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From Nuclear Transfer to Nuclear Reprogramming: The Reversal of Cell Differentiation

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nuclear transplantation, *Xenopus*, nuclear reprogramming, transcription

Abstract

This is a personal historical account of events leading from the earliest success in vertebrate nuclear transfer to the current hope that nuclear reprogramming may facilitate cell replacement therapy. Early morphological evidence in Amphibia for the toti- or multipotentiality of some nuclei from differentiated cells first established the principle of the conservation of the genome during cell differentiation. Molecular markers show that many somatic cell nuclei are reprogrammed to an embryonic pattern of gene expression soon after nuclear transplantation to eggs. The germinal vesicles of oocytes in first meiotic prophase have a direct reprogramming activity on mammalian as well as amphibian nuclei and offer a route to identify nuclear reprogramming molecules. Amphibian eggs and oocytes have a truly remarkable ability to transcribe genes as DNA or nuclei, to translate mRNA, and to modify or localize proteins injected into them. The development of nuclear transplant embryos depends on the ability of cells to interpret small concentration changes of signal factors in the community effect and in morphogen gradients. Many difficulties in a career can be overcome by analyzing in increasing depth the same fundamentally interesting and important problem.

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HOW NOT TO START

Fortunately, the greater part of my career did not follow logically from its exceptionally inauspicious beginning. At my school, students were not taught biology until the age of 15. After just one semester of starting biology, my biology teacher reported at length on my efforts by saying, among other things, that “I believe Gurdon has ideas about becoming a scientist; on his present showing this is quite ridiculous; if he can’t learn simple biological facts he would have no chance of doing the work of a specialist, and it would be a sheer waste of time, both on his part and of those who would have to teach him.” At that post-World War time (1947), there were no textbooks; we were supposed to remember what the teacher said, but I have a bad memory. For the rest of my school time I studied Latin and ancient Greek. At that time, the universities were short of applicants, and I was told that I

could have a place at Oxford so long as I did not study the subjects in which I was examined (Latin and Greek). I was allowed to study Zoology and was given an extra year in which to make up for what I had not done at school. My parents were kind enough to pay for this, in addition to the cost of the (private) school.

I had already acquired a great interest in the color patterns on the wings of butterflies and moths. How such intricate patterns are formed developmentally with great precision (Nijhout 1991), apparently by morphogen gradient interpretation, is still a highly fascinating, unsolved problem (see below). In due course, I applied to the Oxford Entomology Department to do graduate work for a PhD. Fortunately, I was turned down, my student record being judged at that stage to be too weak. Later, I applied, and was fortunately accepted, to do graduate work under the Embryology lecturer at Oxford. This was Michail Fischberg, of Latvian descent, and a student of Hadorn, himself a student of Baltzer, who studied under Spemann and Boveri (Buscaglia & Duboule 2002). Fischberg had done post-doctoral work in the Genetics Department in Edinburgh, headed by C.H. Waddington. At that time, a very high proportion of all who were researching in the area of developmental biology anywhere in Europe could trace some part of their training back to the lineage of Boveri in Germany.

NUCLEAR TRANSPLANTATION IN *XENOPUS*

Fischberg was an excellent choice of mentor. Within a few months after I started work under him in 1956, he encouraged me to try to achieve nuclear transplantation in *Xenopus*, following the first real success in transplanting living cell nuclei to eggs, reported in 1952 by Briggs & King. It is important to appreciate that a major question in developmental biology at that time was whether the genome of cells undergoes any stable changes in the course of cell differentiation. The importance of this question had been already

clear to Weismann, who in 1892 had proposed that as cells embark on a defined developmental pathway, they lose genes no longer needed to be expressed (Weismann 1892). Spemann (1928) made an early attempt to test this idea; his hair loop constriction of a salamander egg at the eight-cell stage demonstrated that at least up to this stage, nuclei are totipotent. But this did not at all exclude the possibility that genetic changes could take place subsequently when cells start to differentiate. Briggs & King's (1952) experiment, in which 30% of transplanted blastula nuclei yielded apparently normal tadpoles, was the first to provide a clear test of the genome equivalence in development. However, Briggs & King (1957) subsequently found that at the relatively early tail-bud stage, endoderm nuclei no longer supported normal development in the way in which blastula nuclei could, leading to the conclusion that irreversible nuclear changes do in fact take place as cells begin to differentiate.

Michail Fischberg was well aware of the value of genetics in developmental biology, and when I joined him, he had just started using *Xenopus* as a laboratory animal, on the grounds that it could be grown to sexual maturity within a year and that, as it is wholly aquatic, it is easy to keep in the laboratory. *Xenopus* can deliver eggs throughout the year, in contrast to the limited-season availability of eggs from *Rana* and European newts, the organisms of choice for European embryologists. The history of how a frog that naturally occurs only in Africa has come to be one of the half-dozen most used animals for research is bizarre (Gurdon & Hopwood 2000).

My first attempts to inject *Xenopus* eggs were frustrated by impenetrable jelly. By good fortune, Michail Fischberg had just bought a new UV microscope. Thinking that this could be a good way of destroying the surface-located egg chromosomes to provide enucleated recipient eggs, we found, surprisingly, that in addition to this, the very low wavelengths of emission (below 2540 Å) of the bulb also dissolved the jelly. Without this, the

extremely elastic jelly, not shared by other amphibian eggs, might have completely prevented success.

By another piece of good luck, Fischberg was also supervising a graduate student working on ploidy, for which the number of nucleoli is a reliable guide (i.e., two nucleoli per diploid nucleus). The anomalous results obtained (one nucleolus in diploid cells) would normally be attributed to the inexperience of a new student. Greatly to his credit, Fischberg traced the particular frog that produced these inexplicable results and found that it always produced one-nucleolated diploid embryos (Elsdale et al. 1958). This phenomenon later turned out to be due to a mutation that had deleted one complete set of ribosomal genes (Brown & Gurdon 1964, Wallace & Birnstiel 1966), but at the time (1958), it was an extremely useful genetic marker of nuclear transplantation, enabling us to prove beyond doubt that the original nuclear transfer embryos obtained in *Xenopus* were from the implanted nucleus (**Figure 1a**) and not from a failed UV enucleation of the egg chromosomes.

We soon obtained numerous fertile adult male and female frogs from transplanted endoderm nuclei (Gurdon 1962a) (**Figure 1b**). My *Xenopus* nuclear transfers, like those of Briggs & King (1957), showed that as cells differentiate, their nuclei become progressively less able to support normal development of enucleated eggs. However, more important in my view was the result that even the fully differentiated cells of the feeding tadpole intestine contained nuclei capable of yielding fertile male and female adult frogs (Gurdon 1962b, Gurdon et al. 1958). I believed that the derivation of entirely normal adult frogs was a more significant result than a large number of abnormal or defective embryos. I therefore took the view that as a general principle, cell differentiation can take place without any stable changes to the genome. Not surprisingly, many senior people in the field were reluctant to accept the conclusions of a graduate student over those of Briggs & King, highly

