Chin, M.H., Pellegrini, M., Plath, K., and Lowry, W.E. (2010). Cell Stem Cell *7*, this issue, 263–269.

Guenther, M.G., Frampton, G.M., Soldner, F., Hockemeyer, D., Mitalipova, M., Jaenisch, R., and Young, R.A. (2010). Cell Stem Cell *7*, this issue, 249–257.

Han, J., Yuan, P., Yang, H., Zhang, J., Soh, B.S., Li, P., Lim, S.L., Cao, S., Tay, J., Orlov, Y.L., et al. (2010). Nature *463*, 1096–1100.

Mikkelsen, T.S., Hanna, J., Zhang, X., Ku, M., Wernig, M., Schorderet, P., Bernstein, B.E., Jaenisch, R., Lander, E.S., and Meissner, A. (2008). Nature *454*, 49–55.

Newman, A.M., and Cooper, J.B. (2010). Cell Stem Cell *7*, this issue, 258–262.

Sharova, L.V., Sharov, A.A., Piao, Y., Shaik, N., Sullivan, T., Stewart, C.L., Hogan, B.L.M., and Ko, M.S.H. (2007). Dev. Biol. *307*, 446–459.

Stadtfeld, M., Apostolou, E., Akutsu, H., Fukuda, A., Follett, P., Natesan, S., Kono, T., Shioda, T., and Hochedlinger, K. (2010). Nature *465*, 175–181.

Tesar, P.J., Chenoweth, J.G., Brook, F.A., Davies, T.J., Evans, E.P., Mack, D.L., Gardner, R.L., and McKay, R.D.G. (2007). Nature *448*, 196–199.

Yamanaka, S., and Blau, H.M. (2010). Nature *465*, 704–712.

Zhao, X.-Y., Li, W., Lv, Z., Liu, L., Tong, M., Hai, T., Hao, J., Guo, C.-L., Ma, Q.-W., Wang, L., et al. (2009). Nature *461*, 86–90.

Note Added in Proof

Recent publications by Kim et al. (Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I., et al. (2010). Nature, in press. Published online July 19, 2010. 10.1038/nature09342) and Polo et al. (Polo, J.M., Liu, S., Figueroa, M.E., Kulalert, W., Eminli, S., Tan, K.Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda, T. et al. (2010). Nat. Biotechnol., in press. Published online July 19, 2010. 10.1038/nbt.1667) extend on the publications previewed here. In brief, Kim et al. and Polo et al. show that mouse iPSCs derived from distinct differentiated cell types show defining transcriptional and epigenetic similarities with their starting cell of origin. This retention of the starting cell's transcriptional program in iPSCs could explain why certain genes are differentially expressed between human iPSCs and ESCs, as discussed here. Furthermore, Polo et al. show that after extended passaging, mouse iPSCs largely censor these cell-of-origin-specific transcriptional programs, thus affirming Chin et al. (2009)'s findings that extended passaging of human iPSC lines increases their transcriptional similarity to human ESC lines.

Getting to the Heart of the Matter: Direct Reprogramming to Cardiomyocytes

Robert Passier[1,*] and Christine Mummery[1,*]
[1]Department of Anatomy & Embryology, Leiden University Medical Center, Einthovenweg 20, 2333 ZC Leiden, The Netherlands
*Correspondence: r.passier@lumc.nl (R.P.), c.l.mummery@lumc.nl (C.M.)
DOI 10.1016/j.stem.2010.07.004

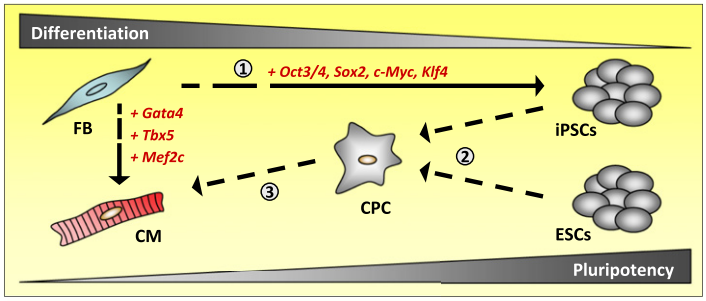
Since MyoD was used to convert fibroblasts directly to skeletal muscle, biologists have tried to apply this strategy to generate other cell lineages. In their recent *Cell* paper, Ieda et al. (2010) use selected cardiac transcription factors to directly reprogram fibroblasts to cardiomyocytes without passing through an intervening pluripotent state.

