(Stadtfeld et al., 2008; Yu and Thomson, 2008). The type of cell chosen for reprogramming appears to determine how many exogenous factors, if any, are needed. Two recent studies have shown that adult mouse neural stem cells express high levels of endogenous *Sox2* and *c-Myc* and can give rise to iPSCs with the addition of just two exogenous factors: *Oct4* and *Klf4* (discussed in Tada, 2008). The CD49f+, laminin-bound germ cells isolated by Conrad et al. (2008) expressed endogenous *OCT4* and *SOX2*, and culture conditions alone reprogrammed the cells into pluripotent haGSC colonies. It is important to note that in both mice and humans, SSCs are the only known adult cell type that express OCT4. Thus, SSCs appear to have a molecular signature that is similar enough to ESCs to allow for their successful reprogramming to a pluripotent state without the need for exogenous factors; endogenous OCT4 might be the key to this success.

The work of Conrad et al. (2008) lays the foundation for vigorous research into elucidating the basic properties of human pluripotent stem cells derived from adult male germ cells. It will be important to identify the range of functional cell types that can be generated from haGSC differentiation in vitro. For example, given the comparatively high numbers of cells expressing pancreatic lineage markers produced after haGSC differentiation in culture, these testis-derived pluripotent stem cells might be a useful resource for diabetes research studies. Only after further experimentation will the potential usefulness of haGSCs be fully revealed. An important caveat to this work, however, is that any autologous therapies utilizing haGSCs would be applicable only to men. The generation of a comparable female haGSC is unlikely, given that most studies support the view that women are born with a lifetime supply of oocytes and that there are no germline stem cells in females. For now, this study demonstrates that human male germline commitment can be altered in vitro and that unipotent cells can become pluripotent without the addition of exogenous transcription factors.

REFERENCES

Conrad, S., Renninger, M., Hennenlotter, J., Wiesner, T., Just, L., Bonin, M., Aicher, W., Bühring, H.J., Mattheus, U., Mack, A., et al. (2008). Nature 10.1038/nature07404.

Dahéron, L., Opitz, S.L., Zaehres, H., Lensch, M.W., Andrews, P.W., Itskovitz-Eldor, J., and Daley, G.Q. (2004). Stem Cells *22*, 770–778.

Guan, K., Nayernia, K., Maier, L.S., Wagner, S., Dressel, R., Lee, J.H., Nolte, J., Wolf, F., Li, M., Engel, W., et al. (2006). Nature *440*, 1199–1203.

Kanatsu-Shinohara, M., Inoue, K., Lee, J., Yoshimoto, M., Ogonuki, N., Miki, H., Baba, S., Kato, T., Kazuki, Y., Toyokuni, S., et al. (2004). Cell *119*, 1001–1012.

Oatley, J.M., and Brinster, R.L. (2008). Annu. Rev. Cell Dev. Biol. *24*, 263–286.

Seandel, M., James, D., Shmelkov, S.V., Falciatori, I., Kim, J., Chavala, S., Scherr, D.S., Zhang, F., Torres, R., Gale, N.W., et al. (2007). Nature *449*, 346–350.

Seydoux, G., and Braun, R.E. (2006). Cell *127*, 891–904.

Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G., and Hochedlinger, K. (2008). Science 10.1126/science.1162494.

Tada, T. (2008). Cell Stem Cell *3*, 121–122.

Yu, J., and Thomson, J.A. (2008). Genes Dev. *22*, 1987–1997.

Cortex Shatters the Glass Ceiling

Edmund Au[1] and Gord Fishell[1,*]
[1]Smilow Neuroscience Program, Department of Cell Biology, NYU Langone Medical Center, 522 First Avenue, New York, NY 10016, USA
*Correspondence: fisheg01@nyumc.org
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Recreating developmental structures in vitro has been a primary challenge for stem cell biologists. Recent studies in *Cell Stem Cell* (Eiraku et al., 2008) and *Nature* (Gaspard et al., 2008) demonstrate that embryonic stem cells can recapitulate early cortical development, enabling them to generate specific cortical subtypes.