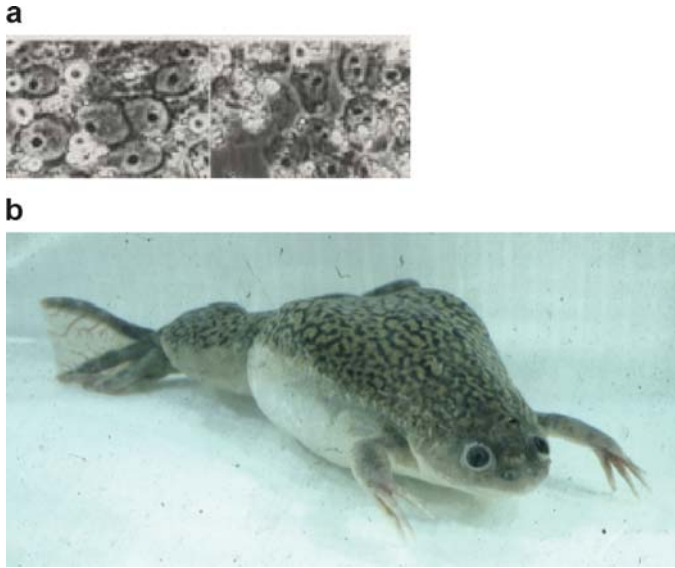


Figure 1

(a) A genetic marker first used for *Xenopus* nuclear transfer experiments. Embryos heterozygous for the complete loss of one set of ribosomal genes have a single nucleolus in their nuclei (left), in contrast to wild-type nuclei (right), which usually have two nucleoli per nucleus representing both nucleolus organizer regions. (b) The first sexually mature adult vertebrate produced by nuclear transplantation. This female frog derived from the transplanted nucleus of a neurula endoderm cell and carried the genetic marker shown in a. From Gurdon et al. (1958).

established researchers whose work was rightly regarded very highly and whose results were entirely correct for *Rana pipiens*. It has turned out that *Xenopus* is better suited for these experiments than other amphibia so far tested. We obtained normal feeding tadpoles with a full range of differentiated cell types from the nuclei of skeletal muscle (Gurdon et al. 1984) (**Figure 2**). With my colleague Ron Laskey, we were also able to obtain normal feeding-stage larvae from a wide range of adult organs such as kidney, lung, and skin (Gurdon et al. 1975, Laskey & Gurdon 1970). Defined cell types such as functional muscle and nerve can be derived by nuclear transfer from a completely unrelated cell type, such as *Xenopus* intestine, with an efficiency of approximately 30%, allowing for the combined effects of serial nuclear transfer and grafts (**Table 1**). Subsequently, DiBerardino & Hoffner (1983)

obtained larvae from adult blood cells in *R. pipiens*. The general principle of genome conservation during cell differentiation was established and has now been born out over the past several decades, except for special exceptions that include antibody-forming cells, with their need to make an enormous variety of proteins.

Recent work in mammals has established the further point that nuclear totipotency extends to the differentiated cells of adults (Hochedlinger & Jaenisch 2002). When this is coupled with gene correction, we can see how nuclear transfer could lead to replacement cell therapy (Rideout et al. 2002).

A second general principle emerged from those early experiments. This was that egg cytoplasm has remarkable powers of reprogramming somatic cell nuclei. This is in sharp contrast to the great stability of differentiation of whole cells (as opposed to nuclei) (Kato & Gurdon 1993).

CLONING

Even in 1958, when the first sexually mature adult nuclear transplant frogs were described (Gurdon et al. 1958), there was press interest and future speculation (Rorvik 1978) about the possibility of cloning humans. But this really took off with the announcement of the cloning of Dolly the sheep (Campbell et al. 1996, Wilmut et al. 1997). In mammals, as in frogs, nuclear transplant embryos, especially from adult cell nuclei, develop abnormally with a bewildering range of defects. In normal human reproduction, approximately 95% of born children appear entirely normal. By nuclear transplantation using adult donor cells, the figure is 1% for mice (Wakayama & Perry 2002) and up to 3% for cows (Tsunoda & Kato 2002). It is for this reason that nearly everyone and nearly all governments disapprove of (or forbid) reproductive cloning in humans. In frogs, nuclear transplant abnormalities are believed to be caused in part because the nucleus of a slow-dividing cell often fails to complete DNA replication in the time (1.5 h) it takes an injected egg always to

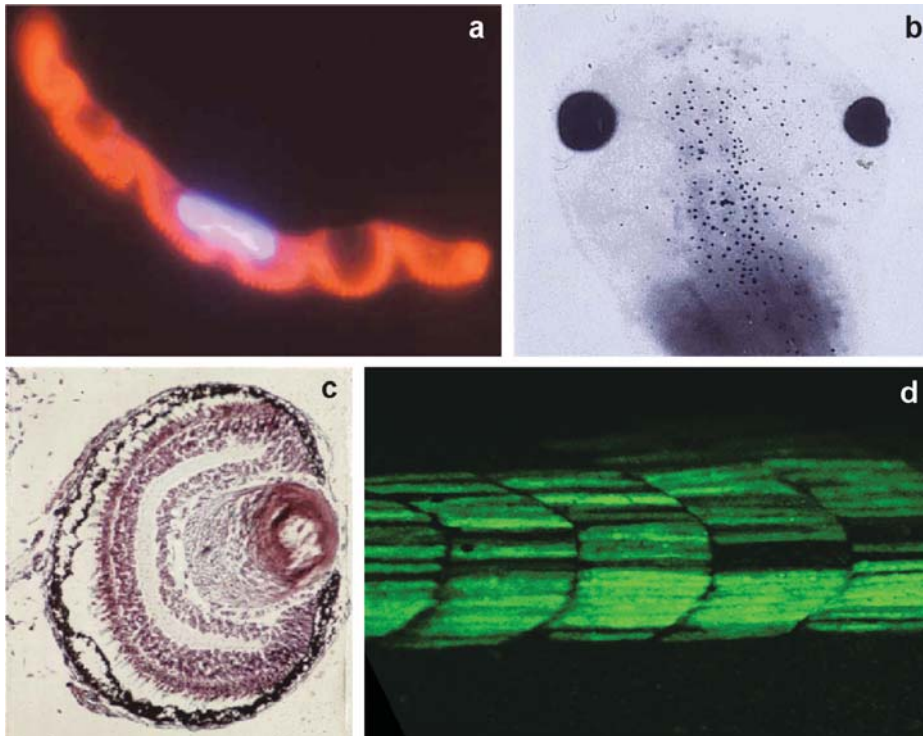


Figure 2

Nuclear reprogramming as judged by cell type morphology. (a) A functional muscle cell from a swimming tadpole; note myofibril in the cell. (b) A swimming tadpole derived from a muscle cell nucleus seen in a. (c) Eye of the muscle nuclear transplant tadpole seen in b; note normal morphology and arrangement of cells in the lens and retina. (d) Green fluorescent protein (GFP) muscle cells in a swimming tadpole obtained by grafting cells from a partial blastula, resulting from an intestine-derived nuclear transplant. GFP cells of the nuclear transplant embryo were grafted to a normal host embryo, some of whose muscle cells are seen, unmarked, intermingled with GFP muscle cells.

divide into two cells, thereby causing breakage of nonreplicated chromosomes. In mammals, there are 24 h before the first cell division, which should permit complete DNA replication. However, several key early zygotic genes, including *Oct4*, are often under-expressed in mammalian nuclear transplant

embryos (Boiani et al. 2002, Bortvin et al. 2003); this may at least partially account for the failure to develop normally.

