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**Caledonian Research Foundation Prize Lecture**  
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***Implementing the Promise of Stem Cells in Science and Medicine***

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It is a great pleasure for me to give this lecture in Edinburgh. I thought I might start off by talking about the origins of the nervous system and explain why I became interested in stem cells.

This story takes place after a lecture at the old medical school, just on top of the hill here, by a man called Michael Gates who was, appropriately enough, interested in the development of the visual system. He had been telling us about how the retina is connected to the rest of the brain and about the experiments of an American called Roger Sperry, who concluded that the initial contact between the axons – the wires going from the eye into the brain – was appropriately mapped. As you look at me here, the image on your retina is mapped directly before it goes into your brain. Sperry's conclusion was that, prior to functional connections – information flowing to the system – these wires from the retinal detectors had a position which must be specified by chemicals that told the brain that this wire was different from the next one. So, as I walked out of the building, I can remember quite distinctly thinking "*these academics they'll say anything*". It seemed that there was no possibility of ever figuring out the biochemistry that Sperry's hypothesis had suggested. Sperry was a very distinguished scientist, got the Nobel Prize, so my irritation was an intellectual one let's just say.

Subsequently, there was a technology developed in Cambridge – hybridoma technology – which allowed the generation of a precise series of chemical probes, even though the initial chemical had not been purified, because it was using the immune system to make specific antibodies. This technology demonstrated that Sperry had been right and that the nervous system is hugely complicated at the level of chemistry.

So now we had another problem. How on earth were we going to understand all of this? What were all these molecules doing? What were they talking to? This seemed to be a much bigger problem than Sperry had suggested initially.

It seemed to me that the solution to this problem was the idea of stem cells. We knew from work in Holland and at CalTech that, in spite of its extraordinary complexity in adult animals, the vertebrate nervous system is derived from a very simple tube of cells. The theory was that different neurones are made in different positions in the nervous system because different signals impinge on this, initially very simple group of identical cells.

The first point that I want to make, therefore, is that the whole idea of stem cells in the nervous system and in another tissues, but I am using the nervous system to make the general point, is old. It is not something that was suddenly dreamed up in the last five years by somebody who wanted to have their name on the front page of the world's newspapers. We knew a long time ago that the nervous system was composed of cells that developmental biologists call an 'equivalence group', which simply means that they all have the same potential. This is one way to define a stem cell.

My next goal was to pull out these cells, to get hold of them and see what they can do. This has taken quite a while but that's the basic underlying idea. The field is presented as if its primary role in medicine is an applied role. But I want to discuss the potential of stem-cell biology in the context of two neurological diseases: stroke, an acute injury to the nervous system; and Parkinson's disease, a chronic, late-onset neurodegenerative disease. But I want you to remember that the field is also hugely important scientifically. That Sperry's initial interest in the way the nervous system was constructed and later contributions were about the fundamental organisation of life and our ability to use the nervous system to detect changes in our environment.

Growing up in Edinburgh, of course, I was exposed to a way of thinking which was not simply a set up to pass exams, but which indicated that I had actually read – or at least could pretend that I had read – things that David Hume and others had written, about the nature of thought. Although I am going to present a lot of this work as if its primary motivation was applied, that's just a ruse really. That's not completely a joke, because it seems to me that it is fundamentally misguided to view this field as threatening our understanding of human dignity. In fact, completely the opposite is true. It is through our understanding of the nervous

system and the development of our abilities that we gain in our knowledge of what makes us human and what makes us able to perceive, not just tragedy or disease and how to respond to it, but all kinds of other wonderful things that human beings do with their nervous systems.

This lecture is going to contain scientific results, but I am going to present them in this sequence talking about stroke, which is an ischaemic injury to the brain with the blood supplies blocked – and the nervous system is hugely sensitive to such a change. In that context, I am going to discuss a very basic cell-survival pathway which regulates the possibility of regenerating adult tissue. Then I am going to talk about Parkinson's disease and three specific issues in the context of Parkinson's disease. How can we generate dopamine neurones? What are the mechanisms that control their survival? Finally, I will also talk about where in the developing organism dopamine neurones actually come from.

The basic idea in stem-cell biology is that if you can control or understand the mechanisms that regulate cell number and cell type, and you understand that well enough to be able to generate differentiated cells which carry out the functions that are found in adult cells, then that's a technology of tremendous promise. So I want to come back to this idea of an equivalence group – that there are cells in the early development of the brain that can give rise to many kinds of cell. The three main cell types in the brain are: neurones, cells that are wired up and can pass information very rapidly from one to the other; and two different kinds of glial cell, the astrocytes and the oligodendrocytes. If you think of a stem cell as giving rise to these three types of cell in a kind of flow-diagram way and has to make binary decisions, you can ask whether it can only do one thing at a time, or are all the fates present in the cell at one time? How does it happen?

To look at questions like that, we built a machine which allows us to image this process over time, so we could see the number of days that it takes for stem cells to turn into the many cell types in the brain. Any individual cell can give rise to all the different cell types and it takes about a week. You can't speed it up. Cells divide, they generate daughter cells – sibling cells – to be politically correct and at the end we can identify which type of cell they are. We can also see that some cells have no real relationship to their siblings, but there are others of the same type which cluster together. This then allowed us to go backwards in time to ask when this restriction occurred. When exactly did these cells acquire this restriction, so they only generated cells of a particular type?

