Nobels and Stem Cells

The work recognised by the Nobel Prize in Physiology or Medicine awarded to Sir John Gurdon and Shinya Yamanaka demonstrated that in the battle of nature versus nurture, nature prevails. Using different techniques many decades apart, both men were able to reveal that it is possible to convert virtually any cell because they retain the fundamental building blocks of life: DNA

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In October, Sir John Gurdon and Shinya Yamanaka shared the Nobel Prize in Physiology or Medicine for "the discovery that mature cells can be reprogrammed to become pluripotent", enabling them to give rise to all adult cell types. Their research has shaped popular culture, leading us to ask fundamental questions about when life begins, and could result in new treatments for many diseases. Additionally, it has demonstrated that in the struggle of nature versus nurture, nature has the upper hand.

Question Time

The discoveries which were recognised by the Nobel Committee were conducted over 40 years apart and began with a question, which as Gurdon frequently points out, is how any scientific experiment should always begin. Gurdon was interested in understanding if different adult cells contained different sets of genes. To address this question, he injected the nucleus of a cell from a frog intestine into the cytoplasm of an enucleated frog egg before stimulating embryogenesis. The injected nucleus was able to successfully develop into an adult frog, demonstrating that adult cells do not only contain genes for a single cell type and that the genetic material in a single adult cell is capable of producing a whole organism (see Figure 1) (1).

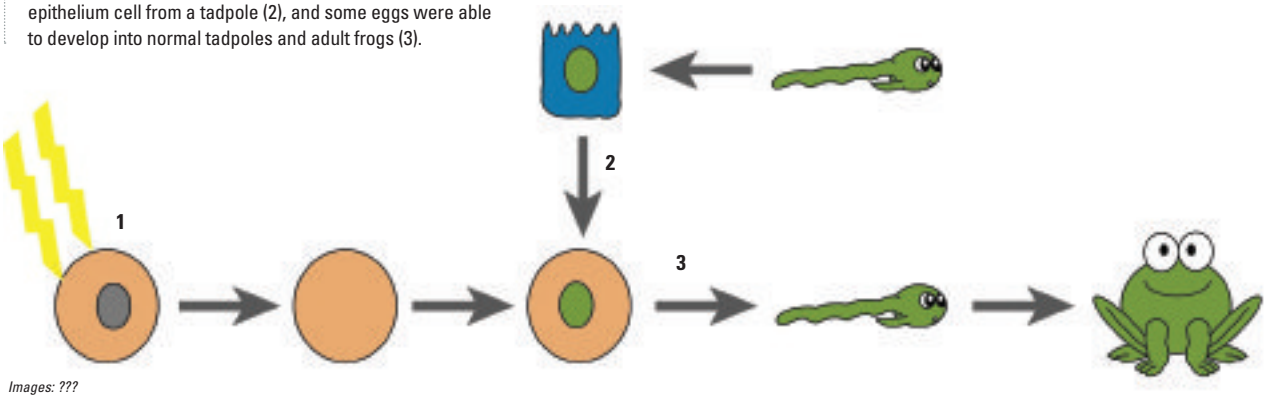
Embryonic Stem Cells

Gurdon's work was initially met with scepticism, despite being built upon the work of the renowned German embryologist Hans Spemann and Briggs and King, who had previously shown than nuclear transfer was possible (2,3). The technique utilised by Gurdon is known as somatic cell nuclear transfer (SCNT). It is the same technique that Sir Ian Wilmut and colleagues used 20 years ago to create Dolly the sheep at the Roslin Institute (4) when 'cloning' entered the zeitgeist.

Embryonic stem cells (ESCs) entered popular culture at around the same time as Dolly the sheep, and it is the lessons which had been learned about ESCs that enabled the Nobel Prize winning work of Shinya Yamanaka. The pluripotentency of ESCs makes them of great interest to researchers, clinicians and the entire industry. ESCs are derived from the blastocyst of a developing embryo; Yamanaka postulated that an adult cell may be able to be directly converted into an ESC-like cell. With his colleague Kazutoshi Takahashi, he discovered that this process was significantly simpler than anyone previously predicted. By introducing three genes expressed in ESCs and c-Myc (a gene which is frequently mutated in cancer and promotes cell growth), they were able to convert adult skin cells, first from a mouse and then from a human, into cells which had all the characteristics of ESCs, erasing all acquired epigenetic memory

Figure 1: Somatic cell nuclear transfer

The nucleus of the frog egg was destroyed by UV light (1); this was then replaced with the nucleus from an intestinal epithelium cell from a tadpole (2), and some eggs were able to develop into normal tadpoles and adult frogs (3).



