Do the protocadherins Fat and Dachsous link up to determine both planar cell polarity and the dimensions of organs?

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Most, perhaps all cells in epithelial sheets are polarized in the plane of the sheet. This type of polarity, referred to as planar cell polarity (PCP), can be expressed in the orientation of cilia and stereocilia, in oriented outgrowths such as hairs, in the plane of cell division, in directed cell movement and possibly in the orientation of axon extension[1,2]. Another popular area in current research is growth: there is an attempt to find systems that fix the shape and size of organs. Although both polarity and growth are subject to overall control by morphogen gradients[3], the mechanisms of this control are almost completely unknown. Here we discuss recent evidence for a 'steepness hypothesis' that links these two apparently disconnected features of animal development.

"In animal development there are deep-seated and regular growth-gradients which appear to be in the first instance correlated with fundamental properties of the animal body such as polarity"

Julian S. Huxley, 1932

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The history of the steepness hypothesis

Grafting experiments by Wigglesworth, Piepho, Bohn, Locke, Lawrence and Stumpf in the 1940s, 50s and 60s first linked morphogenetic gradients, growth and polarity[4]. When pieces of cockroach limb taken from different regions of one leg segment were grafted together, growth was elicited from cells on both sides of the junction to fill

BOX 1 THE STEEPNESS HYPOTHESIS

The steepness hypothesis has been evolving over many years but it remains speculative, unclear and incomplete. We make five propositions. First, the decision to divide or not divide, to live or die, to differentiate or not is made by single cells in a population. These cell-by-cell decisions, summed over the whole, regulate the growth of an organ and fix its size and shape. Second, in each axis, there is a mechanism that senses the dimensions of the organ and this feeds back to regulate these die-or-divide decisions. Third, this dimension-sensing depends on a linear gradient of some signal set up between the boundaries of a defined and growing population of cells whose maximum and minimum is constant; consequently, as the organ grows, the gradient becomes less steep. Thus the steepness of the gradient is effectively a measure of the dimension in one axis that could be conveyed to every cell (Fig. 1). Fourth, the morphogens responsible for the overall pattern of an organ (such as Dpp, Hedgehog and Wingless) set up and orient the Ds/Ft system, which then provides a linear gradient. The Ds/Ft system regulates both growth and PCP. Fifth, in the Ds/Ft system, the direction of a gradient (the vector) determines cell polarity, whereas the steepness of the same gradient feeds into the die-or-divide decisions through the Hippo pathway, which is linked to growth. Note that the Ds/Ft system may be responsible for one axis of growth (for example, anteroposterior in the abdomen) but there may still be other inputs into that axis. Moreover different systems may be responsible for the other axis (dorsoventral in the abdomen).

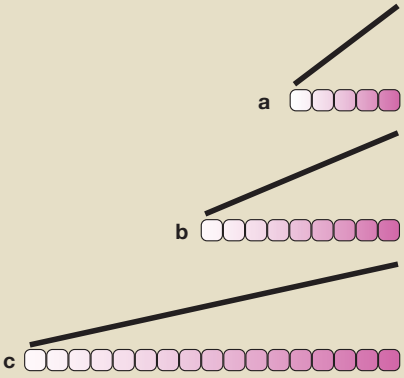

Figure 1 The steepness hypothesis. (**a–c**) The gradient is assumed to be linear. The gradient is also shown by the pink shading of the blocks, which represent cells. As the organ grows, the maximum and minimum limits are conserved while recently divided cells take up intermediate scalar values from their neighbours (some evidence for this can be found in ref. 34). The steepness of the gradient at each point, measured perhaps as a differential across each cell, correlates with one dimension of the organ. Growth would cease when the slope of the linear gradient declines to a certain threshold value, a value that would vary from stage to stage[10]. The steepness hypothesis could help explain the mathematical rules of growth observed in the early twentieth century by D'Arcy Thompson[36] and Huxley[37].

