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THE BODY

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RICHARD M. TWYMAN

Introduction

One Saturday afternoon in the summer of 1997, I answered a knock at my door to find a Jehova's Witness standing under the porch. This was not the first time a Witness had called at my house, and I am sure it will not be the last. My usual reaction to these people, if I have time, is to engage them in polite conversation. I enjoy pitting my unshakeable belief in science against their unshakeable belief in God, and I am genuinely interested in their absolute faith, which makes them willing to give up their time to calling at strangers' houses. On this occasion, I invited the caller in for coffee and we spent the afternoon chatting about our different beliefs. After a while, he said to me that he could never trust science because it could not explain why a cell in your right hand was different from a cell in your left hand, yet God had made it so. Perhaps he was right, but as a teacher of developmental biology to undergraduates in Cambridge, I felt it was my duty to explain at least some of the principles of limb development that scientists have deciphered over the past twenty years. In the end, I promised to read his Bible if he promised to read a text book called *Developmental Biology* by Scott Gilbert, which is almost as big as the Bible, but has more pictures and references. To give the man his due he took the book and returned it a week later, with the comment that he had found it 'pretty hard going' but enlightening. And yes, I also fulfilled my side of the bargain. Developmental biology aims to explain the fundamental cellular and molecular basis of how a living body is constructed, starting with a single cell, the fertilised egg. This process involves growth, the specialization of cells into different types, cells

* The first lecture in this series was given by Christiane Nusslein-Volhard.

organizing themselves into patterns and forming defined structures. It has fascinated and mystified human beings since the dawn of history. However, over the past thirty years or so, many of the fundamental processes involved in development have begun to unravel under the relentless attack of experimental investigation. Some of the remarkable processes that occur during development, and their molecular underpinnings, are discussed in this chapter.

Building the Round Church

Peter Goodfellow (Chapter 2) has likened the body to a large and complex building, where different types of bricks are arranged in patterns rather in the same way that different cell types are organized in the body. This analogy breaks down when we consider how bodies and buildings are built. The Round Church in Cambridge, and all other buildings, were (and continue to be) constructed brick by brick from the foundations upwards. Conversely, a body is not made starting with the cells of the feet and building towards the head. Rather, growth and development occur gradually in all areas simultaneously. The simple reason for this is that the body is alive even as an embryo and must be functional. Conversely, until it is finished, it is quite acceptable for a building to lack a roof or windows, since it is not used until construction is complete.

The miracle of development was appreciated as long ago as the fifth century BC, when Greek philosophers such as Hippocrates, and later Aristotle, debated its mysteries. Until the eighteenth century, scientists argued over two major theories – pre-formation and epigenesis. In the theory of pre-formation, the human body was thought to be ready formed in either the sperm or the egg. Pre-formation was a comfortable theory because it required no explanation for the exquisite structure of the body other than the hand of God. All the organs and systems of the adult body were present in miniature at conception, and development, stimulated by the meeting of sperm and egg, involved nothing more complex than growth. Some scientists at the time even claimed to be able to see tiny human bodies curled up in the head of the sperm! At the time, the existence of cells and atoms was unknown and there was no concept of a 'minimum size'. Each generation of human beings was considered to be pre-formed in ever decreasing dimensions within

the undeveloped sperm and eggs of the embryo. It was thought that the whole of humankind had been created at one point in time, like a series of Russian dolls (Figure 1), beginning with Adam himself.

