The Nobel Prize in Physiology or Medicine 2012

For the discovery that mature cells can be reprogrammed to **become pluripotent jointly to**

Sir John B. Gurdon, Shinya Yamanaka

Press Release on October 08, 2012

The Nobel Prize recognizes two scientists who discovered that mature, specialised cells can be reprogrammed to become immature cells capable of developing into all tissues of the body. Their findings have revolutionised our understanding of how cells and organisms develop. John B. Gurdon discovered in 1962 that the specialisation of cells is reversible. In a classic experiment, he replaced the immature cell nucleus in an egg cell of a frog with the nucleus from a mature intestinal cell. This modified egg cell developed into a normal tadpole. The DNA of the mature cell still had all the information needed to develop all cells in the frog. Shinya Yamanaka discovered more than 40 years later, in 2006, how intact mature cells in mice could be reprogrammed to become immature stem cells. Surprisingly, by introducing only a few genes, he could reprogram mature cells to become pluripotent stem cells, i.e. immature cells that are able to develop into all types of cells in the body.These groundbreaking discoveries have completely changed our view of the development and cellular specialisation. We now understand that the mature cell does not have to be confined forever to its specialised state. Textbooks have been rewritten and new research fields have been established. By reprogramming human cells, scientists have created new opportunities to study diseases and develop methods for diagnosis and therapy.

All of us developed from fertilized egg cells. During the first days after conception, the embryo consists of immature cells, each of which is capable of developing into all the cell types that form the adult organism. Such cells are called pluripotent stem cells. With further development of the embryo, these cells give rise to nerve cells, muscle cells, liver cells and all other cell types - each of them specialised to carry out a specific task in the adult body. This journey from immature to specialised cell was previously considered to be unidirectional. It was thought that the cell changes in such a way during maturation that it would no longer be possible for it to return to an immature, pluripotent stage. **John B. Gurdon** challenged the dogma that the specialised cell is irreversibly committed to its fate. He hypothesised that its genome might still contain all the information needed to drive its development into all the different cell types of an organism. In 1962, he tested this hypothesis by replacing the cell nucleus of a frog's egg cell with a nucleus from a mature, specialised cell derived from the intestine of a tadpole. The egg developed into a fully functional, cloned tadpole and subsequent repeats of the experiment yielded adult frogs. The nucleus of the mature cell had not lost its capacity to drive development to a fully functional organism.

Gurdon's landmark discovery was initially met with scepticism but became accepted when it had been confirmed by other scientists. It initiated intense research and the technique was further developed, leading eventually to the cloning of mammals. Gurdon's research taught us that the nucleus of a mature, specialized cell can be returned to an immature, pluripotent state. But his experiment involved the removal of cell nuclei with pipettes followed by their introduction into other cells. Would it ever be possible to turn an intact cell back into a pluripotent stem cell?

Shinya Yamanaka was able to answer this question in a scientific breakthrough more than 40 years after Gurdon´s discovery. His research concerned embryonal stem cells, i.e. pluripotent stem cells that are isolated from the embryo and cultured in the laboratory. Such stem cells were initially isolated from mice by Martin Evans (Nobel Prize 2007) and Yamanaka tried to find the genes that

