# Morphogen gradient interpretation

**J. B. Gurdon & P.-Y. Bourillot**

*Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, UK, and Department of Zoology, University of Cambridge, Cambridge, UK*

**A morphogen gradient is an important concept in developmental biology, because it describes a mechanism by which the emission of a signal from one part of an embryo can determine the location, differentiation and fate of many surrounding cells. The value of this idea has been clear for over half a century, but only recently have experimental systems and methods of analysis progressed to the point where we begin to understand how a cell can sense and respond to tiny changes in minute concentrations of extracellular signalling factors.**

Morphogens are secreted signalling molecules that organize a field of surrounding cells into patterns. They form a gradient of concentration emanating from a localized source, and determine the arrangement and fate of responding cells according to the different concentrations of the morphogen perceived by the cells. The idea of a morphogen gradient is intimately associated with the concept of positional information[1]. A cell is believed to read its position in a concentration gradient of an extracellular signal factor, and to determine its developmental fate accordingly[2,3].

Morphogen action is of special importance in understanding development. This is because a single event, the emission of morphogen from a source, can lead to the formation of several different cell types in a correct spatial relationship to each other. This is a highly efficient way of creating complex patterns of gene expression and spatial position from a population of uncommitted cells in an embryo.

An understanding of morphogen gradients requires answers to two different questions. The first asks how a desired concentration gradient is formed. We need to know the identity of the morphogen, the shape and absolute concentration of the gradient, and the factors that create and maintain the gradient in its various positions. Much current work is providing some answers to these questions, especially by identifying extracellular molecules that bind morphogens, and that thereby influence the concentration of morphogen free to reach cells whose responses they determine. This subject has been widely reviewed[4,5], and we summarize here, only briefly, some of the principles that are emerging.

The second question asks how cells interpret a morphogen concentration. To understand this, we need to know how cells recognize different threshold concentrations of morphogen through receptors on their surface and how they transduce this information to the nucleus to create the appropriate gene or cell fate response. This second question has so far been little explored, and is the one we primarily address here.

## Examples of morphogens

To be sure that an example of morphogen action exists, several criteria need to be satisfied. Ideally an identified signalling molecule should be present in the right place at the right time. It should be shown to be released from a localized source, forming a concentration gradient over a population of nearby and distant cells that respond directly to the signalling molecule in a concentration-dependent way. Cells in the pathway of the gradient should show two or more qualitatively different responses, such as the expression of different genes, in addition to their default pathway. Over- and underexpression experiments should have the effect of changing gene expression or cell fate in the predicted directions. Thus an increase in the gradient, such that all cells experience an elevated morphogen concentration, should cause them to switch their response to a higher level. Likewise, underexpression should cause cells to move progressively down the scale of responses available to them. A further important characteristic of morphogen action is that it should be direct. This means that wherever a cell is located in the concentration gradient, it should respond directly to the same morphogen, and should not judge its position indirectly through the mediation of other signal molecules or other cells elsewhere in the responding population.

The most convincing and best-analysed examples of morphogen action are listed in Table 1. Examples of morphogens acting during development in *Drosophila* and *Xenopus* are illustrated in Figs 1 and 2. In all of these cases, the morphogens are secreted proteins that are functional only when cut, and often further modified, after synthesis of the primary polypeptide from a gene. Where the most profound analysis has been conducted, it is usually found that a primary morphogen gradient is supplemented, or even created by, other positively or negatively acting factors, such as Short gastrulation (Sog) in the *Drosophila* wing[6] or chordin in the amphibian embryo[7] (see Table 1). These agents affect the concentrations of the factor (in these cases, Decapentaplegic (Dpp) or bone morphogenetic protein (BMP), respectively) that reach the surface of responding cells, and to which cells respond directly when interpreting their position in the gradient. The table does not include Bicoid (*Drosophila* embryo), which has many properties of a morphogen[8], except that it is a transcription factor that can spread through the syncytial embryo, a situation different from other morphogens that are extracellular and spread through multicellular tissue. We concentrate here on the *Drosophila* wing disc and the amphibian blastula animal cap, two systems in which the principles of morphogen gradient interpretation have been extensively studied.

Most of the examples in Table 1 fall short, in one or more respects, of the perfect morphogen. Indeed, few if any of the morphogens so far described obey all the rules laid down by theoreticians for their guidance. Only in a few cases has a natural morphogen gradient been directly visualized (see, for example, refs 9–11); in other cases, the existence of a gradient has to be deduced from its activity, that is, its ability to induce genes in a concentration-related sequence. This difficulty arises because proteins that function as morphogens seem to have activity at extremely low concentrations (for example, activin at 50 pM), and because extracellular diffusible proteins are hard to fix for *in situ* demonstration. For some examples, a read-out of gradient interpretation is satisfactorily provided by directly induced gene expression (such as the *Drosophila* wing and *Xenopus* embryo (Figs 1 and 2)). In others (such as the chick limb) there are no position-related gene or cell-type markers, and gradient effects have to be judged from overall organ or tissue morphology (for example digit number). Here, we concentrate on the former. Other examples of morphogens may exist, such as retinoic acid in the vertebrate anteroposterior neural axis[12], but in most cases they have not been shown to have direct action.

