Growth and patterning from the *engrailed* interface

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ABSTRACT The *Drosophila* wing is divided into anterior and posterior compartments, the latter characterized by the expression of the *engrailed* gene. A comparative analysis is presented here, and suggests that a primary conserved role of *engrailed* is to drive growth of limbs along the proximo-distal axis. The Apical Ectodermal Ridge in vertebrate limbs resembles the Antero/Posterior compartment boundary in fly wings, particularly in molecular aspects. Multiple evidence suggests that the fly wing Antero/Posterior boundary is not the result of differential cell affinities between all anterior and posterior cells, but responds to the area of cell communication between anterior and posterior compartments. Arguments are presented here to support the notion that the compartment boundary is a consequence of *decapentaplegic* function in the control of growth. Patterning, on the other hand, requires the participation of several genes, among which are *engrailed, invected* and *hedgehog*. Finally, regulatory interactions between *en/En-1* and *hh/Shh* may be significant in the context of morphogenetic regulation during normal development.

KEY WORDS: *engrailed, compartments, selector genes, AER, decapentaplegic*

Introduction

When Velázquez painted Las Meninas (Fig. 1) he taught us that the content of an image can flip depending on how we perceive it. So, we can either see a portrait of the princesses, or a portrait of the King and Queen (who are reflected on the mirror) or a portrait of the artist himself. Images are present in living organisms, as whole or part of structures (e.g., eyes) and patterns (e.g., of hairs, of gene expression) which can be seen by naked eye or with sophisticated methods. Developmental geneticists look at these images to try to understand how a fly is made, as an architect from the future would look at our buildings to work out how we made houses from bricks, windows, roofs and doors. Sometimes these images are so astonishing that they make us want to stay within the most immediate plane. The story of the *engrailed (en)* gene and its conceptual trail is a fascinating one in developmental biology, and to Antonio we owe vision in creating a whole area of research.

At a time when no developmental gene had yet been cloned, García-Bellido observed that mutations in the *en* gene produce a transformation of posterior pattern of the wing into anterior (García-Bellido and Santamaría, 1972). Furthermore, he observed that the domain of pattern transformation coincides with a partition along the wing which could only be visualized by clonal analysis, as it restricts the proliferation of cells to either side of it (Fig. 2) (García-Bellido *et al.,* 1973). García-Bellido named compartment each of the wing halves separated by this line. He predicted that compartments would be under the genetic control of selector genes (García-Bellido *et al.,* 1973). A binary switch would determine that a given group of cells would either express one of these genes or not, and this decision would be maintained by all the progeny of these founder cells (García-Bellido, 1975). The beauty of this proposal lied on the fact that we could understand morphology as an addition of lineages, and morphogenesis as the piece-meal process in which these lineages become different from each other in terms of shape, size and pattern by expressing a given combination of selector genes. This proposal was visionary, because it was later shown that only cells of the posterior compartment of the wing express the *en* gene, which like other genes whose mutations cause homeotic transformations, contains a homeobox . Furthermore, it has also been shown that, as predicted, interfaces between compartments are instructive in the control of growth. In fact, work from many labs has described a network of genes that function to establish this interface. Now, following Antonio's frequent exhortation to people working with him, we must look at cell behavior and also beyond *Drosophila* to try to understand how a fly is made.

Conservation of *engrailed* function: enabling proximo-distal growth

engrailed has been studied in leech (Wedeen and Weisblat, 1991; Lans et al., 1993), insects and crustaceans (Patel *et al.,*

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Fig. 1. Las Meninas, by Diego Velázquez (Museo del Prado, Madrid). **Plane 1**: *a portrait of the princesses and their company.* **Plane 2**: *portrait of the King and Queen, who are sitting on our plane, outside the canvas, and are reflected upon the mirror on the wall behind the princesses.* **Plane 3:** *portrait of the painter, Velázquez, on the left as he works on the canvas.* **Plane 4**: *an observer by climbing up the stairs, in the background.*

1989a,b; Scholtz *et al.,* 1993,1994; Scholtz and Dohle, 1996), amphioxus (Holland *et al.,* 1997) and vertebrates (Joyner *et al.,* 1991; Wurst *et al.,* 1994; Loomis et al., 1996; Logan *et al.,* 1997). In segmented organisms, *en* behaves in ways surprisingly similar to how it does in *Drosophila*. Within the trunk, it is invariably expressed in stripes around where each segment boundary will form. These stripes are defined by positional cues, not by lineage (Scholtz et al., 1994; Scholtz and Dohle, 1996). In their onset, the anterior end of the *en* segmental stripe is frequently straight, whereas the posterior end is wiggly as some cells switch off *en* expression (Fig. 2) (Patel *et al.,* 1989a; Scholtz *et al.,* 1993). In *Drosophila* too, *en* expression is not clonal in the embryo, but depends on the distance from wingless (*wg*) signaling cells (Vincent and O'Farrell, 1992). After the establishment of the anterior *non-en / en* interface, more posterior cells switch on *en de novo* within each segment, to define the segment boundary (Patel *et al.,* 1989a; Scholtz *et al.,* 1993) (Fig. 2). These later *en*-expressing cells are not necessarily related by lineage to the more anterior *en*-cells (Scholtz and Dohle, 1996). A dramatic example of the temporal regulation of *en* expression is found in the leech, where *en*-expressing cells demarcate segment boundaries, despite switching *en* on and off at different times along each stripe (Lans *et al.,*

