EMBO reports

meeting point

The numbers behind morphogenesis

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The IHES meeting on Pattern Formation in Morphogenesis covered computational approaches to understanding central developmental processes. Global changes in tissue morphogenesis were attributed to small local asymmetries in protein localization or activity, and the underlying mechanisms for robust patterning of defined signalling pathways were explored.

Watching a field of wheat sway in the wind makes one wonder what intrinsic and extrinsic forces make the field move in unison, in a manner that includes all of the individual elements. It is infinitely more complex to consider the same issues for a developing embryo, as the cells are not all identical to one another and several sequential processes are involved. Although we have identified many of the molecules that orchestrate these amazing processes of morphogenesis, how they are coordinated and integrated, giving rise to an extremely reproducible end result, remains a crucial mystery. We are at a stage in which quantitative understanding of developing systems is required. As we now have the ability to track live embryonic processes even in large sheets of cells, and the capacity to follow the dynamic level and localization of proteins, the data for such analyses is beginning to emerge. The challenge is to apply quantitative approaches in a significant and comprehensive manner to not only characterize the processes but also provide new and testable biological insights.

The meeting on Pattern Formation in Morphogenesis—which took place at the IHES near Paris between 10 and 14 January 2010—attempted to confront these challenges. IHES is an institute for theoretical and applied mathematics that was established 50 years ago. In addition to its permanent members and guests, it hosts meetings that might stimulate new mathematical approaches to tackle the issues addressed. Scientists carrying out experimental work on embryonic development, many of which have already started to collaborate with computational scientists, presented problems that could be amenable to computational analysis. Several themes emerged from these discussions.

Coordinating signals

Olivier Pourquie (Strasbourg U.) described the process of segmentation, by which body segments are formed progressively. The orderly addition of segments involves coordination between three independent processes: oscillations of gene expression—driven by internal clocks—which determine the phase of segment formation; generation of a wavefront that determines the competence zone for generating a new segment at each cycle; and cell division coupled to posterior growth, to generate new precursors for subsequent segments. Microarray analysis of oscillating cells at defined stages has uncovered a broad set of cycling genes. Two opposing phases were identified: one involving Notch and fibroblast growth factor (FGF) pathway genes, and the other Wnt pathway genes. Two external sets of signals interdigitate with the internal clock. The wavefront of competence is defined by a FGF gradient that originates from the posterior region and an opposing retinoic acid (RA) gradient originating from the anterior region. FGF positively regulates RA synthesis, whereas RA represses FGF signalling; hence, a region of bi-stability is defined as the competence zone.

The power of live imaging of an entire cell population during development was evidenced in a talk by Yohanns Bellaiche (Institut Curie, Paris), which focused on the morphogenesis of the pupal notum in flies. Movies following the shape and behaviour of all cells during a 24 h timeframe show discrete behaviours of different cell populations. Computational segmentation using a variety of criteria—such as topology, number of neighbours, or elongated compared with isotropic structure—shows distinct cell populations. Some of these discrete domains correspond to zones of gene expression, raising the question of how gene expression modulates cellular morphology. Future analysis of mutant phenotypes in this global and quantitative system should yield a new level of resolution and understanding of complex and highly orchestrated morphogenetic processes.

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Plant morphogenesis uses a completely different strategy for coordination between intrinsic and extrinsic signals. Ben Scheres (Utrecht U.) presented the global and diverse effects of a single hormone, auxin, in dictating the patterning of both shoots and roots. Auxin is produced broadly, but its dynamic distribution determines various features such as root branching and the regular pattern of flower initiation. So how is the dynamic distribution of auxin regulated? Working backwards from auxin, a linear pathway has been unravelled: auxin transport from cell to cell by polarized PIN proteins gives rise to the local concentration of auxin at sites of cell growth. Auxin positively reinforces local asymmetries in PIN concentration or polarity. PIN transcription is regulated by the PLATHORA family of transcription factors, and its expression is also reinforced by auxin. The challenge

