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Programming and Reprogramming Neuronal Subtypes in the Central Nervous System

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Abstract

Recent discoveries in nuclear reprogramming have challenged the dogma that the identity of terminally differentiated cells cannot be changed. The identification of molecular mechanisms that reprogram differentiated cells to a new identity carries profound implications for regenerative medicine across organ systems. The central nervous system (CNS) has historically been considered to be largely immutable. However, recent studies indicate that even the adult CNS is imparted with the potential to change under the appropriate stimuli. Here, we review current knowledge regarding the capability of distinct cells within the CNS to reprogram their identity and consider the role of developmental signals in directing these cell fate decisions. Finally, we discuss the progress and current challenges of using developmental signals to precisely direct the generation of individual neuronal subtypes in the postnatal CNS and in the dish.

Keywords

directed differentiation; reprogramming; cellular replacement; corticospinal motor neurons

Introduction

Recent discoveries in the fields of nuclear and direct lineage reprogramming have fundamentally challenged the dogma that cells are irrevocably instructed to adopt specific cell fates and that the identity of a terminally differentiated cell cannot be amended. The principle that even differentiated cells can change their identity and acquire new functional roles has profound implications for developmental biology and regenerative medicine, since understanding the molecular mechanisms governing reprogramming makes the possibility of replacing diseased cells with autologous healthy cells of a different type more concrete. Here, we first review relevant work on the programming and reprogramming of cell identity across organ systems. We then focus on the central nervous system (CNS) to consider whether and how reprogramming of cell identity might apply to a system historically considered immutable and hardwired. Finally, we review advances and limitations related to generating specific subtypes of neurons, a challenge that must still be faced to exploit cell programming and reprogramming in the nervous system.

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From stem cells to tissues and back again: changing the identity of differentiated cells

During embryonic development, cells progressively differentiate to acquire specific cell fates and specialized functions. While this is the normal course of events that leads to the formation of somatic tissues, early cloning experiments in frogs and recent nuclear reprogramming experiments in mammals support a model by which developmental acquisition of terminal cell identity appears to be associated with reversible epigenetic modifications rather than permanent genetic changes. Pioneering work by Briggs and King in the 1950's (Briggs and King, 1952; King and Briggs, 1955), Gurdon in the 1960's and 1970's (Gurdon, 1962; Gurdon et al., 1975) and many subsequent groups has demonstrated that the nuclei of late-stage frog embryos transplanted into enucleated oocytes by somatic cell nuclear transfer can support the development of entire frogs. More recently, the cloning of Dolly the sheep and other mammals from differentiated cell types (Wilmut et al., 1997; Hochedlinger and Jaenisch, 2002; Eggan et al., 2004; Inoue et al., 2005), together with the finding that fusion of somatic cells with embryonic stem (ES) cells confers pluripotency (Tada et al., 2001; Cowan et al., 2005), further supports the idea that cell nuclei remain pluripotent even as cell fate is progressively restricted during development. This work primed the field for one of the most exciting discoveries of recent years: the finding by Yamanaka and Takahashi that differentiated cells can be reverted back to a pluripotent, ES-like state by forced expression of four transcription factors, namely *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4* (Takahashi and Yamanaka, 2006). The resulting cells were named induced pluripotent stem cells (iPSC) to reflect their genesis and fate potential, and the process of conversion is usually referred to as nuclear reprogramming. Since this original discovery, iPSC have been produced from a variety of starting cell populations across different organisms, including humans. The finding has challenged dogmas on the immutability of differentiated cell identity in adult tissues and emphasized the powerful role played by transcription factors in instructing identity changes.

From a therapeutic perspective, the ability to generate iPSC from human samples has provided an unprecedented opportunity to model human diseases *in vitro* using disease-relevant populations of cells that are obtained *via* the directed differentiation of patient-specific iPSC (Dimos et al., 2008; Park et al., 2008; Ebert et al., 2009; Soldner et al., 2009; Brennand et al., 2011; Chiang et al., 2011; Mitne-Neto et al., 2011). This is of great relevance for the study of diseases that affect organs and tissues that cannot be easily accessed in patients, first and foremost the central nervous system. Sampling of cells from the brain and spinal cord is invasive, and the isolated cells cannot be expanded or cultured for extensive periods of time – problems that fundamentally limit the study of human neurodegenerative and psychiatric diseases.

The idea behind the use of iPSC to model human diseases of the CNS is quite simple. First, patient-specific iPSC are generated *via* nuclear reprogramming of easily accessible somatic cells of patients (i.e. skin or blood cells), which then are directed to differentiate *in vitro* into the cell types affected by the disease. This approach allows the generation of large numbers of affected cells, since iPSC can be greatly expanded before differentiation. It also enables the generation of diseased cells after a patient has lost many of the endogenous cell counterparts (i.e., during late stages of the disease). Finally, this approach could provide an

interesting model for studying pre-symptomatic stages of diseases, as it is conceivable that iPSC that are induced to differentiate *in vitro* may recapitulate some of the steps normally covered by endogenous cells during disease initiation. A large number of iPSC lines from patients affected by neurodegenerative and psychiatric diseases have been generated. These include lines from patients with Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, spinal muscular atrophy, schizophrenia and Down syndrome (Dimos et al., 2008; Park et al., 2008; Ebert et al., 2009; Soldner et al., 2009; Brennand et al., 2011; Chiang et al., 2011; Marchetto et al., 2011; Mitne-Neto et al., 2011).

As promising as *in vitro* modeling of CNS disease may appear, there are several limitations to consider. First and foremost, neurodegenerative and psychiatric diseases are complex and often involve multiple cell types and circuitries. This must be considered and respected when developing disease models, which are different for different diseases and may require the generation of more than one cell type. If neurons are the cells affected, generation of one neuron type in isolation may not be sufficient to mimic the disease, since neurons work in circuits and not as individual units. It is therefore likely that building of neuronal circuits from iPSC will be necessary, especially to model complex neurodevelopmental (e.g., autism-spectrum disorders) and psychiatric (e.g., schizophrenia) conditions.

Second, generation of the exact cell types affected in a disease is dependent and fundamentally limited by our knowledge of the signals that instruct the fate specification and differentiation of the cell in the embryo. This currently limits the type of neurons that can be generated from pluripotent stem cells to a handful of subtypes (see below). In addition, generation of pure cultures of a given cell of interest depends on the availability of cell type-specific iPSC reporter lines that enable the purification of the relevant cells from unwanted cell types that are typically co-generated in the dish. Building reporter iPSC lines is not trivial when working with human cells, which are notoriously refractory to DNA recombination. However, this has been achieved using zinc finger nucleases to target and modify specific loci in human ES and iPS cells (Klug, 2010), and the process will likely be facilitated by the use of recently discovered TALE proteins, which can be designed to bind specifically to defined sequences of DNA in the genome (Bogdanove and Voytas, 2011).

Finally, there is no firm agreement on how similar iPSC-derived cell types must be to their endogenous counterparts to be considered “equivalent”. Molecular analysis has shown that these cells may in fact be quite different. It is conceivable that the same cell type may be generated following slightly different differentiation programs in the embryo or *in vitro*, and yet the cells may ultimately function similarly. This warrants studies to prove the functionality and value to the cells generated *in vitro*.

Together, these are serious considerations given the extreme diversity of neuron and glia classes that populate the CNS, the complexity of neuronal networks and the fact that many diseases involve multiple cell types. Despite these limitations, iPSC have been used with success to model some aspects of neurodegenerative diseases (Di Giorgio et al., 2007; Nagai et al., 2007; Di Giorgio et al., 2008; Ebert et al., 2009).

