Feedback control of intercellular signalling in development

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The intercellular communication that regulates cell fate during animal development must be precisely controlled to avoid dangerous errors. How is this achieved? Recent work has highlighted the importance of positive and negative feedback loops in the dynamic regulation of developmental signalling. These feedback interactions can impart precision, robustness and versatility to intercellular signals. Feedback failure can cause disease.

Most animal cells develop according to cues in their environment that have been produced by other cells. In the past twenty years many of these signalling molecules and their transduction pathways have been identified, and this has led to the discovery that the same signalling pathways appear in several developmental contexts. For example, a signal that in one instance will cause a cell to differentiate terminally will elsewhere lead another cell type to undergo mitosis and in a third context will trigger cell death. In general, the outcome of the signalling event is not determined by the signal itself but by the developmental state of the cell receiving it. This developmental state consists in the various cellular targets (such as transcription factors and cytoskeletal proteins) that are primed to respond to the signal; whether they are primed depends on the history of the cell.

Obviously, developmental signalling events must be precisely regulated. A signal that is produced in the wrong time or place will lead to inappropriate developmental responses, which can be dangerous—cells must be protected against this. Also, desirable signals must be robust enough to ensure that cells receive them at high enough levels to respond. Just as important is versatility. Not only is there is a wide range of different cell types and tissue environments in which these signals must operate, but they must also function with different spatial and kinetic properties. These three main properties of intercellular signalling in development—precision, robustness and versatility—are stringent requirements and errors are serious. How is this control achieved?

Feedback loops can account for aspects of all these properties. Feedback can be defined as the ability of a system to adjust its output in response to monitoring itself. More than 2,000 years ago, complex systems were designed to incorporate feedback principles (an accurate water clock from the third century BC in Alexandria is the earliest known example of feedback in engineering), although it was only formalized as a theoretical branch of science and engineering by Wiener in the 1940s[1]. Two prominent areas of feedback in biology that have been studied historically have been general growth theory[2], in which cell growth was postulated to be regulated by inhibitory factors produced by cells themselves, and theoretical models of pattern formation[3–5].

More recently, as developmental biology has acquired the molecular tools that allow mechanistic insight, it has become clear that the principles of feedback—both positive and negative loops—are indeed used to produce the signalling properties necessary in animal development. Negative feedback occurs when, for example, a signal induces the expression of its own inhibitor; it serves to dampen and/or limit signalling. Positive feedback occurs when a signal induces more of itself, or of another molecule that amplifies the initial signal, and this serves to stabilize, amplify or prolong signalling. The widespread use of feedback and the variety of its consequences make it an important principle of regulating signals in development.

Here I describe some examples of developmental feedback control, in order to illustrate the strategies in which it participates. The focus is particularly on experimental demonstration of feedback in intercellular signalling, rather than the extensive literature on theoretical modelling of feedback, especially in the context of networks of transcription factors[6]. The issues that will be covered include temporal and spatial control of signalling, and the unification of these into complex patterning and growth control. The relevance of feedback in cancer—where normal developmental controls are disrupted—is also discussed.

Temporal control of signalling

Perhaps the most obvious use of negative feedback is to limit the duration of a signal. In its simplest form, a signal induces its own negative regulator so that when a threshold has been reached, the signal ceases. An example of this is the control of cytokine signalling through the JAK/STAT signalling pathway (Fig. 1). JAKs are soluble tyrosine kinases that bind to cytokine receptors and transduce signals by the STAT proteins: transcriptional activators with SH2 domains that bind to the phosphotyrosine on activated JAKs[7]. Since 1997, a growing family of cytokine-inducible proteins (variously termed SOCS, SSI, JAB and CIS[8–10]) that inhibit cytokine signalling has been identified[11,12]. These proteins participate in negative feedback loops. The physiological significance of the negative feedback is exemplified by SOCS1, which is induced by and inhibits interferon-γ signalling; when the gene for SOCS1 is knocked out, mice die as neonates with defects associated with excess interferon-γ signalling[13,14].

