Development 140, 4119-4124 (2013) doi:10.1242/dev.095141

Studies of morphogens: keep calm and carry on

Angelike Stathopoulos[1,*] and Dagmar Iber[2,*]

Summary

Morphogens are signaling factors that direct cell fate and tissue development at a distance from their source, and various modes of transport and interpretation have been suggested for morphogens. The recent EMBO Workshop on 'Morphogen gradients', which took place in Oxford, UK in June 2013, centered on the formation and interpretation of such morphogen gradients during development. This meeting allowed an exchange of views in light of recent results. Here, we provide a brief overview of the talks, organized in relation to several major themes of discussion at the meeting: (1) morphogen gradient formation; (2) morphogen gradient interpretation; (3) signaling networks and feedback in morphogenesis; (4) emergence of patterns; (5) scaling of patterns; (6) the control of growth; and (7) new techniques in the field.

Key words: Morphogens, Cell fate, Tissue development

Introduction

Most multicellular organisms develop from a single cell. How genetically identically daughter cells are directed to take different developmental paths in a reliable time- and position-dependent manner has fascinated developmental biologists for over a century. The term 'morphogen' has been coined to describe signaling factors, often present in a gradient, that act to provide such positional information to cells within developing tissues (Turing, 1952; Wolpert, 1969). The predominant view is that the graded activity of morphogens in tissues leads to differential gene expression between cells, depending on the local concentrations of morphogen that individual cells encounter (Ashe and Briscoe, 2006; Rogers and Schier, 2011).

A large number of morphogens have been identified, many of which are mentioned in this Meeting Review, with the most studied ones including Hedgehog (Hh), Decapentaplegic (Dpp), Bicoid and Dorsal in *Drosophila*, and sonic hedgehog (SHH), bone morphogenetic protein (BMP), fibroblast growth factor (FGF) and Nodal in vertebrates (Fig. 1). How morphogens are transported has been intensively studied. In addition to simple diffusion, a number of other transport mechanisms have been proposed and are actively being investigated, including shuttling via other proteins, transport via vesicles (e.g. exosomes and exovesicles) and delivery via cellular projections (e.g. cytonemes). A number of model systems have been established to study morphogen transport, gradient formation and interpretation, as well as position-dependent cell differentiation in various biological contexts. For example, the *Drosophila* imaginal discs have been used to study how morphogens are transported and how patterning and growth of tissues may be coordinated. Furthermore, studies of how cells interpret morphogen gradients in the vertebrate neural tube have used molecular and mathematical approaches to decipher how different cell types emerge from a network of transcription factor interactions. By contrast, in the early *Drosophila* embryo, transcription factors are themselves present in gradients and many studies have provided insights into how their interpretation at the *cis*-regulatory level drives differential gene expression. Even within single cells, the influence of molecular gradients on cell length has been a focus of study.

Computational approaches are becoming increasingly more important both for the analysis of the data and to model the complex regulatory networks that result in pattern formation, that sharpen patterns and that translate patterns into a differential cellular response. Recent technological advances now provide quantitative and dynamic spatiotemporal data that allow the thorough testing of old concepts, including the relevance of Turing patterns in biological pattern formation (Turing, 1952). Turing patterns are a very important concept in biology because the Turing mechanism can generate a wide range of patterns from homogenous initial conditions and can thus, in principle, explain how patterns first emerge. However, the mechanism requires very specific interactions between at least two components (e.g. two proteins that act as an activator and inhibitor of each other in a particular way) and these components have to diffuse at substantially different speeds. One hallmark of Turing patterns is their dependency on domain size, as more patterns emerge as the domain grows. Although there are many examples in biology that look like Turing-based patterning, molecular proof is still outstanding and is a focus of intense study in the field.

The recent EMBO Workshop, 'Morphogen gradients', which was organized by James Briscoe (National Institute of Medical Research, London, UK) and Alex Schier (Harvard University, Boston, MA, USA) provided an excellent forum for discussion of recent developments of morphogen systems.

Morphogen gradient formation

Much experimental evidence suggests that morphogen gradients form as a result of diffusion from a source and removal in the target tissue. The relative contributions of fast, unhindered diffusion and of slow, hindered diffusion to the formation of diffusion-based gradients are, however, a matter of controversy. Diffusion constants can be measured using a range of techniques, including fluorescence correlation spectroscopy (FCS), fluorescence recovery after photobleaching (FRAP) and photo-activation. Petra Schwille (Max-Planck-Institute of Biochemistry, Martinsried, Germany) briefly commented on the opportunities to also measure concentrations accurately using FCS, and binding kinetics using fluorescence cross-correlation spectroscopy (FCCS). Using FRAP experiments, Marcos Gonzalez-Gaitan (University of Geneva, Switzerland) proposed a Dpp-GFP diffusion coefficient (D) of 0.1 $\mu m^2/s$ in the *Drosophila* wing disc (Kicheva et al., 2007; Kicheva et al., 2012). Arthur Lander (University of California, Irvine, CA, USA) discussed photoactivation data that, by contrast, support the

[1]Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA. [2]Swiss Institute of Bioinformatics (SIB) and Department of Biosystems Science and Engineering, ETH Zürich, 4058 Basel, Switzerland.