1993). The fact that *en* is switched off in cells that are too distant from an inductive signal and switched on *de novo* in cells not related by lineage is relevant, because it was thought that a key feature of the role of *en* in morphogenesis was that it was switched on once in the embryo and maintained clonally thereafter. That is, the state of expression of *en* was thought to be a mechanism of cell memory essential for the building process. Comparative analysis tells us that *en* functions in the trunk in two conserved processes: the establishment of the antero-posterior interface and the definition of the segment boundary.

In all arthropods examined to date, the anterior straight interface of *non-en (wg)* and *en* cells demarcates the point from which limbs will develop (Fig. 2). This, in *Drosophila*, corresponds to the A/P compartment boundary. In some crustaceans, trunk cells along this anterior-edge of the *en* domain can also cease to express *en* (Scholtz and Dohle, 1996), although it is not known whether this also happens to *en*-expressing cells within the limb. It would be interesting to see if there is a straight compartment boundary in all arthropod limbs. This would tell us whether proximo-distal growth necessarily correlates with the presence of an A/P boundary. Furthermore, we still don't know whether proximo-distal growth in other arthropods is linked to clonal subdivisions. And similarly, whereas *en* expression is maintained in insect and crustacean limbs, there is no knowledge as to whether it is through lineage (Patel *et al.,* 1989a,b). Answers to these comparative questions would be very revealing to our understanding of the control of growth. Unfortunately, we cannot extrapolate our understanding of *en* regulation during segmentation to limb formation, because the latter requires extensive cell division along the proximo-distal axis. Consequently, the contribution of compartments, hence lineage, to morphogenesis has to be evaluated independently in these two different cellular contexts. So far, we rely on *Drosophila* data to unravel the cellular aspects of arthropod limb formation.

In vertebrates, where the trunk is not segmented, *en* is still required for limb formation. However, its expression pattern is somewhat different, and might reveal the general role of compartments in morphogenesis. In vertebrates, *En-1* is expressed along a longitudinal stripe that runs along the length of the embryo and divides the limb into dorsal (*Wint7a*-expressing) and ventral (*En*-1 expressing) domains (Fig. 2) (Logan *et al.,* 1997). This means that expression of *En-1* in vertebrates is shifted by 90° compared to *Drosophila*. The interface between these domains gives rise to the Apical Ectodermal Ridge. The AER is essential for proximo-distal limb growth and induces proliferation in the underlying mesenchymal cells (the progress zone). Recent molecular data have shown that the AER is remarkably similar to the fly A/P compartment boundary. Firstly, they are both established by the interface between homologous *wingless/Wint* and *en/En-1*- expressing cells (Loomis *et al.,* 1996; Logan et al., 1997). Moreover, both the A/P boundary and the AER express *decapentaplegic (dpp)/BMP2* homologous proteins of the TGF-β family, which are otherwise repressed by *en/En-1* in adjacent cells (Loomis *et al.,* 1996). Furthermore, in both cases the expression of *dpp/BMP2* is induced by *hedgehog (hh)/Sonic hedgehog (Shh)* from neighboring cells (Marigo e*t al.,* 1996). In *Drosophila* these are posterior cells, where *hh* expression depends directly on *en*. In the vertebrate limb, *Shh* is expressed in a posterior domain adjacent to and maintained by the AER, still depending indirectly on *En-1* function (Hammerschmidt *et al.,* 1997; Logan *et al.,* 1997). A significant difference between
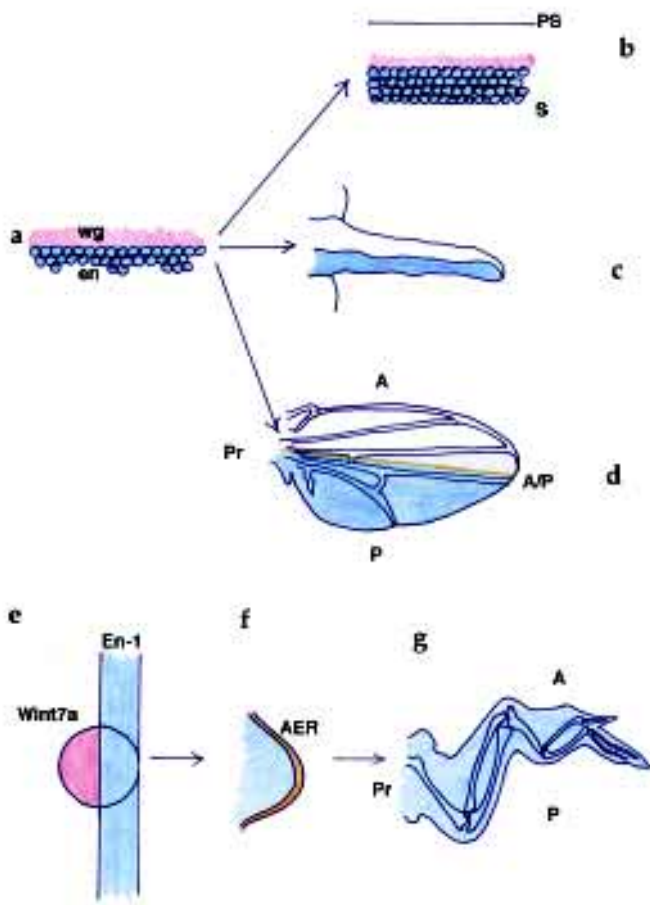
Fig. 2. Conservation of *engrailed* functions. (a) *Neat interface of wingless and engrailed expressing cells, segmentally repeated along the trunk or arthropods.* **(b)** *engrailed is switched on de novo to make the segment boundary. PS, parasegment boundary; S, segment boundary.* **(c)** *Arthropod limb extends from the wg/en interface.* **(d)** *Drosophila wing.* **(e)** *Chick expression of En-1, along the length of the embryo. Subdivision of the limb field into Wint7a and En-1 domains.* **(f)** *Limb outgrowth. The AER is defined by the Wint7a/En-1 interface. En-1 expressed ventrally.* **(g)** *Limb. Red, wingless and Wint7a; Blue, engrailed and En-1; Orange, AER and A/P boundary.*