Other transgenic and knockout experiments have shown that SOCS3 is necessary for the control of erythropoiesis[15], probably through a negative feedback loop controlling the response to the cytokine erythropoietin. SOCS3 also participates in negative feedback control of metabolic factors including leptin and growth hormone[16,17]. Although the mechanisms of SOCS inhibition are not yet fully understood, there are indicators: SOCS1 acts as a pseudosubstrate inhibitor of JAK activity, and the SOCS domain binds to elongins, thereby targeting proteins for proteasomal degradation.

Less obvious than negative feedback control of signalling kinetics is the use of positive feedback loops to prolong signalling. A good example of this occurs in the developing *Drosophila* egg, where epidermal growth factor (EGF)-receptor signalling is crucial for, among other things, specification of dorsal follicle cells[18]. The signal is initiated by the transforming growth factor (TGF)-α-like ligand Gurken, produced by the oocyte, and received by the EGF receptor in the overlying somatic follicle cells (Fig. 2). However, this signal

must be short-lived because soon after it is initiated, an impermeable layer called the vitelline membrane develops between the oocyte and follicle cells. This physical barrier to signalling is overcome when Gurken induces the follicle cells to start producing an autocrine signal—another TGF-α ligand, Spitz—to prolong (and amplify) the initial EGF receptor activity[19].

Spatial control by feedback

Spatial regulation of developmental signalling is also regulated by feedback. A good example is the expression of homeotic genes that control development in all animals. Their tissue-specific expression must be maintained throughout development—long after the initial localized signals that establish them have died away[20]. Positive feedback loops assist this maintenance in a number of cases. This 'autoregulation' of homeotic genes occurs because they activate their own transcription, causing a stabilization and maintenance of their initial expression patterns. In principle, this positive feedback loop could be direct (the homeotic genes encode transcription factors) or indirect, involving intermediate steps. Both types of feedback have been identified: strikingly, not only do homeotic proteins directly activate their own transcription, but they also autoregulate through positive feedback loops that involve intercellular communication.

The most studied example of this is the positive feedback loop that maintains the expression of the Ubx gene in parasegment 7 of the *Drosophila* gut[21,22] (Fig. 3). Here, Ubx controls the expression of the bone morphogenetic protein (BMP)-2/4-like protein Dpp, which in turn causes cells in the adjacent parasegment 8 to express the Wnt protein, Wingless; Wingless then signals back to the parasegment 7 cells. These respond to the combination of Wingless and Dpp signals by activating Ubx transcription, thereby closing the feedback loop. This system appears to play an important part in the spatial fine-tuning of *Ubx* expression, as well as its more obvious function in maintenance and stability. It acts to integrate the consequences of two different signalling molecules from different sources (note that Wingless expression in parasegment 8 is also under other spatial control), and thereby ensures that *Ubx* expression is restricted to the correct region of the gut[23]. The autoregulation of other *Drosophila* and mammalian homeodomain proteins has also been found to involve positive feedback signalling loops[24–27], suggesting that this indirect mechanism is a well-conserved principle.

The *Drosophila* EGF receptor antagonist Argos[28,29] also participates in spatial feedback control, this time in a negative feedback loop. Argos expression is induced by EGF receptor signalling[30], establishing a negative feedback loop—one of the earliest whose developmental significance was understood. In this case, the antagonistic ligand Argos acts at a longer range than the agonist, the TGF-α-like Spitz. This sets up a system of 'remote inhibition' during ommatidial development in the fly eye: cells closest to the source of Spitz respond by producing Argos. These cells, however, are already irreversibly destined to differentiate and therefore unaffected by Argos. Argos, however, diffuses away from its source and prevents more remote cells, which are not yet committed, from responding to the low levels of Spitz to which they are exposed[31,32]. This limits the effective range of EGF receptor signalling and thereby the number of cells that are able to be recruited to the developing eye.

In fact, the *Drosophila* EGF receptor pathway is regulated by a large number of feedback mechanisms (Fig. 4). The signalling inhibitors Argos, Sprouty and Kekkon1 are all induced by EGF receptor activity[33], as are the activating molecules Rhomboid-1 and Vein, at least in some contexts[19,34,35]. This complexity of regulation raises the question of whether this is a case of regulatory overkill, or do these different feedback regulators have distinct functions? The evidence points to the latter—each of these feedback events has its own role and significance. For example, Argos is EGF-receptor-specific, secreted, and can act over many cell diameters[28,29,36]. Sprouty is an intracellular inhibitor of Ras activation and can be induced by, and will inhibit signalling from, a variety of receptor tyrosine kinases[37].