Further insight into the cause of developmental abnormalities has recently resulted in a very unexpected conclusion. In *Xenopus* we transplanted nuclei from embryonic cells that

Table 1 Efficiency of nuclear reprogramming by nuclear transfer in *Xenopus* (Byrne et al 2003, Gurdon 1962b)

Nuclei from larval intestinal epithelial cells transplanted to eggs	Percent of total nuclear transfers to yield tadpoles with functional muscle and nerve cells
First transfers only	15%
First and serial transfers	22%
First and serial transfers and grafts	30%

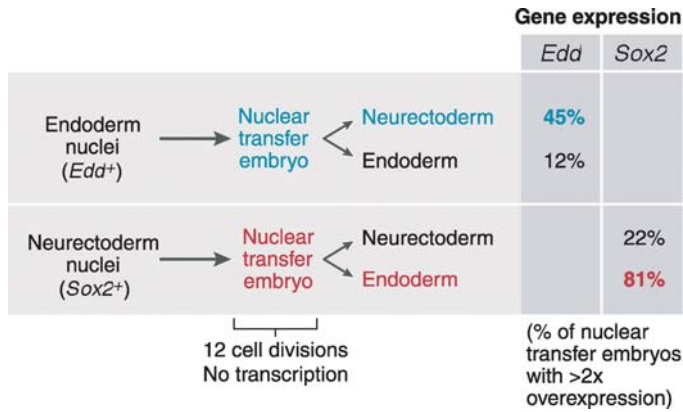


Figure 3

Inheritance of an epigenetically active gene state through nuclear transfer. Endoderm or neurectoderm cells expressing marker genes were used as donors for nuclear transfer. In some nuclear transfer embryos, the original donor marker genes were overexpressed by a factor of two or more in cells of an inappropriate lineage (Ng & Gurdon 2005).

had already started expressing cell-pathway-specific genes such as *Sox2* for neurectoderm. We found that most of the resulting nuclear transplant embryos expressed this neural gene strongly in their endoderm (Ng & Gurdon 2005) (**Figure 3**). This means that this gene, once activated, remembers its activated (but not expressed) state through at least the 12 cell cycles of no transcription up to the mid-blastula transition and in an inappropriate lineage of the embryo. This remarkably long-lasting memory of an active epigenetic state is probably important in stabilizing cell differentiation in normal development and helps to account for the decreasing success of nuclear transfers from differentiating cells.

OXFORD ZOOLOGY AND CALTECH

During my graduate student period, Fischberg was very generous in giving me sole rights to publish research, under my own name, on nuclear transfers from the endoderm lineage, although I was still in his laboratory. I was fortunate to receive offers of postdoctoral fellowships from both Briggs and King, the acknowledged leaders in the field.

However, I also had a fellowship offer from George Beadle at Caltech. Fischberg, who engineered this offer, wisely advised me to work for a while in a new field. So I bought a second-hand Chevrolet in New York and drove to Southern California on the now-famous Route 66. I visited several labs on the way, most notably that of Alexander Brink, the discoverer of paramutation in maize (Brink 1960); in my view, he should have shared the recognition enjoyed by McClintock because they codiscovered transposable elements. I enjoyed a life-long friendship with Alex (and Joyce) Brink, whom I used to visit in their winter home and lab in Florida. I remember his comment on continuing his work after retirement that “there comes a time in life when there ceases to be a distinction between research and occupational therapy.” I also visited Robert Briggs, who had moved to Bloomington, Indiana, and I met the redoubtable Tracy Sonneborn, who did remarkable grafting experiments on the ciliate *Paramecium* (Sonneborn 1977). Sonneborn had a charming but masterful personality, to the extent that all members of his lab, including the women, took up pipe smoking to be in accord with their leader’s lifestyle.

On my arrival at Caltech, I was advised by Ray Owen, the Chairman, to work with a very bright young assistant professor, Bob Edgar, on bacteriophage genetics. Though Edgar was only a couple of years older than me, his prominence was made clear to me later that year when I applied for an assistant professorship at Columbia University. At the end of my visit, the Chairman said that they did not feel able to offer me a position but added that when I got back to Caltech, I should tell Edgar that any time he chose to lift the phone, they had a full professorship waiting for him. I found I was completely inept at phage genetics but nevertheless had a very valuable year at Caltech, then small enough for me to get to know most senior biology professors, including Ed Lewis, James Bonner, Norman Horowitz, and Albert Tyler. I also had the privilege of meeting Sturtevant, one

of the cofounders of biology at Caltech with Morgan. Sturtevant was known to me by repute partly because of his interpretation of the sinistral snail genetics experiments of my uncle, Cyril Diver (Boycott & Diver 1923, Gurdon 2005).

An important part of my year at Caltech was meeting Jim Ebert, the director of the Carnegie Embryology Department in Baltimore and an increasingly influential individual who devoted much energy to placing developmental biologists in appropriate posts throughout the United States and indeed the rest of the world. Through Ebert I met and collaborated with Donald Brown, who had just started work in Baltimore on *R. pipiens*. Brown was, in my view, a founder of molecular embryology. Prior to his time, most molecular analysis of development was essentially descriptive [for example, see the three compendia by Needham (1942)]. Brown saw the need to introduce work on genes and their immediate product RNAs. He and Max Birnstiel were the first to purify a single type of gene in eukaryotes (Brown 1994, Wallace & Birnstiel 1966), well before the era of DNA cloning in bacteria. Brown's work on *Xenopus* 5S genes continued to lead the way in the molecular biology of development for another two decades (Brown 1982, 2004). He was aware of the *Xenopus* O-*nu* mutation referred to above and showed surprisingly that this mutation involved a complete lack of 18S + 28S ribosomal RNA synthesis, the O-*nu* tadpoles developing entirely normally for several days by using their maternal inheritance of ribosomes (and their newly synthesized mRNA) (Brown & Gurdon 1964). Following this early collaboration, I was fortunate enough to have further collaboration with Brown over the next few decades, including a sabbatical for six months in his lab in Baltimore.

I returned to England on having been offered Fischberg's position, he, fortuitously for me, having just accepted a professorship in Geneva, Switzerland, the country of his upbringing. Traveling to England via Japan, I had an introduction from Jim Ebert to

Tokindo Okada in Kyoto, who was one of the first scientists to leave Japan after the war and work in Waddington's Institute in Edinburgh. Okada, more than anyone else, has been instrumental in promoting developmental biology in Japan to reach its very high present level through organizing numerous meetings in Japan, apart from his pioneering work on transdifferentiation (Okada 1991). During this time, I also made contact with Lauri Saxén in Helsinki, who coauthored an outstanding book on primary embryonic induction (Saxén & Toivonen 1962). Saxén and Okada were the leaders, in my generation, of developmental biology internationally.

TOWARD MECHANISMS OF NUCLEAR REPROGRAMMING

Probably the worst stage in an academic career is when starting on one's own as a new assistant professor, with a hefty load of new lectures to be prepared, the need to acquire research support for an independent program, the wish to attract students to form a group, and no one except oneself to do the lab work with which to attract students and research support. I was very fortunate to be joined, within two years of starting at this level, by two outstanding students, Christopher Graham and Ron Laskey.

