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Morphogens, nutrients, and the basis of organ scaling

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SUMMARY The regulation of organ size is a long-standing problem in animal development. Studies in this area have shown that organ-intrinsic patterning morphogens influence organ size, guiding growth in accordance with positional information. However, organ-extrinsic humoral factors such as insulin also affect organ size, synchronizing growth with nutrient levels. Proliferating cells must integrate instructions from morphogens with those from nutrition so that growth proceeds as a function of both inputs. Coordinating cell proliferation with morphogens and nutrients ensures organs scale appropriately with body size, but the basis of this coordination is unclear. Here, the problem is illustrated using the *Drosophila* wing—a paradigm for organ growth and size control—and a potential solution suggested.

INTRODUCTION

Much of the diversity seen in animals is manifested in differences in size and shape. No group exemplifies this more profoundly than insects: the million-or-so species described all possess similar numbers of body segments and limbs, yet insects still vary impressively, due to differing proportions of their homologous body parts. Mammals are no different in this regard. Bone for bone, tarsier and human skeletons are *qualitatively* alike, yet *quantitatively* very different. Morphological evolution, it would seem, commonly progresses through changes in dimension. But via what mechanisms? In the last two decades, studies of organ size regulation have brought us close to an answer to this question. Or rather, to two answers. Because, as outlined below, two views of size control exist. Reconciling them is essential for a cohesive model of organ growth.

MORPHOGENS: ORGAN-INTRINSIC SIZE REGULATION

There is a dogma in developmental biology that organ size is determined intrinsically. Its foundations are early transplantation experiments, most compellingly those of Twitty and Schwind (1931). By exchanging limbs and eyes between small and large salamander species, it was observed that the transplants grew to the size they would normally attain in the donor species. The salamander organs appeared to "know what size to become," implying internal programming to attain the species-specific size. Similar experiments in a variety of animals suggested such autonomous control is the rule (Bryant and Simpson 1984).

The basis of this internal size information was hinted at by regeneration experiments. The cockroach limb regrows after damage: if a proximodistal tibia section is removed and the remaining distal limb grafted back onto the proximal stump, intercalary growth will occur at the join. This restores the tibia to the normal size, the process stopping when the intervening pattern is re-established (Bohn 1970). The interpretation was that growth must be governed by the organ's internal positional information (French et al. 1976). Morphogens—then hypothetical molecules—were posited to impart this kind of information (Lawrence 1970). In the standard model (Wolpert 1969), morphogens emanate from discrete sources in the organ, forming extracellular gradients that position different cell types according to concentration.

Speculation about the existence of morphogens ceased when proteins encoded by the *Drosophila* genes *decapentaplegic* (*dpp*) and *wingless* (*wg*) were revealed to pattern the fly's organs as diffusible, concentration-dependent regulators of gene expression (Lecuit et al. 1996; Nellen et al. 1996; Zecca et al. 1996; Neumann and Cohen 1997). Homologues were shown to function similarly in vertebrates, and a consensus grew that morphogens were real, and important organizers of organ development (Tabata and Takei 2004). Crucially, beyond specifying patterns, the newly identified morphogens appeared to influence organ size. This was demonstrated by experiments conducted on fly legs and wings, where *wg* and *dpp* were expressed in discrete clones of cells:

such clones cause surrounding cells to proliferate, leading to supernumerary limbs (Struhl and Basler 1993; Zecca et al. 1995). Conversely, clones lacking Dpp or Wg receptors fail to proliferate and are excluded from the limb (Burke and Basler 1996; Lecuit et al. 1996; Chen and Struhl 1999). Analogous experiments on vertebrate limbs gave comparable results: ectopic expression of the Hedgehog orthologue Sonic Hedgehog (Shh) reorganizes anteroposterior pattern and induces duplications of digits (Riddle et al. 1993), whereas loss of Shh causes limb truncations (Chiang et al. 1996; Pagan et al. 1996).

Today, substantial evidence supports the view that organ dimensions are dependent on the influence of a small number of conserved extracellular signalling molecules: ligands of the Hedgehog, bone morphogenetic protein/transforming growth factor-β (BMP/TGF-β), wingless/Wnt (Wg/Wnt), epidermal growth factor, and fibroblast growth factor families. As organ-intrinsic regulators of growth, these molecules likely account for the autonomous control of organ size revealed by transplantation experiments. How morphogens influence organ growth remains unclear; relevant issues now include understanding how cells interpret gradients as growth-informative (Box 1), and identifying downstream pathways that control cell proliferation.

INR/TOR SIGNALLING: ORGAN-EXTRINSIC SIZE REGULATION

Separately from the developmental biologists studying intrinsic size regulation, physiologists and endocrinologists were deciphering an entirely different size control system. The finding that growth hormone from the hypothalamus regulates postnatal growth in mammals (Palmiter et al. 1983), and when deficient in humans caused dwarfism (Rimoin et al. 1966), indicated that organ size is also controlled by humoral signals. Growth hormone was shown to act by inducing expression of insulin-like growth factor-1 (IGF-1), a systemic peptide that stimulates growth of most organs (Stewart and Rotwein 1996). IGF-1's net effect is to control body size: mice lacking *IGF-1* attain only 30% of wild type weight (Baker et al. 1993), and IGF-1 production is diminished in Central African pygmies (Merimee et al. 1981).

