by its prior use in autologous bone marrow stem cell transplantation. Two broadly different perspectives are recognizable, focused on cardiac myocytes, as above, or more typically on bone marrow-derived cells' mobilization and homing to injured hearts. Alone, G-CSF impairs homing of bone marrow cells to the heart, perhaps by inhibiting the migratory response to SDF-1; by contrast, G-CSF plus a CD26/dipeptidylpeptidase IV inhibitor that elevates SDF1 levels improved these cells' recruitment to the heart, augmenting vascularization, pump function, and survival (Zaruba et al., 2009). Cardiac-resident progenitor cells are a third potential target, as indicated by the fact that G-CSF may enhance the number of cardiac Sca-1$^+$ cells (Brunner et al., 2008; cf. Harada et al., 2005). Is G-CSFR functionally coupled in the endogenous cardiac progenitor/stem cells resident in adult human hearts? Are salutary effects of G-CSF on the adult heart mediated in part by these cells? Might G-CSF be used to drive cardiopoiesis by adult cardiac progenitor cells, in culture or in situ?

Optimization of any potential cardiopoietin must take into account the effects on proliferation as well as lineage commitment. Proliferation can occur in embryonic cardiomyocytes (Olson and Schneider, 2003), and Shimoji et al. (2010) localized the proliferation evoked by G-CSF to Mef2$^+$ cells, Nkx2.5$^+$ cells, and α-actinin$^+$ cells. However, susceptibility to G-CSF was largely lost by E12.5. It remains to be learned whether other hypoplastic cardiac phenotypes impinge on this axis, e.g., through precocious differentiation, whether cardiac progenitors from both heart fields require G-CSF, and whether the effect of G-CSF is entirely myocyte autonomous.

The unexpected cardiac-lethal phenotype of *gcsfr3*$^{-/-}$ mice differs from the original report of this knockout line, which had no premature lethality and was said to develop normally (Liu et al., 1996). This inconsistency might be explained by the differing genetic background (ultimately backbred to C57Bl/6), but leads to the more general consideration, how many other essential cardiopoietins have been overlooked in ostensibly conclusive models? How might heart-forming factors best be identified in the future? Complementary, higher-throughput approaches to detect novel cardiopoietins have begun to include robotic screens in stem cells and other systems (Sadek et al., 2008). Along with other "high-bandwidth" approaches like saturation mutagenesis, these experimental platforms hold the promise of defining workable triggers for cardiac muscle creation, beyond the insights obtainable in stem cells or model organisms manipulating just one factor or pathway at a time, on an artisanal scale.

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Crossing Boundaries: Direct Programming of Fibroblasts into Neurons

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In a recent paper in *Nature*, Vierbuchen et al. (2010) show that fibroblasts can be directly converted into functional neurons by defined factors. This finding sheds new light on the biology underlying cell-fate restrictions and might offer a new avenue for studying neurological diseases.

The finding that differentiated somatic cells such as fibroblasts can be reprogrammed into induced pluripotent stem cells (iPSCs) by four (or even fewer) transcription factors (TFs) revolutionized the understanding of cellular plasticity and provided a novel tool to study developmental processes and mechanisms of human disease (Takahashi et al.,

2007). Furthermore, there are high expectations that iPSC-derived cells might be a promising source for patient-specific cell-replacement therapies (Hanna et al., 2007).

Vierbuchen and colleagues now take the concept of cell-fate reprogramming one step further and show that fully differentiated embryonic and postnatal fibroblasts can be efficiently converted into functional neurons (called iN cells) without the detour of an uncommitted pluripotent cell (Vierbuchen et al., 2010). They did so by producing lentiviral vectors expressing 19 different TFs that had previously been implicated in neuronal fate determination and cellular reprogramming and tested them for their ability to induce neuronal properties after transduction of fibroblasts. To screen for a large number of TF combinations, they used fibroblasts that were isolated from a transgenic mouse expressing a GFP reporter under the neuronally restricted tau promoter (tauEGFP). Using this approach, they found that the proneural bHLH TF Ascl1 (also called Mash1) on its own was sufficient to induce neuronal properties in cells derived from fibroblasts, although at a low rate. They went on to determine the minimal number of TFs required for efficient fate conversion and neuronal maturation of iN cells and identified two TFs that together with Ascl1 rapidly and efficiently converted fibroblasts into iN cells: Brn2, previously identified as a key gene during embryonic neurogenesis regulating notch signaling, and Myt1l, associated with epigenetic modifications in neural cells. In fact, up to 20% of all fibroblasts targeted with viruses expressing Ascl1, Brn2, and Myt1l expressed neuronal markers within several days after viral transduction.