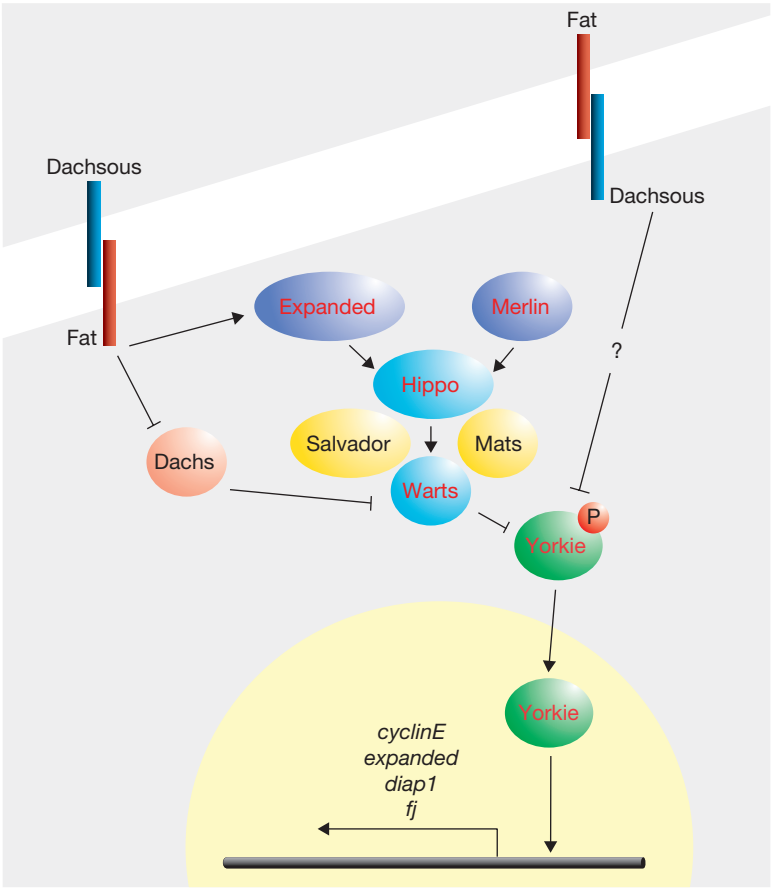

Figure 2 The Hippo pathway[35]. Note that it is not clear how Ft and Ds feed into the pathway, but we imagine the input within one cell to be from both types of Ft/Ds heterodimers in the membrane. Warts is thought to regulate the phosphorylation of the transcription factor Yorkie; unphosphorylated Yorkie enters the nucleus and drives transcription of target genes.

in the missing region. Bohn argued that each leg segment is patterned by a morphogenetic gradient in the proximo-distal axis and that the polarity and length of the new tissue depends on the net difference between the scalar values of the gradient that were juxtaposed by the graft. This idea was then refined further from work on other insects (*Hemiptera*): if, in the wild-type, the direction of slope or vector of the gradient normally determines polarity[5], then the steepness could measure the dimension and regulate growth[4] (see BOX 1).

In the 1960s and 70s, we thought that the gradient would prove to be a morphogen. However, morphogens responsible for patterning, such as Hedgehog in the *Drosophila melanogaster* abdomen, Decapentaplegic (Dpp) and Wingless in the *Drosophila* wing, were later shown to operate upstream of the systems controlling PCP[6,7]. Even later, evidence was gathered from the *Drosophila* abdomen that morphogens act through two independent systems to determine PCP, one depending on Frizzled, Van Gogh and Flamingo and another on the protocadherins Ds and Ft[8]. Mutations in the latter system affect growth as well as PCP, suggesting that the Dachsous/Fat (Ds/Ft) system may provide some measure of dimension to the cells[9–11]. Recently it was also found that if cells with widely different levels of Dpp signalling are apposed, cell division is elicited locally[12], suggesting that disparity of Dpp signalling itself is mitogenic, or that it regulates a subordinate system for controlling growth. As we explain below, and as proposed previously[12,13], it now seems that morphogens control growth indirectly through the Ds/Ft system.

The Ds/Ft system and the Hippo pathway

In the last few years, the concept of a Hippo 'pathway', or 'kinase cassette' (including Merlin, Expanded, Hippo, Salvador, Mats, Warts and Yorkie) has been developed[14] (Fig. 2). A flurry of papers has proposed that the Hippo pathway acts to suppress tumours and is involved in size control. However, these conclusions were based on mutant phenotypes and on experiments that upregulate Yorkie to cause excess growth, leaving open what the Hippo pathway really does during normal development. Nevertheless, it was found recently that *ft*⁻ cells, long known to divide excessively[15], upregulate target genes of the Hippo pathway, such as *cyclin E* and *diap1*. These genes promote growth and inhibit apoptosis, suggesting that the wild-type function of Ft is to regulate cell proliferation through the Hippo pathway[16–19]. Ft has even been called a tumour suppressor[20,21] but it is not clear how it might act in size control.

