1 Life and death of a cell

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This article is not about prison reform, death in police custody or design of medieval monasteries. Instead the cells that it concerns are the living cells that make up our bodies. Most readers will be aware that estimates of the number of human bodies on the planet reached 7 billion in 2011 and none of us has difficulty recognizing 7 billion as a simply enormous number. Therefore it may come as a surprise to discover that 7 billion cells would make up only the terminal joint of my index finger (Figure 1.1). The total number of cells in the human body is best estimated at 100 trillion, 10^{14}. The inevitable conclusion from this is that cells are extremely small, with occasional conspicuous exceptions, like an ostrich egg, which begins as a single fertilized egg and is thus an enormous single cell.

This chapter starts by a simple introduction to the beauty and fascination of living cells. They are responsible for building all the tissues of the body, including blood, nerves, muscle, bone, yet they are all formed by progressive specialization from the cells generated by division of a single fertilized egg. This poses two extraordinary challenges. The first is the nature of the molecular mechanisms that allow cells to diverge and to specialize to fulfil particular functional niches, but the full details of these mechanisms lie outside the scope of this chapter. The second challenge is that of producing stable and balanced numbers of each type of cell within the body. How are the ratios of blood cells to nerve cells or cells that line our gut balanced and managed? This question is made all the

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This lecture is dedicated to César Milstein, 1927–2002, inventor of monoclonal antibodies and Nobel Laureate in Physiology or Medicine 1984, and who recuited me to Darwin College.

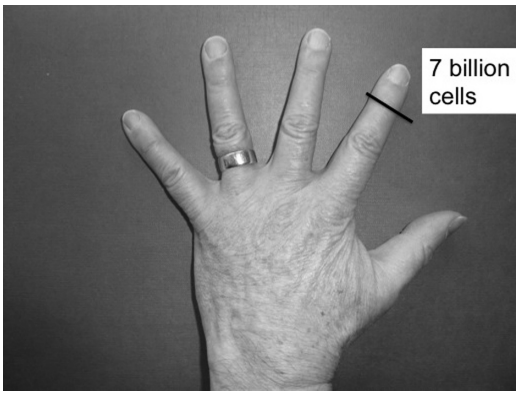

FIGURE 1.1 The number of humans on the planet is 7 billion, yet 7 billion is approximately the number of cells in just the terminal joint of one finger.

more acute by the fact that different types of cell persist in the body for very different lengths of time. Thus the cells that line our intestines, or the cells that line the ducts that carry digestive juices from our pancreas into the gut, survive for only a few days before they are replaced by new counterparts. In contrast most of our nerve cells persist throughout our adult lifetime. Although some types of nerve cell can be formed during our lifetime, others cannot and most remain with us throughout adult life. This poses an extraordinary challenge of bookkeeping and management of cell production and replacement.

After an elementary introduction to the workings of the living cell, this chapter will consider how new cells are formed by the process of cell division, an area in which our understanding has exploded within the last thirty years. It will then argue that death can be good for you, when it is cell death. Programmed cell death is a phenomenon that plays a crucial role in maintenance of the body structure and organization. Not only are worn-out cells constantly replaced and renewed by cell division, but programmed death of specific cells is a mechanism that sculpts the human body and plays an important part in determining the form of our bodies. However, with both cell division and cell death influencing the numbers of each cell population, co-ordination and regulation of cell population sizes becomes crucial. The nature of this challenge will be described and the ways in which it is managed will be outlined.

The two final sections will consider what happens when this tightly managed balance breaks down. Excessive cell proliferation, or inadequate cell death, can both contribute to cancer. These imbalances can be triggered in many different ways, resulting in uncontrolled growth of a selfish cell population. Conversely excess cell death without renewal or replacement can result in a range of degenerative diseases. Some of the most prevalent of these are the neurodegenerative diseases including Alzheimer's and Parkinson's diseases. Both of these, and several other neurodegenerative diseases, arise from disorders of protein folding. Proteins should fold into precise three-dimensional structures but then be unfolded and degraded when they are damaged or have served their purpose. However, errors in protein destruction can give rise to fragments that are capable of forming insoluble aggregates with seriously damaging consequences for the cells that contain them or are surrounded by them. The build-up of toxic insoluble protein aggregates in or around nerve cells can be responsible for death of those nerve cells and several important neurodegenerative diseases.

