# Stem Cell Frontiers

**2011 was a blockbuster year for the stem cell field. Researchers made great progress in growing complex organs in vitro and producing a variety of cell types on demand. But in the last few months, a few studies have markedly propelled the field forward. This Select highlights these recent discoveries, including the first embryonic stem cells (ESCs) made by nuclear transfer, a new type of adult stem cell, and dopamine neurons that reverse symptoms of a neurodegenerative disease.**

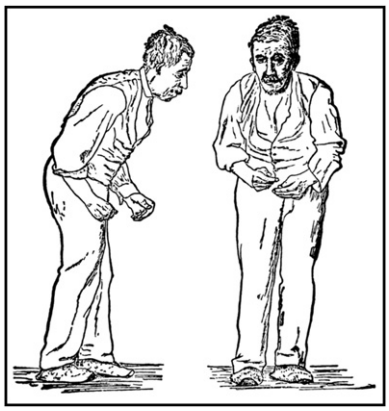


Illustration of an individual with Parkinson's disease. Drawing by William Richard Gowers from "A Manual of Diseases of the Nervous System" (1886).

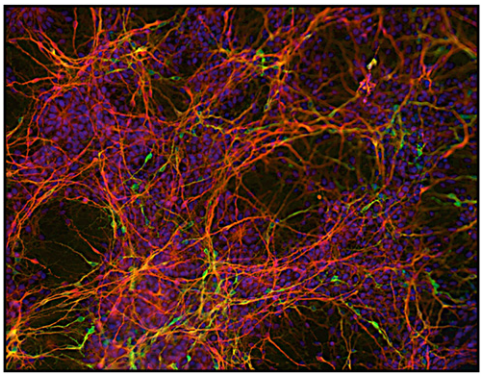
## Functional Dopamine Neurons from ESCs

Parkinson's disease results from the death of dopamine neurons in a region of the midbrain involved in movement, which leads to tremors, stiffness, and difficulty walking. Current therapies for Parkinson's disease are severely limited, and thus, researchers have aggressively sought alternative approaches, such as stem cell therapies. Previous studies have generated dopamine neurons from human pluripotent stem cells (PSCs), but these neurons perform poorly in transplantation experiments: they fail to engraft, display neural overgrowth, or differentiate inappropriately into nondopaminergic neurons. Now, Kriks et al. (2011) develop a new strategy for making dopamine neurons from human ESCs; these neurons efficiently integrate into the brains of monkeys and improve motor function of mouse and rat models of Parkinson's disease.

Dopamine neurons originate from a group of cells in the developing brain, called floor-plate neurons. Recently, the Studer lab reported a protocol for generating precursors of floor-plate cells from human ESCs. When they treat these precursor cells with a specific cocktail of factors (i.e., Sonic Hedgehog activators, canonical WNT activators, and SMAD inhibitors), the neurons express genes that are characteristics of dopamine neurons. The cells also display electrophysiological properties distinct to dopamine neurons of the midbrain. Next, Kriks et al. inject the induced neurons into the brains of mice and rats with lesions in their midbrain dopamine neurons (which mimics Parkinson's disease). The transplanted cells survive for at least 5 months in both animal models, and they reverse some of the motor disabilities caused by the brain damage. Kriks and colleagues then scale up the production of the dopamine neurons 100-fold and transplant the cells into two rhesus monkeys with Parkinsonian-like symptoms. The neurons successfully integrate into the host after 1 month, but future experiments are needed to determine whether the transplanted cells can recover the monkeys' motor function and what the long-term effects of the cell therapy will have.
*Kriks, S., et al. (2011). Nature 480, 547–551.*

## The Chance to Study Patients' Neurons

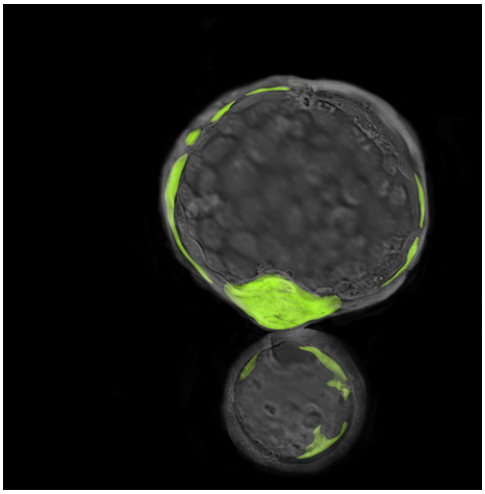
Kriks and colleagues take a significant stride toward developing a cell replacement therapy for Parkinson's disease, but these types of treatments still remain quite far from the clinic. In the meantime, reprogramming technologies are having a more immediate impact in another arena: disease modeling and drug discovery for neuropsychiatric disorders. Now, a new study by Paşca et al. elegantly demonstrates this strategy for Timothy syndrome.



Human neurons (red) differentiate from iPSCs derived from a patient with Timothy syndrome. The patient cultures showed an excess number of neurons expressing tyrosine hydroxylase (green), the rate-limiting enzyme in the synthesis of catecholamines. Nuclei are stained blue. Image courtesy of Dolmetsch Lab, Stanford University.

