

# Evolutionary conservation of mechanisms for neural regionalization, proliferation and interconnection in brain development

Heinrich Reichert

*Biol. Lett.* 2009 **5**, 112-116  
doi: 10.1098/rsbl.2008.0337

## References

This article cites 29 articles, 6 of which can be accessed free  
<http://rsbl.royalsocietypublishing.org/content/5/1/112.full.html#ref-list-1>

Article cited in:  
<http://rsbl.royalsocietypublishing.org/content/5/1/112.full.html#related-urls>

## Subject collections

Articles on similar topics can be found in the following collections

[neuroscience](#) (114 articles)  
[developmental biology](#) (49 articles)  
[evolution](#) (541 articles)

## Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

To subscribe to *Biol. Lett.* go to: <http://rsbl.royalsocietypublishing.org/subscriptions>

## Opinion piece

## Evolutionary conservation of mechanisms for neural regionalization, proliferation and interconnection in brain development

**Comparative studies of brain development in vertebrate and invertebrate model systems demonstrate remarkable similarities in expression and action of developmental control genes during embryonic patterning, neural proliferation and circuit formation in the brain. Thus, comparable sets of developmental control genes are involved in specifying the early brain primordium as well as in regionalized patterning along its anteroposterior and dorsoventral axes. Furthermore, similar cellular and molecular mechanisms underlie the formation and proliferation of neural stem cell-like progenitors that generate the neurons in the central nervous systems. Finally, neural identity and some complex circuit interconnections in specific brain domains appear to be comparable in vertebrates and invertebrates and may depend on similar developmental control genes.**

**Keywords:** brain development; pattern formation; proliferation; neural circuitry

## 1. INTRODUCTION

Despite the extensive history of investigations on the brain, which reaches back over two millennia, fundamental insight into the nature and origin of the brain remained elusive until the nineteenth century when two central theories of the biological sciences were first proposed. These unifying theories were the cell theory of Schleiden and Schwann, which states that all living organisms are composed of and derived from cells, and the evolutionary theory of Darwin, which explains the origin of organismal complexity and diversity through the action of natural selection (Schwann 1839; Darwin 1859). The subsequent successful application of both theories to the analysis of vertebrate brains uncovered fundamental similarities in the neural organization of different vertebrate brains, arguing for a common evolutionary origin of major brain elements in all vertebrates. By contrast, when the nervous systems of vertebrates were compared with those of invertebrates, apparent differences in neuroanatomy were found in both adult structure and embryonic morphogenesis. Based on these differences, a number of investigators proposed an independent evolutionary origin for the nervous systems of vertebrate and

invertebrate animals (gastroneuralia–notoneuralia concept; e.g. Brusca & Brusca 1990).

Contrasting with this notion, and in support of earlier ideas (reviewed by Arendt & Nübler-Jung 1994), are more recent neurogenetic analyses carried out in several vertebrate and invertebrate model systems that reveal striking similarities in the expression and action of regulatory genes that control brain development. Indeed, comparable developmental genetic mechanisms appear to operate in invertebrates and vertebrates during all major stages of brain development from the early specification of the neuroectoderm to the formation of neural circuitry (for recent reviews see Lichtneckert & Reichert 2005; Arendt *et al.* 2008). In the following, I consider four key aspects of brain development as well as the underlying molecular genetic control elements that appear to be conserved.

## 2. GENERATION OF THE NEUROECTODERM

At the earliest stage of brain development, a conserved molecular signalling system polarizes the embryonic ectoderm into a neuroectoderm, from which the central nervous system (CNS) develops, and into a ‘non-neural’ ectoderm (De Robertis 2008). A key element in this signalling system is the Dpp/Bmp protein, a member of the TGF $\beta$  gene family, which is thought to exert an antineurogenic effect that represses neural identity and limits the extent of the neuroectoderm to one side of the embryo. The expression of active Dpp/Bmp on this side is prevented by a second key element in the conserved signalling system, the Sog/Chordin protein, which antagonizes the action of the Dpp/Bmp morphogen. Whenever a CNS develops, in vertebrates or in invertebrates, it is always formed on the side of the body that does not express Dpp/Bmp, be it ventral in invertebrates, or dorsal in vertebrates (Arendt *et al.* 2008).