*Authors for correspondence (angelike@caltech.edu; dagmar.iber.bsse.ethz.ch)

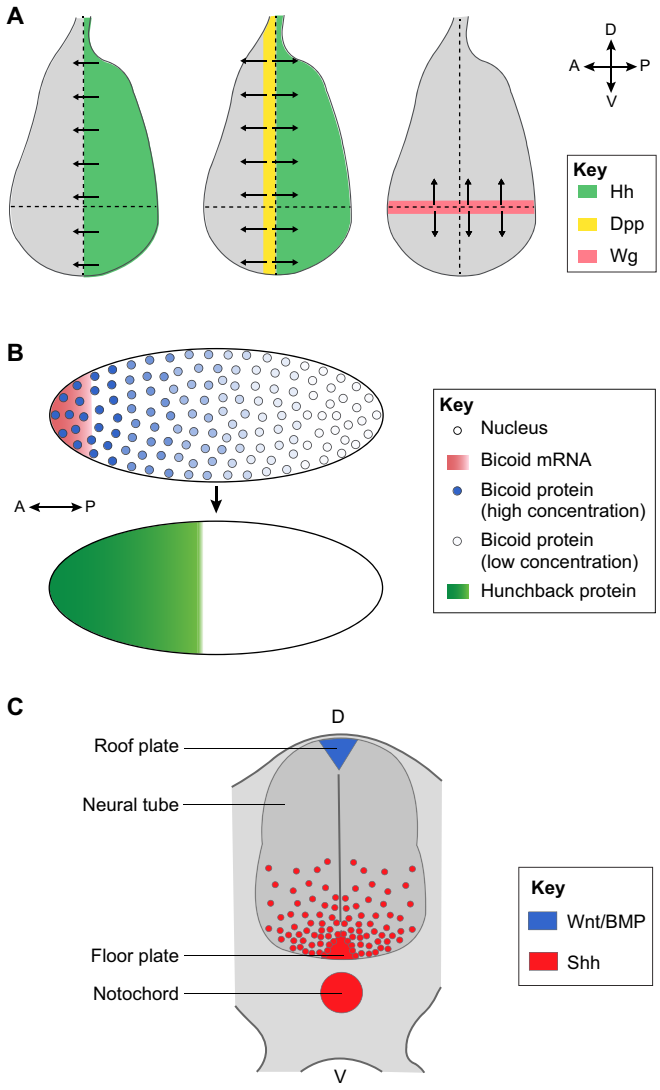

Fig. 1. Examples of morphogens in development. (**A**) Morphogens in the *Drosophila* wing disc. The morphogen Hedgehog (Hh) is expressed in the posterior compartment of the wing disc. At the boundary with anterior cells, Hh activates the expression of Decapentaplegic (Dpp), which disperses into both compartments. Wingless (Wg) is expressed at the dorsal-ventral boundary. Adapted, with permission, from Wolpert (Wolpert, 2003). (**B**) The Bicoid gradient in the syncytial *Drosophila* embryo. The transcription factor Bicoid, which accumulates within nuclei, is present in a gradient that decreases in concentration with distance from the anterior pole. mRNA encoding Bicoid is initially tightly localized to the anterior pole. The extent to which the mRNA encoding Bicoid, which is initially tightly localized to the anterior pole, becomes delocalized is a matter of controversy. Expression of the Bicoid target gene *hunchback* is also shown. Adapted, with permission, from Grimm et al. (Grimm et al., 2010). (**C**) Morphogens in the neural tube. A transverse section of an amniote embryo is shown, highlighting the morphogens Shh (red), which is secreted by the notochord and floor plate, and BMP and Wnt (blue), which are produced dorsally. Adapted, with permission, from Dessaud et al. (Dessaud et al., 2008).

idea that most detectable Dpp in the *Drosophila* wing disc is trapped within cells where it turns over slowly; FCS data support the presence of a small, but significant, pool of rapidly moving Dpp (D=10-21 $\mu m^2/s$) in the intercellular spaces (Zhou et al., 2012; Lander, 2013). Clearly, new assays will need to be developed to clarify the values of the key parameters of Dpp spreading, diffusion, capture and clearance.

Patrick Mueller (Max Planck Institute for Developmental Biology, Tübingen, Germany) presented evidence that the morphogens FGF8 and Nodal move through zebrafish embryos by hindered diffusion (Müller et al., 2012; Müller et al., 2013). Using FCS, FGF- and Nodal-GFP were shown to diffuse rapidly (D≈40-50 $\mu m^2/s$ for FGF8) in small pockets of extracellular space. However, FRAP demonstrated that both Nodal- and FGF8-GFP move slowly (D≈2 $\mu m^2/s$ for FGF8) through the entire tissue, suggesting that movement around cells and reversible binding to extracellular molecules retards their overall movement. Consistent with this idea, when interactions between FGF8-GFP and heparan sulfate proteoglycans (HSPGs, extracellular molecules known to bind to FGFs) were experimentally disrupted, the global diffusivity of FGF8-GFP measured by FRAP increased (to D≈12 $\mu m^2/s$). Given the importance of the extracellular matrix (ECM), questions were raised regarding the extent to which the diffusion kinetics would vary over time. Also looking at the role of heparin sulfate (HS) chains, Laurence Duchesne (University of Rennes, France) used gold nanoparticles to label FGF2, thereby allowing her to study its distribution and dynamics at the cell surface by electron microscopy and photothermal heterodyne microscopy, respectively. She reported that, in Rama 27 fibroblastic cells, more than 99% of the FGF2 is bound onto HS chains, and there is no readily free diffusion of FGF2. According to her results, FGF2 molecules cluster on the cell surface and can move along the HS chain, passing from one chain to another by translocating from one HS-binding site to another (Duchesne et al., 2012). Several motions were identified for a single FGF2 molecule going from high confinement to directed motion, with the latter motion potentially involving the intracellular transport machinery.