Despite its attractive simplicity, the pre-formation theory provided no account for certain observations, such as the intermediate skin colours of

FIGURE 1. Pre-formation versus epigenesis. In pre-formation, the detail of each individual is ready formed and generations are packaged like a series of Russian dolls. In epigenesis, the detailed pattern of the body forms gradually, like a photograph gradually increasing in resolution.

children produced by mating between blacks and whites. If embryos were pre-formed, there should be no mixing of characters in this way. The theory was eventually discarded when it was discovered that living organisms were made of cells, and that development started with a single cell that went on to divide many times. In the light of such revelations, it was no longer possible that entire individuals could be pre-formed within the eggs of the eggs of the eggs of an embryo for countless generations. The alternative theory of epigenesis suggested that development involved a progressive diversification of cells and structures concomitant with an increase in cell number, to form all the organs and systems of the body anew in each generation. The embryo is initially very simple, a few cell types organized in a crude pattern. As development proceeds, more cells are formed and the pattern gradually becomes more finely detailed. This theory fitted the data perfectly, but raised a number of important questions that have begun to be addressed only in the last few decades.

How do cells become different from each other during development?
How do cells become organized into patterns to form individuals with a similar general appearance?
How do cells form particular structures and shapes in developing tissues?

Differentiation – diversifying the body

The human body begins life as a single cell, a fertilized egg. However, the adult human contains many hundreds of specialized cell types, for example neurons in the nervous system, myotubes in the muscles, adipocytes to store fat, erythrocytes to carry oxygen in the blood, keratinocytes to form the outer layer of the skin, hepatocytes in the liver and osteocytes to produce bone. All of these cell types arise from the same egg. At the beginning of development the egg divides a number of times in rapid succession to form a ball of similar cells, a process termed cleavage. After this stage, the cells begin to undergo a gradual process called differentiation, where they diversify into different cell types. Just after cleavage, the human embryo diversifies into only three cell types, called ectoderm, mesoderm and endoderm. These diversify further in a hierarchical manner, eventually producing the full spectrum of cell types in the adult.

How is differentiation achieved? The **important** point to grasp here **is** that

specialized cells are different from **each** other because of the repertoire of proteins they contain. Proteins are macromolecules composed of amino acids. The sequence of amino acids determines the chemical properties of the protein and thus how it functions in the cell. For example, erythrocytes can carry oxygen through the blood because they produce large amounts of the protein haemoglobin. Other cells do not produce this protein, and therefore cannot carry oxygen. Muscle cells owe their extraordinary contractile ability to proteins such as skeletal actin and myosin. Other cells do not produce these proteins and therefore cannot carry out the specialized functions performed by **muscle** cells. In turn, proteins are encoded by genes and with few exceptions every cell **in** the body contains **the** same genes, which are also the same genes as are found in the fertilized egg. The principle of differentiation involves controlling how those genes are used to make proteins in different cells of the **body**. Muscle cells and immature red blood cells contain the same globin genes that make up haemoglobin, but the genes are switched on only in the developing red blood cells. Similarly, the genes encoding the contractile proteins are switched on only in muscle cells. This selective use of information enables the same genome (the full complement of DNA possessed by a given organism) to produce cells with very different functions. Go back and read this paragraph again, but this time read only the bold words. The selection of particular words brings out a sentence with a unique meaning: **how important is each muscle in the body**? The selective use of genetic information during development can similarly bring out a number of unique cell types.

Plate I shows a section through the developing spinal cord of a mouse embryo. The structure in the centre is a dorsal root ganglion, a conglomeration of sensory neurons that receive inputs from sensory organs around the body and feed their axons into the central nervous system. At this stage of development, certain genes are just beginning to be switched on as part of neuronal differentiation, and the corresponding proteins are beginning to be made. Antibodies raised against such proteins can be joined to a fluorescent label and this can show specifically in which areas of the embryo the proteins are made. In this particular example, the protein is called neuron-specific enolase. This is expressed at high levels in neurons relatively late in their differentiation. All over the embryo, and throughout development, different genes are being switched on in different cells to make them structurally and functionally distinct.