hh in *Drosophila* and *Shh* in vertebrates is that the domain of *Shh* expression overlaps but does not coincide with the domain of *En-1*, and is in fact shifted by 90°. It would be interesting to know if there are other arthropods with a similar organization of *en* and *hh* patterns as in the vertebrate limb. This could tell us whether *en* is required in non-vertebrates for the establishment of posterior compartments, or more generally for proximo-distal growth. The observation that in vertebrates both normal digits and ectopic limbs form at the interface between *Wint7a* and *En-1* cells (Logan *et al.,* 1997), just as both branches of crustacean biramous limbs form along the *non-en/en* interface (Panganiban *et al.,* 1995), strongly suggests that a conserved function of *en* is to enable growth.

Consistent with this idea, in *Drosophila*, complete homeotic transformations of posterior (P) into anterior (A) (without an increase in growth) following complete removal of both *en* and *inv* functions have not yet been found. This is because if *en* function is

never present in disc development, *dpp* expression will not be spatially restricted and the disc will not grow normally. This implies that a distinct role of e*n* is to establish the domain of *dpp*, to allow growth and field regulation (reviewed in Hidalgo, 1996). This is reminiscent of the role of *En-1* in the vertebrate limb. Loss of function mutations in *En-1* in mouse cause ventral expansion of the AER, with the consequent formation of distal structures in proximal locations of limbs (Loomis *et al.,* 1996). Also, the AER can be damaged and disrupt proliferation of mesenchymal cells leading to truncation of digits (Wurst *et al.,* 1994). Conversely, ectopic expression of *En-1* in chick affects the AER causing a decrease in proximo-distal outgrowth (Logan *et al.,* 1997). Hence, a primary role of *en* is to enable proximo-distal limb growth.

The pattern of growth

The cellular mechanism by which the wing grows remains a great mystery to *Drosophila* developmental geneticists. It has long been believed that all wing disc cells proliferate equally, because clones of cells can be induced anywhere in the disc at any time (García-Bellido and Merriam, 1971). However, there may be proliferation centres which change with time, because the distribution of clones is not identical at all times (García-Bellido and Merriam, 1971; González-Gaitán *et al.,* 1994). Furthermore, clusters of synchronous cells have been observed at different times during wing development (Milan *et al.,* 1996a, b). Intriguingly, cells in these clusters are not clonally related and in the early phase of wing growth these clusters cannot clearly be correlated to particular wing regions. In vertebrates, the AER is the leading edge of the growing bud, and induces proliferation in the underlying progress zone (Fig. 3). This in turn maintains the neighboring AER, thus establishing a positive feedback loop that controls growth. Cells leaving the progress zone earlier will form proximal structures, whereas cells that divide for a longer time within this zone will make distal structures. Hence, growth is directional.

