From stem cells to neural networks: recent advances and perspectives for neurodevelopmental disorders

NICOLAS GASPARD | PIERRE VANDERHAEGHEN

Université Libre de Bruxelles, Institute for Interdisciplinary Research, Brussels, Belgium.

Correspondence to Pierre Vanderhaeghen at the Université Libre de Bruxelles, Institute for Interdisciplinary Research, Campus Erasme, 808 route de Lennik, B-1070 Brussels, Belgium. E-mail: pierre.vanderhaeghen@ulb.ac.be

PUBLICATION DATA
Accepted for publication 12th August 2010.
Published online 18 November 2010.

ABBREVIATIONS

ESC	Embryonic stem cell
iPS	Induced pluripotent stem cell
SMA	Spinal muscular atrophy

Embryonic or induced pluripotent stem cells, available in mouse and human, have emerged as powerful tools to address complex questions in neurobiology. This review focuses on major advances relating to brain development and developmental disorders. Stem cells can differentiate into many different neuronal subtypes using in vitro models mimicking relevant in vivo developmental processes, and the underlying molecular and cellular mechanisms. Disease–specific human embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells are now available and allow for the study in vitro of the pathophysiology of degenerative and neurodevelopmental hereditary and sporadic disorders, including in the near future those of the human cortex. Finally, some recent studies have shown that stem cell-derived neural progenitors and neurons could help to rebuild damaged brain circuitry, opening the possibility of cell therapy.

Understanding the mechanisms of brain development, especially in humans, remains one of the most fascinating yet challenging questions in neurobiology. Neurodevelopmental disorders are among the most varied and complex conditions, in terms of both pathophysiological mechanisms and possible treatments.

In the last 10 years, embryonic stem cells (ESCs) have emerged as a powerful tool in neurobiology, helping address longstanding questions in an entirely novel way.[1] More recently, the availability of human ESCs and induced pluripotent stem (iPS) cells, reprogrammed from adult cells, has provided a unique opportunity to investigate the mechanisms of human brain development and its disorders. Stem cells also hold great promises for brain repair, whether following direct damage, such as stroke or trauma, or from a developmental or degenerative disease.

This review focuses on recent advances in the use of stem cells as a basis for studying brain development and developmental disorders and discusses some perspectives for cell therapy.

NEURAL DEVELOPMENT FROM PLURIPOTENT STEM CELLS IN VITRO

Embryonic stem cells harbour the unique features of pluripotency, the ability to give rise to any cell type of the organism, and self-renewability. The mechanisms regulating these features as well as the subsequent steps of neural induction, differentiation and specification have been widely studied.[2–4]

This knowledge has been transposed in vitro in protocols designed to generate neural cell types of interest from ESCs (Fig. 1). Thus, neurons of different parts of the neural tube were successfully generated, including spinal motoneurons,[5] midbrain dopaminergic neurons,[6] spinal cord interneurons,[7] Purkinje and granule cells of the cerebellum,[8,9] hypothalamus[10] and, finally, cortical pyramidal neurons.[11–13]

In the case of spinal cord motoneurons,[5] the identity of the ESC-derived motor neurons was assessed by demonstrating the expression of specific molecular markers, electrophysiological properties and, once grafted, the ability to integrate into the spinal cord, extend peripheral axons and form neuromuscular junctions. To achieve this differentiation, ESCs were exposed to two extracellular cues, Sonic Hedgehog and retinoic acid, known to play a major role in the specification of spinal motor neurons in vivo[14] (Fig. 1a,b). This study thus remarkably demonstrated that neural differentiation from ESCs could be directed using exogenous molecules identified in vivo and could lead to a high level of cell specification, including the ability to grow axons and make synapses.

Conversely, the generation of neural progenitors and neurons of the most anterior part of the neural tube, such as cortical pyramidal or hypothalamic neurons, could be achieved by minimizing the presence of extrinsic cues in the differentiation medium, unveiling an intrinsic trend of ESCs to spontaneously differentiate into anterior neural tissue.[10,12] As serum-containing differentiation medium inhibits anterior

neural specification, generation of this cell lineage may also rely on additional antagonists of posteriorizing signals.[11,15] These studies confirmed the currently accepted 'default model' in which neural cells of anterior identity represent the default fate of differentiating embryonic cells.[3,16]

Cortical pyramidal neurons derived from ESCs have been thoroughly compared with their in vivo counterparts: accordingly, they are glutamatergic, display a typical pyramidal morphology, and express typical molecular markers of the six cortical layers. Finally, and perhaps most importantly, they send specific axonal projections to typical cortical targets when grafted in vivo.[11,12]