The essentials of the Ds/Ft system for PCP: the Ds/Ft model

Here follows a simplified summary of our current model of how the Ds/Ft system generates PCP, based on the abdominal epidermis of *Drosophila*[8]; we refer to this as the Ds/Ft model (Fig. 3). Ds and Four-jointed (Fj), a Golgi kinase[22], are expressed in opposing gradients in the anteroposterior axis (set up by the morphogens Hedgehog and Wingless). The heterodimeric bridges formed by Ds and Ft from cell to cell ensure that the amounts of Ft to Ds on the surface of one cell can affect the distribution of Ds and Ft on neighbouring cells[23,24]. According to the Ds/Ft model, when clones of cells are made that express, for example, a large amount of extra Ds, Ft and Ds molecules are redistributed on abutting cells on both sides of the clone/host interface for a few rows of cells. These redistributions of Ds and Ft cause local changes in steepness and/or direction of the Ds/Ft slopes. Of course there can only be a visible reversal of polarity where the effects of the clone oppose, rather than reinforce, the background polarity. For example, in the abdomen, changes in hair orientation are found either anterior or posterior to the clone but not both. Note also and importantly for what follows, the experimental results[8] show that cells with only Ds or Ft can repolarize their neighbours. However these neighbours need both Ds and Ft to be repolarized[8]. These observations argue against a simple ligand–receptor relationship between Ds and Ft[7,17]; instead both Ds and Ft act as ligands and receptors for each other[8].

New evidence for the steepness hypothesis

The steepness hypothesis and the Ds/Ft model have now been supported by two exciting papers[25,26]. As with studies of PCP[8], the method used was to make clones of cells that

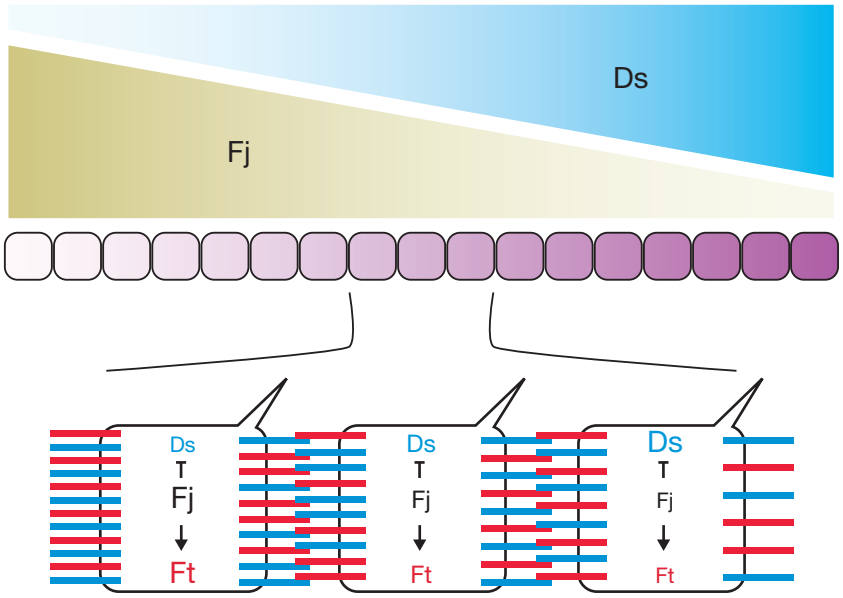

Figure 3 A sketch of the Ds/Ft model. There is evidence that the Ds and Fj gradients are set up by the primary morphogens; they make a Ds/Ft gradient that is responsible for both PCP[7,8], and for activation of Hippo targets that drive growth[16–19]. In the model[8], Ds and Fj concentration gradients span the organ and interact with uniformly expressed Ft molecules to build together, in one axis, a linear gradient of Ds/Ft heterodimers. Putative distributions of Ds and Ft heterodimers are indicated below. In the model, Ds and Ft function as *trans* heterodimers, acting, in effect, as ligands and receptors for each other. This model explains, for example, why ds^- or ft^- cells do not show PCP or growth responses to neighbouring cells — the numbers of Ds/Ft heterodimers could not be compared on the two faces of ds^- or ft^- cells.

set up sharp disparities in the Ds/Ft system but, instead of assessing hair polarity, Rogulja *et al.*[25] and Willecke *et al.*[26] looked at expression of gene targets of the Hippo pathway. What is exciting is their results show that the effects of the Ds/Ft system on Hippo targets are co-extensive with the effects shown earlier on PCP[8], suggesting that growth and PCP are both outputs of the same signalling mechanism. To illustrate this, we choose a few of their many experiments (Fig. 4).

