Cellular Reprogramming – Turning the Clock Back

Nobel Prize in Physiology or Medicine, 2012

Deepa Subramanyam

Deepa Subramanyam is an Assistant Professor at the National Centre for Cell Science in Pune. Her research interests include understanding aspects of cellular trafficking that differ between embryonic stem cells and differentiated cells.

Cellular reprogramming involves the conversion of a specialized cell into a cell which possesses properties similar to an embryonic stem cell. The Nobel Prize for Physiology or Medicine, 2012, was awarded jointly to two researchers, Sir John Gurdon and Shinya Yamanaka, for their creative and brilliant discovery involving the conversion of a mature differentiated cell into an immature cell, capable of giving rise to all the cell types in the body. This article discusses the manifold implications of this discovery.

Historical Perspective

Before moving ahead, it is important to define certain terms and review some historical literature. First things first – what is a differentiated cell? This is a cell that is committed to performing a specific function throughout its lifetime, for example the nerve cell. How can a differentiated cell be produced and how is it different from early embryonic cells? This is a fundamental question that has fascinated scientists for many years. Soon after an egg is fertilized by a sperm, it begins to undergo a large number of changes. Rapid cell division occurs and a number of specialized cell types are produced. While the zygote could give rise to the entire organism, it was unclear at which stage of development, cells lose the capability to give rise to a whole organism.

A number of classic experiments were performed to address this question. As far back as 1885, August Weissmann postulated that as development progressed, subsets of genetic material got segregated between the daughter cells. In 1888, the German embryologist Wilhelm Roux took a two-celled embryo and ablated one of the cells. He observed that the resultant embryo gave rise to only half an embryo, suggesting that even at such an early stage the

Keywords
Embryonic stem cells, pluripotency, reprogramming, differentiation, Nobel Prize 2012.

embryonic cells were non-equivalent. In 1892, Hans Driesch of Germany challenged this idea by demonstrating that in the sea urchin, ablation of one of the cells at the two-cell stage still permitted the formation of the complete organism. Thus, at least up to the two-cell stage, the nuclei of both cells had equivalent potential to give rise to a complete organism. Later, the famous German embryologist Hans Spemann used the newt embryo and extended this equivalence up to the 8-cell stage of the embryo. Spemann was awarded the Nobel Prize in 1935 for his work describing organizer centres. (These organizers were regions of the embryo which, when transplanted into other developing embryos, could result in the development of specific structures). Spemann further proposed an experiment to see whether differentiated cells could be restored to an embryonic state or whether they continued to remain specialized by transplanting their nuclei into the egg [1].

Hans Spemann's breakthrough work led to the discovery of 'Organizer Centers'. He was awarded the Nobel Prize in 1935.

Spemann's proposal was not executed until 1952 when Briggs and King set out to address this exact question. It is important to remember that until this time, it was still believed that as a cell differentiated, it lost parts of its genome in order to acquire specialization. In 1952, Briggs and King demonstrated that the transfer of nuclei from early embryonic cells of a frog (*Rana pipiens*), at a stage where specialization had begun, into eggs resulted in a third of the transfer giving rise to live swimming tadpoles (*Figure* 1). Years later, it was this exact same technique that resulted in the cloning of Dolly the sheep. These experiments suggested that at such an early embryonic stage, no genomic loss

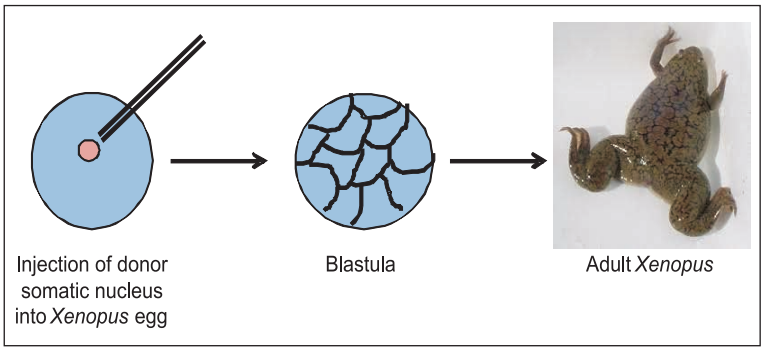


Figure 1. Nuclear transfer into *Xenopus* eggs: Somatic nuclei were injected into an enucleated egg. A certain number of eggs receiving nuclear transfer developed normally and went on to produce adult frogs.