The third known inhibitor, Kekkon1, is EGF-receptor-specific, but is a transmembrane protein, so its action is, unlike Argos, confined to the cell expressing it[38]. Consistent with these theoretical differences, the three known feedback inhibitors of the EGF receptor have distinct phenotypes. Similarly, molecular and genetic analysis has demonstrated that the positive feedback mediated by Rhomboid, an activator of ligand production, and Vein, a neuregulin-like ligand for the EGF receptor, have different roles in controlling signalling.

Integration of feedback events in pattern formation

In reality, the distinction between temporal and spatial control of signalling is artificial. Cells often receive several spatially and temporally restricted signals and must integrate the information to respond appropriately. The most striking developmental examples of this involve pattern formation. How does communication

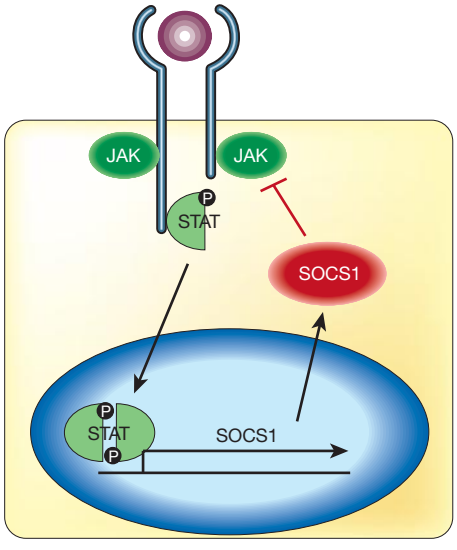

Figure 1 SOCS1 negative feedback loop. Ligand-bound cytokine receptors recruit JAKs, which in turn phosphorylate the transcriptional activator STAT proteins. SOCS/CIS/SSI proteins are consequently expressed and inhibit JAK/STAT signalling.

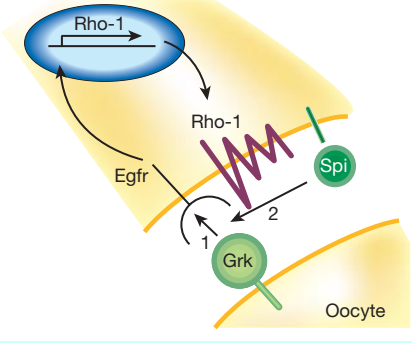

Figure 2 Autocrine amplification by positive feedback in the *Drosophila* oocyte. The TGF-α-like ligand, Gurken (Grk), expressed in the oocyte, activates the EGF receptor (Egfr) in the overlying follicle cells. This activates the expression of Rhomboid-1 (Rho-1), which in turn activates another EGF-receptor ligand, Spitz (Spi). Spitz then amplifies and prolongs EGF-receptor signalling in the follicle cells.

between cells lead to the patterning events needed to produce an animal? Again, positive and negative feedback control emerges as a significant factor. In some cases, the integration of successive feedback loops has been demonstrated so that extensive and complex patterning can now be better understood. Four recently discovered examples will be considered here, two from vertebrates and two from *Drosophila*.

Positive and negative feedback establish left–right asymmetry. Recent progress has been made in understanding how the fundamental property of left–right asymmetry is established in vertebrate embryos. These leaps in understanding are reviewed elsewhere[39] so I will describe only the relevant details here. The principal factor appears to be the TGF-β family signalling molecule, Nodal[40], which is required throughout the left side of the embryo to activate left-specific genes. There are two key events in left–right specification: the initial symmetry breaking and its relay to all cells.

Symmetry breaking is not well understood in all animals but some excellent studies in the mouse embryo have shown that the first event is caused by specialized cilia in cells around the node[41]. These produce a vortical leftward flow of extracellular fluid within which, presumably, are growth factors that lead to the asymmetric expression of Nodal on the left side of the node. Nodal expression then needs to be relayed to the more distal cells of the lateral plate mesoderm (LPM), from which the left–right asymmetric organs differentiate.

