pattern formation draws heavily on recent experiments, demonstrating the role of compartments in *Drosophila* imaginal disks (Lawrence and Struhl, 1996). Key to the model is the existence of two distinct populations of cells: one producing a signal to which they cannot themselves respond and a second population that responds to the signal by producing a morphogen patterning much of the appendage. At the root of this model lies the basic finding by García-Bellido and colleagues that imaginal disks are compartmentalized; a discovery they made by studying the fate of marked cells in the developing fly. Their work used a technique whereby a recombination event induced by X-rays genetically marks single cells and their progeny. In 1968, García-Bellido reported that clones induced by this technique in the first larval instar, before any significant growth has occurred, never cross the dorso-ventral boundary of the wing (García-Bellido, 1968). This was then not considered extraordinary since this boundary corresponds to the wing margin, a well defined morphological landmark. However, a major technical innovation (the minute technique; e.g., Morata and Ripoll, 1975) uncovered the existence of another boundary which would have been very difficult to detect otherwise. The minute technique enables the induction of clones that have a growth advantage over neighboring cells (marked wild type cells growing at a normal rate among Minute/+ background cells which grow slowly). Using this technique, García-Bellido *et al.* (1973) unambiguously demonstrated the existence of a boundary that subdivides the wing primordium into anterior and posterior domains which they called compartments. Although dorso-ventral (D-V) compartmentalization was discovered first, the separation into anterior and posterior (A-P) compartments occurs first in development, during early embryogenesis. Subsequent experiments demonstrated that compartmentalization is a feature of other imaginal disks as well. The questions of how and why quickly arose.

Already in the 1960s, a mutation called engrailed was known to transform partially posterior structures into anterior ones (Tokunaga, 1961) suggesting that the *engrailed* gene product might be required to separate anterior from posterior cells. Indeed, Morata and Lawrence (1975) showed that posterior cells homozygous for the *engrailed*^{1} allele fail to respect the boundary (see also Kornberg, 1981; Lawrence and Struhl, 1982). A key gene required for A-P compartmentalization had thus been identified only two years after A-P compartmentalization itself had been discovered. It was proposed that *engrailed* was only active in the posterior compartment (anterior *engrailed*^{1} cells still respect the boundary) and that somehow, maybe by controlling cell affinities, *engrailed* activity labels posterior cells such that they do not mix with anterior ones. (It is amusing that even before the discovery of the A/P compartments, García-Bellido and Santamaria (1972) had already attempted to study cell affinity among *engrailed*^{1} mutant cells). Part of the model of *engrailed* function was also that it is an indelible marker of posteriorness; that is, its expression would be clonally maintained. Twenty years later, molecular cloning of *engrailed* and detection of its product in posterior cells confirmed that *engrailed* acts in the posterior compartment (DiNardo *et al.,* 1985; Fjose *et al.,* 1985; Kornberg *et al.,* 1985). However, the role of *engrailed* as a direct regulator of genes controlling cell affinities is currently being revised (see below). Also, although *engrailed* expression appears to be stably maintained during larval stages, there is an early period during embryogenesis when expression is unstable.

Compartment boundaries: from embryos to adults

The A-P boundary is established during early embryogenesis. In fact early evidence from García-Bellido and colleagues (García-Bellido *et al.,* 1973) showed that this occurs no later than after the first

Abbreviations used in this paper: A-P, Anterior-Posterior; D-V, Dorso-Ventral.

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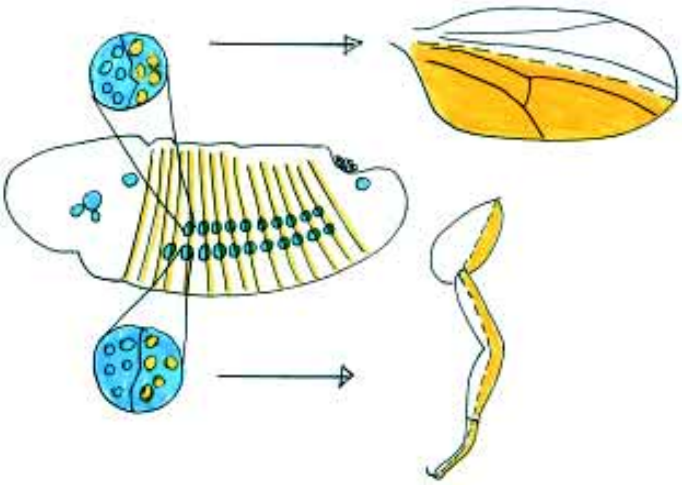