We are used to looking at *Drosophila* wing discs from a frontal view: the flat aspect of the disc towards us, the A/P boundary running within the flat disc. However, if we flip the disc so that we now have the folds of the wing pouch in a frontal view, and the compartment boundary on the edge, the fly disc looks like a vertebrate limb bud (Fig. 3). Now *en/En-1* expression looks the same in both limbs, the AER ridge corresponds to the A/P boundary and *hh/Shh* expression is oriented with regards to veins and digits. In flies, *dpp* is expressed along the A/P boundary, just as BMP2 is along the AER. There is abundant evidence ascribing *dpp* a role in the control of wing disc growth from this expression domain. Perhaps the most elegant demonstration is the induction of clones expressing *dpp* ectopically in wings that practically lack *dpp* function (Fig. 4) (Zecca *et al.,* 1995). *dpp* expressed in these clones alone is able to induce proximo-distal growth and wings of remarkably normal appearance in terms of growth. Similar effects have also been observed when clones lacking *smoothened* function redirect the distribution of *dpp* expression more anteriorly than its normal domain (Chen and Struhl, 1996). Conversely, lack of *dpp* function in mutant flies and in clones along the A/P boundary prevents wing growth (Posakony *et al.,* 1991). And ectopic expression of *dpp* induces cell proliferation (Capdevila and Guerrero, 1994; Burke and Basler, 1996). These experiments demonstrate that *dpp* expressed along the A/P boundary is
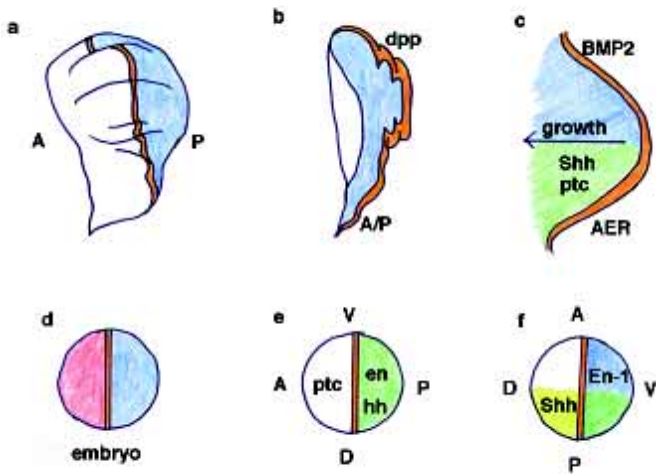
Fig. 3. Growth from the AER and A/P boundary. (a) *Wing disc, classical frontal view.* **(b)** *Wing disc, flipped, lateral view.* **(c)** *Vertebrate limb bud.* **(d)** *Establishment of the wing disc field in the Drosophila embryo by the wingless/engrailed interface.* **(e)** *Wing disc, diagrammatic representation. en and hh are expressed in posterior cells, patched anteriorly, dpp along the A/P boundary.* **(f)** *Vertebrate limb, diagrammatic representation. Shh, ptc and BMP2 expressed posteriorly, En-1 ventrally, BMP2 along the AER. Blue, en and En-1; Red, wingless; Orange, dpp, A/P boundary, AER, BMP2; Yellow, hedgehog, Shh; Green, hh + en, Shh + En-1.*

mitotic recombination induced by X-rays is too low to affect more than one cell within the initial polyclone. However, with the flip-FRT system, the frequency of recombination is increased considerably, and can affect a small group of adjacent cells.

These clones have two important implications: 1) complete wing duplications are only caused by those *en* mutant clones that were able to reproduce an ectopic A/P boundary. 2) That because in order for this to occur the field must be bisected through its border, early (hence older and larger) clones are more likely to span proximal areas than later clones. This situation is surprisingly similar to the one found in vertebrate limbs, where older cells are located in proximal regions. Hence, it would imply that in *Drosophila* growth may also be directional, being equally driven by *dpp* from the A/P boundary. Very remarkably, despite the spatially restricted expression of *dpp* along the A/P boundary, *dpp* receptors are required throughout the wing blade to fulfill the role of *dpp* in the control of cell proliferation (Burke and Basler, 1996). Moreover, this long range requirement for *dpp* changes as the disc grows. Removal of the *dpp* receptor Tkv causes more dramatic effects earlier on in disc development and closer to the source of Dpp (Burke and Basler, 1996). This also suggests that growth may be directional in *Drosophila* as it is in vertebrates. In this context, it is important to bear in mind that the A/P boundary spans the whole disc along its major axis. It is very revealing that ectopic expression of *dpp* can induce growth only in the wing blade (Burke and Basler, 1996). Perhaps dpp regulates growth preferentially as a consequence of the intersection of the A/P axis with the D/V axis at the
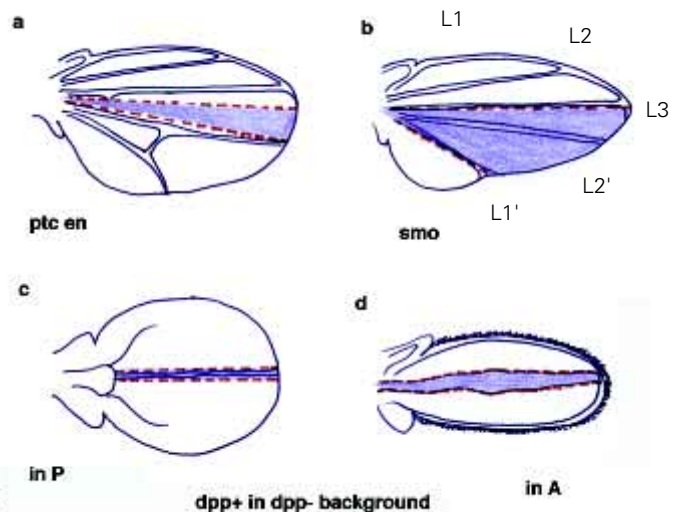
necessary to direct cell proliferation in the wing disc along the proximo-distal axis.

