# 1 Animal Development and Reprogramming

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We all start life as a single fertilised egg cell, but that single cell is then converted into the highly complex organism that we all are. In the first part of this essay, I will address how that happens. In the second part, I will discuss how we can make this process go backwards, and eventually provide new rejuvenated cells when our own deteriorate with age or disease. I will also address the question of whether we really can replace old cells with new cells, and, if so, to which extent. I will finally address the ethical and legal constraints of making this procedure more generally available.

## From Egg to Adult: How Does It Happen?

In most species, excluding mammals and humans, the very early stages of development can be seen. During the development of a frog, a fertilised egg first develops synchronously, and within only a few hours the egg has turned itself into a ball of several thousand cells. Soon after, some of the cells on the outside start to move to the inside of the ball and, within a day or two, the future brain, nervous system, and other parts of the embryo can be seen to form. In mammals, early development takes place in the mother, and the mother's cells help to guide the embryo as it forms. But in all other animals early development takes place entirely independently of the mother. The different kinds of cells of which we consist appear progressively. At an early stage new embryo cells take a decision whether to go in the direction of brain and skin, muscle or intestine. Later, those that have gone in the first direction take another decision and follow separate pathways to reach their eventual fate (Figure 1.1). Once a cell has embarked on a pathway leading to a particular fate, it and

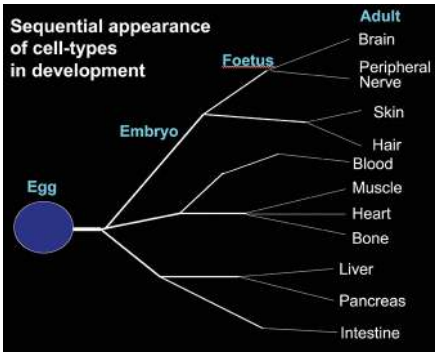


FIGURE 1.1 From egg to adult. As embryo cells divide, they and their daughter cells become progressively restricted in terms of the kinds of cells that they can form.

its daughter cells do not change or go backwards. Therefore, as an embryo grows and differentiates, its cells become progressively committed to particular pathways, leading to specific cell types.

## How Does the Embryo Know What to Do?

We are familiar with frogspawn in a pond. Initially, each egg is a single cell, and in a few days each one has turned itself into a swimming tadpole. How does it know how to do this? The parent frogs have long since disappeared, and give no guidance to the eggs on how to develop.

Before powerful microscopes were invented, it was thought that each egg or sperm might have a little creature inside it, dubbed a homunculus, and it just had to grow (Figure 1.2). Later, in the nineteenth and early twentieth centuries, the very influential German embryologist Ernst Haeckel (1834–1919) was impressed by how, in early life, the embryos of many different kinds of animals look similar. He made the proposition that 'phylogeny is the mechanical cause of ontogeny'. Many people revered Haeckel's global view of development, but the phrase I have quoted did not give any meaningful explanation of how development works. At the time, Haeckel's position as a very senior German professor led others to accept his pronouncements without questioning them. However, later commentators treated him often as what, I think, we call

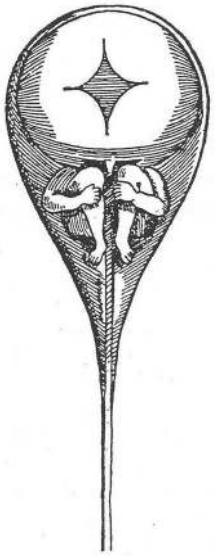

FIGURE 1.2 An early idea that a human sperm might contain a miniature man, a homunculus, which could grow to an adult once in the fertilised egg. (From Hartsoeker (1694).)

the 'whipping boy'. When something did not make sense, they blamed it on Haeckel. For example, to quote one very highly regarded later commentator: 'Haeckel's greatest disservice was not his total ignorance of exception to his rule, but his emphasis on his irrefutable explanation of the mechanical cause of development. He thereby distracted those who might otherwise have made a valuable contribution to this whole field.' (Hamburger, 1988).