Clones overexpressing Ds

Clones overexpressing the *ds* gene were made in the wing disc, creating new Ds/Ft differentials all around the perimeter of the clone. Given that Ds and Ft are present in all cells, the Ds/Ft model predicts that when Ds is expressed strongly in a clone, all around its perimeter, inside and outside, there will be sharp local increases in steepness and, in some places, changes in slope direction (the latter is seen in the earlier PCP[8] experiments). Both new papers[25,26] report that when the Ds/Ft model predicts changes in steepness, a Hippo target gene is upregulated. The ranges of effects on PCP and on Hippo targets are similar, extending to about 2–4 cells from the interface, leaving the centre of the clone relatively unaffected.

If clones containing extra Ds are made in a ft^- fly, the Ds/Ft model predicts that there should be no effects on the Hippo pathway, as all cells in the animal lack Ft, both inside the clone and outside, and thus should be refractory to any incoming Ds/Ft signal. Indeed, there is no effect on the Hippo pathway[25,26]. If clones expressing Ds are made in a ds^- fly, cells outside the clone lack Ds and therefore cannot respond. But just inside the clone, where both Ds and Ft are present, an abrupt, local increase in steepness is predicted by the Ds/Ft model, and indeed an increase in Hippo target expression, as well as growth, is observed[26].

Loss and gain of Fj

Fj modulates Ds/Ft interactions, enhancing Ft activity and reducing Ds activity[8,27]. Removing or adding Fj to clones of cells have opposite effects on hair orientation: in the abdomen, removing Fj reverses hairs behind the clone, whereas adding Fj reverses hairs in front[13]. Although the polarity is changed on only one face of the clones, the Ds/Ft model predicts that in both experiments, steepness of the gradient will be increased all around the clone borders. Accordingly, in the wing and eye, Hippo target genes are upregulated in both

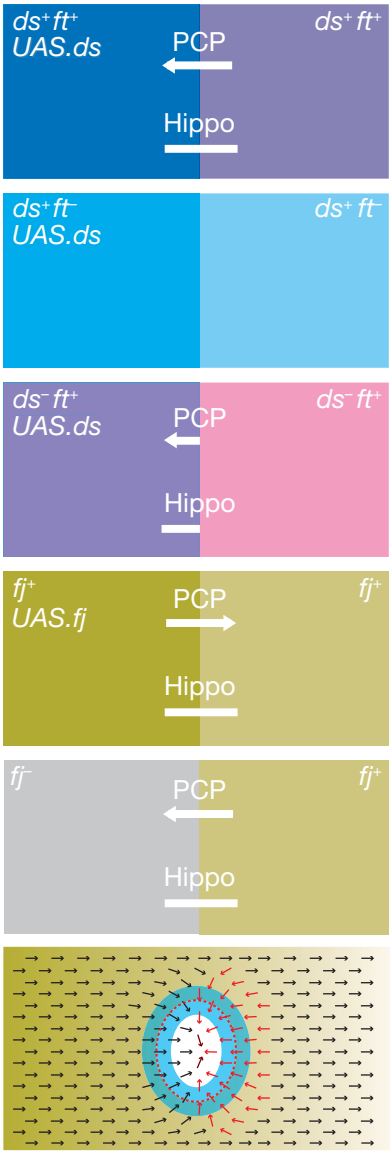

Figure 4 The effects of juxtaposing cells with different levels or states of the Ds/Ft system. The left column shows the genotypes of clones and the right column the background genotypes. The interfaces between cells of these two genotypes drive the effects which produce co-extensive outputs onto PCP and Hippo targets. The arrows show formally the sign as well as the extent of polarity effects that reverse the background polarity on the appropriate sides of the clones[8]. The bars indicate the extent of upregulation of Hippo targets above background levels — these extend a few cell rows on one or both sides of the interfaces as shown[25,26]. Ft is indicated in pink, Ds in blue and Fj in green. In the bottom panel, we show a single example of an ellipsoidal fj^- clone. The clone is outlined with a dotted red line; the arrows indicate the PCP of oriented structures, such as cuticular hairs, red arrows showing where polarity has been changed by the clone-induced Ds/Ft slopes. Blue marks the zone, including both the periphery of the clone and its surroundings, in which Hippo targets are upregulated as a result of local steepening of those slopes.

experiments on both sides of the interface between mutant and wild-type cells[26], even though polarity changes are found on one face[13,28]. Further, in PCP it was observed that effects in *fj*⁻ territory have a longer range[8]; suggestively, the range of effects on Hippo targets seems also to be increased where the Fj concentration is lower[26].