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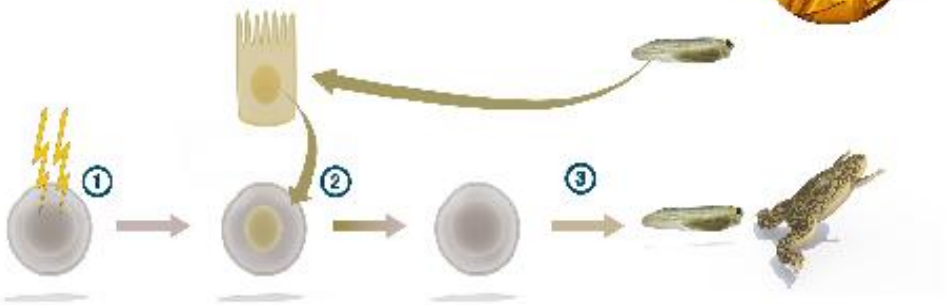
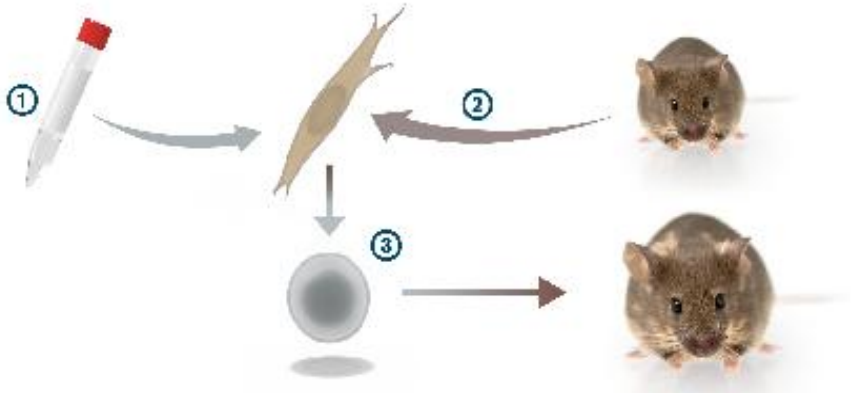
John B. Gurdon

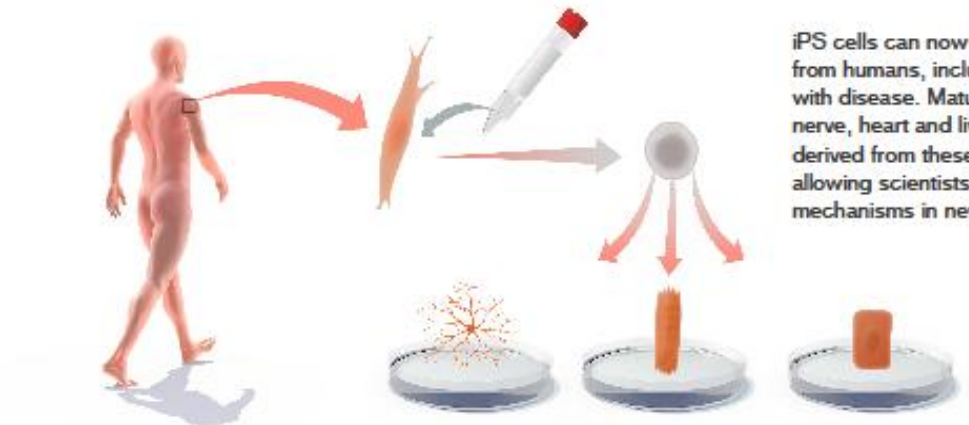


John B. Gurdon eliminated the nucleus of a frog egg cell (1) and replaced it with the nucleus from a specialised cell taken from a tadpole (2). The modified egg developed into a normal tadpole (3). Subsequent nuclear transfer experiments have generated cloned mammals (4).



Shinya Yamanaka

Shinya Yamanaka studied genes that are important for stem cell function. When he transferred four such genes (1) into cells taken from the skin (2), they were reprogrammed into pluripotent stem cells (3) that could develop into all cell types of an adult mouse. He named these cells induced pluripotent stem (iPS) cells.



iPS cells can now be generated from humans, including patients with disease. Mature cells including nerve, heart and liver cells can be derived from these iPS cells, thereby allowing scientists to study disease mechanisms in new ways.

Illustration and layout: Mattias Karlén

kept them immature. When several of these genes had been identified, he tested whether any of them could reprogram mature cells to become pluripotent stem cells.Yamanaka and his co-workers introduced these genes, in different combinations, into mature cells from connective tissue, fibroblasts, and examined the results under the microscope. They finally found a combination that worked, and the recipe was surprisingly simple. By introducing four genes together, they could reprogram their fibroblasts into immature stem cells! The resulting induced pluripotent stem cells (iPS cells) could develop into mature cell types such as fibroblasts, nerve cells and gut cells. The discovery that intact, mature cells could be reprogrammed into pluripotent stem cells was published in 2006 and was immediately considered a major breakthrough.The discoveries of Gurdon and Yamanaka have shown that specialised cells can turn back the developmental clock under certain circumstances. Although their genome undergoes modifications during development, these modifications are not irreversible. We have obtained a new view of the development of cells and organisms.

