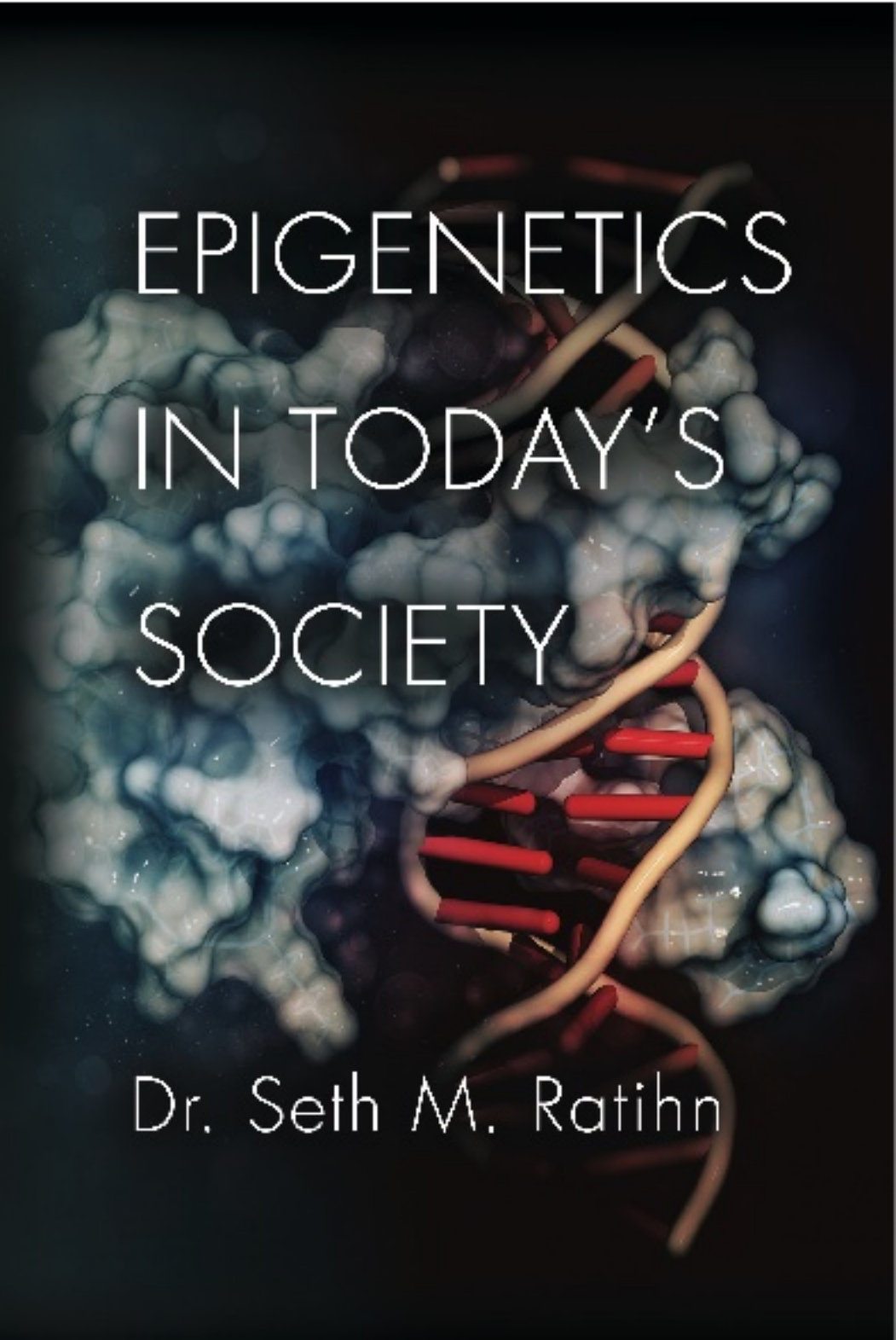
EPIGENETICS IN TODAY'S SOCIETY



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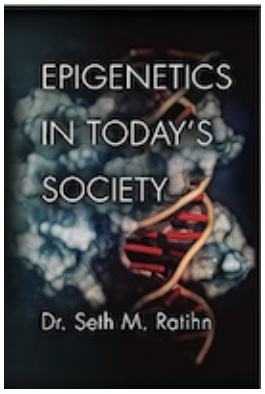

