Development-Inspired Reprogramming of the Mammalian Central Nervous System

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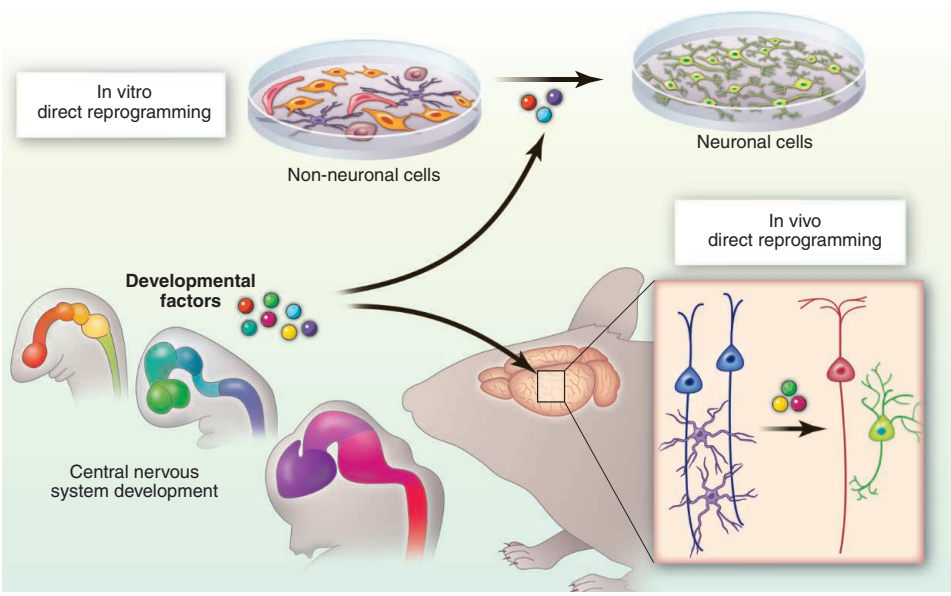
Background: Differentiated cells can be reprogrammed to switch identities from one cell type to another under the direction of powerful transcription factors. In the mammalian central nervous system, this approach has been used experimentally to generate new categories of neuronal cells. The protocols are inspired by what we have learned from normal development, but the applications lie outside of normal embryogenesis. The research is changing how scientists think about regeneration of lost neurons and modeling of neuronal function in the central nervous system. The approaches also allow for new ways to study human neuronal development, a process that cannot be studied in vivo.

Advances: Neurons are a highly specialized cell type, with their ability to transmit electrical signals. Beyond that, though, neurons also specialize into an astonishing diversity of classes. Although reprogramming with known transcription factors is a comparatively blunt tool, researchers have used knowledge of normal neuronal development to identify suites of factors that can convert mouse or human non-neuronal cells into induced neuronal cells showing class-specific features. These protocols have provided a renewable source of neuronal cells for high-throughput studies, which is particularly useful when source tissue is rare or unavailable. One exciting application of lineage reprogramming has been the generation of new neurons in situ by the direct conversion of other cell types already resident within the brain. Astrocytes have been converted into neurons in vivo. Even neurons have been changed from one subtype to another in young animals, indicating that postmitotic neurons may not be as immutable as once thought. These provocative results may foster the development of strategies for neuronal replacement that rely on "code-switching" of neuronal identity on the spot.

Outlook: Direct lineage reprogramming is a nascent but promising field. Although both unspecialized and specialized neuronal cells have already been generated by these methods, we still need more refined understanding of how reprogramming works, how the cellular context constrains reprogramming routes, and what synergistic effects arise with various reprogramming factors. Better-defined criteria are needed to classify neurons obtained by reprogramming and to determine how they differ from their endogenous counterparts. Functional analyses are also necessary to clarify when a new neuron achieves the needed function, even if its other features do not match endogenous neurons. The challenge requires collaborative expertise in stem cell biology, embryology, and fundamental neuroscience. Future ability to reprogram postmitotic neurons in the adult brain will be important for the growth of this field and likely influence the way we think about neuronal stability, regeneration, and function.



Development-inspired signals directly reprogram non-neuronal cells into induced neuronal cells. Pools of transcription factors initially selected based on functional roles during developmental neurogenesis have been reduced to "modules" able to promote the conversion of differentiated cells into neuronal cells. Non-neuronal cells—including astrocytes, fibroblasts, pericytes, and hepatocytes—have been converted into neuronal cells. Young postmitotic neurons and astrocytes have been reprogrammed from one class into another from within the brain.

ARTICLE OUTLINE

ADDITIONAL RESOURCES

T. Vierbuchen, Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035–1041 (2010). DOI:10.1038/nature08797 Medline

C. Rouaux, P. Arlotta, Direct lineage reprogramming of post-mitotic callosal neurons into corticofugal neurons in vivo. *Nat. Cell Biol.* **15**, 214–221 (2013). DOI:10.1038/ncb2660 Medline

N. Heins, Glial cells generate neurons: the role of the transcription factor Pax6. *Nat. Neurosci.* **5**, 308–315 (2002). DOI:10.1038/nn828 Medline

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Development-Inspired Reprogramming of the Mammalian Central Nervous System

Ryoji Amamoto and Paola Arlotta*

In 2012, John Gurdon and Shinya Yamanaka shared the Nobel Prize for the demonstration that the identity of differentiated cells is not irreversibly determined but can be changed back to a pluripotent state under appropriate instructive signals. The principle that differentiated cells can revert to an embryonic state and even be converted directly from one cell type into another not only turns fundamental principles of development on their heads but also has profound implications for regenerative medicine. Replacement of diseased tissue with newly reprogrammed cells and modeling of human disease are concrete opportunities. Here, we focus on the central nervous system to consider whether and how reprogramming of cell identity may affect regeneration and modeling of a system historically considered immutable and hardwired.

