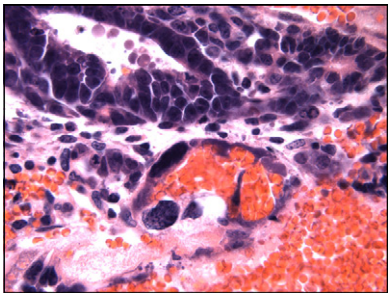
Stem Cells

The understanding of pluripotent stem cells at a molecular level and progress toward applying these cells to tackle human disease are advancing at a rapid pace. This Select, coauthored by *Cell* Editorial Board member Shinya Yamanaka and *Cell* Scientific Editor Karen Carniol, highlights recent work at the frontiers of stem cell research.



Teratomas formed from Tet1-depleted ESCs contain an excess of glandular cells (purple). These are arranged in a ductal structure next to a necrotic region (red) within the teratoma. Image courtesy of Kian Koh.

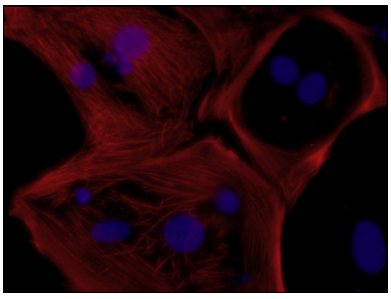
TET-ering between Pluripotency and Differentiation

There's a new kid on the block of epigenetic marks, and several recent papers indicate that it plays an important role in maintaining the balance between pluripotency and lineage commitment. The new player, 5-hydroxymethylcytosine (5hmC), is an oxidized derivative of the classic DNA mark, 5-methylcytosine (5mC), generated by the TET enzymes. Now, Wu et al. (2011), Ficz et al. (2011), Williams et al. (2011), Xu et al. (2011), and Pastor et al. (2011) provide genome-wide views of 5hmC's distribution in mouse embryonic stem cells (mESCs) and find that it is largely associated with promoter regions and gene bodies and, depending on the context, can be associated with either active or repressed transcription states. TET depletion influences 5hmC levels and gene expression patterns. According to Ficz et al., TET deficiency reduces expression of several pluripotency-related genes while facilitating the expression of endoderm markers. Consistent with this, Koh et al. (2011) report that TET levels are high in pluripotent cells and decline during differentiation. These changes appear to be regulated directly by the Oct4-Sox2 complex, a core transcriptional mediator of pluripotency, and depletion of TET genes skews lineage specification from its normal balance of endoderm, mesoderm, and ectoderm during differentiation of mESCs. Thus, 5hmC and the TET enzymes are emerging as key dynamic regulators of pluripotency and differentiation. This knowledge may be useful in ongoing endeavors to direct pluripotent cells to specific cell types.

Wu, H., et al. (2011). Genes Dev. 25, 679–684.
Ficz, G., et al. (2011). Nature 473, 398–402.
Xu, Y., et al. (2011). Mol. Cell, in press. Published online April 20, 2011. 10.1016/j.molcel.2011.04.005.
Williams, K., et al. (2011). Nature 473, 343–348.
Pastor, W.A., et al. (2011). Nature 473, 394–397.
Koh, K.P., et al. (2011). Cell Stem Cell 8, 200–213.

Keeping a Strong Beat in Cardiomyocyte Generation

One such example of directed differentiation that has made great progress recently is the generation of cardiomyocytes. Now, Kattman et al. (2011) have optimized cardiomyocyte differentiation from mouse and human pluripotent stem cells (PSCs). Building on previous work in in vitro systems and embryonic development, the authors establish a marker for cardiac mesoderm, PdfgR-α, which in combination with the known Flk-1/KDR marker, identifies mesodermal cells primed for cardiomyocyte differentiation more robustly than either marker alone. Manipulating the Activin/Nodal and BMP signaling pathways induces the cardiac mesoderm, but importantly, the authors find that the stage at which the pathways need to be manipulated and the concentrations of pathway components required for maximal cardiovascular mesoderm vary between cell lines. The findings suggest that endogenous pathway components must be considered in the generation of specific cell types from PSCs.

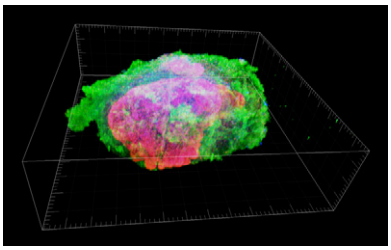


Fibroblasts converted directly into cardiomyocytes express marker proteins of the heart, such as Troponin T (red), the striated pattern of which is a hallmark of differentiated cardiac muscle. Cell nuclei were visualized with DAPI (blue). Image courtesy of Jem A. Efe.