This relay of *nodal* expression is now understood to involve a number of interlocking signalling relays that incorporate positive and negative feedback loops. Although there may be significant species differences in the relay mechanisms, the general and crucial function of Nodal in the left LPM seems to be global. Nodal, in complex with the extracellular cofactor EGF-CFC, signals to maintain its own expression, thereby participating in a positive feedback loop[42–44]. But Nodal also induces the expression of its own inhibitor, Lefty-2/antivin, which competes for Nodal receptors and thus restricts its range of action[45]. In chicks, another negative feedback loop involves the recently discovered Caronte, a TGF-β family factor that relays the localized Nodal expression to more distal cells[46,47]. Thus, feedback control of signalling has a central role in the establishment of widespread Nodal expression, restricted to the left side of the embryo.

Positive feedback can coordinate distinct signals. The vertebrate limb provides a good example of another strategic role for feedback control. The growth and development of the outgrowing limb depends primarily on two organizing centres, the zone of polarizing activity (ZPA) and the apical ectodermal ridge (AER) (Fig. 5)[48]. The ZPA corresponds to a region of mesoderm at the posterior margin of the limb and it moves distally as the limb grows so that it is always on the posterior edge of the limb, close to the distal end. The AER is a ridge of ectoderm that corresponds to the distal tip of the limb. A positive feedback loop maintains the spatial relationship between these two crucial domains[49,50].

The secreted protein sonic hedgehog (Shh) is produced by the ZPA (it is still unclear how this is initiated) and this is transmitted, through a cascade of signalling molecules[51], to the AER, where it maintains the expression of a fibroblast growth factor (this factor has been believed to be FGF-4, although recent evidence from FGF-4 mutants suggests that it may in fact involve other FGFs[52]). The FGF secreted from the AER is then required for the upregulation and maintenance of Shh in the ZPA, thus producing a positive feedback loop between the ZPA and AER. This feedback loop coordinates two essential aspects of limb development: first, growth and proliferation of cells at the growing tip, which are controlled by FGFs; and second, the differentiated fate of cells, which is controlled by Shh from the ZPA.

Negative feedback can restrict ligand range. In the *Drosophila* wing, where much progress has been made in understanding the principles of patterning, a variation on the theme of negative feedback limits signalling by Hedgehog (Hh) to the anterior/posterior compartment boundary. Hh signalling induces the expression of the long-range morphogen Dpp (the fly homologue of BMP2/4), and the global patterning of the wing is therefore dependent on the restricted activity of Hh signalling[53–55]. In this case, the key molecule in the negative feedback loop is Patched[56,57], a component of the Hh receptor that binds Hh but which does not have transducing activity (which is provided by another receptor component, Smoothened[58,59]).

Patched is expressed at low levels in all cells in the anterior compartment, which are Hh-responsive, but is dramatically upregulated in anterior cells near the A/P compartment boundary, when they receive Hh from their posterior compartment neighbours, the source of Hh (Fig. 6). In these cells near the anterior/posterior boundary, now expressing high levels of Patched, Hh is efficiently sequestered, thereby preventing it from diffusing more than a few cells away from the Hh source[60]. Thus, Hh induces the expression of a molecule that restricts the range of Hh signalling.

The dependence of Patched expression on Hh signalling has been conserved throughout evolution[61,62], suggesting that this feedback

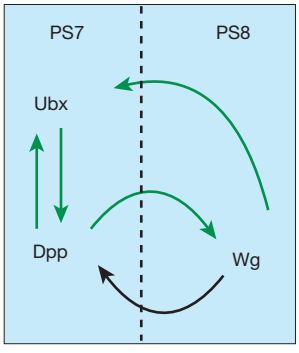

Figure 3 Ubx autoregulation by indirect positive feedback. The regulatory relationship between the three main players in the circuit is shown. The principal feedback loop is highlighted in green: Ubx in parasegment 7 activates the transcription of the signal Dpp; this leads to the expression of Wg in cells of the adjacent parasegment 8; the combination of Dpp and Wg is required to activate Ubx transcription. Ubx is repressed in parasegment 8 by the combined inhibitory action of another homeotic gene, Abd-A, and Wg (which is inhibitory at high concentrations).