The last decade saw IGF-1 and its fly homologues the *Drosophila* insulin-like peptides (DILPs) linked to a conserved signalling pathway: the insulin receptor/target of rapamycin (InR/TOR) pathway (Oldham and Hafen 2003). *Drosophila* mutants with impaired InR/TOR signalling phenocopy the effects of starvation, being small bodied due to fewer and smaller cells (e.g., Bohni et al. 1999). Mosaic animals have shown that the pathway functions cell autonomously to control cell growth and proliferation rates in most organs (Weinkove et al. 1999). Many of the pathway's effects are

Box 1. How do morphogen gradients control cell proliferation?

How morphogens specify patterns is relatively well understood (Ashe and Briscoe 2006). Less is known about how they control growth. In the *Drosophila* wing, proliferation occurs uniformly (Fig. 1A) (Schubiger and Palka 1987), despite being regulated by graded morphogens. How do gradients induce proliferation uniformly? One model conjectures that rather than reading the concentration, cells interpret the gradient slope—constant across a tissue patterned by a linear gradient (Day and Lawrence 2000). The steeper the slope, the greater the momentum to divide. As the tissue expands and the gradient accommodates, the gradient slope would reduce; at a certain point, growth would stop. The slope model thus includes a potential growth termination mechanism. Support comes from experiments in which an activated Dpp receptor (Thickveins; Tkv^{QD}) (Nellen et al. 1996) was expressed in clones using a drug-inducible driver (Rogulja and Irvine 2005). Tkv^{QD} strongly activates the Dpp pathway, creating a marked discrepancy in signalling between the clone and surrounding cells. At these interfaces proliferation was transiently accelerated, and the same effect occurred when a pathway repressor (Brinker) was expressed. Hence, proliferation happens when neighboring cells detect large differences in Dpp signalling. If steep enough, discrepancies sufficient to induce proliferation may exist along the length of the endogenous Dpp gradient. Flattening the gradient by inducing Tkv^{QD} throughout the whole disc reduced wing cell proliferation, again consistent with the slope model (Rogulja and Irvine 2005).

In the alternative "threshold model", cells respond directly to morphogen, but a critical minimum level of morphogen suffices to trigger proliferation. Cells exposed to higher concentrations do not proliferate faster, creating uniform proliferation. This model has found support from studies on the blade territory of the wing imaginal disc (Fig. 1A), which is defined by expression of the selector gene Vestigial (Vg) (Kim et al. 1996). Blade expansion occurs in part through a recruitment process, where a founder population of Vg-positive cells entrains neighboring cells to likewise express Vg (Zecca and Struhl 2007b). Induction of Vg requires a short-range "feedforward" signal coming from Vg-positive cells, but also input from both Wg and Dpp acting at long range. This recruitment process, and the subsequent proliferation of blade cells, both depend on a permissive level of Wg and Dpp—gradient steepness is unimportant (Zecca and Struhl 2007a). Indeed, recent studies where the Wg or Dpp gradients were replaced with moderate, uniform signalling have shown that the wing can grow without gradients (Schwank et al. 2008; Baena-Lopez et al. 2009). A variant of the threshold model posits that cells respond to the absolute concentration of morphogen, with more morphogen driving faster proliferation, but that the graded distribution of morphogen is paralleled by a gradient of growth repressor (Serrano and O'Farrell 1997). The repressor counteracts the morphogen's influence, leading to uniform proliferation. A recent study by Schwank et al. (2011) claims that such a repressor may exist in the wing in the form of Fat: a growth suppressor and upstream regulator of the Hippo/Warts pathway (Pan 2010). The authors note that loss of Fat causes accelerated proliferation particularly in the central blade where morphogen signalling is highest, and suggest that graded Fat activity may oppose the growth-promoting effect of Dpp (Schwank et al. 2011). Unlike the slope model, threshold models do not account for why growth eventually stops. Reconciling support for the threshold model with that for the slope model awaits further investigation.

Box 1. (Contd.)

Recently, two models have been proposed which invoke mechanical forces as regulators of cell proliferation in the wing disc (Aegerter-Wilmsen et al. 2007; Hufnagel et al. 2007). In these models, morphogens induce proliferation in a concentration dependent manner, the effect being strongest in the centre of the blade where morphogen concentration is highest (Fig. 1A). Growth in this central region causes stretching of regions further out; in one model, this stretching effect causes peripheral cells to proliferate, and, since the stretching force is inversely proportional to the influence of morphogens, the outcome is a uniform pattern of proliferation (Aegerter-Wilmsen et al. 2007). Common to both models is the assumption that, by being stretched, peripheral regions experience mechanical stress, and in so doing exert a compressive force on the central region of the disc. At a certain disc size, peripheral regions become large enough that the level of compression in the center rises above a threshold, inhibiting further proliferation there. At this point, where compression overrides the influence of morphogens, disc growth stops. Currently, there is a lack of unequivocal support for mechanical stress-based models of growth control, but their ability to account for a large range of experimental observations (discussed in Aegerter-Wilmsen et al. 2007) means they cannot be dismissed.

mediated by the kinase TOR, a regulator of several processes that are rate-limiting for cell division and cell growth, including translation initiation and ribosome production (Hietakangas and Cohen 2009). Importantly, TOR activity has been shown to depend on organ-extrinsic signals and metabolic compounds generated by nutrition. These include insulin-type peptides transduced via the Insulin Receptor/PI3-Kinase/Akt pathway, glucose, and amino acids (Hietakangas and Cohen 2009). The InR/TOR system thus coordinates cell growth and proliferation with the animal's nutritional status. Acting systemically, it influences the sizes of all organs in concert, thereby regulating body size.