In the earliest stages of embryonic development, a handful of uncommitted cells possess the potential to differentiate into any cell type if given the right cues. During the first half of the 20th century, a major question in developmental biology was whether permanent genomic changes accompany differentiation and are in place to enable such pluripotent cells to attain and maintain terminal, cell-type–specific characteristics (*1*). In 1962, Gurdon first published the results of seminal experiments that challenged the commonly held belief that differentiation was unidirectional and irreversible (*2*, *3*). He used somatic cell nuclear transfer, a technique developed a decade earlier by Briggs and King (*4*), to transplant the nucleus of a differentiated tadpole intestinal cell into an irradiated egg and showed that normal adult frogs could develop from these eggs. This groundbreaking work provided the first proof-of-principle demonstration that it is indeed possible to reprogram differentiated cells back to pluripotency. More recently, similar conclusions were extended to mammalian cells (*5*).

These experiments indicate that barriers that lock these cells into their differentiated state do not involve permanent genomic changes and that there are factors in the egg's cytoplasm that enable fully differentiated cells to "reverse development" and regain pluripotency. One set of such factors was revealed when Yamanaka's group successfully converted fibroblasts into pluripotent stem cells with a cocktail of transcription factors. The resulting cells were thereby named induced pluripotent stem cells (iPSCs) (*6*). Along with Gurdon, Yamanaka received the Nobel Prize for this work, and these findings, more than 40 years apart, contributed to establishing that nuclear reprogramming is possible across a spectrum of organisms, including mammals [see (*7*) for an in-depth review on iPSCs and mechanisms of induced pluripotency] (Fig. 1).

Waddington famously likened the process of cellular differentiation and its associated epigenetic changes during development to a marble traveling along a downward slope and ending up in one of many valleys surrounded by impassable hills (*8*). Reverting differentiated cells back to pluripotency through nuclear reprogramming is comparable to forcibly pushing a marble from a valley back to the starting point, also known as the developmental "ground state." However, it has become evident that it is also possible to push the marble from valley to valley in a process that turns one differentiated cell type directly into another without transitioning through a pluripotent cell state. This process has been termed transdifferentiation or direct lineage reprogramming, and various cell types have been directly reprogrammed to acquire a new differentiated identity, across organ systems and in different species (*9*). Direct lineage reprogramming has several attractive features, including low likelihood of tumor formation and increased speed and efficiency of conversion if starting from a related cell type (*10*). Most notably, this approach carries great potential for applicability in vivo, a key advantage when aiming to rebuild cells of a tissue as complex as the central nervous system. Here, we examine direct lineage reprogramming into neurons and discuss the developmental programs that facilitate conversion of cell identity in the central nervous system.

Generation of Development-Inspired Neurons

In *The Greatest Show on Earth*, Dawkins likened embryology to a concerted, graceful flight murmuration of starling birds, each individual starling in the flock following its own local rules, with no overall goal or blueprint for what the flock

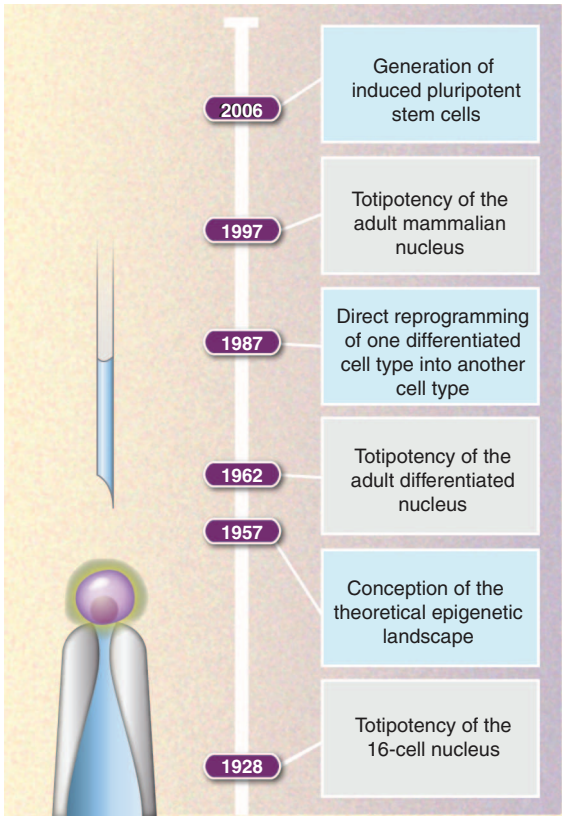

Fig. 1. Historical perspective on nuclear reprogramming. Selected milestone findings from experiments in amphibians first demonstrated that the nucleus of cells at the 16-cell stage (*63*) and differentiated adult cells (*2*) are plastic and capable of generating full organisms. Waddington is credited for theoretically conceiving the epigenetic landscape (*8*). More recent evidence indicates that differentiated mammalian cells are equally able to reprogram to either a pluripotent state (*6*, *64*) or to a new differentiated cell state (*65*).

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should ultimately resemble (*11*). Like starlings in a flock, during development, cells interact according to local guidance cues that converge on the activation of intrinsic programs and ultimately allow for a collection of low-level unspecified units to self-assemble into a high-level configuration. Development is a bottom-up process that requires highly orchestrated and complex signaling mechanisms to produce a whole tissue and organism. Several studies have highlighted the similarities in self-organizing properties between organogenesis in embryos and generation of complex tissues