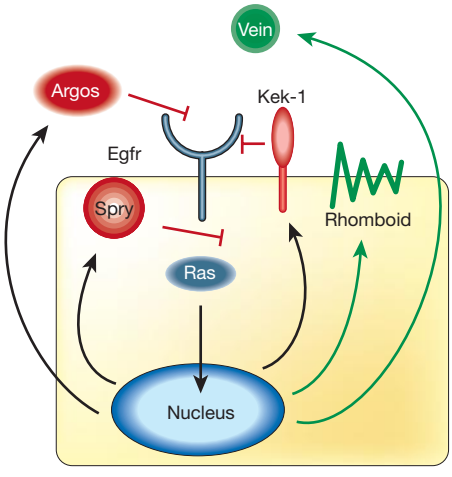

Figure 4 Multiple feedback loops regulate the *Drosophila* EGF receptor. There are three known feedback inhibitors (shown in red) of the fly EGF receptor (Egfr), which signals through Ras: Sprouty (Spry), Kekkon-1 (Kek-1) and Argos. There are at least two components that act in positive feedback, shown in green: Rhomboid and Vein.

loop that limits the signalling range may also be conserved. As there are tissues in which Hh appears to act at long range, it is possible that the sensitivity of this induction of Patched can be tuned to allow different ranges of Hh signalling in distinct developmental contexts. Another negative feedback regulator of Hh signalling, discovered in vertebrates, is Hip, a non-signalling Hedgehog-binding protein, whose expression is regulated by Hh signalling[63].

Integrated feedback cycles can elaborate pattern. The examples described so far in this section have illustrated the importance of feedback control in patterning and how its strategic significance varies in different contexts. The final example of feedback in patterning—the specification of the dorsal/ventral axis and the dorsal appendages in the *Drosophila* egg—highlights how distinct feedback loops can themselves integrate to produce elaboration of pattern.

The anterior–dorsal region of the fly egg has two respiratory appendages that arise from either side of the dorsal midline (Fig. 7). Their differentiation and location is specified by EGF receptor signalling in the somatic follicle cells that surround the egg. As described above, a positive feedback loop occurs when EGF receptor signalling, which is initiated by the TGF-α-like ligand Gurken expressed in the oocyte, triggers the activation of a different TGF-α-like ligand, Spitz, in the follicle cells themselves. This autocrine amplification of an initially paracrine signal causes the peak levels of EGF receptor signalling in cells at the midline to reach a threshold required for the establishment of a negative feedback loop dependent on the expression of the secreted EGF receptor antagonist, Argos (Fig. 7). Argos inhibits signalling at the midline, thereby splitting the initial single peak of receptor signalling into twin peaks, and it is these that specify the position of the appendages[19].

The positive and negative feedback loops are integrated because the amplification of the initial signal is required to induce Argos expression and thus trigger the secondary negative feedback loop. Another point that arises from this example is that the integrated feedback system controls not only the location of signalling but also its timing. The initial single peak at the midline is responsible for the determination of the dorsal/ventral axis, but the displaced twin peaks later determine the appendages. The integrated feedback system ensures that this temporal sequence—first one peak followed by two—occurs correctly.

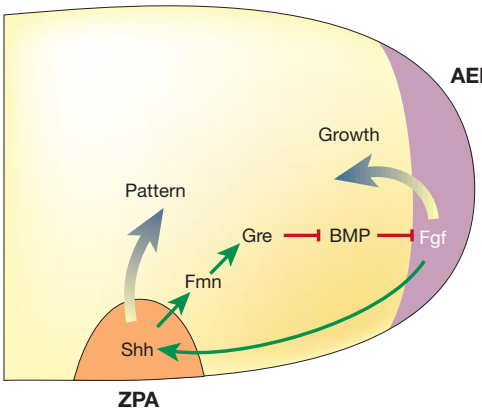

Figure 5 A positive feedback loop coordinates vertebrate limb development. The zone of polarizing activity (ZPA) and the apical ectodermal ridge (AER) are the two principal organizing centres of the limb, patterning the anterior/posterior axis and controlling proliferation at the growing tip, respectively. These two essential organizers of development are coordinated in the growing limb tip by a positive feedback loop that makes them mutually dependent. Sonic hedgehog (Shh) from the ZPA activates the expression of fibroblast growth factor (Fgf) in the AER, via a number of signalling components, Formin (Fmn), Gremlin (Gre) and bone morphogenetic protein (BMP); Fgf maintains Shh expression in the ZPA.