At the fifth cell division, we took away protein FGF because we believed it was keeping the cells in division and when we took it away in a manner that we hadn't understood, we were triggering the cells perhaps to stop dividing so rapidly, and to change from the stem-cell state into the differentiated state. We then asked how quickly the change occurred and the answer was almost immediately following withdrawal of the FGF protein. That, I thought, is "very cool". I am not going to go into this in a lot of detail, but you can imagine how we are looking with more and more precision at this issue. When it happens and what actually happens? Nobody really knows. Nobody really knows how one cell gives rise to another cell.

This raises a lot of interesting issues, but I want to illustrate just one of the potential uses of this approach. When you withdraw the protein and begin to restrict the growth of the cells, they acutely switch the expression of genes which encode for very important proteins. Stem cells have a special protein in the nucleolus – a structure critically important in controlling cell growth. When you initiate the change from the stem cell to the more restricted cells, this protein is switched off.

The nucleolus is an important structure which has been studied for many years. One of the most famous scientists to have worked at the Cold Spring Harbor Laboratories in New York is a woman called Barbara McClintock, a hugely interesting intellectual and eclectic lady. On one of my visits there, when I was still an undergraduate in Edinburgh, I was chatting away to Barbara about what I thought the nucleolus did and she listened to me quietly and then she said to me, "*Well Ron, the reason I called it the nucleolus...*". Barbara knew something important was happening with the nucleolus; that it was regulating growth and organising aspects of nuclear structure and the way this protein is involved in regulating growth looked to be hugely interesting. If you take the protein out of the cell, the cell comes out of the growth state but how? It now seems that it does so by interacting with another protein – P53 – the control of which is important in human cancers.

Mentioning this allows me to make a very general point about the relationship of stem-cell biology and cancer – which is the transition from the stem-cell state to a state where cells have a restricted fate. The growth control switches very rapidly between these two states and involves proteins that are of a very general importance in our understanding of cancer. Although I'm talking about data based on the nervous system, the same rule applies in every tissue. This means that in vertebrates at least, we are beginning to understand the biochemistry that controls the size of the different compartments that regulate the size of our tissues. Stem-cell biology is increasingly viewed as having a very important role in cancer biology and my

view is that understanding this transition between the stem cells and their immediate progeny is going to be extremely important.

I want to take this interesting growth and survival to another dimension for you and talk about a very simple experiment. In this, we took the stem cells from the nervous system at the stage of its development when all the cells would be roughly the same. We put the cells in a dish and then we asked: "*do they live or die*"? We know precisely when the cells die because we were able to photograph them every 15 minutes. This is important, because the cells that die tend to disappear very fast – they just 'blow up' and go away. So they are not there to see if you just look at the end of the experiment. The experiment showed that the cells die, essentially, very early. But if you add a single protein, Delta 4, to the system, you immediately stop this death process.

Delta 4 is a protein with a very special type of receptor, called the notch receptor (discovered in 1919 by people working on the development of the fruit fly, *Drosophila*). In subsequent decades, people became increasingly interested in this receptor because it has the very unusual property of controlling pattern. There are many genes you could study as a developmental biologist, but what you want to understand is the pattern of the organism, "*how is the general organisation of the fruit fly controlled*"? The notch receptor is very important and has taught us a lot about how the overall pattern of flies, mice and men is controlled. It was thought that when the notch receptor is activated when ligands such as Delta 4 bind to it, it is cut by protease and the internal part of the receptor goes to the nucleus, where it turns on genes and the cell responds to that.

But that process takes time, and the response we were seeing in our experiments happens very rapidly. This very rapid death suggested to us that it could not be under the control of the classic function of the notch receptor, so we came up with a story which has important features and I will discuss in more detail later, but summarise for you now.

We showed that the notch receptor directly activates the classic cancer growth pathway, in which proteins are changed by phosphorylation cascade. The receptor is bound and, in most cases people think of these receptors being like the insulin receptor, a series of enzymes carries this information into the cell cytoplasm (not immediately into the nucleus) by modifying the proteins reversibly by adding phosphate groups to them.

It transpired that this positive pathway, which is controlling growth, is inhibited by another well-known pathway. At this point, we are beginning to understand how one might stimulate and regulate the growth of stem cells, because stem-cell biologists need a lot of stem cells! Knowing this is not just something that helps you grow cells in the lab but it also helps you grow cells in tissues.

Using rats with an induced ischaemic injury – which results in a region of the brain being deprived of blood and with very reproducible damage to the underlying brain tissue – we used a pump to introduce the protein, Delta 4, into the space in the middle of the brain. This caused a massive stimulation of stem cells in the brain and a very interesting effect on the behavioural recovery of the animals.

My basic message to you is that you have to do the science to understand what you are doing. But the message is not that you understand it, but it's so complicated that it'll take forever to turn into benefit. The message is that you have to understand it, because if you don't, you will never know what the benefit is. Sometimes, very simple experiments immediately show potential clinical benefits, and this is one.