Somewhat surprisingly, complex temporal and spatial features, such as a specific layer and area identity, could be reproduced in vitro (Fig. 2). During corticogenesis, neurons of the six layers are generated in a specific temporal sequence by the cortical progenitors: deep layer neurons are generated first and then the more superficial neurons are added in an 'inside-out' fashion. This sequence seems to be directed by a cell-intrinsic progressive change in the competence of cortical progenitors. Interestingly, this key feature of corticogenesis is retained by ESC-derived cortical progenitors in vitro[12] and can be efficiently manipulated to enrich the culture in specific neuronal subtypes[11] (Fig. 2a). In addition, using a system where ESCs are cultured as bowls of cells differentiating into cortical-like progenitors,[11] they develop into neural structures that adopt a striking polarized cellular organization, with neural progenitors occupying deeper layers of the bowls, and neurons accumulating at their periphery. This follows an organization highly reminiscent of a nascent cortical primordium (Fig. 2b). These data constitute a first proof that a brain-like structure can emerge as a self-organizing cytoarchitecture

What this paper adds

- A comprehensive review of the actual and the possible applications of pluripotent stem cells in the fields of developmental neurobiology and neurodevelopmental disorders.

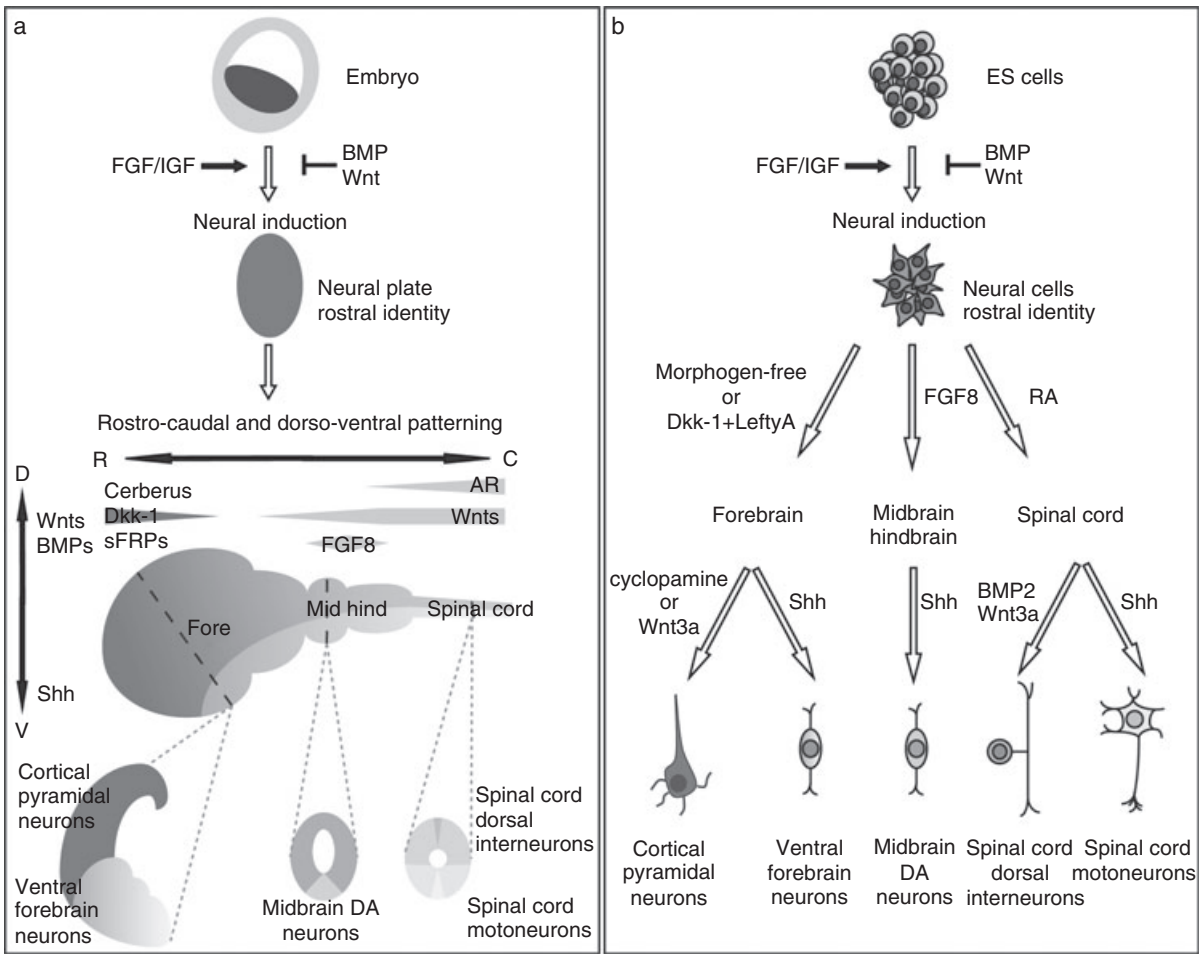

Figure 1: Neural induction and regional patterning in embryonic stem cell neurogenesis. (a) Schematic representation of the mechanisms of neural induction and early patterning of the neural plate/tube. Neural induction is regulated by the coordinated actions of bone morphogenetic proteins (BMP), Wnt and fibroblast growth factors/insulin-like growth factors (FGF/IGF) signalling pathways. The neural plate, initially of anterior identity, is then subsequently patterned by extrinsic morphogens along the rostro-caudal and the dorso-ventral axes into discrete domains. (b) Embryonic stem (ES) cell neural induction and embryonic stem-derived neural progenitor specification follows the same cues as in vivo to give rise to well-defined neuronal populations.