After specification and limitation of the neuroectoderm, a second set of conserved developmental control genes is activated in the neuroectoderm. These are the proneural genes that encode bHLH transcription factors that promote the transition of neuroectodermal cells into neural stem cells (Egger *et al.* 2008; Guillemot 2007). In *Drosophila*, the proneural genes include *achete*, *scute* and *lethal of scute* and in mammals they include *Mash1*, *Ngn1* and *Ngn2*. In both cases, these genes are required for acquisition of neural stem cell properties and initiation of neurogenesis; in mammals they also act in neural differentiation. In *Drosophila*, the evolutionarily conserved Notch signalling pathway subsequently restricts proneural gene expression such that cells expressing proneural genes at high levels adopt a neural stem cell fate whereas adjacent cells adopt alternative fates (Bray 1998). Notch signalling components also act in the embryonic neuroepithelium of mammals, and mutational inactivation or misexpression of these components results in defects in neural stem cell development. Interestingly, both *Drosophila* and mammalian neural stem cells come to lie in a unique cellular microenvironment (‘niche’), which may provide important molecular cues for the differentiation of these progenitors (Doetsch 2003; Dumstrei *et al.* 2003).

One contribution of 10 to a Special Feature on ‘Brain evolution’.

Received 24 June 2008

Accepted 28 July 2008

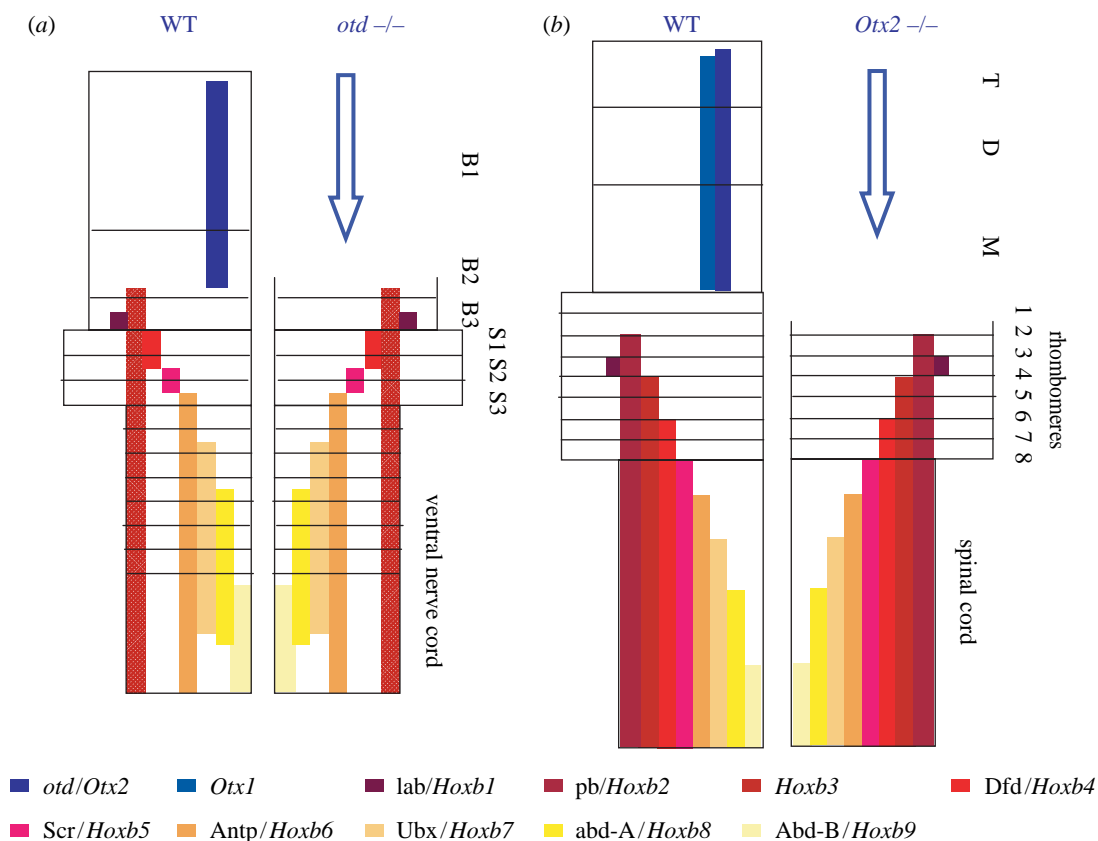


Figure 1. Anteroposterior order of homeotic (*Hox*) and *otd/Otx* gene expression in embryonic brain development of wild-types and brain phenotype of *otd/Otx2* mutation in (a) *Drosophila* and (b) mouse.