Turning one cell type into another *via* direct reprogramming

The fact that transcription factors can convert a differentiated adult cell into a pluripotent stem cell supports the concept that conversion between any two types of cells might be instructed given the right combination of lineage-specific transcription factors. The idea of converting one differentiated cell type into another is not new. It has been known for some time that *MyoD*, which encodes a transcription factor critical in specifying the skeletal muscle lineage during development, can convert fibroblasts (and other cell types) into contracting muscle cells (Davis et al., 1987; Weintraub et al., 1989; Choi et al., 1990). Similarly, C/EBP expression alone is sufficient to convert B lymphocytes (Xie et al., 2004) and, when combined with PU.1, fibroblasts (Feng et al., 2008) into macrophages. *Math1* alone can reprogram inner ear support cells into hair cells and restore hearing (Zheng and Gao, 2000; Kawamoto et al., 2003; Shou et al., 2003; Izumikawa et al., 2005).

Groundbreaking work by the Melton group has recently shown that direct reprogramming by defined factors can also be used to achieve cell conversion *in vivo*. In this study, overexpression of *Pdx1*, *Ngn3* and *MafA* in pancreatic acinar cells was sufficient to reprogram them into functional, insulin-secreting β cells within the environment of the pancreas (Zhou et al., 2008). Since these earlier studies, examples of direct reprogramming have been reported for a variety of cell types. Cocktails of transcription factors have been shown to successfully convert fibroblasts directly into cardiomyocytes (Ieda et al., 2010; Efe et al., 2011), hepatocytes (Huang et al., 2011; Sekiya and Suzuki, 2011), blood progenitors (Szabo et al., 2010) and even neurons (see below), without the need to pass through a pluripotent cell state (for excellent reviews see Zhou and Melton, 2008; Selvaraj et al., 2010; Vierbuchen and Wernig, 2011).

As hurdles to cell identity conversion are overcome, generation of clinically relevant cell types within complex organ systems becomes a more attainable goal. There are reasons to believe that the CNS will be no exception, and that direct reprogramming might be instructed within the brain to generate clinically relevant neuronal and glial cell populations.

Changing cell identity in the adult CNS

The adult CNS, composed of thousands of different subtypes of terminally differentiated and postmitotic neurons as well as astrocytes and oligodendrocytes, has historically been considered an immutable structure. Yet, over the last few years, a tremendous amount of work has demonstrated that cell identity in the brain is much more plastic than was initially thought.

It has long been known that adult neural progenitor cells (AdNPC) continuously generate new neurons, astrocytes and oligodendrocytes throughout adulthood in two distinct brain regions: the subventricular zone of the lateral ventricle and the subgranular zone of the dentate gyrus (Gage, 2000; Alvarez-Buylla and Lim, 2004). Interestingly, recent studies have reported that AdNPC can be manipulated molecularly to change their fate and the type of progeny that they give rise to. For example, manipulation of the PDGF signaling pathways *in vivo* is sufficient to bias AdNPC towards generating oligodendrocytes at the expense of neurons, and vice versa (Jackson et al., 2006) (Figure 1A). In this study,

conditional ablation of the receptor PDGFR α blocked oligodendrocyte generation, whereas PDGF ligand infusion into the lateral ventricle blocked the generation of neurons and induced AdNPC proliferation and hyperplasia, with cells exhibiting glioma-like features. Similarly, overexpression of the transcription factor *Ascl1* (*Mash1*) in subgranular zone AdNPC primarily generates oligodendrocytes at the expense of hippocampal granule neurons (Jessberger et al., 2008) (Figure 1A).

These studies demonstrate that AdNPC can respond to extrinsic and intrinsic signals to alter their cell fate. It is therefore appropriate to ask if AdNPC either in the subventricular/subgranular zones or in non-neurogenic regions might be manipulated to generate clinically relevant cell types, specifically neuronal subtypes that the AdNPC would not normally be fated to make. Insights from experimental and pathological conditions suggest that this might be beyond mere speculation. Induced apoptosis of two projection neuron subtypes in the adult neocortex resulted in the generation of small numbers of the same neuron types, likely from endogenous AdNPC (Magavi et al., 2000; Chen et al., 2004). Furthermore, stroke induction in the adult rat brain has been shown to increase proliferation of subventricular zone AdNPC and generation of newborn immature neurons that migrate to the affected striatal region, where they differentiate into medium spiny neurons (Arvidsson et al., 2002; Thored et al., 2006). Collectively, these studies support the hypothesis that AdNPC might respond to particular molecular cues to acquire neuronal fates that they would normally not have. The findings further suggest that this type of cell plasticity might be exploited to direct the generation of clinically relevant populations of neurons, provided that the signals driving subtype-specific differentiation are available.

In addition to AdNPC, other cell types within the CNS have been shown to retain the ability to change fate or identity. For example, oligodendrocyte precursor cells normally arise from AdNPC and migrate away from the adult germinal zones to terminally differentiate into oligodendrocytes. When cultured sequentially in the presence of PDGF, fetal calf serum and bFGF, on the other hand, oligodendrocyte precursors adopt properties of AdNPC and, upon treatment with additional extracellular signals, give rise to neurons, astrocytes, and oligodendrocytes (Kondo and Raff, 2000). In addition, cytokine stimulation *in vitro* and *in vivo* following ischemic injury can convert oligodendrocytes into astrocytes, a naturally-occurring lineage reprogramming process that is accompanied by cell-intrinsic epigenetic changes triggered in response to cell-extrinsic cues (Kohyama et al., 2008).

Most interestingly, forced expression of *Pax6* or *Ngn2* in astrocytes purified from the postnatal mouse neocortex is sufficient to convert the astrocytes into immature neurons (Heins et al., 2002; Berninger et al., 2007) (Figure 1B). Similarly, following brain injury, overexpression of *Pax6* or down-regulation of *Olig2* induces neuroblast differentiation from *Olig2*-expressing glial cells (astrocytes, oligodendrocytes or their precursors) (Buffo et al., 2005). While the immature neurons that are so generated are unable to fully differentiate and to form functional synapses (Berninger et al., 2007), this limitation has been recently overcome by using *Ngn2* or *Dlx2* instead of *Pax6* to drive direct reprogramming of astrocytes into glutamatergic or GABAergic synapse-forming neurons, respectively (Heinrich et al., 2010) (Figure 1B). These findings demonstrate the intrinsic cellular

plasticity of the adult brain and emphasize how cell-intrinsic and -extrinsic signals might be used to modify cell fate and identity even in the adult CNS.

Can postmitotic neurons of the CNS change their minds?

The mammalian CNS neuron is a classic example of a terminally differentiated cell type. With the exception of the adult neurogenic niches, where specific subtypes of neurons continue to be generated throughout life, neurons are largely made during embryonic development, they are permanently postmitotic cells and, as detected with current methods, they do not normally change identity. Is this immutability due to an intrinsic inability of postmitotic neuronal nuclei to reprogram? Are there genetic barriers that irreversibly preclude an identity change? These possibilities are unlikely for at least a subset of central neurons, given the results of some seminal studies. Somatic cell nuclear transfer experiments have elegantly demonstrated that the nuclei of postmitotic olfactory epithelium neurons, transplanted into enucleated oocytes, can give rise to live mice that have a grossly normal CNS (Eggan et al., 2004; Li et al., 2004). Furthermore, isolated P7 cortical neurons appear to be able to undergo nuclear reprogramming into iPSC upon forced expression of the four reprogramming factors (*Oct3/4*, *Sox2*, *c-Myc* and *Klf4*) combined with loss of *p53* and repression of neuron-specific genes (Kim et al., 2011). These experiments indicate that albeit refractory to change, neurons have the potential to reprogram and that neuronal nuclei, when appropriately primed, can be as plastic as the nuclei of any other cell type.

