

BOUNDARIES IN DEVELOPMENT: Formation and Function

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■ **Abstract** Developing organisms may contain billions of cells destined to differentiate in numerous different ways. One strategy organisms use to simplify the orchestration of development is the separation of cell populations into distinct functional units. Our expanding knowledge of boundary formation and function in different systems is beginning to reveal general principles of this process. Fields of cells are subdivided by the interpretation of morphogen gradients, and these subdivisions are then maintained and refined by local cell-cell interactions. Sharp and stable separation between cell populations requires special mechanisms to keep cells segregated, which in many cases appear to involve the regulation of cell affinity. Once cell populations become distinct, specialized cells are often induced along the borders between them. These boundary cells can then influence the patterning of surrounding cells, which can result in progressively finer subdivisions of a tissue. Much has been learned about the signaling pathways that establish boundaries, but a key challenge for the future remains to elucidate the cellular and molecular mechanisms that actually keep cell populations separated.

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INTRODUCTION

Compartments and Developmental Boundaries

In the early 1970s, new techniques for creating genetic mosaics in *Drosophila* revealed a smooth, linear boundary restricting cell behavior that did not correlate with any morphologically visible landmarks (García-Bellido et al. 1973). The proliferation of the cells that will secrete the cuticle of the adult fly is not governed by a strict cell lineage. Hence, in most cases, the descendants of a single cell that has been genetically marked (a clone) will contribute to variable and irregularly shaped regions of the adult (Figure 1A). However, across the middle of each segment there is a boundary of cell lineage—the anterior-posterior (A-P) compartment boundary. Clones of cells do not cross this boundary, but instead remain restricted to either the anterior or posterior side of the compartment boundary, and form a straight line where they contact it (Figure 1A) (García-Bellido et al. 1973).

Formally, any time different cell types are juxtaposed, a boundary exists. But the revelation of the A-P compartment boundary established a new paradigm in which the subdivision of cells into distinct populations could be viewed as a cause, rather than a consequence, of their development (García-Bellido 1975, García-Bellido et al. 1979). The lack of correlation with visible landmarks suggested that the cell lineage restriction at the A-P compartment boundary reflected the establishment of fundamental differences between adjacent, undifferentiated cells, rather than being a secondary consequence of cell movements during morphogenesis or phenomena associated with differentiation. Moreover, the identification of A-P compartments was soon followed by the realization that important developmental control genes (e.g., *engrailed* and *Bithorax-complex* genes) operated within compartmental units. This led to the concept of boundaries between cell populations serving to delimit the domain of action of genes that specify cell fate (termed selector genes). Finally, the identification of further, successive, compartmental subdivisions [e.g.,

dorsal-ventral (D-V), wing-notum] suggested that cell fate could be specified by a combinatorial, binary code, with cells in different regions of an animal each having a unique genetic address. For example, posterior dorsal wing would be specified by the overlapping expression of a posterior selector gene, a dorsal selector gene, and a wing selector gene.

An equally important concept of boundaries was first elaborated upon in the theoretical work of Meinhardt (Meinhardt 1983a,b). The adult tissues of *Drosophila* develop from clusters of undifferentiated cells termed imaginal discs. Taking into consideration both the compartmentalization of *Drosophila* imaginal discs and their regenerative properties, Meinhardt proposed that compartment boundaries are reference points for positional information during development and regeneration, presumably by serving as sites of morphogen synthesis. He also proposed that boundaries would play similarly important roles in the development of tissues in other animals. To a significant degree, Meinhardt's boundary model has been confirmed by experimental work over the past decade. In addition, this experimental work has made it possible to establish a central dogma for tissue patterning (Lawrence & Struhl 1996). First, a tissue is subdivided into compartments by the interpretation of morphogen gradients and the regulation of selector gene expression. Subsequently, short-range signaling between compartments establishes compartment border cells, and then long-range signals emanating from the compartment border cells regulate the further growth and patterning of the tissue. Although this scheme was developed based on studies of imaginal discs in *Drosophila*, its broad relevance is confirmed by an ever-increasing number of examples in both invertebrates and vertebrates.

This review is devoted to a consideration of the formation and function of developmental boundaries, which we define operationally as boundaries between adjacent cell populations that are instructive with respect to shaping their future development. This encompasses two distinct concepts of boundaries, although in many cases both occur in the same tissue: (a) the subdivision of cells into groups that are the domains of action of regulatory genes and (b) the establishment of distinct fates along the borders themselves. Excellent reviews on compartmentalization have been published recently (Dahmann & Basler 1999, McNeill 2000), and this review is distinguished by its wider scope and inclusion of more recent data from this rapidly advancing field. The wide variety of situations in which boundaries play important roles provides an opportunity to begin to identify common themes in boundary formation and function, and our main goal here is to highlight these general principles.

FROM GRADIENTS TO DISTINCT DOMAINS OF GENE EXPRESSION

Interpreting Morphogen Gradients

In many systems, the process of subdividing a uniform field of cells into distinct cell populations appears to follow an analogous series of steps (Figure 2)

(Lawrence & Struhl 1996). In the first step, a gradient of positional information (a morphogen) is converted into discrete domains of gene expression. This involves distinct downstream target genes with promoters that differ in their sensitivity to the morphogen. Different thresholds of sensitivity result in spatial pattern because cells closest to the morphogen source receive a higher signal than cells farther away. A common second step then appears to be the refinement of the initial pattern through cross-regulatory interactions. For example, genes activated near the morphogen source repress the expression of genes activated farther away. This effectively creates sharp boundaries between cells with different profiles of gene expression and results in distinct, largely complementary sets of genes expressed near to and far from the morphogen source. The first well-documented example of a morphogen gradient that is interpreted to give discrete domains of gene expression was the Bicoid gradient (Driever et al. 1989, Struhl et al. 1989), which sets up the A-P patterning of the early *Drosophila* embryo. This early embryo is a large syncytium, and the Bicoid gradient is established by intracellular diffusion of Bicoid protein within the syncytium. Later, intercellular morphogen gradients of Wingless (WG, a Wnt signal) and Decapentaplegic (DPP, a TGF- β family signal) were identified within *Drosophila* imaginal discs (Lecuit et al. 1996, Nellen et al. 1996, Neumann & Cohen 1997, Zecca et al. 1996).

PATTERNING THE DORSAL-VENTRAL AXIS OF THE VERTEBRATE NEURAL TUBE An elegant example of the interpretation and refinement of a morphogen gradient in vertebrates has come to light from studies of patterning along the D-V axis of the neural tube (Briscoe et al. 2000, reviewed in Goulding & Lamar 2000). The secreted signaling molecule Sonic hedgehog (Shh) is expressed in the ventral neural tube in the notochord and floorplate and forms a morphogen gradient. Shh regulates the expression of different classes of homeodomain transcription factors in the neural tube. *Pax7*, *Dbx1*, *Dbx2*, *Pax6*, and *Irx3* (class I genes) are repressed by Shh signaling, whereas *Nkx2.2* and *Nkx6.1* (class II genes) are activated by Shh signaling. Thus class II genes are expressed in more ventral regions of the neural tube, and class I genes are expressed in more dorsal regions. Different class I and II genes have different sensitivities to Shh, resulting in nested expression patterns. For example, both *Nkx2.2* and *Nkx6.1* are induced by Shh, but a higher Shh concentration is required to activate *Nkx2.2* than *Nkx6.1*. Thus both genes are expressed in the most ventral regions of the neural tube, but only *Nkx6.1* is expressed in more lateral regions. Importantly, class I and class II proteins expressed in adjacent domains negatively cross-regulate each other's expression. This presumably maintains sharp boundaries of gene expression between populations of cells expressing different transcription factors and may even be essential for their initial patterning, if, for example, the repression of class I genes by Shh is mediated indirectly via the activation of class II genes. Ultimately, this patterning mechanism subdivides the ventral half of the neural tube into five distinct cell populations, each specified by a unique combination of transcription factors.