The strongest candidate morphogens are members of the

transforming growth factor-β (TGF-β), Hedgehog (Hh) and Wnt families of secreted proteins. Members of the fibroblast growth factor (FGF) family seem to act as competence factors having permissive rather than concentration-related effects[13].

## Gradient formation

Much recent work has been directed towards the problem of how the necessary amount of morphogen comes to be located in the right place at the right time (see ref. 5 for original work in this field). A difficulty is that we are poorly informed about the actual concentration and shape of the gradient itself. Known morphogens seem to be effective at extremely low concentrations, $10^{-9}$ to $10^{-11}$ M, and are probably not distributed in an even slope across their field of action. Only in a few cases can a glimpse of the morphogen itself be caught by the use of tagged forms containing green fluorescent protein (GFP) or horseradish peroxidase, or by antibody staining. In most cases the source of the morphogen is evident from the distribution of its messenger RNA. But we need to know the rate at which the protein is released from the source, the rate of its spread and its stability. We also need to know whether the gradient is formed with the morphogen in its active state, or whether it is converted into an active condition during or after gradient formation. The complexity of the problem is well exemplified by Hh, which undergoes an endonuclease cleavage and needs cholesterol addition for full activity[14]. Particularly important is the recent finding that, more often that not, the activity of a morphogen is controlled by antagonistic proteins that associate with it and prevent it from binding to its receptors (Table 1). A further level of complexity seems to exist in controlling the distribution and activity of these antagonistic factors. For example, the activity of the BMP gradient in vertebrate embryos is determined primarily by an opposing gradient of chordin and other molecules, the activity of chordin being itself determined by the protease xolloid[7]. In the *Drosophila* wing disc, the different shape of the Wingless (Wg) gradient in the anterior and posterior regions is caused by faster endocytosis and degradation of Wg in posterior cells[11]. It seems that the major factors shaping a gradient are not only different for each morphogen, but may also differ for the same morphogen in different stages of development. Other characteristics of gradients vary greatly from one case to another. For example, the range of a gradient, or the distance over which it has activity, can be as small as a few cell diameters (50 μm for Hh in the wing disc) or as large as 300 μm (for the mouse limb-bud and amphibian embryo). To understand how cells read their position in a gradient, it is important to know its slope. Judging from responses of genes to gradients of Dpp and activin, we estimate that cells read a threefold concentration change over three cell diameters (about 30 μm); however, the involvement of antagonistic factors may make the effective gradient much steeper than this.

There has been much activity in analysing the mechanism of transmission of a morphogen across its field[5]. Three ideas prevail: (1) diffusion in the extracellular matrix (active or facilitated); (2) relay by sequential internalization and re-emission from cell to cell; and (3) cytoplasmic contact by threads of cytoplasm connecting distant cells (cytonemes). The use of transplanted tissues defective for receptors or ligands has shown that TGF-β morphogens such as squint (fish)[15] and activin (frogs)[16] can spread by diffusion over long distances. In contrast, cells defective for endocytosis interfere with the formation of Dpp gradients, a result in support of a relay mechanism. It even seems that both of these mechanisms can have a role in forming the same gradient of Dpp in the wing disc.

Lastly we should point out that the timing of gradient formation is likely to be rapid. In later development, as in the *Drosophila* wing disc, a Dpp gradient is normally formed slowly, extending over 25 cell diameters in 3 days. Nevertheless, the same Dpp gradient can be reformed, after temperature interruption, at the rate of 4 cells in 1 h[9,10]. In *Xenopus* embryos, however, an activin gradient can be formed experimentally over 100 μm in 1 h[17], and natural gradients are normally formed in *Xenopus* and *Drosophila* embryos in 2 h or less. We discuss below how cells interpret such rapid changes in morphogen concentration.

## Perception at the cellular level

A simple mechanism by which a population of cells could make different responses to morphogen concentration would exist if cells have different response thresholds. According to this idea, each cell would have only a binary choice: respond to the morphogen or not. For each cell the actual concentration to which it would respond would be different. In this way, a range of concentration-related responses could be made by a cell population, even though any one cell would be able only to respond or not. This would avoid the