What, then, stops neurons from changing within the brain? How is the identity of neurons maintained in time and space? Can postmitotic neurons undergo reprogramming from one subtype into another or across lineage boundaries? Answering these questions is of fundamental importance for understanding how most central neurons retain their identity and functional roles over the life span of an organism. The implications for the development of new therapeutic strategies for CNS disease are also profound. If neuronal identity could be changed on demand, neuronal subtypes affected in a given disease might be replaced by the direct reprogramming of unaffected neighboring neurons, which could possibly assume the function of the diseased neurons.

Because closely related cell types share much of their developmental history, including some basic gene expression patterns and, possibly, much of their epigenetic landscape (Bernstein et al., 2007), it is likely that direct reprogramming of neurons, if possible, may be more easily and efficiently achieved among neuron subtypes of developmentally related lineages, such as those that are derived from a common progenitor. Should this be attempted *in vivo*, then conversion between two related neuronal classes of neighboring neurons may also be favored by the fact that reprogrammed neurons would be provided with a microenvironment that may be optimal for differentiation, integration into the appropriate micro- and macro-circuitry, and long-term survival.

Programming and reprogramming neuronal diversity using developmental cues

Several experimental precedents reinforce the idea that guided differentiation of ES cells or iPSC into clinically relevant neuronal populations can be realized by developmental signals resembling those that instruct the birth of the same neuronal populations during

development. The first successful example of this strategy enabled Hynek Wichterle and Tom Jessell to generate spinal motor neurons (SMN) from ES cells using a combination of ventralizing and caudalizing signals that are normally used to specify SMN fate in the ventral spinal cord (Wichterle et al., 2002). Recently, this work has been extended to include the generation of specific populations of SMN (Dimos et al., 2008; Peljto et al., 2010).

Importantly, SMN have now been generated not only from healthy ES and iPS cells of mice and humans, but also from iPS cells derived from patients with familial amyotrophic lateral sclerosis (Di Giorgio et al., 2008; Dimos et al., 2008) and spinal muscular atrophy (Ebert et al., 2009), diseases where SMN are specifically affected. This in turn has enabled the first *in vitro* studies aimed at gaining insight into the pathogenesis of these motor diseases (Di Giorgio et al., 2008; Dimos et al., 2008; Ebert et al., 2009).

Beyond SMN, several other types of neurons have been subsequently generated from pluripotent stem cells. Developmental extracellular signals have been used to direct the generation of cerebellar neurons from ES cells (Salero and Hatten, 2007). In the cerebral cortex, one of the most complex and heterogeneous regions of the CNS, developmentally inspired culture conditions have been used to generate both excitatory projection neurons (Watanabe et al., 2005; Eiraku et al., 2008; Gaspard et al., 2008) and cortical interneuron progenitors (Maroof et al., 2010) from ES cells (Figure 2). In some of these studies in particular, exposure of ES cells to morphogens was combined with ES cell culture in tridimensional aggregates that developed inner cavities resembling the architecture of the early developing telencephalon (Watanabe et al., 2005; Eiraku et al., 2008). This led to the generation of different subtypes of projection neurons that “arranged” themselves in concentric structures reminiscent of cortical layers. In agreement with prior elegant work studying the fate potential of cortical progenitors *in vitro* (Shen et al., 2006), ES cells were able, to a certain extent, to mimic the appropriate sequential generation of projection neurons subtypes as observed in the embryo (Eiraku et al., 2008; Gaspard et al., 2008).

More recently, specific culture conditions including successive treatments with selected morphogens enabled the Anderson and Studer groups to derive *Lhx6*-GFP-positive cortical interneuron progenitors from ES cells (Maroof et al., 2010) (Figure 2). Following transplantation, these progenitors migrated within the neocortex, integrated into the local circuitry, and developed morphological and electrophysiological properties of *Lhx6*-expressing cortical interneurons (Maroof et al., 2010).

While the molecular identity of ES-derived excitatory projection neuron and inhibitory neuron subtypes so produced needs to be confirmed by a broader analysis of multiple subtype-specific genes, and the efficiency by which each neuronal population is generated needs improvement, the work demonstrates that cortical neurons can be generated from pluripotent stem cells using extracellular culture conditions inspired by developmental signals. Given that cortical glutamatergic projection neurons and GABAergic interneurons normally integrate into local microcircuitry *in vivo*, the directed differentiation of these two neuronal populations from pluripotent stem cells paves the way for the generation of cortical microcircuitry in the dish. This could be very useful to model circuit-level neurodevelopmental and psychiatric diseases of the cortex.

Changing cell identity from within: the power of transcription factors

In the developing CNS, the establishment of neuron subtype-specific identity is governed by both cell-extrinsic and cell-intrinsic signals. In most cases, directed differentiation of pluripotent stem cells into different classes of neurons has been achieved by the manipulation of extracellular signals. However, manipulation of cell-autonomous signals *via* the forced expression of key transcription factors has been demonstrated to be a very powerful way to affect cell identity in a variety of cellular contexts, and may represent a preferable strategy when aiming to generate very specific subtypes of neurons from complex structures, for example the cerebral cortex (discussed below).

In one of the first successful application of this strategy to generate neurons of the CNS, over-expression of two homeodomain transcription factors, *Lmx1a* and *Msx1*, led to the directed differentiation of ES cells into dopaminergic neurons (Andersson et al., 2006) (Figure 3A). Similarly, simultaneous expression of *Gata2*, *Ascl1* and *Fox2A* instructed the generation of serotonergic neurons from ES cells (Nefzger et al., 2011) (Figure 3A). This work provides a demonstration that specific “codes” of lineage-specific transcription factors, acting within the cell, can drive the acquisition of specific neuronal identity. This also suggests that differentiated cells rather than pluripotent stem cells may respond to similar combinations of transcription factors to undergo reprogramming into neurons.

Fulfilling this prediction, Marius Wernig's team was the first to report the generation of glutamatergic and GABAergic neurons from mouse embryonic fibroblasts using a combination of three transcription factors: *Ascl1*, *Brn2* and *Myt1l* (Vierbuchen et al., 2010) (Figure 3B). The resulting cells were termed induced neurons (iN) to highlight the process of reprogramming that generated them. Building on this seminal work, Wernig and others have subsequently shown that human embryonic fibroblasts behave similarly in response to these three transcription factors (Pang et al., 2011; Pfisterer et al., 2011).

If three transcription factors can convert fibroblasts into neurons, could this basic “cocktail” be integrated with lineage-specific transcription factors to generate defined subtypes of neurons from fibroblasts? In very exciting work, fibroblasts have been directly reprogrammed into dopaminergic neurons by combining *Ascl1*, *Brn2* and *Myt1l* (i.e., the iN factors) with *Lmx1a* and *FoxA2* (Pfisterer et al., 2011) (Figure 3B). Similarly, spinal motor neurons (SMN) have been generated by adding *Ngn2*, *Lhx3*, *Isl1* and *Hb9* to the same set of three iN factors (Son et al., 2011) (Figure 3B). When this cocktail was supplemented with *NeuroD1*, human fetal fibroblasts could also be reprogrammed into SMN albeit at lower efficiencies (Son et al., 2011).

Interestingly, a similar terminal neuronal identity can be reached by using a different cocktail of genes, as demonstrated by Vania Broccoli and colleagues who used *Ascl1*, *Nurr1* and *Lmx1a* (Caiazzo et al., 2011) and Rudolf Jaenisch's group who used *Ascl1*, *Pitx3*, *Lmx1a*, *Nurr1*, *Foxa2*, and *EN1* (Kim et al., 2011) to generate dopaminergic neurons. It is quite exciting that fibroblast-derived dopaminergic neurons were shown to be able to alleviate some symptoms when transplanted in a model of Parkinson's disease (Kim et al., 2011). Human fibroblasts have been converted into neurons by the expression of *miR-9/9** and *miR-124*, two microRNAs that control multiple genes important for neuronal

differentiation and function (Yoo et al., 2011). This process is facilitated by the addition of the neurogenic transcription factors *NeuroD2*, *Ascl1* and *Myt1l* (Yoo et al., 2011) (Figure 3C). These differentiation schemes are summarized in Table 1.