Although the Shh, Bicoid, WG, and DPP gradients are formed in distinct tissues and involve distinct morphogens, they nonetheless all appear to operate according to the same general principles.

MAINTAINING SEPARATE CELL POPULATIONS

Two basic types of mechanisms exist for maintaining discrete adjacent domains of gene expression. One relies on cells continuously receiving positional information, either through a morphogen gradient or through local interactions with neighboring cells. The alternative requires the establishment of a heritable mechanism for the regulation of gene expression, such as positive auto-regulation by transcription factors or chromatin-based maintenance mechanisms. The establishment of a heritable mechanism allows gene expression to become independent of a cell's relative position within the tissue, and thus allows stable gene expression even if cells become displaced far from the original morphogen source, or if a morphogen is only expressed transiently. However, the establishment of a heritable, lineage-based mechanism for regulating gene expression also introduces the necessity of establishing a mechanism for keeping cells with distinct gene expression profiles separated. Otherwise, as cells in adjacent domains continue to proliferate or move, the interface between them could become irregular. This coupling of heritable gene expression to a mechanism for separating cell populations creates a border of cell lineage restriction, as was first identified at the A-P compartment boundary of the *Drosophila* wing.

Detecting Lineage Boundaries

Compartment boundaries were first detected in *Drosophila* because experimental approaches were developed that made it possible to genetically mark single cells and their descendents. These involve inducing mitotic recombination in animals heterozygous for cell-autonomous morphological markers, such as mutations that influence the pigmentation or shape of hairs and bristles. More recently, analysis of lineage restrictions in *Drosophila* has employed molecular markers (Xu & Rubin 1993). This allows ready visualization of clones of cells in developing tissues and direct correlations between clone behavior and gene expression. In vertebrates, clones of cells have been marked by the injection of a dye into a single cell, which then persists through multiple rounds of cell division (Fraser et al. 1990). More recently, a number of other approaches have been used (Cepko et al. 1998, Golden et al. 1995, Zinyk et al. 1998), but all approaches involve the marking of a single cell and examining the distribution of its descendents (a clone). An additional approach that has been employed elegantly in vertebrate systems is the analysis of chick-quail chimeras (Le Douarin 1969). One striking difference between the best-studied systems is that in *Drosophila* imaginal discs there appears to be relatively little cell movement, because a clone of cells will almost always remain together as a contiguous coherent patch. Most of the irregularity in clone shape thus presumably

derives from patterns of growth. By contrast, there appears to be considerably more cell mixing in the vertebrate systems that have been studied, as clones of cells are usually dispersed.

Meaningful lineage boundaries occur between cells that develop immediately adjacent to each other within the same tissue. These cells may appear morphologically similar, but their separation implies that they express different genes, which regulate their distinct behaviors. Clonal analysis can also identify trivial lineage borders. For example, clones marked at the blastoderm stage in *Drosophila* can extend between the wing and the leg (Wieschaus & Gehring 1976), but later in development they are restricted to a single appendage. However, the primordia of the wing and leg form near each other but then migrate apart before fusing again during pupal development (Cohen 1993). A further complication with detecting lineage borders is that in some cases the borders are transient or imperfect. If a clone is induced before the lineage border forms, or its location is examined after a lineage border ceases to exist, then the lineage restriction may be missed.

A-P Compartmentalization in *Drosophila*

A-P compartmentalization of the *Drosophila* wing is established through differential expression of the homeodomain protein Engrailed (EN) (and its homologue Invected, but for simplicity we will use EN to refer to their combined activity) (Blair & Ralston 1997, Lawrence & Struhl 1982, Morata & Lawrence 1975, Tabata et al. 1995, Zecca et al. 1995). During the segmentation of the early embryo, posterior cells, but not anterior cells, become programmed to express EN (Kornberg et al. 1985). Although the patterning mechanisms that initiate EN expression in the embryo are transient, this subdivision is maintained throughout development by the heritable expression of EN in posterior cells of each segment. Genetic studies have demonstrated that EN influences all aspects of posterior fate, including both the patterning and the compartmental location of posterior cells. Thus clones of posterior origin that lack EN function fail to respect the compartment boundary and can end up in the anterior compartment (Blair & Ralston 1997, Lawrence & Struhl 1982, Morata & Lawrence 1975, Tabata et al. 1995, Zecca et al. 1995). When they do so, they can respect the compartment boundary from the anterior side, and behave as though they were anterior cells. The coordinate regulation of both cell type and compartmental location by EN ensures that cells remain in the location appropriate to their fate. Indeed, this appears to be a general property of genes that specify compartmental identity.

Although the role of EN in A-P compartmentalization has been most intensively studied in the *Drosophila* wing, it plays a similar role in other tissues and other segments of the fly (Kornberg 1981, Lawrence & Struhl 1982). Segments in other insects also appear to be subdivided into anterior and posterior compartments, as the posterior-specific expression of EN is conserved throughout the arthropods (Patel et al. 1989).

Non-Lineage-Based Mechanisms for Maintaining Gene Expression Domains

As long as the patterning mechanisms that initiate the subdivision of a field of cells continue to operate, they can continue to define distinct domains of gene expression. Additionally, stable domains of gene expression may be maintained by local cell-cell interactions. In either case, the maintenance of abutting domains of gene expression can be based on position, not lineage. For example, there is a transient period during *Drosophila* embryogenesis when the expression of EN is not yet heritable, but it is also no longer dependent upon the initial segmentation gene hierarchy. Instead, at this stage, EN expression is maintained by a positive feedback loop with neighboring, WG-expressing cells (Heemskerk et al. 1991). When the maintenance of gene expression is not heritable, individual cells may cross a gene expression border, but when they do so their expression profile changes to match that of their new neighbors. Thus a strict A-P compartment border is not established until after EN expression becomes heritable (Vincent & O'Farrell 1992).

PATTERNING THE *DROSOPHILA* LEG Recent studies of proximal-distal patterning in the *Drosophila* leg exemplify how individual cells can intermix, yet stable and sharp borders between distinct domains of gene expression are maintained. The *Drosophila* leg develops from the leg imaginal disc, a circular cluster of undifferentiated epithelial cells. Two morphogen gradients are important for subdividing the developing leg into domains of gene expression along the proximal-distal axis (reviewed in Irvine & Vogt 1997). WG is secreted by a wedge-shaped sector of ventral anterior cells, and DPP is secreted by a wedge-shaped sector of dorsal anterior cells. The combined effect of high levels of DPP and WG signaling in the center of the disc specifies distal fates and promotes the expression of Distal-less (DLL) (Diaz-Benjumea et al. 1994, Lecuit & Cohen 1997). The lower levels of their combined activities in more peripheral regions allows expression of Homothorax (HTH) and Teashirt (TSH), which specify proximal fates (Abu-Shaar & Mann 1998, González-Crespo et al. 1998, Wu & Cohen 1999). WG and DPP also direct the expression of Dachshund (DAC) in medial regions of the leg disc (Lecuit & Cohen 1997).