Epigenetics can potentially revolutionize our understanding of the structure and behavior of biological life on Earth. It explains why mapping an organism's genetic code is not enough to determine how it develops or acts and shows how nurture combines with nature to engineer biological diversity. Surveying the twenty-year history of the field while also highlighting its latest findings and innovations, this volume provides a readily understandable introduction to the foundations of epigenetics. Reaching beyond biology, epigenetics now informs work on drug addiction, the long-term effects of famine, and the physical and psychological consequences of childhood trauma. Finally, it concludes with a discussion of the future directions for this research and its ability to improve human health and well-being.

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Chapter 1

An Ugly Toad and an Elegant Man

Like the toad, ugly and venomous,
Wears yet a precious jewel in his head
William Shakespeare

Humans are composed of about 50 to 70 trillion cells. That's right, 50,000,000,000,000 cells. The estimate is a bit vague but that's hardly surprising. Imagine we somehow could break a person down into all their individual cells and then count those cells, at a rate of one cell every second. Even at the lower estimate it would take us about a million and a half years, and that's without stopping for coffee or losing count at any stage. These cells form a huge range of tissues, all highly specialized and completely different from one another. Unless something has gone very seriously wrong, kidneys don't start growing out of the top of our heads and there are no teeth in our eyeballs. This seems very obvious - but why don't they? It's actually quite odd, when we remember that every cell in our body was derived from the division of just one starter cell. This single cell is called the zygote. A zygote forms when one sperm merges with one egg. This zygote splits in two; those two cells divide again and so on, to create the miraculous piece of work, which is a full human body. As they divide the cells become increasingly different from

one another and form specialized cell types. This process is known as differentiation. It's a vital one in the formation of any multicellular organism.

If we look at bacteria down a microscope then pretty much all the bacteria of a single species look identical. Look at certain human cells in the same way - say, a food-absorbing cell from the small intestine and a neuron from the brain - and we would be hard pressed to say that they were even from the same planet. But so what? Well, the big 'what' is that these cells started out with exactly the same genetic material as one another? And we do mean exactly - this has to be the case, because they came from just one starter cell, that zygote. So the cells have become completely different even though they came from one cell with just one blueprint.

One explanation for this is that the cells are using the same information in different ways and that's certainly true. But it's not necessarily a statement that takes us much further forwards. In a 1960 adaptation of H. G. Wells's *The Time Machine,* starring Rod Taylor as the time traveling scientist, there's a scene where he shows his time machine to some learned colleagues (all male, naturally) and one asks for an explanation of how the machine works. Our hero then describes how the occupant of the machine will travel through time by the following mechanism:

In front of him is the lever that controls movement. Forward pressure sends the machine into the future.

Backward pressure, into the past. And the harder the pressure, the faster the machine travels.

Everyone nods sagely at this explanation. The only problem is that this isn't an explanation; it's just a description. And that's also true of that statement about cells using the same information in different ways - it doesn't really tell us anything, it just re-states what we already knew in a different way.

What's much more interesting is the exploration of *how* cells use the same genetic information in different ways. Perhaps even more important is how the cells remember and keep on doing it. Cells in our bone marrow keep on producing blood cells, cells in our liver keep on producing liver cells. Why does this happen?