Fig. 1. The A-P compartment boundary in imaginal disks traces its origin in early embryo. *Dark lines in the embryo represent the parasegment boundaries (engrailed is expressed in stripes located posteriorly to the boundaries; in orange). It is believed that presumptive imaginal disks (or histoblast nests; in blue) include both engrailed-expressing and non-engrailed-expressing cells and this is maintained throughout growth and patterning of the disks up to the formation of the adult appendages.*

post blastoderm division. The original model of *engrailed* function suggested that, at this early time, *engrailed* expression would be stably maintained. With P. O'Farrell, I have tested this idea (Vincent and O'Farrell, 1992). At the same time we assessed the exact timing of clonal restriction at the A-P border. Using caged fluorescent dyes, we mapped the descendants of single blastoderm cells relative to the stripes of *engrailed* expression. These experiments confirmed that, already at the blastoderm stage, there is a boundary of clonal restriction at the anterior edge of the *engrailed* stripes (the compartment boundary; Fig. 1). But they also showed that maintenance of the boundary does not follow simply from a lineage mechanism. Not all en*grailed*-expressing cells maintain expression stably. In a way this was expected since, by then, *engrailed* expression was known to require a signal, Wingless, secreted by neighboring cells (clearly a non-cell-autonomous mechanism; DiNardo *et al.,* 1988; Martinez-Arias *et al.,* 1988). Indeed, cells at the posterior end of *engrailed* stripe do loose expression if they lie too far from the *wingless* expressing cells (Vincent and O'Farrell, 1992). Considering the requirement for *wingless* and the asymmetry of the Wingless source, the existence of a clonal boundary during early embryogenesis may seem trivial. However, somehow the interface between these two cell populations must be special: it appears straight when compared to the posterior edge of *engrailed* stripes and also, it is there that parasegment grooves (indentation of the epidermis) form at around stage 11. Clearly there must be some interesting cell biology occurring at this interface.

In accordance with the original model, *engrailed* expression does become stable later in embryogenesis; the requirement for *wingless* activity subsides around stage 11, before germ band retraction (Bejsovec and Martinez-Arias, 1991; Heemskerk *et al.,* 1991). It is believed that this stable expression is then carried through imaginal disk growth and patterning. Thus, as disks are being specified in the embryo, a small number of *engrailed*-expressing and non-*engrailed*-expressing cells are put aside (Cohen, 1993; Martinez-Arias, 1993). These two populations of cells expand during disk growth and throughout this time they maintain their state of *engrailed* expression. Clonal maintenance of gene expression is generally thought to follow from a cell autonomous mechanism (although this is not necessarily the case) and all evidence points that this is true for *engrailed* after the initial *wingless*-dependent period. But the compartment boundary is a signaling center: in the *Drosophila* wing, posterior, *engrailed*-expressing cells secrete Hedgehog to which only anterior cells can respond (Basler and Struhl, 1994; Tabata and Kornberg, 1994). Since Hedgehog acts at a short range, only a narrow band of cells respond and, as a consequence, begin to express *decapentaplegic (dpp)*. The Dpp product is believed to pattern much of the wing disk (in both anterior and posterior directions; Zecca *et al.,* 1995; Lecuit *et al.,* 1996). Since *dpp* expression continuously requires the Hedgehog signal (Nellen *et al.,* 1996), it could be that the maintenance of the boundary depends in part on these exchanges of signals (see below).

Why have compartment boundaries?

In discussing what compartment boundaries are for, one can only conjecture. Clearly, one important role for a stable compartment boundary relates to homeotic gene function. The state of expression of such genes and their realm of action respects the compartment boundaries (e.g., Struhl, 1984). To ensure that no cell expresses a given homeotic gene in the wrong compartment, it is essential that the boundary of homeotic gene expression always co-aligns with the compartment boundary (see, Lawrence, 1992). This will be achieved if homeotic gene expression is stably maintained through cell division and if compartment boundaries are clonally maintained, although one cannot exclude mechanisms based on mutual cell interactions. In fact, patterns of homeotic gene expression do change during development, suggesting that non-clonal mechanisms are at work and hence that the basis of the coincidence between compartment boundaries and the zones of homeotic gene action is more complex than anticipated (see for example Martinez-Arias, 1993)

Compartments have also been proposed to act as units of growth control but so little is known about how growth and cell divisions are regulated during imaginal disk development that this proposal still seems abstract and will not be discussed further here. This promises nevertheless to constitute a fertile ground for future investigation (e.g., Weigmann *et al.,* 1997, see also García-Bellido and García-Bellido, this issue).