The expression of HTH and TSH forms a sharp border with more distal cells, which initially express DLL and later express DAC (Lecuit & Cohen 1997). However, no cell lineage restrictions exist along the proximal-distal axis of the leg. Moreover, experiments in which TSH-expressing cells were specifically marked revealed that a significant number eventually came to occupy more distal positions in the leg (Weigmann & Cohen 1999). Because HTH- and TSH-expressing cells are restricted to proximal regions throughout leg development, we can infer that these proximal cells must change their gene expression profile, ceasing to express HTH and TSH, and instead expressing DAC or DLL when they occupy more distal regions. Although distal cells do not cross as readily into the proximal domain, they can do so if given a growth advantage (Weigmann & Cohen 1999).

The ability of proximal cells to switch their gene expression can be related to the manner in which the WG and DPP morphogen gradients pattern the proximal-distal axis of the leg. HTH is repressed in distal cells as a consequence of WG and DPP signaling (Abu-Shaar & Mann 1998, Wu & Cohen 1999). Thus the expression of HTH can be repressed in cells that move or grow toward the distal leg. Moreover, because HTH represses expression of DAC (Abu-Shaar & Mann 1998, Wu & Cohen 1999, Wu & Cohen 2000), DAC can become expressed in cells that lose HTH expression. Conversely, DLL and DAC have been reported not to require continuous WG and DPP signaling for their expression (Lecuit & Cohen 1997), which may account for the relative infrequency with which distal cells cross into the proximal leg.

CREATING SPECIALIZED BORDER CELLS

One of the most important consequences of creating separate cell populations is that it allows the establishment of specialized cells along the border between them. Specialized border cells can be induced by short-range signaling between cells in adjacent domains both at lineage-restricted (compartment) and non-lineage-restricted borders. The unique fates of border cells can contribute directly to morphogenesis. For example, cells along the D-V compartment boundary of the *Drosophila* wing secrete the specialized bristles of the wing margin. However, the most important role of border cells is as a signaling center that directs further tissue patterning.

Specialized Border Cells Can Function as Organizers

Direct experimental evidence for signaling centers along compartment borders first came from studies of patterning in *Drosophila* imaginal discs (reviewed in Blair 1995, Irvine & Vogt 1997, Lawrence & Struhl 1996). In the wing, signaling centers form along both the A-P and D-V compartment boundaries. Both function as organizers, in that they influence the fate of surrounding tissues by virtue of their expression of long-range graded morphogens.

ESTABLISHING COMPARTMENT BORDER CELLS IN THE *DROSOPHILA* WING: A-P Specialized cells are established along the anterior side of the A-P compartment border in the wing as a consequence of Hedgehog (HH)-mediated signaling from posterior cells (Figure 1B) (Basler & Struhl 1994, Tabata & Kornberg 1994). EN plays a key role in establishing and positioning these compartment boundary cells by simultaneously promoting the expression of HH (Tabata et al. 1992, Zecca et al. 1995) and repressing the expression of *Cubitus interruptus* (CI) (Eaton & Kornberg 1990, Schwartz et al. 1995). CI is a transcription factor that mediates responses to HH signaling (reviewed in Aza-Blanc & Kornberg 1999), hence its repression by EN limits HH to signaling across the compartment border from posterior cells to anterior cells. Although in other contexts, such as described above for the neural

tube, HH signals can act at long range, in the wing HH acts as a short-range signal (reviewed in Chuang & Kornberg 2000). HH signaling promotes the expression of the secreted signaling molecule DPP (Basler & Struhl 1994, Diaz-Benjumea et al. 1994), which acts in the wing as a long-range graded morphogen (Lecuit et al. 1996, Nellen et al. 1996).

The interpretation of the DPP morphogen gradient results in further subdivision of the wing and the formation of additional borders of gene expression. Genes such as *spalt* and *spalt-related* are expressed in broad domains in response to the DPP morphogen gradient (de Celis et al. 1996a). The borders of expression of these genes then participate in positioning stripes of specialized cells, the wing veins, that form at discrete positions along the A-P axis of the wing (de Celis & Barrio 2000, Sturtevant et al. 1997).

ESTABLISHING COMPARTMENT BORDER CELLS IN THE *DROSOPHILA* WING: D-V Specialized cells are established along the D-V compartment border of the wing by activation of the Notch receptor (Figure 3A) (de Celis & García-Bellido 1994, Diaz-Benjumea & Cohen 1995, Kim et al. 1995, Rulifson & Blair 1995, reviewed in Irvine & Vogt 1997). In contrast to HH signaling at the A-P border, Notch activation is positioned in cells along both sides of the D-V compartment border. Notch activation in ventral cells is primarily dependent upon one ligand, Serrate, whereas Notch activation in dorsal cells is primarily dependent upon another ligand, Delta (Couso et al. 1995, de Celis et al. 1996b, Diaz-Benjumea & Cohen 1995, Doherty et al. 1996, Panin et al. 1997). The Notch ligands are transmembrane proteins, and although there is some evidence that they can be processed (Klug et al. 1998, Qi et al. 1999), they have only been found to signal at short range. The ability of these ligands to activate Notch is restricted to the D-V border by the action of Fringe. Fringe is expressed specifically by dorsal cells (Irvine & Wieschaus 1994) and encodes a glycosyltransferase (Bruckner et al. 2000, Moloney et al. 2000, Munro & Freeman 2000) that potentiates the ability of cells to respond to Delta and inhibits cells' ability to respond to Serrate (Fleming et al. 1997, Panin et al. 1997). A key target of Notch activation at the D-V border of the wing is WG, which, like DPP, acts as a long-range graded morphogen (Neumann & Cohen 1997, Zecca et al. 1996).

Boundary Organizers in Vertebrates

Organizers have also been identified along borders in vertebrates. For example, the isthmic organizer forms along the border between the midbrain and hindbrain and exerts a key influence on A-P patterning of this region of the brain. Both Wnt1 and FGF8 are expressed by cells adjacent to the midbrain-hindbrain border and are implicated in its patterning activity (reviewed in Joyner et al. 2000, Simeone 2000).

D-V COMPARTMENTALIZATION IN THE VERTEBRATE FLANK One classic example of a vertebrate organizer comes from the developing limb bud. As the limb bud grows

out, a region of thickened ectoderm, termed the apical ectodermal ridge (AER), becomes visible. Surgical manipulations first identified the AER as the source of signals essential for limb outgrowth (Saunders 1948), and subsequent work has identified members of the FGF family, especially FGF8, as being responsible for the action of the AER in promoting limb outgrowth (reviewed in Martin 1998).

The AER coincides with a lineage restriction between dorsal and ventral cells (Altabef et al. 1997, Michaud et al. 1997). Notably, this D-V lineage restriction also occurs in the ectodermal tissue between the limbs (the flank) (Altabef et al. 1997), but no morphologically distinct structure forms at the D-V border in the flank. In addition to the coincidence between the AER and the D-V lineage border, two additional observations imply that the lineage border is the site of a key organizer that promotes limb formation. First, transplants between dorsal and ventral cells can induce limb-like outgrowths that are centered along the novel juxtapositions between dorsal and ventral cells (Laufer et al. 1997, Tanaka et al. 1997). Second, beads soaked in FGFs can induce ectopic limbs at novel A-P locations along the flank, but these ectopic limbs always grow out from the normal D-V location, regardless of the placement of the bead (Altabef et al. 1997, Cohn et al. 1995).