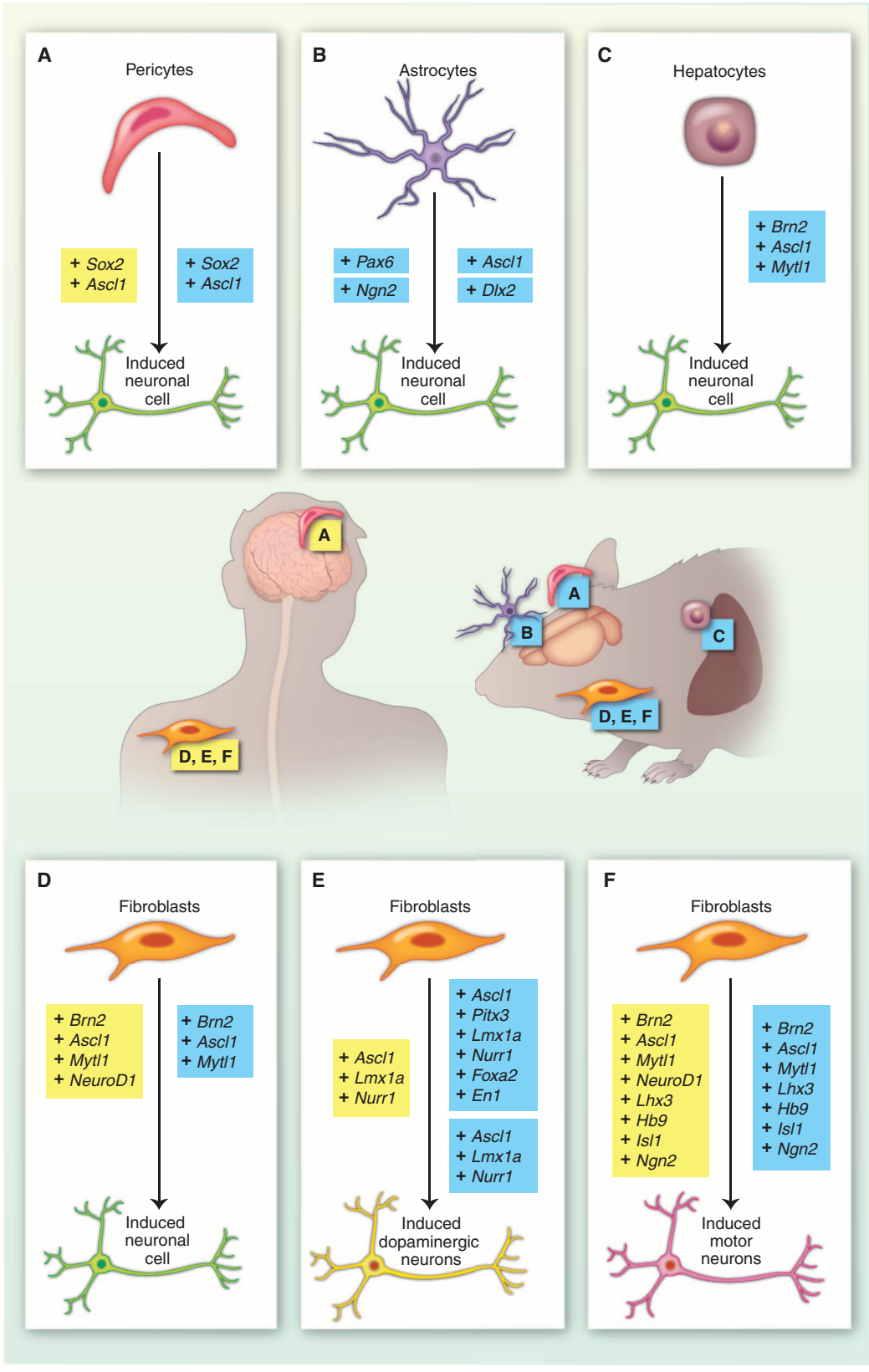

Fig. 2. Direct reprogramming of various cell types into induced neuronal cells in vitro. (**A**) Cultured pericytes (*20*), (**B**) astrocytes (*15*–*18*), (**C**) hepatocytes (*19*), and (**D**) fibroblasts (*22*, *23*) are reprogrammed into induced neurons by defined factors. (**E**) Fibroblasts are reprogrammed into iDA neurons (*38*, *39*) and (**F**) iMNs (*34*). Selected methods for the direct conversion into these neuronal subtypes are illustrated. Blue box, mouse reprogramming factors; yellow box, human reprogramming factors.

from pluripotent stem cells in a dish. In the nervous system, investigators have demonstrated that a three-dimensional (3D) culture of pluripotent stem cells in defined differentiation media could induce self-directed organization of complex tissues ("organoids"), which developed into structures highly similar to the optic cup (*12*, *13*) and the cerebral cortex (*14*). Such an approach may become a useful strategy to generate the complexity of neural tissue beyond individual neurons.

Developmental studies have also identified master transcription factors that alone are able to instruct signature features of neuronal classes as they develop in the central nervous system. This has fueled top-down experiments in which researchers dictate the use of a handful of master regulators to generate specific neurons from other types of cells. Scientists are venturing into daring territories where neuronal cells in their own brand-new category are generated outside the context of embryogenesis following development-inspired protocols. For the first time, protocols to generate predefined neuronal classes from human embryonic stem cells (ESCs), fibroblasts, and other cell types are rapidly expanding, and this progress is likely to have a major impact not only in the clinics but also on our understanding of human neural development, a process that cannot be studied in vivo.

Potency of Developmental Transcription Factor Modules to Generate Neurons

The idea of direct lineage reprogramming with transcription factors is not new, and over the years, overexpression of key transcription factors has been used to successfully convert the identity of various cell types, both in vitro and in vivo (*9*). One of the first indications that intrinsic modulation of transcription factors may be sufficient to generate neurons from non-neuronal cells came from experiments in which *Pax6* was overexpressed in young glial cells isolated from the early postnatal brain (*15*). These results are in line with the known developmental role of *Pax6* in the cerebral cortex, where its loss results in reduced numbers of neurons generated from radial glia cells (*15*). Subsequent studies have demonstrated that other neurogenic factors, namely *Ngn2*, *Ascl1*, and *Dlx2*, can also reprogram early-postnatal astrocytes into neurons in vitro (*16*–*18*). Neurons have been subsequently produced from many differentiated cell types, including hepatocytes (*19*), pericytes (*20*), adult astrocytes (*21*) and, most often, fibroblasts (*22*) (Fig. 2).