Pyramidal cells are the principal excitatory neurons within the cortex, comprising approximately 80% of all neurons within this structure. They are derived from radial glial progenitors within the pallium and are generated in a temporally controlled manner. Within the cortex, progenitors first generate Cajal-Retzius cells, which reside in the most superficial layer, followed by deep layer neurons. Subsequent neurogenesis then generates successively more superficial populations, as cortical neurogenesis proceeds in an inside-out manner. Gaspard et al. (2008) and Eiraku et al. (2008) have both recently demonstrated that mouse embryonic stem cells (ESCs) can be induced to follow a program of neurogenesis similar to that observed in the developing cortex.

Birthdating studies by both groups showed that the first neurons generated in their cultures are reelin-positive Cajal-Retzius cells. Next observed were neurons exhibiting deep layer characteristics, followed by the progressive appearance of neurons displaying more superficial

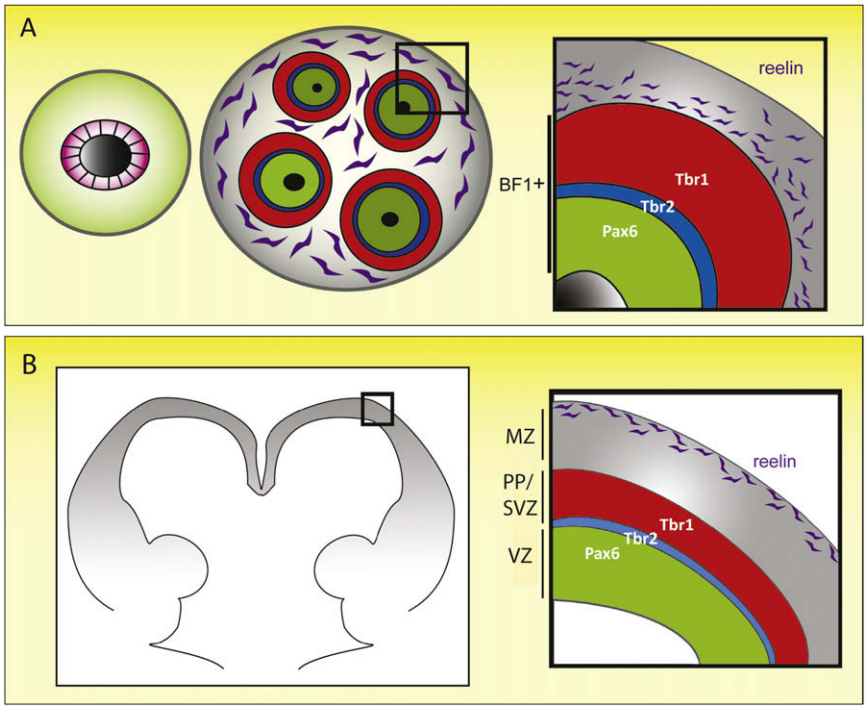

Figure 1. ESC-Derived Telencephalic Precursors Self-Organize into Rosette Structures Reminiscent of Early Cortical Development

(A) Early embryoid bodies are hollow spheres with proliferative cells (pink) lining the lumen. Over time, the sphere collapses into numerous rosettes. Pax6+ VZ cells are deep, surrounded by Tbr2+/Pax6+ SVZ-like cells and a superficial layer of Tbr1+/Tuj1 neurons. Reelin+/calretinin+ Cajal-Retzius cells are found at the outer edge of the rosettes.