As of yet, a selector gene that participates both in the establishment of the D-V lineage restriction and in specifying and positioning the AER has not been identified. One candidate was a homologue of *Drosophila engrailed*, *En-1*, which is expressed in ventral cells of the limb buds and flank and can influence AER formation (Laufer et al. 1997, Logan et al. 1997, Loomis et al. 1996, Rodriguez-Esteban et al. 1997). However, by combining ectopic expression with cell lineage analysis, Altabef et al. (2000) concluded that *En-1* is not required for the D-V lineage restriction in chicks. Kimmel et al. (2000) have combined ectopic expression of *En-1* with cell lineage analysis in mice. Their results are more complex because they identified two distinct lineage boundaries at the AER and a role for *En-1* in proper maintenance of sharp boundaries. Nonetheless, their results do not support a role for *En-1* in the initial subdivision of the limb and flank into dorsal and ventral compartments.

Mechanisms that Sharpen Boundaries

Because of the role that some border cells can play as organizers, the width of the border cell population can be of crucial importance. In general, limiting the width of the border is accomplished by having specialized border cells induced by short-range signals. For example, the spread of HH is influenced by a number of factors including its modification by cholesterol, the presence of specific proteoglycans, and the levels of its receptor, Patched (reviewed in Chuang & Kornberg 2000).

In the case of D-V signaling, multiple mechanisms help to restrict Notch target gene expression to a narrow domain at the D-V compartment border. One involves WG, which has been reported to repress the ability of Notch signaling to activate downstream genes (Rulifson et al. 1996). This helps to limit the expression of WG to the D-V border, where the levels of Notch activation are highest. Another involves the autonomous inhibition of Notch signaling by Notch ligands (Micchelli et al.

1997). Notch signaling is also down-regulated by *nubbin*. *nubbin* encodes a DNA-binding protein that is broadly expressed in the wing disc (Ng et al. 1995). In the absence of *nubbin*, Notch targets, including WG, become expressed in cells much farther from the compartment boundary than would normally occur in wild-type, and the stripe of WG expression also becomes irregular (Neumann & Cohen 1998). Apparently, during normal development, the peak of Notch activation at the D-V compartment boundary is sufficient to override the repressive effects of *nubbin* and WG, whereas farther from the border, where Notch activation is weak, the repressive effects of *nubbin* and WG prevail.

IMPORTANCE OF COMPARTMENT BORDERS IN POSITIONING ORGANIZERS The observation that compartment boundaries are the source of graded morphogens that pattern the wing has also led to the realization that a crucial outcome of the separation of cells into distinct compartments is the influence on morphogen distribution (Dahmann & Basler 1999). For example, because the compartment border is a smooth straight line, even as the *Drosophila* wing primordia grows about a thousand-fold in size, WG and DPP expression remain in a straight line. Thus the patterning regulated by them can be stable throughout development. Conversely, if the borders between compartments were squiggly, then the distribution of WG and DPP would be irregular, resulting in alterations and variability in the growth and patterning of the wing. Thus one key function of establishing a sharp border between compartments appears to be its influence on the position and shape of organizers that are induced there.

COMPARTMENT BORDERS CAN LIMIT GROWTH Although much attention has been focused recently on the importance of compartment borders as a source of morphogens that promote growth and patterning, earlier studies emphasized the apparent role of compartment borders in limiting growth (García-Bellido 1975, Simpson & Morata 1981). Using mutations in *Drosophila* called *Minutes*, which affect protein synthesis, it is possible to give a clone of cells a growth advantage over its neighbors. This growth advantage will enable a clone to occupy a much greater proportion of a tissue than it otherwise would. Nonetheless, all clones respect the compartment boundary, and the final size of the compartment is unaffected. These observations indicate that in some way a compartment is a unit of growth control.

The limitation of growth within a compartment presumably derives from the still poorly understood mechanism by which patterning dictated by compartment boundary organizers regulates tissue growth. Intriguingly, the DPP activity gradient can adjust to compartment size. Compartment size can be altered by manipulating genes involved in an insulin-related pathway (Leevers et al. 1996). In a smaller compartment, the DPP activity gradient is steeper, but all positional values are maintained (Teleman & Cohen 2000). This maintenance of positional values even occurs if only some cells within a compartment are given a growth advantage, suggesting that size regulation occurs throughout the compartment unit, as opposed to locally within cells with perturbed growth rates. Ultimately, compartments may

help to ensure that a properly proportioned organism develops even if cell growth is altered through diet, infection, or injury.

HOW ARE CELLS KEPT SEPARATED?

The formation of compartments depends upon the linking of heritable gene expression to a mechanism for separating cell populations. One of the most important mechanisms for separating cells has long been thought to be the generation of adhesive differences, and the first evidence linking specific cell adhesion molecules to compartmentalization is beginning to emerge from studies of the vertebrate brain.

The Differential Adhesion Hypothesis

The idea that specific adhesive interactions could play an important role in organizing tissues was first emphasized in the classic experiments of Holtfreter (Townes & Holtfreter 1955). However, Steinberg, with his differential adhesion hypothesis, illustrated that tissue separation could be driven by differences in the quantity, as well as the quality, of adhesive interactions among cells (Steinberg 1962, 1978). Steinberg modeled cells in a tissue after molecules in a liquid. They both are composed of discrete units that cohere yet are mobile. Given these basic properties, the configurations that a mixture of cells will adopt can be predicted according to thermodynamic principles: They may intermix, envelope, adhere yet remain in distinct phases, or separate, depending upon the relative strengths of adhesive interactions between and among different cell types (Figure 4). Indeed, the differential

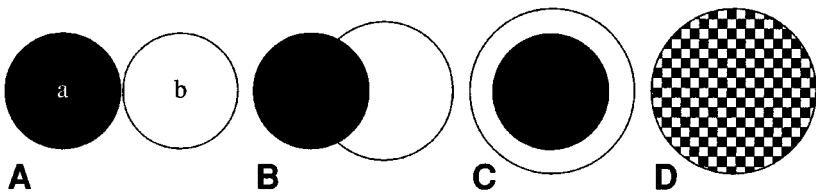


Figure 4 The differential adhesion hypothesis predicts cell behavior. If two populations of adhesive cells are juxtaposed (black “a” cells and white “b” cells in this example), they will adopt reproducible configurations that depend upon the relative strengths of homophilic and heterophilic adhesion (Steinberg 1978). (A) When there is no heterophilic adhesion, the cells remain separate. (B) When the strength of heterophilic adhesion is less than either of the homophilic adhesions, the less adhesive cells will partially envelope the more adhesive cells. (C) When the strength of heterophilic adhesion is greater than the weakest homophilic adhesion, the less adhesive cells will completely envelope the more adhesive cells. (D) When the strength of heterophilic adhesion is greater than the average of the homophilic adhesions of the two cell populations, then the cells will intermix.

adhesion hypothesis is supported by measurements of tissue surface tension (Foty et al. 1996) and reliably predicts the behavior of cells that are forced to express different levels of a single homophilic cell-adhesion molecule, cadherin (Steinberg & Takeichi 1994).