ORGAN SCALING: INTEGRATING MORPHOGENS WITH INR/TOR SIGNALLING

The fact that studies of organ size control can be split into two spheres of research emphasises that morphogens and nutrition are key but separable parameters governing growth. Yet, taken at face value, the coexistence of these distinct size control mechanisms presents something of a paradox. In the morphogen-centric view, organ size depends on an internal self-organizing system that coordinates cell proliferation with patterning; it is the range and distribution of morphogens, which primarily affect organ size—altering these parameters expands or shrinks organs accordingly. In the nutrient-centric view, the extent of organ growth depends on external signals that drive cell proliferation as a consequence of nutrition. In this latter view, the level of InR/TOR activity experienced by an organ is primary. Each view supposes that one system is the primary size-limiting factor, and implicitly assumes that the other system is merely growth-permissive. How can we reconcile these two views of organ size control?

The two systems must somehow be integrated. Animals develop in environments of varying nutritional quality, creating a range of body sizes. To ensure organismal viability, mechanisms have evolved to allow organs to *scale* appropriately with nutrient levels (Huxley 1932). In this way, the relative sizes of organs are constrained across the body size spectrum, ensuring that a functional whole animal develops. The phenomenon of organ scaling requires morphogen activity to be coordinated with systemic InR/TOR signalling, so that cells proliferate, and organs grow, as *a function of both inputs*. So how are these two systems related?

Many studies have focused on how organ-intrinsic *or* organ-extrinsic systems function to control organ size. Far less attention has been paid to deciphering how these systems are integrated to control organ scaling. This is the challenge highlighted here. In what follows, I use certain observations from the literature to construct a hypothetical scenario for scaling of the *Drosophila* wing blade: a model system for studying organ growth (Neto-Silva et al. 2009).

THE *DROSOPHILA* WING: WINDOW INTO A RESOLUTION?

The wing blade develops during the 4-day larval phase as part of an internal epithelium called an imaginal disc (Fig. 1A). From approximately 30 cells, the disc grows to number $\sim$ 50,000 at the onset of metamorphosis, when proliferation stops and morphogenetic processes shape the disc into the mature wing. Wing blade cell proliferation depends on the activities of the morphogens Dpp and Wg (Fig. 1A), which spread from localized sources to delimit the size of the blade as well as its shape and venation pattern. Loss of either Dpp or Wg abrogates wing development, whereas altering their distributions changes wing size and pattern in predictable ways (Fig. 1B). Together, Wg and Dpp are thought to be both necessary and sufficient to organize the growth and patterning of the wing blade (Zecca et al. 1995; Zecca and Struhl 2007a, b).

Throughout the period of disc growth, the animal feeds constantly and increases in mass; if nutrient-deprived, InR/TOR signalling is lowered and growth rates throughout the organism are reduced (Shingleton et al. 2005). The larva must attain a threshold mass to trigger the physiological switch which leads to metamorphosis—if starved prior to attaining this "critical weight," development will simply be delayed until the weight is achieved. On reaching the critical weight, a hormonal cascade is set in motion, which lasts approximately 2 days until pupariation. Importantly, if the larva is starved