One possible and very attractive explanation is that as cells become more specialized they rearrange their genetic material, possibly losing genes they don't require. The liver is a vital and extremely complicated organ. The website of the British Liver Trust[1] states that the liver performs over 500 functions, including processing the food that has been digested by our intestines, neutralizing toxins and creating enzymes that carry out all sorts of tasks in our bodies. But one thing the liver simply never does is transport oxygen around the body. That job is carried out by our red blood cells, which are stuffed full of a particular protein, hemoglobin. Hemoglobin binds oxygen in tissues where there's lots available, like our lungs, and then releases it

when the red blood cell reaches a tissue that needs this essential chemical, such as the tiny blood vessels in the tips of our toes. The liver is never going to carry out this function, so perhaps it just gets rid of the hemoglobin gene, which it simply never uses.

It's a perfectly reasonable suggestion - cells could simply lose genetic material they aren't going to use. As they differentiate, cells could jettison hundreds of genes they no longer need. There could of course be a slightly less drastic variation on this - maybe the cells shut down genes they aren't using. And maybe they do this so effectively that these genes can never ever be switched on again in that cell, i.e. the genes are irreversibly inactivated. The key experiments that examined these eminently reasonable hypotheses - loss of genes, or irreversible inactivation - involved an ugly toad and an elegant man.

Turning back the biological clock

The work has its origins in experiments performed many decades ago in England by John Gurdon, first in Oxford and subsequently Cambridge. Now Professor Sir John Gurdon, he still works in a lab in Cambridge, albeit these days in a gleaming modern building that has been named after him. He's an engaging, unassuming and striking man who, 40 years on from his groundbreaking work, continues to publish research in a field that he essentially founded.

John Gurdon cuts an instantly recognizable figure around Cambridge. Now in his seventies, he is tall, thin and has a wonderful head of swept back blonde hair. He looks like the quintessential older English gentleman of American movies, and fittingly he went to school at Eton. There is a lovely story that John Gurdon still treasures a school report from his biology teacher at that institution which says, 'I believe Gurdon has ideas about becoming a scientist. In present showing, this is quite ridiculous.'[2] The teacher's comments were based on his pupil's dislike of mindless rote learning of unconnected facts. But as we shall see, for a scientist as wonderful as John Gurdon, memory is much less important than imagination.

In 1937 the Hungarian biochemist Albert Szent-Gyorgyi won the Nobel Prize for Physiology or Medicine, his achievements including the discovery of vitamin C. In a phrase that has various subtly different translations but one consistent interpretation he defined discovery as, 'To see what everyone else has seen but to think what nobody else has thought'[3]. It is probably the best description ever written of what truly great scientists do. And John Gurdon is truly a great scientist, and may well follow in Szent-Gyorgyi's Nobel footsteps. In 2009 he was a co-recipient of the Lasker Prize, which is to the Nobel what the Golden Globes are so often to the Oscars. John Gurdon's work is so wonderful that when it is first described it seems so obvious, that anyone could have done it. The questions he asked, and the ways in which he answered them, have that

scientifically beautiful feature of being so elegant that they seem entirely self-evident.

John Gurdon used non-fertilized toad eggs in his work. Any of us who has ever kept a tank full of frogspawn and watched this jelly-like mass develop into tadpoles and finally tiny frogs, has been working, whether we thought about it in these terms or not, with fertilized eggs, i.e. ones into which sperm have entered and created a new complete nucleus. The eggs John Gurdon worked on were a little like these, but hadn't been exposed to sperm.

There were good reasons why he chose to use toad eggs in his experiments. The eggs of amphibians are generally very big, are laid in large numbers outside the body and are see-through. All these features make amphibians a very handy experimental species in developmental biology, as the eggs are technically relatively easy to handle. Certainly a lot better than a human egg, which is hard to obtain, very fragile to handle, is not transparent and is so small that we need a microscope just to see it.