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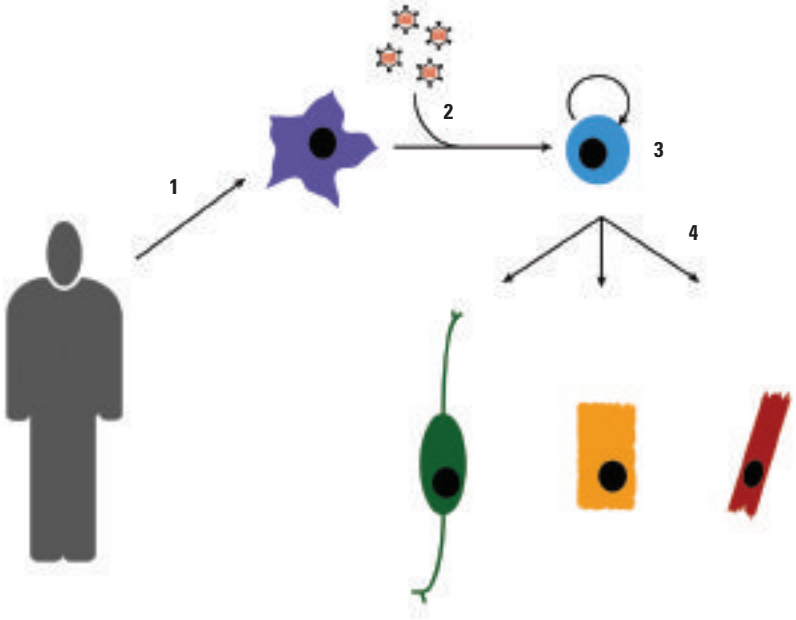

Figure 2: Induced pluripotent stem cells

Skin cells grown from adult skin (1) were given four transcription factors (Oct4, Sox2, Klf4 and c-myc) (2). They were cultured for up to four weeks, during which time pluripotent cells emerged (3). These pluripotent cells could be maintained in culture and differentiated to all adult cell types (4). The differentiated cells have been used for cell therapy and drug discovery.

(see Figure 2) (5,6). Yamanaka dubbed these cells induced pluripotent stem cells (iPSCs) (see Figure 3). These iPSCs can be differentiated *in vitro* into potentially any adult cell type (see Figure 2).

Many companies are already looking to capitalise on the research of Gurdon and Yamanaka to uncover new therapies for a constellation of different diseases. The opportunities for new therapies fall into two broad categories, cell-based therapeutics and drug discovery, with a third group of companies providing a host of products and services to support these activities.

Cell Based Therapy

Cell based therapeutics, as the name implies, involves the use of cells for therapy. The cells can directly replace damaged, defective or diseased cells and tissues (cell replacement therapy), or the cells can act as a type of drug delivery vehicle, where the trophic factors secreted by the cells provide therapeutic benefit.

Many of the cellular therapeutics that are being developed by UK companies involve the use of tissue stem and progenitor cells which have been directly isolated from donors, rather than the differentiated progeny of cells which have been reprogrammed. This includes companies such as ReNeuron, Intercytex, Cell Medica, Azellon Cell Therapeutics and Shire, which recently acquired Advanced Biohealing Inc, the makers of Dermagraft for $750 million. iPSCs could be the cell source for many of these products, should that be deemed an appropriate strategic fit. However, iPSC derived cells for cell replacement therapy still carry concerns due to their potential tumorgenicity and immuno-compatibility. A number of academic groups are investigating the use of iPSC derived cells for cell replacement therapy, including Professor Anthony Hollander at the University of Bristol and Professor Pete Coffey at UCL. The only approved clinical trial using iPSC derived cells is currently under way in Kobe, Japan, where Masayo Takahashi is examining the use of iPSC derived rod cells to treat age-related macular degeneration (AMD).

Drug Discovery

iPSCs may have their greatest near-term utility in drug discovery. Multiple iPSC lines have been derived from patients with a variety of different diseases. The iPSCs can then be differentiated into the cells which are affected in the disease of interest. This has two principal applications; the cells can be used to understand the fundamental mechanisms which go awry in the disease, and they can be used as a screening tool to identify leads which have a functional

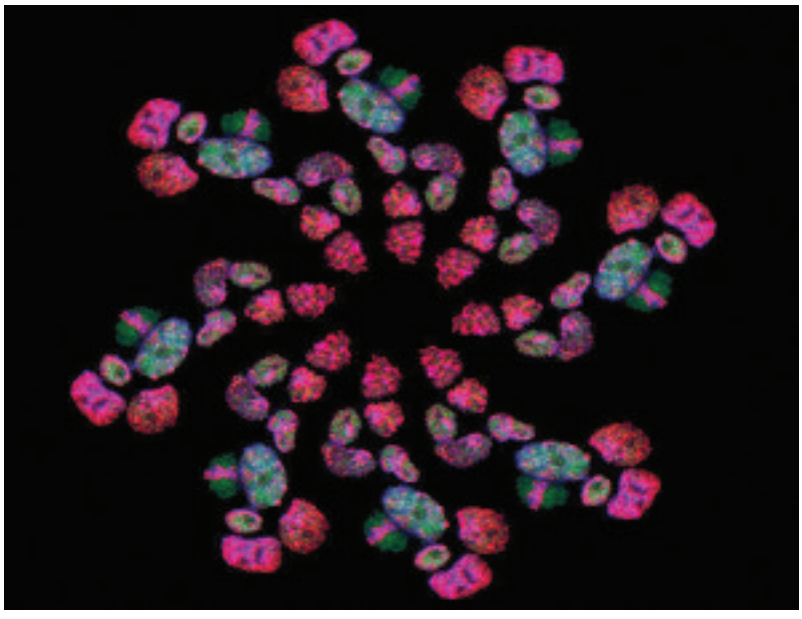

Figure 3: iPSCs growing in culture

Immunocytochemistry shows presence of proteins in cell nucleus; green Oct4, red H3K9me3 and blue DapI (DNA stain).