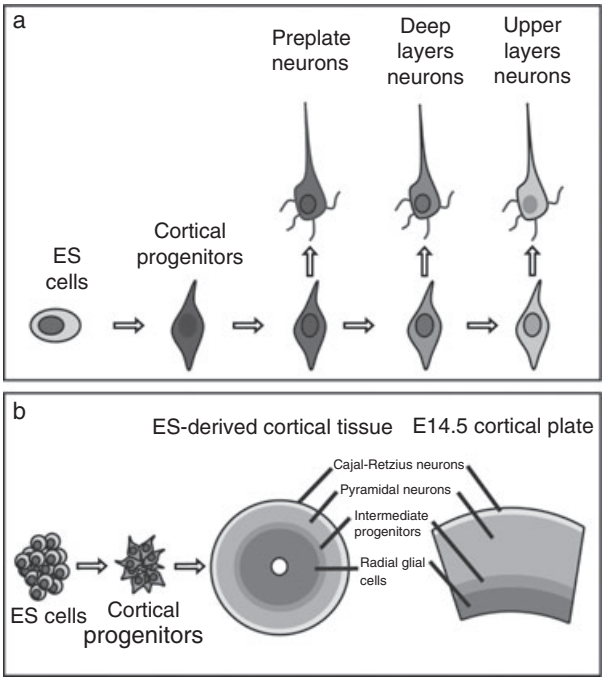

Figure 2: Temporal and spatial patterning during embryonic stem cell neurogenesis. (a) Embryonic stem (ES)-derived cortical progenitors undergo a sequential shift in competence and successively generate different subtypes of neurons. (b) Schematic representation of polarized laminar structures developed embryonic stem-derived cortical progenitors in floating aggregates and reminiscent of the cytoarchitecture of the developing cortex.

in vitro, and provide a promising system to decipher some of the underlying mechanisms of cortical specification, generation, and patterning.

While in vitro systems of corticogenesis thus display remarkable similarities with in vivo developmental processes, they differ in several important aspects. Even if neurons with all six layer identities are specified in vitro, one never sees the formation of a six-layered organization. Also, there is an under-representation of upper-layer neurons, suggesting that extrinsic factors are lacking in vitro to generate a full cortex. Interestingly, the ability to form a six-layered structure is a unique property of the neocortex, the part of the cortex that is the most recent in evolution, suggesting that in vitro cortical neurogenesis may display most similarities to a primitive pathway of corticogenesis corresponding to an ancestral form of cortex (such as the paleo- and archicortex). This hypothesis is in part supported by the observation that, despite their relatively broad laminar representation, ESC-derived pyramidal neurons seem to belong to very specific cortical areas, mainly visual and limbic, which correspond to the phylogenetically oldest cortical areas.[17]

Despite these shortcomings, models of corticogenesis from ESCs represent an invaluable tool to study the molecular mechanisms underlying cortical development. Indeed, in addition to their pluripotency and self-renewability, ESCs are easily accessible to genetic and biochemical manipulation, allowing the design of high-throughput genetic or small molecules functional screens, that would otherwise be very difficult to achieve in vivo.