Cellular context is centrally important, and not all starting cell populations reprogram to the same final identity upon expression of the same transcription factors. Elegant work by Oliver Hobert's laboratory illustrates this concept by demonstrating that single *Caenorhabditis elegans* transcription factors can reprogram mitotic germ cells into defined neuron types *in vivo* only upon concomitant removal of LIN-53 (RbAp46/48 in humans), a component of histone remodeling complexes. This work demonstrates that even powerful terminal selector genes able to regulate broad programs of subtype-specific neuronal identity can only work in the right chromatin context (Tursun et al., 2011).

Taken together, these studies demonstrate several important principles. Under the right culture conditions, transcription factors that instruct the developmental generation of neuronal classes can play very powerful roles in the establishment of similar neuronal fates from both pluripotent stem cells and differentiated cell types. In addition, despite the complexity of the overall signaling scheme necessary to generate a given neuronal population in the embryo, it may not be necessary to fully recapitulate every developmental step *in vitro*. Rather, a small number of “master” signals may serve as switches to activate broader pathways, ultimately leading to the generation of similar classes of neurons. These findings are very exciting and justify further investigation into both the molecular mechanisms underlying neuronal fate specification in the embryo and reprogramming in the dish.

In the future, it will be important to determine how similar are neurons derived from pluripotent stem cells or differentiated fibroblasts, and how they compare to their endogenous counterparts. It is conceivable, for example, that neurons derived from fibroblasts may retain epigenetic features of the starting cell type, a “memory” of their original state. Molecular comparison of ES- versus fibroblast-derived SMN and midbrain dopaminergic neurons confirm to some extent this prediction by showing that characteristics of the terminal neuron types depend on the identity of the cell population from which they are derived (Son et al., 2011; Caiazzo et al., 2011). In this regard, molecular profiling and comparison of ES-derived, fibroblast-derived and endogenous neurons will likely highlight pathways that are only partially reactivated during reprogramming and may inform strategies to improve the reprogramming process by adding some of the missing genes. Finally, induced neurons will have to be thoroughly tested at the functional level. How similarly can they “act” to their endogenous counterparts? Although more extensive tests are necessary, the fact that induced dopaminergic neurons appear to ameliorate symptoms in a model of Parkinson disease sends an optimistic message that these neurons may be useful (Kim et al., 2011).

Directing the generation of projection neuron subtypes in the cerebral cortex

The CNS is composed of an outstanding diversity of neuron types. Here, like nowhere else in the body, the field of reprogramming is faced with a fundamental question: will it be possible to generate only defined subtypes of neurons? Will this be necessary to meet the

clinical needs of cellular repair and drug screening? The ground-breaking work published so far demonstrates that “broad” classes of neurons can be generated in the dish. However, in the brain these classes are *per se* very heterogeneous. For example, dopaminergic neurons of the midbrain are not a single population; rather they are composed of neurons located in different regions, expressing different markers, connecting to different targets and ultimately having different functions and susceptibility to disease (Ang, 2009). Similarly, spinal motor neurons are very heterogeneous and are organized in columns and pools that connect to different muscle groups (Dasen and Jessell, 2009). These considerations are important, especially when aiming at regenerating neurons of complex CNS regions such as the cerebral cortex.

The mature neocortex contains an astonishing number of projection neuron and interneuron subtypes. Elegant work has demonstrated that both projection neurons and cortical interneurons can be generated from pluripotent stem cells (Watanabe et al., 2005; Eiraku et al., 2008; Gaspard et al., 2008; Maroof et al., 2010) (Figure 2). However, can individual subtypes of neurons be uniquely made? Here, we discuss this possibility with regards to cortical projection neurons.

Excitatory projection neurons represent the largest portion (approximately 80%) of all cortical neurons. They are born from neural progenitors in the dorsal telencephalon and are classified into numerous subtypes based on their location within different cortical layers and areas; their axonal projections to distinct intracortical, subcortical and subcerebral targets; and the combinatorial expression of different neuron type-specific genes (Bayer et al., 1991; Molyneaux et al., 2007). Among them, corticofugal projection neurons include corticothalamic projection neurons, which are found in layer VI and project to the thalamus, as well as corticospinal motor neurons (CSMN), corticotectal projection neurons and several other types of subcerebral projection neurons, which are located in different areas of layer V and project to the spinal cord, the superior colliculus and other targets in the brainstem and below the brain, respectively (Molyneaux et al., 2007). In addition, the cortex includes several types of callosal projection neurons (CPN), which are located in layers II/III and V (and in small numbers in layer VI) and connect to targets in the contralateral hemisphere (i.e. *via* the corpus callosum), the striatum and the frontal cortex (Richards et al., 2004; Mitchell and Macklis, 2005; Lindwall et al., 2007; Molyneaux et al., 2009; Fame et al., 2011).

The signals that drive the early steps of fate specification and overall development of individual types of neurons are largely undefined for most populations. However, CSMN and, to a smaller extent, callosal projection neurons are notable first exceptions (Arlotta et al., 2005; Molyneaux et al., 2009). CSMN and related subcerebral projection neurons are probably the best-characterized molecularly. These neurons constitute one subpopulation of subcerebral projection neurons, and are located in layer Vb of the neocortex. They extend their axons *via* the internal capsule, the cerebral peduncle and the pyramidal tract to the brainstem and the spinal cord (Canty and Murphy, 2008). Several genes have been identified that in combination mark CSMN at the molecular level and distinguish them from other related subtypes of cortical projection neurons, even within the same layer (Arlotta et al., 2005; Molyneaux et al., 2007; Belgard et al., 2011). These include transcription factors (e.g.,

Ctip2, *Bcl6*, *Sox5*, *Fezf2*); cell surface proteins (e.g., *Encephalopsin*, *Itm2a*, *Daf1*); calcium signaling proteins (e.g., *Pcp4*, *S100a10*); cell adhesion proteins (e.g., *Cdh22*, *Cdh13*, *Cntn6*), axon guidance molecules (e.g., *Neto1*, *Netrin-G1*) (Arlotta et al., 2005) and lincRNAs (Belgard et al., 2011). Most importantly, beyond their roles as CSMN molecular markers, some of these genes have been shown to control central steps of development of CSMN, including the timing of birth, fate specification and axonal connectivity (Arlotta et al., 2005).

Among key transcription factors, *Fezf2*, expressed in all subcerebral projection neurons from early stages of development through adulthood (Arlotta et al., 2005; Inoue et al., 2005), has been shown to be essential for *in vivo* CSMN early specification (Chen et al., 2005; Chen et al., 2005; Molyneaux et al., 2005). In the absence of *Fezf2* in null-mutant mice, CSMN and all related subcerebral projection neurons do not specify, and axonal projections from the cerebral cortex to either the spinal cord or the brainstem fail to form (Chen et al., 2005; Molyneaux et al., 2005).

A second set of genes has been identified that controls later aspects of subcerebral projection neuron development, possibly acting downstream of *Fezf2*. The transcription factor *Ctip2*, for example, is important for the establishment of appropriate axonal connectivity by CSMN to the spinal cord (Arlotta et al., 2005; Chen et al., 2008). Similarly, *Sox5* have been demonstrated to control the timing of generation, migration and connectivity of the CSMN population (Joshi et al., 2008; Kwan, 2008) and *Bhlhb5* its arealization (Lai et al., 2008).