An important consequence of directional growth is that cells that fall beyond the reach of *dpp* should make proximal structures, whereas cells that continue to divide closer to the source should make distal structures. A glimpse of this situation is revealed by the features of clones of *en* mutant cells. A very intriguing aspect of the homeotic transformations caused by loss of *en* function in P compartments of the wing is that they are complete only if clones are extremely large, reach the wing margin and hinge region and duplicate a compartment boundary (see below) (Fig. 5) (Tabata *et al.,* 1995). That is, a new morphogenetic field (generated from ectopic both anterior and posterior compartments) must be established in order for controlled growth and patterning to take place. The limb field in vertebrates is defined by the homogeneous expression of *Wint7a* (Parr and McMahon, 1995) (Fig. 2), and similarly in the fly wing the homolog *wg*, is expressed throughout the wing field in the early stages of the wing disc development (Couso *et al.,* 1993). Later on, *wg* expression is restricted to several domains, which include a ring of cells at the base of the wing (Couso *et al.,* 1993), perhaps defining the edge of the wing field. *en/En-1* expression bisects the field in vertebrates and flies, defining the domain of expression of *BMP2/dpp* (Sanicola *et al.,* 1995; Loomis *et al.,* 1996) (Fig. 3). In order to induce an ectopic anterior compartment within the normal posterior, clones of *en* mutant cells must bisect again the field in two points across its border and consequently induce a new stripe of *dpp* expression (Fig. 5). These two boundaries function as centers for the organization of normal and duplicated wing growth. This situation has never been reported with X-rays induced clones, presumably because the frequency of

Fig. 4. *dpp* directs growth and establishes the A/P boundary. (a) *Clone of ptc-en double mutant cells causing an anterior ectopic compartment boundary. This boundary is not flanked by en expressing cells on either side.* **(b)** *Clone of smo mutant cells between the normal compartment boundary and vein 3. This clone duplicates the area between L3 and L1 and establishes an ectopic compartment boundary just posteriorly to L3. The normal boundary lies at the posterior edge of the clone, posterior to ectopic L1'.* **(c)** *Clone of cells expressing dpp ectopically within the posterior compartment in a background mutant for dpp. Notice the remarkable straightness of the clone. The normal A/P boundary (both in c and d) lies proximally, although it is practically imperceptible.* **(d)** *Clone of cells expressing dpp ectopically in A, in a dpp mutant background. Clones in purple.*
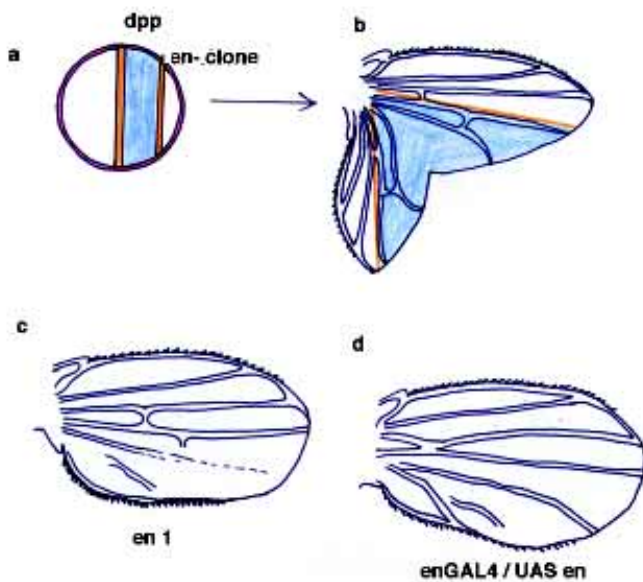
Fig. 5. Pattern transformations caused by alterations in engrailed expression. (a) *Hypothetical transection of the wing field early in development by a clone of en/inv mutant cells that will later on give rise to organized growth and pattern transformation.* **(b)** *Observed FRT-hsFLIP induced clone of en/inv mutant cells causing the formation of an ectopic A/P boundary, a duplicated wing with A (mutant) and P (wild-type) compartments.* **(c)** *Homeotic transformation observed with en[1,] a neomorphic allele of en.* **(d)** *Similar transformation caused by overexpressing en in the posterior compartment. Blue, engrailed expression; Orange, A/P boundary; Pink, clone; Red, edge of the wing field.*

presumptive wing margin (Meinhardt, 1986; Diaz-Benjumea and Cohen, 1993; Williams *et al.,* 1994; Neumann and Cohen, 1997) or the P/D axis in the hinge region (Neumann and Cohen, 1996). This situation is similar to the vertebrate limb, in which the AER is restricted distally. It is extremely intriguing that both in flies and vertebrates proximo-distal limb growth requires the intersection of A/P and D/V axes, having conserved the molecules, even if their distribution has changed. In flies, and subsequently to the establishment of the limb primordia, *en* and *hh* define the P compartment, whereas *wg* and *fringe* are restricted to the ventral compartment, opposing the expression of *apterous* in dorsal cells. In vertebrates, *En-1* is expressed in ventral cells, opposing dorsal cells expressing *Wint7a* and the *fringe (r-Fng)* and *apterous (Lmx-1)*homologs, whereas *Shh*is expressed in posterior cells (Johnson and Tabin, 1997). These comparable orthogonal distributions reveal constraints for proximo-distal growth.

