CyclinD2 at the edge: splitting up cell fate

Federico Calegari

DFG-Research Center for Regenerative Therapies, Dresden University of Technology, Dresden, Germany.
Correspondence to: federico.calegari@crt-dresden.de

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Asymmetric cell division and cell cycle length are two fundamental mechanisms that influence the fate of neural stem cells during mammalian brain development. In this issue of *The EMBO Journal*, the team of Noriko Osumi proposes an elegant link between the two by showing that the mRNA of cyclinD2, a positive regulator of G1 progression, is confined to the basal end-foot of radial glial cells and is asymmetrically distributed upon mitosis to the two resulting daughter cells (Tsunekawa *et al*, 2012). According to this model, the daughter cell inheriting cyclinD2 mRNA maintains its self-renewal capability, while lengthening of G1 and differentiation would occur in the sibling cell.

All neurons and glia of the mammalian central nervous system derive from highly elongated radial glial cells spanning the apical (ventricular) to basal (pial) axis of the neural tube. As development proceeds, an increasing proportion of radial glial cells switches from divisions that generate two additional radial glial cells (proliferative divisions) to divisions that generate one radial glial plus one differentiated cell (differentiative divisions) (Gotz and Huttner, 2005). The observation that radial glia retain their basal process while undergoing apical mitosis (Miyata *et al*, 2001; Noctor *et al*, 2001) has made it evident that these cells are intrinsically prone to divide asymmetrically, since only one daughter would inherit the process upon cytokinesis. Reinforcing the view that asymmetric inheritance of cellular components may underlie asymmetric cell fate change, this observation has added a new level of complexity to asymmetric division of neural progenitors that was previously thought to involve mainly apical, as opposed to basal, components (reviewed by Kosodo and Huttner, 2009). *De facto*, formal demonstration that asymmetric inheritance of the basal process is a

prerequisite for differentiative divisions is still missing and it is even debated whether the daughter inheriting the process is the radial glial cell, her differentiated sibling, or if in some

Figure 1 CyclinD2 crowns stem cell identity. Loss of stemness represents a revolutionary event allowing the generation of differentiated cells. According to the model by Tsunekawa *et al*, radial glial cells during mammalian cortical development ensure that daughter cells without basal process would lengthen G1 upon cytokinesis thereby triggering asymmetric cell fate change and neurogenesis. This model elegantly links the subcellular localization of a cell cycle regulator with asymmetric cell division and fate. Note that the deposed cell must not necessarily need to be a neuron but could also be an intermediate/basal progenitor. Bystanders watch aghast while undergoing interkinetic nuclear migration waiting for their turn to divide.

instance the process itself could be split in two (Kosodo and Huttner, 2009). Nevertheless, it is well recognized that the peculiar morphology of radial glial cells can provide the means to control asymmetric cell division and fate and many laboratories are investigating the mechanisms underlying the inheritance of the basal process and its role in neurogenesis.

Similar to asymmetric cell division, cell cycle length has long been recognized as an important parameter for neural differentiation, since lengthening of G1 in radial glial cells was shown to correlate with neurogenesis at the temporal, spatial, and cellular level (reviewed by Salomoni and Calegari, 2010). Interestingly, manipulations that lengthened G1 induced differentiative divisions and neurogenesis while, conversely, shortening G1 promoted proliferative divisions and progenitors expansion supporting the notion (*the cell cycle length hypothesis*) that longer cell cycles are more likely to allow the accumulation of factors necessary for cell fate change to occur (Salomoni and Calegari, 2010). It is still unclear whether cell cycle regulators influence differentiation solely through a change in G1 length or, indirectly, through still unknown additional functions. Nevertheless, an overwhelming number of reports have pointed out a strong correlation between the activity of G1-specific cdk/cyclin complexes, G1 length, and cell fate change in various paradigms of stem cell differentiation including embryonic, neural, and hematopoietic stem cells (Lange and Calegari, 2010).

It now comes as an interesting surprise that the mRNA of cyclinD2, a positive regulator of G1 progression mediating the transition from radial glial cells to intermediate/basal progenitors (Glickstein *et al*, 2009), is almost exclusively localized to the basal end-foot of radial glial cells (Tsunekawa *et al*, 2012). Tsunekawa and Osumi must have thought that this cannot be a mere coincidence and embarked on a detailed characterization of the subcellular distribution of cyclinD2 mRNA and protein, its inheritance upon mitosis, and the effects of manipulating the levels of cyclinD2 on neurogenesis. By using *in vivo* electroporation either in whole embryo culture or *in utero*, two techniques that the Osumi group has pioneered (Osumi and Inoue, 2001), Tsunekawa *et al* now show that a 50 bp *cis*-acting transport element is present in the 3'UTR of cyclinD2 that is responsible for its localization at the basal end-foot of radial glial cells (Figure 1). From fixed samples, the authors concluded that the majority of basally localized siblings resulting from a radial glial division appeared to be the ones inheriting the process and having higher levels of cyclinD2. Moreover, disrupting the asymmetry in cyclinD2 by forcing its overexpression or downregulation resulted in decreased or increased neurogenesis, respectively. From these observations, the authors