### 3. SELF-RENEWAL AND PROLIFERATION OF NEURAL STEM CELLS

Following their generation, neural stem cells initiate proliferation by self-renewing and producing neural progeny. There are remarkable similarities in the action of specific developmental control genes during this process in vertebrate and invertebrate brains (Doe 2008). For example, the Activin, Notch, Hedgehog and Fibroblast growth factor signalling pathways are active and have comparable roles in maintaining neural stem cell self-renewal and/or proliferation in the developing CNS of *Drosophila* and mouse. Moreover, the molecular components that regulate the asymmetric nature of the neural stem cell divisions, including regulators of cell polarity and determinants of daughter cell fates, have highly conserved counterparts in invertebrates and mammals (Knoblich 2008; Zhong & Chia 2008). In addition to these molecular biological similarities, there are striking parallels in the cell biology of neural stem cell proliferation in vertebrates and invertebrates.

In the mammalian embryonic CNS, neural stem cells can self-renew while producing secondary progenitors that each divide once to form two post-mitotic neurons (Götz & Huttner 2005). This corresponds to the proliferation of insect neural stem cells ('neuroblasts') that can also self-renew while producing secondary progenitors ('ganglion mother cells') that divide once to form two neurons (Egger *et al.* 2008). However, in mammalian brain development, neural stem cells can also self-renew and generate amplifying intermediate progenitors, each of which retains some

self-renewal potential and can generate multiple neural progeny (Kriegstein *et al.* 2006; Merkle & Alvarez-Buylla 2006). Remarkably, a similar mode of amplifying neural stem cell proliferation has recently been discovered in *Drosophila*. Specific brain neuroblasts in the fly brain divide asymmetrically in stem cell mode to self-renew and generate intermediate progenitors that subsequently divide multiple times in stem cell mode, thus amplifying the number of progeny produced (Bello *et al.* 2008).

### 4. PATTERNING THE DEVELOPING BRAIN

During early embryogenesis, the brain primordium in vertebrates and invertebrates is subdivided into compartment-like domains by the regionalized expression of developmental control genes (figure 1). This regionalization is especially prominent along the anteroposterior axis of the developing brain. Posterior parts of the brain primordium are patterned by the action of *Hox* genes (Kiecker & Lumsden 2005). These genes are involved in the regional specification of neural identity; in the absence of specific *Hox* genes, unspecified or mis-specified cells are generated. Anterior parts of the brain primordium are patterned by control genes such as the segmentation genes, exemplified by the *otd/Otx* cephalic gap gene family (Simeone *et al.* 2002; Acampora *et al.* 2005). Functional studies carried out in *Drosophila* and mouse indicate that *otd/Otx* gene activity is essential for the formation of the anterior brain primordium; in the absence of these genes, formation of the anterior brain is severely defective (figure 1).

