Two become one

Developmental biologists and cell biologists have long ploughed their separate furrows. But now these two disciplines are coming together in unexpected and exciting ways, says Helen Pearson.

Kathryn Anderson wasn't looking to embark on an affair with cell biology — it just happened. Anderson, a developmental biologist at the Sloan-Kettering Institute in New York, was studying 'open-brain' mice — which have a fatal genetic mutation that prevents their brain from folding correctly during embryonic development — when her research took a surprising turn.

She had expected the gene involved to be one of the usual suspects implicated in severe developmental abnormalities — those encoding proteins that regulate the expression of other genes, or that are involved in key cellular signalling pathways known to direct embryological development. Instead, she found that the gene produced a protein that carries materials around the cell[1]. Such 'housekeeping' proteins had long been ignored by developmental biologists — deemed of interest only to researchers fascinated with the detailed mechanics of a cell.

Anderson is just one of many developmental biologists who are now realizing that apparently mundane aspects of cells' internal workings can help to illuminate some of the mysteries of development. "For whatever reason, developmental geneticists have encountered basic cell biology in the past few years," she says. Cell biologists are likewise coming to recognize that their obsession with the mechanics of processes such as cell division, and the transport of proteins around the cell, can contribute to the study of development. Helping to broker this marriage between fields are techniques of video microscopy, which use fluorescent markers to film proteins moving around in cells and tissues (see 'Lights, camera, action!', overleaf).

For the past two decades, developmental geneticists have been busy identifying 'big hitting' developmental genes. These encode the proteins involved in signalling pathways that direct the organization of animals' tissues and organs. But knowing that the brain, for instance, is directed to grow in the way it does because gene A turns on gene B turns on gene C, and so on, still leaves much to be learned about the developmental processes that underlie the complexity of the finished organ.

"Over the past five years there has been a shift away from the formal logic of genetic pathways," says Stephen Cohen, who studies the development of wings in the fruitfly *Drosophila* at the European Molecular Biology Laboratory in Heidelberg. This has seen many developmental biologists migrate into the territory staked out by their counterparts in cell biology. In retrospect, says Cohen, it was an obvious move. Developmental biologists consider how tissues and organs form — but these are simply populations of cells working together. So gaining a complete picture of how organisms are built will require an understanding of cells' internal workings.

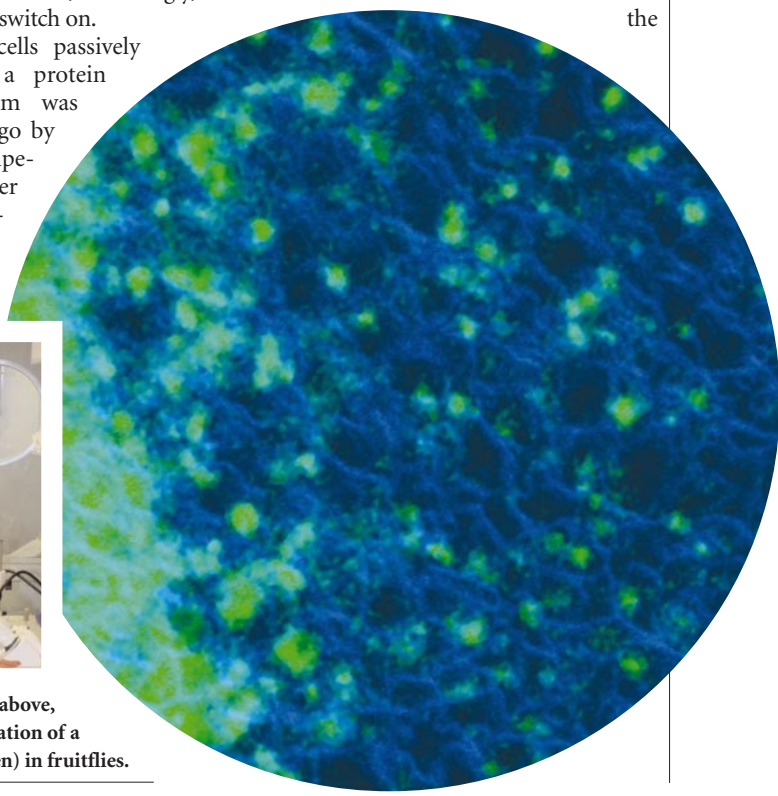
Steeped in gradients

One area of developmental biology being revitalized by this new wave of thinking is the study of morphogens. These are secreted proteins, many of which trigger the signalling pathways that developmental geneticists have been teasing apart. According to the textbooks, morphogens work their magic by diffusing from a source to form a high-to-low gradient. Cells lying in the path of the diffusing morphogen sense its concentration in order to work out where they sit in a developing organ or tissue and, accordingly, what genes they need to switch on.