Another function for a stable boundary could be to provide a link between the embryo and the adult. Presumptive imaginal disks contain only about 20 cells at embryonic stages (Cohen, 1993, Martinez-Arias, 1993). It is important that both anterior and posterior cells are included in any one disk such that an antero-posterior axis is passed on to the adult and that this axis is oriented coordinately with the rest of the animal. This would be achieved by including *engrailed*-expressing and non-expressing cells in the disk primordium (the mechanism of disk specification ensures this) and by maintaining these two populations of cells in a stable clonal fashion. Of course, non-clonal mechanism could achieve this too, provided that the boundary is stable.

Are compartments a universal feature of animal design?

If compartment boundaries are only a bridge between the embryo and the adult in metamorphosing insects, they would seem of limited

universality. Of course, in any embryo, one expects that at given developmental stages, certain cells should not mix with others. For example, the separation between rhombomeres in vertebrates could correspond to compartmentalization as defined in flies. The boundaries between rhombomeres are a barrier to cell mixing and correspond to boundaries of *Hox* gene expression (Fraser *et al.,* 1994). But, contrary to the situation in *Drosophila*, clonal restriction at these boundaries is not absolute (Birgbauer and Fraser, 1994). Also, the boundaries of *Hox* gene expression are initially fuzzy and become sharp only when morphological segmentation takes place (see Lumsden and Krumlauf, 1996 for a review). So, there are similarities between the situations in flies and vertebrates but they may not be exact and actual mechanisms of compartmentalization might differ (see below the molecules involved). It might be worth developing, in vertebrate embryos, a method similar to the minute technique to find out whether other clonal boundaries exist there, and possibly to uncover units of growth.

One gene does it all?

Much has been made of the key role of Engrailed in distinguishing posterior from anterior cells. The most important observation is that clones of cell lacking *engrailed* function no longer respect the boundary (Morata and Lawrence, 1975; Fig. 2). When clones are given a growth advantage, as with the minute technique, wild type posterior clones often fill most of the posterior compartment. If their only restriction to grow into the anterior compartment were due solely to e*ngrailed* activity, one would expect clones lacking *engrailed* function to fill most of the wing in a minute experiment. This is not seen (Morata and Lawrence, 1975). Initially this negative result was discounted because the clones lacking *engrailed* function still had the activity of the highly homologous gene *invected*. Although this gene is normally dispensable, it was thought to partially "fill in" for *engrailed* in the *engrailed*- clone. However, Hidalgo (1994) subsequently reported that *engrailed invected* double mutant clones do not fill the wing disc even with the minute technique, suggesting that other gene products contribute significantly to compartmentalization. Which are these genes and what is the cell biology behind compartmentalization? These questions are now ripe for the picking.

In the past, most of the attention has been paid on the importance of posterior cells in the maintenance of the A-P boundary since those are the ones that express *engrailed*. Two recent papers (Blair and Ralston 1997; Rodriguez and Basler, 1997) have focused on the role of anterior cells. They reasoned that, in addition to acting in the posterior compartment, engrailed might also affect the behavior of cells at the other side of the boundary. This influence would be mediated by Hedgehog (which is secreted by *engrailed*-expressing cells. In both papers, anterior clones of cells were prevented from responding to Hedgehog by being mutant for *smoothened*. Such clones end up on the wrong side of the border, in the posterior territory, clearly indicating a role for the Hedgehog pathway in boundary maintenance (Fig. 2). Blair and Ralston report that these cells do not behave exactly like posterior cells: they do not mix readily with their new neighbors as wild type clones do. One suggestion they make is that maybe anterior *smoothened* clones are pushed (unwillingly) into the posterior territory without acquiring "posterior characters" (normally imparted by *engrailed* expression). Therefore, there may be two mechanisms at work. One would require *engrailed* expression to ensure maximal affinity among posterior cells and the