Another morphogen, Hedgehog (Hh), is a dually lipid-modified protein that associates with the membrane. How can it be released and how does it spread? Pascal Therond (University of Nice, France) described the role of ESCRT (endosomal sorting complexes required for transport) proteins during the secretion of Hh in the *Drosophila* wing disc. He showed data supporting the view that long-range Hh activity is associated with the apical side of the disc, whereas the basolateral pool of Hh has a more limited range. Ana Citlali Gradilla from Isabel Guerrero's lab (Centro de Biología Molecular Severo Ochoa, Madrid, Spain) presented results that showed a spatial and temporal correlation between cytoneme length and Hh signaling activity, and proposed that a network of cytonemes exists in the *Drosophila* wing disc in which, for example, the apical pool of Hedgehog can be reinternalized to the basal side of the disc. RNAi screens identified Flotillin and actin dynamics proteins as targets that disrupt cytonemes without affecting Hh production, and ESCRT proteins as targets that affect the number of exovesicles. Furthermore, she reported a Hh co-receptor, Ihog, localizes in exovesicles and cytonemes. Both these cell structures, exovesicles and cytonemes, can affect Hh signaling; presumably gradient length scale shortens as a result of effects on cytoneme length or exosome number. However, Patrick Mueller noted that, although cytoplasmic bridges of unknown function have been observed between cells in zebrafish embryos, filopodia acting as cytonemes have so far not been described in zebrafish embryos.

Finally, Martin Howard (John Innes Centre, Norwich, UK) discussed how intracellular concentration gradients are constructed within a single cell, namely in fission yeast by the kinase pom1p. The cortical pom1p gradient involved highly dynamic formation of pom1p clusters that grew and then disintegrated over timescales of

seconds. Although such dynamic gradients formed by biochemical reactions and diffusion are subject to noise, time averaging can correct for this (Howard, 2012; Saunders et al., 2012).

Morphogen gradient interpretation

A series of talks focused on how morphogen gradients are interpreted at the cis-regulatory level. Multiple inputs into a gene are responsible for its regulation, not only at distal regulatory elements but also at promoters. Mike Levine (University of California, Berkeley, USA) discussed the role of the minimal promoter in supporting timing of gene expression along the dorsal-ventral axis of *Drosophila* early embryos. He described recent studies showing that promoter sequence can function in an autonomous manner to regulate differential pausing of RNA polymerase and, in turn, that levels of pausing relate to the timing of gene expression. Specifically, he discussed how a spectrum of paused RNA polymerase determines the time it takes to support coordinated gene expression across cells of a tissue (Lagha et al., 2013). Manu from Martin Kreitman's lab (University of Chicago, IL, USA) described a modeling-based approach for reverse engineering the *cis* regulation of genes from high-throughput data, developed in collaboration with Eric Bertolino (University of Chicago, IL, USA). This approach overcomes a major obstacle to inferring gene regulation – the concerted regulation of *cis*-regulatory modules by many transcription factors – by using a transcriptional model that is capable of incorporating multiple inputs. Their study uncovered the complex regulation of the hematopoietic gene *Cebpa* (CCAAT/enhancer-binding protein α) by both myeloid and non-myeloid factors.

Nathalie Dostatni (Institut Curie, Paris, France) discussed the relationship between the Bicoid transcription factor and the robustness of axial patterning. Bicoid acts in the very early *Drosophila* embryo to regulate expression of the *hunchback* (*hb*) gene. Beautiful live *in vivo* movies of expression driven by one *hb*-associated enhancer were obtained using the MS2-MCP system, which enables mRNA expressed in transgenic animals (i.e. a reporter) to be labeled *in vivo* with fluorescent proteins (Weil et al., 2010). Expression was supported predominantly in anterior regions of the embryo, and the pattern became more precise (less noisy) with time. However, noise in the system can sometimes be beneficial and in some cases used to generate patterns. For example, Angela Stathopoulos (California Institute of Technology, Pasadena, USA) discussed how low levels of the Dorsal transcription factor present in lateral and dorsal regions supports stochastic expression, which can actively contribute to pattern formation when integrated in time (Reeves et al., 2012).

Transcriptional repressors also play an important role in specifying outputs of morphogen gradients. For example, positioning of the dorsal boundary for the gene *intermediate neuroblasts defective* (*ind*) along the *Drosophila* embryo dorsal-ventral axis involves dorsally acting repression. Angela Stathopoulos discussed how the dorsal boundary is regulated by a two-tier repression system involving transcriptional repressors acting downstream of both EGFR and TGFβ signaling (Garcia and Stathopoulos, 2011) as well as evidence for input by other repressors. Stas Svartsman (Princeton University, NJ, USA) provided insight into the molecular mechanisms supporting this *ind* gene repression, obtained from studying diphosphorylated ERK (extracellular-regulated kinase) (dpERK) gradient dynamics in the early *Drosophila* embryo. He showed evidence that ERK-dependent relief of gene repression through the Capicua repressor works as a two-step process, in which fast reduction of repressor activity is followed by slower changes in nuclear localization and overall protein levels (Lim et al., 2013).