But what have we learned about the principles of development, what can we say now about how an egg can turn itself into a complete organism? If we look at the inside of a frog egg, there is no indication of any kind of organism, or type of cell, inside. All we can see is a tiny nucleus at the top in a huge mass of the so-called cytoplasm, most of which is yolk platelets, a source of nutrition for the embryo (Figure 1.3). We now know that there are two fundamental mechanisms by which this mass of apparently disorganised cytoplasm turns itself into cells of different kinds.

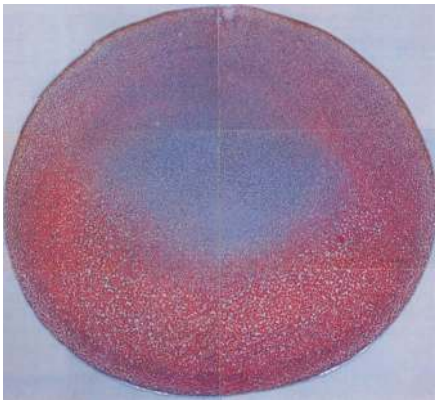

FIGURE 1.3 The unfertilised egg of a frog. Most of the egg consists of yolk (red), and apparently structureless cytoplasm (blue) in the middle. (Plate 8 of Hausen and Riebesell (1991). With permission of Springer Nature.)

## Asymmetric Distribution of Parental Molecules

As long ago as 1905, Conklin made a detailed description of the marine mollusc Styela. He could see that the yellow-coloured cytoplasm of the undivided egg gradually became localised in one part of the egg, and subsequently in some of the cells derived from that region of the egg (Figure 1.4). This yellow material eventually became part of the muscle of the embryo. The basic concept that resulted from this observation is that the undivided egg has various substances in its cytoplasm and that these are gradually distributed in the early embryo so that each of them goes to different parts of the future embryo. The yellow pigmented material happens to mark those substances that become muscle, though the yellow pigment is itself only indicative of other muscle-forming substances (Figure 1.4). As development proceeds, these formative substances, originally present in the undivided egg, gradually become localised to those parts of the early embryo which will turn into various kinds of cells. Various external influences cause these substances to move to where they need to be. These include the exact position in which the sperm enters the egg, and the movement of substances under the influence of gravity. As a result of the progressive localisation of these formative substances, cells

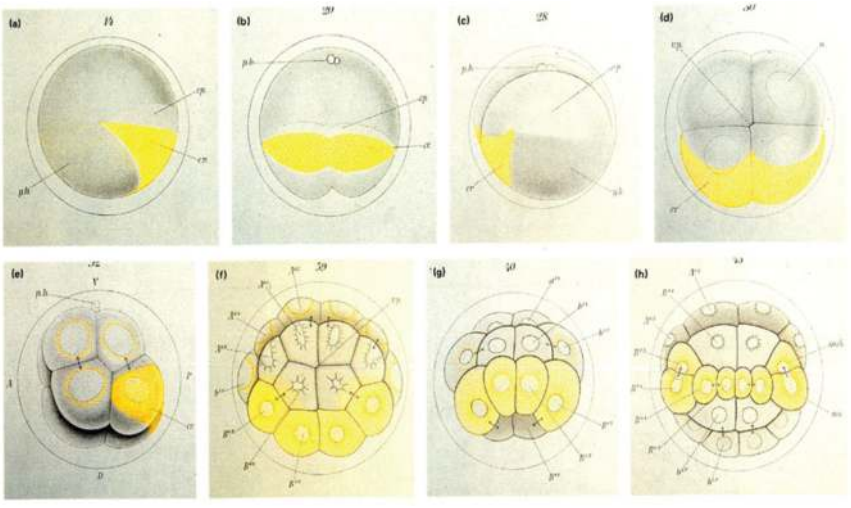

FIGURE 1.4 Localised substances in the early fertilised egg become progressively localised to the part of the embryo that will form a particular kind of cell. The muscle-forming substances of the egg of the mollusc are occupied by a yellow pigment. (From Conklin (1905).)

gradually become committed to their eventual fate, and alternative pathways are eliminated (Figure 1.1).

