Reconciling competence and transcriptional hierarchies with stochasticity in retinal lineages

Henrik Boije, Ryan B MacDonald and William A Harris

Recent advances suggest that there is a stochastic contribution to the proliferation and fate choice of retinal progenitors. How does this stochasticity fit with the progression of temporal competence and the transcriptional hierarchies that also influence cell division and cell fate in the developing retina? Where may stochasticity arise in the system and how do we make progress in this field when we may never fully explain the behavior of individual progenitor cells?

The conflict

More than two decades ago, clonal analysis in the retina revealed the multipotency of retinal progenitor cells (RPCs) [1–3]. The widely accepted competence model proposed by Livesey and Cepko [4] put multipotency into the context of the previously described evolutionarily conserved order of retinal histogenesis correlated to the fact that clones generated early, produce both early and late generated cell types, while clones generated later produce only late cell types [5,6]. The competence model suggests that RPCs acquire and then lose the ability to make various cell types as retinal development proceeds (Figure 1a). It was proposed that the progression of competence might be largely regulated by extrinsic signalling — that instructive environmental cues could be changing as a function of development [5,7]. However, no convincing instructive cues have been found. Indeed, cell-mixing and transplant experiments revealed that young RPCs in older environments do not change their temporally appropriate fates [8–10]. More recently, it was shown that RPCs grown in isolation give rise to clones that are similar both in size and composition to clones in vivo [11*,12**]. Thus, a changing external environment is neither essential, nor adequate, to achieve histogenetically appropriate fates (although it should be noted that environmental cues may nevertheless provide negative feedback to fine-tune the proportions of cells that acquire particular fates [13–15]). The competence model must therefore rely on an intrinsic progression in fate potential. Indeed, the intrinsic nature of cellular diversification in the developing retina is consistent with a large and growing literature on various of transcription factors (TFs), often working together within hierarchies, that are involved in specifying cell fates [16,17].

A puzzling aspect of retinal development in light of these transcriptional cascades has come from recent theoretical treatments of the statistical properties of retinal clones, which are variable in cell number and fate composition. This work shows that the variability of cell numbers among clones can be accurately accounted for by assuming that RPCs are equipotent and their proliferation is in part stochastic [12**,18*,19**]. This work also shows that cell fate variability among clones is likely to have a partially stochastic explanation [12**,19**]. The fact that proliferation and fate might be in part stochastic does not mean that these processes are uncontrolled, random or unregulated, but rather that they operate according to defined probabilities and predictable ensemble behaviors that are statistically well behaved. Consistent with the predictions of these stochastic models, live imaging studies have shown that the daughters of individual RPCs do not appear to obey a strict temporal program of fates. Rather they sometimes give rise to cell types within a clone that are reversed in their order of appearance to the overall order of histogenesis, and are thus contrary to the predictions of a strict competence model [12**,19**,20] (Figure 1b). These findings raise questions about how our understanding of intrinsic progression of RPCs, and TF hierarchies, can be reconciled with the stochastic nature of clonal lineages.

The transcriptional circuitry of retinal cell fate

It is clear that numerous TFs expressed in RPCs play roles in the specification of retinal cell types. In a number of vertebrates, there is a core transcriptional hierarchy

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Figure 1

The competence model. The classical view sees retinal progenitor cells progressing through competence windows during which a particular cell type is generated (a). Recent studies suggest that although a unidirectional transition of competence occurs, progenitor cells choose from multiple fates at any one time (b).