Research during recent years has shown that iPS cells can give rise to all the different cell types of the body. These discoveries have also provided new tools for scientists around the world and led to remarkable progress in many areas of medicine. iPS cells can also be prepared from human cells.

For instance, skin cells can be obtained from patients with various diseases, reprogrammed, and examined in the laboratory to determine how they differ from cells of healthy individuals. Such cells constitute invaluable tools for understanding disease mechanisms and so provide new opportunities to develop medical therapies.

Source: www.nobelprize.org/nobel_prizes/medicine/laureates/2012/press.html

Unique bond between SAP102 and GluN2B enables learning and memory

Published on October 31, 2012

Two proteins have a unique bond that enables brain receptors essential to learning and memory to not only get and stay where they're needed, but to be hauled off when they aren't, researchers say.
NMDA receptors increase the activity and communication of brain cells and are strategically placed, much like a welcome center, at the receiving end of the communication highway connecting two cells. They also are targets in brain-degenerating conditions such as Alzheimer's and Parkinson's.

In a true cradle-to-grave relationship, researchers have found the scaffolding protein, SAP102, which helps stabilize the receptor on the cell surface, binds with a subunit of the NMDA receptor called GluN2B at two sites, said Bo-Shiun Chen, neuroscientist at the Medical College of Georgia at Georgia Health Sciences University.While one binding site is the norm, these proteins have one that's stronger than the other. When it's time for the normal receptor turnover, the stronger bond releases and the lesser one shuttles the receptor inside the cell for degradation or recycling."One binding site is involved in stabilizing the receptor on the cell surface and the other is important in removing the receptor. We think it's a paradigm shift; we've never thought about the same scaffolding protein having two roles," said Chen, corresponding author of the study in the journal *Cell Reports.*"We believe by understanding the normal turnover of these receptors, we can learn more about how to prevent the abnormal receptor loss that occurs in debilitating diseases such as Alzheimer's." In Parkinson's, the receptors inexplicably move away from where the synapse, or information highway connects to the neuron, making them less effective. NMDA receptors are supposed to cluster where the synapse hooks into the receiving neuron; in fact, it's part of what anchors the synapse, Chen said.

Interestingly, this pivotal protein, SAP102, a member of the MAGUK family of scaffolding proteins, is the only family member known to directly contribute to maladies: its mutation causes intellectual disability.While all cells have a system for managing the number of receptors on their surface, in Alzheimer's, this removal process appears accelerated, with increased engulfing of receptors and less neuron-to-neuron communication. The neurotransmitter glutamate helps establish and maintain the synapse and also binds with GluN2B.GluN2B-containing NMDA receptors stay open to receive information for a long time, enabling the type of vigorous and sustained communication that enables learning and memory. In fact the number of these receptors naturally decreases with age, which may be one reason young people learn easier. When it's time to remove a receptor, phosphorus gets added to GluN2B, changing its function so it no longer binds to the scaffolding protein.

Source: *www.news-medical.net/.../Unique-bond-between-SAP102-and-GluN2B-enables-learning-and-memory.aspx*

New pathway appears to play a major role in information processing in the brain

Published on November 9, 2012

Scientists at The Scripps Research Institute (TSRI) have identified a new pathway that appears to play a major role in information processing in the brain. Their research also offers insight into how imbalances in this pathway could contribute to cognitive abnormalities in humans.

The study, published in the November 9, 2012 issue of the journal *Cell*, focuses on the actions of a protein called HDAC4. The researchers found that HDAC4 is critically involved in regulating genes

essential for communication between neurons. They found that HDAC4 represses these genes, and its function in a given neuron is controlled by activity of other neurons forming a circuit.

Synapses, specialized junctions that allow neurons to exchange information, are incredibly complex and built with hundreds of genes. Many of these genes become induced when neurons receive excitatory input from other neurons, including those activated by sensory experiences such as vision, hearing and smell. This process influences the assembly of neural circuits during development, and plays a fundamental role in learning and memory.