**Table 1 Examples of morphogens**

| Developmental process | Signal source | Morphogen (range)* | Anti-factor | Receptors | Gene response† (concentration) | Gene response (time, h) | Reference |
|---|---|---|---|---|---|---|---|
| *Drosophila* embryo dorsoventral axis | Dorsal region | Scw (short)<br>Dpp (long) | Sog<br>Brinker | Tkv<br>Sax<br>Punt | *Race* (high)<br>*zen* (middle)<br>*pannier* (low) | 3 | 19, 21, 47 |
| *Drosophila* imaginal wing disc | Anteroposterior compartment boundary | Dpp (long) | Brinker | Tkv<br>Punt | *sal* (high)<br>*omb* (low) | 24–72‡ | 6 |
| *Drosophila* imaginal wing disc | Dorsoventral compartment boundary | Wg (long) | | Fz | *neur* (high)<br>*Dll* (middle)<br>*vg* (low) | 24–72‡ | 34, 50 |
| *Xenopus* mesoderm formation | Nieuwkoop centre | Activin (long)<br>Xnr1, -2, -6 (short) | Antivin<br>Follistatin | Activin receptors I, II | *gsc*, *eomes* (high)<br>*Xbra*, *apod* (low) | 3 | 7 |
| *Xenopus* axis formation | Organizer (Spemann) | BMP2, -4 | Chordin<br>Noggin<br>Follistatin | BMP receptors | *Xvent* (high)<br>*MyoD* (middle)<br>*Xnot* (low) | 3 | 7 |
| Zebrafish axis formation | Organizer | Squint (long)<br>Cyclops (short) | Lefty | Activin receptors I, II<br>Oep (coreceptor) | *gsc* (high)<br>*Ntl* (*Xbra*) (low) | 3 | 15, 45 |
| Vertebrate neurogenesis | Notochord; neural floor plate | Shh (long) | BMP4 and TGF-β family | Ptc<br>Smoothened | *Nkx2.2* (high)<br>*Dbx* (middle)<br>*Pax7* (low) | 12 | 42, 43 |

*Short range, 20 μm or less; long range, 100 μm or more.
† Includes repression as well as activation.
‡ Only after 50 h is *omb* expression further from the source than *sal*.