Affinity Differences at the A-P Compartment Boundary

Although affinity differences have long been proposed as a basic mechanism underlying the separation of cells into distinct compartments, the molecules that actually mediate these proposed affinity differences have not yet been identified. Instead, their existence has been inferred from the behavior of cells upon experimental manipulation. For example, clones of cells in the posterior compartment that are mutant for *engrailed* can violate the compartment border and end up in the anterior compartment (Figure 1C) (Blair & Ralston 1997, Lawrence & Struhl 1982, Morata & Lawrence 1975, Tabata et al. 1995, Zecca et al. 1995). Alternatively, in some cases they may remain in the posterior compartment, but when they do so they form a circular cluster that has a smooth interface with surrounding cells. Parallel behaviors can be elicited in anterior cells that are forced to express EN ectopically (Zecca et al. 1995). Whenever EN-expressing and EN non-expressing cells are confronted, they have been seen to sort out from each other and adopt geometries that minimize the interface between them, which leads to the inference that EN-expressing and EN non-expressing cells have different affinities.

Consistent with the differential adhesion hypothesis, overexpression of *Drosophila* E-cadherin in a clone of cells in the wing can cause them to sort out from neighboring cells, which results in circular clones with smooth borders (Dahmann & Basler 2000). Strikingly, the influence of E-cadherin expression is sufficient to override normal A-P compartmentalization, as cells of anterior and posterior compartmental origin can intermix within clones of cells expressing high levels of E-cadherin (Dahmann & Basler 2000). However, E-cadherin is not required for normal A-P compartmentalization, as *E-cadherin* mutant cells still respect the compartment boundary.

HEDGEHOG SIGNALING INFLUENCES AFFINITY DIFFERENCES AT THE A-P COMPARTMENT BORDER It had long been assumed that the affinity differences between anterior and posterior cells would simply be an EN-dependent property of all posterior cells. However, HH-mediated signaling between anterior and posterior cells actually plays a major role in generating the affinity differences at the compartment border (Figure 1C) (Blair & Ralston 1997, Rodriguez & Basler 1997). Clones of cells in the anterior compartment that are unable to respond to HH because they lack the *smoothened* gene (*smo*) form a smooth interface with other anterior cells and can end up in the posterior compartment. Further analysis has revealed that the influence of HH signaling is dependent upon CI and hence must be mediated by the transcriptional regulation of its target genes (Dahmann & Basler 2000). Thus

providing CI to posterior cells can be sufficient to cause them to end up on the anterior side of the compartment boundary (Dahmann & Basler 2000). However, not all of the affinity difference between anterior and posterior cells actually depends upon the establishment of a signaling system between them—there is also a component that depends directly on EN. Thus even though *smo* mutant anterior cells can violate the compartment border, they still form a relatively smooth interface with posterior cells (Blair & Ralston 1997, Lawrence et al. 1999). Moreover, EN can influence the compartmental sorting of cells even in the absence of HH signaling (Dahmann & Basler 2000, Lawrence et al. 1999).

The observation that both HH signaling and EN make independent contributions to the affinity differences between anterior and posterior cells raises the questions of why two apparently redundant systems are utilized and whether they regulate distinct cell affinity mechanisms or converge upon a common system. HH is made exclusively by posterior cells, and the spread of HH into the anterior compartment establishes a gradient of HH signaling. At least in the *Drosophila* abdomen, this gradient appears to establish a gradient of cell affinity, which may help maintain stable tissue patterning (Lawrence et al. 1999). Thus it is possible to view the HH- and EN-dependent regulation of cell affinities as two separate systems that originated for two distinct purposes: an EN-dependent cell affinity that distinguishes posterior cells from anterior cells and a HH-dependent cell affinity that establishes a gradient of cell affinity within the anterior compartment. Because the absence of CI in posterior cells prevents HH responsiveness, at the A-P compartment border both systems act in parallel to establish the sharp difference in affinity that keeps cells separated.

It is currently unclear whether EN and CI regulate cell affinity through the same or distinct molecular mechanisms. Normally, if sorting were driven by quantitative differences in a single adhesion molecule, then the differential adhesion hypothesis would predict that the interface between anterior and posterior cells should be curved, with the less adhesive cells at least partially enveloping the more adhesive cells (Figure 4B) (Steinberg 1978). Because the interface between anterior and posterior cells is actually quite straight across much of an imaginal disc, it may be that additional mechanisms are involved in maintaining the shape of the compartment boundary or that distinct adhesion systems are actually used to balance the adhesiveness among cells on each side of the compartment boundary. Alternatively, it could be that having adhesion regulated by signaling rather than by lineage allows the compartment boundary to remain straight despite differences in adhesiveness between anterior and posterior cells. In the modeling and experiments that have been done on the differential adhesion hypothesis, cell adhesion is stable. But if adhesion is regulated by signaling, then as cells begin to rearrange to try to adopt configurations that maximize contacts among the more adhesive population, their adhesiveness will vary with their distance from the signaling source. Although the consequences of such dynamic adhesion have not been modeled, we speculate that it may allow straight borders to persist despite quantitative differences in adhesion.

Cadherins and Compartments in the Vertebrate Brain

Both morphological studies and gene expression patterns that define subregions of the brain have led to the suggestion that the vertebrate brain is segmented (Figure 5) (reviewed in Lumsden & Krumlauf 1996, Puelles & Rubenstein 1993). Some regions are subdivided by cell lineage restrictions, providing evidence for the existence of compartments in the neuroepithelium (see below). However, recent studies demonstrate that some of the proposed neuromere boundaries in the forebrain do not correspond to cell lineage boundaries (Larsen et al. 2001); nor does the midbrain-hindbrain border correspond to a cell lineage border (Jungbluth et al. 2001), despite the sharp borders of gene expression that occur there and its role as an organizer of brain patterning.

Within the ventricular cells of the telencephalon, cell labeling studies have demonstrated the existence of two neighboring cell-lineage-restricted compartments, the lateral ganglionic eminence (lge) and the presumptive cerebral cortex (ctx) (Fishell et al. 1993, Inoue et al. 2001). As in other systems, cells belonging to the same compartment mix freely with each other, whereas cells belonging to adjacent compartments do not mix and a smooth boundary forms between them. Recent studies have implicated the cadherin family of cell adhesion molecules as important in maintaining this compartment boundary (Inoue et al. 2001). More than 30 different cadherin family members are expressed in the vertebrate brain, and their expression often coincides with segmental domains and boundaries (reviewed in Redies 2000). Homophilic interactions are thought to predominate among cadherins, although heterophilic interactions can also occur. Thus a cell population expressing one type of cadherin can sort out from a cell population expressing another type (reviewed in Takeichi 1995). Cadherin-6 (*cad6*) and R-cadherin (*Rcad*) are expressed in complementary patterns in the lge and ctx, respectively, and a sharp interface of their expression exists at the ctx/lge compartment boundary (Figure 5C) (Inoue et al. 1997, 2001).

Notably, *cad6* and *Rcad* are expressed in these complementary domains at the time that cell dispersion becomes restricted, which is prior to the formation of a morphologically visible border between the lge and ctx (Inoue et al. 2001). Moreover, these cadherins can direct sorting of neuroepithelial cells to one compartment or the other (Inoue et al. 2001). When cells at the ctx/lge boundary are forced to express *cad6*, they will preferentially sort to the *cad6*-expressing lge. Conversely, if cells at the ctx/lge boundary are forced to express *Rcad*, they will tend to sort to the *Rcad*-expressing ctx, although the sorting effect is less pronounced. In *cad6* mutant mice, cells forced to express *cad6* no longer sort to the lge, but instead remain at the interface of the ctx/lge boundary (Inoue et al. 2001). Together these results imply that homophilic interactions between cadherins can cause cell sorting in the neuroepithelium. However, it remains unclear how these cadherins relate to the cell lineage restriction at the ctx/lge boundary, because in *cad6* mutant mice, the ctx and lge compartments are unperturbed and a ctx/lge compartment boundary still forms (Inoue et al. 2001). Thus if cadherins have a role in compartmentalization, there must be some redundancy in the system.