(Figure 2), which can explain some of the molecular decisions that retinal cells must make to achieve particular fates. The TF Atoh7 is required for the generation of GCs [21,22] and prevents PR fate by inhibiting genes required for their development [21,23]. Loss of Atoh7 leads to an increase in cone PRs suggesting that the absence of Atoh7 provides a permissive environment for a fate shift to cones [24]. Ptf1a can inhibit Atoh7 expression and is necessary for the specification of HCs and ACs [25,26]. Misexpression of Ptf1a causes an increase in HCs and ACs at the expense of GCs, PRs and BCs indicating that Ptf1a is sufficient for the re-specification of these cell types [20,26]. Vsx2 is initially expressed throughout the RPC pool, and represses the expression of Atoh7, FoxN4 (an upstream regulator of Ptf1a expression) and Vsx1 [27,28]. Vsx2 is down-regulated in all but a small population of RPCs that will give rise to a subset of BCs and MCs. The Vsx1-lineage gives rise to a subset of BCs distinct from the Vsx2-lineage derived BCs [27]. Loss of FoxN4 or Ptf1a prevents HC genesis, severely reduces the number of ACs, and leads to an increase of PRs and GCs [25,29,30].

While this core hierarchy may explain how the major cell types arise, a number of studies, too many to review here, have revealed that several additional factors that also influence particular retinal fates (Figure 3, revised from [31]). Moreover, the simultaneous expression of two or more TFs can synergistically influence fate suggesting that combinatorial coding also plays an influential role cell fate diversification [32–34]. From these studies, it appears that the intrinsic core hierarchy of retinal cell determination is overlaid with a complex weave of transcriptional circuitry that makes it challenging to predict which cell types will arise from particular progenitors.

Many of the TFs discussed above are expressed only when cells exit or are about to exit the cell cycle and seem to act by specifying one fate over another. In other words, most of them control what the daughter cells of RPCs will become once they exit the cycle but not the competence of RPCs. Competence controlling factors should be expressed in dividing RPCs during the time that they are making particular cell types. They should also act upstream of the fate determining genes, perhaps by increasing the chance that particular sets of these fate determining genes are turned on or off. Such temporal competence factors are clearly seen in Drosophila CNS neuroblasts, where a sequence of fate-influencing TFs starting with Hunchback are expressed [35]. There is some evidence for similar temporal competence factors playing a role in vertebrate neurogenesis. For example, in the mouse, RPCs pass through an early stage in which they express Ikaros, the vertebrate orthologue of Hunchback. Ikaros, when overexpressed, biases the production of early fates, while Ikaros mutant mice have
Core transcriptional hierarchies. During the early proliferative phase of retinal development all RPCs express Vsx2, which inhibit factors such as Atoh7 and Vsx1. As development progress this inhibition is abolished and genes influencing cell fate are expressed. Depending on the level of Atoh7, and presence or not of Ptf1a, the progenitor follows different paths giving rise to different cell fates. The Vsx1-lineage gives rise to a distinct population of BCs from the population expressing Vsx2.

reduced numbers of early-born cell types [36,37]. One cannot rule out the possibility that an entire sequence of competence factors homologous to those found in Drosophila neuroblasts will be found in the vertebrate retina, but at present there is scant evidence for this.

**Clonal stochasticity**

The statistical distribution of clone sizes seen in both late rat RPCs in vitro and zebrafish RPCs in vivo fits well with a model that assumes RPCs are equipotent but that the mode of division (proliferative (PP) versus asymmetric (PD) versus differentiative (DD)) is stochastic [12**,19**]. Layered on top of this stochasticity, however, is a progressive program in which the probability for particular modes of division ontogenetically evolves. For example, in the zebrafish retina at early stages, all divisions are proliferative. This is followed by a period where each division mode (PP, PD and DD) occurs with approximately equal probability. The final stage of retinal proliferation is another stochastic period dominated by DD divisions (Figure 4) [19**]. This simple model not only accounts for the distribution of clone sizes from RPCs at different stages of development, it also accurately predicts division patterns observed in a population of individual RPCs in vivo.