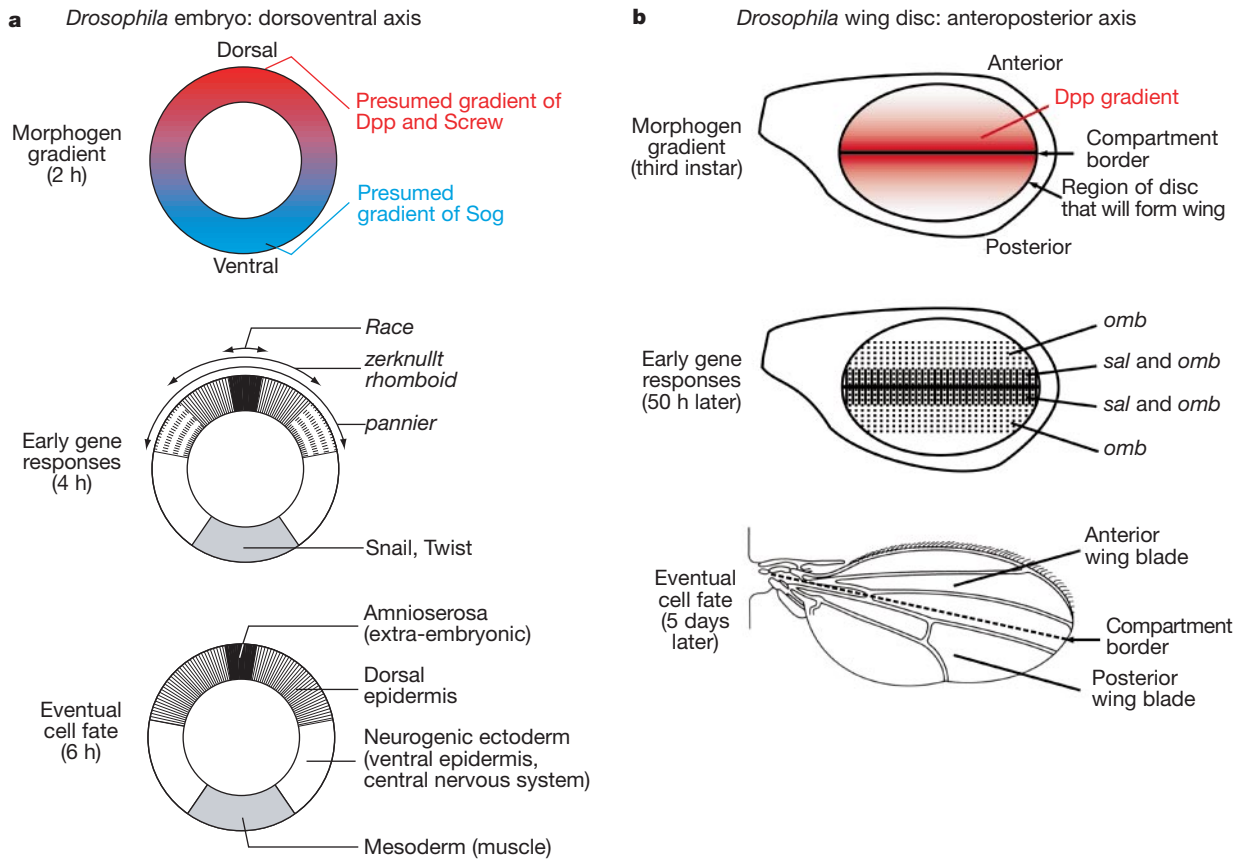


**Figure 1** Morphogen action during development in *Drosophila*. **a**, Diagrams of cross-sections of *Drosophila* embryos, showing the presumed morphogen gradients, early gene responses and cell fates[48]. Early gene responses overlap in the dorsal region. Gradients of Dpp and Screw, and their inhibitor Sog, are formed as an effect of a maternal ventral-to-dorsal gradient of the nuclear protein Dorsal. An intracellular gradient of the repressor Brinker coincides in position and cooperates functionally with the Sog gradient. **b**, Diagrams looking down on a *Drosophila* wing disc. The repressor Brinker is present in the wing-forming regions not occupied by Dpp. The transition from a third-instar disc to a complete wing involves complicated folding and extension movements.

need to credit cells with the ability to make any more sophisticated a response than an on/off switch.

In most cases it is not possible to be sure that any one cell has more than a binary choice of response. In a few examples, however, it seems clear that an individual cell chooses between at least three responses. One of these examples is when activin-loaded beads are placed in a spherical cell reaggregate of two face-to-face animal caps of a *Xenopus* blastula (Fig. 2b). There is no movement, and very little cell division, in the responding cell population; nevertheless a ripple of *Xbrachyury* (*Xbra*) gene expression spreads out radially from the activin beads, and this is followed 1–2 h later by the expression of *goosecoid* (*gsc*) and *eomesodermin* (*eomes*) in more centrally located cells. These gene responses are direct (cycloheximide insensitive) and expand radially as the concentration of activin on the beads, or as the time over which the beads are left in place, is increased[17]. In the same cells, *Xbra* expression is followed by *gsc* and *eomes* expression, and subsequently by *sox* expression. *Xbra* suppression at higher activin concentrations is an indirect effect of *gsc* and other high-response genes[18]. The same choice of response seems to apply to the *Drosophila* embryo. A two- to fourfold change in Dpp by RNA injection is sufficient to cause the ectoderm cells, which do not change position relative to each other at this stage, to adopt one of three different fates[19,20]. Over- or underexpression of Dpp causes the expression of *spalt* (*sal*) and *optomotor blind* (*omb*) genes to move nearer or further from the signal source in the wing disc[21]. Therefore, in all of these cases, the same cell has a choice of at least three different responses to gradient concentration.

Another important question concerning the cellular basis of morphogen perception asks whether a cell needs its neighbours to determine its position in a gradient, or whether it can measure concentration on its own. In regeneration, such as occurs in newt limbs or cockroach legs, a missing structure is replaced by cells according to their position. Cells must therefore be guided by their neighbours to follow a particular fate, a concept commonly illustrated by Wolpert's French flag model[22]. The most decisive answer to this question requires a test in which isolated cells, unable to receive information from their neighbours, are exposed to different concentrations of morphogen, and tested for gene response. The only system in which this has been done is with *Xenopus* blastula cells exposed to activin, and then cultured either as single cells, confluent monolayers (lateral contact), or as reaggregates (contact on all sides)[23]. These configurations have no effect on the type or amount of *Xbra* or *gsc* gene expression, and therefore show that, at least in this case, a cell can read its position in a concentration gradient without reference to its neighbours.

The fact that isolated cells have the ability to measure concentration independently of their neighbours does not necessarily mean that they do so in normal tissues. Almost certainly the spread of morphogen, and hence the exposure of a cell to the appropriate concentration of morphogen, is greatly affected by its neighbours. For example, underexpression of the Hh coreceptor Patched (Ptc) will increase the availability of Hh to nearby cells[24]. However, the

