Published in final edited form as:

Nat Biotechnol. 2010 April ; 28(4): 333–335. doi:10.1038/nbt0410-333.

Stem cell biologists sure play a mean pinball

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Abstract

Mouse fibroblasts are reprogrammed to functional neurons by expression of a few transcription factors.

Developmental biologists have traditionally considered lineage commitment and differentiation to be unidirectional and irreversible processes enforced by stable epigenetic patterns. But recent advances in stem cell biology have shown that, at least under certain conditions, mature adult cells are capable of changing cell type in response to ectopic cues. In the latest example, Vierbuchen *et al.*¹ report in *Nature* that cells from the mesodermal lineage (fibroblasts) can be switched to an ectodermal lineage (neurons) using a simple cocktail of transcription factors. These results promise to open new avenues for the regeneration of neural tissues and provide further evidence that lineage reprogramming might be harnessed to create a variety of desired cell types for regenerative medicine and drug discovery.

The idea that each somatic cell has the potential to regenerate an entire organism arose from pioneering studies of cloning, first in amphibians and then in mammals. The recent discovery that ectopic expression of *Oct4*, *Sox2*, *Klf4* and *c-Myc* can push terminally differentiated cells back in time to a pluripotent state^{2, 3} has both confirmed this basic idea and changed the way that biologists think about cellular differentiation. In particular, the simplicity of creating induced pluripotent stem (iPS) cells with a small number of transcription factors has led to a reassessment of the plasticity of somatic cells.

As iPS cells can be differentiated to virtually any tissue of the body, they represent a new source of autologous tissue for cell therapy. An alternative approach to the generation of desired cell types is lineage reprogramming, in which one type of mature, differentiated cell is transformed into another. Important insights into this process came from studies showing that exocrine pancreatic acinar cells were reprogrammed to endocrine beta-islet cells using the transcription factors Pdx1, Ngn3 and MafA⁴, that expression of Atoh1 in nonsensory cochlear cells generated functional auditory hair cells⁵ and that B lymphocytes could be converted to macrophages using C/EBP genes⁶. In neural reprogramming, expression of the neural-specific transcription factors Pax6, Ngn2 and Ascl1 (also Mash1) switched astroglial cells to neurons⁷ and, expression of Nurr1, Ngn2 and Mash1 generated dopaminergic neurons from restricted neural progenitors⁸.

Vierbuchen *et al.*¹ began by compiling a list of 19 important transcription factors known to co-regulate the neurogenic program during development. They infected embryonic and 3-day-old tail-tip fibroblasts isolated from mice expressing green fluorescent protein (GFP)

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Competing financial interests

The authors declare no competing financial interests.

under the control of the neural *Tau* gene with viruses expressing all 19 factors and their permutations. By studying different combinations they found that only five genes—*Ascl1*, *Brn2/4*, *Myt1l*, *Zic1* and *Olig2*—were capable of repatterning fibroblasts to GFP-expressing neurons, termed induced neuronal (iN) cells. The only gene absolutely required was *Ascl1*. However, *Brn2* and *Myt1l* or *Zic1* were needed to increase transformation efficiency, maturation and generation of more complex neuronal phenotypes. Similarly, the efficiency of reprogramming to iPS cells can be improved by increasing the number of exogenous transcription factors.

Vierbuchen *et al.*¹ found that neurotransmitters elicited responses from the iN cells that are characteristic of excitatory glutamatergic neurons and inhibitory GABAergic neurons. However, more restricted dopaminergic, cholinergic and serotonergic projection neurons of the central nervous system were not obtained. Importantly, purified iN cells were capable of firing repetitive action potentials and formed functional synapses. Analysis of the synaptic activity showed a preponderance of excitatory cortical neurons. The majority of the new neural cells were born early and over time developed elaborate fibers and specialized electrophysiology. Nonetheless, it remains to be determined whether iN cells made a complete and stable phenotypic switch or whether a partially intact fibroblast epigenetic pattern survived after incomplete neural reprogramming.