In the vertebrate neural tube, sonic hedgehog (SHH) acts as a morphogen to control the pattern of different neuronal cell types. Karen Page (University College London, UK) presented an ordinary differential equation-based model for the SHH-dependent transcription network in the neural tube, as well as a bifurcation analysis of the network, which delineated the conditions for which the network could provide spatial patterns, based on hysteretic switches, or oscillations (Balaskas et al., 2012). This model provides an explanation for how spatially and temporally changing levels of SHH signaling can be interpreted by cross-regulatory interactions between transcription factors, activators and repressors; future work will focus on building spatial models on growing domains and the role of stochastic effects. Fengzhu Xiong from Sean Megason's lab (Harvard Medical School, Boston, USA) explored the impact of cell migration on gradient interpretation. Morphogen gradients are noisy, and cell movement relative to the morphogen source will further randomize the exposure of a cell to morphogen over time. The position-dependent cellular response to SHH in the zebrafish neural tube was indeed found to be initially heterogenous, but cells subsequently rearranged according to their gene expression state, resulting in sharply defined domains (Xiong et al., 2013). Finally, Ruth Diez del Corral (Cajal Institute, Madrid, Spain) described studies that demonstrate a role for FGF signaling in shaping the initiation of SHH-dependent patterning of the neural tube. Expression of a dominant-negative FGF receptor leads to dorsal expansion of the domain of *Olig2* gene expression, but did not correlate with an obvious expansion of the SHH gradient. Instead, it seems that FGF upregulates the *Ptch2* gene, which encodes a negative regulator of the SHH signaling pathway. This result highlights the important role of interactions between signaling pathways in helping to define gene expression domains.

Alexander Medvinsky (University of Edinburgh, UK) used an *ex vivo* re-aggregation assay to model tissue interactions required for the specification of hematopoietic stem cells (HSCs), which derive from the ventral region of the dorsal aorta in early embryos. He described studies showing that the ventral domain can induce formation of definitive HSCs from the dorsal domain of the dorsal aorta and, vice versa, the dorsal domain can enhance formation of HSCs from the ventral domain of the dorsal aorta. *In vivo*, SHH emanates from the notochord overlaying the dorsal aorta, whereas BMP4 emanates ventral to the aorta. The aorta, therefore, presents as an 'upside down' neural tube with the same signals specifying positional specific cell responses. He proposed that BMP and SHH signaling, as well as other genes differentially expressed in the dorsal and ventral domains, can underpin specification of definitive HSCs in the embryonic dorsal aorta.

Signaling networks and feedback in morphogenesis

A number of talks emphasized the importance of a network view in understanding the cellular response to morphogens. Fernando Casares (Centro Andaluz de Biologia del Desarrollo, Seville, Spain) presented work on how the Hh gradient is interpreted intracellularly by a gene regulatory network to control the specification, patterning and size of the *Drosophila* ocelli (the 'other' eye type in insects, aside to the compound eyes) and associated mechanosensory bristles. In the ocellar complex, Wg/Wnt and Hh morphogen gradients establish a double-negative feedback loop to precisely regulate ocellar size. Analysis of a mathematical model of this Hh-driven gene network suggests that

the gene network architecture constrains the morphological evolvability of the ocelli. Preliminary analysis of ocellar complexes in a sample of dipterans agrees with the model predictions.

Konrad Basler (University of Zurich, Switzerland) described studies in which a combination of *Drosophila* genetics in the wing disc and computational modeling was used to investigate how the ligand-dependent regulation of receptor expression shapes the Wingless (Wg) gradient. The combination of modeling and experiments showed that the emerging patterns can be explained with Wg signaling transcriptionally repressing its receptors Arrow (Arr) and Frizzled 2 (Fz2), and inducing the expression of its receptor Frizzled 3 (Fz3).

Feedback between morphogen-dependent signaling and cell processes can also ensure proper coupling during morphogenetic change. Shinya Matsuda from Osamu Shimmi's lab (University of Helsinki, Finland) presented evidence that BMP transport and morphogenesis of the *Drosophila* posterior crossvein (PCV) are tightly coupled. RhoGAP Crossveinless-C (Cv-C) is induced in PCV primordial cells by BMP signaling and mediates PCV morphogenesis cell-autonomously by inactivating members of the Rho-type small GTPase family. Cv-C is also required for BMP transport, suggesting that a feed-forward mechanism coordinates the spatial distribution of extracellular instructive cues and morphogenesis (Matsuda et al., 2013).

