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Cortical neurogenesis from pluripotent stem cells: complexity emerging from simplicity

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The cerebral cortex contains dozens of neuronal subtypes grouped in specific layers and areas. Recent studies have revealed how embryonic and induced pluripotent stem cells (PSC) can differentiate into a wide diversity of cortical neurons *in vitro*, while recapitulating many of the temporal and spatial features that characterize corticogenesis. PSC-derived neurons can integrate into the brain following *in vivo* transplantation and display patterns of morphology and connectivity specific of cortical neurons. PSC-corticogenesis thus emerges as a robust model that provides new ways to link cortical development, evolution, and disease.

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Introduction

The cerebral cortex is arguably the most complex structure in our brain, and cortical neuron number and diversity are thought to be at the core of its powerful computational capacities. Most (>85%) cortical neurons are excitatory pyramidal neurons, while the remaining 15% are inhibitory interneurons. Pyramidal neurons and interneurons can be further subdivided into many subtypes, characterized by specific patterns of gene expression, morphology and connectivity [1].

Pluripotent stem cells (PSC), whether embryonic (ESC) [2] or induced (iPSC) [3,4], have emerged as a promising tool to model normal brain development and diseases.

Here we will review recent data that demonstrate that a substantial fraction of cortical neuron diversity and complexity can be generated *in vitro* from PSC, while mimicking much of *in utero* development, revealing that many features of corticogenesis can result from self-organization. We will put special emphasis on studies that used human cells, and the insights that they provide on human brain development, evolution, and disease.

Starting-up: regional patterning and neuronal specification

The cortical primordium emerges in the telencephalon, the anterior-most part of the forebrain. Interestingly, the telencephalic/forebrain identity first develops largely in the *absence* of any extrinsic morphogenic cues, and is even enhanced through active *inhibition* of morphogen signals such as Wnts or BMPs [5]. The telencephalon then undergoes patterning along the dorso-ventral axis, leading to the parcellation into several neurogenic niches, including the dorsal telencephalon and the ventrally located ganglionic eminences, which will generate cortical pyramidal neurons and most interneurons, respectively [6,7,8]. These basic features of corticogenesis are essentially recapitulated during directed differentiation of cortical neurons from PSC. Indeed when PSC are cultured without any added (caudalizing) morphogen or in the presence of selected morphogen inhibitors, most of them differentiate into neural precurors displaying a forebrain/telencephalic identity [9••,10–13,14••,15••,16•]. Moreover, if PSC-forebrain differentiation takes place with little or no SHH signalling, it mostly leads to the generation of dorsal telencephalic progenitors and glutamatergic, cortical pyramidal neurons [9••,14••,15••,17••,18,19,20••]. In contrast, addition of SHH leads to specification of ventral telencephalic progenitors that will differentiate into both GABAergic and cholinergic neurons [19,21•,22,23•]. Since the majority of cortical GABAergic interneurons in humans, as in rodents, originate in the subcortical telencephalon [24•,25•], ventralized telencephalic differentiation of human PSC also give rise to cortical interneurons [26,27••,28••].

Modelling temporal patterns of corticogenesis

Following early patterning, cortical neurogenesis will start to take place leading to the generation of six different neuronal layers, each characterized by specific patterns of gene expression and connectivity [1]. The layer-identity of a cortical neuron is tightly linked to the timing of its generation: this temporal patterning results in the sequential generation of layer-specific types of cortical neurons and is a fundamental process of neuronal diversification [29]. Remarkably, PSC-derived corticogenesis recapitulates this temporal patterning *in vitro*, leading to

the sequential generation of a repertoire of neurons displaying specific molecular markers of all six layers [9••,15••,17••,20••,30], similarly to what was previously demonstrated using *ex vivo* cultures of early cortical progenitors [31]. Intriguingly, the proportion of each layer-specific neuronal subtype varies considerably depending on differentiation conditions. ESC-derived pyramidal neurons obtained in minimal culture conditions (low cell density without any added morphogens) are strongly skewed towards a deep layer identity [9••,15••], while a higher proportion of upper layer neurons are obtained when PSC are first differentiated at high density [20••] or as cell aggregates [17••,32], or when the PSC-derived cortical progenitors are transplanted in the mouse brain [15••]. These differences should be explored much further, and it may yield new insights on the mechanism that control the timing and rates of production of specific pyramidal neuron subtypes.