One possible shortcoming of this study is that the authors used only embryonic and neonatal tail tip fibroblasts as the starting material. Although there was little evidence of neuronal production from these tissues, neural crest derivatives that migrate from the neural tube into peripheral tissues during development are known to permeate many skin structures⁹. Indeed, they have been shown to persist until adult development as multipotent skin-derived precursors¹⁰. To determine whether rare neural crest cells rather than fibroblasts responded to *Ascl1* and the other reprogramming factors, it would be useful to perform clonal studies and to evaluate fibroblasts for an assortment of neural crest markers. Interestingly, *Sox10*, an early neural crest marker, was expressed in tail-tip fibroblasts. Nevertheless, the authors did not rule out the possibility that the iN cells could represent dorsal root ganglion or sympathetic neurons of the peripheral nervous system, evidenced by expression of peripherin in some iN cells. If true, this would further suggest the possibility of a neural crest derivative contaminating the primary fibroblast cultures from which the iN cells were generated.

If iN cells do arise from differentiated fibroblasts, it will be important to work out the underlying mechanisms of reprogramming. Both Vierbuchen *et al.*¹ and the authors of a recent commentary¹¹ suggest that there may have been a direct transformation from the mesodermal to the ectodermal lineage. Alternatively, it is possible that *Ascl1* and the other transcription factors pushed the cells back to a transient primitive (but not pluripotent) state before they differentiated down a different lineage. It seems unlikely that a fully differentiated fibroblast switched fate directly to a fully differentiated neuron. Rather, the fibroblast may have first transitioned back to a neural progenitor state. Vierbuchen *et al.*¹ did not examine the role of cell division in the reprogramming process. Does the fibroblast have to divide in the presence of *Ascl1* before acquiring a neural fate? This may allow it to 'reset' crucial expression profiles to a more primitive state on which other exogenous transcription factors would act. Examining neural progenitor markers such as *Pax6* and *Tbr2* and inhibiting proliferation during reprogramming would help address these issues.

To illustrate the concept of lineage reprogramming via a transient primitive state, we have developed a model based on the metaphor of a pinball machine (Fig. 1). In the classic model of C.H. Waddington, a cell moving toward terminal differentiation is represented as a ball rolling down along branching valleys of an epigenetic landscape. In our more interactive

model, development of a cell ('ball') begins at the zygote stage and progresses through the blastocyst stage in the 'ball launch lane' (bottom right). As cells move down from the top of the panel, they differentiate into the three germ-cell lineages. A cell is driven to ectoderm, mesoderm or endoderm depending on what cues—'bumpers' or 'flippers'—it strikes. Bumpers represent endogenous transcription factors, whereas flippers represent ectopically expressed transcription factors. The latter can push the cell across epigenetic 'edges' to more primitive stages higher up on the panel or all the way to pluripotency (iPS cells) in the ball launch lane (as shown by *the* pinball wizard, Shinya Yamanaka^{2, 3}) as well as down specific differentiation pathways. Some master-regulator transcription factors, such as Oct4, can propel the cell a long distance toward pluripotency, whereas lineage-specific transcription factors can only tap it within a single lineage.

In the new work by Vierbuchen *et al.*¹, putative mesodermal fibroblasts were converted to an ectodermal neuronal fate with the Ascl1, Brn2 and Myt1l flippers (blue). For simplicity, we illustrate only two other examples of reprogramming: iPS cells and the intra-lineage conversion of pancreatic endocrine to exocrine cells⁴.

In the future it will be of great interest to assess how this model compares to the alternative view that a cell can move laterally on the pinball machine (or through a tunnel in Waddington's model), thus converting directly to a new lineage without passing through a more primitive state. This will be a fundamental principle to understand as the field moves forward.

It will also be important to evaluate the implications of the present study for cell therapy for diseases of the brain. Intra-lineage *in vivo* fate switching within the pancreas or the ear may have immediate impact for diseases like type 1 diabetes or hearing loss. Any newly generated pancreatic beta cells or auditory hair cells would be in a suitable location. However, switching fibroblasts to neurons *in vivo* would be unrewarding as any new neurons produced would not be in their nervous system niche and would therefore be functionally ineffective. On the other hand, *in vitro*—generated neurons or neural progenitor cells may be useful in transplantation-based therapies, and the possibility of generating these cells from patient fibroblasts without going through a pluripotent stage would have the advantage of circumventing tumorigenicity concerns associated with iPS cells. For most clinical applications, however, it would be necessary to be able to expand the cells, as large numbers of cells are typically required for transplantation. Additional work is needed to determine whether the approach of Vierbuchen *et al.*¹ can be modified to produce expandable neural progenitor cells.

Whatever the future holds for this fascinating and fast-moving field, it is apparent that stem cell biologists, in the words of The Who's "Pinball Wizard", sure play a mean pinball.

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be pushed directly sideways to another cell type (transdifferentiation) without being flipped to a less differentiated state.