(B) Coronal plane of E11.5 mouse brain. The layers of the cortex form with a VZ found deep, preplate (PP) and SVZ in the middle and a marginal zone (MZ) found superficially.

layer markers. Both groups demonstrated that the resulting neuronal populations are largely excitatory and glutamatergic—Gaspard et al. through electrophysiology and Eiraku et al. by using calcium imaging. Functionally, both groups observed that ESC-derived pyramidal cells preferentially integrated within cortical rather than striatal tissue in coculture experiments and established either cortico-cortico or cortico-fugal projections when transplanted into neonatal cortex. In a particularly compelling experiment, Gaspard et al. employed a *Tau*^{EGFP} reporter knockin ESC line to demonstrate that the cortical populations they generated tended to project along specific pathways after in vivo transplantation. Notably, these transplant studies suggested that the identity of the cells were primarily visual and limbic deep layer pyramidal neurons, a fate they maintained even when transplanted into the prefrontal region. Similar data from the Sasai group also suggested that the fate of the resulting neurons was determined in vitro prior to transplantation, arguing against a requirement for in vivo cues being required for the establishment of the observed cell identities.

Although their findings were similar, there are some key differences between the two studies. Most importantly, the Sasai group differentiated their mouse ESCs through embryoid body (EB) formation (Osakada et al., 2008; Watanabe et al., 2005), whereas Vanderhaegen's group used an adherent monolayer approach (Ying et al., 2003). EBs form spontaneously when ESCs are cultured in suspension and in the absence of leukemia inhibitory factor. Floating ESCs readily form aggregates that, when neuralized, arrange themselves into structures that have been termed neural rosettes (Lee et al., 2000).

The simplicity of the methods utilized by Gaspard and colleagues is striking when compared with the Sasai approach. For example, Eiraku et al. took considerable pains to regulate the starting size of their EB aggregate, while Vanderhaegan ostensibly achieved the same end goal by using relatively standard tissue culture methods. Thus, one might conclude that the more convoluted approach used by the Sasai group is overly cumbersome. However, the ability of the Sasai group to prod ESCs to self-organize into a structure that resembles that seen in vivo may provide important insights as to which developmental steps contribute in the determination of precise cortical fates. By using an EB approach, Eiraku et al. were able to analyze not only the end product, but could, in a three-dimensional context, also examine the neural progenitors and immature neurons that gave rise to the more complex structures.

In their study, Eiraku et al. employed a *FoxG1*^{venus} knockin ESC line to serve as a reporter of telencephalic differentiation. By standardizing EB formation through aggregating a defined number of ESCs per well, the authors more than doubled their previous differentiation efficiency to ~66% (Watanabe et al., 2005). Using these optimized conditions, Eiraku et al. found that most EB aggregates started off as hollow spheres, which resembled developing neuroepithelium. As seen in vivo, the mitotically active cells were confined largely to the lumen of the sphere and expressed CD133 (prominin), characteristic of ventricular zone neural stem cells. Over the course of a week in culture, the spheres collapsed to form smaller spheres termed neural rosettes (Figure 1A). Each rosette maintains the same basic organization of the original sphere, with CD133+ ventricular cells restricted to the luminal surface, nested within sequentially broader domains of Pax6 and Tbr1 expression extending outward. Further mimicking the organization observed in vivo, the earliest born reelin+ Cajal-Retzius cells are found along the outside of these rosettes. Thus, at this stage, after approximately 10 days in culture, the organization of these rosettes is strikingly similar to that observed in the embryonic mouse cortex at E11.5 (Figure 1B). Remarkably, the authors also demonstrate that the same holds true for human ESCs cultured under these conditions, albeit forming at a considerably slower rate than their murine counterparts (Eiraku et al., 2008).

For as yet unknown reasons, EBs, whether mouse or human, fail to recapitulate later aspects of cortical development.

Pyramidal neurons, such as layer V Ctip2+/Emx1+ and upper layer Brn2+ neurons that are born later during development were produced in smaller numbers and were randomly distributed in the EBs rather than in an inside/out fashion. Similarly, Gaspard et al. also had better success generating early born neurons (Cajal-Retzius and layer VI and V pyramidal cells) than in generating upper layer pyramidal cells in vitro. Consistent with these findings, in vitro analysis of wild-type cortical stem cells was observed to produce upper layer pyramidal neurons in fewer than deep layer pyramidal neurons (Shen et al., 2006).