In stroke there are very few available treatments for people who have suffered this kind of ischaemic damage to the nervous system. There is some acute clinical care that can be applied to the injury itself, but subsequently, it's very hard for individuals to be treated in a general way after an injury of this type. But these kinds of results suggest that there is indeed a regenerative process in the nervous system and that perhaps we can look forward to a systematic understanding of the mechanisms that might underlie therapies for stroke.

Now I will discuss some of the data and give you a sense of how we came to these conclusions. If you add the ligands, Delta 4 or Kappa 1 to the notch receptor, you get an immediate activation of the enzyme AKT. This activation is represented by a change in the phosphorylation status of two particular amino acids, which goes up and down in 5 minutes. We are thus able to map how the whole pathway is activated sequentially over time, in pulses, in minutes. Downstream, however, there are changes that take place days later, which is of great interest to those involved in either stem-cell biology or cancer biology.

So for example, there is the growth factor sonic hedgehog, which activates another receptor pathway of major interest in contemporary research in cancer biology. Our results have shown that the notch receptor is activating the sonic hedgehog system. A further experiment showed that other growth factors can inhibit the pathway at between 30 and 60 minutes. This information allows us to manipulate in both positive and

negative pathways, something we have done using the cells which give rise to the pancreatic islets, the insulin-producing cells and were able to increase their numbers. This is important because, although the biochemistry is a little complicated, the conclusions are obvious. Further, these pathways are activated in tissue and that's also an obvious extension of interest.

In another experiment, we inserted a needle into the space that exists in all of our brains and deposited the Delta 4 protein once only into the space. Five days later, there was a massive increase in the stem cells in that region of the adult central nervous system. What are these cells? Are they present all over the brain? Are they influencing all kinds of other behaviours that the brain performs? We are just going to ask one question: *"if we give this rat a stroke, what happens if we pump in these proteins for a few days?"*

What we are doing here is something very simple. A lot of the time when people think about measuring behaviour in animals, they think it is complicated but if it was complicated, we couldn't do it in a Federal facility in the United States. So we do very simple things. For example, you give a rat an injury of this type, you hold it up, you place it up against a table and you see which paw does it puts down first. If you injured one side of the brain, it tends not to use the paw that is regulated by that side of the brain.

So you can develop a very simple set of tests to look at the motor behaviour of an injured rat. What you see is that the animals get progressively worse over the next six weeks. In the control group, we put artificial cerebral spinal fluid (ACSF) on its own into the brain; but when we added either Delta 4 or FGF2 to the ACSF, the animals were stabilised. When both proteins were added together, there was a very clear behavioural benefit that lasted for several weeks.

Now we need to be clear about this. We are not saying that we have found a therapy for stroke. What we are saying is that we have a test to ask questions about why these animals are showing this behavioural benefit. Further, there are a multiple cell types that could be involved and only one type is of the nervous system. There are other major cell types to consider. First, those of the vascular system – after all, it was this which was damaged initially to cause the stroke. The second very important tissue system that is very likely to be involved here is the immune system. It is increasingly clear that in almost every area of medicine, that there are multiple cell types interacting to generate the disease, or available to be manipulated to generate the benefit.

A major component of the benefit is the manipulation of the immune response that follows this kind of injury. But I am trying to take you through a set of experiments that end up in this behavioural test after injury, to give you a sense of where this field might be going. It seems very exciting that it is possible to control the numbers and activity of stem cells in adult tissues by manipulating such a fundamental pathway. One way to view these responses is that regenerative biology – my main interest – is actually just the flip-side of cancer biology. The pathway that I have discussed is the classic cancer pathway. If you invited one of your colleagues interested in the signalling mechanisms that regulate cancer she/he would understand every feature of that pathway. The exciting conclusion is that, if this is the case, then we already know a lot about the molecular mechanisms that regulate regeneration in tissues, which would suggest that perhaps this field is going to start moving even faster.

Now I want to talk about a different kind of injury. In this case, it's one that comes on in an expected way. You don't suddenly develop Parkinson's disease. You slowly find that you cannot perform certain tasks that previously you took for granted. I asked one woman I know who has Parkinson's disease when she first knew that there was something wrong. She said that she goes riding all the time and because the saddle is heavy, she picked it up in a particular way to put it on a fence. One day she realised she simply couldn't stand and swing it to her right, but had to turn round and do it the other way. She thought this completely weird so went to the doctor, who called in the neurologist, who told her she had this progressive motor disease, Parkinson's disease. So, Parkinson's disease very often first appears in an asymmetric way and the main advances in our understanding about Parkinson's disease have come in recent years from human genetics.

I now want to tell you a story about human genetics and use it as a way of setting your imagination. I want to set your expectations in a particular way. When I was a student, I went to lectures and eventually got captured by the idea of DNA. There was a particular group that took pity on me and showed me how to do things. One of those in the group was a person called Ed Southern – who became famous because he developed a technology that made it possible to measure the chemical distance between genes for the first time. Before that, very complicated and tedious genetics experiments were needed, which required breeding animals to know where genes were and how the resultant animals were related to each other. Ed had developed this technology, which came to be named after him, the Southern Blot. The Southern Blot measured the chemical distances between genes and was the beginning of the Human Genome Project. Now we know where all human genes are in relation to one another and their sequences. We also now

know that many individuals in the population carry mutations in specific genes that influence their risk of getting Parkinson's disease.