Sir John B Gurdon, born in 1933 in Dippenhall, United Kingdom, studied zoology at Christ Church, Oxford. His seminal work demonstrated that genomic composition remained intact through various stages of development and differentiation.

had occurred. In 1957 they published another set of results demonstrating that the transfer of nuclei belonging to more specialized cells into eggs, failed to promote normal development into tadpoles, lending credence to the hypothesis that permanent genetic changes occur during development and specialization.

John Gurdon and 'Nuclear Stemness'

It was at this point in time that John Gurdon entered this field of research. Interestingly enough, Gurdon's entry into biology was not as smooth as one would expect of someone who went on to win the Nobel Prize. Biology was not taught in his school until the age of 15. Within a single semester of studying biology, his teacher commented on John's intention to become a biologist, saying that he thought it would only be a sheer waste of time for both John and his teacher. Gurdon went on to study Latin and ancient Greek. While applying for university, he was told that as long as he did not apply for either Greek or Latin, he would be accepted at Oxford. He opted for Zoology and spent a year taking extra lessons. By the time he applied for the graduate program at Oxford he was already fascinated by biology and was especially interested in how butterfly wing colour patterns were specified. He applied to the Oxford Entomology Department to pursue his graduate studies, but was turned down [2].

In due course of time he joined the laboratory of Michail Fischberg, a lecturer in embryology, and was urged to try his hand at nuclear transplantation. Using *Xenopus* as a model system, Gurdon diligently performed thousands of nuclear transfers using nuclei from differentiated tadpole cells as donors and frog oocytes as the recipient. He observed that as the differentiation status of the donor cell increased, the efficiency of nuclear transfers decreased. However, it was still possible to obtain live adults from even highly differentiated cell nuclei [3]. This was a landmark result and demonstrated emphatically that the genomic composition remained intact through various stages of development and differentiation, and that specification does not depend on the loss of genetic material. However, it still remained unknown as to what

molecules present in the oocyte possess the remarkable ability to turn back the clock and restore a cell back to an embryonic state. In recognition of Gurdon's incredible contributions to the field of early development, an institute was created in his name for pursuing research activities in developmental biology and cancer.

The ramifications of Gurdon's research are huge. In the years that followed, numerous attempts were made to perform nuclear transplants in mammals. Over the last few decades, mice, sheep, pigs, cows and monkeys have all been cloned using nuclear transplantation. Of these the most famous was the sheep, Dolly. Following this flurry of activity in the cloning field, James Thomson and his colleagues in the USA reported deriving the first embryonic stem cell line from the human embryo [4]. Extensive efforts were made to understand the gene networks that maintained a cell in the embryonic state and the changes it had to undergo during the process of differentiation. It was during this time that the idea emerged that perhaps patient-specific embryonic stem cells could be produced by performing nuclear transfers from patient donor cells into human oocytes. While nuclear transfers have been successfully performed in other mammals, conducting such experiments in humans is fraught with several problems, both ethical and technical. The efficiency of nuclear transfer is extremely low even in other mammalian systems and is likely to be even lower in humans. Thus a large number of attempts would have to be made before a single good-quality embryo is obtained from which it is possible to isolate embryonic stem cells. Additionally, a number of people were possessed of the fear that scientists would start cloning humans. While this is far from the truth, a number of regulations were put in place that restricted the usage of stem cells.