It seemed clear to me that an analysis of nuclear transplantation at a molecular level was potentially rewarding. What are the molecules and mechanisms that can reverse the gene expression pattern of a differentiated cell to an embryonic state in a remarkably short time of a few hours? I did not realize that I would still be preoccupied with the same question 40 years later. I was attracted then, as I still am today, to the principle that it pays to simplify one's problem so as to analyze one step at a time, a reductionist approach, out of favor at present with the current appeal of the so-called systems analysis. At that time it was known that the first genomic activity following normal fertilization in Amphibia is the induction of DNA synthesis 20 min

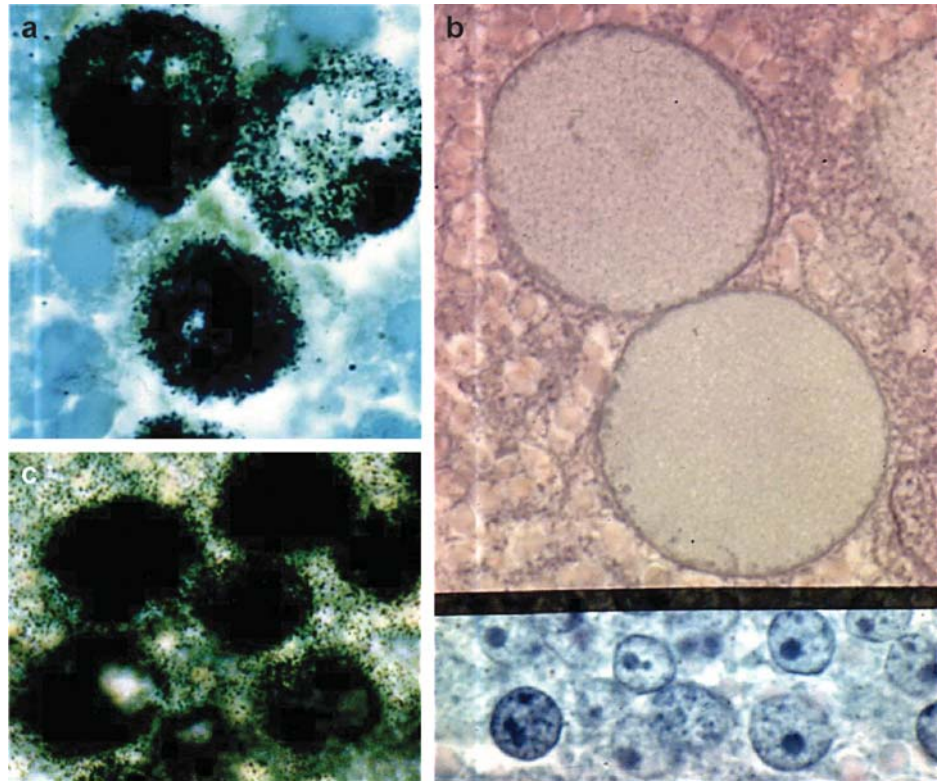


Figure 4

Early changes in the activity of transplanted nuclei. (a) Nuclei from adult frog brain are induced to synthesize DNA (3H-TdR incorporation) within 1 h of injection into unfertilized eggs. From Graham et al. (1966). (b) Somatic nuclei injected into oocytes undergo a massive enlargement and chromatin dispersal; the nuclei are shown immediately after transfer (*above*) and two days after transfer (*below*). From Gurdon (1986). (c) Adult brain nuclei injected into oocytes show intense RNA synthesis (H3-U incorporation). From Gurdon (1986).

after fertilization. Working with Graham, we showed that DNA replication is induced in transplanted nuclei within 30 min, even when these are taken from nondividing adult (frog) brain cells (Graham et al. 1966). A massive nuclear enlargement, chromatin dispersal, and the induction of DNA synthesis are the first changes that somatic cell nuclei undergo after transplantation to eggs (**Figure 4**). An important strategic step at this time was to try to simplify nuclear transplantation analysis by injecting purified molecules rather than whole nuclei into eggs. Protein-free DNA molecules were efficiently replicated after injection into eggs (Gurdon et al. 1969). This problem was

later taken on with great success by Laskey and his students (Blow & Laskey 1986, Coverley & Laskey 1994), who took advantage of egg extracts to induce DNA replication *in vitro*. Marcel Méchali (who worked with Laskey) has recently demonstrated the striking ability of M-phase cytoplasmic extracts of eggs to reset the frequency of DNA replication origins of a somatic nucleus to that of embryo cells (Lemaitre et al. 2005). We had shown, as indeed had DiBerardino & King (1967), that transplanted somatic cell nuclei often fail to complete DNA replication as fast as zygote nuclei and that this may account in part for abnormal nuclear transplant embryo

development. It now seems that M-phase egg cytoplasm contributes to nuclear reprogramming in part by resetting the initiation of DNA replication.

Other work at that time did as much as the prevailing methodology for transcript analysis allowed. This was to show that already by the blastula stage, nuclear transplant embryos had changed their gross pattern of ribosomal and transfer RNA synthesis to that typical of normal embryos from fertilized eggs (Gurdon 1986).

Contacts with senior researchers in a field can open unexpected new directions of work. I had the good fortune to get to know Professor Jean Brachet from the University of Brussels. It was he who first established the connection between protein synthesis and the RNA content of cells and who wrote an excellent book (Brachet 1957) that influenced many in addition to me. Brachet took a very positive view of our work, and although I never worked with him, he was one of the most influential and supportive developmental biologists of his time. It was he who introduced me to his colleague Professor Chantrenne, who was one of the first to purify the mRNA for a known gene (globin). I had the idea that not only DNA but also other macromolecules might be harmlessly injected with frog eggs, where they might resume their natural function. If I had proposed the injection of purified mRNA into *Xenopus* eggs in a grant application, it would surely have been rejected because it was known that a *Xenopus* egg is full of ribonuclease activity. Nevertheless, with Brachet's help, the experiment was done and succeeded outstandingly well (Gurdon et al. 1971). *Xenopus* eggs and oocytes translate injected mRNAs very efficiently (Gurdon et al. 1971) and for up to one month in cultured oocytes. We found no constraints for the cell type or species of injected mRNA. *Xenopus* embryo muscle cells translate injected rabbit globin mRNA very well (Woodland et al. 1974) (**Figure 5**). Since the work of Krieg & Melton (1984), the injection of in vitro-synthesized mRNA to eggs has become a very widely used procedure for

analyzing gene function by overexpression. The *Xenopus* mRNA injection procedure was later to prove of great value in identifying genes that encode neural receptors (Morales et al. 1995, Soloviev & Barnard 1997). The wider use of injected living eggs of *Xenopus* was followed by the work of my students Alan Colman and Charles Lane, the son of Dame Miriam Rothschild, famous for flea taxonomy and nature conservation, showing secretion and other gene expression steps that injected eggs perform (Drummond et al. 1985; Gurdon & Melton 1981; Lane et al. 1971, 1980).

It is interesting that, in spite of the intricate architecture of the cytoplasm and nucleus of a living cell, the crude deposition of macromolecules into such structures is so often followed by normal function. In most cases, purified macromolecules, such as mRNA, are unaccompanied by their normal (usually protein) partners and are injected at the wrong concentration into an inappropriate and physically damaged compartment. Yet they are soon assembled correctly and located properly, and the cell accepts these invasions as if they belong to its own estate.

THE LABORATORY FOR MOLECULAR BIOLOGY IN CAMBRIDGE

There can be little doubt that the United Kingdom's Medical Research Council (MRC) Laboratory for Molecular Biology is the most successful research institute in the world. With a current tally of 15 Nobel Laureates who largely earned their recognition there, and an annual budget (in 1980) of less than 1% of that of the National Institutes of Health in Bethesda, it doesn't seem to me to have much competition. When I was invited by its founding and long-term chairman, Max Perutz, to accept a group leader position there, this was a unique opportunity. Nevertheless, it was a hard decision. The families of myself and my wife were fully settled in Oxford, where I had also established close personal