Emergence of patterns

Gradients often result from a pre-pattern. Patterns can, however, also emerge or refine spontaneously during development. A number of examples were presented at the meeting. Shuttling mechanisms, in particular, can be employed to refine signaling patterns, such that sharp gradients can arise within the source of a broadly expressed morphogen. Benny Shilo (Weizmann Institute of Science, Israel) presented a shuttling mechanism for the extracellular protein Spätzle (Spz), a ligand for the Toll receptor, which acts to shape dorsal-ventral patterning in the *Drosophila* embryo. Distinct roles of N- and C-terminal fragments of Spz as inhibitor and activator, respectively, coupled with their capacity to associate with each other in multiple forms, leads to dynamic relocalization of these protein segments to support formation and sharpening of the Toll activation gradient (Haskel-Ittah et al., 2012).

In plants, auxin gradients are important during morphogenesis and result from the polar positioning of auxin pumps (Grieneisen et al., 2007). Veronica Grieneisen (John Innes Centre, Norwich, UK) presented a model according to which the polarity arises through a mechanism of wave-pinning in an intracellular signaling network. This network can be further coupled to other cells via indirect signaling (auxin) achieving cell-cell communication and tissue polarity (Abley et al., 2013).

Turing mechanisms have been suggested for many patterning events based on similarity of patterns in simulations and nature. James Sharpe (Centre for Genomic Regulation, Barcelona, Spain) showed that the wavelength of the digit patterns changes in several Hox mutants as required by a Turing mechanism for digit definition (Sheth et al., 2012). Indeed, the wavelength appears to be smoothly tuned by the dose of distal Hox genes. He further presented computational modeling studies on accurate 2D limb bud shapes that explored the collaboration between local self-organization and global positional information (from gradients such as Fgf signaling) in digit patterning. Future work will need to focus on the identification of the Turing components.

Using 3D simulations on embryonic geometries, Dagmar Iber [Swiss Federal Institute of Technology (ETH), Zurich, Switzerland] showed that a receptor-ligand-based Turing mechanism correctly predicts the embryonic growth fields during lung branching morphogenesis; the branch points and branch modes that are observed during wild-type and mutant lung and kidney branching morphogenesis can also be explained with such a receptor-ligand-based Turing mechanism (Menshykau et al., 2012; Menshykau and Iber, 2013). Receptor-ligand based Turing mechanisms can be implemented with any receptor-ligand pair as long as: (1) ligands diffuse faster than receptors; (2) receptors and ligands interact cooperatively; and (3) receptor-ligand binding upregulates the receptor concentration on the membrane. These conditions are met by many receptor-ligand networks, and although the signaling networks that control branching morphogenesis in lung and kidney are different, both can give rise to a receptor-ligand based Turing mechanism.

Direct experimental evidence for Turing mechanism based on the measurement of kinetic parameters is still missing. Petra Schwille presented reconstitution experiments with bacterial Min proteins on model membranes. This self-organizing system yielded travelling waves on large domains (Loose et al., 2008) and produced oscillations in closed compartments (Zieske and Schwille, 2013). The protein concentrations and kinetics were all measured (Loose et al., 2008; Loose et al., 2011).

Scaling of patterns

Often the same patterning mechanism is employed in embryos of very different sizes, but it is an open issue how the relative patterns are preserved on domains of different lengths. Marcos Gonzalez-Gaitan had previously shown that the Dpp gradient scales with the size of the growing wing disc (Wartlick et al., 2011). Gonzalez-Gaitan now further showed that, in zebrafish, the Bmp gradient (Bre-GFP) scales with the size of the pectoral fin bud, and that, in *Drosophila*, the Dpp gradient scales with the size of the anterior compartment in the eye disc. The length of the anterior side first expands and subsequently shrinks during eye development, and according to Gonzalez-Gaitan the gradient scales at all times. Inna Averbukh from Naama Barkai's group (Weizmann Institute of Science, Rehovot, Israel) presented a model in which local coupling of cell division to morphogen signaling resulted in spatially uniform growth and the scaling of tissue pattern with the growing tissue size.

Scaling of the Dpp gradient in wing discs has previously been suggested to be mediated, at least in part, by Pentagone (Pent), which is repressed by Dpp and expands the Dpp gradient (Ben-Zvi et al., 2011; Hamaratoglu et al., 2011). Arthur Lander presented evidence that Pent does not diffuse far from its site of production (distant from the Dpp source), a result that argues against one model for scaling, the expander-repressor integral feedback model (Ben-Zvi and Barkai, 2010). He pointed out, however, that because the Dpp receptor *tkv* is also preferentially expressed far from the Dpp source (Dpp signaling represses its expression), Pent might not need to diffuse far to control the shape of the entire Dpp gradient. For this to work as a mechanism for scaling, there would need to be strong uptake of Dpp to buffer its concentration near its source of production, and he presented evidence that this occurs. He noted that this mechanism of scaling is similar to the one originally proposed by Lewis Wolpert for simple source-sink gradients (Wolpert, 1969).

Scaling is observed also during somitogenesis, such that differently sized embryos develop the same number of somites. Somitogenesis is controlled by oscillations in Notch, WNT and FGF signaling activity. Alexander Aulehla (EMBL Heidelberg,

Germany) used *in vitro* cultures of mouse presomitic mesoderm (PSM) cells to show that the maximal phase difference within the PSM (i.e. the phase gradient amplitude) remains constant on differently sized domains, thus resulting in scaling of the phase gradient (dφ/dx) with the size of the domain; the oscillation frequency (dφ/dt) remains constant, and the wave velocity, v=(dφ/dt)/(dφ/dx), is therefore also scaled (Lauschke et al., 2013). The mechanistic basis of the constant phase gradient amplitude is unknown.