Furthermore, the transposition of these approaches to human ESCs, as recently described with some adjustments,[11] together with the development of new transgenesis tools,[18,19] now offer the exciting opportunity to study human brain development, compare it with lower mammals and begin to unravel the specificity of human neurodevelopment.

There are, however, limitations. The availability of human blastocysts from which ESCs are derived is tightly linked to ethical and logistical considerations that differ widely between countries. Biological uncertainties also remain regarding the variability between the human ESC lines available. Their transcriptional signature in the undifferentiated state and their ability to differentiate into neural tissue vary significantly, probably reflecting the heterogeneity in the way they were generated.[20,21] This inconsistency needs to be taken into account in subsequent studies.

STEM CELL-BASED MODELS OF BRAIN DISEASE

The availability of human ESCs harbouring mutations for monogenic disorders opens the door to the development of in vitro models of such diseases. Cell lines have been derived for Duchenne and Becker muscular dystrophies, Huntington disease,[22] fragile-X syndrome,[23] adrenoleukodystrophy and neurofibromatosis-1.[24] As these cells are usually obtained from embryos discarded after pre-implantation genetic diagnosis, they raise ethical questions which may limit their widespread use.

Alternative solutions exist and an obvious one would be to genetically modify human ESCs to harbour the desired mutation. Although new transgenesis methods are being developed,[18,19] it remains a major technical challenge. Furthermore, the genetic background on which these mutations will act may be of importance and modified human ESCs might never develop a relevant phenotype, akin to the incomplete penetrance and variable expressivity of the phenotype of a genetic disease observed among different patients sharing the same mutation. Finally, genetic methods to develop models for polygenic diseases have yet to be discovered.

For all these reasons, iPS cells might represent today's best alternative. Induced pluripotent stem cells are adult or embryonic somatic cells that have been genetically reprogrammed to a stem cell state close to ESCs. Their reprogrammation has been achieved with several cell types, for example, from an adult or embryonic mouse,[25] and also from human cells.[26] Initial methods to achieve this relied on integrating retroviruses, which carry a risk of insertional mutagenesis. Newer techniques have been developed,[27–31] however, these are in need of improvement to become as efficient as retroviral vectors.[32]

In addition to wild-type iPS cells, several disease-specific human iPS cell lines have been generated from patients suffering from various disorders, such as, amyotrophic lateral sclerosis,[33] spinal muscular atrophy (SMA),[34] familial dysautonomia,[35] Parkinson disease,[36,37] Huntington disease,[37] Down syndrome,[37] and Rett syndrome.[27] With the exception

of the dysautonomia and SMA cell lines, little is known about the effect of the carried mutation in the relevant differentiated cell types.

Cholinergic motor neurons derived from the SMA iPS cell line exhibited a selective survival deficit which could be reversed by small chemical compounds known to raise the level of the protein encoded by the gene mutated in SMA.[34] A study of the dysautonomia cell line by Lee et al.[35] revealed multiple molecular cellular defects which could be partially corrected by a plant hormone known to restore the level of the normal splice form of the inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP), the gene responsible for the disease. These two examples highlight the opportunities offered by iPS cells for disease modelling and in vitro drug discovery.

Induced pluripotent stem cells also appear to be well-suited to the modelling of diseases whose genetic basis is not easily reproduced in animal models. Examples of such diseases in child neurology are numerous and include monogenic disorders in which the gene is not present or may be structurally different in the mouse, such as some cases of non-syndromic intellectual disability, as well as microdeletion and microduplication syndromes.

Here there are also limitations, and differentiation of iPS cells into various neuronal cell types has still to be documented. So far, only differentiation into spinal motor neurons,[33,34,38] dopaminergic neurons[36] and neural crests[35] have been demonstrated. Epigenetic modifications observed in stem cells and especially after reprogrammation to the iPS state might interfere with the development of genetic diseases linked to gene imprinting, such as the Angelman and Prader-Willi syndromes. Complex diseases caused by the interaction of multiple genetic defects with poorly determined environmental factors will be difficult to model. The ability to model disorders due to compromised intercellular connectivity and signalling depends on the ability to precisely reproduce cellular networks in vitro. This could also benefit from the generation of mouse or human chimeric experiments, grafting human iPS or embryonic stem-derived neurons into the mouse brain.