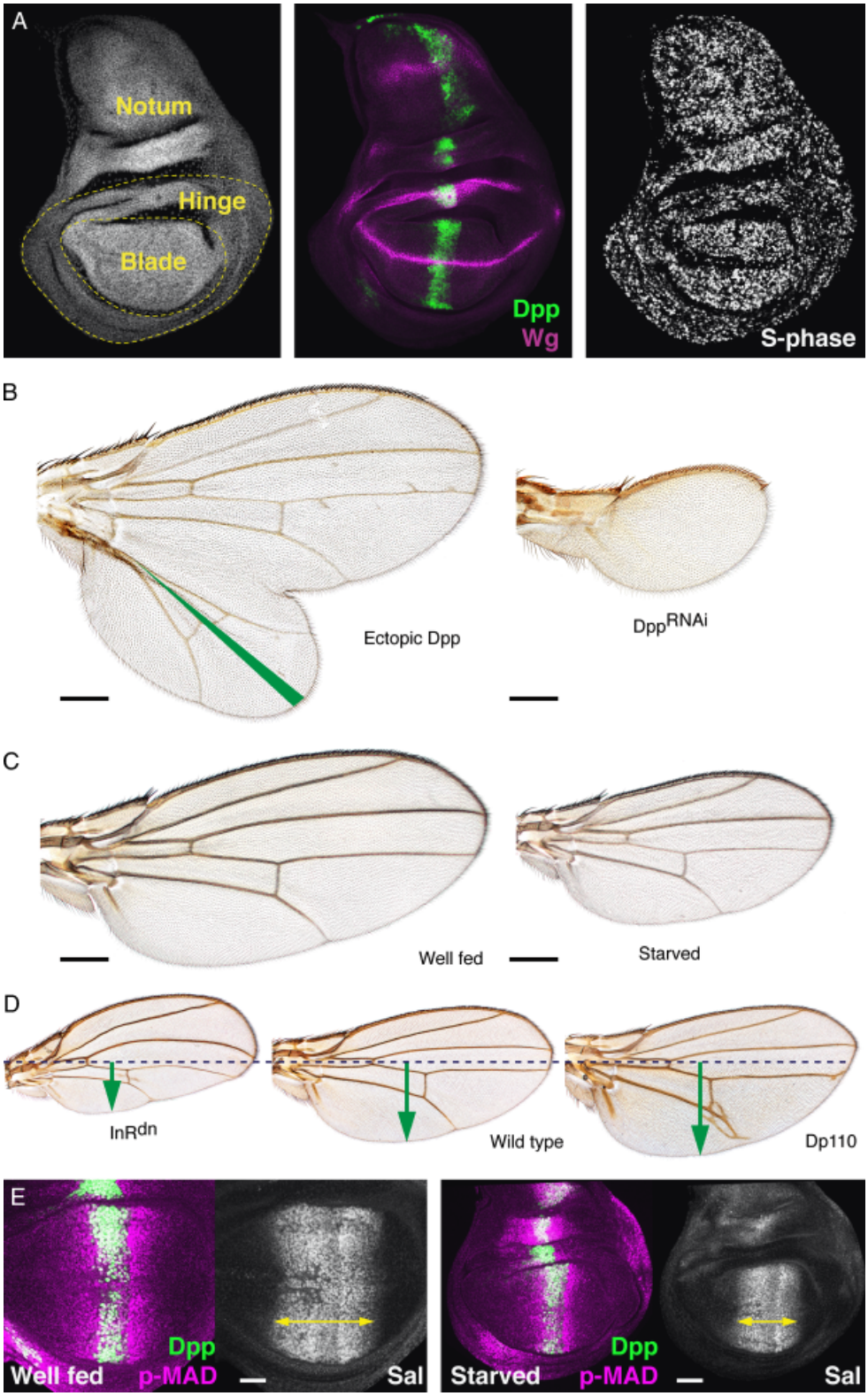

Fig. 1. Control of wing size by morphogens and nutrients. (A) The wing imaginal disc is subdivided into territories fated to become wing blade, hinge, and notum (the thoracic box). Orthogonal morphogen gradients of Dpp and Wg direct wing blade growth and patterning, and together create a uniform pattern of cell proliferation (shown here by the pattern of S-phase cells). (B) Morphogens define wing size. A clone of ectopic Dpp (approximate position in green) induces proliferation in surrounding cells, creating an enlarged wing (compare with the well fed fly's wing in (C). Inhibiting Dpp production by expressing Dpp^{RNAi} specifically in the wing reduces it in size. Note that in each case there is also a change in wing pattern: ectopic Dpp reorganizes pattern to generate a duplicated wing with cell types corresponding to all levels of Dpp activity (Zecca et al. 1995). Dpp^{RNAi} creates a wing comprised solely of cell types specified by low Dpp activity. (C) Nutrition influences wing size. Raising larvae on nutrient-poor food systemically impairs growth, leading to a small but perfectly patterned wing. (D) Experimentally altering InR/TOR signalling in the posterior half of the wing (the P compartment) shows that InR/TOR signalling defines wing size. Expressing a dominant negative Insulin Receptor reduces compartment size, whereas the catalytic subunit of PI3-Kinase (Dp110) stimulates InR/TOR signalling and increases compartment size (Weinkove et al. 1999). In both cases, as in (C), patterning is unaffected; the venation pattern simply scales with tissue size. (E) Dpp gradient scaling. Dpp emanates from a stripe in the middle of the wing, forming an extracellular gradient mirrored by an intracellular activity gradient of phosphorylated MAD (pMAD). Dpp activates target genes such as Spalt (Sal) according to concentration. Starvation reduces disc size, but narrowing of the pMAD gradient shows that the Dpp gradient scales to fit the tissue. The Sal domain contracts, preserving wing pattern (Teleman and Cohen 2000).

after reaching the critical weight, systemic InR/TOR activity during the 2-day window is decreased (Shingleton et al. 2005), and a miniature fly with scaled down but relatively normally patterned wings will develop (Fig. 1C; the same is true for other organs). Likewise, if InR/TOR signalling is experimentally reduced specifically in the wing, a small but correctly patterned wing will result (Fig. 1D). Conversely, overactivating the InR/TOR pathway increases wing size above normal, but again, the pattern expands appropriately (Fig. 1D).

If one looks at the morphogen gradients in the wing disc of a starved fly, they are reduced in range to fit the smaller tissue (Fig. 1E). The expression domains of morphogen target genes are likewise contracted (Fig. 1E). The conclusion is that even though Wg and Dpp control the size and pattern of the wing, the system is plastic, and is normally scaled according to the level of InR/TOR signalling received post-critical weight. The experimental manipulations of InR/TOR activity show that the pathway acts directly on the disc, and also that the system can be pushed to scale both up and down. If Dpp and Wg are so instrumental for wing growth and patterning, how does InR/TOR cause this near-perfect scaling of the wing?