Negative feedback in the TGF-β family

The extensive TGF-β family of growth factors, which includes the activins, BMPs, GDFs, Nodal-related proteins and many others, controls a very large range of processes in development and growth control. A well conserved negative feedback loop regulates these important signals. The TGF-βs all signal through a class of serine/threonine kinase receptors and the signal is then transduced to the nucleus by the Smad family of proteins. These were first identified in *Drosophila*, and have since been found to be general TGF-β transducers. Receptor-binding Smads are phosphorylated and then complex with a co-Smad, Smad4, allowing them to transduce to the nucleus where they form transcriptional complexes (Fig. 8)[64–66]. A subclass of inhibitory Smads has been identified in *Drosophila* (Dad) and vertebrates (Smad6 and Smad7)[67–70].

Although there is some controversy about how these inhibit signalling, in general the inhibitory Smads compete for binding either to the receptor (they lack a phosphorylation site) or to Smad4 (refs 64–66). Their expression is activated by TGF-β signalling, so they form a classic negative feedback loop. The conservation of this feedback loop from flies to humans emphasizes its significance, as does the discovery of tumours with elevated levels of the inhibitory Smads[71] (TGF-β signalling generally inhibits proliferation).

Recently, another negative feedback loop relevant to TGF-β and cancer has been discovered. The SnoN oncoprotein has been shown[72] to be a co-repressor that blocks Smad4-mediated transcriptional activation (Fig. 8). Upon TGF-β signalling, activated Smad3 enters the nucleus and relieves this repression, allowing target genes, which include *snoN* itself, to be transcribed. This SnoN negative feedback loop probably ensures that these target genes are kept stably off in the absence of significant TGF-β signalling. In its oncogenic form, SnoN is no longer susceptible to Smad3 de-repression, so it cannot escape from the negative feedback loop and TGF-β growth inhibition is lost.

Negative feedback generates stability

Some of the above examples illustrate one of the general consequences of negative feedback loops—the generation of systems that are stable even when confronted with environmental fluctuations[73]. This property has recently been quantified in an artificially engineered gene network, where it was shown that negative feedback provided a twofold increase in stability over a broad range of input values[74]. This homeostatic function of negative feedback has an important role in protecting cells from the uncontrolled growth and developmental aberrations that can lead to cancer following environmental damage.

p53 is a tumour suppressor whose activity is lost in the majority

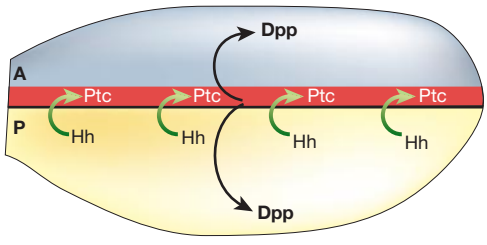

Figure 6 Variation on a negative feedback loop in the fly wing. The signalling protein Hedgehog (Hh) is expressed in all posterior (yellow) wing cells. The signal can only be transduced by anterior (grey and red) cells. High levels of Hh signalling induces the expression of the Hh binding protein, Patched (Ptc), in cells (red) near the Hh source. Patched sequesters Hh and prevents it from spreading further, causing Hh signalling to be restricted to cells close to the anterior/posterior (A/P) border. These cells express Dpp, which patterns the wing. The region in red is thus established as an organizing centre.

of human cancers. Its normal function is to induce cell-cycle arrest and/or cell death in damaged cells[75]. In healthy cells, p53 is maintained safely below its functional threshold by the action of a negative feedback loop—it is inhibited by the protein Mdm2, whose own transcription is activated by p53 (refs 76, 77). When stressed, a cell needs to break out of this negative feedback loop, so that p53 can perform its cellular policing function. As with the inability to break out of the SnoN feedback loop discussed above, cells that cannot escape the Mdm2/p53 feedback loop are in danger of losing control of normal growth and development, as they cannot respond normally to potentially carcinogenic damage. Thus, a number of tumours contain amplified or overexpressed Mdm2. In these cases, the p53 gene is usually the wild type, indicating that the failure to escape from Mdm2 is sufficient to block the p53 response to cell damage, which leads to tumour progression.