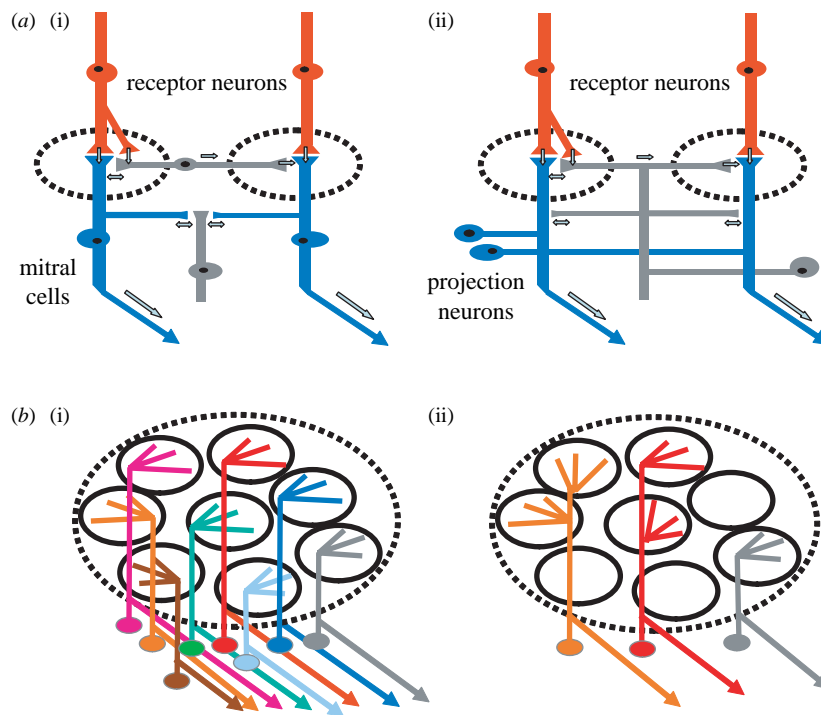


Figure 2. (a) Circuit organization of first olfactory relay centre in (i) mammalian and (ii) insect brains. (b) Schematic of projection neurons and their dendritic arbours in glomeruli of the *Drosophila* brain in (i) wild-type and (ii) *ems* mutant; dotted circles, glomerular neuropil.

Underscoring the functional conservation of *odt/Otx* genes are cross-phylum genetic rescue experiments in which the endogenous gene in one animal group can be replaced in a functionally equivalent manner by the homologous gene from the other animal group (Reichert & Simeone 2001).

Three sets of homeobox genes control the formation of columnar domains along the dorsoventral axis (Cornell & Ohlen 2000). In *Drosophila* these are the *vnd*, *ind* and *msh* genes, which are expressed in adjacent longitudinal columns and are required for the formation and specification of neural stem cells and their progeny in the ventral nerve cord. These genes also have comparable expression domains and functional roles in the developing brain (Urbach & Technau 2003; Sprecher *et al.* 2006). In vertebrates, gene homologues belonging to the *Nkx* (*vnd*), *Gsh* (*ind*) and *Msx* (*msh*) gene families are expressed in comparable columnar domains in the neural plate and neural tube, and are involved in controlling regional identity in these domains. Recent studies also provide evidence for conserved expression of *Pax6* and *Pax3/7* genes in columnar domains of the developing CNS in the annelid *Platynereis* and in vertebrates (Denes *et al.* 2007).

## 5. MAKING NEURONS AND NEURAL CIRCUITS IN THE BRAIN

In some cases, neurons that emerge from neural stem cells with comparable molecular genetic coordinates are similar in vertebrates and invertebrates. For example, in insects, nematodes and vertebrates, somatic motor neurons exhibit the same transcription factor signature involving the *hb9*, *lim3* and *islet1/2* genes (Thor & Thomas 2002). Moreover, in vertebrates and in *Platynereis*, cholinergic motor

neurons come from the *Pax6/Nkx6*-expressing progenitor domains while pioneering serotonergic projection neurons emerge from the *Nkx2*-expressing medial columns (Denes *et al.* 2007). Similarly, in zebrafish and *Platynereis* early differentiation of vasotocinergic and RFaminergic neurons are comparable in terms of morphology and cell type-specific gene expression including expression of a tissue-specific microRNA (Tessmar-Raible *et al.* 2007).

Could the development of neural circuits in the brain also be controlled by similar genetic elements in vertebrates and invertebrates? Initial evidence for this possibility comes from studies of the olfactory system, which manifests a remarkable similarity in circuit organization in insects and mammals (figure 2a). In both insects and mammals, genes of the *ems/Emx* family act early in patterning of the CNS, like the *odt/Otx* genes, and are also important for the later development of specific olfactory neurons, namely the projection neurons in insects and the mitral cells in mammals. In *Drosophila*, the *ems* gene is expressed in neural stem cells that produce the projection neurons and is required for correct cell number and dendritic targeting of these neurons (Lichtneckert *et al.* 2008). In the absence of *ems*, the number of projection neurons is severely reduced and the remaining projection neurons innervate inappropriate olfactory glomeruli (figure 2b). In the mouse, the *Emx1/2* genes are expressed in developing mitral cells, and the loss of these genes leads to marked defects in the mitral cell layer (Mallamaci *et al.* 1998; Bishop *et al.* 2003). Similar roles of the *ems/Emx* genes in the development of second-order olfactory neurons, together with a comparable requirement of *ems/Emx* genes in olfactory sense organ development, argue for evolutionarily



conserved roles of the *ems/Emx* genes in olfactory system development in insects and mammals. If this is the case, then the similar circuit organization of the olfactory system in insects and mammals may not only be the result of functional convergence but could also reflect the conservation of molecular mechanisms for olfactory system development.