But this view of cells passively receiving cues from a protein diffusing around them was challenged two years ago by Tom Kornberg and Felipe-Andrés Ramírez-Weber of the University of California, San Francisco. Far from just lying down and taking their instructions from morphogens, it seems that cells often play an active role in determining how the gradient forms.

Kornberg and Ramírez-Weber were looking at the disc of developing tissue that ultimately gives rise to a fruitfly wing. They labelled cells at the edge of the disc with green fluorescent protein (GFP), a commonly used fluorescent marker. This revealed long thread-like fingers, which the researchers dubbed 'cytonemes', extending from these cells towards the

The mutated gene found in open-brain mouse embryos (right) offers a surprising link between cell biology and development.



Marcos González-Gaitán (above, rear) has imaged the formation of a morphogen gradient (green) in fruitflies.

K. MARGITUDIS/MPI-CBG

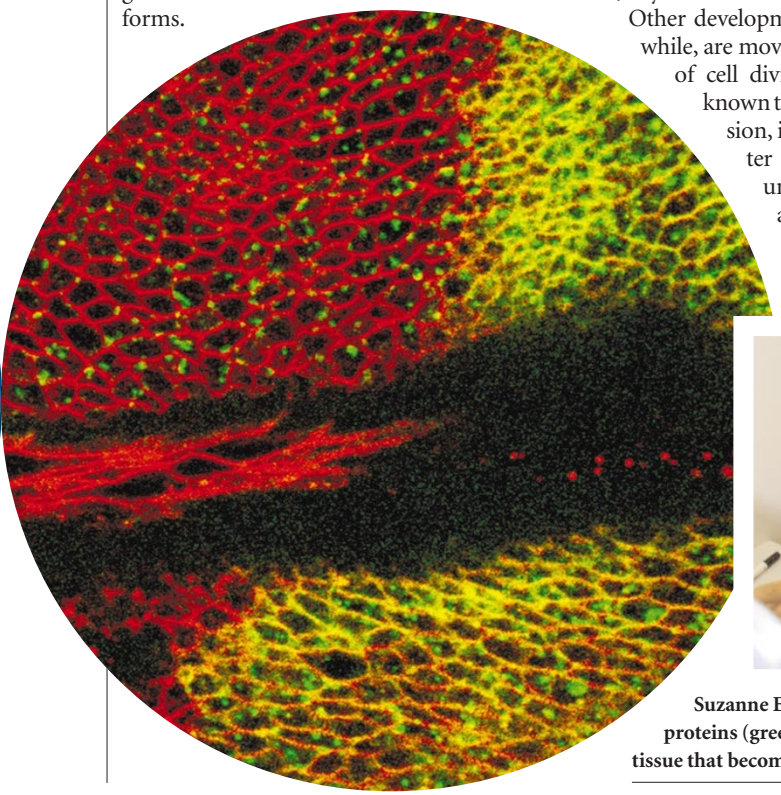
disc's dark, unlabelled centre, where a morphogen called Decapentaplegic (Dpp) is produced[2]. Cells actively seek out morphogens by extending these protrusions, Kornberg and Ramírez-Weber proposed — although they have yet to prove that morphogens are actively channelled along the cytonemes.

Disc drive

Other researchers have labelled key fruitfly morphogens such as Hedgehog, Wingless and Dpp with fluorescent markers and watched the concentration gradients form. "It has changed the way I look at things," says Marcos González-Gaitán of the newly opened Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (see this issue's *NatureJobs*, pages 4–5), which is concentrating on the interface between cell biology and development.

By fusing Dpp to GFP, González-Gaitán and his colleagues were able to watch a gradient form in the fruitfly wing disc. Some of the Dpp could clearly be seen in small round dots inside cells that the researchers think are endosomes — membrane-bound vesicles that form when substances are actively taken up by cells. And in discs containing patches of mutant cells unable to form endosomes, the flow of the morphogen through the tissue was impeded[3]. "Instead of going between cells it's going through them," says González-Gaitán.

This means that the uptake and subsequent release of morphogens by individual cells may help to determine how quickly and how far they move through a tissue, and so determine the 'shape' of the concentration gradient that forms.



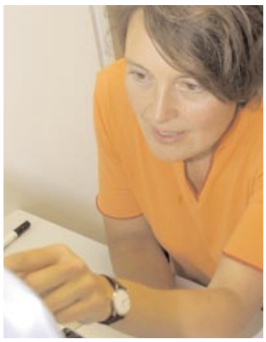
Suzanne Eaton saw membrane proteins (green) migrate across the tissue that becomes a fruitfly's wing.

A related cellular process — the speed at which morphogens are broken down after being taken up into endosomes — may also be key in shaping morphogen concentration gradients. Wingless is a morphogen that normally accumulates in a series of graded stripes across the body of a developing fruitfly embryo, and is involved in specifying the insect's segmented body plan. But when Jean-Paul Vincent of the National Institute for Medical Research in London blocked the protein's breakdown using a drug called chloroquine, the concentration gradient changed, with the Wingless stripes extending into areas that do not normally contain the morphogen[4].