Shinya Yamanaka, born in 1962 in Japan revolutionized the concept of 'Stemness'. Yamanaka factor, i.e, *Oct4*, *Sox*, *Klf4* and *c-Myc* can make pluripotent stem cells from somatic cells

Yamanaka and 'Reverse Differentiation'

Shinya Yamanaka, a researcher based in Japan, had been working on identifying various genes that maintained the 'stemness' state in embryonic stem cells or ESCs. Along with his graduate student Kazutoshi Takahashi, they shortlisted a set of genes that could be

responsible for maintaining the pluripotent state of embryonic stem cells. (The word pluripotent refers to the ability of a cell to produce many different types of cells.) The plan was to introduce these genes into fibroblasts, (a type of differentiated cell which is the major constituent of connective tissue) in the hope that some combination of these ESC genes might trigger a conversion in the fibroblasts to a more pluripotent state. Starting with a list of 24 genes, Takahashi introduced these into mouse fibroblasts in combination. Amazingly enough, this led to their conversion into cells that morphologically and transcriptionally resembled embryonic stem cells, displaying markers of pluripotency (genes that are expressed only in the pluripotent cells and thereby can mark these cells) [5]. Thus, differentiated somatic cells could be successfully reprogrammed to a pluripotent state.

Takahashi and Yamanaka took this one step further. Numerous combinations of these 24 genes were tried and finally a combination of four genes was found to be sufficient to reprogram somatic cells to pluripotency. These four genes are *Oct4*, *Sox2*, *Klf4* and *c-Myc* and are now famously referred to as the Yamanaka factors. The cells were dubbed "induced pluripotent stem cells" or iPSCs (*Figure* 2). A year after this initial discovery, Yamanaka and colleagues followed this up by demonstrating that the same combination of four factors could successfully reprogram human fibroblasts to pluripotency. Simultaneously, James Thomson and colleagues reported a second combination of factors which could also result in the reprogramming of human cells [6,7]. What this

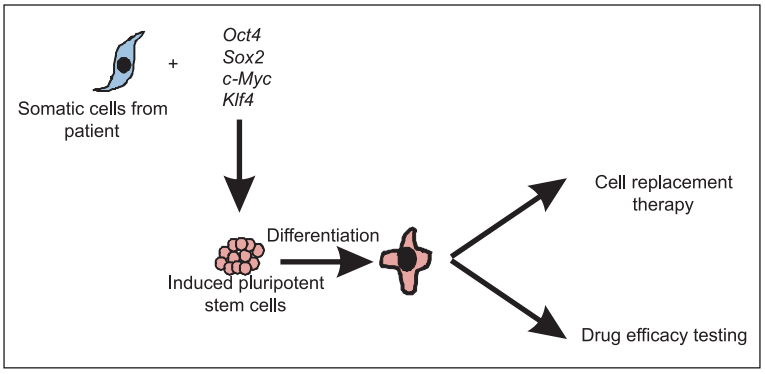
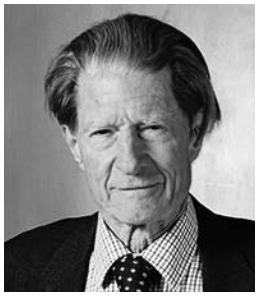


Figure 2. Outline of the Yamanaka technique of reprogramming: Introduction of the 4 Yamanaka factors – *Oct4*, *Sox2*, *Klf4* and *c-Myc* into somatic cells results in the formation of induced pluripotent stem cells. Such iPSCs can be generated from patient somatic cells which can be differentiated down specific lineages for use in cell replacement therapy or for testing drug efficacy.

basically meant to the biomedical community was that, for the first time one could envisage the generation of patient-specific pluripotent cells for cell-based therapies. Indeed, following these reports, hundreds of laboratories across the globe turned their attention to creating patient-specific iPSCs. Personalized medicine was fast becoming a reality.

While the contributions of Gurdon and Yamanaka occurred in different millennia, they both demonstrated that every cell retains the ability to be converted back to the pluripotent state. Together they challenged the prevalent view that differentiation was a one-way process and showed that under certain specialized conditions mature cells could revert to a pluripotent state.



Sir John B Gurdon

Shinya Yamanaka

Figure 3. The winners of the 2012 Nobel Prize in Physiology or Medicine.