John Gurdon worked on the African clawed toad *(Xenopus lae- vis,* to give it its official title), one of those John Malkovich ugly- handsome animals, and investigated what happens to cells as they develop and differentiate and age. He wanted to see if a tissue cell from an adult toad still contained all the genetic material it had started with, or if it had lost or irreversibly inactivated some as the cell became more specialized. The way he did this was to take a nucleus

from the cell of an adult toad and insert it into an unfertilized egg that had had its own nucleus removed. This technique is called somatic cell nuclear transfer (SCNT), and will come up over and over again. 'Somatic' comes from the Greek word for 'body'.

After he'd performed the SCNT, John Gurdon kept the eggs in a suitable environment (much like a child with a tank of frog- spawn) and waited to see if any of these cultured eggs hatched into little toad tadpoles. The experiments were designed to test the following hypothesis: 'As cells become more specialized (differentiated) they undergo an *irreversible* loss/inactivation of genetic material.' There were two possible outcomes to these experiments:

Either

The hypothesis was correct and the 'adult' nucleus has lost some of the original blueprint for creating a new individual. Under these circumstances an adult nucleus will never be able to replace the nucleus in an egg and so will never generate a new healthy toad, with all its varied and differentiated tissues.

Or

The hypothesis was wrong, and new toads can be created by removing the nucleus from an egg and replacing it with one from adult tissues.

Other researchers had started to look at this before John Gurdon decided to tackle the problem - two scientists called Briggs and King using a different amphibian, the

frog *Rana pipiens*. In 1952 they transplanted the nuclei from cells at a very early stage of development into an egg lacking its own original nucleus and they obtained viable frogs. This demonstrated that it was technically possible to transfer a nucleus from another cell into an 'empty' egg without killing the cell. However, Briggs and King then published a second paper using the same system but transferring a nucleus from a more developed cell type and this time they couldn't create any frogs. The difference in the cells used for the nuclei in the two papers seems astonishingly minor - just one day older and no froglets. This supported the hypothesis that some sort of irreversible inactivation event had taken place as the cells differentiated. A lesser man than John Gurdon might have been put off by this. Instead he spent over a decade working on the problem.

The design of the experiments was critical. Imagine we have started reading detective stories by Agatha Christie. After we've read our first three we develop the following hypothesis: 'The killer in an Agatha Christie novel is always the doctor.' We read three more and the doctor is indeed the murderer in each. Have we proved our hypothesis? No. There's always going to be the thought that maybe we should readjust one more to be sure. And what if some are out of print, or unobtainable? No matter how many we read, we may never be entirely sure that we've read the entire collection. But that's the joy of *disproving* hypotheses. All we need is one instance in

which Poirot or Miss Marple reveal that the doctor was a man of perfect probity and the killer was actually the vicar, and our hypothesis is shot to pieces. And that is how the best scientific experiments are designed - to disprove, not to prove an idea.

And that was the genius of John Gurdon's work. When he performed his experiments what he was attempting was exceptionally challenging with the technology of the time. If he failed to generate toads from the adult nuclei this could simply mean his technique had something wrong with it. No matter how many times he did the experiment without getting any toads, this wouldn't actually prove the hypothesis. But if he *did* generate live toads from eggs where the original nucleus had been replaced by the adult nucleus he would have *disproved* the hypothesis. He would have demonstrated beyond doubt that when cells differentiate, their genetic material isn't irreversibly lost or changed. The beauty of this approach is that just one such toad would topple the entire theory - and topple it he did.

John Gurdon is incredibly generous in his acknowledgement of the collegiate nature of scientific research, and the benefits he obtained from being in dynamic laboratories and universities. He was lucky to start his work in a well set-up laboratory, which had a new piece of equipment, which produced ultraviolet light. This enabled him to kill off the original nuclei of the recipient eggs without causing too much damage, and also 'softened up' the cell so that he could use tiny glass hypodermic

needles to inject donor nuclei. Other workers in the lab had, in some unrelated research, developed a strain of toads which had a mutation with an easily detectable, but non-damaging effect. Like almost all mutations this was carried in the nucleus, not the cytoplasm. The cytoplasm is the thick liquid inside cells, in which the nucleus sits. So John Gurdon used eggs from one-strain and donor nuclei from the mutated strain. This way he would be able to show unequivocally that any resulting toads had been coded for by the donor nuclei, and weren't just the result of experimental error, as could happen if a few recipient nuclei had been left over after treatment.