The Southern Blot paper was published in 1975; 30 years later we are in a completely transformed world. I am asking you to use that measurement to calibrate your imagination and not expect necessarily that we're going to have a cure for Parkinson's disease, or radically improve our understanding of Parkinson's disease tomorrow, but I hope the next 20 minutes will allow you to see that human genetics and stem-cell biology may interact in a very interesting way as we go forward to understand the mechanisms that give rise to human disease.

What happens is that you find the gene, but then it is very often a long process to understand why that particular protein is influencing the disease. How can we help?

One of the reasons Parkinson's disease became a focus for attention in the stem-cell field is that it affects a particular group of neurones – the dopamine neurones – very dramatically. Dopamine is a neurotransmitter that has very interesting effects on our nervous system, one of which is that it regulates our sense of mood, our sense of whether this experience is rewarding or not. If I could, I would give you a lot of drugs to make you think that this was a rewarding experience. I could send you out to Princes Street at the weekend and you could find a source of drugs of this type, because most recreational drugs target the dopaminergic system. Dopamine is a very important neurotransmitter, regulating the behaviour of many other regions of the brain. But the dopamine neurones themselves, the cells that make dopamine, are a very small group. They are generated in the ventral mid-brain and they send their axons all over the brain, so they have 25 times more synaptic connections than the classic types of glutaminergic neurones which sit in your frontal cortex. Dopamine neurones are a very unusual type of cell.

It is quite clear that these neurones die in Parkinson's patients. But because they are such a small group of neurones with such a big effect, the idea arose that perhaps, if the cells die, why can't you replace them? That idea has some merit and one of the reasons that our group has become well known is that we've shown that you can indeed replace missing dopamine neurones, by growing them in the lab. I am going to show you data that support the conclusion that these lab-generated neurones actually work in the brain of an animal. I am not going to try to persuade you that grafting dopamine neurones grown in the lab is the only, or perhaps the major, reason to develop our interest in where dopamine neurones come from.

What I am going to try to persuade you is that we have to be able to grow the neurones in order to study them intensely, to understand how they work and control dopamine release. How is it that when you feel something good, I understand that it is dopamine saying to other cells in your brain that this is something good? There are many more interesting things to find out about dopamine neurones than simply to grow them and stick them in an unthinking way into the heads of patients. I am going to try to illustrate that by talking about three issues around whether we can indeed look forward to growing dopamine neurones from stem cells in large numbers.

What might human genetics mean in terms of understanding the survival and function of the dopamine neurones? I am going to tell you that dopamine neurones come from events that happen very early in the development of a mammalian embryo. We will look at the idea of making clinically important cells from embryonic stem cells.

But first I want to talk about why embryonic stem cells? The answer really is very simple. Embryonic stem cells have a very special property, which is that they can be grown for a long period in a laboratory outside the animal and retain their extraordinary potential to generate all the cells of the body. It has transpired that, by rather simple manipulations, you can elicit this developmental potential in a laboratory, in a controlled culture system. You might imagine that this is not a trivial thing to do, but it's not hopelessly complicated.

In the first sets of experiments from our group we showed that we could make dopamine neurones and oligodendrocytes, which are the cells at risk in multiple sclerosis and demyelinating diseases. We could also make pancreatic islets which secrete insulin in an appropriate way when they are exposed to glucose. We did all this using mouse embryonic stem cells. Just briefly, I want to discuss the developmental potential of the group of cells that comprise the very early (8-cell stage) human embryo. Depending on your particular cultural background there are different kinds of ethical problems associated with these cells, but ethical issues clearly exist here.

I am going to talk about the developmental and clinical potential and then I will make another couple of comments about these embryonic cells and how they arise during embryonic development in something called the epiplast.

At present, we don't really know how human and mouse embryonic stem cells fit in the normal path of the early embryonic development, but we will find that out before too long. That is clearly going to be of great importance to us in terms of understanding these cells and their properties, and also of great importance to us clinically. Very important things happen early in development and which are of great importance subsequently. One of them, for example, is at the stage where the epiblast exists and some cells have already shifted away from the early pluripotent stage and are moving and specifying themselves as the different cells of the body.

One thing that has happened in female embryos is that one of the X chromosomes is inactivated. The inactivation process takes place very rapidly and with a whole set of molecules involved in it, but we know very little about it because it happens so early normally. It is, however, likely to be of great importance in different areas of medicine, for example in understanding breast cancer, where there are clear data suggesting that the X chromosome becomes reactivated with dreadful consequences for that cell and, of course, for the individual affected. I am making the point that understanding these very early events is of clear general interest.

Now I am going to discuss how we know that, using mouse embryonic stem cells, we can make dopamine neurones. Doing a very simple series of manipulations over the course of about three weeks in culture, we can generate different populations of cells from a starting group of embryonic stem cells. These manipulations result in highly enriched populations of cells. Although the body contains hundreds of different kinds of cells, if these experiments are done at different stages, you essentially get one or two cell types. This is because the cells have some kind of self-organising property. They are alive and if you get the conditions right, they grow and expand and take over the system. So by the fifth stage, we have a set of cells which seem the same as if you had simply taken out a piece of the brain and put the cells into culture.