This spatial fit between the PCP results and upregulation of Hippo targets in these experiments (and others described in the two recent papers[25,26]) argue that PCP and the Hippo pathway are both outputs of the same Ds/Ft landscape; the orientation of hairs depending on the vectors of local gradients and the activation of Hippo targets correlate with the steepness of these slopes.

Hippo pathway and cell division

There is good evidence that upregulating Hippo targets increases mitosis. Both new articles[25,26] describe the effects of clones expressing Ds and Fj and they find stimulation of mitosis in the vicinity of the clone borders, both outside, and in some cases, inside the clone. They report a greater stimulation when the Ds- and Fj-expressing clones are located near the nadirs of the Ds and Fj gradients, respectively, as is also the case for the activation of Hippo targets[25,26] and PCP[8,13,28]. Again these results argue that it is the degree of difference across the interface in Ds/Ft activity that drives Hippo targets and cell division, as well as changes in PCP. The effects on cell division were blocked in *dachs*⁻ flies[25], a gene needed downstream of *fat* for both growth and, possibly PCP[29]. These experiments all support the steepness hypothesis — the steepness of the Ds/Ft gradient regulating Hippo target expression and cell proliferation, and its direction providing information used to polarize the cells.

Problems with the main conclusions and some unanswered questions

Some results do not fit: first, according to the Ds/Ft model, *ft*⁻ clones should show a uniform level of expression of Hippo targets inside the clone and should induce non-autonomous upregulation in the surrounding wild-type cells. Although *ft*⁻ clones do cause non-autonomous effects on PCP[8,13], the same non-autonomous effect has not (yet) been observed on Hippo targets or growth[19]. If this latter result proves to be correct, there will be a problem with the straightforward case we have presented.

Second, the Ds/Ft model predicts that the effect of Ft on neighbouring cells, the key element of PCP, depends on its extracellular domain interacting with Ds in the next cell. It follows that the intracellular domain should be ineffective on its own, and we found that when this domain was overexpressed locally, it had no detectable PCP activity[8]. However, uniform overexpression of the intracellular domain can partially rescue the *ft*⁻ overgrowth phenotype[30]. These divergent results between PCP and growth seem to argue in different directions and are currently unresolved.

Third, some results suggest that the steepness hypothesis is insufficient. As expected, substituting uniform expression of both Ds and Fj in place of the normal, opposing gradients of Fj and Ds (that is, flattening the slope of the Ds/Ft system) does reduce growth, but the effects, as monitored by BrdU incorporation, are only transient[25] and the resulting wings are only modestly reduced to about half their normal size[25,26,31]. Removing either Ds or Ft (and activating the Hippo pathway) results in enhanced growth, but again, the effects are weak, as the rare surviving adult flies make wings that are only moderately enlarged[30]. If the Ds/Ft model were as central as we like to believe, then perhaps flattening or removing the Ds/Ft gradient should have more catastrophic effects.

Fourth, the precise nature of the Ds/Ft gradient is unknown; although our Ds/Ft hypothesis posits that the numbers of Ds/Ft *trans* heterodimers are the key variable, this is not proven. In the model[8], a difference in the number of heterodimers between the two faces of a cell may be the cue for planar polarity and the amount of that difference could represent the steepness, but there is no direct evidence.

Finally, it seems important for the steepness hypothesis that the gradient be more or less linear, as only a linear gradient could convey consistent information of dimension to all cells. But it is not known whether the gradient is linear.

The steepness hypothesis has other implications, as yet unresolved. For example, it may seem that growth should be related to organ size — faster, early in development, when primordia are small and the slope steep, and slower later on, as the cells increase in number and the linear gradient becomes shallow (BOX 1). Yet, the growth rate, for example of the Drosophila wing, is relatively constant. But here we begin to enter a continent beyond the scope of this commentary — there it may be found that the controls of size and shape depend on many inputs and feedback mechanisms[4,32,33] operating in two or even three axes.

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COMPETING FINANCIAL INTERESTS

The authors declare they have no competing financial interests.

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