The compartment boundary as a consequence of *dpp* function and growth

Because posterior clones of cells mutant for *en[1]* can cross the A/P boundary into anterior territory, it was proposed that *en* regulates cell-autonomously the expression of cell adhesion molecules which prevent anterior cells from mixing with posterior cells (García-Bellido, 1975). Hence, cells lacking *en* function acquire anterior features, and mingle with cells of the anterior compartment. Consequently, the compartment boundary was considered

a line which results from differential cell affinities expressed by all anterior or posterior cells (García-Bellido, 1975; Morata and Lawrence, 1975).

Over recent years we have seen that the A/P boundary does not simply behave as an adhesion barrier to proliferating cells. In fact, clones of cells mutant for *en* can cross the boundary from the posterior side, but they can also cross it from the anterior side, and they can equally respect it and not cross it at all (Hidalgo, 1994; Blair and Ralston, 1997). Furthermore, clones of cells lacking the function of either *smoothened* or *cubitus-interruptus*, two genes which are expressed in the anterior compartment, can cross into posterior territory, also indicating that the A/P boundary is not established exclusively by genes downstream of *en* (Domínguez *et al.,* 1996; Blair and Ralston, 1997; Rodriguez and Basler, 1997). Moreover, when clones of cells lacking *en* function are found in their entirety within A, they do not mingle with anterior cells (Blair and Ralston, 1997). Instead, they have round edges, as if avoiding contact with surrounding cells. The same clone shapes are found when cells lacking *smo* or *ci* functions are located within P (Domínguez *et al.,* 1996; Blair and Ralston, 1997). Finally, to date no cell adhesion molecules have been found to be expressed differentially in A or P compartments. These results suggest that, whereas it cannot be ruled out that *en* may regulate expression of adhesion molecules, differential cell affinities expressed by A and P posterior cells are not clearly part of the compartmentalization process. This provokes the question of why then should normal clones never cross the compartment boundary.

There is genetic evidence indicating that the compartment boundary depends on *dpp* function (Hidalgo, 1994) (Fig. 4). The most dramatic is the fact that, when clones of *dpp* expressing cells in a *dpp* mutant background induce the formation of wings, these clones are unusually long, thin and straight (Fig. 4) (Zecca *et al.,* 1995). The straightness of these clones resembles the normal A/P boundary. Furthermore, ectopic expression of *dpp* in *patched-engrailed*double mutant clones can induce an ectopic boundary within A (Hidalgo, 1994) (Fig. 4). And similarly, *smo* clones within A can displace the expression of *dpp* more anteriorly, also generating an ectopic compartment boundary within A (Chen and Struhl, 1996) (Fig. 4). And finally ectopic expression of the *dpp* activated receptor *tkv* generates clones of straight boundaries that avoid contact with neighboring cells (Burke and Basler, 1996). In all these cases, the ectopic boundaries are flanked on both sides by cells that do not express e*n*. Conversely, loss of *dpp* function alone damages the integrity of the A/P boundary (Hidalgo, 1994). Remarkably, ectopic boundaries are present, and the endogenous boundary can be damaged, despite the fact that *en* expression remains unaltered. This means that, rather than *en*, *dpp* function is necessary and sufficient to establish the A/P compartment boundary.

This view implies that clones of mutant cells that cross the A/P boundary must in some ways affect *dpp* expression. This is consistent with the observations that *en[1]* mutations cause the ectopic expression of *dpp* throughout P (Raftery *et al.,* 1991). Ectopic expression of *dpp* is also caused by complete loss of *en/inv* functions and by *ptc en* double mutant clones (Hidalgo, 1994; Sanicola *et al.,* 1995; Tabata *et al.,* 1995). Similarly clones of *smo* and *ci* also interfere with the regulation of *dpp*, either by reducing or displacing it from its normal position (Chen and Struhl, 1996; Domínguez *et al.,* 1996). Clones from all these mutant genotypes can cross the A/P boundary.
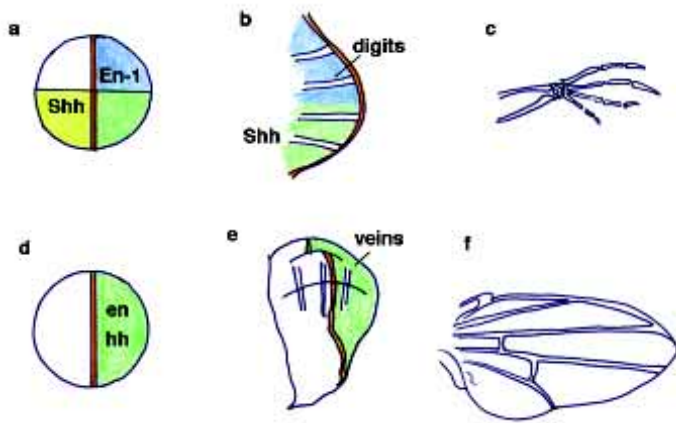
Fig. 6. Patterning of digits and veins from the source of *Shh/hh*. (a) *Vertebrate limb field, frontal view.* **(b)** *Vertebrate limb bud, lateral view. Digits are specified perpendicularly to the AER. Shh is expressed in a posterior domain, En-1 ventrally.* **(c)** *Adult vertebrate limb. Final skeletal morphology of digits.* **(d)** *Fly wing disc field. en and hh are coexpressed in P.* **(e)** *Fly wing disc. Veins appear in the wing disc parallel to the A/P boundary.* **(f)** *The adult fly wing, vein pattern. Blue, engrailed and En-1; Yellow, Shh and hh; Green, Shh and En-1, hh and en; Orange, AER and A/P boundary.*

Blair has shown that the A/P boundary can also be visualized in terms of cell morphology, as these cells are more elongated than their neighbors (Blair, 1992).