Fibroblasts have been extensively used for reprogramming experiments. In a first ground-breaking study, a module of three factors—*Brn2*, *Ascl1*, and *Myt1l*—collectively known as the BAM factors, has been used successfully to reprogram mouse embryonic fibroblasts (MEFs) and tail-tip fibroblasts into induced neuronal cells (iN cells), albeit at a low efficiency (<20%) (*22*). iN cells generated by these methods display neuronal morphology and gene expression, as well as functional electrophysiological properties. Integration of the BAM module with NeuroD1 extended this reprogramming capacity to human fibroblasts (*23*). Each of the BAM factors has demonstrated functions in neuronal development. *Ascl1* in particular is necessary for neuronal differentiation in the ventral telencephalon, neurogenesis in the olfactory epithelium, and development of the sympathetic ganglia (*24*). In line with such a powerful developmental role, *Ascl1* has also been shown to be the primary driver, among the BAM factors, of the MEFs to iN cell conversion (*25*). Notably, the presence of a specific trivalent chromatin signature at *Ascl1* binding sites on the genome appears to alone predict the capability of different cell types to reprogram into iN cells (*25*). How these mechanisms relate to *Ascl1* function during normal development is not known; however, it is interesting that *Ascl1* DNA binding sites in MEFs and ESC-derived neural progenitor cells largely overlap. This data suggests that *Ascl1* may instruct neuronal reprogramming of MEFs by binding to the same genomic loci that it occupies in neural progenitor cells during development, which in turn would make this reprogramming protocol a useful platform to explore principles of developmental neurogenesis.

Fibroblast-to-neuron conversion has enabled subsequent studies aimed at improving the efficiency and precision by which iN cells are generated. A long road lies ahead; however, it seems that manipulation of transcription factors may be combined with extrinsic, development-inspired cues to enhance direct reprogramming. Overexpression of *Ascl1* and *Ngn2* or *Ngn2* alone was combined with small-molecule inhibitors of pathways normally repressed during developmental neurogenesis to generate iN cells at a high efficiency (*26*, *27*). The mechanisms by which patterning signals modify the fate of a differentiated cell remain unexplained. During development, these signals act at early stages of neural induction on progenitors that are in a plastic epigenetic state. Therefore, it is plausible that *Ascl1* and *Ngn2* might synergistically facilitate the process of direct reprogramming of differentiated cells by inducing chromatin remodeling. Such a role in chromatin remodeling by master regulators might result in the establishment of a plastic cellular environment in fibroblasts, which in turn makes them sensitive to extracellular patterning signals. In line with this concept, it was shown that *Pax6* directly interacts with the chromatin remodeler Brg1-containing BAF complex to regulate a transcriptional cross-regulatory network that can reprogram glia into neurons (*28*). Interestingly, ectopic expression of brain-enriched microRNAs that promote the

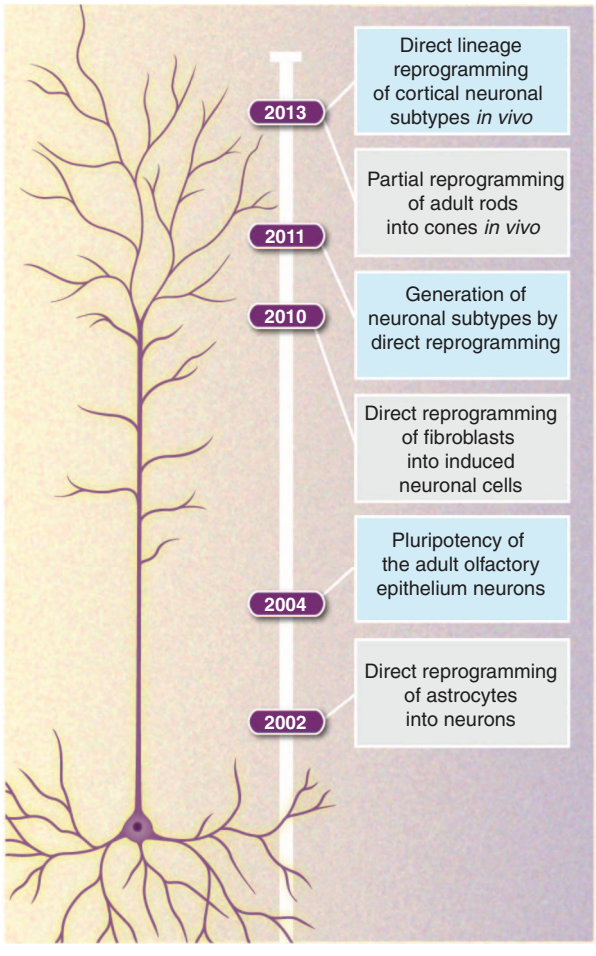

Fig. 3. Historical perspective on neuronal reprogramming and reprogramming into neurons. Selected milestone experiments that collectively supported the view that neurons are amenable to be reprogrammed and that non-neuronal cell types can be reprogrammed into neurons. Neurons could be generated from non-neuronal cells in vitro (*15*), and successive studies have shown that lineage-distant fibroblasts could be used as the starting cells for direct reprogramming into generic neurons (*22*) and specific neuronal subtypes (*34*, *38*). Somatic cell nuclear transfer experiments have determined that adult neurons can undergo nuclear reprogramming (*51*, *52*). Studies have induced neuronal class switch in vivo (*58*–*60*), suggesting that some neurons can undergo lineage reprogramming, although this capacity drastically decreases with neuronal age.

assembly of neuron-specific BAF complexes has successfully converted fibroblasts into neurons, indicating that active alteration of the epigenetic landscape of non-neuronal cells can be sufficient to generate neuron-like cells (*29*).

Inducing Neuronal Diversity

The human brain consists of ~100 billion neurons, which are grouped in a large number of neuronal classes (*30*). Classification of neuronal diversity in the mammalian brain is far from complete, but it is clear that defined differences exist among different classes of neurons and that susceptibility to neurological conditions is strongly neuronal class–specific. Therefore, generation of specific neuronal subtypes for the purpose of cell replacement therapy or in vitro disease modeling becomes important.