The Maximov laboratory is interested in understanding how synapses are formed and regulated. Previous studies have identified several factors necessary for activity-dependent transcription in the brain (transcription is a process of converting genetic information from DNA to RNA), but Maximov notes many puzzles remain to be solved. For example, the majority of synapse-related genes are silent in the embryonic brain, which does not receive direct sensory input from an external world. These genes become de-repressed shortly after birth, yet scientists still know little about the underlying mechanisms of how this happens.

Richard Sando III, a graduate student at the TSRI Kellogg School of Science and Technology, a member of the Maximov lab and the first author of this study, noted the team become interested in class IIa histone deacetylases (HDACs), which include HDAC4, in part because they have been implicated in regulation of transcription of non-neuronal tissues. Class IIa HDACs are also known to change their cellular localization in response to various signals. He said that there were hints that, in neurons, the translocation of HDAC4 from the nucleus to cytoplasm may be triggered by synaptic activity. They found that mutant mice lacking excitatory transmitter release in the brain accumulate HDAC4 in neuronal nuclei. But what was really exciting was our discovery that nuclear HDAC4 represses a pool of genes involved in synaptic communication and memory formation.Coincidentally, Maximov had been familiar with these same genes since his postdoctoral training with Tomas Sudhof, a neuroscientist whose pioneering work resulted in the identification of key elements of the transmitter release machinery. "It was truly astonishing when their names came up in our *in vitro* genome-wide mRNA profiling screens for neuronal HDAC4 targets," Maximov said.

To learn more about the function of HDAC4 in the brain, the team wanted to study its role in a mouse model. First, however, the scientists had to overcome a serious technical obstacle—HDAC4 also appears to protect neurons from apoptosis (programmed cell death), so complete inactivation of this gene would lead to neurodegeneration. To solve this problem, the team generated mice carrying a mutant form of HDAC4 that could not be exported from the cell nucleus. This mutant repressed transcription independently of neuronal activity.Another surprise came after the team had already initiated their experiments. Underscoring the team's findings, a human genetic study was published linking mutations in the human HDAC4 locus with a rare form of mental retardation.

"One of these human mutations produces a protein similar to a mutant that we introduced into the mouse brain," said Maximov. "Furthermore, our studies revealed that these mice do not learn and remember as well as normal mice, and their memory loss is associated with deficits in synaptic transmission. The pieces came together."

Other contributors to the study, "HDAC4 Governs a Transcriptional Program Essential for Synaptic Plasticity and Memory," were Natalia Gounko and Simon Pieraut from the Maximov Laboratory; John

Yates III, professor in the Department of Chemical Physiology at TSRI; and Lujian Liao, a staff scientist in the Yates Laboratory.

Source: http://www.scripps.edu/news/press/2012/20121109maximov.html

Study identifies essential mechanism that regulates calcium flow into mitochondria

Published on October 26, 2012

Most healthy cells rely on a complicated process to produce the fuel ATP. Knowing how ATP is produced by the cell's energy storehouse - the mitochondria -- is important for understanding a cell's normal state, as well as what happens when things go wrong, for example in cancer, cardiovascular disease, neurodegeneration, and many rare disorders of the mitochondria.

Two years ago, Kevin Foskett, PhD, professor of Physiology at the Perelman School of Medicine, University of Pennsylvania, and colleagues discovered that fundamental control of ATP is an ongoing shuttle of calcium to the mitochondria from another cell compartment. They found that mitochondria rely on this transfer to make enough ATP to support normal cell metabolism.

Now, Foskett's lab and the lab of co-corresponding author Muniswamy Madesh, PhD, at Temple University, have discovered an essential mechanism that regulates the flow of calcium into mitochondria, described in the October 26 issue of *Cell*. They demonstrated that the mitochondrial protein MICU1 is required to establish the proper level of calcium uptake under normal conditions. Maintaining the correct levels of calcium in the mitochondria plays an important role in cellular physiology: Calcium flux across the inner mitochondrial membrane regulates cell energy production and activation of cell-death pathways, for example. In MICU1's absence, Madesh and Foskett found that mitochondria become overloaded with calcium, generating excessive amounts of reactive oxygen molecules and eventually cell death. Mitochondrial calcium has been studied for nearly five decades at Penn, starting with observations made by the late Britton Chance, the Eldridge Reeves Johnson Professor Emeritus of Biochemistry and Biophysics, in the 1960s, and physiologist Tony Scarpa, in the early 1970s. Calcium uptake is driven by a voltage across the inner mitochondrial membrane and mediated by a calcium-selective ion channel called the uniporter. While the proper level of calcium influx is required for mitochondria to produce enough ATP to support cellular processes, too much influx overloads mitochondria and is toxic. Because producing ATP generates a large negative voltage that attracts the positively charged calcium ion, mitochondria face an ongoing risk of becoming overloaded with calcium.