The living cell

Figure 1.2 illustrates the extraordinary beauty of cells. It is a photograph of a single cell viewed in a fluorescence microscope after three different cellular components have been stained red, green or blue. The blue stain reveals DNA packaged within the cell nucleus, which serves as the information archive of the cell. The red stain reveals mitochondria, the powerhouses of the cell that metabolize ingested food and convert it into a form of energy that can be used throughout the cell. In addition, mitochondria play an essential regulatory role in the control of cell death. The green stain reveals components of the cytoskeleton, namely microtubules that are essential for distribution of other large components around the cell. They play a central role in the process of cell division and the segregation of chromosomes to the two progeny cells that arise from cell division.

The packing of DNA within the cell nucleus is truly remarkable. Each cell nucleus contains 2 m of DNA and this encodes the information for the structure and function of all the cells in the body. Yet these 2 m of DNA

Table 1.1. *The problem of packing DNA into the cell nucleus. The extraordinary scale of the problem is seen more clearly by a scale model in which all dimensions are increased 1-million-fold.*

Dimensions of DNA and the cell nucleus, actual and magnified 1 million times		
		$\times 10^6$
DNA diameter	2 nm	2 mm
DNA length in nucleus	2 m	2000 km
Diameter of nucleus	5 μ	5 m

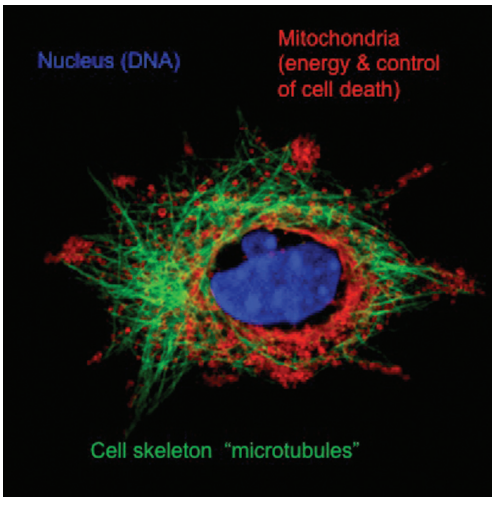

FIGURE 1.2 A fluorescent micrograph of a human cell stained to reveal DNA in the cell nucleus (blue), mitochondria that are the power units of the cell (red) and microtubules that are dynamic components of the cell skeleton (green). Image reproduced with permission from Peter Laskey.

are packed into a cell nucleus of only a few microns in diameter in such a way that all the DNA is available for duplication to produce the nuclei of the progeny cells after cell division. The scale of this packing problem can be understood more clearly by a scale model in which everything is increased 1-million-fold, as shown in Table 1.1. On this scale the nucleus

would have a diameter of about 5 m and DNA would have a diameter of 2 mm, the diameter of thin string, but the length of DNA enclosed in each of these 5-metre nuclei would reach from St Pancras to St Petersburg, namely 2000 km. Remarkably this must all be packed in in such a way that it is accessible for copying in readiness for cell division. A second example illustrates the extraordinary length of DNA packaged in each cell. I stated that the terminal joint of my index finger contains approximately 7 billion cells. All five complete digits from one hand would have approximately 100 billion cells and each of these contains 2 m of DNA. The conclusion is, therefore, that if DNA was extracted from all five digits of one hand and joined end to end the resulting DNA molecule would be 200 million km long, enough to reach the sun. Remember that this example is not a scale model; it is reality.