MORPHOGEN GRADIENT SCALING

For the following hypothesis, it is important to consider the process of morphogen gradient formation. Following secretion from their source cells, movement of Dpp and Wg across the disc depends on cell surface molecules such as heparan sulfate proteoglycans (HSPGs) (Belenkaya et al. 2004; Franch-Marro et al. 2005; Han et al. 2005). HSPGs bind and possibly stabilize morphogens in the extracellular space (Hufnagel et al. 2006; Akiyama et al. 2008), facilitating morphogen diffusion across tissue. As they spread out, morphogens can be captured by receptors and internalized—an event that leads to signalling pathway activation. Internalization is often followed by degradation of the ligand–receptor complex, removing morphogen from the system. Moving away from the morphogen source, each cell internalizes and degrades a proportion of available morphogen, making less available to the next cell. It is this degradation process which shapes the extracellular morphogen distribution into a gradient, mirrored by an intracellular signalling activity gradient. Patterns of target gene expression arise as gene promoters are activated or repressed at different thresholds of morphogen signalling activity (Ashe and Briscoe 2006).

As outlined in Box 1, it is unclear how morphogens drive cell proliferation—cells may measure the slope or threshold of the gradient, or perhaps even respond to mechanical stretching or other secondary systems set up by morphogens which stimulate growth. But however they do it, by promoting tissue growth, Wg and Dpp increase the size of the field they pattern. This poses a problem: as the wing enlarges, target gene expression domains must expand so that the pattern scales with the growing organ. Moreover, all cells in the wing must be continually supplied with Wg and Dpp, because failure to transduce either morphogen impairs proliferation, and leads to apoptosis (Burke and Basler 1996; Lecuit et al. 1996; Chen and Struhl 1999). So, as Dpp and Wg induce proliferation, more Dpp and Wg needs to be made available to sustain the pattern and growth of the expanding wing cell population. This demands that the ranges of the morphogen gradients *scale with tissue size*, keeping all cells in receipt of sufficient levels of each morphogen. What mechanism enables the gradients to extend their ranges with the growing tissue?

Morphogen gradients that adjust to altered tissue size are known to occur elsewhere—the Bicoid gradient in the syncitial fly embryo for example (Gregor et al. 2005), or the dorsoventral BMP2/4/7 gradient in the *Xenopus* embryo (De Robertis 2009). Scaling of both these gradients has been studied in some detail (Lewis 2008), and the mechanisms responsible are currently the subject of debate. Bicoid forms a gradient along the anteroposterior axis of the embryo that scales in embryos from fly species with different egg sizes (Gregor et al. 2005). However, Bicoid is a transcription factor, which spreads through a sea of nuclei. The scaling mechanism likely differs to that for Dpp and Wg, which travel across a cellular epithelium.

The *Xenopus* dorsoventral gradient may be more informative. Famously, bisecting an amphibian embryo into ventral and dorsal halves results in a disordered "belly piece" from ventral tissue, whereas the dorsal half develops into a normally patterned, albeit miniature tadpole (Spemann 1938). The dorsal half can do this because the BMP gradient adjusts to pattern a field half the normal size. Scaling in this case is thought to depend on interactions between the BMP gradient and certain target genes which contribute to shaping the gradient. In particular, one target gene repressed by BMPs—ADMP—is itself a BMP. By repressing a protein which can itself contribute to BMP activity, the gradient is subject to negative feedback. It is this property of the gradient which is thought to enable it to scale with embryo size (Reversade and De Robertis 2005; Ben-Zvi et al. 2008).

Dpp and Wg transcriptionally repress their receptors (Cadigan et al. 1998; Lecuit and Cohen 1998) and HSPGs (Fujise et al. 2003; Han et al. 2005), suggesting equivalent negative feedback interactions may exist in the wing. Classically, these feedback interactions have been invoked to explain how morphogen gradients are buffered against physiological fluctuations in morphogen secretion (Chen and Struhl 1996; Cadigan et al. 1998; Eldar et al. 2003). The first example of this phenomenon came from studies of the morphogen Hedgehog, which, by inducing expression of its receptor, acts to restrict its own mobility (Chen and Struhl 1996). In the case of Dpp and Wg, elevated morphogen