Affinity Differences at Non-Compartmental Borders: *Drosophila* Appendages

In principle, special mechanisms for separating cells are not needed at non-lineage restricted borders because distinct domains of gene expression are maintained by regulatory interactions. In fact, however, affinity differences are not restricted to compartment borders but instead appear to contribute to maintaining sharp borders even in the absence of a lineage restriction.

An example of this occurs during the proximal-distal patterning of the *Drosophila* leg. As noted above, no lineage restriction exists between proximal and distal cells. Nonetheless, if distal cells, which normally express DLL, are forced to express HTH, they will sort out of the distal disc epithelium (Abu-Shaar & Mann 1998, Wu & Cohen 1999). Conversely, if proximal cells, which normally express HTH, are forced to express DLL, they will sort out of the proximal disc epithelium (Wu & Cohen 1999). Thus proximal and distal cells appear to have distinct affinities in the leg, and the same also appears to hold true for the wing (Azpiazu & Morata 2000, Liu et al. 2000). Indeed, the normal geometry between proximal and distal cells in imaginal discs is consistent with the observation that distal cells are more adhesive than proximal cells. The entire distal region of the appendage is essentially circular, these distal cells are encircled by proximal cells, and a smooth interface forms between proximal and distal cells (e.g., Figure 3A; cf Figure 4C).

Although there is no normal lineage restriction between proximal and distal cells in the wild type, if a clone of proximal or distal cells is forced to constitutively express HTH or DLL, respectively, that clone will remain restricted to the proximal or distal leg (Wu & Cohen 1999). Thus by experimentally linking affinity to cell lineage, it is possible to create the functional equivalent of a compartment border for those cells.

Compartmentalization in the Hindbrain: Stripes of Repulsion?

Visible bulges, termed rhombomeres, appear transiently in the vertebrate hindbrain during embryonic development. Each rhombomere is a developmental compartment: Cell labeling experiments first demonstrated that rhombomeres are separated by lineage-restricted borders (Fraser et al. 1990). In transplantation and cell mixing studies, cells from alternate rhombomeres can mix freely, but cells from adjacent rhombomeres sort out and form sharp borders (Guthrie et al. 1993, Wizenmann & Lumsden 1997). A number of genes have been identified whose expression coincides with rhombomere boundaries, and the formation of borders between rhombomeres appears to be important for maintaining the proper region-specific expression of genes along the A-P axis (reviewed in Lumsden & Krumlauf 1996).

The separation of cells in adjacent rhombomeres is influenced by the action of type B Eph receptor tyrosine kinases and their ephrin-B ligands. Type B Ephs and ephrins can signal bidirectionally, so each member of an interacting pair actually

functions as both a ligand and a receptor (reviewed in Mellitzer et al. 2000). Ephs and ephrins are expressed in complementary patterns at several points in development, including the developing hindbrain (Gale et al. 1996, reviewed in Mellitzer et al. 2000). Odd rhombomeres (r3 and r5) express EphB receptors, whereas even rhombomeres (r2, r4, and r6) express ephrin-B ligands (Figure 5A). Eph signaling was first implicated in the separation of rhombomeres by the observation that expression of a truncated EphA4 receptor (which also interacts with ephrin-Bs) can disrupt normal rhombomere segmentation (Xu et al. 1995). Intriguingly, ectopic expression of ephrin-B2 in odd rhombomeres, or of EphA4 in even rhombomeres, causes the cells mis-expressing these proteins to sort to the borders between even and odd numbered rhombomeres (Xu et al. 1999). Deletion of the cytoplasmic tail of ephrin-B2 or Eph4A does not abolish this sorting behavior. Hence, it seems to reflect their action as ligands rather than as receptors. Further demonstration that Eph-ephrin signaling can direct cell separation comes from experiments in which an EphB receptor and an ephrin-B ligand are expressed separately in zebrafish animal caps, and then the animal caps are fused (Mellitzer et al. 1999). When wild-type animal caps are fused, cells will intermix, but when animal caps expressing an Eph and an ephrin are fused, they remain separate with a sharp border between them. However, in contrast to experiments in which Ephs or ephrins are mis-expressed in the hindbrain, bidirectional signaling is required to separate animal cap cells.

The mechanism by which Eph-ephrin signaling separates rhombomeres remains to be determined but has been suggested to involve mutual de-adhesion at the interface between Eph and ephrin-expressing cells (Mellitzer et al. 1999, Xu et al. 1999). This suggestion derives in part from the determination that Eph-ephrin signaling mediates repulsive cues during axon guidance (reviewed in Flanagan & Vanderhaeghen 1998) and is consistent with the fact that an enlarged intercellular space is observed at rhombomere boundaries (Heyman et al 1993).

A Fence at the D-V Compartment Border of the *Drosophila* Wing

Aside from differences in cell affinity, other mechanisms have also been proposed to account for the separation of cells at compartment borders. These include a zone of cells that die or fail to proliferate, a mechanical barrier, or oriented mitoses. Although no clear evidence that any of these mechanisms participates in compartmentalization has been established, studies of D-V compartmentalization in the *Drosophila* wing argue for the existence of a mechanism for cell separation that does not rely on the establishment of adhesive differences.

Both dorsal cell fate and dorsal cell location in the wing are specified by a homeodomain transcription factor, Apterous (Diaz-Benjumea & Cohen 1993). Apterous also influences signaling between dorsal and ventral cells, and thus Notch activation, by virtue of its regulation of Fringe and Serrate expression (Figure 3A)

(Diaz-Benjumea & Cohen 1995, Irvine & Wieschaus 1994, Kim et al. 1995). The primary mechanism by which *Apterous* influences compartmentalization is through its influence on D-V signaling. Clones of cells mutant for Notch or its ligands disrupt the compartment border (Micchelli & Blair 1999, Rauskolb et al. 1999). Moreover, repositioning the stripe of Notch activation that normally occurs along the D-V border, whether through absence of *Fringe* in dorsal cells or ectopic expression of *Fringe* or *Serrate* in ventral cells, can effectively reposition the compartment border such that a straight line of Notch activation occurs across the middle of the wing imaginal disc, but the interface between *Apterous*-expressing and non-expressing cells is displaced from this line and can be irregular (Figure 3B) (Rauskolb et al. 1999).

Notch is normally activated along both sides of the compartment border and thus could not make a compartment border by conferring a distinct dorsal-type or ventral-type cell affinity. Moreover, when Notch signaling is disrupted, cells can intermix in either direction, and both wild-type and mutant cells can violate the compartment border (Figure 3B) (Micchelli & Blair 1999, Rauskolb et al. 1999). Thus it appears as though a stripe of Notch activation makes a kind of fence that prevents cell intermingling; when the fence is broken, cells on either side can intermix in either direction. Moreover, Notch activation does not seem to confer a distinct border-cell affinity either, as clones of cells in which Notch signaling is constitutively activated do not sort to the D-V border (Rauskolb et al. 1999).