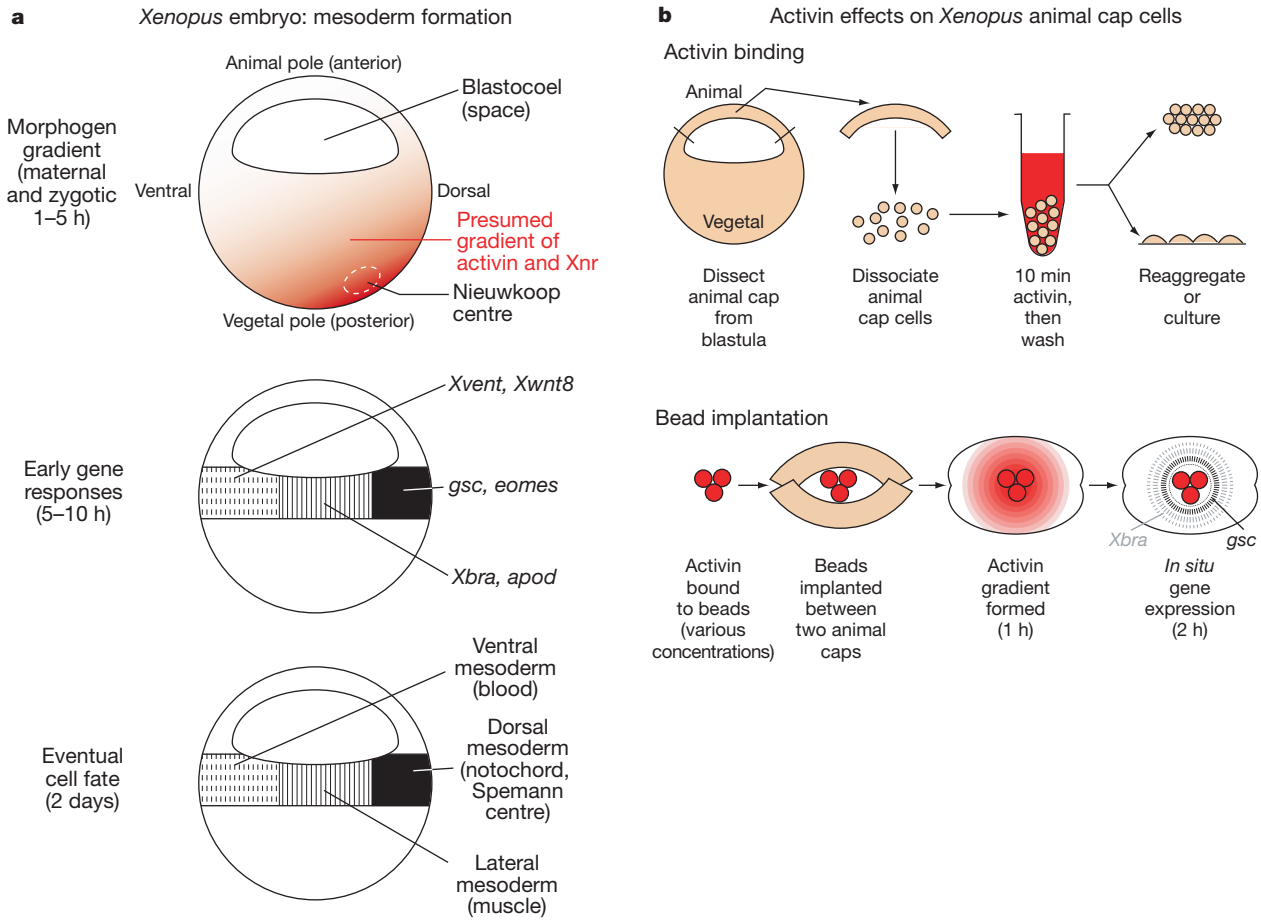


**Figure 2** Examples of morphogen action during development in *Xenopus*. **a**, Diagrams of cross sections of an amphibian embryo. Gene responses are shown only for the equatorial mesodermal region, which is most directly responsive to Nieuwkoop signalling and to an activin or Xnr morphogen gradient. **b**, Assays used with amphibian animal cap cells to analyse interpretation of morphogen gradients. Activin is currently believed to simulate the action of endogenous *Xenopus* nodal proteins.

reading or interpretation of concentration by a cell seems to be independent of its neighbours. In *Drosophila* wing discs, clones of cells that overexpress the Dpp receptor Thickveins (Tkv) or other components of the Dpp pathway can be created in the Dpp gradient. In spite of this, cells express the induced genes *sal* and *omb* in accord with their genetic constitution and not with their position in the gradient[25]. Therefore cells respond to Dpp signalling according to their own interpretation of gradient concentration, and not according to any influence from their neighbours. In *Xenopus*, activin can elicit a normal concentration response when passing through cells unable to respond to activin (endodermal cells) and through tissue inhibited for protein synthesis by cycloheximide[17]. These cells can not depend on their normal neighbours or engage in relay signalling in these cases. All these experiments argue that cells interpret position in a concentration gradient independently of their neighbours.

Similar transplantation or mosaic experiments have demonstrated, at least in some cases, the important point that morphogen action is direct. An example of indirect action is the release of Hh from a row of cells along the anteroposterior compartment border in *Drosophila* wing discs. Hh travels a very short distance and induces the release of the long-range morphogen Dpp. One could imagine a sequence of such indirect effects so that a cell has to make only a binary response to its neighbour before handing on another signal. Genetic or embryological mosaics of cells carrying dominant negative or mutationally defective receptors have shown, at least for the *Drosophila* wing[25], *Xenopus* embryo[16] and fish embryo[15], that near and distant cells respond directly to the same morphogen.