Pattern formation – organizing the body

The Round Church in Cambridge is constructed from different types of brick, and the body is similarly made of different cells, but different cells are not enough. In the Round Church, different types of brick are found in specific places, such as around windows and doors, and similarly, in the body, particular cell types are restricted to certain locations. For example, the eye contains various specialized cell types that are found nowhere else in the body, such as retinal ganglion cells, rods and cones. Therefore, development cannot rely solely on the creation of different types of cells – they must also be organized in a distinct pattern. Both differentiation and pattern formation are essential for development. It is all very well having rods and cones and retinal ganglion cells, but if these are dispersed randomly throughout the body, there would be no eyes and no-one would be able to see! For correct function, these cell types must be localized in the head and organized in a specific manner to form the eye.

Pattern formation in development has fascinated scientists and lay people alike, for when the process goes wrong it generates the most bizarre and unusual effects. In humans, this ranges from mild conditions such as polydactyly, where individuals may have six, seven or even more fingers on each hand, to severe defects such as cyclopia (having a single eye), which in previous centuries used to provide the major attractions in barbaric and exploitative freak shows. Scientists work on organisms that are simpler than humans to establish the principles of development, and the species that has provided the most information concerning the process of pattern formation is the fruitfly *Drosophila melanogaster*. Indeed, I think the thing that most attracted me to developmental biology when I finished my A-level examinations was the *Drosophila* mutant called *Antennapedia* pictured on the genetics course leaflet for Newcastle University. As its name suggests, this unfortunate insect has legs growing out of its head in place of antennae. The cells in the antennae-forming region of the fly larva somehow 'think' that they belong further down the body, and so develop as though they were sticking out of the thorax rather than the head.

Pattern formation is all about telling a cell where it is in the embryo in relation to other cells, so that it can behave in the appropriate manner for its position and form the correct structure. If you are lost in a strange city,

you would consult a map to find your whereabouts, and the map is based on a framework of coordinates, in turn based on the compass points north, east, south and west. Similarly, the cells in the embryo are organized in relation to a framework of coordinates based on the principal axes of the body. The craniocaudal axis runs from head to tail, the dorsoventral axis from back to belly, and a third axis runs from left to right. If a cell is 'aware' of its position along all three axes, it can be unambiguously assigned a location in the embryo and can behave in accordance with that position to generate the correct regional structure. The first aspect of pattern formation in development is therefore the establishment of these axes. Remember that the egg is basically a symmetrical cell, so axis specification must involve some symmetry-breaking process. A wide variety of different mechanisms is used. In *Drosophila*, and many other invertebrates, the egg develops surrounded by maternal cells and these place molecules inside the egg that become asymmetrically distributed to define the head and tail ends, and the dorsal and ventral surfaces. In amphibians, the place where the sperm enters the egg plays a major role in determining both the craniocaudal and dorsoventral axes. In chickens, the tail end of the embryo is determined by gravity in a critical two hour period as the egg rotates on its way down the oviduct. In mammals, the dorsoventral axis of the embryo is thought to be defined by the orientation of the embryo with respect to the cells that will form extra-embryonic membranes. The left–right axis in mammals is the most intriguing of all, since it is established after the other axes have formed by the beating of tiny hairs in a central region of the embryo called the node, moving tiny amounts of fluid to the left-hand side of the embryo and activating different genes on different sides.

Once the axes are set up, cells need to be made aware of their position along the axis (Figure 2). This is often achieved by cells at one end of the axis producing a certain molecule that forms a gradient, so that high concentrations are formed at one end of the axis and low concentrations at the other, and all possible intermediate values in between. The surface membranes of animal cells bristle with receptors for various molecules, allowing them to react to proteins and other molecules in the environment. This, for example, is how cells respond to hormones. The receptors are usually linked to internal signalling pathways that eventually result in particular genes being switched on. Cells may switch on certain genes only at high doses of a par-