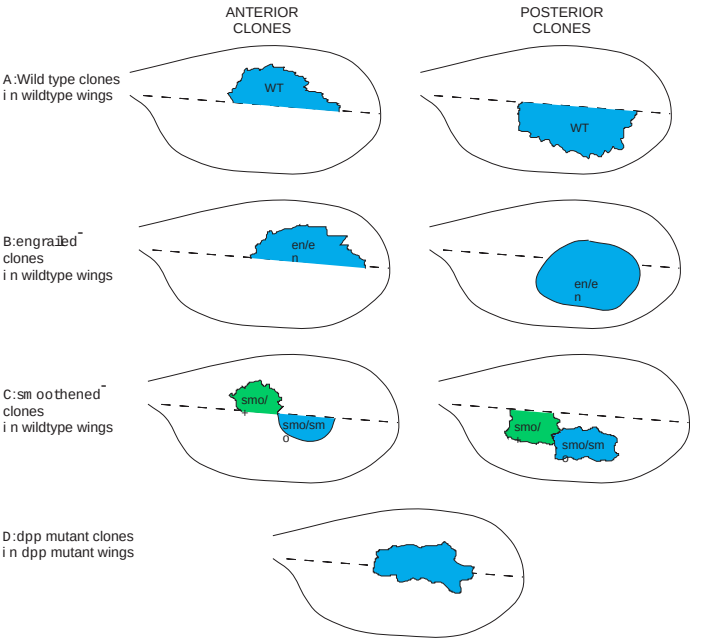

Fig. 2. Diagrammatic behavior of various types of clones. (A) *Wild type clones in either compartment respect the compartment border (based on Garcia-Bellido et al., 1973). Clone boundaries are shown wiggly to indicate that cells intermingle normally (except at the A-P border) irrespectively of their clonal origin.* **(B)** *Anterior engrailed mutant clones respect the compartment border while posterior engrailed mutant clones do not, at least sometimes (loosely based on Morata and Lawrence, 1975; Hidalgo, 1994; and Blair and Ralston, 1997). The engrailed mutant clone of posterior origin shown here straddles the border but some of these clones are found entirely in the anterior compartment (not shown, see Blair and Ralston). The boundary of posterior clones is shown smooth to indicate that the cells of the clones do not intermingle normally with either anterior or posterior cells.* **(C)** *Anterior clones mutant for smoothened are "pushed" into the posterior compartment; the compartmental origin of the clone is known from the position of the "wild type" twin (based on Blair and Ralston, 1997; and Rodriguez and Basler, 1997). Again the edge of mutant clone is not ragged (according to Blair and Ralston, 1997). Clones in a viable dpp mutant combination no longer form a straight boundary at the A-P border (after Hidalgo, 1994). The experiment on which this diagram is based does not allow the identification of the clone's compartmental origin.*

other (in response to Hedgehog) would specify a specialized band of cells to form a barrier along the boundary. The maintenance of *engrailed* expression would be cell autonomous (and this would guarantee coordinate expression with homeotic genes) while signaling mechanisms would create a relatively straight barrier. This latter aspect could be under the direct influence of Dpp: Hidalgo (1994) has reported that in one viable *dpp* allele, the boundary is no longer straight and well defined.

Dpp is not required at all compartment boundaries. Other candidates are becoming worthy of attention. For example, at the D-V boundary in wing imaginal disks, both Wingless and Notch are active (e.g., Couso and Martinez-Arias, 1994) and either could potentially be involved in boundary maintenance (*dpp* is not expressed there). Notch and its ligand, Delta have been shown to mediate cell adhesion in tissue culture cells (Fehon *et al.,* 1990), an activity which could conceivably prevent cell mixing at the boundary. Wingless also could be modulating cell adhesion since it controls the stability of Armadillo,

the fly homolog of β-Catenin (Peifer *et al.,* 1994; van Leeuwen *et al.,* 1994). Further work is needed to investigate the role of the adhesion activity (if any) of either of these gene products at the D-V boundary. Parasegment grooves of the early *Drosophila* embryo are another interesting boundary to study. Again, dpp is almost certainly not involved. In contrast, Wingless is required since the grooves do not form in *wingless* mutant embryos (Lawrence and Vincent, unpublished). Again, it will be interesting to find out how *wingless* might control, directly or indirectly the cell behavior which leads to these epithelial indentation. In vertebrate embryos, a completely different class of molecules are thought to keep cells separate at the rhombomere boundaries. These are the receptor tyrosine kinases of the Eph family. Some of these family members are expressed in alternating segments and expression of a dominant negative Eph member leads to a breakdown of the boundaries (e.g., Xu *et al.,* 1995). So far no role for an Eph like molecule in boundary maintenance has been ascribed in *Drosophila*. In summary, our molecular understanding of boundary maintenance is still sketchy but the gene products mentioned in this paragraph provide a lead for more detailed studies.

Conclusion

As initially demonstrated by García-Bellido and colleagues, boundaries of clonal restriction exist at various places in the developing fly, most notably at the D-V and A-P boundaries in imaginal disks and at the parasegmental borders in embryos. Clonal boundaries may be a common feature of animal design although the role of compartments in vertebrates remains incompletely explored. So far the molecular mechanisms for boundary maintenance do not seem conserved. In *Drosophila*, *engrailed* has long been the only gene clearly required for boundary maintenance. More candidates have now emerged and we can look forward to a cell biological understanding of how cell populations are kept separate during development.

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