An alternative approach to generating specific cell types in vitro is to reprogram a differentiated cell, such as a fibroblast, directly to the cell type of interest. This is the strategy taken by Efe et al. (2011) to produce cardiomyocytes, though with an interesting twist: the factors that they apply to the fibroblasts are those employed to generate induced pluripotent stem cells (iPSCs). In general, expression of Oct4, Sox2, Klf4, and c-Myc in mouse fibroblasts induces a pluripotent state, but when Efe et al. transiently express these factors and remove the cytokine leukemia inhibitory factor (LIF) from the culture media, the cells pass through state in which they produce cardiac precursor markers. Tweaking culture conditions at key times and ultimately adding BMP triggers efficient transdifferentiation to beating cardiomyocytes. A number of additional experiments strongly suggest that a pluripotent intermediate is not involved in the transdifferentiation program. More recently, Kim et al. (2011) applied this approach to reprogram

fibroblasts directly into expandable neural precursor cells. It will be exciting to see what other cell types can be directly programmed starting with the iPSC-inducing factors.

Kattman, S.J., et al. (2011). Cell Stem Cell 8, 228–240.
Efe, J.A., et al. (2011). Nat. Cell Biol. 13, 215–222.
Kim, J., et al. (2011). Proc. Natl. Acad. Sci. USA, in press. Published online April 26, 2011. 10.1073/pnas.1103113108.



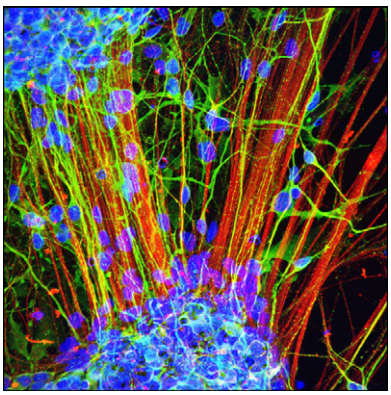
3D rendering of an intestinal organoid after 28 days of culture. Red represents epithelium, and green represents mesenchyme. Image courtesy of Jason Spence.

Pluripotent Cells Go 3D

Efficient generation of specific cell types in vitro has tremendous therapeutic potential, and of equal excitement is the generation of complex tissues in culture. Remarkable progress has been made on this front, as highlighted by two recent papers. First, Spence et al. (2011) succeed in generating three-dimensional (3D) intestinal tissue in vitro starting from human PSCs. Administration of developmental signaling factors at particular stages of culturing led first to definitive endoderm and then to the hindgut lineage with markers for intestinal tissue. The flat sheets of cells then spontaneously form tubes, which bud to release gut tissue spheroids. When the authors transfer these spheroids to a 3D matrigel culture that is known to support intestinal growth from tissue-specific progenitor cells, the spheroids undergo morphogenesis reminiscent of that seen in fetal gut development. This culminates in the formation of columnar epithelia with specialized structures and cell types that are characteristic of intestine, such as villi, enterocytes with functional peptide transport systems, mucin-producing cells, and progenitor cell niches. The authors took advantage of this in vitro organogenesis to test whether the *NEUROG3* gene is responsible for a congenital disorder defined by loss of enteroendocrine cells. Leveraging the system for transplantation-based therapies is also a tantalizing potential application of this system. Another notable achievement in generating complex 3D tissues in vitro was reported by Eiraku et al. (2011), who used a 3D culturing system to generate a complex retinal tissue, the optic cup, from mouse embryonic stem cells, as described in the Select on Vision in the April 29th issue of *Cell*.

Spence, J.R., et al. (2011). Nature 470, 105–109.
Eiraku, M., et al. (2011). Nature 472, 51–56.

Taking on Complex Disease with iPSCs



Neurons derived from hIPSCs generated from the fibroblasts of a patient diagnosed with schizophrenia. The neurons express βIII-tubulin (red) and the dendritic marker MAP2AB (green). Nuclei are visualized with DAPI (blue). Image courtesy of Kristen Brennand.

The potential of stem cells lies not only in the ability to generate complex cell types and tissues, but also in their ability to allow us to study human phenotypes at a molecular level in human cells without the potentially confounding differences between humans and model organisms. iPSCs have been derived from the fibroblasts of patients with several disorders and then have been effectively differentiated to create cellular models of the corresponding disease. While most of these studies have focused on monogenic disorders, Brennand et al. (2011) take a bold step by establishing a patient iPSC-based model of the complex neuropsychiatric disorder schizophrenia. They differentiate iPSCs from four schizophrenia patients to neurons and then characterize the neurons' gene expression and ability to make synaptic connections. The patient-derived neurons exhibit reduced connectivity compared to controls. Expression profiles of these patient-derived neurons are consistent with this observation, as they show changes in genes associated with forming synaptic connections, such those in the gluatamate, cAMP, and Wnt signaling pathways. Approximately one-quarter of the genes with altered expression had been previously associated with schizophrenia, and the drug loxapine, which is prescribed for this disorder, ameliorates the defects in synaptic connectivity. The expression profiling also suggests that pathways not previously associated with schizophrenia might be important players, such as Notch signaling, cell adhesion, and Slit/Robo axon guidance. The authors postulate that follow-up studies, including those with larger numbers of patient-derived neurons, will reveal a core set of pathways and/or genes common to all patients, hopefully enabling a focused mechanistic dissection and therapeutic targeting effort for this debilitating disease.

Brennand, K.J., et al. (2011). Nature 473, 221–225.

Shinya Yamanaka and Karen Carniol