### Signalling between Different Cells

We now know that cells of one kind in one part of an embryo send signals, in the form of molecules, to other cells elsewhere in the embryo. In the frog embryo, for example, the earliest distinction is between those cells at the top end of the embryo and those at the bottom (Figure 1.5). Substances which originated at the top end of the undivided egg, seen in yellow, become localised in the cells which will eventually form skin and brain, whereas substances at the other end of the egg, seen in blue, are localised in cells which will form the intestine and internal organs. After only a few hours, the blue cells send signal molecules upwards to the nearby yellow cells and cause the yellow cells to change their fate by making different substances, seen in green. These commit those cells to

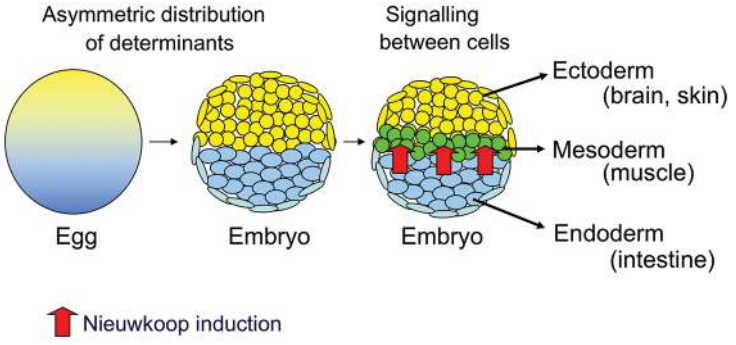


FIGURE 1.5 The principle of signalling between cells. The yellow and blue substances are asymmetrically distributed to different poles of the egg, and then to different cells of the early embryo. When many cells have been formed, the 'blue' cells (the endoderm, giving rise to the intestine etc.) send molecular signals to the early 'yellow' cells (the ectoderm, giving rise to skin, brain etc.), causing them to change direction and become 'green' cells (the mesoderm, giving rise to muscle, bones, etc.).

become different parts of the body such as muscle, bone, etc. (Figure 1.5). This process of signalling between cells in one position and their neighbours elsewhere continues through development, and in later life.

The same principle lies behind the fundamental process of how stem cells operate. For example, deep in our skin are skin stem cells. As seen in Figure 1.6, the stem cell gradually accumulates skin-forming substances, and these are asymmetrically distributed as the stem cell grows and eventually divides (in the same way as this happens in eggs). The stem cell divides, typically asymmetrically, into one small daughter cell and another larger daughter cell. The small cell has accumulated a high concentration of these skin-forming substances, which cause that cell to activate genes resulting in the formation of skin. This process continues and repeats itself as the larger daughter cell, which remains a stem cell, undergoes subsequent divisions (Figure 1.6).

Very often the process of signalling works in a concentration-dependent way. As the signalling molecules spread across a cell or a group of cells from one part of the embryo, they become dilute as they spread further away. Consequently, cells near the source receive a high concentration of this substance and those further away a lower concentration. Amazingly, cells are able to sense the concentration of substance

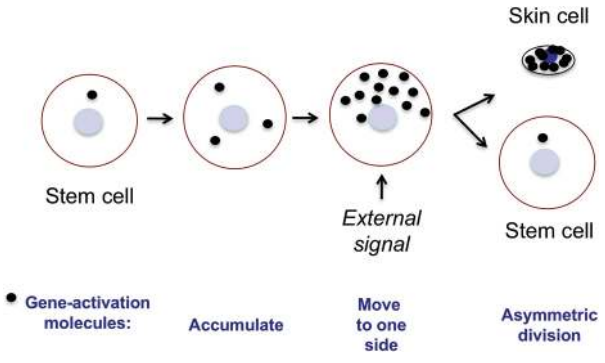


FIGURE 1.6 The principle of stem cell function. A stem cell makes skin-forming substances, which then become highly concentrated at one side of this cell (these gene-activating molecules are symbolised by black dots in the figure). After asymmetric cell division, they are present in the smaller daughter cell, which forms a skin cell. The larger daughter cell continues life as another stem cell.