This month, Suzanne Eaton, also at the Dresden Max Planck Institute, published another observation that may help to explain morphogen trafficking. She was studying how proteins are sorted to one side or the other of the fruitfly wing disc. She tagged GFP to a membrane protein expressed on only one side, but she noticed tiny glowing dots inside cells on the other, unlabelled side. These dots seem to form as tiny membrane-bound packages within larger endosomes. Eaton calls them 'argosomes'[5], and speculates that they are released when endosomes fuse with cells' outer membranes — allowing morphogens to be passed from one cell to another.

The mechanisms being touted to explain the formation of morphogen gradients are not mutually exclusive, and it is possible that different processes come into play for different morphogens, and for the movement of the proteins over different distances. "Everyone would like to be the one who figures it out," says Eaton.

Other developmental biologists, meanwhile, are moving into detailed studies of cell division. It has long been known that asymmetric cell division, in which the two daughter cells that arise from an unequal cell division acquire different fates, is a fundamental way of creating cell diversity during development.



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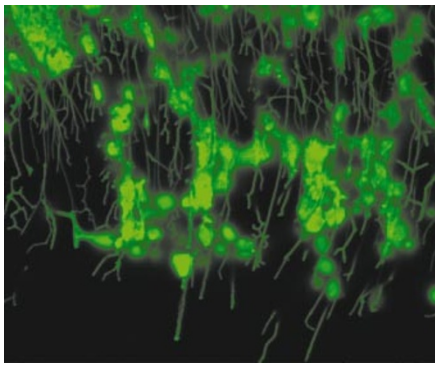


F.-A. RAMÍREZ-WEBER

Green-fingered: are the thread-like cytonemes that reach out from cells searching for morphogens?

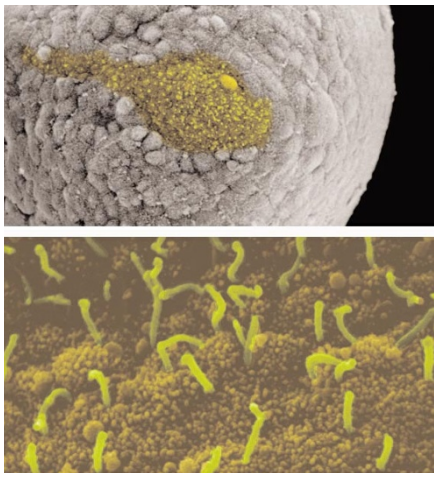
For this to achieve the desired results, the cells must be 'polarized' — they must have a 'top' and a 'bottom' in relation to the developing body plan. The fate of the daughter cells depends on the distribution of key proteins called determinants. Over the past few years, researchers have shown that asymmetric cell division depends both on the localization of these proteins within the parent cell before division, and on the orientation of a structure called the mitotic spindle, along which replicated chromosomes move to opposite ends of the cell before it divides. The spindle determines the plane of cell division, and its positioning influences the relative sizes of the resulting daughter cells, and the quantities of determinants that each receives.

Determined efforts

Back in 1994, a protein determinant called Numb was shown to accumulate in a crescent at one end of cells in fruitfly embryos that give rise to neurons[6]. Since then, researchers have identified a host of other determinants that also become localized to one or the other end of asymmetrically dividing cells, and they are busy investigating how this localization occurs[7].

Meanwhile, other groups are investigating the orientation of the mitotic spindle. Andrea Brand and her team at the University of Cambridge, for instance, have revealed that a sudden shift in the spindle's axis is crucial to the development of the fruitfly nervous system. The cells that give rise to neurons develop from a simple layer of cells in which Numb, and another determinant called Prospero, are concentrated at the base of each cell. In the divisions that form this layer, the plane of cell division runs from top to bottom of the parent cell — which means that equal quantities of the determinants end up in the daughter cells. But some cells, called neuroblasts, detach from the layer and divide asymmetrically into a large neural stem cell and a smaller cell that inherits Prospero and Numb and subsequently divides to give rise to two neurons.

Brand's team showed that the change between symmetric and asymmetric division involves a simple switch of spindle



Y. OKADA/UNIV. TOKYO

Cilia (right) on cells in the 'node' (top right) may direct the asymmetrical development of important organs, says Nobutaka Hirokawa.

alignment. The researchers fused GFP to tau, a protein component of the mitotic spindle, and tracked the spindles using time-lapse video microscopy. In neuroblasts, they found that the spindle rotates by 90° in 60 seconds just before an asymmetric division[8]. "When I show the movies there are often gasps from the audience," says Brand.