Progress has been made to generate a small number of neuronal classes by direct reprogramming. These classes were typically chosen based on contribution to disease and knowledge of the factors controlling their neuron class–specific development. Spinal motor neurons are a notable example. They are susceptible to selective degeneration in pathologies like spinal muscular atrophy and amyotrophic lateral sclerosis (ALS) (*31*), and several of the transcription factors governing spinal motor neuron development are known (*32*). In 2002, a pioneering study provided proof-of-principle evidence that spinal motor neurons can be made from ESCs using developmental patterning signals (*33*). Subsequently, a cocktail of transcription factors made of BAM and motor neuron transcription factors (*Brn2*, *Ascl1*, *Myt1l*, *Lhx3*, *Hb9*, *Isl1*, and *Ngn2*) reprogrammed mouse fibroblasts into induced motor neurons (iMNs) (*34*). Although these iMNs did not show clear anterior-posterior (A-P) motor neuron identities, two different sets of programming factors—NIL (*Ngn2*, *Isl1*, and *Lhx3*) and NIP (*Ngn2*, *Isl1*, and *Phox2a*)—succeeded at differentiating ESCs into two groups of motor neurons with spinal and cranial identity, respectively. Interestingly, by performing chromatin immunoprecipitation sequencing analysis of *Isl1* genome binding in NIL- and NIP-programmed neurons, it was shown that *Isl1* binds to different genomic loci depending on whether *Lhx3* or *Phox2a* are coexpressed, indicating that synergistic binding of programming factors may be crucial to drive the generation of different types of motor neurons (*35*). Indeed, during the MEF to iMN conversion, application of *Lhx3*, *Hb9*, *Isl1*, and *Ngn2* enhanced efficiency, whereas the addition of *Sox1*, *Pax6*, *Nkx6.1*, and *Olig2* decreased the efficiency rather than being neutral. These data suggest that the composition of each transcription factor module is critical to the success of reprogramming and that the most effective modules may be those composed of transcription factors that are expressed synchronically within the desired cell lineage in the embryo. In the future, deeper understanding of the synergism between individual transcription factors will clarify the mechanisms of reprogramming into specific neuronal cells. This knowledge should lead to a more informed choice of transcription factors and better predictions of reprogramming outcome to ultimately replace the current candidate screening approach.

Researchers have also extensively invested in generating midbrain dopaminergic (mDA) neurons because of their degenerative phenotype in patients with Parkinson's disease (*36*). *Nurr1* and *Lmx1a*, transcription factors necessary to generate mDA neurons during development (*37*), were coexpressed with *Ascl1* in mouse and human fibroblasts in an attempt to generate induced dopaminergic (iDA) neurons in vitro (*38*). Since then, several different combinations of factors have also been used to reprogram fibroblasts to a similar neuronal fate (*39*–*41*). Notably, the resulting neuronal cells displayed functional characteristics of endogenous dopaminergic neurons, including

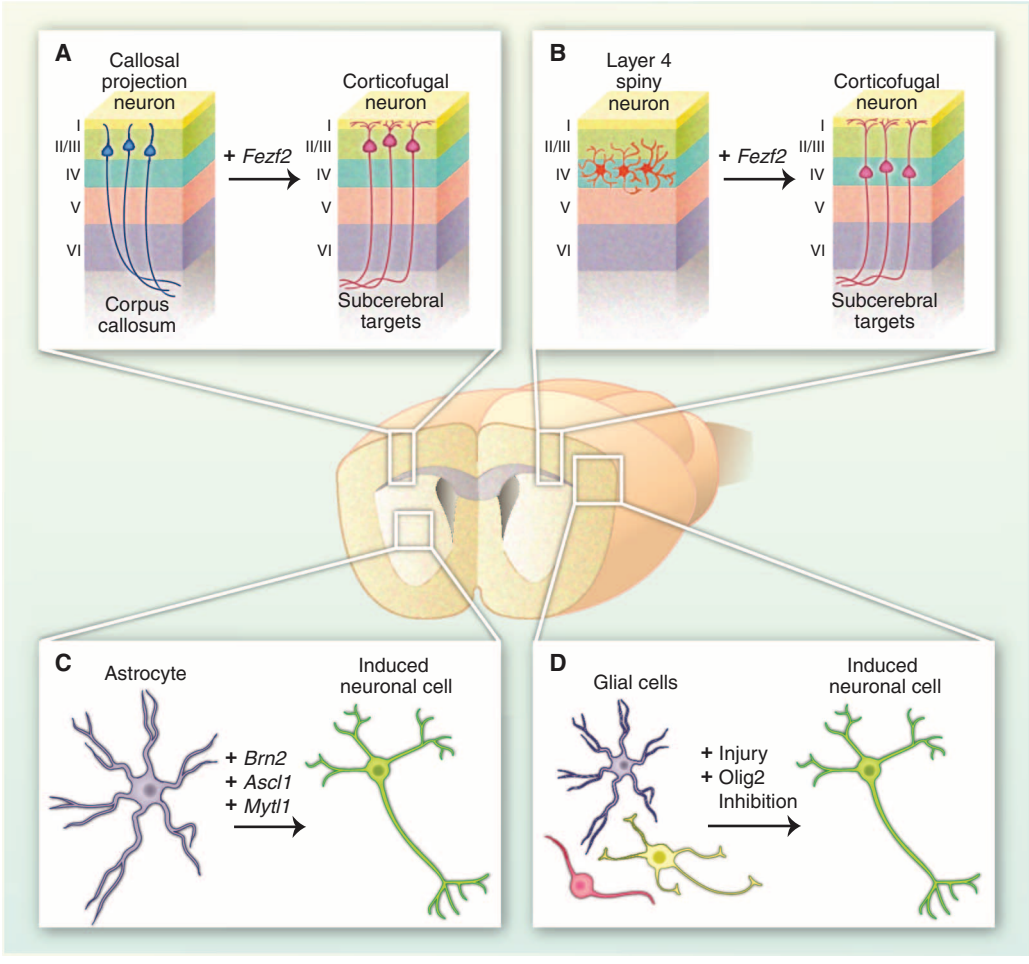

Fig. 4. In vivo direct reprogramming of various cell types into neurons. (**A**) Endogenous callosal projection neurons of early postnatal mice are directly reprogrammed into corticofugal projection neurons (*58*). (**B**) Layer IV spiny neurons are reprogrammed into neurons with electrophysiological properties of corticofugal neurons (*59*). (**C**) Adult striatal astrocytes are reprogrammed into induced neuronal cells by overexpression of the BAM factors (*48*). (**D**) Cortical OLIG2$^+$ glial cells give rise to neuronal cells upon injury, combined with either inhibition of *Olig2* or overexpression of *Pax6* (*47*).