that they receive, and respond accordingly. In this way, many different kinds of cell-types are formed by the concentration-dependent effects of one molecule along the concentration gradient of this molecule.

In summary, the way a single undivided egg cell turns itself into a complex embryo depends on two processes. One is the asymmetric distribution of cell-type-forming substances, and the other is the signalling from one kind of cell to other cells nearby. These two fundamental mechanisms enable an egg to turn itself progressively into an embryo, and into the many different kinds of cells that there are in the body.

## Can Cells Be Made to Go Backwards in Development?

I have argued above that the process of normal development is a one-way process as cells gradually become more restricted in terms of what they can form; they and their daughter cells do not go backwards. This results in very stable cell-types of different kinds. Fortunately, we therefore do not find brain cells in our intestine or muscle cells in our skin. However, it is now possible, by experimental means, to make cells go backwards in life, and so to reverse this normally stable process of cell differentiation.

We need now to go back some 50 years to the 1950s to trace this technological development from the time when I first started as a

graduate student. At that time it was not known whether all the different kinds of cells of our body have the same genes. A perfectly plausible idea at the time was that, as the embryo forms brain, skin, muscle, and all the other tissues, the genes needed for brain, for example, are lost from those cells that will form muscle.

The pioneering work of Briggs and King in 1952 succeeded in the technical achievement of moving the nucleus out of one kind of cell into an egg which itself had not been fertilised and had no nucleus. They were able to take the nucleus from an embryo cell, put it into the cytoplasm of an egg, and grow a normal tadpole. This opened the way to the process now called nuclear transplantation. My own involvement also started in the 1950s by doing these experiments with the South African frog *Xenopus laevis*, which was particularly favourable for this kind of work. I eventually found that it was possible to take the nucleus out of a functional intestine cell and implant that into an unfertilised egg whose own nucleus had been removed. As a result, it was possible to obtain an entirely normal adult, sexually mature frog from the combination of the nucleus of an intestine cell with an egg. This proved decisively that, as cells differentiate, in this case into intestine, they do not lose any genes needed for all the other kinds of cells that compose an adult animal. This gave rise to the fundamental conclusion that, as cells undergo specialisation into different tissue types, all genes of the body are retained in all cells. This makes it possible, in principle, to generate embryo cells from the nucleus of a skin cell, and hence to generate all the other kinds of cells that embryos normally give rise to. Thus the normally stable process of specialisation into intestine or other cell-types can be reversed experimentally by taking the nucleus from a specialised cell and transplanting it to an egg. The egg then forms the whole range of other kinds of cells that make up the body. In effect, the intestine cell nucleus is rejuvenated and made to go back to the beginning of life and start all over again (Figure 1.7).

There were all sorts of technical difficulties in achieving this basic experiment. First of all, the species of frog I used had eggs covered with an intensely elastic jelly, which made them impenetrable by any kind of needle that might be used to transplant a nucleus into the egg. Eventually this problem was solved by finding an appropriate wavelength of ultraviolet light that softened the jelly, and, by good luck, killed the nucleus of