The mechanistic basis of scaling may be more complex than appreciated by current models. Thus, Aysu Uygur from Clifford Tabin's lab (Harvard Medical School, Boston, USA) presented data from the neural tubes of finch, chick and emu, which show that, contrary to current models, protein concentrations and thresholds do not necessarily remain constant during the evolution of scaled structures. Thus, different levels of *Shh* are expressed in the differently sized neural tubes of finch, chick and emu, and different SHH concentration thresholds evolved in the different species. Furthermore, Angela Stathopoulos presented data from studies of scaling along the dorsal-ventral axis of *Drosophila melanogaster* embryos, suggesting that although the dorsal gradient scales, not all of its target genes do. Therefore, scaling behaviors of genes expressed along the dorsal-ventral axis appear to be gene specific and varied (Garcia et al., 2013).

The control of organ growth

During organ development, growth rates slow down, thereby limiting the size of the organ. Gonzalez-Gaitan showed that this decrease in growth rate is the result of a longer G2 phase. According to Gonzalez-Gaitan, growth rates in the wing and eye discs are controlled by Dpp. Thus, Gonzalez-Gaitan proposes that cells divide as soon as the Dpp concentration has increased by 40% and Dpp-dependent signaling by 50% since the last cell division event (Wartlick et al., 2011). Hence, the higher the Dpp concentration, the longer it will take to achieve the relative increase, thus resulting in a declining growth rate. Experiments by the Basler lab now showed that clones in the wing disc that cannot respond to Dpp ($mad^{-/-}$, $brk^{-/-}$) grow at the same speed as wild-type cells (Schwank et al., 2012); according to Gonzalez-Gaitan, the same correlation between increasing Dpp-dependent signaling levels [as judged by measuring the Dpp downstream signaling protein Daughters against Dpp (Dad)] and growth is still observed in these Dpp-unresponsive clones (Wartlick et al., 2012). Dagmar Iber presented evidence for an alternative mechanism based on dilution of a cytokine to explain the declining growth rate in the eye disc and thus growth control.

New techniques for studying morphogens

Sydney Brenner once famously remarked, 'Progress in science depends on new techniques, new discoveries and new ideas, probably in that order'. New techniques are continuously emerging and may help to address the controversial points. Benoit Sorre, working jointly in Eric Siggia and Ali Brivanlou's groups (Rockefeller University, New York, USA), has employed microfluidics to analyze how the temporal profile of TGFβ signals influences cellular responses. He showed that the cell response, monitored by nuclear Smad4 concentration levels, was adaptive: after each stepped increase in TGFβ concentration, the cell response went back to a baseline level despite continuous stimulation (Warmflash et al., 2012). On the contrary, when stimulated with pulsed TGFβ signals, cells responded fully to each pulse and the transcriptional response kept increasing. He argued that pulsed signals provide a mean to bypass the inhibitory intracellular circuits that otherwise limit cell response. Gaining further insights into the temporal dynamics of signaling pathway activation and target gene activation is an important future direction. The ability to assay morphogen gradient outputs *in vivo* live was achieved in work described by Nathalie Dostatni using the MS2-MCP system to visualize gene expression in real time within the *Drosophila* embryo. The MS2-MCP system shows great promise to provide more definitive temporal information about morphogen gradient outputs.

Markus Affolter (University of Basel, Switzerland) presented recent advances on using nanobodies to study issues regarding morphogen distribution. Nanobodies are short, 117 amino acid sequences derived from single chain antibodies made in camels, and recognize proteins much like the more complicated heavy and light chain containing antibodies do (Muyldermans, 2013). Specific nanobodies recognizing *Drosophila* proteins have not been isolated yet, but a GFP-specific nanobody can be fused to different membrane tethering sequences and used to capture GFP-tagged proteins in *Drosophila* (see Caussinus et al., 2011). This novel tool can now be used in *dpp*-null flies to manipulate the Dpp-GFP gradient length by expressing different levels of the nanobody fusion protein. This should enable a detailed analysis of the impact of Dpp levels and gradient lengths on scaling and growth control.

Finally, Radek Erban (University of Oxford, UK) presented computational methods for molecular-based (stochastic, Brownian dynamics) modeling of morphogen gradients, with a special focus on multiscale methods, which use models with a different level of detail in different parts of the computational domain.

Conclusions

The meeting showed the immense progress that has been made in the 60 years since morphogens were first proposed (Turing, 1952). Advances in experimental techniques and computing now provide us with a detailed, and often dynamic, view on morphogen transport and response mechanisms in tissues. Almost every talk involved analysis of quantitative data, and was critical of itself and of the field. In addition, James Briscoe and Alex Schier led discussions daily that were also very helpful in defining where researchers agree, as much as in highlighting the important unanswered questions and controversies. For example, it seems clear now that morphogens can be transported in several ways, but it remains unclear to what extent the different routes contribute to patterning. A number of studies highlighted the dynamic nature of morphogen read-out and the importance of feedbacks and a network context. Important open problems concern the mechanistic basis of scaling and growth control – as well as the role of Turing patterns. The field is growing and changing, as evidenced by the many cases of differing ideas in almost every area discussed.