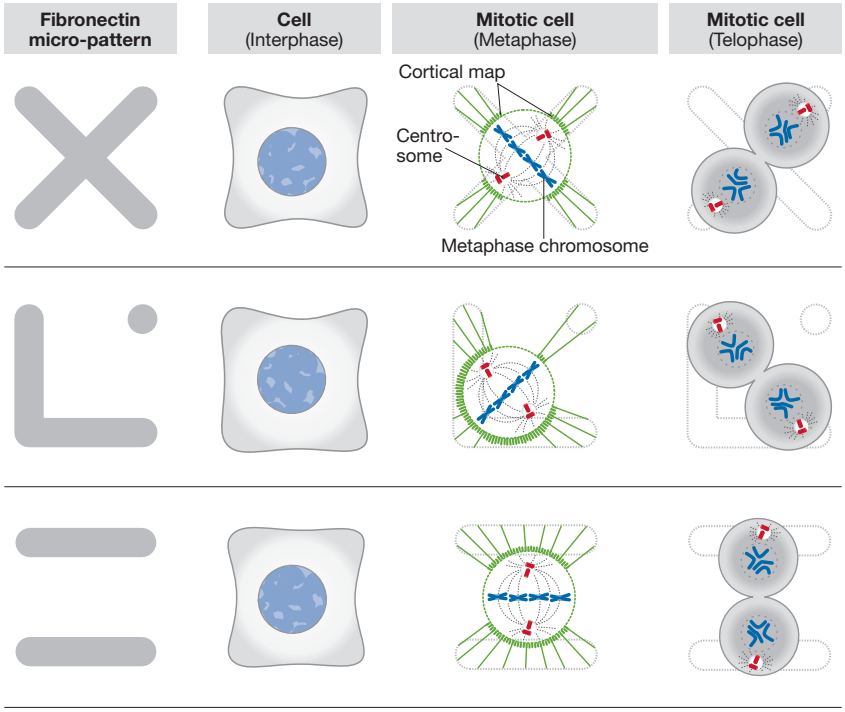

Fig 1 | Cell adhesion patterns guide cell division. Cells spread with a similar square shape on three different adhesive patterns, whereas the orientation of the division axis is different for each pattern. Actin-binding proteins are maintained on the cortex of the mitotic cell, where they are transported along actin-containing retraction fibres that form during mitotic rounding. A cortical map—like a topological memory of the premitotic adhesive pattern—is thereby formed and controls mitotic spindle orientation. Figure adapted from Théry *et al*, 2005, 2007.

in this system is to model the processes in the context of the whole plant and to understand how local asymmetries—such as root bending—give rise to an accumulation of auxin. As positive feedback loops are operating to enhance small local asymmetries, it is challenging to elucidate how a balanced situation is maintained without giving rise to 'runaway' overactivation.

Cell boundaries and contacts

The contacts between cells, and those between the cells and the matrix, are crucial to the behaviour of a cell layer within a tissue. Global tissue anisotropy signals should eventually be translated to asymmetries at the level of junctions between individual cells.

The interplay between extrinsic signals and intrinsic cell processes during germ band extension in the *Drosophila* embryo were discussed by Thomas Lecuit (IBDML, Marseilles). An unknown external signal gives rise to myosin II anisotropy, such that it is enriched at the cell junctions between anteroposterior neighbours. Modelling the system shows that low anisotropy values give rise to maximal rates of germ band extension. Thus, a relatively small bias at the cellular level is sufficient to execute global directional morphogenic movements. Attempts to quantitate the tension generated by myosin II anisotropy were made by analysing the correlation between cell deformation and myosin II distribution, and through the ablation of junctions in a highly directed manner to monitor the relaxation after ablation. A conclusion from these studies is that cortical apical myosin II is not the only force-generating element; a dynamic exchange occurs between pulsatile medial and cortical myosin II populations. Cross-correlation studies show that contraction of the medial myosin II network coincides with shrinkage of the junctions, and contraction of cortical myosin lags shortly behind and could stabilize junction length. The anisotropic flow of medial myosin II to the cortex is, in turn, influenced by the polarized distribution of E-cadherin, to which myosin II is anchored. The reorganization of junctions at the single-cell level gives rise to convergence–extension movements that drive germ band extension at the level of the whole embryo. In relation to cell junctions, it is interesting to consider studies carried out on soap bubbles, which impinge on the final structure of cell–cell contacts. In both cases, the forces drive minimization of the contact area. However, in live tissues, the process is not driven simply by physical forces but rather facilitated by biasing the distribution of myosin II and E-cadherin.

One of the most dramatic manifestations of small asymmetries at cell–cell contacts relates to planar cell polarity, as presented by Jeff Axelrod (Stanford U.). In response to a global cue, the asymmetries between adjacent cell surfaces are amplified to generate a stable pattern in which the cells in an epithelial monolayer are polarized orthogonally to their apical–basal axes, producing molecular asymmetries oriented uniformly in the same direction along the apical surface. One result is the uniformly distal orientation of hairs on the *Drosophila* wing. A set of equations can describe the proposed interactions between the different components involved in planar cell polarity, and parameter space can then be explored to identify those parameters that not only generate the normal pattern but also recapitulate the well-characterized mutant clonal phenotypes. The solutions obtained demonstrate the feasibility of a model that relies simply on communication between adjacent cells to transmit polarity through the tissue. The local accumulation of a 'distal' group of membrane proteins at one end of the cell triggers the accumulation of the 'proximal' proteins in the membrane of the adjacent cell, and vice versa, thus reinforcing the asymmetry in each cell. This could be mediated by preferential endocytosis at one end and movement of vesicles carrying the crucial proteins to the opposite side.

Understanding the effect that asymmetries in the extracellular matrix have on the generation of actin cables and, eventually, on the shape of the cell, was beautifully investigated in a system presented by Michel Bornens (Institut Curie, Paris). By printing an adhesive substrate in squares, triangles or different letter shapes, one can monitor the structure of the actin network that will form in the attached cells and their relation to the final shape of the cell (Fig 1).