the ability to release dopamine and class-specific electrophysiological properties. Moreover, although additional work will be required to determine the clinical utility of these iDA neurons, encouragingly, transplantation experiments showed integration into mouse striatum and a mild amelioration of Parkinsonian symptoms (*39*). It should be noted, however, that cells generated by these methods did not acquire a distinct midbrain dopaminergic neuron identity. Clear persistence of epigenetic memory of fibroblast origin was observed, and subtype-specific markers of mDA neurons were not present to the extent observed in the endogenous counterparts (*38*, *39*). This result highlights that, despite the necessary role of the reprogramming transcription factors during mDA development, these modules were not sufficient to impart the cells with class-specific traits of mDA neurons. However, the data also suggest that, even if lacking lineage-specific features, iDA neuronal cells may mimic key functional features of mDA neurons and therefore be clinically relevant. The fact that different combinations of factors lead to neuronal cells with similar functional properties may reflect the fundamental nature of such induced traits, which in vivo are shared by most types of dopaminergic neurons, not only mDA neurons. Alternatively, different molecular pathways may direct reprogramming to the same final cellular identity.

Considerable questions and challenges remain; nonetheless, pioneering studies in both direct lineage reprogramming and directed differentiation of defined neuronal classes indicate that production of patient-specific, defined classes of human neurons with clinical value is becoming a reality.

Challenges of Generating Neuronal Diversity

Direct lineage reprogramming is a nascent, but promising, field. Although both unspecialized and specialized neuronal cells have been generated, the extent of reprogramming is largely undefined, and this young field is collectively in need of better-defined criteria to classify the neurons obtained by these approaches. Some broad questions remain unanswered. How similar are reprogrammed neurons to their endogenous counterparts? Or, perhaps more relevantly, how close do these neurons need to be for applications such as disease modeling and cell replacement therapy? Do neurons obtained by direct reprogramming always maintain a memory of their original identity, and how does that influence their functionality? Some of these questions are beginning to be answered, whereas others remain a challenge for the future. We propose that some criteria for classifying reprogrammed neurons may be universal; however, others should take into account the intended use of the neurons.

Guidelines for defining neurons derived from non-neuronal cells in vitro have been suggested (*10*). The criteria include acquisition of neuronal morphology, expression of pan-neuronal markers, and functional synaptic inputs and outputs (*10*). We agree that these criteria are well suited to broadly define iN cells as they test the acquisition of fundamental, basic traits that distinguish all neurons from fibroblasts and other non-neuronal cells. iN cells with these properties are valuable cells for a variety of applications, including disease modeling and therapeutic screening. For example, direct reprogramming of fibroblasts into "generic" populations of induced neuronal cells through overexpression of the BAM factors may be sufficient to model pathologies that affect a broad spectrum of neurons, with limited class specificity. In one example, fibroblasts isolated from human patients with a familial form of Alzheimer's disease have been used to generate iN cells, which in turn could model some features of the disease, including modified amyloid precursor protein processing (*42*).

However, within the brain and spinal cord, neurons differ greatly from each other, and numerous subtypes can be recognized. Neuronal classification is based on many distinguishing features, which include global molecular identity, morphology, ultrastructural traits, electrophysiological properties, and connectivity. All of these traits collectively (and not in isolation) allow class distinction. Some neuronal subtype-specific traits—most prominently morphology, target-specific connectivity, and the ability to functionally integrate into circuitry—can only be examined in vivo and remain largely untested for reprogrammed neurons. Transplantation into developing or early postnatal brain, when the corresponding endogenous neuronal classes are acquiring defining traits, should allow for a clean assessment of the true potential of reprogrammed neurons to acquire class-specific features and developmentally "behave" like their endogenous counterparts. In particular, axonal connectivity to specific targets is a key, defining feature of many classes of neurons in the central nervous system and a prime predictor of functional integration. The trajectory of axons of reprogrammed neurons could easily be determined in vivo using genetic labels and standard retrograde tracing experiments. Reporter labeling of reprogrammed neurons would also allow for morphological measurements, including the establishment of stereotyped dendritic trees, which differ in shape, size, complexity, and position among different neuronal classes. Finally, modulation and recording of neuronal activity, facilitated by the use of optogenetic tools, could clarify the functional contribution of reprogrammed cells within a network of neurons.

Comparative analysis of the transcriptomes among reprogrammed neuron classes, endogenous neurons of the same class, and the starting non-neuronal cells (e.g., fibroblasts) has been initiated for iDA neurons and iMNs (*34*, *38*). Transcriptional profiling and hierarchical clustering of fibroblast-derived spinal motor neurons showed that they cluster more closely to endogenous motor neurons than fibroblasts or ESCs (*34*). Similarly, iDA neurons resemble to some extent endogenous dopaminergic neurons (*38*). However, notable differences exist. The iDA neurons obtained in vitro exhibited expression profiles that were distinguishable from those of endogenous mDAs (*38*, *39*). Furthermore, expression of several fibroblast genes was retained after reprogramming into dopaminergic neurons. It is unclear how this memory affects the functionality of iN cells, and mechanistic studies are necessary to understand how fibroblast identity is maintained in order to inform the development of more complete reprogramming strategies. It is likely that, within a dish, neurons have achieved distinct levels of reprogramming. Induced neuronal cells have thus far been profiled as populations, which may have led to underestimating differences and similarities present at the single-neuron level. Major technological progress now enables single-cell RNA sequencing (*43*), and these methods are being rapidly interfaced with high-throughput platforms to allow the automatic sequencing of large numbers of individual cells. Single-cell molecular profiling of reprogrammed neurons should in the near future help define the molecular underpinnings that drive the acquisition of neuronal subtype-specific identity by these methods and allow the selection of better reprogrammed cells for downstream applications.