effect directly upon the cells of interest. Either approach has the potential to generate new therapeutic agents. Pfizer is using this approach in its only remaining European research centre, Neusentis, based just south of Cambridge. It is using iPSCs which carry mutations in a number of receptors associated with chronic pain disorders. The company is collecting iPSCs from patients with a variety of different molecular lesions and then differentiating the iPSCs to the neurons affected in the particular condition that is being studied. A variety of compound libraries are being screened against the neurons in an attempt to identify the molecules which have the greatest therapeutic potential for different genotypes, long before the compounds enter clinical trials. In effect, Pfizer is conducting *in vitro* clinical trials which will stratify patient populations and can predict how patients with the same condition but different genotypes may be affected by the same or different compounds.

Tackling Toxicity

The current cost of bringing a drug to market can be up to $11 billion (7). Between 2008 and 2010, 19 per cent of drugs in clinical trials failed due to safety concerns during Phases 2 or 3 (8). Safety problems are often associated with toxicity; heart and liver cells are often most prone to toxic effects. There is increasing interest from a variety of pharmaceutical companies including GSK and Roche to screen their compounds for toxicity *in vitro*, before they enter clinical trials (9). This could potentially save billions in wasted development costs. Human cells which are physiologically equivalent to the cells which are most prone to toxic side-effects can be derived from human iPSCs. A number of companies are already making iPSC derived cells for *in vitro* toxicity analysis including DefiniGEN, a company recently spun-out from the University of Cambridge and backed by £75,000 from the University of Cambridge Enterprise Fund and other angel investors including Jonathon Milner, CEO of Abcam. DefiniGEN is able to derive liver cells from human iPSCs which are being marketed for drug discovery for Inherited Metabolic Disorders (IMD) but could also be used for toxicity and safety studies. GE Healthcare Life Sciences has taken a different approach from its stem cell research unit just outside Cardiff, marketing Cytiva Cardiomyocytes, heart cells differentiated from human ESCs. These cardiomyocytes have been used to assess the safety of a panel of drugs from Genentech. In a blind trial using the Cytiva cells, it was able to identify the compounds which failed due to toxicity during clinical trials, but also others which were withdrawn post-marketing due to toxicity concerns among patient groups.

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iPSC generation, maintenance and differentiation requires various reagents and equipment. A number of companies based in the UK are providing services to support research and development activities. TAP Biosystems based in Royston has manufactured a number of cell handling systems which are used for the expansion and differentiation of various stem cells, including iPSCs. The company has sold over 100 of its Cellmate systems since 1989, including to Shire for growth of its human dermal fibroblasts. Now it is developing other systems for high-throughput expansion of cells, which include features such as the IncuCyte imaging system from Essen BioSciences, enabling cells to be monitored in real-time as they expand.

Differentiation of pluripotent cells to the desired destination cell can be challenging; often this is achieved using an iterative process which involves an understanding of the molecular mechanisms controlling the development of the desired target cell *in vivo*. Plasticell, based in London, is taking a different approach. Plasticell has developed Combicult; a method by which many thousands of differentiation protocols are simultaneously screened to identify a protocol which most effectively produces the cell type of interest. This is done randomly by exposing the starting cells to many thousands of differentiation protocols, then identifying the cells which have differentiated to the desired destination cell. The protocol which most efficiently induced this differentiation can then be identified. Plasticell is working with Pfizer among others to develop differentiation protocols and have licensed its media for mesenchymal stem differentiation to EMD Millipore.

Conclusion

The work of Sir John Gurdon and Shinya Yamanaka was truly ground-breaking; they rewrote scientific dogma by asking very simple questions about how a cell is defined. Thousands of scientific studies have validated the findings of these discoveries and they are being commercialised by dynamic start-ups and FTSE 100 companies. The UK has been at the centre of this boom; this is the second Nobel Prize for stem cell science for UK scientists; in 2007, Sir Martin Evans and Olivier Smithies shared the Nobel Prize in Medicine or Physiology for "principles for introducing specific gene modifications in mice by the use of embryonic stem cells". The UK has a vibrant research community which is building on these discoveries, and both home-grown and overseas companies operating in the UK are successfully commercialising discoveries from this new scientific field which began with a simple question in Oxford in the 1960s.

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