The failure to date to reproduce later events in cortical neurogenesis in vitro could stem from a number of causes. Eiraku et al. suggested that the problem may be structural, resulting perhaps from the lack of a supportive framework. An alternative possibility may lay in the suggestion that while early pyramidal cell populations are generated by neural stem cells of the ventricular zone (VZ), subsequent populations are derived from the subventricular zone (SVZ) (Leone et al., 2008; Tarabykin et al., 2001). Perhaps then the failure of EBs to produce upper layer cortical pyramidal neurons reflects a failure in the in vitro environment to robustly generate an SVZ. In support of this notion, similar problems have been reported in ex vivo brain slices, where proliferation is strongly curtailed (Haydar et al., 2000). Indeed, a recent study has demonstrated that notch signaling is differently utilized in VZ and SVZ progenitors, suggesting fundamental differences between these two progenitor pools (Mizutani et al., 2007). Such a line of reasoning is consistent with the observation that Eiraku et al. did not significantly enrich for Brn2+ upper layer pyramidal cells by sorting for FoxG1^{Venus}+ cells at later time points in the presence of DAPT, an inhibitor of notch signaling.

Going forward, it will be necessary to demonstrate that ESC-derived cortical progenitors are capable of generating all pyramidal cell lineages in large numbers and not just the earliest born populations. Moreover, it will be necessary to demonstrate that specific lineages can be preferentially produced and isolated and that the whole process can be accomplished with high efficiency. Clearly, obstacles still exist for the robust generation of upper layer pyramidal cells. There seems little doubt, however, that the ability to recapitulate and monitor events associated with cortical development in vitro provides an invaluable tool for systematically refining culture conditions, ultimately to replicate what Claude Bernard famously termed the "internal milieu."

REFERENCES

Eiraku, M., Watanabe, K., Matsuo-Takasaki, M., Kawada, M., Yonemura, S., Matsumura, M., Wataya, T., Nishiyama, A., Muguruma, K., and Sasai, Y. (2008). Cell Stem Cell *3*, this issue, 519–532.

Gaspard, N., Bouschet, T., Hourez, R., Dimidschstein, J., Naeije, G., van den Ameele, J., Espuny-Camacho, I., Herpoel, A., Passante, L., Schiffmann, S.N., et al. (2008). Nature *455*, 351–357.

Haydar, T.F., Wang, F., Schwartz, M.L., and Rakic, P. (2000). J. Neurosci. *20*, 5764–5774.

Lee, S.H., Lumelsky, N., Studer, L., Auerbach, J.M., and McKay, R.D. (2000). Nat. Biotechnol. *18*, 675–679.

Leone, D.P., Srinivasan, K., Chen, B., Alcamo, E., and McConnell, S.K. (2008). Curr. Opin. Neurobiol. *18*, 28–35.

Mizutani, K., Yoon, K., Dang, L., Tokunaga, A., and Gaiano, N. (2007). Nature *449*, 351–355.

Osakada, F., Ikeda, H., Mandai, M., Wataya, T., Watanabe, K., Yoshimura, N., Akaike, A., Sasai, Y., and Takahashi, M. (2008). Nat. Biotechnol. *26*, 215–224.

Shen, Q., Wang, Y., Dimos, J.T., Fasano, C.A., Phoenix, T.N., Lemischka, I.R., Ivanova, N.B., Stifani, S., Morrisey, E.E., and Temple, S. (2006). Nat. Neurosci. *9*, 743–751.

Tarabykin, V., Stoykova, A., Usman, N., and Gruss, P. (2001). Development *128*, 1983–1993.

Watanabe, K., Kamiya, D., Nishiyama, A., Katayama, T., Nozaki, S., Kawasaki, H., Watanabe, Y., Mizuseki, K., and Sasai, Y. (2005). Nat. Neurosci. *8*, 288–296.

Ying, Q.L., Stavridis, M., Griffiths, D., Li, M., and Smith, A. (2003). Nat. Biotechnol. *21*, 183–186.