In particular, there was a sense that the true basis of morphogen gradients is being critically evaluated and, in some cases, tested. There was also a feeling that there is a way to go to address some of the important issues involving decision making during development, whether through morphogens or through other mechanisms. However, the current trend to look critically at the problems using quantitative or mathematical modeling tools, for example, seems like a good forward driving force. In summary, this meeting provided an exciting range of new data and ideas and promises interesting times ahead in the field of morphogens to define the fundamental (or alternative) mechanisms that enabled the evolution of complex life.

Acknowledgements
We thank the organizers and speakers for contributing to this Meeting Review, and apologize that the need for brevity did not always allow us to capture the full scope of all their interesting studies.

Funding
The authors' research is supported by the National Institutes of Health, and by SNF Sinergia and SNF SystemsX RTD. Deposited in PMC for release after 12 months.

Competing interests statement
The authors declare no competing financial interests.

References

Abley, K., De Reuille, P. B., Strutt, D., Bangham, A., Prusinkiewicz, P., Marée, A. F. M., Grieneisen, V. A. and Coen, E. (2013). An intracellular partitioning-based framework for tissue cell polarity in plants and animals. *Development* **140**, 2061-2074.
Ashe, H. L. and Briscoe, J. (2006). The interpretation of morphogen gradients. *Development* **133**, 385-394.
Balaskas, N., Ribeiro, A., Panovska, J., Dessaud, E., Sasai, N., Page, K. M., Briscoe, J. and Ribes, V. (2012). Gene regulatory logic for reading the Sonic Hedgehog signaling gradient in the vertebrate neural tube. *Cell* **148**, 273-284.
Ben-Zvi, D. and Barkai, N. (2010). Scaling of morphogen gradients by an expansion-repression integral feedback control. *Proc. Natl. Acad. Sci. USA* **107**, 6924-6929.
Ben-Zvi, D., Pyrowolakis, G., Barkai, N. and Shilo, B.-Z. (2011). Expansion-repression mechanism for scaling the Dpp activation gradient in Drosophila wing imaginal discs. *Curr. Biol.* **21**, 1391-1396.
Caussinus, E., Kanca, O. and Affolter, M. (2011). Fluorescent fusion protein knockout mediated by anti-GFP nanobody. *Nat. Struct. Mol. Biol.* **19**, 117-121.
Dessaud, E., McMahon, A. P. and Briscoe, J. (2008). Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. *Development* **135**, 2489-2503.
Duchesne, L., Octeau, V., Bearon, R. N., Beckett, A., Prior, I. A., Lounis, B. and Fernig, D. G. (2012). Transport of fibroblast growth factor 2 in the pericellular matrix is controlled by the spatial distribution of its binding sites in heparan sulfate. *PLoS Biol.* **10**, e1001361.
Garcia, M., Nahmad, M., Reeves, G. T. and Stathopoulos, A. (2013). Size-dependent regulation of dorsal-ventral patterning in the early Drosophila embryo. *Dev. Biol.* **381**, 286-299.
Garcia, M. and Stathopoulos, A. (2011). Lateral gene expression in Drosophila early embryos is supported by Grainyhead-mediated activation and tiers of dorsally-localized repression. *PLoS ONE* **6**, e29172.
Grieneisen, V. A., Xu, J., Marée, A. F. M., Hogeweg, P. and Scheres, B. (2007). Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* **449**, 1008-1013.
Grimm, O., Coppey, M. and Wieschaus, E. (2010). Modelling the Bicoid gradient. *Development* **137**, 2253-2264.
Hamaratoglu, F., de Lachapelle, A. M., Pyrowolakis, G., Bergmann, S. and Affolter, M. (2011). Dpp signaling activity requires Pentagone to scale with tissue size in the growing Drosophila wing imaginal disc. *PLoS Biol.* **9**, e1001182.
Haskel-Ittah, M., Ben-Zvi, D., Branski-Arieli, M., Schejter, E. D., Shilo, B.-Z. and Barkai, N. (2012). Self-organized shuttling: generating sharp dorsoventral polarity in the early Drosophila embryo. *Cell* **150**, 1016-1028.
Howard, M. (2012). How to build a robust intracellular concentration gradient. *Trends Cell Biol.* **22**, 311-317.
Kicheva, A., Bollenbach, T., Wartlick, O., Jülicher, F. and Gonzalez-Gaitan, M. (2012). Investigating the principles of morphogen gradient formation: from tissues to cells. *Curr. Opin. Genet. Dev.* **22**, 527-532.
Kicheva, A., Pantazis, P., Bollenbach, T., Kalaidzidis, Y., Bittig, T., Jülicher, F. and González-Gaitán, M. (2007). Kinetics of morphogen gradient formation. *Science* **315**, 521-525.
Lagha, M., Bothma, J. P., Esposito, E., Ng, S., Stefanik, L., Tsui, C., Johnston, J., Chen, K., Gilmour, D. S., Zeitlinger, J. et al. (2013). Paused Pol II coordinates tissue morphogenesis in the Drosophila embryo. *Cell* **153**, 976-987.