The choice of fate also appears to have a stochastic element. Gomes et al. [12**], found that the cell fates in more than one hundred clones from a rat retina were largely consistent with the hypothesis that these late progenitors were equipotent but choosing their fates stochastically, with the relative possibility for each cell type being equivalent to the proportions of these cells in the mature retina. However, it has to be said that a few combinations of fate within clones appear more or less frequently than expected, indicating that in addition to the overriding stochasticity, there may also be some preprogrammed motifs operating according to underlying, but as yet unknown, rules. For example, it was recently found that a subset of RPCs express the TF Olig2 and were biased toward production of rod PRs and ACs [38]. Similarly, GCs that respond to vertical motion arise from progenitors that express Cdh6 [39]. In the zebrafish analysis [19**], there were also some patterns that could not be explained by a stochastic mechanism, such as the fact that at late stages of retinogenesis, most PRs, BCs and HCs come in pairs. All of these instances, however, may reflect the action of TFs operating very close to the last division to specify particular fates. Asymmetrically inherited Numb may also be at play here. If, for example, Numb, is inherited by one of the two daughters at a terminal division, the two daughter will chose two different fates (e.g. a dominant fate taken by the Numb inheriting cells and a secondary fate taken by the other). This could explain how some terminal divisions are partially patterned, though it may be impossible to predict in advance of the division which daughter will inherit Numb and thus which daughter will take which fate [40].

**Why stochasticity?**

It is interesting to speculate about the mechanisms that generate stochasticity within retinal lineages. We can imagine that levels of TFs themselves might be variable, due to dynamic changes in transcription rate, translation efficiency, or mRNA and protein stability (as reviewed in [41]). There may even be mechanisms for generating a stochastic outcome. For example, the choice of red versus green opsin in the primate retina relies on the random looping of DNA to bring a single promoter region adjacent to one of the two protein coding regions [42,43]. Variability may also arise through post-transcriptional mechanisms involving mi-RNAs and long noncoding RNAs, or post-translational mechanisms such protein phosphorylation and ubiquitination through interaction with cell cycle enzymes [44,45]. It is also likely that epigenetics, the packing and remodeling of chromatin in the nucleus, will affect the chance that a specific locus will fire or not [46].
The Notch-Delta signaling mechanism may also contribute to the stochastic decisions that RPCs make [47–49]. This mechanism can magnify small fluctuations in fate potential and may also lead to oscillations. Indeed the expression of the Notch downstream target, Hes1, is known to oscillate at rates much shorter than the minimal cell cycle time in neural progenitor cells in culture, and pairs of interacting cells may oscillate out of phase with each other.

The complexity of transcriptional regulation. Although key factors can explain some of the diversification there are numerous factors affecting fate outcome. Cross-talk between branches increase the complexity of the system.
each other [50]. The pattern generator for this rhythm may lie within individual progenitors due to a cell intrinsic double negative feedback loop in which miR-9 controls the stability of Hes1 mRNA, while Hes1 represses the transcription of miR-9 [51]. Recent studies in the mouse telencephalon have shown that proneural TFs also oscillate in progenitor cells possibly in response to the oscillations of the Hes1 repressor [52]. Interkinetic nuclear migration along the apico-basal axis of the neuroepithelium may also contribute to stochasticity through this pathway. For example, Notch signaling tends to be apical, and cells whose nuclei are more apical may be influenced to a greater extent [53]. But as the apico-basal movements of RPC nuclei throughout most of interphase are themselves stochastic [54], the efficacy of Notch signaling could be affected by this random one-dimensional walk. Similarly, as mentioned above, the asymmetric inheritance at the last division of Numb, a negative regulator of Notch signaling, may contribute to stochasticity by influencing which daughter cell which chooses a dominant fate and which will choose a secondary one [39].

Finally, it is unknown to what extent the multiple transcriptional hierarchies present within RPCs interact. In the face of combinatorial coding mechanisms where different TFs have non-additive influences on fate choice, asynchronous, loosely coupled, or independently firing networks could mean that such combinations of TFs may appear probabilistically within single RPCs. All of these stochasticity-generating mechanisms may be going on simultaneously within RPCs, suggesting that a high level of uncertainty is inherent in this system. This is not necessarily a bad thing. Complex systems in which many variables interact often produce robust and well-behaved distributions such as the relative proportions of ‘snake eyes’ versus ‘lucky sevens’ in a large population of dice throws. Similarly, although individual RPCs give rise to clones that are highly variable, the total number of differentiated retinal cells generated from the 2000 or so RPCs of the zebrafish optic vesicle will always be very close to 22,000, and within this large set of differentiated retinal cells, all the major neuronal types will proportionally represented [19**].