A landmark paper from Takahashi and Yamanaka (2006) showed that adult mammalian cells could be reverted to a pluripotent state with just four transcription factors. Before that time, pluripotent cell reprogramming was thought to require either somatic cell nuclear transfer into an unfertilized egg cell or fusion of somatic cells with pluripotent embryonic stem cells (ESCs) (Hochedlinger and Jaenisch, 2006). Direct reprogramming of terminally differentiated cells, also called lineage reprogramming, had been limited to skeletal muscle via MyoD. This transcription factor became recognized as a "master regulator gene," because it was able to convert fibroblasts, chondrocytes, and retinal epithelium into contracting muscle in culture (Choi et al., 1990). Subsequent examples included the conversion of B lymphocytes into macrophages by CEP/B (Xie et al., 2004) and inner ear support cells into sensory hair cells by Math1 (Izumikawa et al., 2005). Yet, despite years of research, master regulators for other lineages have remained elusive. Now, with an approach similar to Yamanaka's, Srivistava and colleagues demonstrate that fully functional cardiomyocytes can be derived from cardiac and skin fibroblasts (Ieda et al., 2010).

Master Regulators: A Team Approach

Yamanaka demonstrated that a selected group of transcription factors was sufficient to direct somatic cells to adopt an immature pluripotent state and that this conversion involved the loss of the original imprint that determined the cells' functional characteristics. This finding helped shift the field's approach to lineage reprogramming. Instead of performing modest searches for single master regulator genes, hundreds of critical developmental factors were screened in multiple combinations with lineage reporter cells as readouts. Yamanaka's protocol reduced several hundred pluripotency candidate genes to just four. Why not try the same direct reprogramming paradigm to generate specific differentiated cell lineages?

Melton and colleagues applied this technique successfully to identify a small set of genes capable of converting



Targeted cells	Inducing factors	Reprogrammed cells	Efficiency	Reference
embryonic, adult fibroblasts	Oct3/4, Sox2, c-Myc, Klf4	pluripotent stem cells	<0.1%	(Takahashi and Yamanaka, 2006)
pancreatic exocrine cells	Ngn3, Pdx1, Mafa	pancreatic β cells	1.8-7.7% up to 19.5%	(Zhou et al., 2008)
embryonic, adult fibroblasts	Ascl1, Brn2, Myt1l	neurons	>20%	(Vierbuchen et al., 2010)
embryonic, adult fibroblasts	Gata4, Mef2c, Tbx5	cardiomyocytes	20%	(Ieda et al., 2010)

Figure 1. Cardiomyocyte Formation by Direct Programming or Differentiation of Pluripotent Stem Cells

Top: Transcription factors Gata4, Mef2c, and Tbx5 directly reprogram fibroblasts (FB) to cardiomyocytes (CM) without intervening cardiac progenitor cell (CPC) or pluripotent stem cell stages (left). Alternatively, fibroblasts are reprogrammed to induced pluripotent stem cells (iPSCs) by inducing factors Oct3/4, Sox2, c-Myc, and Klf4 (1). Cardiomyocyte production requires specific induction protocols, which support differentiation through sequential developmental stages from CPCs (2) to CMs (3). Pluripotent embryonic stem cells (ESCs) offer an alternative source to yield cardiomyocytes via similar differentiation paradigms.
Bottom: Somatic cells are reprogrammed to induced pluripotent stem cells (dark gray) or to specific cell types (direct reprogramming, light gray) with varying efficiencies.

exocrine pancreas cells into endocrine pancreas, the β-islet cells essential for regulated insulin production (Zhou et al., 2008). By using genome-wide expression analysis, the authors narrowed down >1100 transcription factors expressed in the developing mouse pancreas to ~30 associated with pancreatic and endocrine progenitors, 9 of which were known to be required for β-cell fate specification. After an in vivo process of elimination, a trio of factors proved capable of converting exocrine pancreas tissue into functional endocrine cells. Although present only as single cells and not in β-cell clusters, the results demonstrated the principle that combinations of developmentally relevant genes can direct lineage reprogramming in vivo.