Timothy syndrome is caused by a missense mutation in the *CACNA1C* gene, which encodes a subunit of the calcium channel Cav1.2. This mutation alters the electrophysiological properties of the channel, leading to cardiac arrhythmia, delays in neurodevelopment, and autism spectrum disorders. Paşca et al. take fibroblast skin cells from two individuals with Timothy syndrome and three controls. They convert the fibroblasts to induced pluripotent stem cells (iPSCs) by standard reprogramming techniques (i.e., they infect the cells with retroviruses expressing *SOX2*, *OCT3/4*, *KLF4*, and *C-MYC*). They then coax the cells into forming neurons. The Timothy syndrome neurons display dramatic changes in calcium signaling compared to the control neurons. But, more surprisingly, they also show abnormal expression of tyrosine hydroxylase, an enzyme critical for making the key neurotransmitters dopamine and norepinephrine. Paşca and colleagues then engineer a mouse model of Timothy syndrome. Although the animals display some of the phenotypes observed in the human neurons, the animals don't generate neurons that overproduce dopamine and norepinephrine. Finally, Paşca and colleagues use the Timothy syndrome neurons as a drug testing ground. They identify a new class of compounds that reduces the extra dopamine-producing

neurons by ~70%. Such results highlight the importance of studying human neurons from patients—an approach made immensely more accessible by advances in reprogramming technologies.
*Paşca, S.P., et al. (2011). Nat. Med. 17, 1657–1662.*



**A human blastocyst obtained after transfer of a skin cell genome into a human oocyte. The green fluorescence originates from the skin cell genome. The bright cluster of cells in the center is the inner cell mass from which stem cells are derived. Image courtesy of The New York Stem Cell Foundation, S. Paull.**

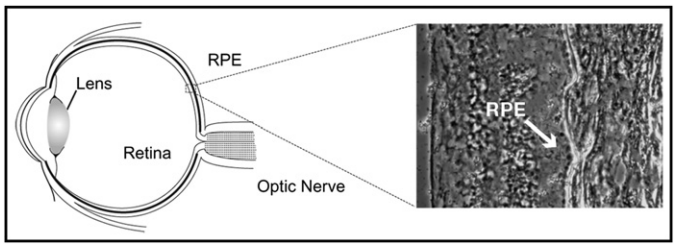
## Nuclear Transfer Clears Epigenetic Memory

Paşca et al. reprogram skin cells by overexpressing four master transcription factors linked to pluripotency, but this isn't the only approach for converting differentiated cells into stem cells. In many animals, "personalized" stem cells can be made through a technique called "somatic cell nuclear transfer." Specifically, a diploid genome from a differentiated cell is injected into an oocyte, and the oocyte's haploid genome is removed. When this "fertilized" egg starts to develop, stem cells can be harvested from the blastocyst. Now, Noggle et al. (2011) report the first human ESC lines derived from somatic cell nuclear transfer. But there's one hitch: the cells have three copies of the genome.

Noggle et al. begin by switching out the oocyte genome with that from a fibroblast cell at metaphase II of meiosis because this strategy has been successful in other mammalian species. However, the human zygote stops dividing after only a few rounds of mitosis (i.e., with only six to ten cells). A series of control experiments suggest that the oocyte genome is required to initiate active transcription from the transplanted genome; as transcription fails, the zygote stops dividing. When the oocyte genome is kept after the fibroblast nucleus is injected, ~20% of the zygotes develop to the blastocyst stage. Noggle and colleagues then derive two stem cell lines from these embryos. Although the cells are triploid, they are still pluripotent (i.e., they can give rise to all three germ layers). Most impressively, the nuclear transfer method appears highly efficient at removing the epigenetic memory of the fibroblast cell, possibly more so than the standard reprogramming method via forced expression of transcription factors. Many obstacles—both technical and political—still remain before this technique catches up to the standard reprogramming method. Nevertheless, this study takes a major stride toward reaching the paramount goal of producing a reprogrammed stem cell that is indistinguishable from "natural" ESCs.
*Noggle, S., et al. (2011). Nature 478, 70–75.*

## The Eye's Secret Stem Cells

2011 was also a big year for research on adult stem cells. Like embryonic stem cells, adult stem cells have the ability to self-renew, but they typically can give rise to only a few different types of cells. In addition, adult stem cells reside in organs throughout the lifetime of an animal, where they repair and regenerate tissues. Adult stem cells have been identified in a handful of organs, such as the skin, gut, and brain, but for many tissues, these repair factories still await discovery. Now, Salero et al. (2012) discover a new population of adult stem cells in the human eye, specifically in the retinal pigment epithelial (RPE) cells.




**RPE cells form a pigmented layer of the human retina, which is required to keep the rods and cones healthy. Image from Salero et al. (2012).**

RPE cells form a layer in the retina that helps keep the rods and cones healthy. Degeneration of RPEs is the leading cause of age-related vision loss. In some amphibians, damage to the retina triggers RPE cells to proliferate and regenerate numerous parts of the retina, including the lens and neurons. To search for similar cells in humans, Salero et al. isolate single RPE cells from human donors and culture them with serum. Most of the cells don't divide or produce only a few progeny, but ~10% of the clones display strong self-renewal properties; they form stem cell-like spheres that can be serially propagated. These self-renewing RPE cells are also multipotent. They can differentiate into cells derived from both the neuroectoderm lineage (i.e., a neuron phenotype) and the mesoderm lineage (i.e., adipocyte, osteogenic, and chondrocyte phenotypes), which is quite rare for a cell derived from the CNS. More experiments are needed to characterize the full differentiation capacity of the RPE stem cells, but clearly, these cells offer a valuable new resource for modeling RPE-related diseases and developing treatments for age-related blindness.
*Salero, E., et al. (2012). Cell Stem Cell 10, 88–95.*

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