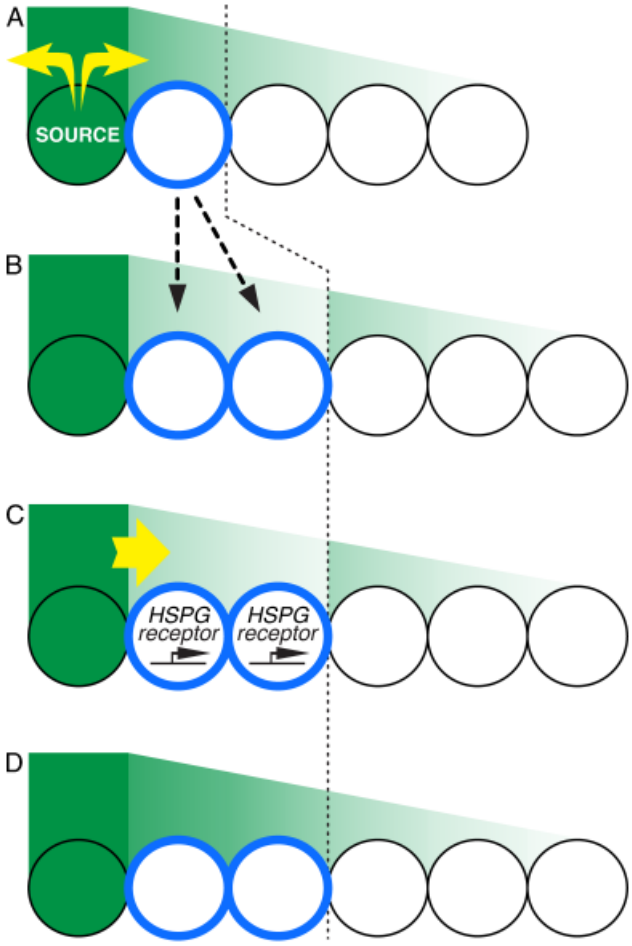

Fig. 2. A hypothetical model of morphogen gradient scaling. (A) Morphogen emanates from source cells and spreads out, forming both extracellular protein and intracellular signalling gradients (green). (B) Cell proliferation produces two daughter cells (in blue). The increase in tissue area locally dilutes extracellular morphogen concentration per daughter cell relative to the mother cell (note the lower concentration of morphogen around each cell). (C) Daughter cells experience reduced signalling levels, and respond by increasing receptor/HSPG transcription. Increased receptor/HSPG levels makes cells more attractive to morphogen, sensitizing cells to morphogen and enhancing the traffic of morphogen toward and over them (yellow arrow). (D) The enhanced attractiveness of cells to morphogen restores signalling levels in daughter cells to a level closer to that experienced by the mother cell. The process is posited to reiterate throughout the disc each time a cell divides.

production may repress receptors and HSPGs to inhibit transduction and spreading, respectively. A drop in morphogen output would reduce signalling and increase receptor/HSPG expression, sensitizing cells to available morphogen.

Could these kinds of negative feedback interactions explain how the ranges of the Dpp and Wg gradients scale with the growing tissue? The hypothesis here is that they might. To understand how, consider the following scenario (schematized in Fig. 2, A–D). Dpp and Wg induce cell proliferation uniformly across the wing as it grows (Schubiger and Palka 1987). It is conceivable that, by increasing tissue area, proliferation tends to dilute the extracellular morphogen concentration per cell (Fig. 2B). The effect on individual cells would be a drop in intracellular morphogen signalling. This could be countered by increased expression of receptors and HSPGs (Fig. 2C). Such a response would sensitize the cells to morphogen, making them more efficient at drawing from the extracellular morphogen pool. In essence, cell proliferation may parallel the effect of a drop in morphogen production, in which receptor/HSPG upregulation strives to restore normal signalling levels.

For this feedback process to enable the gradient range to scale with size, the tissue-wide supply of morphogen must be matched to the increasing demand of the growing tissue. Importantly, in the case of Dpp, the morphogen appears to be transported more efficiently from the source if HSPG expression is experimentally raised in cells abutting the source (Crickmore and Mann 2007). It is as though HSPGs make cells more "attractive" to morphogen. For this reason, proliferation of cells juxtaposing the morphogen source could, by diluting extracellular morphogen concentration and alleviating repression of HSPGs, increase their attractiveness, making them better at receiving morphogen from its point of secretion (Fig. 2C). The cells could internalize some morphogen and leave the remainder available to subsequent cells. The process would repeat in these more distant cells, and indeed in cells along the length of the growing tissue. Through this reiterating process, morphogen could be transported from the source and trafficked increasingly longer distances. Described here as ratchet-like, such a process would most likely operate at a steady state, maintaining equilibrium morphogen signalling levels in cells.

Though hypothetical and quite possibly incorrect in its specifics, the purpose of this model is simply to illustrate that conceivably, extending the ranges of the Dpp and Wg gradients could arise as a direct consequence of tissue expansion. This idea is somewhat similar to one proposed recently by Ben Zvi and Barkai (2010), in which a so-called "expander" molecule facilitates morphogen diffusion. Through simulations, it was shown that, in signalling circuits in which morphogen represses the level of the expander, the range of the gradient can scale with tissue size. Such an expander could plausibly be an HSPG, the main difference between the model here and that of Ben Zvi and Barkai being that their expander molecule is diffusible. HSPGs possess a glycosyl-phosphatidylinositol anchor that can be cleaved to release the extracellular portion of the HSPG, but whether these diffusible forms are needed for morphogen spreading, or whether HSPGs can remain membrane-anchored is unclear. Either way, HSPGs appear to function as expanders, which, in the case of the wing at least, are also repressed by morphogen activity.

A SCENARIO FOR WING SCALING BY INR/TOR ACTIVITY

Having considered formation of the Dpp and Wg gradients, and how negative feedback mechanisms can explain how their ranges scale with tissue size, how might InR/TOR signalling modulate this process to cause organ scaling? Conceivably, InR/TOR could influence the Wg and Dpp gradients in a number of ways. One might imagine that InR/TOR signalling stimulates morphogen production, and that by reducing InR/TOR signalling, too little Wg and Dpp is secreted. This seems unlikely, however, because altering InR/TOR signalling activity specifically in the Dpp-producing cells changes the size of this region of the wing, yet leaves the size of the surrounding wing unaffected (Leevers et al. 1996). Furthermore, the relative imperviousness of wing size to moderate increases in Dpp production has been documented experimentally (Morimura et al. 1996). These results make it unlikely that altered morphogen production has a prominent role in InR/TOR's ability to scale wing size and pattern.