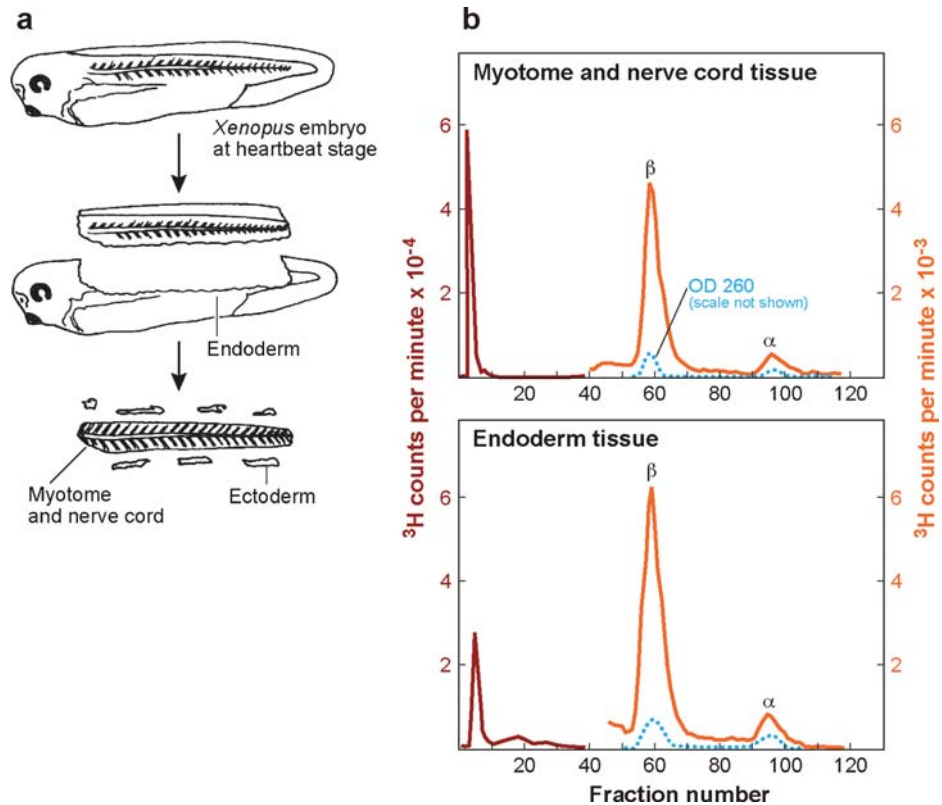


Figure 5

Purified mRNA is efficiently translated after injection into *Xenopus* fertilized eggs. Mouse globin mRNA was injected into fertilized eggs that were grown to a heartbeat stage when the endoderm and the myotome and nerve cord were isolated and analyzed to show α and β globin synthesis in both tissues (Woodland et al. 1974). (a) Dissection of swimming tadpoles. (b) Mouse globin synthesis in *Xenopus* tadpole regions, analyzed by carboxymethyl cellulose chromatography.

connections with senior Oxford figures not in my department, most notably with the biochemist Rodney Porter, with whom I shared horticultural and alpine interests. Oxford was very strong scientifically, and I was commonly included in a lunch cafeteria group containing Rodney Porter, James Gowans, Henry Harris, and others. I had been very generously treated by these senior colleagues and by my college (Christ Church), so why leave? On the other hand, Perutz had assembled, and even more remarkably retained throughout their working lives, a remarkable group of scintillating luminaries. These included Francis Crick, with a brilliant ana-

lytical mind, but never, they say, able to remember whether the hairs on the back of a fly point forward or backward; Fred Sanger, whose ultramodest style led him often to be confused with a janitor, unless you happened to know that he had two Nobel Prizes under his belt; Aaron Klug, whose extraordinary memory enabled him to know more about your older published papers than you yourself could recall; and many others such as Cesar Milstein, Sydney Brenner, and Hugh Huxley. So I moved to Cambridge, where I enjoyed a personal friendship with Perutz, partly because of our shared interests in ice skating and alpinism (he did original research on



Figure 6

Colleagues working with J.B. Gurdon and R.A. Laskey at the MRC Laboratory for Molecular Biology in Cambridge in 1980 (composite photo). From left: Doug Melton (Harvard), Marvin Wickens (Madison), Bill Earnshaw (Edinburgh), Eddy De Robertis (UCLA), Ruth Longthorne, Richard Harland (Berkeley), Laurence Korn (Protein Design Laboratories), Kazuko Nishikura, Stuart Weisbrod (Merlin BioMed Group), John Gurdon (Cambridge), Julian Wells (Adelaide), Ron Laskey (Cambridge).

glaciology) and partly because of my admiration for his personal style of leadership. He always insisted on his position as chairman, not director.

Largely, no doubt, on account of the eminence of that institute, I was joined by several outstanding colleagues, including Doug Melton, who with characteristic modesty asked to join our group to do weekend glass washing; Eddy De Robertis from Argentina, who later analyzed, to a greater extent than anyone else, the molecular basis of the Spemann organizer; Laurence Korn, who later created the highly successful biotech company, Protein Design Laboratories; Marvin Wickens; and many others (**Figure 6**). I was also rejoined by Ron Laskey, who developed his own group.

My work at that time took a new direction, although it still addressed the same original problem of how a somatic cell nucleus is reprogrammed to an embryonic pattern of gene expression. Nuclear transplant embryos, just like embryos from fertilized eggs, are transcriptionally quiescent until they reach the mid-late blastula stage consisting of 4000 cells. We had no evidence to say whether the reprogramming of gene expression took place in the egg or only at the 4000-cell stage. I was well aware, through

personal friendship with H.G. Callan of St. Andrew's University, of the intense transcriptional activity of the growing first meiotic prophase oocyte, with its spectacular Lampbrush chromosomes (Callan 1982, Callan & Lloyd 1960). It occurred to me that it might be possible to transplant somatic cell nuclei to a meiotic prophase oocyte (**Figures 7 and 4c**). It has even been shown that sperm nuclei will adopt a lampbrush-like configuration when injected into oocyte germinal vesicles (Gall & Murphy 1998). If this cell would reprogram gene expression, this would greatly simplify attempts to analyze transcription, especially because the growing oocyte is inactive in DNA replication. With De Robertis, who carried out extensive and painful two-dimensional protein analyses, we found that these growing oocytes did indeed reprogram somatic nuclei and did so directly and without DNA replication. Moreover, *Xenopus* oocytes could reprogram mammalian nuclei, apparently to an oocyte-specific pattern of protein synthesis (De Robertis & Gurdon 1977) (**Figure 8**). The technology of transcriptional analysis available at that time did not permit pursuit of how the oocyte could directly reprogram transplanted nuclei, although advances in methodology some 20 years later have made it possible to return

a Two kinds of nuclear transfer in Amphibia

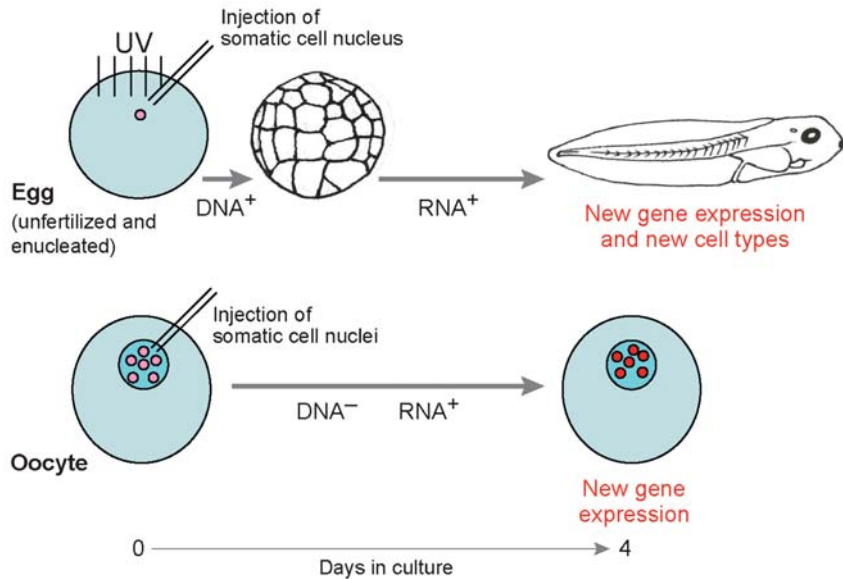
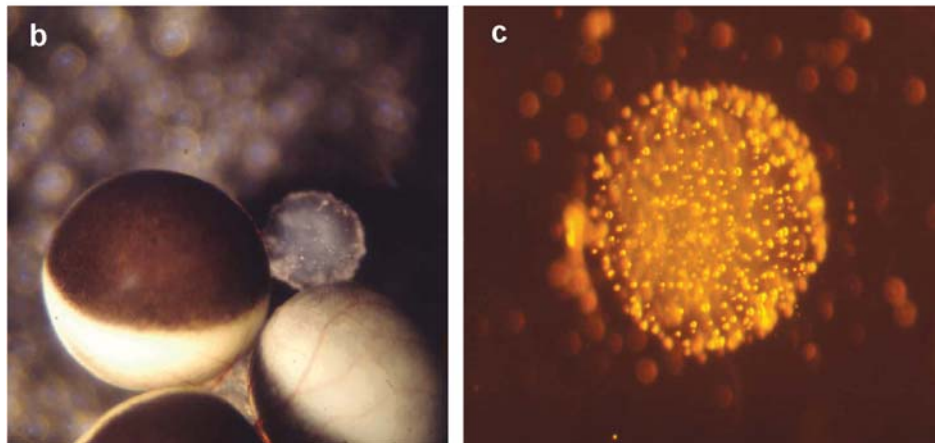