This is a highly efficient system and the cells are alive – and that is why it is so interesting. We don't know how all these molecules interact. What we're interested in is how they interact. They grow and, if you treat them right, they make dopamine neurones. They seem to do this by closely mimicking what normally happens in development. You can then take such cells and put them into animal models of Parkinson's disease and do various kinds of tests – look at the electrical properties of individual neurones, or at the way the animals use their limbs and move.

We have shown that these grafted cells really have the functions that you would expect of normal dopamine neurones. We have also used PET to measure the dopaminergic functions of the animals. Using a radiolabel you can see that the projections of the dopamine neurones in the striatum are intense, but we can 'blow' them away with our drug. We can then see them replaced by a smaller, but quite clearly present group of dopamine projections derived from our graft. There is no question that these grafted cells have dopamine functions, measurable by electrophysiology, by behavioural tests, by direct inspection and by reviewing the responses of the cells that are 'listening' to the dopamine.

So, are we going to go around sticking cells of this type into patients? It's possible that we might do that and it seems more likely that we might do it in the case of diabetes, where the cells we are interested in form very small, localised structures – pancreatic islets – which secrete a protein that goes all over the body. But in the case of something like the nervous system, where there is a whole circuitry involved, I am sure that clinical grafting will occur. But, would it not be much simpler to know enough about these cells so that we could stop the whole process in the first place? Or slow it down significantly. Would that not be a more plausible goal?

I am now going to discuss three different kinds of data to help you think about this. One is about making dopamine neurones from a stem-cell source in the first place; the second is to explore the relationship between human genetics and our understanding of the developmental biology and function of dopamine neurones; and, finally, I'll go back to the issue of where they come from. I will talk about the first two at some length because I think you will find it interesting and I am trying to give you a sense of where, in our group, we think where we want to go with this kind of technology.

Can we make dopamine neurones from human embryonic stem cells? We take human embryonic stem cells, grow and differentiate them in much the same way as we do with mouse embryonic stem cells. You can listen to the electrical activity of the resultant neurones, about 40% of which are expressing the enzymes required to make the transmitter, dopamine. After a month in these conditions, all the neurones are firing trains of action potentials and some are hooked up to each other synaptically. They are talking to each other as neurones should. These might be considered as 'young adult' neurones, clearly functional, able to stand on their own two feet so to speak. So the answer would appear to be yes.

There are lots of questions you might ask about optimising the system, how would you show that these are exactly the same type of dopamine neurones and so on, but the most important thing to remember is that these are human cells and that this is a hugely efficient process. Meaning that, as it takes place, all the neurones are behaving in the same way. So it could be relatively easy to understand control mechanisms for the acquisition of function in these cells.

Alongside all the other things we are doing, there is information coming from the Human Genome Project. If you recall my earlier reference to the Southern Blot paper of 1975, when I named several genes as being identified quite clearly in modulating the progression of Parkinson's disease. Now there is another gene, Etya20. This came to our attention because the growth factor is selectively expressed in the dopamine neurones in adult rats, so we searched the online databases and found one at Duke University in North Carolina, which suggested that there was a change in the region of the genome close to Etya20 in human patients with Parkinson's disease. We wondered whether Etya20 is affected by this mutation and so contacted the people in Duke and asked them to look a bit closer at Etya20 among the 10-15 genes in this region. After initial reluctance, they agreed and we were fortunate.

It did seem, statistically, as if Etya20 carries this change. However, whether we are talking about Parkinson's, Alzheimer's, cancer or diabetes, we need some understanding of the underlying biology in addition to statistical arguments, to be sure that the gene is specifically affecting dopamine neurones. So one of my colleagues started growing dopamine neurones to clarify how Etya20 influences their behaviour.

It transpired that Etya20 is a survival factor for a specific sub-set of dopamine neurones in the substantia nigra. The dopamine neurones appear in the ventral tegmental area, project down in the bottom of the brain and up to the frontal cortex and their function is to control mood. They are disrupted in schizophrenia and mood disorders, for example, but the more lateral cells are selectively sensitive to the injuries that cause Parkinson's disease. They are selectively responsive to Etya20 because they carry a growth factor receptor which, when activated, causes these cells to survive and, importantly, to make more dopamine. This is exactly what we want for Parkinson's patients – something that helps them remain more active and also protects them.

We are deeply involved in this kind of analysis. The dopamine that is made in this system is packaged and secreted when the cell is firing, releasing more dopamine. Those of you interested in neuroscience, and who think that I am absolutely serious about quoting David Hume at the beginning of this lecture, will understand that this is actually very interesting. It's not just that we're thinking about keeping dopamine neurones alive, but as we do that, we are getting deep insights into how the cell works and what kinds of mechanisms it uses to function.

So now I move to my third and final point – where do dopamine neurones come from? It turns out that dopamine neurones come from a very unusual place, from the most ventral regions of the brain. We have mapped out different domains in the ventral mid-brain and it is clear that these dopamine neurones come from a site that does not generate neurones in any other region of the brain. Further, this site produces a growth factor, a morphogen, which controls the morphology or differentiation of adjacent cells. The morphogen is sonic hedgehog.