This suggests that *dpp* or *hedgehog* (acting directly or via *dpp*), rather than directly *en*, may regulate the expression of A/P boundary cell adhesion molecules (Blair and Ralston, 1997; Rodriguez and Basler, 1997). The existence of enhancer trap lines expressed along this boundary suggest that this is not unlikely. The compartment boundary could thus be stabilized by cell adhesion between cells at either side of the boundary, but not necessarily beyond.

A puzzling aspect of cell behavior is revealed by twin spot analysis. Mutant e*n* clones have been found in their entirety within A, whereas their twin lied in P. According to the compartment hypothesis, *en* mutant cells crossed completely into A territory (Blair and Ralston, 1997). Although migration of wing clones has not been observed *in vivo*, if the A/P boundary were to behave like the AER, it would be difficult to understand how twin clones could be found in different compartments other than through cell migration. The AER is a specialized group of cells that can be easily distinguished from surrounding cells and that does not exchange cells with its surroundings. Consequently, twin clones should not be found in different compartments as a consequence of cell proliferation. On the other hand, if differential cell affinities between all A and P do not play a role in compartmentalization, it is unclear why should mutant clones migrate at all.

Because *dpp* function has been shown to induce cell proliferation (Posakony *et al.,* 1991; Zecca *et al.,* 1995), this implies that the straight A/P compartment boundary is a consequence of the control of growth. That is, the A/P boundary of the wing is telling us how the wing disc grew. In *dpp* mutants in which the wing disc does not grow to its normal size, the A/P boundary is not defined (Hidalgo, 1994). Furthermore, very suggestive is the observation that a proximal *nubbin* clone causing a dramatic reduction in wing

size does not respect the A/P boundary (Ng *et al.,* 1995). It would be interesting to know if further mutations that reduce the normal growth of the wing also show abnormal cell behavior at the compartment boundary. To conclude, the A/P boundary is a footprint of the pattern of growth. In the near future, it will be very exciting to learn more about this mysterious line, its relationship with growth and about how far we can generalize our understanding of the control of growth to different morphologies.

Patterning: shifting *hh* and *en* domains according to morphology

en also plays an important role in patterning posterior cells (see Hidalgo, 1996). Complete loss of *en* and *inv* functions in small clones reproduces anterior pattern elements (Hidalgo, 1994; Tabata *et al.,* 1995). Also, homeotic transformations not linked to alterations in growth have been observed when *en* is expressed ectopically in P, indicating that *en* can repress its own expression to mimic partially the loss of function phenotype (Fig. 5) (Guillén *et al.,* 1995). Finally, duplicated wings produced by the ectopic expression of *dpp* within A grow well but do not reproduce posterior patterning (Fig. 4) (Zecca *et al.,* 1995). However, the patterning role of *en* is not restricted to the posterior compartment. In fact, *en* is expressed in anterior cells lying between L3 and L4 (Blair, 1992), where it affects the size of this region and represses enervation at the wing margin (Hidalgo, 1994). This implies that during normal development patterning is not absolutely restricted by compartmental subdivisions. Furthermore, A vs P identity is not a clear cut cell-autonomous consequence of *en/inv* functions. In fact *hh*, expressed in P and direct target of *en*, is necessary to define vein 3, which lies within A (Mullor *et al.,* 1997). This role cannot be accomplished by *dpp*, a frequent target of *hh* (Mullor *et al.,* 1997).
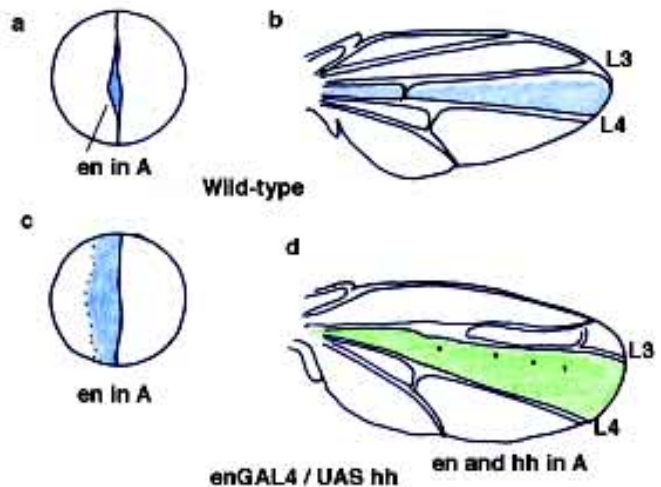