Another key issue is understanding how the spindle positions itself asymmetrically in cells that divide unequally. Earlier this year, a team led by Tony Hyman of the Dresden Max Planck Institute shed light on the process by studying the first cell division in embryos of the nematode worm *Caenorhabditis elegans*. In this species, early asymmetric cell divisions determine cell fates for the entire organism.

Hyman's team severed the mitotic spindle with an ultraviolet laser and used time-lapse video microscopy to watch what happened to structures called centrosomes, to which the two ends of the spindle are attached. Both began to move off towards the ends of the cell, but one moved farther and more rapidly than the other[9]. This indicated that the asymmetric positioning of the spindle occurs as a result of differential pulling forces exerted on the centrosomes by microtubules in the cell's internal skeleton. By studying mutant worms, the researchers also revealed that these forces are controlled by the genes that determine cell polarity.

Two-way traffic

Although many of the players in developmental cell biology are migrating from traditional developmental biology, they are being joined by cell biologists moving in the opposite direction. For instance, until three years ago, Nobutaka Hirokawa of the University of Tokyo was tightly focused on the proteins that transport cargoes of other proteins around the cell.

But when Hirokawa and his colleagues engineered mice to lack one such protein, called KIF3B, they encountered a developmental quirk. The mice died during gestation — but in half of them, the heart, liver and other organs had begun to form on the opposite side of the body to their usual position[10]. "Until this moment we were not developmental biologists," says Hirokawa. But he knew that the formation of body-plan asymmetries was a hot topic in developmental circles, so he pitched in.

Hirokawa and his colleagues took a fresh look at the node, a small structure formed at the end of what eventually becomes the spinal cord — and which is thought to be involved in establishing left–right asymmetry. Cells in the node each have hair-like cilia protruding from their surface. Hirokawa's team revealed that these rotate at 600 revolutions per minute. Fluorescent beads thrown into the surrounding fluid were tugged from one side to another by the current generated.

In mice lacking KIF3B, the cilia do not form, because the protein transports components needed for their construction. Hirokawa suggests that the rotating cilia set up a morphogen gradient that directs the normal pattern of asymmetric gene activity and causes organs to form on the correct side of the body. If the cilia are absent, he argues, this asymmetry develops at random. But many developmental biologists remain unconvinced because the putative morphogen remains unknown. "It's provocative," admits Hirokawa. "Cell biologists like that."

Despite occasional differences in perspective, the distinctions between cell biologists who are becoming interested in development and developmental biologists who are discovering cell biology are becoming blurred. "Merging is inevitable," says Andy McMahon, who studies mouse development at Harvard University. He believes the biggest potential impediments are technical, such as the difficulty of transferring cell-biology techniques into more complex systems. Although it has proved easy, for instance, to transfer cellular imaging techniques to fruitfly embryos, mouse and chick embryos are more bulky, making it difficult to visualize individual cells within a developing tissue or organ.

To get round this problem, groups such as McMahon's are establishing methods to grow individual organs or tissues in culture. If they succeed, developmental and cell biology should be poised for a long and happy marriage. Hyman says that for years he endured the scorn of developmental colleagues who could not understand his interest in cell biology. "Not any more," he says. ■

Helen Pearson works in *Nature*'s science writing team.

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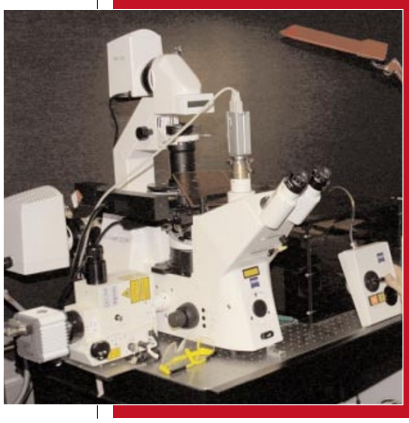
Lights, camera, action!

Attend any conference on developmental cell biology, and you'll spend some of your time watching videos. "Everyone wants to make movies," says Bruce Bowerman, who works on the mechanics of cell division at the University of Oregon in Eugene. "It's like Hollywood."

Indeed, the field's emergence has been facilitated by technology to make videos that reveal how development is unfolding at the cellular level in living tissues.

The key to this technology was the isolation of green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*. Using genetic engineering, GFP can be fused to various cellular components without disrupting their normal function. And biologists bored with green can now extend their palette. GFP has been genetically tweaked into shades of yellow, blue and cyan — and a red version has been isolated from *Discosoma* mushroom anemones, a type of soft coral.

Tracking the movement of fluorescent proteins involves time-lapse video imaging of living cells or tissue slices using sophisticated cameras and microscopes (see left). Lasers are used to excite the fluorescent dyes, cutting down on background fluorescence and allowing researchers to focus on thin slices within a larger sample of tissue. Images of adjacent slices can also be stacked up together to give a three-dimensional picture.



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