Experiments in which *Fringe* or *Serrate* were expressed in dorsal cells in *apterous* mutant animals led to the suggestion that Notch activation was not sufficient to establish the normal D-V border because dorsal and ventral cells intermixed despite the presence of a stripe of Notch activation (Milan & Cohen 1999). However, this conclusion has been contradicted by a more recent study (O'Keefe & Thomas 2001), which suggests that the absence of a compartment border in the initial work derived from inadequate levels of Notch activation. Nonetheless, it is likely that a secondary mechanism can also contribute to compartmentalization, as the D-V border is maintained even within broad regions of Notch activation (Rauskolb et al. 1999), and the borders of clones that violate the compartment border after disruption of Notch activation can be smoother than normal clone borders (Micchelli & Blair 1999, Rauskolb et al. 1999).

The Role of *Fringe* and Notch Signaling at Other Developmental Borders

Intriguingly, borders of some sort appear to form along edges of *Fringe* expression in many distinct tissues, both in invertebrates and in vertebrates (reviewed in Irvine 1999). These include borders between leg segments and at the D-V midline of the eye in *Drosophila*, and during segmentation of the presomitic mesoderm and formation of the AER in vertebrates. The repeated deployment of the same signaling pathway may derive from the mechanisms that exist to restrict Notch activation

to a narrow stripe and hence to define a precise border. The ligands are short-range signals, Notch activation is inhibited cell-autonomously in ligand-expressing cells, and Fringe expression further positions and restricts Notch activation. However, the role of Notch signaling and its contribution to boundary formation in these different tissues vary. For example, although lineage restrictions form at the D-V border of the *Drosophila* eye and the vertebrate limb, in contrast to the situation in the wing, it does not appear that Notch activation plays a significant role in generating these lineage restrictions (Altabef et al. 1997, Yang et al. 1999). Moreover, it has recently been suggested that the role of Notch signaling in somitogenesis does not involve making a boundary per se, but rather synchronizing the oscillatory behavior of the somite clock (Jiang et al. 2000). One place where Fringe does seem to play a key role in compartmentalization is the zona limitans intrathalamica.

THE ZONA LIMITANS INTRATHALAMICA The zona limitans intrathalamica (zli) was initially identified as a boundary of cell-lineage restriction in the vertebrate brain between the ventral thalamus and the dorsal thalamus (Figdor & Stern 1993). However, the zli is not simply a boundary cell population but rather a compartment that initially constitutes about a third of the forebrain and then narrows dramatically to form the zli. Labeled polyclones originating in the zli do not mix with cells from the neighboring compartments, rather they define two cell restriction boundaries: one where the zli abuts the dorsal thalamus and the other where the zli abuts the ventral thalamus (Zeltser et al. 2001).

Lunatic Fringe (Lfng), one of three vertebrate homologs of *Drosophila* Fringe, is repressed in the zli but expressed throughout the rest of the forebrain, and Notch ligands are expressed by cells flanking the zli (Figure 5B) (Cohen et al. 1997, Johnston et al. 1997, Laufer et al. 1997, Zeltser et al. 2001). The lineage boundaries of the zli correspond precisely to Lfng expression borders. Moreover, ectopic expression of Lfng in the zli suppresses zli formation, as assayed by the zli-specific expression of Shh (Zeltser et al. 2001). Importantly, Lfng expression can also direct cell sorting. Cells ectopically expressing Lfng in the zli preferentially sort out of the zli into the dorsal or ventral thalamus (Zeltser et al. 2001). Because this cell-sorting behavior occurs at a single-cell level, it is most easily explained if Lfng affects cell adhesion. Thus even though Fringe affects cell location in both the *Drosophila* wing and the vertebrate forebrain, the mechanisms by which it does so appear to differ.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Since the detection of the A-P compartment boundary in *Drosophila* almost 30 years ago, tremendous progress has been made in identifying borders and establishing their importance during development. The subdivision of tissues into

distinct cell populations establishes sharply delimited domains of action for regulatory control genes, and signaling between these adjacent cell populations in many instances establishes signaling centers that organize growth and further patterning. However, these key features of compartmentalization can also occur at gene expression borders that do not correspond to cell lineage borders. Thus whereas lineage restriction continues to be an important indicator of developmental boundary formation, the absence of lineage restriction does not necessarily indicate that no developmental boundary exists. Rather, a lineage restriction is really a reflection of the regulatory mechanisms that are employed to define distinct domains of expression. When gene expression is regulated by a heritable mechanism, a cell lineage border can exist. But when gene expression is regulated by signaling, a strict lineage border is not needed.

Despite the progress in identifying borders and the genes that establish them, we still understand relatively little of the molecular mechanisms that actually keep cells separated. In many instances, signaling between adjacent cell populations plays a key role in maintaining a sharp separation between them, but what are the targets of this signaling? The analysis of genes required for border formation has implied that in many cases cells are separated at borders by adhesive differences, but what are these adhesive molecules, and how are they regulated? The recent studies on cadherins in the vertebrate forebrain are an exciting beginning (Inoue et al. 2001), but the degree to which they contribute to normal compartmentalization remains unclear. The key challenge for the field in the next years will be to identify adhesive molecules with a clearly demonstrated role in compartmentalization. Identification of these molecules and molecules that participate in other cell-separation mechanisms will be a landmark advance for the field and will form a foundation for many future studies.

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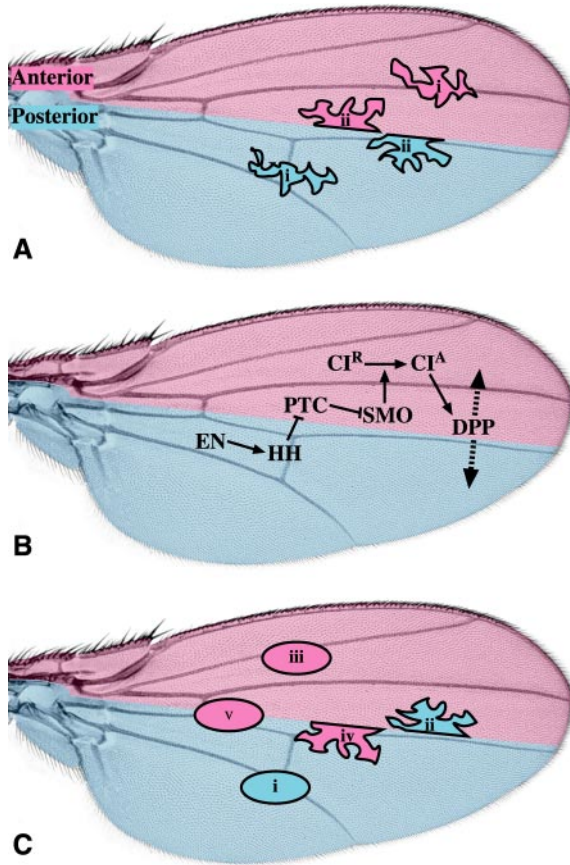


Figure 1 A-P compartmentalization of the *Drosophila* wing. Anterior cells are shaded magenta and posterior cells are shaded cyan. (A) Schematized clones of wild-type cells are indicated by solid fill. Within a compartment, they have irregular boundaries and occupy random positions (i). They never cross the compartment border, and they have straight edges where they contact it (ii). (B) Simplified schematic of HH-mediated signaling across the A-P compartment boundary (reviewed in Murone et. al. 1999). HH signaling ultimately results in the conversion of CI from a transcriptional repressor to a transcriptional activator. CI regulates the expression of DPP, which spreads from its site of synthesis at the A-P boundary (dashed arrows). CI and EN also regulate cell affinity (not shown). (C) Simplified schematic of the influence of *engrailed* and HH pathway mutations on clone shape and location. Clones of posterior provenance that are mutant for *engrailed*, or ectopically express CI, can form a smooth rounded clone within the posterior compartment (i) or cross over to the anterior compartment (ii). Clones of anterior provenance that are mutant for *smo*, or ectopically express EN, can form a smooth rounded clone within the anterior compartment (iii) or cross over to the posterior compartment (iv). Clones of cells that lack CI and EN can straddle the compartment boundary and form smooth borders with both anterior and posterior cells (v).