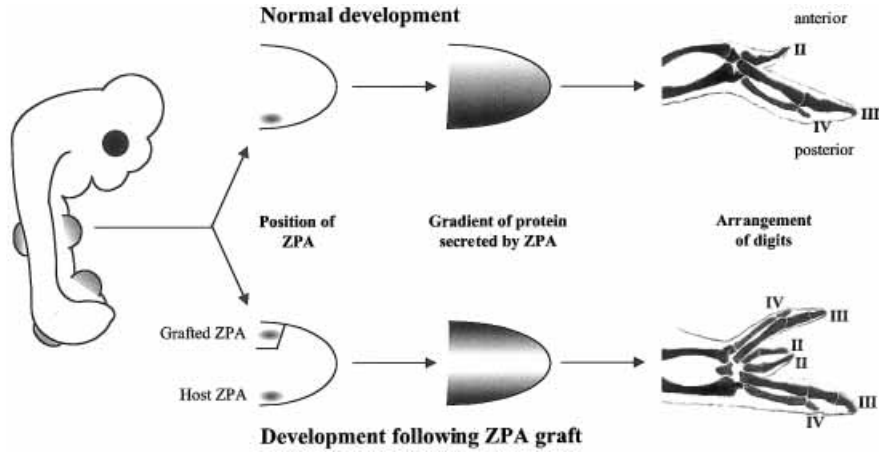

FIGURE 2. An experiment showing how cells get to know their position in a developing embryo. A chicken wing has three digits, conventionally termed II, III and IV, with II anterior and IV posterior. Normal development involves a signalling centre called the zone of polarizing activity (ZPA) that specifies the posterior of the limb. A protein synthesized here forms a gradient, and cells respond to this gradient at different concentrations by forming different digits (at high concentrations, digit IV is formed, at low concentrations, digit II is formed). If a ZPA is grafted from one limb bud to the normal anterior side of a host limb bud that already has its own ZPA, a double gradient is established, and cells that would normally form anterior digits are respecified to form posterior ones. The limb that develops has double the normal number of digits arranged in a symmetrical pattern.

ticular activator and other genes at low doses. Therefore, cells at different positions in a concentration gradient of an activating molecule may begin to produce different proteins and this is what causes them to behave differently according to their position. By artificially manipulating such gradients, scientists can reorganize the pattern of developing structures such as limbs, for example reversing the orientation of the digits and generating extra fingers or toes (Figure 2).

Cells in different positions along an axis switch on different combinations of genes that guide cell behaviour, causing them to generate structures appropriate for their position. If these genes are disrupted by mutation and the cell cannot switch them on, it will be forced into the behaviour appropriate for a different region of the body, resulting in the development of a misplaced

structure, such as legs in place of antennae. This is known as a homeotic mutation, a mutation that causes one body part to develop with the likeness of another (Figure 3).

In one of the most exciting discoveries in the history of developmental biology, the genes responsible for homeotic mutations in *Drosophila* were found to be grouped closely together in one cluster and encode proteins that control how other genes are switched on and off. The genes were expressed in overlapping patterns so that each particular cell could be given a unique 'positional value' based on the number of genes that were expressed. The simplest way to explain this is to liken the system to a binary code, in which each expressed gene is represented by 1 and each silent gene is represented by 0. In this way, individual cells can be given different binary codes according to their position along the axis, and this will enable them to behave in the appropriate manner to generate the correct regional structure (see Plate II). Mutations in the genes would change the code, so the cell would think it was in an entirely different position, and a different body part would develop. Even more exciting was the discovery that all animals possess a similar set or sets of genes that control cells' positional values. In humans and most