## Perception at the cell surface

A cell may respond to morphogen concentration through its receptors in two ways. One is to be armed with receptors having different binding characteristics; for example, high- and low-affinity receptors and their transduction pathways could operate at low and high concentrations of ligand, respectively. The other is to vary the occupancy of one type of receptor, and hence its signalling activity, according to ligand concentration. We therefore need to know whether different morphogen responses are transmitted by one or more kinds of receptor. The *Drosophila* morphogen Dpp is bound initially by the type II receptor Punt, which phosphorylates type I receptors Saxophone (Sax) and Tkv. Overexpression of Tkv can substitute for Sax, and increasing amounts of activated Tkv induce the full range of concentration-dependent cell fates[26,27]. It seems that the two type I receptors use the same intracellular transduction pathway, Sax being used in normal development to amplify the effects of Tkv. The specificity of these receptors has also been examined by the effects of dominant negative variants; dominant negative Tkv blocks all activity of Dpp, and it is clear that Dpp alone can specify all positional values in a concentration-dependent way[6]. The receptors for *Drosophila*

Wg are of two kinds, with a tenfold difference in ligand affinity measured *in vitro*[28], but this difference is not related to Wg concentration response in the wing, and these receptors are functionally redundant[29].

Vertebrate TGF-β factors including activin are also bound initially by a type II receptor; this phosphorylates an associated type I receptor, which in turn phosphorylates Smad2, causing it to enter the nucleus. From binding studies on isolated cells from the *Xenopus* animal cap[30], activin binding is known to be limited only by type II receptors, at all concentrations, and that these receptors have only one class of affinity, judged by Scatchard analysis. The inactivation of type I activin receptors by dominant negative overexpression eliminates response to all concentrations of activin, showing that these same receptors are necessary and sufficient components of the pathway, at all concentrations[31]. All of these results indicate that cells read different concentrations of a morphogen by varying the activity of one type of receptor and not by making use of different receptors for the same morphogen.

We need eventually to understand morphogen perception in terms of receptor occupancy. To understand how a cell can measure morphogen concentration using only one kind of receptor, we first need to know whether it is measuring the absolute number of occupied receptors or a proportion of these, such as the ratio of occupied to unoccupied receptors. This last idea could operate if, for example, unoccupied receptors have a phosphatase activity that counteracts the known serine-threonine kinase activity of TGF-β type II receptors. The overexpression, by up to tenfold, of the activin type II receptor in *Xenopus* embryo cells shows that the choice of gene response depends on the absolute number of occupied receptors, entirely independently of how many unoccupied receptors are present[30].

An understanding of receptor signalling with time would be complicated if receptors turn over or are recycled rapidly. In the case of activin, it seems that receptors bind their ligand with high affinity so that it remains bound for several hours[30]. Over this timescale, receptors and their bound ligand are stable and seem not to be internalized. This situation is certainly not universal. Many receptors are internalized and downregulated soon after ligand binding. In the particular case of major histocompatibility complex (MHC) receptors, these continue to signal after the ligand, which binds with low affinity, has dissociated[32,33]. For morphogen interpretation that takes many hours or days (such as in the vertebrate limb), receptor turnover must certainly be taken into account.

## From receptor to gene

We need next to know at what stage in the pathway from receptor to gene there is a change from a quantitative difference in the number of occupied receptors to a qualitative choice of gene expression. In the rather few cases so far analysed, this switch does not happen in the known transduction pathways. Thus Armadillo is necessary and sufficient for the induced expression of genes that respond to both short- and long-range Wg signalling[34]. Likewise, Smad2 is required for all responses to activin signalling. Furthermore, the threefold difference in extracellular activin concentration that causes a switch in *Xbra/gsc* gene expression is accompanied by only a threefold increase in the level of nuclear Smad2 concentration[35,36]. Thus, surprisingly, morphogen concentration seems to be transmitted from outside the cell to the nucleus by a single pathway with no amplification.

The examination of activated transduction molecules is proving valuable in visualizing the effects of morphogen gradients. Antibodies against activated (phosphorylated) Smad1 reveal a gradient distribution with a high point on the ventral side of the embryo, as expected if this reflects the BMP gradient[37]. Over- and under-expression of BMP and activin changes the gradient distribution, as predicted for Smad1 and Smad2 (ref. 35). Likewise, a gradient of phosphorylated Mad is seen in *Drosophila* embryos with a high point in the most dorsal region of the blastoderm embryo, and this is influenced in accord with an up- or downregulation of Dpp and Screw morphogen expression[38]. The direct demonstration of these gradients of activated TGF-β transduction molecules provides valuable support for the existence of morphogen gradients in embryos, as these presumed gradients are very hard to see directly.

The next step for the determination of the threshold responses takes place at the level of transcription. In no case so far has the mechanism by which changes in extracellular morphogen concentration activate different genes at the transcriptional level been exactly deduced. Several ideas exist, however, and two of these are presented in Fig. 3.