John Gurdon spent around fifteen years, starting in the late 1950s, demonstrating that in fact nuclei from specialized cells *are* able to create whole animals if placed in the right environment i.e. an unfertilized egg[4]. The more differentiated/specialized the donor cell was, the less successful the process in terms of numbers of animals, but that's the beauty of disproving a hypothesis - we might need a lot of toad eggs to start with but we don't need to end up with many live toads to make our case. Just one non- murderous doctor will do it, remember?

So John Gurdon showed us that although there is something in cells that can keep specific genes turned on or switched off in different cell types, whatever this something is, it can't be loss or permanent inactivation of genetic material, because if he put an adult nucleus into the right environment - in this case an 'empty' unfertilized egg

- it forgot all about this memory of which cell type it came from. It went back to being a naive nucleus from an embryo and started the whole developmental process again.

Epigenetics is the 'something' in these cells. The epigenetic system controls how the genes in DNA are used, in some cases for hundreds of cell division cycles, and the effects are inherited from when cells divide. Epigenetic modifications to the essential blueprint exist over and above the genetic code, on top of it, and program cells for decades. But under the right circumstances, this layer of epigenetic information can be removed to reveal the same shiny DNA sequence that was always there. That's what happened when John Gurdon placed the nuclei from fully differentiated cells into the unfertilized egg cells.

Did John Gurdon know what this process was when he generated his new baby toads? No. Does that make his achievement any less magnificent? Not at all. Darwin knew nothing about genes when he developed the theory of evolution through natural selection. Mendel knew nothing about DNA when, in an Austrian monastery garden, he developed his idea of inherited factors that are transmitted 'true' from generation to generation of peas. It doesn't matter. They saw what nobody else had seen and suddenly we all had a new way of viewing the world.

The epigenetic landscape

Oddly enough, there was a conceptual framework that was in existence when John Gurdon performed his work.

Go to any conference with the word ‘epigenetics’ in the title and at some point one of the speakers will refer to something called ‘Waddington’s epigenetic landscape’. They will show the grainy image seen in Figure 1.1.

Conrad Waddington was a hugely influential British polymath. He was born in 1903 in India but was sent back to England to go to school. He studied at Cambridge University but spent most of his career at the University of Edinburgh. His academic interests ranged from developmental biology to the visual arts to philosophy, and the cross fertilization between these areas is evident in the new ways of thinking that he pioneered.

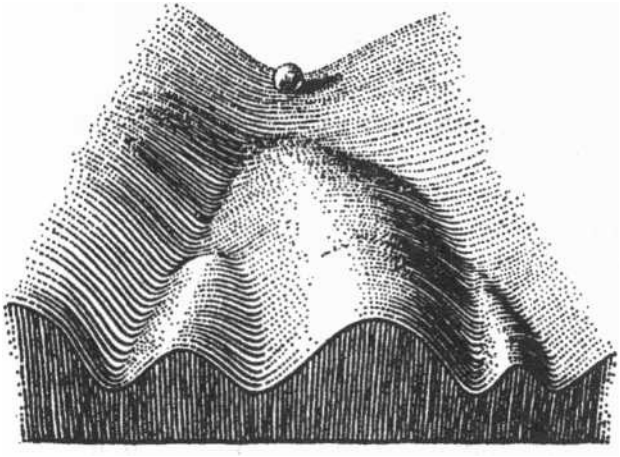


Figure 1.1 The image created by Conrad Waddington to represent the epigenetic landscape. The position of the ball represents different cell fates.