Lander, A. D. (2013). How cells know where they are. *Science* **339**, 923-927.
Lauschke, V. M., Tsiairis, C. D., François, P. and Aulehla, A. (2013). Scaling of embryonic patterning based on phase-gradient encoding. *Nature* **493**, 101-105.
Lim, B., Samper, N., Lu, H., Rushlow, C., Jiménez, G. and Shvartsman, S. Y. (2013). Kinetics of gene derepression by ERK signaling. *Proc. Natl. Acad. Sci. USA* **110**, 10330-10335.
Loose, M., Fischer-Friedrich, E., Herold, C., Kruse, K. and Schwille, P. (2011). Min protein patterns emerge from rapid rebinding and membrane interaction of MinE. *Nat. Struct. Mol. Biol.* **18**, 577-583.
Loose, M., Fischer-Friedrich, E., Ries, J., Kruse, K. and Schwille, P. (2008). Spatial regulators for bacterial cell division self-organize into surface waves in vitro. *Science* **320**, 789-792.
Matsuda, S., Blanco, J. and Shimmi, O. (2013). A feed-forward loop coupling extracellular BMP transport and morphogenesis in Drosophila wing. *PLoS Genet.* **9**, e1003403.
Menshykau, D. and Iber, D. (2013). Kidney branching morphogenesis under the control of a ligand-receptor-based Turing mechanism. *Phys. Biol.* **10**, 046003.
Menshykau, D., Kraemer, C. and Iber, D. (2012). Branch mode selection during early lung development. *PLOS Comput. Biol.* **8**, e1002377.
Müller, P., Rogers, K. W., Jordan, B. M., Lee, J. S., Robson, D., Ramanathan, S. and Schier, A. F. (2012). Differential diffusivity of Nodal and Lefty underlies a reaction-diffusion patterning system. *Science* **336**, 721-724.
Müller, P., Rogers, K. W., Yu, S. R., Brand, M. and Schier, A. F. (2013). Morphogen transport. *Development* **140**, 1621-1638.
Muyldermans, S. (2013). Nanobodies: natural single-domain antibodies. *Annu. Rev. Biochem.* **82**, 775-797.
Reeves, G. T., Trisnadi, N., Truong, T. V., Nahmad, M., Katz, S. and Stathopoulos, A. (2012). Dorsal-ventral gene expression in the Drosophila embryo reflects the dynamics and precision of the dorsal nuclear gradient. *Dev. Cell* **22**, 544-557.
Rogers, K. W. and Schier, A. F. (2011). Morphogen gradients: from generation to interpretation. *Annu. Rev. Cell Dev. Biol.* **27**, 377-407.
Saunders, T. E., Pan, K. Z., Angel, A., Guan, Y., Shah, J. V., Howard, M. and Chang, F. (2012). Noise reduction in the intracellular pom1p gradient by a dynamic clustering mechanism. *Dev. Cell* **22**, 558-572.
Schwank, G., Yang, S.-F., Restrepo, S. and Basler, K. (2012). Comment on "Dynamics of dpp signaling and proliferation control". *Science* **335**, 401, author reply 401.
Sheth, R., Marcon, L., Bastida, M. F., Junco, M., Quintana, L., Dahn, R., Kmita, M., Sharpe, J. and Ros, M. A. (2012). Hox genes regulate digit patterning by controlling the wavelength of a Turing-type mechanism. *Science* **338**, 1476-1480.
Turing, A. (1952). The chemical basis of morphogenesis. *Philos. Trans. R. Soc. B* **237**, 37-72.
Warmflash, A., Zhang, Q., Sorre, B., Vonica, A., Siggia, E. D. and Brivanlou, A. H. (2012). Dynamics of TGF-β signaling reveal adaptive and pulsatile behaviors reflected in the nuclear localization of transcription factor Smad4. *Proc. Natl. Acad. Sci. USA* **109**, E1947-E1956.
Wartlick, O., Mumcu, P., Julicher, F. and Gonzalez-Gaitan, M. (2012). Response to comment on "Dynamics of dpp signalling and proliferation control". *Science* **335**, 401.
Wartlick, O., Mumcu, P., Kicheva, A., Bittig, T., Seum, C., Jülicher, F. and González-Gaitán, M. (2011). Dynamics of Dpp signaling and proliferation control. *Science* **331**, 1154-1159.
Weil, T. T., Parton, R. M. and Davis, I. (2010). Making the message clear: visualizing mRNA localization. *Trends Cell Biol.* **20**, 380-390.
Wolpert, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* **25**, 1-47.
Wolpert, L. (2003). Cell boundaries: knowing who to mix with and what to shout or whisper. *Development* **130**, 4497-4500.
Xiong, F., Tentner, A. R., Huang, P., Gelas, A., Mosaliganti, K. R., Souhait, L., Rannou, N., Swinburne, I. A., Obholzer, N. D., Cowgill, P. D. et al. (2013). Specified neural progenitors sort to form sharp domains after noisy Shh signaling. *Cell* **153**, 550-561.
Zhou, S., Lo, W.-C., Suhalim, J. L., Digman, M. A., Gratton, E., Nie, Q. and Lander, A. D. (2012). Free extracellular diffusion creates the Dpp morphogen gradient of the Drosophila wing disc. *Curr. Biol.* **22**, 668-675.
Zieske, K. and Schwille, P. (2013). Reconstitution of pole-to-pole oscillations of min proteins in microengineered polydimethylsiloxane compartments. *Angew. Chem. Int. Ed. Engl.* **52**, 459-462.