Once these limitations are overcome, application of iPS technology to neurodevelopmental disorders should be productive. For instance, Williams syndrome is a multisystemic neurodevelopmental disorder caused by a hemizygous deletion on chromosome 7, spanning 28 genes.[39] Hemizygosity in several of these genes, including genes coding for transcription factors or proteins involved in cell migration, neurite outgrowth and synapse formation, has been implicated in the typical cognitive profile of the affected children.[40] However, the molecular mechanisms involved remain obscure and available animal models do not fully capture the complexity of this genomic disorder.[41] Induced pluripotent stem cells are a promising approach to faithfully replicate the genetic defect in an experimental setting. Once differentiated into cortical progenitors and neurons, they could be used to study alterations in gene expression, neurite formation and synaptic activity. Furthermore, once abnormalities are revealed in any of those processes, the simplicity of the in vitro setting could allow an efficient screen of chemical or genetic treatments able to restore normal cellular function that could be translated to clinical trials.

Whereas iPS generation from somatic cell is considered as dedifferentiation, transdifferentiation refers to the reprogramming of an adult somatic cell type into another. This has been recently achieved by reprogramming adult skin fibroblasts into neurons.[42] Although the identity of these neurons needs to be thoroughly characterized, this approach might alleviate the problem of neural differentiation observed with some iPS cell lines.[43]

STEM CELL-BASED THERAPY

It has long been considered that damaged neural connections in the central nervous system could not be easily regrown. This notion was recently challenged in a series of works culminating in a promising study by Gaillard et al.[44] In this study, the authors reported that embryonic cortical neural progenitors and neurons grafted in the lesioned motor cortex of adult mice could extend axons along cortico-cortical and cortico-subcortical pathways, including down to the spinal cord. Remarkably, the growing axons have the ability to make connections with specific targets and no major overgrowth was observed, a critical issue in neural repair. Also, the reconstruction of the damaged networks resulted in a significant functional improvement.

The next step is to extend these findings to ESCs and iPS cells. Conclusive evidence is still lacking but preliminary data are available. In two recent studies of cortical development from ESCs, grafting experiments were used to demonstrate cortical neuronal identity.[11,12] Both studies reported extensive but selective axonal growth from the graft to various cortical targets. Although the grafts were performed in utero or in neonates, they suggest that ESC-derived grafts might be used for brain repair in adult damaged brains. Induced pluripotent stem-derived dopaminergic neurons have been recently shown to improve behaviour in a rat model of Parkinson disease upon transplantation into the adult brain.[45]

CONCLUSIONS

The availability of ESC-based models offers an entirely new approach to studying normal cortical development in animal, but also importantly in human species.

Neurodevelopmental disorders will particularly benefit from the generation of disease-specific human ESC and iPS cell lines, in particular for dissecting pathophysiological mechanisms, or screening for potential treatments. In the future, some of these disorders might also be treated by ESC or iPS cell-based therapy.