Fig. 7. *en* is induced non-autonomously by hh in anterior cells. (a,b) *Wild type.* (a) *Wing disc, diagram. Expression of en in anterior cells only* **(b)**. *Wing, expression of en between veins L3 and L4.* **(c,d)** *enGAL4/UAS hh.* (c) *Diagram of disc. Expansion of en expression as a consequence of expressing hh in the anterior domain of en.* (d) *Expression of hh and en anteriorly pushes L3 more anteriorly, leaving the sensilla slightly behind. Blue, expression of en; Green, expression of en and hh.*

In vertebrates, loss of function and ectopic expression of *En-1* cause partial transformations of dorso-ventral patterning, suggesting that although *En-1* is involved in D/V patterning, it is not sufficient (Wurst *et al.,* 1994; Loomis *et al.,* 1996; Logan *et al.,* 1997). Furthermore, *En-1* also regulates expression of *Shh* in neighboring cells, where it plays an important role patterning the digits (see (Hammerschmidt *et al.,* 1997). Consequently, specification of digits is linked to D/V patterning and assigning patterning roles exclusively to *En-1* is not straight forward.

Reminiscent of the situation in flies, *Shh* regulates *BMP2* expression in posterior cells, but digit patterning cannot be achieved by *BMP2* . *Shh* is expressed in a posterior domain, only partly overlapping the *En-1* domain, and perpendicular to the AER (Hammerschmidt *et al.,* 1997) (Fig. 6). Digits are also perpendicular to the AER. In the fly wing, veins are instead parallel to the A/P boundary and they are at least partly specified by *hh* (Mullor *et al.,* 1997) (Fig. 6). Intriguingly, this might at least partly explain why *hh* expression coincides with the domain of *en*. It is fascinating that these patterning roles of *Shh/hh* have been conserved, correlating with a shift in their expression domains to allow patterning of digits (perpendicular to the AER) or veins (parallel to the A/P boundary) (Fig. 6). Further comparative studies will tell us how far the patterning roles of *hh* can be separated from its role in growth control.

The intimate relation between *en, inv* and *hh* is not only interesting to our notion of identity, but also to our understanding of how compartments are established, and, in essence, of what they really are. Compartments are considered units of lineage. Consequently, *en* expression is thought to be maintained cell autonomously (García-Bellido, 1975). However, it has been shown that *en* expression between veins L3 and L4 within A is induced by *hh* from posterior cells (de Celis and Ruiz-Gómez, 1995; Guillén *et al.,* 1995) (Fig. 7). Furthermore, ectopic expression of *hh* in all *en* cells causes the anterior domain of *en* to expand dramatically (Mullor *et al.,* 1997) (Fig. 7). And finally, anterior *smo* clones in this region, which cannot receive *hh* signaling, produce neurons typical of loss of e*n* expression (Blair and Ralston, 1997). These data show that *en* expression in A is induced non-autonomously by *hh*. So far, although it is thought that *en* expression in posterior cells is maintained cell autonomously through lineage, this has not been shown.

 It is not known how *En-1* expression is maintained in ventral cells of the vertebrate limb either, nor for how long. However, it is known that *En-1* protein is not required for *En-1* expression, suggesting that cell-autonomous autoregulation is not employed (Logan *et al.,* 1997). Remarkably, despite the regulation of *Shh* expression by a homeobox gene (Johnson and Tabin, 1997), no clonal restrictions have been found in vertebrates within the dorsal *(non-en)* or ventral *(en)* compartments. That is, expression of *Shh* does not seem to be restricted by a clonal mechanism either. It would be interesting to know how the expression of *en* and *En1* are maintained in fly and vertebrate limbs.

The plasticity of morphogenetic fields

Growth and patterning in the wing disc may take place in a manner more similar to vertebrates than had been anticipated. Rather than lineage and irreversible determination of cell fate, cell communication seems to be the driving force of morphogenesis.

Not surprisingly, cell communication provides cell populations with the plasticity required to adjust to change (regulation within a morphogenetic field). Upon interference with normal development, a field can regulate cell number and cell size to ensure that global normal proportions, size and shape are reached. The wing disc behaves as a field, because it can regulate autonomously when grown in the larval abdomen. Compartments can also regulate, but only as long as there is an A/P interface (Wilcox and Smith, 1980; Weigmann *et al.,* 1997) - that is, their regulation is not autonomous. Regulation is not restricted by compartmental subdivisions either, since following removal or transplantations of fractions of wing disc, the disc regulates by inducing cell proliferation and patterning irrespectively of where compartment boundaries lie (Bryant, 1975). Nevertheless, there is abundant evidence showing that the A/P and D/V boundaries and the proximal edge of the wing field are important in the control of growth during normal development (see Edgar and Lehner, 1996). Cells must sense the dimensions of the field from these three regions.

Whereas a non-autonomous view of compartment regulation would make the notion of lineage dispensable to our understanding of morphogenesis, it would not turn compartments into less stable domains. On the contrary, regulation insures morphological stability. In the future, it will be very enlightening to learn more about the regulation of cell proliferation and size. As Antonio has also foreseen, perhaps we may soon be able to look at a wing and see beyond the image of the geometric design the now mysterious plane of controlled growth.

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