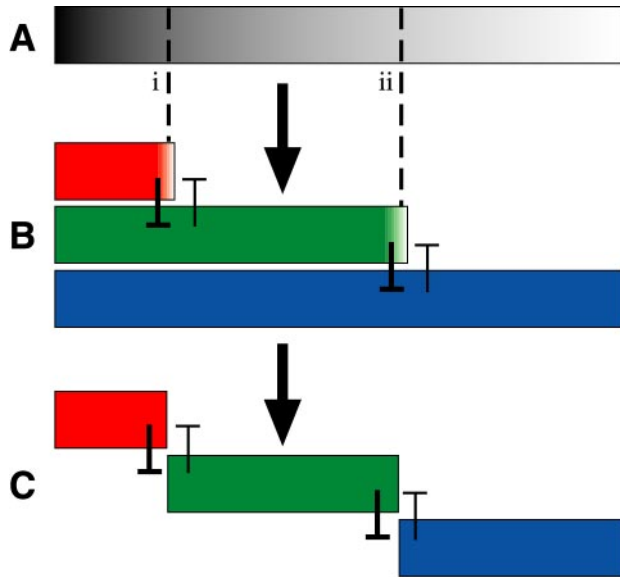


Figure 2 Establishment of different domains of gene expression by interpretation of a morphogen gradient. (A) Different threshold levels (*dashed lines*) of a morphogen (*gray shading*) are required to promote the expression of the red target gene (i) and the green target gene (ii). (B) Mutual repression between these genes can then establish distinct, non-overlapping domains of gene expression. (C) In this schematic, a third domain is established because red and green genes repress the expression of the gene that is not directly regulated by the morphogen gradient (*blue*).

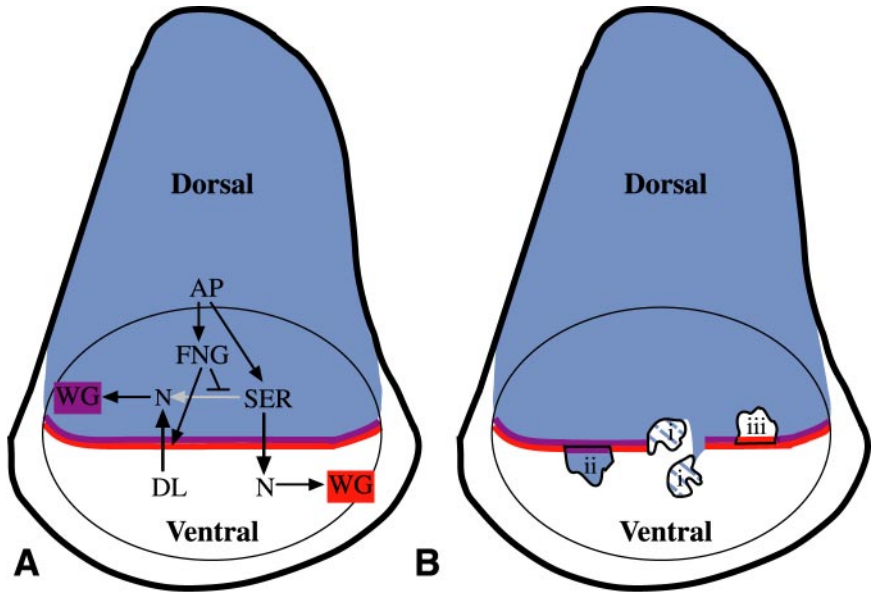


Figure 3 D-V compartmentalization in the *Drosophila* wing imaginal disc. Ventral WG-expressing cells at the D-V boundary are red, dorsal WG-expressing cells at the D-V boundary are purple, other dorsal cells are blue. The oval encompasses the distal region of the disc, which will give rise to the wing blade. (A) Notch (N) activation is positioned at the D-V boundary by signaling from its ligands, Delta (DL) and Serrate (SER), and is modulated by Fringe (FNG). Expression of FNG and SER in dorsal cells is promoted by Apterous (AP). (B) The D-V compartment border is established by a stripe of Notch activation. Clones of cells mutant for Notch eliminate the compartment border. Consequently, *Notch* mutant clones of dorsal or ventral provenance (*blue slashes*) can result in wild-type or mutant cells violating the compartment border from either the dorsal or ventral side (i). Clones of dorsal cells mutant for *fng* can reposition the compartment border, resulting in the mutant clone adopting a ventral location and an apparent border forming within cells of dorsal provenance (ii). Clones of ventral cells ectopically expressing FNG or SER can reposition the compartment border, resulting in the clone adopting a dorsal location and an apparent border forming within cells of ventral provenance (iii).

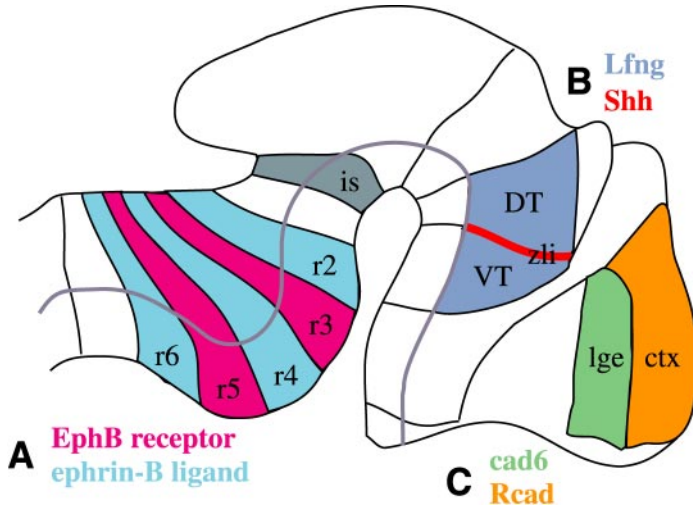


Figure 5 Compartments in the vertebrate brain. Schematic illustrates compartments in three regions of the brain. Work described in the text includes experiments on chicks, mice, and fish, but only a mouse brain is schematized here. The isthmus organizer (is; gray) forms at the midbrain-hindbrain border. No cell lineage border exists here, but the cells at the border have a major influence on brain patterning. (A) In the hindbrain, EphB receptors (*magenta*) are expressed in rhombomeres r3 and r5, and ephrin-B ligands (*cyan*) are expressed in r2, r4, and r6. Eph-ephrin signaling influences the separation of cells into distinct rhombomeres. (B) In the thalamus, the zli is a compartment between the dorsal thalamus (DT) and the ventral thalamus (VT). Lfng (*blue*) and Notch ligands are expressed in the DT and VT (and elsewhere in the brain, not shown), but are excluded from the zli, which expresses Shh (*red*). (C) In the telencephalon, a cell lineage restriction occurs between the lateral ganglionic eminence (lge), which expresses cad6 (*green*), and the cortex (ctx), which expresses Rcad (*orange*).