Mitochondria somehow manage to keep the concentration of calcium in the mitochondrial matrix at beneficial levels. Remarkably, these levels are 100,000 to a million times lower than expected if calcium was simply in equilibrium with the cytoplasm. The molecular mechanisms for how this is accomplished have remained unclear.

Foskett and Madesh discovered that MICU1 interacts with the uniporter calcium channel protein MCU and sets a brake for calcium uptake by the mitochondria. This regulation is essential to prevent an overload of calcium in the mitochondria and associated cellular stress. Until recently, the molecular identity of the uniporter was unknown. MICU1 was identified as a protein found at the inner mitochondrial membrane and seemed to be required for uniporter calcium uptake. Subsequently, MCU

was identified as the likely ion-conducting part of the uniporter. MICU1 and MCU interact biochemically and their expression patterns are tightly coupled across tissues and species. Nevertheless, the relationship between them was unknown.

The Penn-Temple team found that loss of MICU1 leads to an accumulation of calcium in the mitochondria through MCU-mediated calcium uptake. Rather than being required for MCU-mediated mitochondrial calcium uptake, as previously thought, they found that MICU1 acts as the gatekeeper. MICU senses the concentration of calcium in the matrix of the mitochondria, establishing a set point that prevents calcium uptake under normal, resting concentrations of calcium.

These findings reveal a previously unknown role for MICU1 in preventing an overload of calcium in the mitochondria. Foskett and Madesh speculate that the interaction between MICU1 and MCU may be an important site for regulating cellular bioenergetics and oxygen molecule signalling. They also suggest that disrupting the balance of the two molecules could lead to damage in neurons and cells of the heart, liver and other organs. Mitochondrial calcium is important for metabolic and cardiovascular functions, and maintaining this homeostasis is crucial. Cells lacking the set point will lead to mitochondrial dysfunction and cell death.
"Our findings suggest new therapeutics in a variety of pathophysiological conditions," notes first author Karthik Mallilankaraman from Temple.

"If we could discover molecules or mechanisms to impinge on the biochemical or functional interactions of MICU1 and MCU, or to modify their activities, it may be possible that metabolic dysfunctions observed in many diseases could be manipulated with beneficial therapeutic outcomes," concludes Foskett.

Source:*www.news-medical.net/.../Study-identifies-essential-mechanism-that-regulates-calcium-flow-into-mitochondria.aspx*

Diabetics-with-low-vitamin-D-levels-more-likely-to-develop-clogged-arteries

Published on November 14, 2012

People with diabetes often develop clogged arteries that cause heart disease, and new research at Washington University School of Medicine in St. Louis suggests that low vitamin D levels are to blame. In a study published Nov. 9 in the *Journal of Biological Chemistry*, the researchers report that blood vessels are less like to clog in people with diabetes who get adequate vitamin D. But in patients with insufficient vitamin D, immune cells bind to blood vessels near the heart, and then trap cholesterol to block those blood vessels

In earlier research, Bernal-Mizrachi, an assistant professor of medicine and of cell biology and physiology, and his colleagues found that vitamin D appears to play a key role in heart disease. This new study takes their work a step further, suggesting that when vitamin D levels are low, a particular class of white blood cell is more likely to adhere to cells in the walls of blood vessels. Vitamin conspires with immune cells called macrophages either to keep arteries clear or to clog them. The macrophages begin their existence as white blood cells called monocytes that circulate in the bloodstream. But when monocytes encounter inflammation, they are transformed into macrophages, which no longer circulate.