Positive feedback loops can generate instability

In contrast to the stability produced by negative feedback, positive feedback can cause instability. In engineering applications, positive feedback is usually avoided as a dangerous phenomenon that can lead to system failure (think of the famous Tacoma Narrows Bridge disaster). Nevertheless, organisms have evolved safeguards against this and I have discussed several essential and benign examples of positive feedback in developmental signalling. But a striking counter-example, which highlights the potential instability, is the autocrine, positive feedback loops that occur in tumour formation and progression.

The Ras/MAP kinase pathway is activated by the EGF receptor, and it has been proposed that autocrine secretion of ligands is a significant cause of EGF receptor hyperactivity in cancer. Recently, EGF receptor ligands have been shown to be induced by Ras/MAP kinase signalling, providing a direct demonstration of this positive feedback cycle (A. Schulze and J. Downward, personal communication). Interestingly, this pathogenic strategy directly parallels the positive EGF receptor feedback loops discovered in normal development—an example of the widespread phenomenon of tumorigenesis 'hijacking' normal developmental programmes.

Canalization

At the beginning of the 20th century, the embryologist Hans Spemann observed that development was robust: small perturbations were corrected so as not to cause lasting damage. He referred to this correction mechanism as "double assurance". In 1942, Waddington wrote about this developmental robustness at greater length and coined the term "canalization" to refer to what are really two separate but related phenomena[80]. The first is the buffering potential of normal development, allowing animals to develop normally under a range of environmental conditions. The second

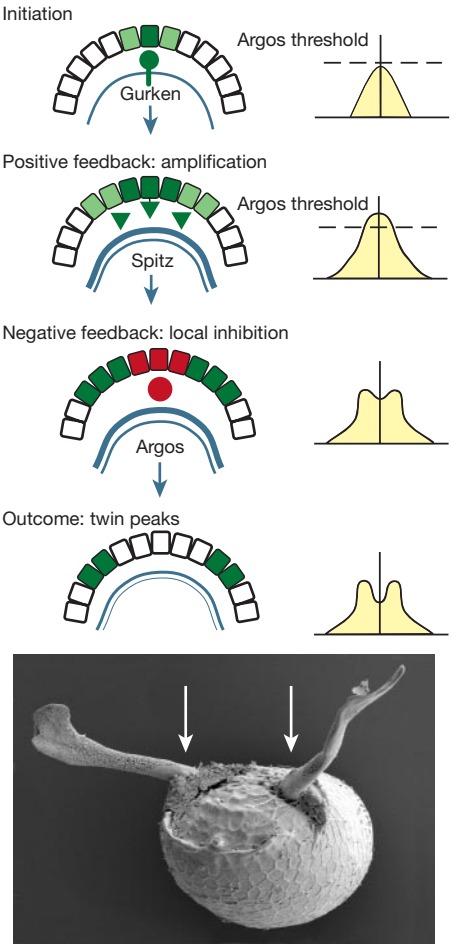

Figure 7 Integrated positive and negative feedback pattern the *Drosophila* egg. EGF receptor signalling in the follicle cells is initiated by Gurken in the underlying oocyte. This initiates a positive feedback loop that amplifies EGF receptor signalling via the ligand Spitz (see Fig. 2). The amplified signalling then induces the expression of the inhibitor Argos, which blocks signalling near its source at the midline, thereby splitting the peak of signalling into two. The resultant twin peaks of EGF receptor signalling specify the lateral position of the prominent respiratory appendages, marked with arrows in the lower panel. The yellow curves indicate net EGF receptor signalling at each stage in the process.