Medical Implications of iPSCs

The importance of induced pluripotent stem cells cannot be stressed enough. One can imagine a scenario where a patient suffers from a condition where a particular cell type is destroyed or damaged. Through the process of reprogramming, patient-specific iPSCs can be created which can then be differentiated to produce the particular cell type that can then be reintroduced into the patient. In patients who have a particular gene mutated, which affects a particular cell type, it is also possible to perform gene therapy to correct the mutation in patient-specific iPSCs and then introduce the corrected cells post differentiation into patients. In situations where it is unclear whether a patient will respond to a particular drug regimen or not, one can also use patient-specific iPSCs to screen drugs and chemicals in order to determine which combination will be the most efficacious. iPSCs can also be used to determine the best dosage at which the required effect is obtained while minimizing side effects. These are only a few examples of iPSCs and one can be certain that these cells can be used in many more conditions and situations in the years to come.

Future of Stem Cell Research

While it may appear that little is left to be done, in reality we still know very little about iPSCs. Even though iPSCs closely resemble

The brilliant discoveries of Gurdon and Yamanaka have provided a glimmer of hope to those people for whom personalized medicine is the only avenue for leading a normal life.

ESCs, there are still many differences between these two cells types. Can these differences result in tumorigenesis or improper differentiation, endangering the patient? Numerous researchers are investing time and effort to improve the quality and efficiency of producing these iPSCs. Efforts are being made to generate iPSCs without the introduction of any extraneous genetic material which may intergrate into the host genome. Researchers are also making an effort to grow these cells on substrata that is completely free of animal products in an attempt to make iPSCs safe to re-introduce back into patients. However, in order to gain a better understanding of iPSCs, it is important to continue to study embryonic stem cells in parallel. In due course of time, it may indeed be possible to generate safe iPSCs which can be used in patients. It is important to understand that many years of research still need to be done to completely ensure that iPSCs are safe for introducing into patients. The same can be said for stem cells. We need to exercise caution before jumping ahead and using these cells to cure diseases. If stem cells or iPSCs are not treated properly, they can result in the development of tumors which can be more debilitating than the actual disease itself. However, there is great hope for the utilization of stem cells and iPSCs to cure a number of life-threatening conditions, and research in hundreds of laboratories across the world is ongoing to make this a reality.

The brilliant discoveries of Gurdon and Yamanaka have not only provided glimpses into the intricacies of early development, but also provided a glimmer of hope to those people for whom personalized medicine is the only avenue for leading a normal life.

Suggested Reading

[1] G Q Daley, Cellular alchemy and the golden age of reprogramming, *Cell.*, Vol.151, No.6, pp.1151–1154, 2012.

[2] J B Gurdon, From nuclear transfer to nuclear reprogramming: the reversal of cell differentiation, *Annu. Rev. Cell. Dev. Biol.*, Vol.22, pp.1–22, 2006.

[3] J B Gurdon, The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles, *J. Embryol. Ex. Morphol.*, Vol.10, pp.622–640, 1962.

[4] **J A Thomson, J Itskovitz-Eldor, S S Shapiro, M A Waknitz, J J Swiergiel, V S Marshall and J M Jones, Embryonic stem cell lines derived from human blastocysts, *Science*, Vol.282, No.5391, pp.1145–1147, 1998.**

[5] **K Takahashi and S Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, *Cell*., Vol.126, No.4, pp.663–676, 2006.**

[6] **K Takahashi, K Tanabe, M Ohnuki, M Narita, T Ichisaka, K Tomoda and S Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, *Cell*, Vol.131, No.5, pp.861–872, 2007.**

[7] **J Yu, M A Vodyanik, K Smuga-Otto, J Antosiewicz-Bourget, J L Frane, S Tian, J Nie, G A Jonsdottir, V Ruotti, R Stewart, I I Slukvin and J A Thomson, Induced pluripotent stem cell lines derived from human somatic cells, *Science*, Vol.318, No.5858, pp.1917–1920, 2007.**

Address for Correspondence
Deepa Subramanyam
National Centre for Cell Science
University of Pune Campus
Ganeshkhind
Pune 411 007, India.