Importantly, Vierbuchen and colleagues used electrophysiological recordings to prove that iN cells are indeed capable of firing action potentials and forming functional synapses as soon as 12 days after fate conversion when plated with other iN cells or cocultured with embryonic neurons. When analyzing the neuronal subtype generated by iN technology, Vierbuchen et al. found that the majority of cells differentiated into excitatory, glutamatergic neurons (which is surprising given the predominant role of Ascl1 for the generation of GABAergic, inhibitory cells or oligodendrocytic cells in vivo; Casarosa et al., 1999; Jessberger et al., 2008).

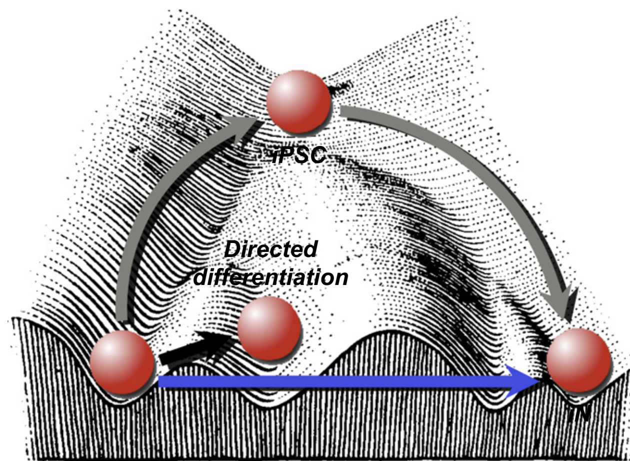

Figure 1. Directing Cell Fate with Ectopic Master Gene Expression
Waddington's epigenetic landscape with uncommitted pluripotent cells at the summit and distinct, fully differentiated cells at the bottom of the valleys predicted that the ball (here standing for cell lineage) could only roll downhill. However, with iPSC technology differentiated cells can be reprogrammed into a pluripotent cell that can in turn give rise to most (if not all) tissues, e.g., brain cells (gray arrows). Lineages within germline boundaries can also be crossed with single or multiple-factor expression (directed differentiation, black arrow) (e.g., Jessberger et al., 2008 or Zhou et al., 2008). Vierbuchen et al. now showed that iN technology with three defined TFs can convert cells from one lineage into another, even across germline boundaries (blue arrow).

What are the implications of the direct conversion of fibroblasts into neurons both for our understanding of the basic biology underlying cell-fate specification and for potential therapeutic applications with iN cells?

A former concept regarding the mechanisms underlying cell specification was that cells, which have terminally differentiated, are bound in their cell fate because of irreversible epigenetic modifications that prevent the transcription of genes specific to other cell lineages. The development of iPSC technology has challenged this concept by showing that in principle every cell retains the potential to dedifferentiate into a pluripotent ground state by overexpression of a few TFs (Figure 1). However, reprogramming of somatic cells into an iPSC involves a thorough eradication of epigenetic marks, thereby allowing the cell to "start all over again" with an at least partially naive chromatin (Maherali et al., 2007). The findings by Vierbuchen et al. show that directed conversion from one differentiated cell type into another (in this case fibroblasts into neurons) with only three TFs can be achieved very quickly (within days) and efficiently, without going back to an uncommitted pluripotent cell state. Future studies will have to analyze whether the three TFs used merely impose the neuronal phenotype over the fibroblast phenotype, which could still be hidden within the cell, e.g., on an epigenetic level. Alternatively, the three TFs could activate transcriptional networks required for neuronal differentiation and at the same time erase the epigenetic signature of fibroblasts while converting them into neurons.