The InR/TOR pathway could possibly signal to downstream components involved in morphogen signalling; perhaps InR/TOR alters cells' sensitivities to morphogen by transcriptionally regulating receptors and HSPGs, or modulates the internalization of ligand–receptor complexes. Notably, however, organ scaling also occurs when disc growth is altered by other more generic means—increasing the amount of cell death for example (Yoshida et al. 2001), or partially decreasing the protein translation rate of each cell (Miron et al. 2001). This argues that a sophisticated signalling relationship between InR/TOR and morphogens need not underlie wing scaling. Rather, it supports a far simpler explanation: InR/TOR activity scales the wing solely by influencing cell proliferation rate.

As explained in the previous section, Dpp and Wg induce wing growth, but as they do so, their ranges must be continually broadened to sustain the pattern and growth of the expanding tissue. Any impairment of cell proliferation reduces tissue growth—the very process posited to bring about gradient scaling. If the ability of Dpp and Wg to drive proliferation were somehow contingent upon extrinsic InR/TOR activity, then reducing InR/TOR signalling—for example through starvation—would constrain the ability of Dpp and Wg to expand the wing. Their gradients would scale with wing size as a result of negative feedback mechanisms, and the result would be a smaller wing patterned by gradients of shorter range, as in Fig. 1E. Similarly, experimentally increasing InR/TOR activity to accelerate tissue growth rate would—again, through the size-sensitivity of the gradient-forming mechanisms—spur the Dpp and Wg gradients to extend with the tissue (Teleman and Cohen 2000).

In summary, if the ranges of the Wg and Dpp gradients are sensitive to the size of the tissue, as they are during the normal development of the wing disc as it enlarges, then InR/TOR need only influence tissue growth rate to scale the wing up or down. Of course, the scale of the wing would also be limited by the duration of growth: at the end of larval development, growth might be terminated by metamorphic cues, or, in well-fed animals, by unidentified internal constraints inferred to halt proliferation when the disc reaches the maximal possible size (Bryant and Levinson 1985). At this size, it may be that morphogen output can no longer supply the whole disc, so signalling drops below a threshold. Alternatively, the gradient slope could become too shallow for further proliferation (Day and Lawrence 2000) (note that even in the slope model, cells still need to be supplied with morphogen to proliferate). Still another possibility is that mechanical compression builds up throughout the epithelium, preventing the further stimulation of growth (Aegerter-Wilmsen et al. 2007; Hufnagel et al. 2007).

COMPARISON WITH SELECTOR GENE-CONTROLLED ORGAN SCALING

The model of InR/TOR-controlled wing scaling contrasts with another case in which wing size is scaled: the reduction in size of the fly hind wing to produce the haltere. Most extant winged insect orders possess fore and hind wings, but in dipterans, the hind wings have been reduced in size to form tiny club-shaped organs, which aid balancing during flight. The extreme size reduction is due to the activity of the homeotic gene *ubx*, which is expressed in the haltere but not the wing (Lewis 1978). The mechanism by which Ubx transforms wing to haltere has been revealed, in part, by studies of Ubx's influence on morphogen signalling (Weatherbee et al. 1998; Crickmore and Mann 2006, 2007; de Navas et al. 2006). Ubx does not cell-autonomously reduce the rate of cell proliferation: in the haltere, clones of *ubx*—cells do not grow larger than control clones (Crickmore and Mann 2006). Instead, Ubx, a transcription factor, represses transcription of Dpp and Wg, thereby decreasing morphogen output. Simultaneously, Ubx also represses HSPG expression, while strongly increasing expression of morphogen receptors. What little morphogen is secreted is thus prevented from diffusing effectively, because most of it binds receptor and not HSPG, preventing it spreading further through the tissue.

Hence, Ubx reduces wing size not by affecting cell proliferation directly; instead, it influences growth indirectly, by transcriptionally targeting specific components involved in gradient formation. This is the opposite to how InR/TOR is posited to elicit wing scaling: InR/TOR is unlikely to directly modulate the levels of morphogens, receptors and HSPGs, but *is* cell-autonomously rate-limiting for cell proliferation. And it is this effect on growth, which—by indirectly influencing morphogen/receptor/HSPG feedback

relationships—accommodates gradients to tissue dimensions, causing the wing to scale in size.

COMBINING MORPHOGEN AND INR/TOR INPUTS INTO CELL PROLIFERATION

This model posits that integration of morphogens with InR/TOR signalling could conceivably occur solely at the level of cell proliferation. A question thus arises over how InR/TOR and morphogens interact to control this process. A significant observation is that loss of either InR/TOR, Wg or Dpp inputs impairs wing cell proliferation (Burke and Basler 1996; Lecuit et al. 1996; Chen and Struhl 1999; Weinkove et al. 1999; Zhang et al. 2000). This argues that cells autonomously need simultaneous input from both InR/TOR *and* morphogens to grow and divide normally. Hence, the convergence point between and InR/TOR and morphogens may lie in the pathways downstream of Wg and Dpp, which control tissue growth. A strong candidate for such a pathway is the Hippo/Warts (Hpo/Wts) pathway. This is a network of proteins that control the activity of a transcriptional coactivator, Yorkie (Yki; homologous to vertebrate YAP) (Huang et al. 2005). When active, the Hpo/Wts pathway phosphorylates Yki, sequestering it in the cytoplasm (Dong et al. 2007; Zhao et al. 2007; Oh and Irvine 2008), whereas blocking the pathway sends Yki to the nucleus where it binds to several transcription factors, including Scalloped (Sd/TEAD) (Goulev et al. 2008; Wu et al. 2008; Zhang et al. 2008) Homothorax (Peng et al. 2009) and MAD (Oh and Irvine 2011). In conjunction with these transcription factors, Yki induces tissue growth by transcribing a range of target genes with roles in cell proliferation and cell survival (reviewed in Pan 2010).