Although similar square cells might be induced by matrixes in the shape of an X or an = sign, the subsequent cell division patterns vary. Mitotic cells round up at the centre of mass of the adhesive patterns. A map of these patterns on the cortex of mitotic cells would determine spindle orientation by preferentially activating motors that pull on astral microtubules. An interesting question is whether the cell adhesion pattern in a stem-cell niche could convert symmetrical to asymmetrical division patterns. A movie depicting the progeny of a single cell grown on an L-shaped adhesive substrate provided a real tale of suspense: the original cell is triangular and each of the two daughter cells resides on a different arm of the L, but they will eventually take a balanced position in the adhesive pattern, with one cell occupying the triangular base of the L and the other occupying the remaining territory.

Robustness of morphogen gradients

A central issue addressed at the meeting was the robustness of patterning. Most cell-fate decisions are a result of signalling between cells, which is mediated by a handful of conserved signalling pathways. Surprisingly, the patterns that are generated are highly reproducible, despite fluctuations in the levels of the different signalling components—including the morphogen itself—owing to biological noise, loss of one allele or alterations in temperature. As morphogen patterning systems are not simply on/off switches, but rather respond to the absolute level of the signal at each position, how this robustness is maintained remains a central open question.

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In the absence of quantitative information on the relevant parameters of the patterning system, Benny Shilo (Weizmann Institute of Science) presented an approach to study the system by using its robustness as the key insight into the quantitative mechanism. By examining a restricted system comprising the extracellular components of the bone morphogenetic protein (BMP) patterning pathway in the early *Drosophila* embryo, a wide range of parameters can be screened and one can identify those that will not only provide a pattern, but are also robust to fluctuations. Such an analysis identifies the values or ratios between values that must be fixed and gives an insight into the mechanism for robustness, which can then be experimentally tested. In the case of BMP signalling, an active ligand shuttling mechanism—which leads to concentration of the ligand at the dorsal midline—was suggested by the computational screen, and indeed demonstrated experimentally. The same pathway is utilized in *Xenopus* embryos, in which an additional ligand, termed ADMP, endows the system with the capacity to scale pattern with size. Studying the same system, Michael O'Connor (U. Minnesota, Twin Cities), described the role of CV2—an extracellular protein that binds to both BMP and its receptor—in refining patterning in the wing veins. CV2 is a transcriptional target of BMP signalling; thus, the precise level of CV2 fine-tunes patterning and integrates signals over time. An interesting question concerns the scaling of BMP-induced patterning in embryos from different fly species. There is up to a fivefold difference in embryo size between species, although the number of nuclei per embryo and their presumed biosynthetic capacities are fixed. Computational studies show that these features might account for scaled patterning. However, it is also possible that during evolution, mutations that alter the binding affinities or diffusion features of patterning elements contribute to scale the pattern in these species.

Evolutionary perspectives

On a broader evolutionary perspective, a central question is when the main patterning pathways emerged and how they facilitated the growing complexity of developing organisms. Cnidarians are the true metazoans that are closest to the base of the animal kingdom; they have only two body layers and one axis. The cells are in a continuous state of proliferation, differentiation and turnover, similar to the cells that line the gut of higher organisms. An experiment carried out by Ethel Browne in 1909 at Thomas Morgan's lab, preceded conceptually the Spemann *Xenopus* transplantation experiment by 15 years. Browne transplanted the 'organizer' containing the region of the single opening from a white hydra strain to a pigmented strain and observed the formation of an ectopic opening comprised mostly of the cells of the recipient. This experiment demonstrated that 'induction'—a crosstalk between cells that can lead to repatterning—is already operating in Cnidarians.

> **…the dialogue and tight collaboration between experimental and computational approaches […] will continue to take place at many levels**

Thomas Holstein (Heidelberg U.) presented the results of the recent genome sequencing of *Cnidaria*, demonstrating an amazing degree of representation of the five main signalling pathways, including for example 10 Wnt genes. Some of these Wnt genes were shown to be crucial for *Cnidaria* regeneration. Hans Meinhardt (MPI, Tubingen) suggested that the single hydra body axis was converted to more complex patterns by two evolutionary inventions, the trunk and the midline. The midline is generated by a moving spot—known as Hensen's node—which eventually specifies the dorso-ventral axis.

As became clear at the meeting, the dialogue and tight collaboration between experimental and computational approaches to elucidate the basis for morphogenesis will continue to take place at many levels. The analysis of morphogenetic processes with a critical and insightful quantitative outlook has been able to rule out and point to molecular mechanisms in several instances. The continuous development and improvement of microscopic techniques for live imaging, in parallel to computational approaches to segment the images, and the strengthening of a constant and ongoing dialogue between the experimental and computational disciplines, ensure many surprising discoveries ahead.

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