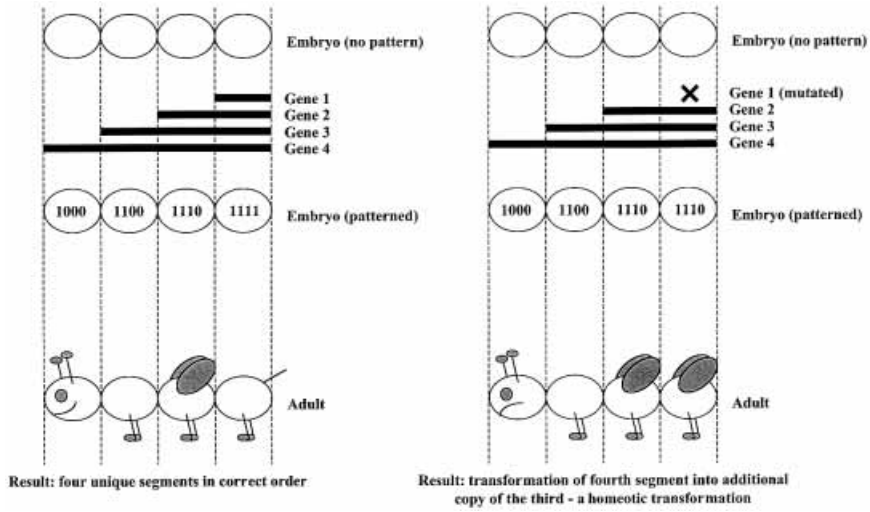

FIGURE 3. Patterning in a hypothetical insect: how cells know where they are and what to do. Combinations of genes are expressed in overlapping patterns to give each segment of the insect a unique code (shown as a binary code (ones and zeros)), which controls how the cells behave to make appropriate structures (left panel). If these genes are mutated (right panel), the code is changed and cells behave inappropriately for their position, generating body structures in the wrong place.

other vertebrates, there are four distinct sets of *Hox* genes, but the characteristic overlapping expression patterns are similar to those seen in flies, and mutations in the genes also affect the position of different body structures. For example, disrupting certain *Hox* genes in mouse embryos causes the cells of the lumbar vertebrae to believe they are actually part of the thorax, resulting in mice with an extra set of ribs! The remarkable conservation of the *Hox* genes among animals suggests that all animals, however diverse, are simply variations on a similar developmental theme.

Morphogenesis – structuring the body

Morphogenesis means the creation of form and this can be regarded in many ways as the last piece in the developmental puzzle. We have discussed above how a developing cell decides what sort of cell it will become in the adult, and how it finds out where it is in relation to other cells. Now, endowed with this information, the cell goes on to behave in a manner that generates the particular structure appropriate for that part of the body. The way in which cells behave to form particular structures is termed morphogenesis.

The way in which cells form different shapes is quite remarkable. Plates II and III show the structures formed by cells in the developing fruit fly and mouse brains, respectively. In both cases, there is an exquisite architecture. Cells simply dividing and dividing to produce more cells would form an amorphous blob, so the behaviour of cells must be precisely controlled to allow such beautiful yet functional organs and tissues to form. There is a wide variety of morphogenetic mechanisms used during development, some of which are listed below:

Cell division – cells can divide at different rates in different parts of the embryo, and can divide in different planes to generate particular structures. The plane of cell division is particularly important in plants, where other types of cell movement are restricted by the cell walls.

Changes in cell size and shape – this can cause the folding and buckling of cell sheets to generate curves and hollows.

Cell fusion – cells fuse together, for example, during muscle development.

Cell adhesion – the way in which cells stick together, through adhesion molecules displayed on their surfaces, plays a predominant role in maintaining tissues and tissue boundaries, and causing cells to move in relation to each other. The loss of cell adhesion causes cells to disperse.

The extracellular matrix – this is a network of molecules secreted by cells into their immediate environment. Interactions between cells and this substrate can maintain cell sheets and provide a surface over which cells can migrate.

Cell death – surprisingly, cell death also plays an important role in development, as discussed below.

Cell behaviour can therefore take many forms, and as an example, we return to Peter Goodfellow's original analogy of the Round Church (Chapter 2). The church is made of bricks stuck together with cement. Similarly, a body is made of cells that have special molecules on their surfaces that enable them to stick together, producing tough and durable tissues. While most bricks are rectangular, occasionally there are bricks that are wider at one end than the other, and these are used to make specialized structures such as arches. Similarly, by changing the shape of a cell, the tissue can be made to bend and distort to form a tube. In exactly this way, many of the tubular structures of the body are formed, for example, the neural tube that gives rise to the brain and spinal cord.