I have already mentioned sonic hedgehog and it is in a diffuse cloud in the ventral region of the brain. All the cells in this region express a transcription factor called LMX1B and all the LMX1B-positive cells all express another transcription factor, FOXA2. These cells have a very unusual function in other regions of the brain. In the hind-brain, sonic hedgehog-positive cells generate progeny which just exist in a line in the middle of the brain; but in the mid-brain they form a cloud of cells around the ventral mid-line. All of these cells have once expressed sonic hedgehog and all of them express tyrosine hydroxylase, the enzyme that is the marker for, and responsible for generating dopamine. Thus, it is clear that the tyrosine hydroxylase-positive dopamine neurones come from the floorplate – the name given to this special region.

In my introduction, I talked about the idea that the nervous system comes from an equivalence group, a group of cells which are initially equivalent, but which respond to a growth factor or a signal from another source. This is what is going on here and these floorplate cells are induced in this region by sonic hedgehog, which is first produced in the notochord. This is interesting because it shows us that the origins of the dopamine system are in a very unusual class of cells which are FOXA2-positive. FOXA2 function is required for the differentiation of the floorplates and it continues to be expressed in adult dopamine neurones.

Now I want to return to the genes which we know are influencing human Parkinson's disease, but if you knock them out in a mouse, there's not much wrong with the dopamine neurones. This is because these genes don't actually *cause* Parkinson's disease, they *influence* Parkinson's disease – as is the case for

many of the genes of great clinical interest. There is currently a lot of 'hoo-ha' in the field of Parkinson's genetics, but why? People go to an enormous amount of trouble to knock out these genes. But they have only got a gene, they don't know what the gene is doing and, in particular, they don't know why, as you age, Parkinson's disease occurs and why dopamine neurones, in particular, seem to be so sensitive.

If you knock out both copies of the FOXA2 gene it is lethal, very early and you know this because critical structures for organising the embryo are missing. But if you knock out only one copy of the gene, initially everything seems to be perfectly normal, but then very interesting things happen. As soon as the animal starts to walk, it does something that, if you were just naïve about it, you would say is a bit like Parkinson's disease. When you put the front feet of a mouse in red ink and the back feet in black ink and let the mouse walk on a sheet of paper, you see that the mouse strides across the paper, but in the animals only lacking one copy of the FOXA2 gene, they move in a sort of hopping, shuffling way – characteristically seen in Parkinson's patients. So it is clear there is something wrong with these animals early on. But they have normal numbers of dopamine neurones and are not completely messed up. As they age, however, they begin to show some very odd behavioural features. In one case, the animal had a very stiff foot, with a tail that was completely stiff and held to the side – and because the intercostal muscles were absolutely rigid, the animal could not move its muscles correctly. This is another feature found in Parkinson's disease and other diseases that affect the nigra striatal system. Finally, and most importantly, there is an asymmetric loss of dopamine neurones in these animals.

So it now seems that we have an animal model, not necessarily of Parkinson's disease, but one which will teach us why dopamine neurones are so sensitive to these diseases. We can take these other genetic tools and start mapping them on to this problem. I just want to mention a group of studies of Parkinson's patient which found a polymorphism right next to the FOXA2 gene. It seems, therefore, that some people carry a mutation which affects FOXA2 function; it is not just something that happens in mice.

So let me close by doing two things. First, I want to remind you about the different issues that I have discussed and to say that one of the great pleasures in science is that you work with a range of people who come from countries all over the world and move around the world in search of the 'ah ha' feeling that motivates scientists – the sort of intellectual motivation, which is so important in scientific progress. Then I want to come back to the general issue – why is stem-cell biology important?

It is important because we understand exactly how different genes act in these pathways. We know the FOXA2 gene is directly in the pathway, which is activated by Notch and DJ1. Another gene involved in Parkinson's disease is thought to interact with a negative regulator of AKT and, as I mentioned, FGF looks as if it is interacting with the pathway in another way.

What is emerging from our studies is a coherent understanding of the disease because we can identify specific cell types and focus our attention on them, in the cascade of cell types that are generated during development. Also, this idea is an extension of one that was very important in the origins of molecular biology and captured by the phrase attributed to Jacques Monod, a very famous French molecular biologist, namely, "*What is true for E. coli is true for the elephant*". Now of course, the opposite of this is not true. What's true for elephant is not necessarily true for *E. coli*. I mean, if you want to understand how large mammals walk across the plains of East Africa and you restrict yourself to studying *E. coli*, we might be here for a very long time. But if you want to understand the behaviour of mammals at the cellular level, then it would be very useful if you could isolate all the different cell types of a mammal and look at their properties in the same intensely-focused way that molecular biologists were able to do by using a simple organism like *E. coli*. I think that that captures in my view of the promise of stem-cell biology. This is that it is not magic, but when used in combination with other advances in contemporary medicine, it may lead us – if we are patient enough and imaginative enough and positive enough – to very interesting new therapies.

Once again, thank you very much for giving me this opportunity to come.