While the sequential generation of pyramidal neurons from PSC is a robust feature, observed from ESC and iPSC of mouse and human origin, direct comparison between mouse and human PSC-corticogenesis revealed that it is greatly extended in time with human PSC [15••,17••,19,20••,32], even when using identical culture conditions [15••]. Consistent with the protracted period of cortical neurogenesis in humans, human ESC-cortical progenitors start to generate postmitotic neurons after about four weeks instead of six to eight days in the mouse, which is correlated with appearance of radial glia (RG)-like progenitors, the main neurogenic cortical progenitor [33]. Thereafter, mouse ESC-corticogenesis takes two to three weeks to be completed, while it takes 10–15 weeks starting from human ESC (Figures 1 and 2) [9••]. These temporal specificities are strikingly similar to *in vivo* corticogenesis [34–38], and they may be directly relevant to the links between development and evolution of the cortex. Indeed, many of the species-specific features of the primate/human cortex, including a larger number and diversity of neurons, are thought to be linked to differences in the mechanisms underlying the generation of cortical neurons [36,38,39]. The mechanisms by which the primate embryonic brain can generate more neurons for prolonged periods might be linked to species-specific properties intrinsic to cortical progenitors, such as differential cell cycle control or tuning of self-renewal versus terminal differentiation [40]. The emergence of other types of progenitors may also contribute to evolutionary changes in cortical neurogenesis. These progenitors include the recently described 'outer' radial glial (oRG) cells [35,37,41–44], which share many features with RG cells, including the potential for self-renewal, but they lack any apical projection. Most strikingly, while human oRG cells can generate neurons directly, their progeny undergoes multiple rounds of divisions before final differentiation, thus providing a mechanism for increased neuronal output and cortical expansion. Importantly, the detection of oRG-like cells was reported following *in vitro* differentiation from human PSC [20••,45••,46••] but not from mouse PSC [46••], providing further evidence of species-specific features of PSC-corticogenesis directly relevant to evolution.

A third aspect of corticogenesis that appears to be species-specific is neuronal maturation: once generated human cortical neurons display much prolonged patterns of morphological and functional maturation, such as dendrite patterning and synaptogenesis [47,48]. Similarly, PSC-derived human cortical neurons mature very slowly at the molecular and functional levels [15••,20••,32]. Even more strikingly, comparison of human versus mouse PSC-derived cortical neurons transplanted into the mouse neonatal cortex revealed that the human pyramidal neurons follow a species-specific programme of delayed neuronal maturation and synaptogenesis [15••,49]. For instance, while ESC-derived mouse pyramidal neurons develop full blown and specific axonal and dendritic projections after four weeks [9••], similarly differentiated and transplanted human neurons take more than six months to develop subcortical projections and at least nine months to develop complex dendrite arborization pattern, dendritic spines, and functional synaptic activity [15••]. Similar results are found with GABAergic cortical interneurons derived from human PSCs. While synaptogenesis and reasonably mature-appearing action potentials can be detected within one month of co-culturing with mouse cortical pyramidal neurons [27••], transplantation studies into the neocortex of neonatal mice show limited terminal differentiation of these cells even six months later [28••].

Overall, these data point to cortex-intrinsic mechanisms that control the clock of several key aspects of corticogenesis, for which PSC-based models may provide attractive experimental set-ups to dissect the underlying mechanisms [30], with potentially important relevance to basic mechanisms linking cortical development and evolution.

Importantly, the very slow rate of maturation of cortical cells from human PSC presents a major challenge to the widespread application of this system for the study or treatment of human disease.

Modelling spatial patterns of corticogenesis

The cytoarchitecture of the cortex is crucial to its function, and despite its apparent complexity, key aspects of the patterned, three dimensional (3D) organization of the developing cortex can also be recreated *in vitro* (Figure 1b). When PSC are cultured as bowls of cells and differentiated into cortical-like progenitors, this leads to robust polarized cellular organization [17••], with progenitors occupying deeper layers of the bowls, and neurons accumulating at their periphery, following an organization highly reminiscent of a nascent cortical