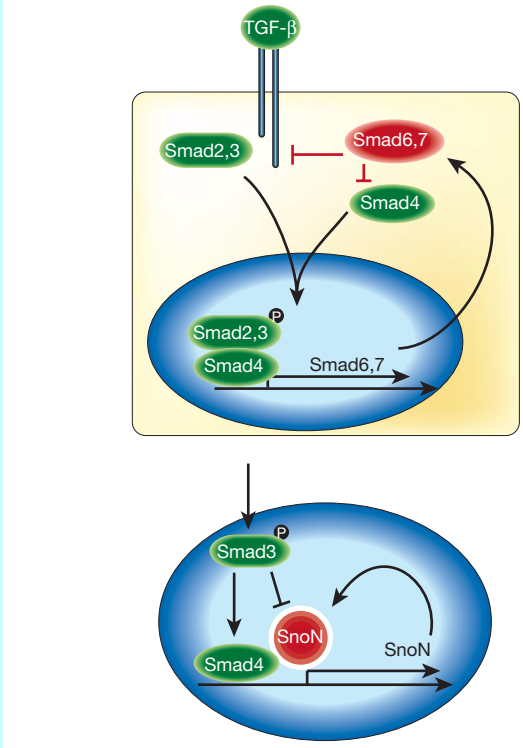

Figure 8 Two negative feedback loops that regulate TGF-β signalling and are implicated in cancer. Top panel, activated TGF-β receptors recruit and phosphorylate specific Smad proteins which, in complex with Smad4, activate transcription of target genes. These include the inhibitory Smad6 and Smad7 (Dad in *Drosophila*). TGF-β signalling inhibits cell proliferation, and, consequently, overexpression of the inhibitory Smads is associated with tumours. Bottom panel, the SnoN oncoprotein is a co-repressor that prevents transcriptional activation by Smad4 until phosphorylated Smad3 enters the nucleus. SnoN transcription is activated by Smad4, forming a negative feedback loop. Oncogenic SnoN is unable to break out of this feedback cycle as it is no longer sensitive to Smad3. P, phosphate.

is the robustness of developmental decisions—choices are amplified to all-or-nothing states rather than hovering around some intermediate.

These two phenomena lead to a marked invariance of the wild-type phenotype of animals. Redundancy of gene function after gene duplication during evolution provides one explanation for the relative stability of the wild-type state[81]. It is now, however, possible to suggest feedback mechanisms as another underlying principle that leads to canalization of development. And in fact the two aspects of the phenomenon—buffering and robustness—can neatly be explained by negative and positive feedback, respectively. Negative feedback allows self-adjustment of developmental signalling systems when they are perturbed[82]; positive feedback causes initially small changes to become irreversibly large, the classic example being the amplification of small or even stochastic differences to select a single cell as a neural precursor in Notch-mediated lateral inhibition[83,84].

Perspectives

Over a rather short period of time, the importance of feedback control in normal development has become clear; it is also increasingly obvious that failure of feedback can lead to disease. Most pathways and a broad range of developmental decisions are regulated by feedback, both in vertebrates and invertebrates. It therefore seems reasonable to suggest that feedback loops provide a fundamental strategy for controlling cell signals in growth and development.

The recognition of negative feedback has paralleled the discovery in the past few years of a wide variety of signalling inhibitors. Although a reasonable proportion of these do participate in negative feedback control (that is, their expression is dependent on the signalling pathway they inhibit), there are also many that appear not to be regulated in this way. However, it can be difficult to determine whether a particular protein's activity is, directly or indirectly, controlled by a given signalling pathway, and it may turn out that more of these inhibitors than currently thought do participate in negative feedback. Negative feedback loops are relevant to disease as well as normal development. For example, the discovery of inhibitory Smad overexpression in tumours, and the p53/Mdm2 feedback loop are two examples of a theme that seems likely to become increasingly common.

Positive feedback, beyond its well-studied role in transcription factor autoregulation, has been less recognized but is nonetheless of real significance in development. In its simplest form, positive feedback can prolong and amplify the response to a weak signal. As described above, in its more intricate manifestations, it has a role in quite complex signalling outcomes, such as coordination of developmental events, defining precise domains of gene expression and pattern formation. As in engineering, however, positive feedback in biology always carries with it the risk of loss of control of signalling, and a subsequent breakdown of homeostasis that can lead to disease. □

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Acknowledgements

I am grateful to M. Bienz, T. Casci and S. Munro for their help with the manuscript.

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