concluded that the active transport of cyclinD2 mRNA to the basal end-foot of radial glial cells can ensure that only one daughter would inherit high levels of this positive regulator of G1 progression. As a result, and consistent with *the cell cycle length hypothesis*, the sibling cell that did not inherit the cyclinD2 mRNA-containing basal process would lengthen G1 in the subsequent cell cycle leading to a change in her fate and consequent differentiation (Tsunekawa *et al*, 2012).

Importantly, this model implies that the basally localized daughter cell is the one inheriting the process and remaining as a proliferative radial glial cell while her apical sibling would undergo differentiation. Whether this is consistent with other studies is difficult to say because inheritance of the basal process is a controversial issue with opposite views proposed by different groups (Kosodo and Huttner, 2009). Nevertheless, in support to their model Tsunekawa *et al* show that the apically localized sibling, the one with lower levels of cyclinD2, tends to express higher levels of the neurogenic factor Ngn2. Interestingly, the group of Anna Philpott has recently shown that G1-cdk/cyclins can destabilize Ngn2 by direct phosphorylation (perhaps an 'additional function' for these cell cycle regulators?) thereby inhibiting differentiation concomitantly to shortening G1 (Ali *et al*, 2011). Reinforcing each other, these two recent observations (Ali *et al*, 2011; Tsunekawa *et al*, 2012) depict an elegant interplay between cell cycle regulators and cell fate determinants that mechanistically link asymmetric cell division, G1 length, and neurogenesis at the molecular and cellular levels.

Several points still need to be addressed to fully validate the model proposed by the team of Noriko Osumi. In fact, it is fair to say that the use of fixed samples to infer about differentiation is suboptimal and that time-lapse microscopy becomes necessary when asymmetric cell division and cell fate change are correlated at the single cell level. Moreover, while the analysis of cell cycle parameters performed by Tsunekawa *et al* revealed to be entirely consistent with the model proposed, no direct measurement of G1 length has been provided neither upon overexpression nor downregulation experiments. Finally, active transport of cyclinD2 mRNA, as opposed to other possibilities including passive diffusion or selective degradation, was only deduced. Yet, no step in science is typically complete *per se* and Tsunekawa *et al* can certainly be granted the rare merit of providing solid evidence for the proposal of an original, elegant, and simple model that will likely stimulate and inspire the field in the years to come.

Conflict of interest

The author declares that he has no conflict of interest.

References

- Ali F, Hindley C, McDowell G, Deibler R, Jones A, Kirschner M, Guillemot F, Philpott A (2011) Cell cycle-regulated multi-site phosphorylation of Neurogenin 2 coordinates cell cycling with differentiation during neurogenesis. *Development* **138**: 4267–4277
- Glickstein SB, Monaghan JA, Koeller HB, Jones TK, Ross ME (2009) Cyclin D2 is critical for intermediate progenitor cell proliferation in the embryonic cortex. *J Neurosci* **29**: 9614–9624
- Gotz M, Huttner WB (2005) The cell biology of neurogenesis. *Nat Rev Mol Cell Biol* **6**: 777–788
- Kosodo Y, Huttner WB (2009) Basal process and cell divisions of neural progenitors in the developing brain. *Dev Growth Differ* **51**: 251–261
- Lange C, Calegari F (2010) Cdks and cyclins link G(1) length and differentiation of embryonic, neural and hematopoietic stem cells. *Cell Cycle* **9**: 1893–1900

- Miyata T, Kawaguchi A, Okano H, Ogawa M (2001) Asymmetric inheritance of radial glial fibers by cortical neurons. *Neuron* **31**: 727–741
- Noctor SC, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR (2001) Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409**: 714–720
- Osumi N, Inoue T (2001) Gene transfer into cultured mammalian embryos by electroporation. *Methods* **24**: 35–42
- Salomoni P, Calegari F (2010) Cell cycle control of mammalian neural stem cells: putting a speed limit on G1. *Trends Cell Biol* **5**: 332–342
- Tsunekawa Y, Britto JM, Takahashi M, Polleux F, Tan S-S, Osumi N (2012) Cyclin D2 in the basal process of neural progenitors is linked to non-equivalent cell fates. *EMBO J* **31**: 1879–1892