## QUESTION & ANSWER SESSION

**Unidentified Questioner** : *You showed that Delta 4 increased the numbers of stem cells. Are we to conclude that in a normal rat brain, stem cells are actually subject to a high degree of cell death when they are attempting to self-renewal or regenerate?*

**RM**: In fact, we know very little about the mechanisms that normally control the regenerative processes in the brain and often, when I talk about stem cells in the adult brain, people ask, well why do we have them? I think that the place we are now, is that it's like the situation where you get given an expensive motor car to drive, but you're told you can only drive it around in 1<sup>st</sup> gear, so that under normal conditions we're not really able to boost the numbers of stem cells in the brain, but what I'm saying to you is that with this new insight, it looks as if we can at least get this car into 3<sup>rd</sup> gear, and drive up to the higher gears. We can know what this system does; we really don't know what these precursors are doing in the adult brain.

*My concern was whether what you see in vitro in terms of this very dramatic death of stem cells, actually occurs in vivo and the implication of your experiment would be that this is the case. Is there any evidence other than that experiment for this?*

**RM**: You are right to ask that question, but I don't have a detailed answer for it. Remember I mentioned that multiple cell types could be involved. It looks as if not only are we going to have to develop assays to look at the numbers of nervous system stem cells that are alive or dead, but we are currently looking at the very interesting vascular system responses which are occurring alongside this. As those of you interested in stem-cell biology will know, there is a lot of interest in the possibility that cells of the vascular system are intimately involved with these niches in different tissues.

**Donald Bruce, Church of Scotland** : *Our recent General Assembly passed a report on the ethical issues of all this and we have taken the view that cellular research should be allowed. However, there is a strong preference for adult cell research, if at all possible. One of the case studies the 18-month working group looked at was on Parkinson's and what would you say are the current prospects of not needing to use embryo stem cells. Is there some realistic way of being able to use adult cells only or is this still really a 'blue skies' idea?*

**RM**: As you can see, for example, in my response to the previous question, I am perfectly capable of entertaining blue sky ideas about adult stem cells in the context of stroke. It looks extremely interesting. I am not proposing that we will rebuild the injured ischaemic adult brain, but that I am showing you that, at least, I am not somehow pathologically averse to working in adult tissues. It is very exciting, but then as I talked about Parkinson's disease, it looks to us as if dopamine neurones come from this very special and very rare kind of precursor cell and that, at present, there is no reason to think that you can generate those cells in significant numbers from any other source than embryonic stem cells. I ended with that quote from Monod about what is true for *E. coli* being true for the elephant. The first time I heard Jacques Monod talk, I was a student in Edinburgh. I knew nothing about what Monod was talking about, I just turned up at the lecture because you felt you had to, and here's this guy talking about gene expression, from the Pasteur Institute, completely commanding presence. It turns out that Jacques Monod was in the French Resistance in the Second World War and he wasn't in the French Resistance because he didn't have anything else to do, he was taking some very serious decisions as a senior member of the French Resistance – life-threatening decisions both for himself and others. At the end of his life he wrote a book called '*Chance and Necessity*' and, in the last chapter, he is concerned with ethics and he points out that everything you do has an ethical cost, an ethical measure. So it seems to me, that in the case of human embryonic stem cells, there is going to be an increasingly powerful argument that there is going to be real clinical benefit from manipulating these cells. I am not making this as an argument that scientists should do anything they want, I am saying that this is an issue that needs to be seriously addressed and that there is going to be a real need to create practical conditions which allow the cells to be obtained and studied and I, myself, don't see that as being a particular problem, not in Scotland, anyway.

**Unidentified questioner** : *Just to follow that. We hear on this side of the water that in the United States, it is difficult to carry out research in this area. How do you do it in your land?*

**RM**: They don't ban it completely. This is one of the skills of being a good politician. The current President of the United States, in one of his first television addresses to the nation, dealt with the subject of stem cells and what was said was that all embryonic stem cell lines that existed at that particular stage, in August in 2000, could be used and that Federal plants could be used to manipulate them. At NIH one of the things that a group of us are doing, is growing those cells and trying to figure out what their properties are and in my view, the current restrictions on using new cells, are not totally inhibitory. But I imagine that as the arguments get stronger for generating new cells – and I must say even those arguments are going to involve

having new cells with particular genomes in them, so that we can look at the way particular genes are interacting to regulate early developmental processes – the United States Government will generate a new policy. Whether that will happen in the next few years, I rather doubt, but I think the arguments will grow in strength.

**Questioner:** *It might even come here?*

**RM:** Precisely. I mean that is also true, this is a worldwide effort.

**Another unidentified questioner:** *You are supposed to ask big, grand questions in these talks. I have got five different specific science questions, so I'll try to avoid asking those and I will ask them later, but it's really interesting for me, you mentioning human genetics and you talk about FGF20 and the FOX gene as well. So what wasn't clear in either case was how robust the evidence is to suggest that variants in those genes do have a role in this case and it's great if somebody like you understands the biology involved and will work with a human geneticist to prove these things actually are playing a role. It wasn't clear to me whether in the first case, the FGF20, you were not looking at Mendelian forms of Parkinson's, but what cases were you looking at, what sort of variants of information and whether you think this is causative and how, in that case?*

**RM:** If you can ask a grand question, can I give a grand answer?

**Questioner:** *That wasn't a grand question, I wish it was.*

**RM:** I think it is a grand question, actually. My response to you is that there are very few genes which have very simple penetrance and cause disease, but there are a few. We need a general way of putting all of these genes that influence your risk of getting disease into a kind of context. Most people who do genetics, forgive me for saying this, think that genes cause disease because they have arrows connected to them which go the disease.