Research after reconciliation
Stochasticity can be seen as a problem. It may be disappointing to think that we may never be able to predict exactly what a set of RPCs will do; which cells will divide how many times and what the fate outcomes of these divisions will be. But while this kind of stochasticity is like a cloud that obscures the answers to certain questions, it is a cloud that has a silver lining, in that it focuses our attention on other questions that may be easier to address, and even perhaps more interesting. For example, recent studies show that eliminating certain TFs leads to fate switches in daughter cells rather than the death of particular cell types. As a result, such retinas may have vastly altered cell fate distributions while the number of cells in such retinas may be very similar to wild type retinas. Such results suggest that proliferation and fate may therefore be best explained by independent and largely uncoupled stochastic mechanisms, and this makes sense as many of the TFs that have major roles in cell fate are not expressed until cells are about to leave or have just left the cell cycle. Another important issue is that, in spite of the stochastic noise, retinal development clearly progresses through distinct phases of proliferation and cell fate probabilities (i.e. at each stage of development we can accurately predict the population distributions of proliferative/differentiative divisions and the cell fate distributions). Clearly, the next step is to understand what it is that determines the transition between these phases. What is the timer and how does it work? We would also like to know more about how the probability profiles at each phase are themselves controlled. In the developing retinas of some animals, for example, it is likely that probability of asymmetrical divisions during the middle phases will be higher than in other animals, or the probability of rods may be much higher than the probability of cones. What are the factors that set these probabilities and are they the same factors that are at the heart of the evolution of retinal size and cellular composition within vertebrates? Finally, we would like to know more about the extrinsic versus intrinsic influences on cell proliferation and fate. For example, do large clones tend to have small clones as neighbors, or are the decisions that are made within each clone independent of the behavior of neighboring clones? Therefore, crucially, while the mechanisms that generate stochasticity are interesting to consider, it may be more productive to investigate those features of retinal development that are independent of stochasticity and remain salient in spite of it. Lastly, it will be useful to know if the concepts outlined here for the retina are also applicable to other parts of the nervous system, or even other tissues.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Holt CE, Bertsch TW, Ellis HM, Harris WA: Cellular determination in the Xenopus retina is independent of lineage and birth date. Neuron 1988, 1:15-26.

2. Turner DL, Cepko CL: A common progenitor for neurons and glia persists in rat retina late in development. Nature 1987, 328:131-136.
3. Wets R, Fraser SE: Multipotent precursors can give rise to all major cell types of the frog retina. Science 1989, 239:1142-1145.

4. Livesey FJ, Cepko CL: Vertebrate neural cell fate determination: lessons from the retina. Nat Rev Neurosci 2001, 2:109-118.

5. Cepko CL, Austin CP, Yang X, Alexiades M, Ezzedine D: Cell fate determination in the vertebrate retina. Proc Natl Acad Sci U S A 1996, 93:589-595.

6. Wong LL, Rapaport DH: Defining retinal progenitor cell competence in Xenopus laevis by clonal analysis. Development 2009, 136:1707-1715.

7. Harris WA: Cellular diversification in the vertebrate retina. Curr Opin Genet Dev 1997, 7:651-658.

8. Watanabe T, Raff MC: Rod photoreceptor development in vitro: intrinsic properties of proliferating neuroepithelial cells change as development proceeds in the rat retina. Neuron 1990, 4:461-467.

9. Belliveau MJ, Young TL, Cepko CL: Late retinal progenitor cells show intrinsic limitations in the production of cell types and the kinetics of opsins expression. J Neurosci 2000, 20:2247-2254.