## Kinetics of interpretation

A proper understanding of morphogen action requires a knowledge of timing. An interesting 'sequential cell context' hypothesis proposes that a low dose of morphogen first activates a 'low-concentration' response, which, at a later time, induces a 'high-concentration' response[39]. The difficulty with this idea seems to be that the subsequent response is indirect, and this model cannot therefore explain those morphogen responses known to be direct

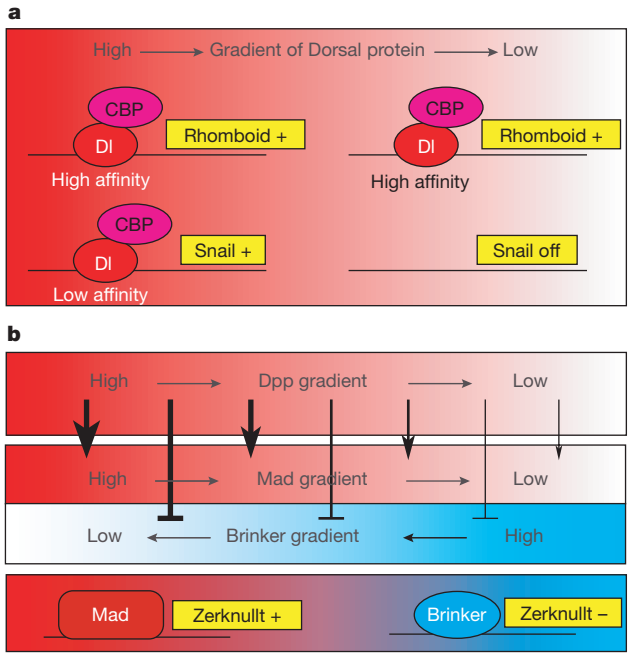


**Figure 3** Two possible mechanisms of concentration-dependent transcription. Dorsal (Dl) is a maternal cytoplasmic protein that moves to the nucleus of ventral cells in the *Drosophila* embryo, and activates the promoters of ventral (mesodermal) genes. It is present in nuclei in a gradient from high (ventral) to low (dorsal) concentration, opposite to the gradient of Dpp (Fig. 1a). **a**, Promoter binding affinity. In the *Drosophila* embryo, Snail (mesoderm) has weak binding sites that respond only to high concentrations of Dl, whereas Rhomboid has high-affinity binding sites for Dl and so is expressed at both high and low concentrations of Dl[47]. **b**, Competition between activator and repressor. In *Drosophila* Dpp signalling, Brinker, the transcription of which is repressed by Dpp, acts as a repressor of Dpp target-gene transcription. Several Mad- and Brinker-binding sites have been described in the promoter of *zerknullt* (*zen*) and many of these overlap. In the early embryo, competition between these two factors determines the expression domain of *zen*. In cells responding to a high concentration of Dpp, the high concentration of phosphorylated Mad outcompetes Brinker for binding to the promoter of *zen*, which is therefore expressed. In the cells receiving a lower concentration of Dpp, Brinker, which is not downregulated, represses *zen* expression by preventing the binding of Mad and by recruiting co-repressor to the promoter[49].

(cycloheximide insensitive). Here we discuss three ideas on how cells make direct responses to morphogen gradients.

Our first proposition is that the availability of ligand (morphogen) is the limiting factor in determining the level of response to concentration. In theory any component of a pathway, such as the availability of receptors, the supply of transduction molecules, and so on, could prevent or restrict the ability of a cell to recognize the concentration of an extracellular molecule. For example, if all of a cell's receptors were occupied with a low morphogen concentration, the cell could not perceive a higher concentration until more receptors had been synthesized, and the availability of these would determine the ability of a cell to know its position in a gradient. The following results indicate that ligand supply is usually limiting. In the *Drosophila* wing disc, twofold increases in Dpp extend the range of concentration-related responses[6]; similar increases in receptor abundance have little effect. Dpp represses the level of its receptor Tkv[40], so that Dpp is probably not limiting near its source, and both response genes are activated equally during the first 50 h. However, further from the Dpp source, receptor levels are higher, and it is presumed that Dpp is limiting in this more distant region where cells read the gradient to differentially activate *sal* and *omb*. Also, in the wing disc, Wg downregulates its receptor Frizzled (Fz), so that it is in lower abundance near the source than further from it[41]. Ptc absorbs Hh and Sonic hedgehog (Shh) near their source, thereby making their supply limiting further away[24,42,43]. We know in *Xenopus* that small increases in activin concentration result in stepped changes in the types of gene expressed[44], as expected if morphogen concentration is limiting. We also know that an increase in receptor abundance does not change gene response[30]. Moderate increases in Smad2 supply do not affect response, although massive increases activate the signalling pathway in the absence of ligand. Because less than 10% of the activin receptors are occupied when gene switches at low concentration take place, it is hard to understand why the available supply of ligand is not all absorbed by the cells with empty receptors near the signalling source. This apparent inconsistency can be explained, however, by supposing that most of the morphogen molecules remain in the intercellular space between cells, either free or attached with low affinity and high capacity to the extracellular matrix[30]. Although activin can be bound rapidly at a high concentration (1–4 nM) in dissociated cells, at *in vivo* concentrations (50–200 pM), the time required for binding is long and should permit unbound ligand molecules to pass out of reach of receptors on cells near the source.