As more is learned about the functional roles played by additional CSMN-genes, it is conceivable that instructive developmental signals might be used to direct the differentiation of CSMN and other subcerebral projection neuron types in the dish. Prior work supports this prediction directly. For example, *Ngn2* is sufficient to ectopically instruct the birth of glutamatergic neurons when overexpressed in progenitors of the lateral ganglionic eminence (LGE), which normally give rise to GABAergic medium spiny neurons of the striatum and interneurons of the olfactory bulb (Mattar et al., 2008; Wichterle et al., 2001) (Figure 4C). This proneural gene also has a similar effect when overexpressed in astrocytes and neural progenitors in culture (Berninger et al., 2007; Heinrich et al., 2010). *Fezf2* is sufficient to instruct the birth of subcerebral projection neurons including CSMN *in vivo* when overexpressed in cortical progenitors fated to become upper layer neurons (Chen et al., 2005; Molyneaux et al., 2005; Lodato et al., 2011) (Figure 4B). Demonstrating the powerful “master” role played by *Fezf2*, we have found that overexpression of *Fezf2* is sufficient to also switch LGE progenitors into corticofugal projection neurons *in vivo* (Rouaux and Arlotta, 2010) (Figure 4C). In this study, despite developing ectopically within the embryonic striatum, *Fezf2*-expressing neurons survived, acquired molecular features of corticofugal neurons and developed distinct pyramidal-like morphology and connectivity to subcortical and subcerebral targets (Rouaux and Arlotta, 2010). Notably, in this cellular context, *Fezf2* is sufficient to instruct a corticofugal fate without the need to first specify LGE progenitors to a cortical neural progenitor identity (Rouaux and Arlotta, 2010). This finding supports the idea that recapitulation of every step of normal development may not be necessary for acquisition of subtype-specific neuronal identity. Rather, “master” transcription factors may be sufficient to elicit broad programs of identity acquisition.

Equally important for inducing genetic programs specifying one cortical projection neuron subtype versus another is the concurrent inhibition of genes directing alternative fates. For example, the down-regulation of *Satb2*, a gene critical for callosal projection neuron development (Alcamo et al., 2008; Britanova et al., 2008) and responsible for *Ctip2* repression (Alcamo et al., 2008; Britanova et al., 2008; Leone et al., 2008), may be useful to prevent the acquisition of a callosal neuron fate. Similarly, down-regulation of *Tbr1*, a gene important for the development of corticothalamic projection neurons (Hevner et al., 2001; Han et al., 2011; McKenna et al., 2011), may further refine the generation of subcerebral neurons versus corticofugal neurons of layer VI.

While it is difficult to predict what combination of induced and repressed genes can drive CSMN differentiation from pluripotent stem cells (or via the direct lineage reprogramming of differentiated cells types), a molecular logic based on developmentally meaningful signals may represent a reasonable start. Basic patterning of ES-derived neural progenitors into *Pax6* and *Bf1* positive, “cortical” NPC has been already described from both mouse and human cells. It is plausible to think that these progenitors may have undergone at least some level of dorsal telencephalic patterning and thus may be responsive to dorsal proneural genes (e.g. *Ngn2*) to begin to generate dorsal glutamatergic neurons. In this basic blueprint, lineage-specific transcription factors (e.g. *Fzf2*) may initiate and possibly maintain a cascade of gene expression that leads to the activation of “effector genes” necessary for subcerebral projection neuron development. Further refinement of neuronal subtype-specific identity to generate subpopulations of subcerebral neurons, like CSMN, may subsequently require expression of area-specific transcription factors (e.g. *Bhlhb5*). The complexity of lineage bifurcation decisions and the development of morphological and fine connectivity features of CSMN may be beyond what can be done *in vitro*. It is likely that transplantation of the neurons generated *in vitro* will be necessary to fully understand their identity, connectivity and potential functional roles.

Conclusion

The discovery that even terminally differentiated cells can be reprogrammed to acquire a new cellular identity demonstrates that cell differentiation is not an irreversible process. Developmental signals that shape cell diversity in the embryo have been shown to play powerful roles in directing the generation of new cell types both *in vitro* and in the adult organism. This now offers an unprecedented opportunity to model complex diseases in the dish, as well as to attempt cell replacements *in vivo*. Like other organs before it, reprogramming of CNS cells is fast becoming a reality. Important challenges lay ahead, not the least the need to instruct and control the generation of the unparalleled diversity of neuron subtypes and complex circuitry that characterize the CNS. For as difficult as this may seem, it is exciting that current work has already challenged dogmas on the immutability of the CNS, demonstrating the value and potential of reprogramming to model and study a spectrum of presently untreatable neurodegenerative and psychiatric diseases.

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References

- Alcamo EA, Chirivella L, Dautzenberg M, Dobрева G, Farinas I, Grosschedl R, McConnell SK. *Satb2* regulates callosal projection neuron identity in the developing cerebral cortex. *Neuron*. 2008; 57:364–377. [PubMed: 18255030]
- Alvarez-Buylla A, Lim DA. For the long run: maintaining germinal niches in the adult brain. *Neuron*. 2004; 41:683–686. [PubMed: 15003168]
- Andersson E, Tryggvason U, Deng Q, Friling S, Alekseenko Z, Robert B, Perlmann T, Ericson J. Identification of intrinsic determinants of midbrain dopamine neurons. *Cell*. 2006; 124(2):393–405. [PubMed: 16439212]
- Ang SL. *Foxa1* and *Foxa2* transcription factors regulate differentiation of midbrain dopaminergic neurons. *Adv Exp Med Biol*. 2009; 651:58–65. [PubMed: 19731550]
- Arlotta P, Molyneaux BJ, Chen J, Inoue J, Kominami R, Macklis JD. Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. *Neuron*. 2005; 45:207–221. [PubMed: 15664173]
- Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med*. 2002; 8:963–970. [PubMed: 12161747]
- Bayer SA, Altman J, Russo RJ, Dai XF, Simmons JA. Cell migration in the rat embryonic neocortex. *J Comp Neurol*. 1991; 307:499–516. [PubMed: 1856333]
- Belgard TG, Marques AC, Oliver PL, Abaan HO, Sirey TM, Hoerder-Suabedissen A, Garcia-Moreno F, Molnar Z, Margulies EH, Ponting CP. A transcriptomic atlas of mouse neocortical layers. *Neuron*. 2011; 71:605–616. [PubMed: 21867878]
- Berninger B, Costa MR, Koch U, Schroeder T, Sutor B, Grothe B, Gotz M. Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. *J Neurosci*. 2007; 27:8654–8664. [PubMed: 17687043]
- Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. *Cell*. 2007; 128:669–681. [PubMed: 17320505]
- Bogdanove AJ, Voytas DF. TAL effectors: customizable proteins for DNA targeting. *Science*. 2011; 333:1843–1846. [PubMed: 21960622]
- Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH. Modelling schizophrenia using human induced pluripotent stem cells. *Nature*. 2011; 473:221–225. [PubMed: 21490598]
- Briggs R, King TJ. Transplantation of Living Nuclei From Blastula Cells into Enucleated Frogs' Eggs. *Proc Natl Acad Sci U S A*. 1952; 38:455–463. [PubMed: 16589125]
- Britanova O, de Juan Romero C, Cheung A, Kwan KY, Schwark M, Gyorgy A, Vogel T, Akopov S, Mitkovski M, Agoston D, Sestan N, Molnar Z, Tarabykin V. *Satb2* is a postmitotic determinant for upper-layer neuron specification in the neocortex. *Neuron*. 2008; 57:378–392. [PubMed: 18255031]
- Buffo A, Vosko MR, Erturk D, Hamann GF, Jucker M, Rowitch D, Gotz M. Expression pattern of the transcription factor *Olig2* in response to brain injuries: implications for neuronal repair. *Proc Natl Acad Sci U S A*. 2005; 102:18183–18188. [PubMed: 16330768]
- Caiazzo M, Dell'Anno MT, Dvoretzkova E, Lazarevic D, Taverna S, Leo D, Sotnikova TD, Menegon A, Roncaglia P, Colciago G, Russo G, Carninci P, Pezzoli G, Gainetdinov RR, Gustincich S, Dityatev A, Broccoli V. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature*. 2011; 476:224–227. [PubMed: 21725324]
- Canty AJ, Murphy M. Molecular mechanisms of axon guidance in the developing corticospinal tract. *Prog Neurobiol*. 2008; 85:214–235. [PubMed: 18378059]
- Chen B, Schaevitz LR, McConnell SK. *Fez1* regulates the differentiation and axon targeting of layer 5 subcortical projection neurons in cerebral cortex. *Proc Natl Acad Sci U S A*. 2005; 102:17184–17189. [PubMed: 16284245]