## 6. CONCLUSIONS

Comparative studies of different aspects of brain development in vertebrates and invertebrates reveal remarkable similarities in expression and function of key developmental control genes. Indeed, vertebrates and invertebrates share a complex set of control genes and molecular genetic interactions that are responsible for neural induction, regionalized patterning, progenitor proliferation and circuit formation in the developing brain. This suggests that many of the molecular mechanisms involved in building the brains of extant bilaterians may already have been present in their common urbilaterian ancestor.

This work was supported by the Swiss National Science Foundation (SNSF).

Heinrich Reichert\*

Biozentrum, University of Basel,  
4056 Basel, Switzerland

\*heinrich.reichert@unibas.ch

- Acampora, D., Annino, A., Turoto, R., Puelles, E., Lucchesi, W., Papalia, A. & Simeone, A. 2005 *Otx* genes in the evolution of vertebrate brain. *Brain Res. Bull.* **66**, 410–420. (doi:10.1016/j.brainresbull.2005.02.005)
- Arendt, D. & Nübler-Jung, K. 1994 Inversion of dorsoventral axis? *Nature* **371**, 26. (doi:10.1038/371026a0)
- Arendt, D., Denes, A. S., Jékely, G. & Tessmar-Raible, K. 2008 The evolution of nervous system centralization. *Phil. Trans. R. Soc. B* **363**, 1523–1528. (doi:10.1098/rstb.2007.2242)
- Bello, B. C., Izergina, N., Caussinus, E. & Reichert, H. 2008 Amplification of neural stem cell proliferation by intermediate progenitor cells in *Drosophila* brain development. *Neural. Dev.* **3**, e5. (doi:10.1186/1749-8104-3-5)
- Bishop, K. M., Garel, S., Nakagawa, Y., Rubenstein, J. L. R. & O'Leary, D. D. M. 2003 *Emx1* and *Emx2* cooperate to regulate cortical size, lamination, neuronal differentiation, development of cortical afferents and thalamocortical pathfinding. *J. Comp. Neurol.* **457**, 345–360. (doi:10.1002/cne.10550)
- Bray, S. 1998 Notch signalling in *Drosophila*: three ways to use a pathway. *Semin. Cell Dev. Biol.* **9**, 591–597. (doi:10.1006/scdb.1998.0262)
- Brusca, R. C. & Brusca, G. J. 1990 *Invertebrates*. Sunderland, MA: Sinauer Associates.
- Cornell, R. A. & Ohlen, T. V. 2000 *Vnd/nkx*, *ind/gsh*, and *msh/msx*: conserved regulators of dorsoventral neural patterning? *Curr. Opin. Neurobiol.* **10**, 63–71. (doi:10.1016/S0959-4388(99)00049-5)
- Darwin, C. 1859 *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London, UK: John Murray.
- De Robertis, E. M. 2008 Evo–devo: variations on ancestral themes. *Cell* **132**, 185–195. (doi:10.1016/j.cell.2008.01.003)
- Denes, A. S., Jékely, G., Steinmetz, P. R., Raible, F., Snyman, H., Prud'homme, B., Ferrier, D. E., Balavoine, G. & Arendt, D. 2007 Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in Bilateria. *Cell* **129**, 277–288. (doi:10.1016/j.cell.2007.02.040)
- Doe, C. Q. 2008 Neural stem cells: balancing self-renewal with differentiation. *Development* **135**, 1575–1587. (doi:10.1242/dev.014977)
- Doetsch, F. 2003 A niche for adult neural stem cells. *Curr. Opin. Genet. Dev.* **13**, 543–550. (doi:10.1016/j.gde.2003.08.012)
- Dumstrei, K., Wang, F. & Hartenstein, V. 2003 Role of DE-cadherin in neuroblast proliferation, neural morphogenesis, and axon tract formation in *Drosophila* larval brain development. *J. Neurosci.* **23**, 3325–3335.