Figure 7

The use of eggs or ovarian oocytes as recipients for nuclear transfer in *Xenopus*. (a) Diagram to illustrate the differences between an egg (second meiotic metaphase) and an ovarian oocyte (first meiotic prophase). (b) Oocytes encased in gonadal follicular tissue, including blood vessels, and a germinal vesicle (GV). (c) An isolated GV; the light particles are mainly extrachromosomal nucleoli.



productively to this type of analysis (see below).

Following, as before, the principle of simplification, we found that purified DNA is very efficiently transcribed after injection into oocytes (Brown & Gurdon 1977, Mertz & Gurdon 1977) (**Figure 9**). Oocytes thus provide a living test tube for DNA transcription by RNA polymerase II in a way that is still not achieved by in vitro systems.

CAMBRIDGE ZOOLOGY AND THE CANCER RESEARCH CAMPAIGN

I found myself offered the named Research Professorship, from which Sir Alan Hodgkin had just retired and which would require moving, with my group, to the Cambridge Zoology Department. Perutz advised me against the move on the ground that I would be

crippled by university administration. Cleverly, the head of the Zoology Department, a well-known neuroscientist, Sir Gabriel Horn, had arranged at the same time to offer another professorship to Laskey, with whom I had worked closely for more than 20 years. The MRC, where we worked, provided no postdoctoral or other scientific posts to its group leaders in spite of its preeminence, so we needed continually to depend on those who joined our lab to bring their own support. After much hesitation, Laskey and I decided to seek support from the Cancer Research Campaign (CRC), a major national cancer charity, for a well-funded dual group in a separate part of a new building. It could well be said that no one in their right mind would leave the MRC Molecular Biology Laboratory to take up a university teaching post. However, the offer of considerably enhanced research support was persuasive, as was the dictum that it is always better to leave a research institute while you are still wanted than to wait until you need to be phased down or out. I also have the personal philosophy that research-protected posts should be accorded to those in mid-career so that those who enjoy the benefit of such positions can repay the system with teaching and administration in their latter part of their careers.

The generous CRC funding for our two groups in the Cambridge Zoology Department coincided with my research interest moving progressively toward the earliest stages of development. What are the processes that generate a spatially organized array of different cell types in early development, a time when nuclear transplant embryos from differentiated cells so commonly fail? It is interesting that the celebrated magnum opus of E.B. Wilson (1925) devotes none of its 1232 pages to embryonic induction, or signaling, as we now call it. Most would now regard signaling between cells as the most important mechanism in early development. Even in the early 1920s, no one had identified a natural inducer molecule, in spite of concerted efforts by Needham, Brachet,

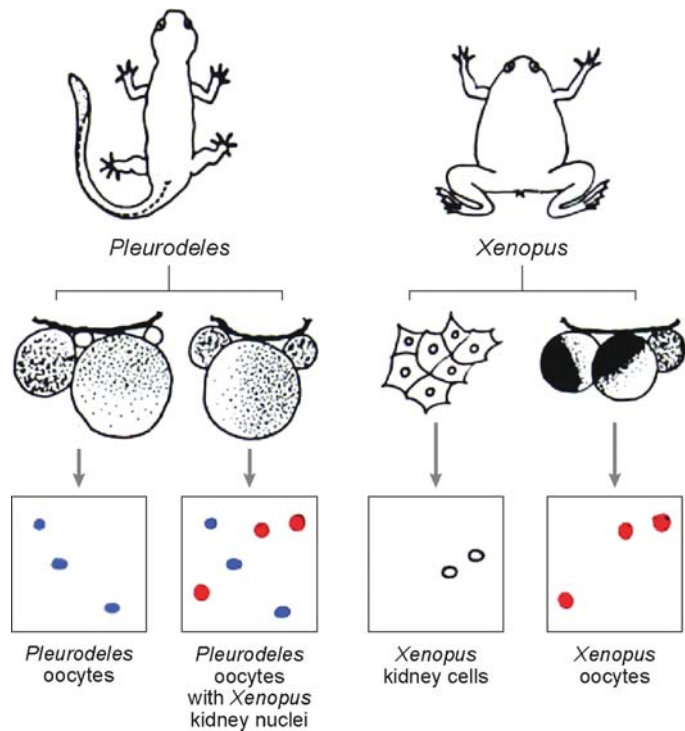


Figure 8

Somatic nuclei injected into oocytes of *Xenopus* are induced to express oocyte-specific genes. The patterns of gene expression in oocytes of *Xenopus* and the other amphibian *Pleurodeles* are distinguished by two-dimensional protein analyses. The transfer of nuclei between two species makes it possible to detect the activation of oocyte-specific genes above the background of the recipient oocytes (De Robertis & Gurdon 1977).

and Waddington in the 1930s (Nakamura & Toivonen 1978, Waddington et al. 1936) and a lifetime of work by Tiedemann, one of the last students of Warburg. It was J.C. Smith and colleagues (Smith et al. 1990) who first isolated and purified the TGF β -family member activin. He followed the wise principles used by Cohen and Levi-Montalcini (Levi-Montalcini 1987) in Hamburger's laboratory in St. Louis to isolate the first growth factor, nerve growth factor. They started from an abundant source (the mouse submaxillary salivary gland instead of mocassin snake venom first used by them) and a simple assay, rather than struggle with a difficult source (newt gastrulae) and a troublesome assay (precipitated pellets for implantation), as had Tiedemann.

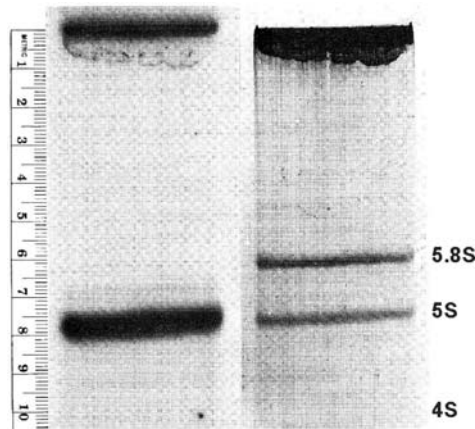


Figure 9

Purified DNA, genomic or plasmid, is efficiently transcribed after injection into the germinal vesicle of *Xenopus* oocytes. The oocyte's RNA polymerase III is used to transcribe 5S genes. Gel electrophoresis of ^{32}P -RNA synthesized by oocytes injected with 5S DNA (^{32}P , *left*; stained gel, *right*) (Brown & Gurdon 1977).