Our second proposition is that cells respond to ligand concentration according to the absolute number of receptors occupied at any time. Most importantly, there is no integration of the number of occupied receptors multiplied by time. Thus a given number of receptors occupied for 1, 2 or 4 h will all elicit the same choice of response, even though a two or four times greater number of occupied receptors induces qualitatively different gene responses. After only a 10-min exposure to activin, the number of occupied receptors in dissociated cells from the *Xenopus* animal cap remains constant for a few hours, at, for example, 300 of a total 5,000 receptors per cell. Under these conditions, gene expression does not change, whether tested 2 or 4 h later[30]. This is entirely consistent with the complementary observation that there is indeed an integration of receptor occupancy and time as cells fill receptors with available surrounding morphogen: a cell in 4 nM activin will fill twice as many receptors in 20 min as in 10 min, and twice as many in 8 nM activin as in 4 nM activin for 10 min. This principle has also been demonstrated by varying the duration of signalling by the Nodal cofactor Oep in zebrafish axis formation[45]. We therefore propose that the loading of receptors by ligand increases progressively with time, but that, once loaded, the activity of a receptor remains constant with time.

Our third proposition concerns gene read-out. We suggest that a cell with a particular number of occupied receptors will continue to express the same gene until either the occupancy of receptors goes up or the period of competence terminates. Bead exchange experiments have indicated a ratchet effect by which a cell can rapidly change its response to morphogen concentration in an upward direction, by filling a higher proportion of its receptors, but can change downwards only very slowly, as receptors become vacated. In the particular case of the gene *Xbra*, it is transcribed less strongly as activin concentration goes up and higher response genes such as *gsc* or *eomes* are expressed. This is, however, an indirect effect[18], and much more generally a low-concentration-response gene such as *omb* in the *Drosophila* wing disc continues to be expressed when a high response gene such as *sal* is activated. Likewise, *neuralized* (*neur*), *Distalless* (*Dll*) and *vestigial* (*vg*) are all transcribed at the highest Wg concentrations. It is not yet clear how the co-expression of two or more morphogen response genes at the same time, and presumably in the same cells, generates the appropriate morphological or developmental effect.

Finally, we comment on a particularly intriguing future problem in the interpretation of morphogen gradients: how cells respond to an extracellular concentration that changes with time. In development, signalling centres start to emit their signalling factor or morphogen at a particular stage. On the assumption that the supply of morphogen is limiting (above), cells nearest the source will at first experience a low concentration of the morphogen, but this will increase until it reaches the much higher level to which cells near the source make their correct response. We want to know how these cells near the source know when to interpret the factor concentration around them. There are two ways in which cells

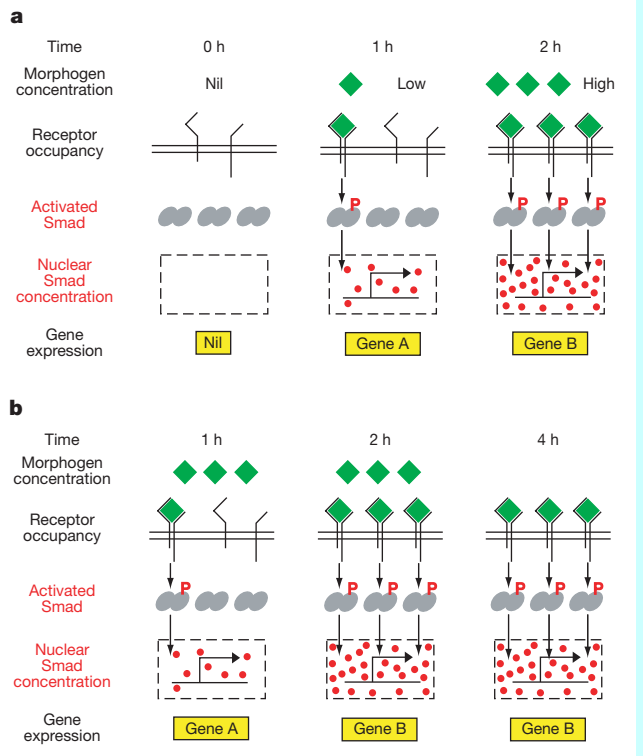


**Figure 4** Concepts of morphogen gradient interpretation. **a**, Morphogen concentration that increases with time. The model proposes that the number of occupied receptors, and of phosphorylated Smad molecules, increases with time. **b**, Morphogen concentration that is constant or declines with time. The model proposes that, with constant morphogen concentration, receptor occupancy and signalling activity increases. With a constant number of occupied receptors, it is proposed that the level of intracellular signalling activity also remains constant.

might solve this problem. One is to wait until a steady-state gradient has been reached and respond only at that time; the other is to respond continuously to their ambient concentration and to change their gene response accordingly with time. The latter is more likely, because it is known from bead exchange experiments that cells can change their expression upwards but not downwards during the course of an activin response[46]. Our current ideas on how cells interpret gradient concentrations that change with time are summarized in Fig. 4. To provide clear answers to these and other questions, future work should endeavour to monitor the transduction, and perhaps even gene transcription, response to morphogen interpretation in single cells in real time. □


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Correspondence should be addressed to J.B.G. (e-mail: j.gurdon@welc.cam.ac.uk).