The capacity for information storage within DNA is remarkable and is superbly illustrated by an exhibit in the Wellcome Collection at the Headquarters of The Wellcome Trust in London. The four bases A, C, T and G, read in groups of three, encode information in DNA. The exhibition in the Wellcome Collection prints out the human genome in a normal type font and it is bound into a large volume on display with the letters A, C, T and G repeated many times on each page. The volume that is opened on display is the size of a large encyclopaedia but remarkably it is one of 118 such volumes that represent the complete human genome. They occupy a large bookcase stretching from floor to ceiling, yet this is the information encoded in each copy of the human genome and stored in each of the 100 trillion cells in our bodies. In micro-fabrication, we clearly still have a lot to learn.

Figure 1.3 shows how information flows from DNA in the cell nucleus to produce the many types of specific protein that are made outside the nucleus, in the cytoplasm. The information in one strand of DNA is copied into a related molecule, called RNA, that is exported from the nucleus through pore complexes in the nuclear envelope, to the cytoplasm. There it serves as a template to be translated by specialized machinery, called ribosomes, to produce the many proteins of the cell. It is the proteins that then build the structure and do the work, including catalysing the many biochemical reactions within the cell. In summary, the cell is an extraordinarily organized information storage and interpretation machine that

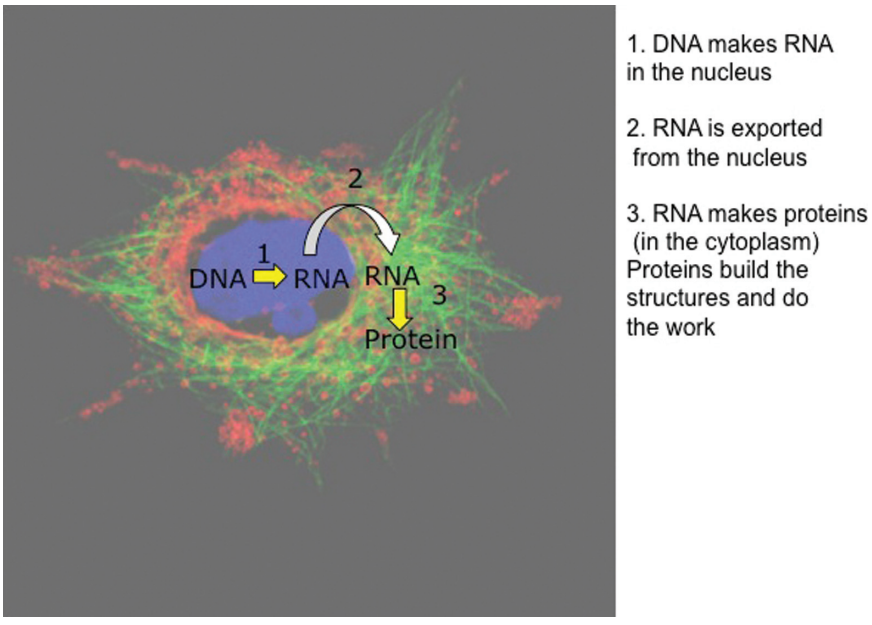

FIGURE 1.3 Information flow from DNA in the cell nucleus for synthesis of proteins in the cytoplasm.

encodes vast amounts of information in very small spaces and yet selectively retrieves subsets of that information for expression in different types of cell. The reason why cells differ from each other in the body is not that they contain different DNA, but that they copy different subsets of the DNA into RNA and therefore make different proteins. A sophisticated network of controls determines which regions of DNA, which genes, are copied into RNA to make proteins. After a subset of genes have been copied from DNA into RNA, there are further levels of control before the proteins are made. However, these necessarily lie outside the scope of this chapter.