Activin behaves as a perfect morphogen in that it can direct amphibian blastula cells into different cell-fate pathways of the early embryo according to its concentration (Green & Smith 1990) and appears to be a natural signaling molecule (Piepenburg et al. 2004). Indeed, activin-loaded beads implanted into blastula animal cap sandwiches create ripples of gene expression according to their concentration (**Figure 10**). That a single morphogen

molecule, released from one point in an embryo, can create a spatially organized arrangement of different cell types makes activin's mode of action of exceptional interest and importance.

I was fortunate enough to be joined by Tim Mohun, who had great expertise and experience in muscle development. This was a very good choice of readout for work on the mechanism of morphogen action (Mohun et al. 1984). Over the next decade, we were able to analyze to some extent the way in which a cell perceives and interprets a concentration of extracellular activin, an astonishing feat when one bears in mind that a cell can distinguish threefold changes in the level of activin at 10^{-10} M concentrations after a 10-min exposure. We found that the mechanism depends on the absolute number of occupied receptors, a value interpreted by the steady-state concentration of the transduction molecule Smad2 in the nucleus of a responding cell (Bourillot et al. 2002, Dyson & Gurdon 1998, Gurdon & Bourillot 2001, Jullien & Gurdon 2005).

To simplify analysis according to the reductionist principle, I tried to work with the response of single cells to a morphogen concentration. This led in time to the discovery of the community effect, by which a group of cells that have made an initial interpretation of morphogen concentration must collectively signal to other, like, nearby cells to



Figure 10

Morphogen gradient expression. Chromatographic beads loaded with different amounts of activin are implanted into blastula animal cap sandwiches. A ring of Xbrachyury expression is seen to have moved progressively further away from the beads, according to activin concentration. This gene response is seen approximately 2 h after bead implantation. Because there is very little cell division or cell movement, the same cells change their response to activin as the morphogen spreads away from the beads. From Gurdon et al. (1994).

activate a downstream cell-fate-determining gene (Gurdon 1988, 1993; Standley et al. 2001) (**Figure 11**). It now seems clear that morphogen gradient interpretation involves numerous steps, the precise response being continually adjusted by, for example, extracellular ligand-binding materials and antifactors (Ashe & Briscoe 2006). Furthermore, the mechanisms involved seem more elaborate the later in development they take place. The fly wing disc appears to be complicated by cell division, cell movement, and a two-day time course (Martinez Arias 2003). Signaling in early embryos may be simpler and usually needs to be completed in just a few hours or less.

There is much that remains to be understood in development about signaling from secreted extracellular factors and especially about a cell's interpretation of morphogen concentration. Much of the current literature on signaling aims at describing the (often indirect) effects of signal factor depletion and at identifying the downstream components of a signal transduction pathway. Useful as this is, I believe we will soon need to move to a far more quantitative analysis involving concentration and time. Living cells are extremely sensitive to concentration. In many cases, one copy of a gene, as opposed to the usual two, leads to abnormality (the haploinsufficiency phenomenon), and threefold changes in morphogen concentration elicit different cell fates. Likewise, the duration of signaling is crucial. The number of receptors occupied by ligand is proportional to time. The accumulation of a stable gene product will increase in proportion to the duration of a signaling process. Signaling processes in early development often generate the production of a transcription factor whose function is to activate a downstream gene; but this will only succeed when the intranuclear concentration of that transcription factor reaches a sufficient level (often 10^4 molecules per nucleus) to turn on its downstream genes reliably. Thus, a full understanding of signaling in development will require knowledge of

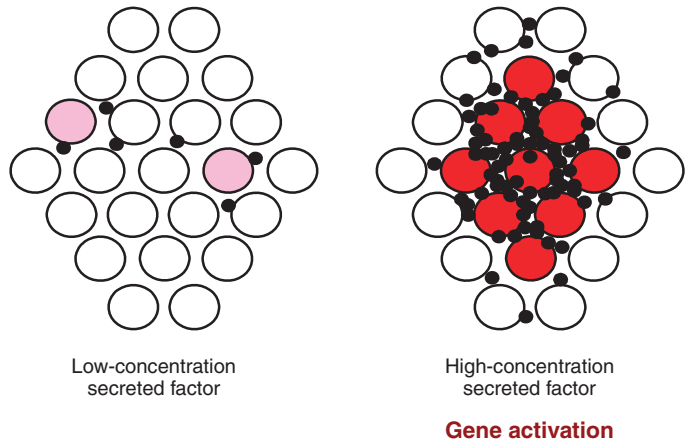


Figure 11

Design of experiment to show a community effect in *Xenopus* muscle development. MyoD is expressed only when the approximately 100 cells that have already received an appropriate concentration of activin are located close to each other, thereby raising the concentration of eFGF above a threshold level (Standley et al. 2001). On the left, two pink cells have received sufficient activin to embark on a muscle pathway; on the right, many similar cells are located next to each other. All cells influenced by activin secrete a community factor (*small black dots*). Only those cells in close proximity to each other create a sufficient concentration of the community factor to reach the threshold for muscle differentiation (*red*) (Standley et al. 2001).

times, concentrations, half-lives, dissociation constants, and other variables to explain how a necessary concentration is achieved at the right place for the right length of time. This level of understanding may be particularly important in explaining more complex developmental phenomena, such as the lepidopteran wing patterns referred to above (**Figure 12**) (Bard & French 1984, Brunetti et al. 2001, Monteiro et al. 2001).

THE WELLCOME TRUST/CANCER RESEARCH CAMPAIGN INSTITUTE

My career has tended to unfold in aliquots of ten years. Toward the end of my time in Cambridge Zoology, the London-based Wellcome Trust was looking for ways to increase its spending. Sir Henry Wellcome's will in 1936 left £1 million for "the improvement of human and animal health." In 50 years, the



Eye spots, enlarged

Figure 12

Lepidoptera wing patterns. (Left) Underside of hind wing of *Morpheus achilles*. (Right) Eye spots in many Lepidoptera consist of concentric rings of different colors best explained by the spread of a morphogen from the central spot during late larval and early pupal stages.

capital value of the Trust had increased by 10,000 times, largely owing to the outstanding financial skills of Sir Roger Gibbs, its chairman from 1989 to 2000. The Trust's annual expenditure on research was now slightly greater than that of the Howard Hughes Medical Institute in the United States. Ron Laskey and I were asked by the Trust whether we would be interested in proposing an expansion of our two-group lab by bringing in other groups, possibly into a new building. We invited Martin Evans and Michael Akam from Cambridge as well as Chris Wylie and Janet Heasman from St. George's Medical School in London to join us. We were awarded a new building in the Cambridge science area at the modest cost of £4 million. We set ourselves up with a chairman, as opposed to a director, in the style of Perutz, and appointed some younger group leaders, including Daniel St. Johnston, Stephen Jackson, and Tony Kouzarides. Thanks largely to the diplomacy of Gabriel Horn, head of the Zoology Department in Cambridge, we were incorporated painlessly into the university while remaining an externally funded research institute. This was the start of the Wellcome/CRC Institute in Cambridge. We were soon joined by Azim Surani and his group.