10. Rapaport DH, Patheal SL, Harris WA: Cellular competence plays a role in photoreceptor differentiation in the developing Xenopus retina. J Neurobiol 2001, 49:129-141.

11. Cayouette M, Barres BA, Raff M: Importance of intrinsic mechanisms in cell fate decisions in the developing rat retina. Neuron 2003, 40:687-804.

This study highlights the importance of intrinsic factors during fate commitment of retinal progenitor cells. Embryonic day 16-17 rat retinal progenitor cells were grown in serum-free or serum-containing clonal-density cultures. The clones generated were similar regarding their proliferative behaviour, fate assignment and birth order suggesting that extracellular cues may be less important than previously assumed.

12. Gomes FL, Zhang G, Carbonell F, Correa JA, Harris WA, Simons BD, Cayouette M: Reconstruction of rat retinal progenitor cell lineages in vitro reveals a surprising degree of stochasticity in cell fate decisions. Development 2011, 138:227-235.

Long-term videomicroscopy allowed the reconstruction of clones generated by single RPCs in vivo. The results revealed that the mode of division of RPCs was strikingly consistent with a stochastic pattern of behavior in which the division to multiply or differentiate is set by fixed probabilities. It further emphasized the importance of cell-intrinsic properties during fate commitment.

13. Kim J, Wu HH, Lander AD, Lyons KM, Matzuk MM, Calof AL: GDF11 controls the timing of progenitor cell competence in developing retina. Science 2005, 308:1927-1930.

14. Wallace VA: Proliferative and cell fate effects of Hedgehog signaling in the vertebrate retina. Brain Res 2008, 1192:61-75.

15. Waid DK, McLoon SC: Ganglion cells influence the fate of dividing retinal cells in culture. Development 1998, 125:1059-1066.

16. Xiang M: Intrinsic control of mammalian retinogenesis. Cell Mol Life Sci 2012, 70:2519-2532.

17. Bassett EA, Wallace VA: Cell fate determination in the vertebrate retina. Trends Neurosci 2012, 35:565-573.

18. Slater JL, Landman KA, Hughes BD, Shen Q, Temple S: Cell lineage tree models of neurogenesis. J Theor Biol 2009, 256:164-179.

The paper introduces mathematical models which are able to explain the stochasticity during proliferation seen in experimental data during mouse cortical neurogenesis.

19. He J, Zhang G, Almeida AD, Cayouette M, Simons BD, Harris WA: How variable clones build an invariant retina. Neuron 2012, 75:785-799.

Imaging of clones generated during zebrafish retina development allowed the reconstruction of lineage-trees in vivo. The lineage data was consistent with a simple model in which the retina is derived from a set of equipotent retinal progenitor cells that are subject to stochastic factors controlling lineage progression. The data also reveal that the overlapping histogenesis of fates seen in the overall population is accounted for by the variability of cell determination within clones.

20. Jusuf PR, Almeida AD, Randlett O, Joubin K, Poggi L, Harris WA: Origin and determination of inhibitory cell lineages in the vertebrate retina. J Neurosci 2011, 31:2549-2562.

21. Yang Z, Ding K, Pan L, Deng M, Gan L: Math5 determines the competence state of retinal ganglion cell progenitors. Dev Biol 2003, 264:240-254.

22. Feng L, Xie Z-H, Ding Q, Xie X, Libby RT, Gan L: MATH5 controls the acquisition of multiple retinal cell fates. Mol Brain 2010, 3:36.

23. Le TT, Wroblewski E, Patel S, Riesenberg AN, Brown NL: Math5 is required for both early retinal neuron differentiation and cell cycle progression. Dev Biol 2006, 295:764-778.

24. Brown NL, Patel S, Brzezinski J, Glaser T: Math5 is required for retinal ganglion cell and optic nerve formation. Development 2001, 128:2497-2508.