For the first time, neuroscientists and stem cell biologists alike are faced with the notable challenge of classifying an ever-growing number of "man-made," reprogrammed neurons that did not exist a mere 5 years ago. It is possible that incompletely reprogrammed neurons of a specific class might be functionally equivalent to their endogenous neuronal subtypes. However, when aiming to generate neurons for goals as ambitious as neurological disease modeling and circuit replacement, we propose that a useful starting point would be to determine how iN cells relate to "nature-made" neurons and, further, that the endogenous neuronal complexity should be respected and emulated to a feasible extent.

In Vivo Neuronal Reprogramming

In organs and tissues such as the blood, the heart, and the pancreas, it is possible to directly reprogram one cell type into another in vivo by overexpression of defined factors (*44*–*46*). However, researchers have questioned whether all cells are endowed with such plasticity.

Resident non-neuronal cells of the central nervous system have been reprogrammed into induced neuronal cells in vivo. Due to the ability to divide, abundance in the brain, and proximity in lineage distance, astrocytes have been the ideal starting candidate cell type to generate new neurons. Early work showed that, in the adult mouse neocortex, $OLIG2^+$ cells (which include oligodendrocytes, their progenitors, and astrocytes) could give rise to neurons upon injury, combined with either overexpression of *Pax6* or inhibition of *Olig2* (*47*). Since then, glial fibrillary acidic protein–positive ($GFAP^+$) cells could be turned into morphologically identifiable neurons in the adult striatum upon expression of the BAM cocktail

(48), and overexpression of *Sox2* was sufficient to reprogram adult striatal astrocytes into neuroblasts, which in turn were able to form neurons (21). These studies illustrate the feasibility of directly reprogramming non-neuronal cells into neurons in situ, which may become a therapeutic option in the future.

In addition to non-neuronal cells, an important question in the field has been whether neurons themselves could be turned from one class into another and whether this could become an optimal strategy to generate neuronal subtypes susceptible to disease with enhanced precision. The plasticity of neurons has been the subject of much debate. Once generated, neurons become postmitotic and do not change their identity for the life span of the organism, suggesting that neurons cannot be converted into other cell types. Much effort has been directed to generate live mice using somatic cell nuclear transfer from primary neurons, with varying results. Some studies led to conclude that postmitotic neurons may indeed have largely lost their developmental pluripotency (49, 50). However, a first sign that neurons may be capable of reprogramming their identity came from pioneering experiments in which a live mouse was obtained from the nucleus of an olfactory epithelium neuron (51, 52). Using a similar approach, viable mice were subsequently produced by somatic cell nuclear transfer using the nuclei of postmitotic neurons from the cerebral cortex of juvenile mice (53). These experiments provided a proof-of-principle demonstration that the nucleus of at least some classes of neurons is plastic and that no irreversible genetic or epigenetic changes have taken place that preclude the acquisition of a new cellular identity. For a historical perspective on neuronal reprogramming and on reprogramming into neurons, see Fig. 3.

If neurons retain the capability to reprogram their identity, could neurons then be converted from one class into another within the central nervous system? This field is only emerging, but some studies have begun to explore this strategy to build new neurons and circuits, in vivo (Fig. 4). Similar to the work on reprogramming non-neuronal cells, master selector genes able to drive the acquisition of class-specific neuronal identity can be powerful tools to instruct neuronal class switch in vivo. In a first application to neurons of the cerebral cortex, we have used the transcription factor *Fezf2*, a master gene capable of instructing multiple features of identity of corticospinal motor neurons (CSMN), to investigate whether reprogramming other cortical neurons to become CSMN is possible within the brain. *Fezf2* is developmentally required for the birth of CSMN, and in its absence all CSMN fail to generate (54–56). In agreement, *Fezf2* alone can cell-autonomously instruct the acquisition of CSMN-specific features when expressed in a permissive cellular context in vivo (57). We have demonstrated that overexpression of *Fezf2* is sufficient to directly reprogram embryonic and early postnatal callosal projection neurons (CPN), a class of cortical neurons making interhemispheric connections via the corpus callosum, into corticofugal neurons, including CSMN. Reprogrammed callosal neurons acquire molecular properties of CSMN and change their axonal connectivity from interhemispheric intracortical projections to corticofugal projections directed below the cortex, including to the spinal cord (58). In line with these findings, endogenous electrophysiological features of CSMN were induced when *Fezf2* was force-expressed in layer IV stellate interneurons of the cortex (59).

What is notable about callosal neuron reprogramming is that postmitotic neuronal identity could be changed at postmitotic day 3 (P3) and P6 when callosal neurons have already reached their layer location, have connected to their targets in contralateral cortex, and have acquired defined, class-specific features. The data indicate that young neurons retain some ability to change and that the postmitotic nature of the cell does not per se preclude reprogramming. However, neuronal plasticity progressively declines, and reprogramming capabilities in response to *Fezf2* have been exhausted by P21 (58).

These results indicate that mechanisms are in place postmitotically to progressively restrict neuronal fate potential and reprogramming capabilities as neurons age. Molecular studies are now needed to extend the critical period of postmitotic neuron reprogramming to the mature brain, and this will be an important challenge for the growth of this field. To this end, one long-term goal will be to investigate the mechanisms that contribute in the first place to maintaining neuron class–specific identity during brain development and maturation. Such studies will help understand normal mechanisms used by neurons to refrain from changing but also will inform strategies to facilitate direct reprogramming of neuronal identity in the adult. It is likely that, beyond fate specifying transcription factors, "opening" the permissive temporal window of neuronal reprogramming will require additional manipulations. Epigenetic status, one of the major barriers to reprogramming somatic cells, may be modulated by chemical and genetic approaches. These manipulations may increase the plasticity of the target neurons and enable fate-specifying transcription factors to reprogram neuronal identity at later developmental stages.