A house without windows and doors is not much use, and similarly there is as much developmental potential in leaving gaps in the body as there is in filling it with cells. In the case of the house, the bricks are simply left out of the window frames. However, since development begins with a single cell, it is not possible to leave gaps where gaps are required in the adult. Gaps must be formed by controlling cell behaviour. A good example of this process is the formation of the gaps between your fingers. The hand, indeed the whole arm, begins development as a small protruberance from the side of the embryo's body known as the limb bud. As development proceeds, the limb bud extends and begins to form the structures of the mature limb. The hand itself is initially a circular pad with no fingers. Between the fourth and eighth weeks of gestation, cells in the interdigital regions are instructed to die as part of the overall developmental programme. These cells are in essence no different from the cells that eventually form the fingers, but because of their position in the hand their role in development is to die rather than form the bone, muscle and skin of the finger. Cell death is an important feature of development generally, particularly in the nervous system, where over half of the neurons originally created during development are killed off in the process of establishing and refining the complex circuitry required for a functioning nervous system. Therefore, despite the claims made by Captain

James T. Kirk of the Starship *Enterprise*, in development at least, the needs of the few do not outweigh the needs of the many.

The future

In the past, individual genes with important roles in development were identified on the basis of their mutant phenotypes, i.e. the effect on the organism when the gene was mutated. Scientists have slowly pieced together the immense puzzle of development using the fragments of information generated by the analysis of individual genes in various model organisms, including the fruitfly *Drosophila*, a nematode worm called *Caernorhabditis elegans*, amphibians such as the frog *Xenopus laevis*, the chicken, the mouse and most recently, a highly versatile zebrafish, *Danio rerio*. The puzzle is not yet complete, but the future for developmental biology looks bright, since genome projects are underway for a number of these model organisms, and in the case of *C. elegans*, the genome sequence is complete. I believe that, one day, it will be possible to describe in detail the entire process of development, from egg to adult, in terms of genes, proteins and cell–cell interactions, and perhaps manipulate developmental processes for our own ends. This will make it possible to put right birth defects, regenerate amputated limbs, replace burned skin, and cure diseases of ageing, such as Parkinson's disease and Huntington's disease. The molecular biologist Edwin Chargraff once expressed his concerns that tinkering with biological processes was akin to a child testing a toy to destruction. However, by learning how a toy is built, it will be possible not only to repair broken toys but to make the toys better and more resilient in the first place.

FURTHER READING

Gould, S. J., *Hen's Teeth and Horse's Toes*, Middlesex: Penguin Books, 1983. [Two chapters in this book provide very entertaining and accessible discussions on development: Chapter 14 (Hen's teeth and horse's toes) and Chapter 15 (Helpful monsters).]

Lawrence, P. A., *The Making of a Fly*, Oxford: Blackwell Scientific Publications, 1992. [An excellent book delving a little deeper into *Drosophila* development; much used by students.]

Nusslein-Volhard, C., 'Gradients that organize embryo development', *Scientific American* **275** (1996) 54–55. [A nice account of pattern formation in *Drosophila*.]

Slack, J. M. W., *From Egg to Embryo*. Cambridge: Cambridge University Press, 1991. [A comprehensive text for those seeking a little more information.]

Twyman, R. M., *Instant Notes Developmental Biology*, Oxford: BIOS Scientific Publishers, 2000. [A brief summary of major research areas in developmental biology, with a guide to current literature on the subject.]

Wolpert, L., *The Triumph of the Embryo*, Oxford: Oxford University Press, 1991. [This book provides a wealth of accessible information on the importance of developmental biology and how it is studied.]