The connection between Hpo/Wts signalling and morphogens stems from the finding that upstream of this pathway are the atypical cadherins Fat (Ft) and Dachsous (Ds). Known for their roles in planar polarity (Lawrence et al. 2007), these large transmembrane proteins form heterodimeric bridges between cells. In various organs, including the wing, morphogens create opposing gradients of Ds and Ft signalling activities (Casal et al. 2002; Yang et al. 2002; Simon 2004; Rogulja et al. 2008; Zecca and Struhl 2010). It has been proposed that the resulting asymmetries in heterodimeric interactions perceived by each cell provide a polarizing vector (Lawrence et al. 2007), and may also regulate growth (Rogulja et al. 2008; Willecke et al. 2008). Uniformly overexpressing Ds in the wing to flatten the putative Ft–Ds heterodimer gradient suppresses growth and reduces wing size, whereas clonal removal or overexpression of *ds*—both of which should create artificially steep heterodimer differentials in cells at clone boundaries—lead to local Hpo/Wts inactivation and cell proliferation (Rogulja et al. 2008; Willecke et al. 2008).

It is not clear how a Ft–Ds heterodimer asymmetry could impede Hpo/Wts signalling, but this scenario may provide a molecular foundation for a "slope model," relating growth to morphogen gradient steepness (as discussed in Box 1) (Lawrence et al. 2008). If morphogens control proliferation by indirectly regulating Yki in this way, then explaining how cells integrate Yki activity with InR/TOR input would be important for understanding organ scaling. Yki is a potent inducer of proliferation (Huang et al. 2005), a metabolically demanding process, which burdens a cell's biosynthetic capacity as it doubles in mass and divides. Considering InR/TOR's diverse roles in metabolism (Hietakangas and Cohen 2009), it is likely that InR/TOR signalling is in some way required by Yki to promote growth. *Drosophila* cell culture experiments found no effect of InR/TOR signalling on Yki activation, and vice versa (Dong et al. 2007), so it may be that both pathways act in parallel to drive cell proliferation.

It may of course be that Hpo/Wts signalling does not mediate the proliferative effect of Wg and Dpp; indeed, recent work by Zecca and Struhl demonstrates a different relationship between morphogens and the Hpo/Wts pathway. Previously, the authors had shown that wing growth occurs in part by wing cells recruiting surrounding cells into the wing primordium (Box 1) (Zecca and Struhl 2007a). Their new study finds that morphogens use Hpo/Wts signalling as part of this recruitment mechanism, and they argue against it being used as a cell proliferation inducer (Zecca and Struhl 2010). Clearly, knowing which pathways are used by morphogens to trigger cell proliferation is a prerequisite for understanding how, molecularly, InR/TOR and morphogens combine to cause tissue growth.

However, the hypothesis here is simply that InR/TOR is needed to facilitate morphogen-driven cell proliferation, and that this link alone, in conjunction with the size-sensitivity of morphogen gradients, suffices to explain organ scaling in response to nutrient levels. Scaling is a near-universal property of metazoans, and since organ growth in all animals depends on InR/TOR signalling and morphogens, this tentative model may apply more generally.

MORPHOGEN GRADIENT SCALING AND ALLOMETRY

Often, scaling relationships between an organ and body size, or between axes within the same organ, are not directly proportional. These "allometric" relationships cause animal or organ shape to change with size. For instance, the mandibles of lucanid beetles are disproportionately enlarged and more elaborate in bigger-bodied males (Huxley 1932), and the dimensions of arthropod genitalia are generally less affected than other structures by nutrient deprivation (Eberhard 2009) or InR/TOR inhibition (Shingleton et al. 2005). Organ–body

or organ–organ allometries such as these have been explained as possibly resulting from organs being differentially sensitive to changes in InR/TOR activity (Nijhout 2003; Shingleton et al. 2008).

Based on the model for organ scaling described here, allometry might equally result from variation in the ability of different morphogen gradients to scale with tissue size. For example, morphogens controlling growth in one organ might scale more effectively with size than morphogens functioning in another. Furthermore, a morphogen delimiting the anteroposterior axis of an organ might scale more effectively than a gradient influencing the dorsoventral axis. The result would be a relative lengthening of the anteroposterior axis with increasing organ size. Hence, variation in the scaling capacity of different gradients might also underlie size-dependent architectural variation *within* organs. Artificial selection experiments indicate that allometries are the products of continuous purifying selection to maintain scaling relations between different body regions (Weber 1990; Frankino et al. 2005). An attractive possibility is that natural selection can maintain or modify allometries by fine-tuning the tissue size sensitivity of different morphogen gradients.

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