In support for the existence of epigenetic blocks to neuronal reprogramming in vivo, work in the retina demonstrates that failure of reprogramming of adult rods into cone photoreceptors may be at least partly due to DNA methylation at key, class-specific loci (60). This study is notable, as the authors were able to induce several molecular, ultrastructural, and physiological properties of cones upon conditional removal of the rod-specifying transcription factor *Nrl* from adult rods. Although the cells retained some rod-specific traits, the work suggests that partial conversion of diseased rods into cones may be feasible in adults.

There are advantages to neuron-to-neuron conversion that make this approach worth pursuing. Closely related neurons could be chosen that share some pan-neuronal features, most prominently the use of the same neurotransmitter, ability to send long-distance axons, similar morphology, and location into circuit. This method is likely to facilitate the generation of highly specialized neurons, ease their integration into circuitry, and possibly reduce off-target connectivity. Because neurons do not divide, this process is also unlikely to become tumorigenic. It is generally accepted that a small percentage of new neurons of a given class can be sufficient to regain some functionality without visible effects on the behavior mediated by the starting neuronal population. Should in vivo reprogramming of adult neurons become a reality, this approach could be used to generate neurons that are affected by disease by the conversion, in situ, of a small percentage of neighboring neurons that are naturally resistant to the same pathology.

Looking into the Future of Induced Neuronal Cells

This is an exciting time for the field of cellular reprogramming in the central nervous system. Generation of neurons by direct reprogramming holds great promise for both cell replacement therapy and disease modeling. However, challenges remain. This field is just beginning to understand how differentiated cells are turned into neuronal cells. Mechanistic studies will be of fundamental importance to be able to predict the effect of different transcription factors and of the starting cellular context on the success of direct reprogramming into neuronal cells. This knowledge should help the field move away from current strategies that screen multiple-factor permutations. In addition, better criteria to classify induced neuronal cells are needed, especially when aiming to obtain specific classes of neurons. We propose that as more "realistic" and complex replicas of endogenous central neurons and tissue are generated, successful reprogramming in the central nervous system will require that expertise in reprogramming and embryology meet those in fundamental neuroscience to drive the choice of neurons to generate and to classify and functionally test the final neuronal products.

Given the speed of reprogramming and the ease of access to patient-derived fibroblasts, direct reprogramming of neurons is a manageable, alternative approach to generating neurons by directed differentiation of human iPSCs. We foresee that comparative characterization of neuronal cells obtained by these two methods will soon define similarities and differences between these sources. Newer approaches to achieve genome editing have also emerged over the past few years that will enable large-scale genome modification of different cell types to insert (or repair) disease-associated mutations (61, 62). These state-of-the-art technologies allow for scalable, highly specific insertion of somatic mutations in the

genome and could ultimately add a new dimension to modeling and understanding human disease using iN cells. For neuroscientists in particular, generation of an unlimited supply of human neuronal cells (neurons are notoriously difficult to obtain from patients and cannot be expanded in culture) was merely an ambition, far from reality until this opportunity emerged a few years ago. It is now possible to design clinical trials in the dish that may revolutionize preclinical screening of therapeutic compounds by testing them in a high-throughput manner on human neuronal cells, in parallel to mouse modeling.

One application of direct lineage reprogramming in the nervous system is the generation of new neurons in situ by the direct conversion of cells that are resident within the central nervous system. Given the highly specialized nature of neurons and the complexity of the connections they make and receive, it may be advantageous to generate new neurons by direct conversion of other classes of neurons. The starting neurons may have already acquired basic pan-neuronal features that are functionally critical. However, a major challenge will be to identify and overcome barriers that currently hamper reprogramming of neurons in the adult nervous system. The mechanisms that maintain neuronal class–specific identity throughout the life span of an organism are largely unknown. Although it is speculative at this stage, we propose that neurons might maintain their identity using unique mechanisms. It is intriguing that the closure of the temporal window of nuclear plasticity of neurons loosely corresponds to their integration into circuit. This suggests the provocative possibility that elements of neuronal identity are sustained by the network in which each neuron integrates.

It remains to be determined whether and how local (or even long-distance) circuitry would react in response to a change in neuronal class–specific identity induced by reprogramming. Should adult neuronal reprogramming become a reality, this in vivo application could be informative in elucidating aspects of circuit plasticity and understanding some of the rules that shape circuit maintenance in vivo. With the knowledge of development and cell identity of all neurons present in the nervous system of *Caenorhabditis elegans*, this organism may be a perfect first model system to determine whether circuit remodeling accompanies the process of direct reprogramming in vivo. Investigation of invertebrate organisms that are endowed with natural reprogramming capabilities will also facilitate understanding of reprogramming in mammals.

As evidenced by the presentation of the Nobel Prize in 2012, nuclear reprogramming is an exciting, rapidly growing field with the potential to transform basic science and clinical research. Direct reprogramming from one cell into another may be particularly advantageous for the central nervous system because of its in vivo applicability, in addition to neuronal production in the dish. Although the progress in the field has generated as many unresolved questions as answers, direct reprogramming has shown promise to revolutionize the way the field thinks about neuronal stability and repair.

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Development-Inspired Reprogramming of the Mammalian Central Nervous System

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Repairing the Brain

Research with stem cells and reprogramming of cellular fates is leading to improved understanding of neurodevelopmental events, as well as opening doors to possible cellular replacement therapies. **Amamoto and Arlotta** (10.1126/science.1239882) review recent progress in this field and highlight the discoveries made and the remaining challenges as stem-cell technologies are applied to cells of the central nervous system.