**Questioner:** *We don't believe that.*

**RM:** No, but how do they cause disease? They cause disease because they are acting in particular cells. The frequency of an allele in the population can be measured. What regulates the frequency of that allele in the population? The selective co-efficient of the gene is the force that's keeping that allele at that frequency, but that selective co-efficient isn't something that's assigned to that gene, it is in the context, in the particular gene-connected context and that is the biology, which why I believe having access to many different kinds of human cells will rapidly influence us. It will resolve this difficulty because it will give us the idea that in a particular patient, FGF20 is important and let's imagine, for example, that the mutation is an F1 regulatory site and that now we start talking about an ALS and that you can start beginning to put real mechanism on this human genetics. So that's the world I am looking forward to.

**Questioner:** *I think your talk is very relevant in the whole question of translational work which we are really being encouraged to do today. My points are these. That the neuroscientist in you is being slightly sotto voce tonight but is actually critical to your argument, which is that what makes the brain so different from many other organs in the body is that the cells are connected to each other, often by quite long-range actions. Deep down, you are slightly sceptical about our ability, either in the short term or perhaps even the long term, to repair all of that. Third, that the general public is being sold the idea that stem cells are going to cure Alzheimer's disease, or stroke, or whatever else and, fourth, that you are telling us that this is probably not going to happen, but that we are going to learn things about the fundamental biology of cells by looking at stem cells and, from that, we are going to help prevent the diseases happening in the first place.*

**RM:** Let's come back to the question that Nick asked. I am talking about a growth factor FGF20 and Nick is saying 'wrong, are you serious about this, is this really involving Parkinson's disease? What I am saying is that the genetics gives us a hint, that in some patients, this is a point of susceptibility. But in all patients this receptor is present in the most susceptible cells. So I think it is much more plausible that in 5 or 10 years, we will have ways of activating this receptor in the patient's cells, rather than going around sticking new cells into the patient's brain; particularly because I believe all these pathways are actually speaking to a common survival pathway of cells. So it is all Adam Smith actually. It is that if you want an effective economy, you have a very complex information system – actually this is Chapter One of 'The Wealth of Nations'. It is remarkable, he talks about the division of labour and what he is actually talking about, essentially in a Scottish culture and Scottish educational system, is that you have to have a remarkable amount of information flowing through your society if you want this person to be a blacksmith, this person to be a wheelwright, and this person to make barrels. He says it is amazing how much talking they do and how much education you need. So, that is to say that there are hundreds of genes all doing this, that and the next thing, but actually, if you want it to work, you have to have a common currency and there has to be an agreement about a legal system and a value of the currency. So, it seems to me that this is going to be true

in biology and that FGF20 or growth factors like this, will be understood as fast in Parkinson's disease as they will be understood in other diseases, because there is going to be a very general model about cell survival. Remember, I asked you to calibrate your imagination by a fairly dramatic length of time. I think in 20 years, here in The Royal Society of Edinburgh, this will all seem totally obvious.

**VOTE OF THANKS by Professor Nick Hastie FRS FRSE, Director, MRC Human Genetics Unit, Western General Hospital, Edinburgh.**

I really do want to thank you for a very inspiring talk, which was very clear and a great deal of circumspection in an area which has got so much hype – that was tremendous. What particularly impressed me is that you put up a slide of quotes of David Hume, but you could quote Adam Smith without having slides. Either you are really smart and ready for that question, or you're just a polymath – as we expect of Edinburgh scientists who trained here and then went away.

I suppose there are many messages from your talk, all music to my ears and to many of us here. The first is that stem-cell biology is fascinating if done right. We really do need to understand the mechanisms which control stem-cell renewal and what makes them differentiate in many ways and to understand where they come from in the first place. Unless you know that, and what regulates them, you're not really going to do anything intelligent with them in terms of therapeutic protocols. The second thing is that in some contexts, like the pancreas, it might be appropriate to put cells back – and that's what most of the public thinks we are talking about – but in others it's going to be far more likely that you would use the biological knowledge to either prevent the thing happening in the first place or to stimulate those with factors that you understand from the biology. The third thing that I love, is that you're introducing human genetics into the equation. I would say that, because we do human genetics, but what I liked at the end relates to what people in human genetics have known for some time now, that you can even study the rare in human genetics and learn about this common pathway.

It's the same arguments for outstanding Alzheimer's and many other diseases and you're talking about the common pathways. If you're wrong, often we can find out by studying the rare or the biology you're talking about and then come up with intelligent ways of treating it. As you say, it might be 15 to 20 years. So we need you out there, people like you, telling the public about it. In Edinburgh, they've bought the same arguments, to have strong developmental biology alongside stem cell work. We could go on forever and ask you more questions, but it's great to see somebody trained in Edinburgh come back as a Caledonian Prizewinner. I think you're the second, I think Ian Mattaj was the first. It's a testament to the wonderful golden era that we have had in Edinburgh science and I hope that we will be seeing some others of the young ones coming back in 20 years, or even a shorter timeframe, to be the third. On behalf of all of us, I want to thank you for your great lecture and I've got lots of questions for you over dinner and drinks.

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