To bring this story up to date, the Wellcome Trust in 2001 invited proposals for new scientific buildings or the upgrading of old ones. Although we had occupied our building for only ten years, we had expanded to overflow capacity, and the building was not designed to handle our increased technical needs. My ten years' principle encouraged another move, and owing to the success of our group leaders, we were awarded funds for a larger and more highly equipped building. Our new institute progresses under the chairmanship of Jim Smith, whose work on morphogen gradients was referred to above. What started as two moderately sized research groups has now evolved into an institute containing 17 research groups.

NUCLEAR REPROGRAMMING AND THE PROSPECT OF CELL REPLACEMENT

Within the past decade, nuclear transfer in mammals has achieved success. Dolly the sheep has attracted wide public attention because cloning of sheep predicts much more clearly than that of frogs the possibility of human cloning. As mentioned above, hardly anyone favors the application of reproductive cloning in humans because of the high proportion of defects resulting from current technology. Likewise, nearly everyone appreciates the potential benefits of therapeutic cloning in humans (i.e., isogenic cell replacement). For the purposes of cell replacement, it is unnecessary to be able to produce a fertile adult by nuclear transfer. Cells defective in one respect may be therapeutically valuable for replacing certain other types of cells. The low rate at which fertile adults are obtained by nuclear transfer is not therefore a constraint for therapeutic cloning.

The inherent stability of the differentiated state of cells (Kato & Gurdon 1993) can be reliably overcome in only two ways, namely by cell fusion and nuclear transplantation. In each case, the nucleus of a cell is taken out of its own cytoplasmic environment and placed

in that of another cell. The most dramatic reprogramming takes place when a nucleus is incorporated into a recipient cell containing a huge excess of cytoplasm such as a multinucleate myofibril or a large egg. Conceptually, this is understandable if we suppose that cells are subject to continual intracellular reprogramming. Thus, a regulatory gene produces a message that is translated into protein in the cytoplasm; such proteins, which are often autoregulatory, enter the nucleus, where they activate their own and other genes that promote the continuation of that cell's differentiated pathway or state. If regulatory factors are continuously cycling between nucleus and cytoplasm, gene expression should be changed when a nucleus finds itself in an overwhelmingly abundant foreign cytoplasm.

For example, a particularly impressive cell hybrid experiment is that where a human hepatocyte nucleus was induced to express muscle genes when fused as a heterokaryon to a mouse myofibril (Blau et al. 1983). Terminally differentiated erythrocytes are reactivated when fused to cultured cells (Harris 1970). Mouse thymocyte nuclei express *Oct4* when hybridized to embryonic stem (ES) cells (Kimura et al. 2004), and embryonic germ cells can demethylate DNA of thymus cells (Tada et al. 1997). Eggs and oocytes of frogs and mammals can also induce profound changes in injected somatic nuclei. Mouse thymus nuclei express *Oct4* and other mammalian stem cell genes, and the activation of *Oct4* is accompanied by demethylation of the *Oct4* promoter DNA (Byrne et al. 2003, Simonsson & Gurdon 2004) (**Figure 13**). Mouse oocytes also have remarkable reprogramming abilities (Rideout et al. 2001), although these are not always completely effective (Humpherys et al. 2002).

An altogether more difficult problem will be to identify the molecules and mechanisms involved in nuclear reprogramming. Frog oocytes have an advantage here. The most obvious activity of eggs, whether activated by fertilization or by a transplanted nucleus, is to induce nuclear DNA synthesis. Transcrip-

tional reprogramming is not seen until the *Xenopus* late blastula stage (8 h) or the two-cell stage in mice (24 h). The finding that *Xenopus* oocytes in first meiotic prophase can directly induce transcriptional reprogramming in the absence of DNA replication considerably simplifies the analysis (Byrne et al. 2003). The amount of material in *Xenopus* is also very advantageous. One *Xenopus* ovary is equivalent to approximately 10^{10} embryonic stem (ES) cells or 10^4 mouse ovaries. Cell-free systems based on *Xenopus* eggs or oocytes, like all other cell-free systems, do not have the ability to reinitiate transcription in nuclei and therefore cannot reveal transcriptional reprogramming. Nevertheless, these systems have permitted the identification of a Tata-binding protein (Kikyo & Wolffe 2000) and nucleoplasmin (Gonda et al. 2003, Kikyo et al. 2000, Wade & Kikyo 2002) as likely to play a part.

Looking ahead, I envisage three steps in achieving successful nuclear reprogramming as a route toward cell replacement. First, it will be necessary to identify the genomic and epigenetic changes that cells undergo as they differentiate. These include DNA

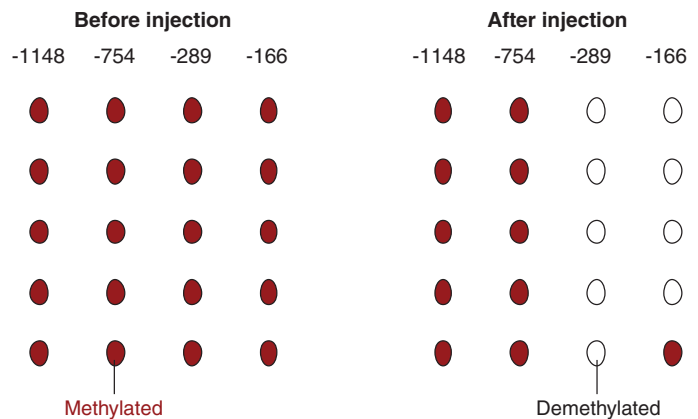


Figure 13

DNA demethylation in *Xenopus* oocytes. DNA or whole nuclei injected into the germinal vesicle of *Xenopus* oocytes undergo selective demethylation of the DNA comprising the promoter region of the stem cell marker gene *Oct4*. Mouse thymus nuclei or plasmid DNA was injected into *Xenopus* oocytes (Simonsson & Gurdon 2004). Only the minimal promoter from 0 to -350 is demethylated.

demethylation and histone modifications. Eggs and oocytes have mechanisms that can efficiently reverse these changes (Gao et al. 2004, Simonsson & Gurdon 2004, Teranishi et al. 2004). Because these mechanisms exist, it must be possible to identify, and put to use, the molecules responsible. This first step is the reversal of differentiation marks.

I regard the second stage of nuclear reprogramming as the provision of transcription factors necessary for activation of embryo and stem cell genes so that adult cells are converted to a multipotent and proliferative state. I assume that these factors will soon be identified for all genes in the genome. It is not yet clear, in my view, whether protein transcription factors will function properly if introduced in the appropriate concentration into an unrelated cell type. The provision of such factors, even if they need modification or association with other cell components, should not be impossible.

The third requirement, it seems to me, is to make inactive or repressed genes (i.e., their regulatory elements) accessible to regulatory proteins. As cell differentiation proceeds, genes no longer required become increasingly inaccessible in the form of higher-order complexes, heterochromatin, etc. Judged from

morphological criteria (above), eggs and oocytes seem to have a special capacity to unravel condensed chromatin. We know little about this decondensation step. I suppose that the provision of gene regulatory molecules in a cell will be insufficient for gene reprogramming in the absence of decondensation.

Looking far ahead, it may become possible to convert cells of an adult to an embryonic state without needing to use eggs. Overexpression of a DNA demethylase and other nuclear reprogramming molecules in an accessible adult human cell may be sufficient to generate ES-like cells. If all these steps are identified and made to function in a somatic cell so that rejuvenated cells can be created and made to proliferate as ES cells, will this be sufficient for cell replacement therapy? Cells introduced into the bloodstream seem to spread very widely, but much has to be learned in cell biology about their integration and continuing function. Nevertheless, it is hard for me to believe that when all is known about the molecular mechanisms that explain the success of gene reprogramming by nuclear transfer, such knowledge, in addition to having inherent scientific interest, will not contribute to the field of therapeutic cell replacement.

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