Cell division

In 1855 a Prussian pathologist, Rudolph Virchow, wrote: 'Omnis cellula e cellula' [All cells come from cells], indicating that all cells are

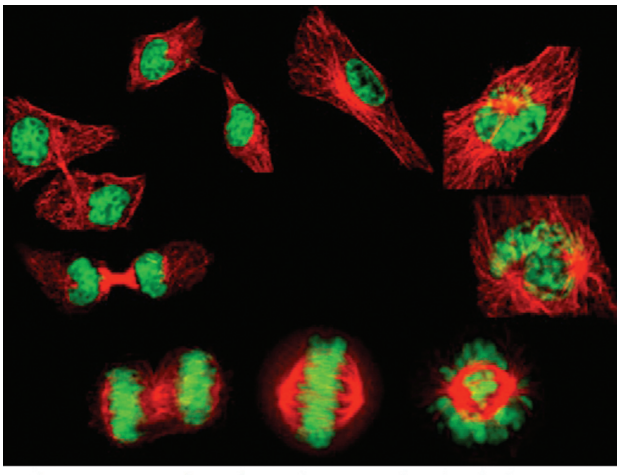

FIGURE 1.4 Stages of cell division starting with a single cell at 1 o'clock and proceeding clockwise to form 2 cells at 11 o'clock. Chromosomes are green and microtubules are red. Image reproduced with permission from Matthew Daniels.

derived by division of existing cells rather than assembled de novo. Cell division is the most dramatic event that takes place within a cell's life-cycle. It involves condensation of the long DNA threads in the nucleus into discrete, microscopically visible, chromosomes and their division longitudinally by a mechanical spindle apparatus. Between consecutive divisions DNA is dispersed throughout the cell nucleus, but complexed to specific proteins that neutralize the acidic charges of its many phosphate groups and that enable it to be folded into a hierarchy of structures in the nucleus. This is the state in which DNA is copied to make RNA and also duplicated to make more DNA. Once the DNA has been copied to produce two complete copies, it then becomes coiled into visible chromosomes for division between the two progeny cells. In human chromosomes the DNA is compacted 10,000-fold allowing it to be divided in two within the boundaries of the cell.

The mechanism of cell division requires attachment of the chromosomes to the ends of microtubules (stained green in Figure 1.2 and red in Figure 1.4), with one side of each chromosome attached to microtubules

extending from each pole of the spindle. Microtubules have functions in cells between divisions. They serve as dynamic rails along which other cellular components are distributed, but they become fundamentally reorganized during division, by two microtubule organizing centres, from which they grow. The two halves of each chromosome become attached to the ends of microtubules ready to be divided longitudinally for distribution into the two progeny cells. At this time each of the two DNA molecules arising from DNA synthesis is coiled into one of the two parallel halves ('chromatids') and these are held together by ring shaped proteins called cohesins that appear to physically hold the two halves together until all chromosomes are equally attached to both spindle poles. Division is delayed until each chromosome is firmly attached to both poles of the microtubule spindle. This delay causes chromosomes to align in a flat plate experiencing equal forces pulling them towards each pole, and only then can they split longitudinally for division, a process that is achieved by cleaving the cohesin protein rings that link the two new DNA molecules together. In addition, chromosome condensation and division of the chromosome in two are both delayed until all DNA synthesis is complete. Incomplete DNA synthesis would result in chromosome breakage when the chromosomes are pulled apart during cell division. The 'checkpoint' mechanisms that delay events during cell division to ensure that each event is complete before the next one starts are crucial in maintaining the integrity of the genetic information in each cell. Maintaining the integrity of the genome in these ways is equally crucial to prevent cancer. Mutations in the genes that regulate cell proliferation either positively or negatively are the raw materials for the pseudo-Darwinian selection that selects for the fastest dividing cells in cancer. I say 'pseudo-Darwinian' because, as explained later, real Darwinian selection acts at the level of the individual, rather than at the level of the selfish cancer cell. True Darwinian selection selects against excessive proliferation of selfish cancer cells and thus against genetic instability and other causes of cancer. Instead it selects for genome stability and checkpoint mechanisms that defend the individual against instability of the genome and thus defend against the risk of cancer.