Waddington presented his metaphorical epigenetic landscape in 1957 to exemplify concepts of developmental biology[5]. The landscape merits quite a bit of discussion. As you can see, there is a ball at the top of a hill. As the ball rolls down the hill, it can roll into one of several troughs towards the bottom of the hill. Visually this immediately suggests various things to us, because we have all at some point in our childhood rolled balls down hills, or stairs, or something.

What do we immediately understand when we see the image of Waddington's landscape? We know that once a ball has reached the bottom it is likely to stay there unless we do something to it. We know that to get the ball back up to the top will be harder than rolling it down the hill in the first place. We also know that to roll the ball out of one trough and into another will be hard. It might even be easier to roll it part or all of the way back up and then direct it into a new trough, than to try and roll it directly from one trough to another. This is especially true if the two troughs we're interested in are separated by more than one hillock.

This image is incredibly powerful in helping to visualize what might be happening during cellular development. The ball at the top of the hill is the zygote, the single cell that results from the fusion of one egg and one sperm. As the various cells of the body begin to differentiate (become more specialized), each cell is like a ball that has rolled further down the hill and headed into

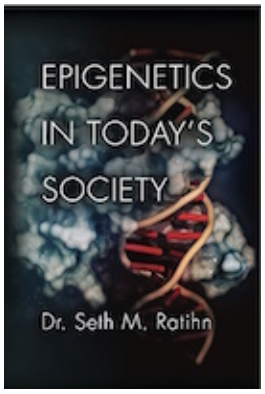
one of the troughs. Once it has gone as far as it can go, it's going to stay there. Unless something extraordinarily dramatic happens, that cell is never going to turn into another cell type (jump across to another trough). Nor is it going to move back up to the top of the hill and then roll down again to give rise to all sorts of different cell types.

Like the time traveller's levers, Waddington's landscape at first just seems like another description. But it's more than that, it's a model that helps us to develop ways of thinking. Just like so many of the scientists in this chapter, Waddington didn't know the details of the mechanisms but that didn't really matter. He gave us a way of thinking about a problem that was useful.

John Gurdon's experiments had shown that sometimes, if he pushed hard enough, he could move a cell from the very bottom of a trough at the bottom of the hill, right the way back up to the top. From there it can roll down and become any other cell type once more. And every toad that John Gurdon and his team created taught us two other important things. The first is that cloning - the recreation of an animal from the cells of an adult - is possible, because that's what he had achieved. The second thing it taught us is that cloning is really difficult, because he had to perform hundreds of SCNTs for every toad that he managed to generate.

That's why there was such a furor in 1996 when Keith Campbell and Ian Wilmot at the Roslin Institute created the first mammalian clone, Dolly the sheep[6]. Like John

Gurdon, they used SCNT. In the case of Dolly, the scientists transferred the nucleus from a cell in the mammary gland of an adult ewe into an unfertilized sheep egg from which they had removed the original nucleus. Then they transplanted this into the uterus of a recipient ewe. Pioneers of cloning were nothing if not obsessively persistent. Campbell and Wilmut performed nearly 300 nuclear transfers before they obtained that one iconic animal, which now revolves in a glass case in the Royal Scottish Museum in Edinburgh. Even today, when all sorts of animals have been cloned, from racehorses to prize cattle and even pet dogs and cats, the process is incredibly inefficient. Two questions have remained remarkably pertinent since Dolly tottered on her soon to be prematurely arthritic legs into the pages of history. The first is why is cloning animals so inefficient? The second is why are the animals so often less healthy than 'natural' offspring? The answer in both cases is epigenetics, and the molecular explanations will become apparent as we move through our exploration of the field. But before we do, we're going to take our cue from H. G. Wells's time traveller and fast-forward over thirty years from John Gurdon in Cambridge to a laboratory in Japan, where an equally obsessive scientist has found a completely new way of cloning animals from adult cells.



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