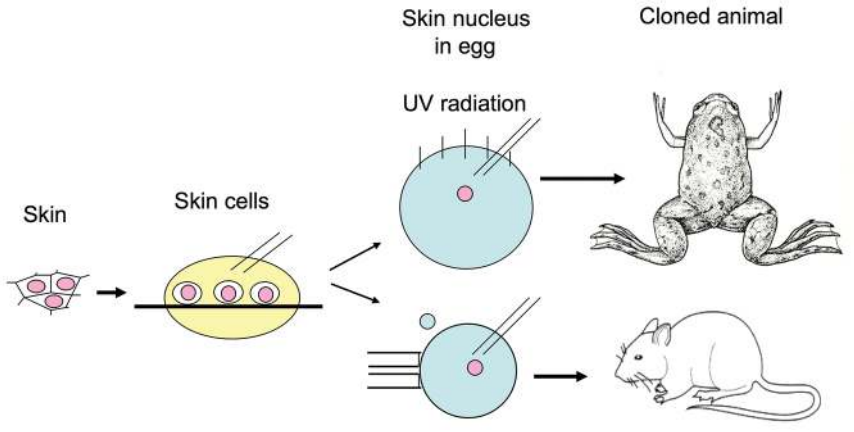


FIGURE 1.7 The principle of a nuclear transplantation experiment. The nucleus of an adult skin cell (pink) is transplanted into an egg (light blue), whose own chromosomes have been killed by ultraviolet irradiation (top), or removed from the egg (bottom). In some cases, the resulting embryo can become an adult cloned animal.

the egg. The next problem was to find a way of decisively marking the nucleus of the intestine cell so that it could be proved that the resulting adult frog did indeed come from the intestine cell nucleus and not from a failure to remove the nucleus of the egg. At that time, my PhD supervisor had a student who was struggling with a difficult problem and obtained an inexplicable result concerning the number of nucleoli in a nucleus in these frog cells. Dr Michael Fischberg, my supervisor, had the extraordinary wisdom not to ask the student to repeat all the experiments with new cells and new reagents. Instead, he said 'Please go back and find the actual animal which gave you these inexplicable results.' It turned out that a particular mutation had occurred, which altered the number of nucleoli in a cell. This was a natural genetic mutation, which turned out to be extremely valuable in enabling us to mark the descendants of a transplanted nucleus, as opposed to nuclei from an egg whose own nucleus had not been successfully removed. The adult frogs resulting from transplanted intestine nuclei eventually became males and females, and they were reproductively fertile. This proved that every single cell-type, including eggs and sperm, can be derived from the nucleus of an intestine cell.

Some decades later, the cloning of Dolly the sheep was announced. This was the first successful application of this nuclear transfer technique to mammals. Dolly has become very famous. Some people asked why did we not give names to our intestine-derived adult frogs. The answer was that we had so many of them that this would not have been realistic. Dolly the sheep was from the only successful nuclear transplant at that time, though subsequently nuclear transfer in mammals has been successful in a whole range of different species, even including humans.

### Embryonic Stem Cells

The next major advance in this field, going from nuclear transfer to the eventual possibility of cell replacement, depended upon the discovery of embryonic stem cells by Martin Evans. He discovered, contrary to expectation, that it is possible to take cells from an early mouse embryo and place them in culture in such a way that these cells go on dividing indefinitely but nevertheless may remain embryonic. Amazingly, these permanent embryonic stem cells, as they are called, can be made to specialise when required. Special cell-type-forming substances, of the kind I have mentioned above when discussing signalling, can be added to these embryonic stem cells, and they can be made to form all different kinds of cells, including those of brain, heart, and other tissues. This extraordinary discovery, most appropriately awarded a Nobel Prize in 2006, has led to the current great interest in cell replacement therapy. The critical discovery made by these experiments of Martin Evans can be summarised as follows.

1. The cells can be grown from the early embryos of mice and humans, and are known as embryonic stem cells.
2. They can be grown indefinitely in the laboratory to make billions of unspecialised stem cells.
3. They can be made, at any time, to differentiate into heart, brain, and all other kinds of cells.

### Induced Pluripotent Stem Cells

The next key advance in this field, leading to cell replacement therapy, was announced by Takahashi and Yamanaka (2006). As a result of this work, Yamanaka received a Nobel Prize for Physiology or Medicine in