- Egger, B., Chell, J. M. & Brand, A. H. 2008 Insights into neural stem cell biology from flies. *Phil. Trans. R. Soc. B* **363**, 39–56. (doi:10.1098/rstb.2006.2011)
- Götz, M. & Huttner, W. B. 2005 The cell biology of neurogenesis. *Nat. Rev. Mol. Cell Biol.* **6**, 777–788. (doi:10.1038/nrm1739)
- Guillemot, F. 2007 Spatial and temporal specification of neural fates by transcription factor codes. *Development* **134**, 3771–3780. (doi:10.1242/dev.006379)
- Kiecker, C. & Lumsden, A. 2005 Compartments and their boundaries in vertebrate brain development. *Nat. Rev. Neurosci.* **6**, 553–564. (doi:10.1038/nrn1702)
- Knoblich, J. A. 2008 Mechanisms of asymmetric stem cell division. *Cell* **132**, 583–597. (doi:10.1016/j.cell.2008.02.007)
- Kriegstein, A., Noctor, S. & Martinez-Cerdeno, V. 2006 Patterns of neural stem and progenitor cell division may underlie evolutionary cortical expansion. *Nat. Rev. Neurosci.* **7**, 883–890. (doi:10.1038/nrn2008)
- Lichtneckert, R. & Reichert, H. 2005 Insights into the urbilaterian brain: conserved genetic patterning mechanisms in insect and vertebrate brain development. *Heredity* **94**, 1–12. (doi:10.1038/sj.hdy.6800664)
- Lichtneckert, R., Nobs, L. & Reichert, H. 2008 *empty spiracles* is required for the development of olfactory projection neuron circuitry in *Drosophila*. *Development* **135**, 2415–2424. (doi:10.1242/dev.022210)
- Mallamaci, A., Iannone, R., Briata, P., Pintonello, L., Mercurio, S., Boncinelli, E. & Corte, G. 1998 *EMX2* protein in the developing mouse brain and olfactory area. *Mech. Dev.* **77**, 165–172. (doi:10.1016/S0925-4773(98)00141-5)
- Merkle, F. T. & Alvarez-Buylla, A. 2006 Neural stem cells in mammalian development. *Curr. Opin. Cell Biol.* **18**, 704–709. (doi:10.1016/j.ceb.2006.09.008)
- Reichert, H. & Simeone, A. 2001 Developmental genetic evidence for a monophyletic origin of the bilaterian brain. *Phil. Trans. R. Soc. B* **356**, 1533–1544. (doi:10.1098/rstb.2001.0972)
- Schwann, T. 1839 *Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachstum der Tiere und Pflanzen*. Berlin, Germany: Sander.
- Simeone, A., Puelles, E. & Acampora, D. 2002 The *Otx* family. *Curr. Opin. Genet. Dev.* **12**, 409–415. (doi:10.1016/S0959-437X(02)00318-0)
- Sprecher, S. G., Urbach, R., Technau, G. M., Rijli, F. M., Reichert, H. & Hirth, F. 2006 The columnar gene *vnd* is required for tritocerebral neuromere formation during embryonic brain development of *Drosophila*. *Development* **133**, 4331–4339. (doi:10.1242/dev.02606)
- Tessmar-Raible, K., Raible, R., Christodoulou, F., Guy, K., Rembold, M., Hausen, H. & Arendt, D. 2007 Conserved

- sensory–neurosecretory cell types in annelid and fish forebrain: insights into hypothalamus evolution. *Cell* **129**, 1389–1400. (doi:10.1016/j.cell.2007.04.041)
- Thor, S. & Thomas, J. 2002 Motor neuron specification in worms, flies and mice: conserved and ‘lost’ mechanisms. *Curr. Opin. Genet. Dev.* **12**, 558–564. (doi:10.1016/S0959-437X(02)00340-4)
- Urbach, R. & Technau, G. 2003 Segment polarity and DV patterning gene expression reveals segmental organization of the *Drosophila* brain. *Development* **130**, 3607–3620. (doi:10.1242/dev.00532)
- Zhong, W. & Chia, W. 2008 Neurogenesis and asymmetric cell division. *Curr. Opin. Neurobiol.* **18**, 4–11. (doi:10.1016/j.conb.2008.05.002)