A subsequent study from Wernig and colleagues used a similar approach to produce neurons from both mouse embryonic and postnatal fibroblasts (Vierbuchen et al., 2010). A pool of 19 candidate developmental genes expressed in neural tissue yielded a combination of three transcription factors Ascl1, Brn2, and Mytl1 that was sufficient for the induction of functional neuronal cells.

Lineage Reprogramming to Cardiomyocytes

In their current *Cell* paper, Srivastava and colleagues endeavoured to reprogram committed fibroblasts to cardiomyocytes and started with a pool of 14 candidate cardiomyocyte-inducing factors (Ieda et al., 2010). Cardiomyocyte induction of mouse neonatal cardiac fibroblasts was observed 1 week after retroviral transduction in 1.7% of the cells. After fine tuning the cocktail to combine crucial cardiac-inducing factors and exclude inhibitory or ineffective factors, a best set was identified, consisting of the transcription factors Gata4, Mef2c, and Tbx5 (Figure 1, top). Use of this trio of factors increased the efficiency of direct cardiomyocyte induction to a remarkable 20%. Although induced cardiomyocytes (iCMs) displayed expression of cardiac proteins within a week, their further maturation occurred in the weeks thereafter: sarcomeric organization and contractility increased and electrical properties "matured." As expected for true reprogramming, epigenetic resetting of a selected group of genes to that of a cardiomyocyte state was evident. Furthermore, by using inducible lentiviral expression, the authors showed that iCMs were stable for at least 1 week after the three factors were switched off.

Because the cardiomyocytes derived from a starting population of cardiac fibroblasts could in principle have originated from rare, contaminating cardiac progenitor subpopulations, the procedure was repeated with dermal fibroblasts isolated from tail tips of adult mice. Direct reprogramming of somatic cells to cardiomyocytes was confirmed in this manner and in fact, conversion efficiencies were comparable between the two targeted fibroblast populations.

Most interestingly, in comparison to reprogramming of somatic cells to induced pluripotent stem cells (iPSCs), lineage reprogramming (both for cardiomyocytes as well as neuronal cells) appears to be rapid and relatively much more efficient (Figure 1, bottom). Srivastava and colleagues propose that the difference in effciencies may be explained by the fact that the cells are directly converted to cardiomyocytes without reverting to precardiac developmental stages such as mesoderm or cardiac progenitor cells (Figure 1, top).

Where Next and Which Applications?

Based on current and previous findings, Gata4, Mef2c, and Tbx5 are clearly crucial for cardiac development and can promote cardiac differentiation. However, it is surprising to see that Nkx2-5, another core cardiac transcription factor, inhibited rather than enhanced cardiomyocyte reprogramming efficiency. A recent study by Takeuchi and Bruneau (2009) found that Gata4 and Tbx5 with Baf60c, a subunit of the Swi/Snf-like BAF chromatin-remodelling complex, was sufficient to transdifferentiate noncardiogenic mouse mesoderm to cardiomyocytes. In this example, Nkx2-5 was not one of a minimal set of factors required for cardiac differentiation, but its expression was induced by the combination of Gata4 and Baf60c. Interestingly, Takeuchi and Bruneau's cocktail of Gata4, Tbx5, and Baf60c was not sufficient to reprogram fibroblasts in the

Srivastava group's system. An obvious difference between the two studies is the differentiation status of the targeted cells: fully differentiated fibroblasts versus unspecified mesodermal cells. Fibroblast conversion to cardiomyocytes can proceed without an intervening mesodermal or progenitor stage (Ieda et al., 2010), so it is conceivable that distinct starting cells may respond differently to the same factors. It would be of interest to investigate whether inducible and temporal activation of selected factors would affect reprogramming efficiency, epigenetic status, maturation, function, and stability of the resulting cardiomyocytes, especially because reprogrammed cardiomyocytes were similar but not identical to neonatal cardiomyocytes.