In the new study, researchers looked at vitamin D levels in 43 people with type 2 diabetes and in 25 others who were similar in age, sex and body weight but didn't have diabetes.

They found that in diabetes patients with low vitamin D - less than 30 nanograms per millilitre of blood - the macrophage cells were more likely to adhere to the walls of blood vessels, which triggers cells to get loaded with cholesterol, eventually causing the vessels to stiffen and block blood flow. They looked at blood pressure, cholesterol, diabetes control, body weight and race. But only vitamin D levels correlated to whether these cells stuck to the blood vessel wall."Riek and Bernal-Mizrachi say what's not yet clear is whether giving vitamin D to people with diabetes will reverse their risk of developing clogged arteries, a condition called atherosclerosis. They now are treating mice with vitamin D to see whether it can prevent monocytes from adhering to the walls of blood vessels near the heart, and they also are conducting two clinical trials in patients. In one of those studies, the researchers are giving vitamin D to people with diabetes and hypertension to see whether the treatment may lower blood pressure. In the second study, African Americans with type 2 diabetes are getting vitamin D along with their other daily medications, and the research team is evaluating whether vitamin D supplements can slow or reverse the progression of heart disease. Sometime in the next several months, the scientists hope to determine whether vitamin D treatment can reverse some of the risk factors associated with cardiovascular disease."In the future, we hope to generate medications, potentially even vitamin D itself that help prevent the deposit of cholesterol in the blood vessels. Previous studies have linked vitamin D deficiency in these patients to increases in cardiovascular disease and in mortality. Other work has suggested that vitamin D may improve insulin release from the pancreas and insulin sensitivity. Our ultimate goal is to intervene in people with diabetes and to see whether vitamin D might decrease inflammation, reduce blood pressure and lessen the likelihood that they will develop atherosclerosis or other vascular complications."

Source: *www.news-medical.net/.../Diabetics-with-low-vitamin-D-levels-more-likely-to-develop-clogged-arteries.aspx*

Research-shows-reduced-plasticity-in-brains-of-preterm-teens

Published on November 14, 2012

New research at the University of Adelaide has demonstrated that teenagers born prematurely may suffer brain development problems that directly affect their memory and learning abilities.The research, conducted by Dr Julia Pitcher and Dr Michael Ridding from the University of Adelaide's Robinson Institute, shows reduced 'plasticity' in the brains of teenagers who were born preterm (at or before 37 weeks gestation). The results of the research are published today in the *Journal of Neuroscience*. "Plasticity in the brain is vital for learning and memory throughout life," Dr Pitcher says. "It enables the brain to reorganize itself, responding to changes in environment, behavior and stimuli by modifying the number and strength of connections between neurons and different brain areas. Plasticity is also important for recovery from brain damage."We know from past research that preterm-born children often experience motor, cognitive and learning difficulties. The growth of the brain is rapid between 20 and 37 weeks gestation, and being born even mildly preterm appears to subtly but significantly alter

brain microstructure, neural connectivity and neurochemistry. However, the mechanisms that link this altered brain physiology with behavioral outcomes - such as memory and learning problems - have remained unknown.

The researchers compared preterm adolescents with those born at term, and also with term-born adults. They used a non-invasive magnetic brain stimulation technique, inducing responses from the brain to obtain a measure of its plasticity. Levels of cortisol, normally produced in response to stress, were also measured to better understand the chemical and hormonal differences between the groups.

Teenagers born preterm clearly showed reduced neuroplasticity in response to brain stimulation. Surprisingly, even very modest preterm birth was associated with a reduced brain response. On the other hand, term-born teenagers were highly 'plastic' compared with adults and the preterm teens.

Preterm teens also had low levels of cortisol in their saliva, which was highly predictive of this reduced brain responsiveness. People often associate increased cortisol with stress, but cortisol fluctuates up and down normally over each 24-hour period and this plays a critical role in learning, the consolidation of new knowledge into memory and the later retrieval of those memories. This might be important for the development of a possible therapy to overcome the neuroplasticity problem.

Source:*www.news-medical.net/.../Research-shows-reduced-plasticity-in-brains-of-preterm-teens.aspx*