REFERENCES

1. Gaspard N, Vanderhaeghen P. Mechanisms of neural specification from embryonic stem cells. *Curr Opin Neurobiol* 2010; **20:** 37–43.
2. Altmann CR, Brivanlou AH. Neural patterning in the vertebrate embryo. *Int Rev Cytol* 2001; **203:** 447–82.
3. Levine AJ, Brivanlou AH. Proposal of a model of mammalian neural induction. *Dev Biol* 2007; **308:** 247–56.
4. Chambers I, Tomlinson SR. The transcriptional foundation of pluripotency. *Development* 2009; **136:** 2311–22.
5. Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. *Cell* 2002; **110:** 385–97.
6. Lee SH, Lumelsky N, Studer L, Auerbach JM, McKay RD. Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat Biotechnol* 2000; **18:** 675–9.
7. Murashov AK, Pak ES, Hendricks WA, et al. Directed differentiation of embryonic stem cells into dorsal interneurons. *FASEB J* 2005; **19:** 252–4.
8. Su HL, Muguruma K, Matsuo-Takasaki M, Kengaku M, Watanabe K, Sasai Y. Generation of cerebellar neuron precursors from embryonic stem cells. *Dev Biol* 2006; **290:** 287–96.
9. Salero E, Hatten ME. Differentiation of ES cells into cerebellar neurons. *Proc Natl Acad Sci USA* 2007; **104:** 2997–3002.
10. Wataya T, Ando S, Muguruma K, et al. Minimization of exogenous signals in ES cell culture induces rostral hypothalamic differentiation. *Proc Natl Acad Sci USA* 2008; **105:** 11796–801.
11. Eiraku M, Watanabe K, Matsuo-Takasaki M, et al. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* 2008; **3:** 519–32.
12. Gaspard N, Bouschet T, Hourez R, et al. An intrinsic mechanism of corticogenesis from embryonic stem cells. *Nature* 2008; **455:** 351–7.
13. Gaspard N, Bouschet T, Herpoel A, Naeije G, van den Ameele J, Vanderhaeghen P. Generation of cortical neurons from mouse embryonic stem cells. *Nat Protoc* 2009; **4:** 1454–63.
14. Jessell TM. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat Rev Genet* 2000; **1:** 20–9.
15. Watanabe K, Kamiya D, Nishiyama A, et al. Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat Neurosci* 2005; **8:** 288–96.
16. Gaspard N, Gaillard A, Vanderhaeghen P. Making cortex in a dish: in vitro corticopoiesis from embryonic stem cells. *Cell Cycle* 2009; **8:** 2491–6.
17. Kaas JH. The evolution of isocortex. *Brain Behav Evol* 1995; **46:** 187–96.
18. Hockemeyer D, Soldner F, Beard C, et al. Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. *Nat Biotechnol* 2009; **27:** 851–7.
19. Lacoste A, Berenshteyn F, Brivanlou AH. An efficient and reversible transposable system for gene delivery and lineage-specific differentiation in human embryonic stem cells. *Cell Stem Cell* 2009; **5:** 332–42.
20. Osafune K, Caron L, Borowiak M, et al. Marked differences in differentiation propensity among human embryonic stem cell lines. *Nat Biotechnol* 2008; **26:** 313–5.
21. Adewumi O, Aflatoonian B, Ahrlund-Richter L, et al. Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat Biotechnol* 2007; **25:** 803–16.
22. Mateizel I, De Temmerman N, Ullmann U, et al. Derivation of human embryonic stem cell lines from embryos obtained after IVF and after PGD for monogenic disorders. *Hum Reprod* 2006; **21:** 503–11.
23. Eiges R, Urbach A, Malcov M, et al. Developmental study of fragile X syndrome using human embryonic stem cells derived from preimplantation genetically diagnosed embryos. *Cell Stem Cell* 2007; **1:** 568–77.
24. Frumkin T, Malcov M, Telias M, et al. Human embryonic stem cells carrying mutations for severe genetic disorders. *In Vitro Cell Dev Biol Anim* 2010; **46:** 327–36.
25. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126:** 663–76.
26. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131:** 861–72.
27. Hotta A, Cheung AY, Farra N, et al. Isolation of human iPS cells using EOS lentiviral vectors to select for pluripotency. *Nat Methods* 2009; **6:** 370–6.
28. Woltjen K, Michael IP, Mohseni P, et al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* 2009; **458:** 766–70.
29. Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature* 2009; **458:** 771–5.
30. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science* 2008; **322:** 945–9.
31. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 2008; **322:** 949–53.
32. Yusa K, Rad R, Takeda J, Bradley A. Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. *Nat Methods* 2009; **6:** 363–9.
33. Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 2008; **321:** 1218–21.
34. Ebert AD, Yu J, Rose FF Jr, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 2009; **457:** 277–80.
35. Lee G, Papapetrou EP, Kim H, et al. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature* 2009; **461:** 402–6.
36. Soldner F, Hockemeyer D, Beard C, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* 2009; **136:** 964–77.
37. Park IH, Arora N, Huo H, et al. Disease-specific induced pluripotent stem cells. *Cell* 2008; **134:** 877–86.
38. Hu BY, Zhang SC. Differentiation of spinal motor neurons from pluripotent human stem cells. *Nat Protoc* 2009; **4:** 1295–304.
39. Pober BR. Williams-Beuren syndrome. *N Engl J Med* 2010; **362:** 239–52.
40. Tassabehji M. Williams-Beuren syndrome: a challenge for genotype-phenotype correlations. *Hum Mol Genet* 2003; **12:**(Suppl. 2) R229–37.
41. Osborne LR. Animal models of Williams syndrome. *Am J Med Genet C Semin Med Genet* 2010; **154C:** 209–19.
42. 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:** 1035–41.
43. Hu BY, Weick JP, Yu J, et al. Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc Natl Acad Sci USA* 2010; **107:** 4335–40.
44. Gaillard A, Prestoz L, Dumartin B, et al. Reestablishment of damaged adult motor pathways by grafted embryonic cortical neurons. *Nat Neurosci* 2007; **10:** 1294–9.
45. Wernig M, Zhao JP, Pruszak J, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc Natl Acad Sci USA* 2008; **105:** 5856–61.