- Chen B, Wang SS, Hattox AM, Rayburn H, Nelson SB, McConnell SK. The Fezf2-Ctip2 genetic pathway regulates the fate choice of subcortical projection neurons in the developing cerebral cortex. *Proc Natl Acad Sci U S A*. 2008; 105:11382–11387. [PubMed: 18678899]
- Chen J, Magavi SS, Macklis JD. Neurogenesis of corticospinal motor neurons extending spinal projections in adult mice. *Proc Natl Acad Sci U S A*. 2004; 101:16357–16362. [PubMed: 15534207]
- Chen JG, Rasin MR, Kwan KY, Sestan N. Zfp312 is required for subcortical axonal projections and dendritic morphology of deep-layer pyramidal neurons of the cerebral cortex. *Proc Natl Acad Sci U S A*. 2005; 102:17792–17797. [PubMed: 16314561]
- Chiang CH, Su Y, Wen Z, Yoritomo N, Ross CA, Margolis RL, Song H, Ming GL. Integration-free induced pluripotent stem cells derived from schizophrenia patients with a DISC1 mutation. *Mol Psychiatry*. 2011; 16:358–360. [PubMed: 21339753]
- Choi J, Costa ML, Mermelstein CS, Chagas C, Holtzer S, Holtzer H. MyoD converts primary dermal fibroblasts, chondroblasts, smooth muscle, and retinal pigmented epithelial cells into striated mononucleated myoblasts and multinucleated myotubes. *Proc Natl Acad Sci U S A*. 1990; 87:7988–7992. [PubMed: 2172969]
- Cowan CA, Atienza J, Melton DA, Eggan K. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science*. 2005; 309:1369–1373. [PubMed: 16123299]
- Dasen JS, Jessell TM. Hox networks and the origins of motor neuron diversity. *Curr Top Dev Biol*. 2009; 88:169–200. [PubMed: 19651305]
- Davis RL, Weintraub H, Lassar AB. Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell*. 1987; 51:987–1000. [PubMed: 3690668]
- Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat Neurosci*. 2007; 10:608–614. [PubMed: 17435754]
- Di Giorgio FP, Boulting GL, Bobrowicz S, Eggan KC. Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. *Cell Stem Cell*. 2008; 3:637–648. [PubMed: 19041780]
- Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H, Henderson CE, Eggan K. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 2008; 321:1218–1221. [PubMed: 18669821]
- Ebert AD, Yu J, Rose FF Jr, Mattis VB, Lorson CL, Thomson JA, Svendsen CN. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature*. 2009; 457:277–280. [PubMed: 19098894]
- Efe JA, Hilcove S, Kim J, Zhou H, Ouyang K, Wang G, Chen J, Ding S. Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. *Nat Cell Biol*. 2011; 13:215–222. [PubMed: 21278734]
- Eggan K, Baldwin K, Tackett M, Osborne J, Gogos J, Chess A, Axel R, Jaenisch R. Mice cloned from olfactory sensory neurons. *Nature*. 2004; 428:44–49. [PubMed: 14990966]
- Eiraku M, Watanabe K, Matsuo-Takasaka M, Kawada M, Yonemura S, Matsumura M, Wataya T, Nishiyama A, Muguruma K, Sasai Y. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell*. 2008; 3:519–532. [PubMed: 18983967]
- Fame RM, MacDonald JL, Macklis JD. Development, specification, and diversity of callosal projection neurons. *Trends Neurosci*. 2011; 34(1):41–50. [PubMed: 21129791]
- Feng R, Desbordes SC, Xie H, Tillo ES, Pixley F, Stanley ER, Graf T. PU.1 and C/EBPalpha/beta convert fibroblasts into macrophage-like cells. *Proc Natl Acad Sci U S A*. 2008; 105:6057–6062. [PubMed: 18424555]
- Gage FH. Mammalian neural stem cells. *Science*. 2000; 287:1433–1438. [PubMed: 10688783]
- Gaspard N, Bouchet T, Hourez R, Dimidschstein J, Naeije G, van den Ameel J, Espuny-Camacho I, Herpoel A, Passante L, Schiffmann SN, Gaillard A, Vanderhaeghen P. An intrinsic mechanism of corticogenesis from embryonic stem cells. *Nature*. 2008; 455:351–357. [PubMed: 18716623]

- Gurdon JB. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J Embryol Exp Morphol.* 1962; 10:622–640. [PubMed: 13951335]
- Gurdon JB, Laskey RA, Reeves OR. The developmental capacity of nuclei transplanted from keratinized skin cells of adult frogs. *J Embryol Exp Morphol.* 1975; 34:93–112. [PubMed: 1102625]
- Han W, Kwan KY, Shim S, Lam MM, Shin Y, Xu X, Zhu Y, Li M, Sestan N. TBR1 directly represses *Fezf2* to control the laminar origin and development of the corticospinal tract. *Proc Natl Acad Sci U S A.* 2011; 108:3041–3046. [PubMed: 21285371]
- Heinrich C, Blum R, Gascon S, Masserdotti G, Tripathi P, Sanchez R, Tiedt S, Schroeder T, Gotz M, Berninger B. Directing astroglia from the cerebral cortex into subtype specific functional neurons. *PLoS Biol.* 2010; 8:e1000373. [PubMed: 20502524]
- Heins N, Malatesta P, Cecconi F, Nakafuku M, Tucker KL, Hack MA, Chapouton P, Barde YA, Gotz M. Glial cells generate neurons: the role of the transcription factor Pax6. *Nat Neurosci.* 2002; 5:308–315. [PubMed: 11896398]
- Hevner RF, Shi L, Justice N, Hsueh Y, Sheng M, Smiga S, Bulfone A, Goffinet AM, Campagnoni AT, Rubenstein JL. *Tbr1* regulates differentiation of the preplate and layer 6. *Neuron.* 2001; 29:353–366. [PubMed: 11239428]
- Hochedlinger K, Jaenisch R. Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature.* 2002; 415:1035–1038. [PubMed: 11875572]
- Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, Wang X, Hui L. Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature.* 2011; 475:386–389. [PubMed: 21562492]
- Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell.* 2010; 142:375–386. [PubMed: 20691899]
- Inoue K, Wakao H, Ogonuki N, Miki H, Seino K, Nambu-Wakao R, Noda S, Miyoshi H, Koseki H, Taniguchi M, Ogura A. Generation of cloned mice by direct nuclear transfer from natural killer T cells. *Curr Biol.* 2005; 15:1114–1118. [PubMed: 15964276]
- Izumikawa M, Minoda R, Kawamoto K, Abrashkin KA, Swiderski DL, Dolan DF, Brough DE, Raphael Y. Auditory hair cell replacement and hearing improvement by *Atoh1* gene therapy in deaf mammals. *Nat Med.* 2005; 11:271–276. [PubMed: 15711559]
- Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, Roy M, Quinones-Hinojosa A, VandenBerg S, Alvarez-Buylla A. PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. *Neuron.* 2006; 51:187–199. [PubMed: 16846854]
- Jessberger S, Toni N, Clemenson GD Jr, Ray J, Gage FH. Directed differentiation of hippocampal stem/progenitor cells in the adult brain. *Nat Neurosci.* 2008; 11:888–893. [PubMed: 18587391]
- Joshi PS, Molyneaux BJ, Feng L, Xie X, Macklis JD, Gan L. *Bhlhb5* regulates the postmitotic acquisition of area identities in layers II–V of the developing neocortex. *Neuron.* 2008; 60:258–272. [PubMed: 18957218]
- Kawamoto K, Ishimoto S, Minoda R, Brough DE, Raphael Y. *Math1* gene transfer generates new cochlear hair cells in mature guinea pigs in vivo. *J Neurosci.* 2003; 23:4395–4400. [PubMed: 12805278]
- Kim J, Lengner CJ, Kirak O, Hanna J, Cassady JP, Lodato MA, Wu S, Faddah DA, Steine EJ, Gao Q, Fu D, Dawlaty M, Jaenisch R. Reprogramming of postnatal neurons into induced pluripotent stem cells by defined factors. *Stem Cells.* 2011; 29:992–1000. [PubMed: 21563275]
- Kim J, Su SC, Wang H, Cheng AW, Cassady JP, Lodato MA, Lengner CJ, Chung CY, Dawlaty MM, Tsai LH, Jaenisch R. Functional integration of dopaminergic neurons directly converted from mouse fibroblasts. *Cell Stem Cell.* 2011; 9(5):413–9. [PubMed: 22019014]
- King TJ, Briggs R. Changes in the Nuclei of Differentiating Gastrula Cells, as Demonstrated by Nuclear Transplantation. *Proc Natl Acad Sci U S A.* 1955; 41:321–325. [PubMed: 16589674]
- Klug A. The discovery of zinc fingers and their applications in gene regulation and genome manipulation. *Annu Rev Biochem.* 2010; 79:213–231. [PubMed: 20192761]