There will be obvious important clinical implications if generic and robust iCM production protocols can be developed and applied for adult human cells. Although many hurdles remain to be overcome in order to establish effective cell-based therapies (Passier et al., 2008), patient-derived iCMs may be used in the future for treatment of heart disease and would offer advantages over iPSC-derived cardiomyocytes. First, direct reprogramming avoids residual pluripotent stem cells in the transplanted populations, eliminating the risk of teratoma formation. Second, higher yields and faster kinetics of cardiomyocyte production would lower the costs and reduce the delivery time to patients and may permit the use of autologous cells even for acute conditions. Nevertheless, other safety issues would still need to be addressed, including the risk of genomic integration of foreign genetic material, spontaneous transformation during cell expansion in culture, and cardiac arrhythmias after transplantation to the human heart. Besides future therapeutic applications, however, much more immediate clinical relevance is found in the biotechnology and pharmaceutical industries. These companies are actively seeking predictive human disease models for target and drug discovery. Future comparisons between human pluripotent stem cell-derived (both from iPSCs or genetically manipulated human ESCs) or directly reprogrammed cardiomyocytes (or other specialized cell types) with native populations will be needed to determine the relevance of any of these "manufactured" cells for high-throughput drug and compound screening.

REFERENCES

Choi, J., Costa, M.L., Mermelstein, C.S., Chagas, C., Holtzer, S., and Holtzer, H. (1990). Proc. Natl. Acad. Sci. USA *20*, 7988–7992.

Hochedlinger, K., and Jaenisch, R. (2006). Nature *441*, 1061–1067.

Ieda, M., Fu, J.-D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B.G., and Srivastava, D. (2010). Cell *142*, in press.

Izumikawa, M., Minoda, R., Kawamoto, K., Abrashkin, K.A., Swiderski, D.L., Dolan, D.F., Brough, D.E., and Raphael, Y. (2005). Nat. Med. *11*, 271–276.

Passier, R., van Laake, L.W., and Mummery, C.L. (2008). Nature *453*, 322–329.

Takahashi, K., and Yamanaka, S. (2006). Cell *126*, 663–676.

Takeuchi, J.K., and Bruneau, B.G. (2009). Nature *459*, 708–711.

Vierbuchen, T., Ostermeier, A., Pang, Z.P., Kokubu, Y., Südhof, T.C., and Wernig, M. (2010). Nature *463*, 1035–1041.

Xie, H., Ye, M., Feng, R., and Graf, T. (2004). Cell *117*, 663–676.

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J., and Melton, D.A. (2008). Nature *455*, 627–632.

There's No Place Like Home for a Neural Stem Cell

Julie A. Siegenthaler[1] and Samuel J. Pleasure[1,*]
[1]Department of Neurology, Programs in Neuroscience and Developmental Biology, Institute for Regenerative Medicine, University of California, San Francisco, San Francisco, CA 94158, USA
*Correspondence: sam.pleasure@ucsf.edu
DOI 10.1016/j.stem.2010.07.001

Neural precursor cells (NPCs) reside in the subventricular zone in association with blood vessels and ependymal cells. In this issue of *Cell Stem Cell*, Kokovay et al. (2010) show that SDF1 directs the association of NPCs with this niche and regulates their lineage progression in a stage-specific manner.

In the adult brain, a few areas of active neurogenesis churn out new neurons that will ultimately incorporate into the existing circuitry of specific brain structures. One of these areas is the subventricular zone (SVZ) lining the lateral ventricles, where the progression from quiescent stem cell to migrating neuroblast follows an orchestrated program (Figure 1). Quiescent multipotential neural stem cells (NSCs), called B cells, are found in the SVZ. When activated they produce rapidly dividing type C transient-amplifying cells that then give rise to type A neuroblasts. The neuroblasts then migrate away from the lateral ventricles to the olfactory bulbs via the rostral migratory stream (Miller and Gauthier-Fisher, 2009). In addition to understanding the lineage relationship between neural cells in the SVZ, there is increasing focus on how the SVZ microenvironment both helps maintain the stem cell pool and facilitates neuroblast production. Understanding