Not only is it essential to ensure that all of the DNA has been synthesized to make two complete copies before the cell can divide, but it

is also essential that no part of the DNA is copied twice. The genetic imbalances that arise from either incomplete DNA synthesis or repeated DNA synthesis of the same piece of DNA both provide the raw materials for carcinogenic changes resulting in cancer. Duplicating the immensely long threads of DNA that exist in our cells poses another serious problem. DNA is a double helix. One strand wraps around the other once for each ten letters of the genetic code. Therefore as a human cell contains 6×10^9 letters (3×10^9 from each parent), there are 6×10^8 (600 million) helical turns to be removed to unwind the DNA strands before they can be separated into the progeny cells. This generates important roles for two classes of proteins that will feature later in this chapter. Unwinding one strand from the other requires unwinding activities called DNA helicases, because they unwind the DNA double helix, but as they do so they simply move the helical twists from the DNA that is undergoing synthesis and pass them on to the DNA that has not yet been synthesized generating a serious topological tangle. Fortunately the cell has two classes of activity that ingeniously solve this problem. They have the ability to remove torsional stress from DNA by cutting and rejoining it. Unfortunately they have the off-putting names of 'DNA topoisomerases', but what they do is remarkable. Type 1 topoisomerases cut one strand of DNA, hold on to the end by a chemical bond and pass the other strand through a gap that they have made, removing helical twists one at a time. Each time they do this they repair the gap that they made, restoring the DNA to its original structure but with less torsional stress. Type 2 topoisomerases do something even more remarkable. They cut through both strands of the DNA, holding on to the cut ends, and pass a second double helical DNA molecule through the temporary gap that they have made. This can untangle the most troublesome tangle. Both these extraordinary activities will feature later in the chapter.

Death can be good for you, when it's cell death

During the time it will take to read this chapter, approximately a billion of your cells will have died. Fortunately approximately a billion will also have been produced by new cell division. The balance of these processes is remarkable as the population of each of the many types of cell in our

bodies is very stably regulated. Cell death is not a random or haphazard event. It is precisely regulated and executed with extraordinary efficiency. Programmed cell death (otherwise known as apoptosis) is mediated by a cascade of molecular assassins that destroy the cell completely so that only residual remnants remain to be consumed by its neighbours. Once a signal for a cell to die is received, it is amplified by a series of enzymes that culminate in chopping the essential proteins of the cell into non-functional fragments as well as cleaving DNA into irreparable fragments. Mitochondria (stained red in Figure 1.2) modulate the damage level and amplify the death signal in a condemned cell. They do this by releasing the protein cytochrome C out of the mitochondrion. Its release activates formation of a disc-shaped protein complex called the 'apoptosome' or 'wheel of death' that accelerates a cascade of destructive enzymes, resulting in the systematic disintegration of the cell. Apoptotic cell death is so decisive that there is no risk of a genetically damaged cell persisting in a state that could allow it to divide and become cancerous. Damaged or wounded cells are destroyed absolutely and irreversibly, as seen in progress in Figure 1.5. In addition to contributing to turnover and renewal of cells in the mature body, programmed cell death also plays an important role in shaping and sculpting tissues. For example, the human hand is sculpted from a flat paddle in the embryo by death of cells between the digits rather than just by growth of the digits themselves. They are literally carved out of a flat paddle-shaped limb, just as a sculptor would carve them from a block of stone (Figure 1.6).

In addition to the tight regulation of a cell's lifespan by programmed cell death, the length of time and number of generations for which a cell is able to divide are also tightly regulated. When cells are grown in culture they normally divide for only a limited number of generations and then stop. They become senescent. The timing of senescence is sometimes correlated with erosion of the specialized ends of chromosomes called telomeres. These have specialized DNA structures that are not duplicated by the normal DNA synthesis machinery, but extended by a dedicated enzyme called telomerase. This enzyme becomes inactivated in mature cells as they differentiate or senesce. It is maintained in an active state in stem cells or reactivated in cancer cells.