The finding by Vierbuchen et al. supports the notion that there are powerful master genes controlling cell fate. This has previously been implied by showing that a single TF (or the combination of a few) was sufficient to direct the fate of cells from one lineage into another, even in vivo (Figure 1) (Davis et al., 1987; Jessberger et al., 2008; Zhou et al., 2008). However, the important advancement of the current study is that previous studies converted cell fates within germ-layer boundaries (e.g., fibroblasts into muscle; exocrine pancreas cells into β-cells). Vierbuchen et al. now converted cells from the mesoderm (fibroblasts) into neurons that originate from the ectoderm (Figure 1). With the novel finding of cell-type conversion, it is clear that despite extensive and functionally important regulation of gene transcription by chromatin modifications or noncoding RNAs, a handful of genes can control the fate of cells even across germline boundaries. Thus, pushing the right button (or as in the Vierbuchen study, three buttons) can have dramatic effects on cell fate and differentiation. In this context, the iN technology might together with classical developmental neurobiology, embryonic stem cell (ESC) research, and iPSC technology be useful in identifying

mechanisms underlying neuronal differentiation and specification.

Anticipating that iN technology also works for human cells (which awaits confirmation), patient-specific iN cells might provide a novel platform for disease modeling and might also be a source for cell-replacement therapies. This is in principle already possible with iPSC strategies. However, a major roadblock preventing the clinical use of iPSCs for therapeutic transplantation is the danger of transplanting single undifferentiated cell clones prone to cancerous growth (Miura et al., 2009). This substantial side effect of iPSCs should be nonexistent in iN cells, even though long-term transplantation studies will have to formally show this. In addition, it seems reasonable to speculate that the "trick" of conversion not only works for neurons but also for many other cell types such as liver or pancreas, which could also be useful for disease modeling or cell replacement.

The findings presented by Vierbuchen et al. offer a novel technology and source to generate neurons, but the real challenge in replacing lost neurons in most degenerative human diseases will be to identify the mechanisms that regulate and orchestrate the meaningful and functional integration of transplanted cells, so that replacing neurons can truly take over the function of injured areas. In addition, it could turn out that neuronal diversity even within the same neuronal lineage, which seems to be a cardinal feature of neuronal circuitries at least in the primate brain, cannot be achieved without context-dependent cues that are missing in the culture dish (Muotri and Gage, 2006). Be that as it may, the study by Vierbuchen et al. adds a novel and important piece to the existing toolbox that will be instrumental to understanding and potentially treating human neurological diseases.

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Niche Today, Gone Tomorrow—Progenitors Create Short-Lived Niche for Stem Cell Specification

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Establishing tissue-specific adult stem cells during organogenesis is important for maintenance of tissue homeostasis throughout the lifetime of the organism. In a recent study in *Science*, Mathur et al. (2010) describe how progenitor cells in the *Drosophila* larval midgut create a temporary niche to maintain stem cell fate during development.

Stem cells are generally thought to reside in niches, or specialized local microenvironments, which prevent differentiation (Fuller and Spradling, 2007). Although both stem cells and their niches arise during development, the mechanisms governing their specification are poorly understood (Slack, 2008). In a recent report in *Science*, the Ohlstein lab provides an interesting glimpse into this process in the *Drosophila* midgut by showing that stem cell precursors (known as adult midgut progenitors [AMPs]) specify their own transient "niche" (peripheral cells [PCs]) during morphogenesis (Mathur et al., 2010).

The *Drosophila* adult midgut is an epithelial monolayer composed of absorptive enterocytes (ECs) interspersed with secretory enteroendocrine (ee) cells. Although this tissue had long been considered quiescent, groundbreaking work in 2006 revealed that it is constantly renewed by a pool of intestinal stem cells (ISCs). ISCs divide asymmetrically to produce both ISCs and daughters (enteroblasts [EBs]) that differentiate into ECs or ee cells in a Notch-dependent manner (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006) (Figure 1C).