- Kohyama J, Kojima T, Takatsuka E, Yamashita T, Namiki J, Hsieh J, Gage FH, Namiyama M, Okano H, Sawamoto K, Nakashima K. Epigenetic regulation of neural cell differentiation plasticity in the adult mammalian brain. *Proc Natl Acad Sci U S A*. 2008; 105:18012–18017. [PubMed: 19004774]
- Kondo T, Raff M. Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science*. 2000; 289:1754–1757. [PubMed: 10976069]
- Kwan KY, Lam MM, Krsnik Z, Kawasaki YI, Lefebvre V, Sestan N. SOX5 postmitotically regulates migration, postmigratory differentiation, and projections of subplate and deep-layer neocortical neurons. *Proc Natl Acad Sci U S A*. 2008; 105(41):16021–6. [PubMed: 18840685]
- Lai T, Jabaudon D, Molyneaux BJ, Azim E, Arlotta P, Menezes JR, Macklis JD. SOX5 controls the sequential generation of distinct corticofugal neuron subtypes. *Neuron*. 2008; 57:232–247. [PubMed: 18215621]
- Leone DP, Srinivasan K, Chen B, Alcamo E, McConnell SK. The determination of projection neuron identity in the developing cerebral cortex. *Curr Opin Neurobiol*. 2008; 18:28–35. [PubMed: 18508260]
- Li J, Ishii T, Feinstein P, Mombaerts P. Odorant receptor gene choice is reset by nuclear transfer from mouse olfactory sensory neurons. *Nature*. 2004; 428:393–399. [PubMed: 15042081]
- Lindwall C, Fothergill T, Richards LJ. Commissure formation in the mammalian forebrain. *Curr Opin Neurobiol*. 2007; 17:3–14. [PubMed: 17275286]
- Lodato S, Rouaux C, Quast KB, Jantrachotechatchawan C, Studer M, Hensch TK, Arlotta P. Excitatory projection neuron subtypes control the distribution of local inhibitory interneurons in the cerebral cortex. *Neuron*. 2011; 69:763–779. [PubMed: 21338885]
- Magavi SS, Leavitt BR, Macklis JD. Induction of neurogenesis in the neocortex of adult mice. *Nature*. 2000; 405:951–955. [PubMed: 10879536]
- Marchetto MC, Brennand KJ, Boyer LF, Gage FH. Induced pluripotent stem cells (iPSCs) and neurological disease modeling: progress and promises. *Hum Mol Genet*. 2011; 20:R109–115. [PubMed: 21828073]
- Maroof AM, Brown K, Shi SH, Studer L, Anderson SA. Prospective isolation of cortical interneuron precursors from mouse embryonic stem cells. *J Neurosci*. 2010; 30:4667–4675. [PubMed: 20357117]
- Mattar P, Langevin LM, Markham K, Klenin N, Shivji S, Zinyk D, Schuurmans C. Basic helix-loop-helix transcription factors cooperate to specify a cortical projection neuron identity. *Mol Cell Biol*. 2008; 28:1456–1469. [PubMed: 18160702]
- McKenna WL, Betancourt J, Larkin KA, Abrams B, Guo C, Rubenstein JL, Chen B. Tbr1 and Fezf2 regulate alternate corticofugal neuronal identities during neocortical development. *J Neurosci*. 2011; 31:549–564. [PubMed: 21228164]
- Mitchell BD, Macklis JD. Large-scale maintenance of dual projections by callosal and frontal cortical projection neurons in adult mice. *J Comp Neurol*. 2005; 482:17–32. [PubMed: 15612019]
- Mitne-Neto M, Machado-Costa M, Marchetto MC, Bengtson MH, Joazeiro CA, Tsuda H, Bellen HJ, Silva HC, Oliveira AS, Lazar M, Muotri AR, Zatz M. Downregulation of VAPB expression in motor neurons derived from induced pluripotent stem cells of ALS8 patients. *Hum Mol Genet*. 2011; 20:3642–3652. [PubMed: 21685205]
- Molyneaux BJ, Arlotta P, Fame RM, MacDonald JL, MacQuarrie KL, Macklis JD. Novel subtype-specific genes identify distinct subpopulations of callosal projection neurons. *J Neurosci*. 2009; 29:12343–12354. [PubMed: 19793993]
- Molyneaux BJ, Arlotta P, Hirata T, Hibi M, Macklis JD. Fez1 is required for the birth and specification of corticospinal motor neurons. *Neuron*. 2005; 47:817–831. [PubMed: 16157277]
- Molyneaux BJ, Arlotta P, Menezes JR, Macklis JD. Neuronal subtype specification in the cerebral cortex. *Nat Rev Neurosci*. 2007; 8:427–437. [PubMed: 17514196]
- Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat Neurosci*. 2007; 10:615–622. [PubMed: 17435755]
- Nefzger CM, Haynes JM, Pouton CW. Directed expression of Gata2, Mash1, and Foxa2 synergize to induce the serotonergic neuron phenotype during in vitro differentiation of embryonic stem cells. *Stem Cells*. 2011; 29:928–939. [PubMed: 21472823]

- Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, Citri A, Sebastiano V, Marro S, Sudhof TC, Wernig M. Induction of human neuronal cells by defined transcription factors. *Nature*. 2011; 476:220–223. [PubMed: 21617644]
- Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ. Disease-specific induced pluripotent stem cells. *Cell*. 2008; 134:877–886. [PubMed: 18691744]
- Peljto M, Dasen JS, Mazzoni EO, Jessell TM, Wichterle H. Functional diversity of ESC-derived motor neuron subtypes revealed through intraspinal transplantation. *Cell Stem Cell*. 2010; 7:355–366. [PubMed: 20804971]
- Pfisterer U, Kirkeby A, Torper O, Wood J, Nelander J, Dufour A, Bjorklund A, Lindvall O, Jakobsson J, Parmar M. Direct conversion of human fibroblasts to dopaminergic neurons. *Proc Natl Acad Sci U S A*. 2011; 108:10343–10348. [PubMed: 21646515]
- Richards LJ, Plachez C, Ren T. Mechanisms regulating the development of the corpus callosum and its agenesis in mouse and human. *Clin Genet*. 2004; 66:276–289. [PubMed: 15355427]
- Rouaux C, Arlotta P. Fezf2 directs the differentiation of corticofugal neurons from striatal progenitors in vivo. *Nat Neurosci*. 2010; 13:1345–1347. [PubMed: 20953195]
- Salero E, Hatten ME. Differentiation of ES cells into cerebellar neurons. *Proc Natl Acad Sci U S A*. 2007; 104:2997–3002. [PubMed: 17293457]
- Sekiya S, Suzuki A. Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. *Nature*. 2011; 475:390–393. [PubMed: 21716291]
- Selvaraj V, Plane JM, Williams AJ, Deng W. Switching cell fate: the remarkable rise of induced pluripotent stem cells and lineage reprogramming technologies. *Trends Biotechnol*. 2010; 28:214–223. [PubMed: 20149468]
- Shen Q, Wang Y, Dimos JT, Fasano CA, Phoenix TN, Lemischka IR, Ivanova NB, Stifani S, Morrissey EE, Temple S. The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells. *Nat Neurosci*. 2006; 9:743–751. [PubMed: 16680166]
- Shou J, Zheng JL, Gao WQ. Robust generation of new hair cells in the mature mammalian inner ear by adenoviral expression of *Hath1*. *Mol Cell Neurosci*. 2003; 23:169–179. [PubMed: 12812751]
- Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, Hargus G, Blak A, Cooper O, Mitalipova M, Isacson O, Jaenisch R. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell*. 2009; 136:964–977. [PubMed: 19269371]
- Son EY, Ichida JK, Wainger BJ, Toma JS, Rafuse VF, Woolf CJ, Eggan K. Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell*. 2011; 9:205–218. [PubMed: 21852222]
- Szabo E, Rampalli S, Risueno RM, Schnerch A, Mitchell R, Fiebig-Comyn A, Levadoux-Martin M, Bhatia M. Direct conversion of human fibroblasts to multilineage blood progenitors. *Nature*. 2010; 468:521–526. [PubMed: 21057492]
- Tada M, Takahama Y, Abe K, Nakatsuji N, Tada T. Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. *Curr Biol*. 2001; 11:1553–1558. [PubMed: 11591326]
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
- Thored P, Arvidsson A, Cacci E, Ahlenius H, Kallur T, Darsalia V, Ekdahl CT, Kokaia Z, Lindvall O. Persistent production of neurons from adult brain stem cells during recovery after stroke. *Stem Cells*. 2006; 24:739–747. [PubMed: 16210404]
- Tursun B, Patel T, Kratsios P, Hobert O. Direct conversion of *C. elegans* germ cells into specific neuron types. *Science*. 2011; 331(6015):304–8.
- Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*. 2010; 463:1035–1041. [PubMed: 20107439]
- Vierbuchen T, Wernig M. Direct lineage conversions: unnatural but useful? *Nat Biotechnol*. 2011; 29:892–907. [PubMed: 21997635]
- Watanabe K, Kamiya D, Nishiyama A, Katayama T, Nozaki S, Kawasaki H, Watanabe Y, Mizuseki K, Sasai Y. Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat Neurosci*. 2005; 8:288–296. [PubMed: 15696161]

- Weintraub H, Tapscott SJ, Davis RL, Thayer MJ, Adam MA, Lassar AB, Miller AD. Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced expression of MyoD. *Proc Natl Acad Sci U S A*. 1989; 86:5434–5438. [PubMed: 2748593]
- Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. *Cell*. 2002; 110:385–397. [PubMed: 12176325]
- Wichterle H, Turnbull DH, Nery S, Fishell G, Alvarez-Buylla A. In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development*. 2001; 128:3759–3771. [PubMed: 11585802]
- Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997; 385:810–813. [PubMed: 9039911]
- Xie H, Ye M, Feng R, Graf T. Stepwise reprogramming of B cells into macrophages. *Cell*. 2004; 117:663–676. [PubMed: 15163413]
- Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y, Lee-Messer C, Dolmetsch RE, Tsien RW, Crabtree GR. MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature*. 2011; 476:228–231. [PubMed: 21753754]
- Zheng JL, Gao WQ. Overexpression of Math1 induces robust production of extra hair cells in postnatal rat inner ears. *Nat Neurosci*. 2000; 3:580–586. [PubMed: 10816314]
- Zhou Q, Melton DA. Extreme makeover: converting one cell into another. *Cell Stem Cell*. 2008; 3:382–388. [PubMed: 18940730]
- Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature*. 2008; 455:627–32. [PubMed: 18754011]

A Reprogramming of Adult Neural Progenitors *in vivo* **B Reprogramming of Glia Cells** **Figure 1. Neural cells can change fate potential and terminal identity**

(A) Adult neural progenitors located in the SVZ and the SGZ can acquire different cell fates in response to extracellular or cell autonomous signaling. (B) Astroglia isolated from the postnatal cortex can reprogram into neurons following overexpression of cell intrinsic cues.

Figure 2. Generation of cortical neurons from pluripotent stem cells

Manipulation of developmental morphogens instructs the differentiation of ES cells into progenitors of telencephalic identity that sequentially give birth to different subtypes of excitatory projection neurons (A, B). Both monolayer (A) and aggregate (B) culture methods enable the generation of projection neurons of different cortical layers, which are generated in a temporal order resembling that of the endogenous projection neuron counterparts. Modified culture conditions enable the generation of neural progenitors resembling those of cortical interneurons (C).

Figure 3.

Directed differentiation of neuron subtypes. Specific combinations of transcription factors instruct the generation of neuron subtypes from ES cells (A) and via the reprogramming of differentiated cell types (B).

Figure 4. Master transcription factors can instruct the birth of new neuronal subtypes from neural progenitors of the developing cortex and striatum, in vivo. Ctx, cortex; LV, lateral ventricle; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence.

Summary of transcription factor “codes” used to direct the differentiation of ES cells or the reprogramming of differentiated cell types into neuron subtypes.

Table 1

Starting Cell Type	Transcription Factors	Induced Neurons	References
ES cells	<i>Lmx1a, Msx1</i>	Dopaminergic neurons	Andersson <i>et al.</i> , 2006
	<i>Gata2, Ascl1, Fox2a</i>	Serotonergic neurons	Nefzger <i>et al.</i> , 2011
Cortical astroglia	<i>Ng2</i>	Glutamatergic neurons	Heinrich <i>et al.</i> , 2010
	<i>Dlx2</i>	GABAergic neurons	Heinrich <i>et al.</i> , 2010
Fibroblasts	<i>Ascl1, Brn2, Myt1l</i>	Mixed neurons	Vierbuchen <i>et al.</i> , 2010
	<i>Ascl1, Brn2, Myt1l, Lmx1a, Fox2a</i>	Dopaminergic neurons	Pfisterer <i>et al.</i> , 2011
	<i>Ascl1, Nurr1, Lmx1a</i>	Dopaminergic neurons	Caiazzo <i>et al.</i> , 2011
	<i>Ascl1, Pitx3, Lmx1a, Nurr1, Foxa2, EN1</i>	Dopaminergic neurons	Kim <i>et al.</i> , 2011
	<i>Ascl1, Brn2, Myt1l, Ng2, Lhx3, Isl1, Hb9</i>	Cholinergic spinal motor neurons	Son <i>et al.</i> , 2011