25. Fujitani Y, Fujitani S, Luo H, Qiu F, Burlison J, Long Q, Kawaguchi Y, Edlund H, MacDonald RJ, Furukawa T et al.: Ptfla determines horizontal and amacrine cell fates during mouse retinal development. Development 2006, 133:4439-4450.

26. Leleivre EC, Lek M, Boije H, Houille-Vernes L, Brjeuil V, Slembruck A, Roger JE, Sahel JA, Matter JM, Sennlaub F et al.: Ptfla and Rbpj complex inhibits ganglion cell fate and drives the specification of all horizontal cell subtypes in the chick retina. Dev Biol 2011, 358:296-308.

27. Vitorino M, Jusuf PR, Mauros D, Kimura Y, Higashijima S-I, Harris WA: Vsx2 in the zebrafish retina: restricted lineages through derepression. Neural Dev 2009, 4:14.

This study reveals how the multipotency of retinal progenitors is regulated by a few core factors working as either inhibitors or activators. The continued proliferation is ensured and differentiation is prevented by Vsx2 inhibiting the expression of fate determinants such as Atoh7, FoxN4 and Vsx1.

28. Clark AM, Yun S, Veien ES, Wu YY, Chow RL, Dorsky RI, Levine EM: Negative regulation of Vsx1 by its paralog Chx10/ Vsx2 is conserved in the vertebrate retina. Brain Res 2008, 1192:99-113.

29. Dullin JP, Locker M, Robach M, Henningfeld KA, Parain K, Afelsk S, Pieler T, Perron M: Ptfla triggers GABABergic neuronal cell fates in the retina. BMC Dev Biol 2007, 7:110.

30. Li S, Mo Z, Yang X, Price SM, Shen MM, Xiang M: FoxN4 controls the genesis of amacrine and horizontal cells by retinal progenitors. Neuron 2004, 43:795-807.

31. Zhang X, Serb JM, Greenlee MH: Mouse retinal development: a dark horse model for systems biology research. Bioinform Biol Insights 2011, 8:99-113.

32. Hatakeyama J, Tomita K, Inoue T, Kageyama R: Roles of homeobox and bHLH genes in specification of a retinal cell type. Development 2001, 128:1313-1322.

33. Hatakeyama J, Kageyama R: Retinal cell fate determination and bHLH factors. Semin Cell Dev Biol 2004, 15:83-89.

34. Wang JCC, Harris WA: The role of combinational coding by homeodomain and bHLH transcription factors in retinal cell fate specification. Dev Biol 2005, 285:101-115.

35. Ishikawa T, Pearson B, Holfbrook S, Doe CQ: Drosophila neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. Cell 2001, 106:511-521.

36. Elliott J, Jolicoeur C, Ramamurthy V, Cayouette M: Ikarsos confers early temporal competence to mouse retinal progenitor cells. Neuron 2008, 60:26-39.

This paper reveals the partially conserved intrinsic machinery conveying temporal competence to retinal progenitors. The presence of Ikarsos, the ortholog of Drosophila transcription factor Hunchback, is necessary and sufficient to specify early-born neuronal identity.

37. Tarchini B, Jolicoeur C, Cayouette M: In vivo evidence for unbiased ikarsos retinal lineages using an ikarsos-cre mouse line driving clonal recombination. Dev Dyn 2012, 241:1973-1985.
38. Hafler BP, Surzhenko N, Beier KT, Punzo C, Trimarchi JM, Kong JH, Cepko CL: Transcription factor Olig2 defines subpopulations of retinal progenitor cells biased toward specific cell fates. Proc Natl Acad Sci U S A 2012, 109:7882-7887.

39. De la Huerta I, Kim IU, Voinescu PE, Sanes JR: Direction-selective retinal ganglion cells arise from molecularly specified multipotential progenitors. Proc Natl Acad Sci U S A 2012, 109:17663-17668.

40. Kechad A, Jolicoeur C, Tufford A, Mattar P, Chow RW, Harris WA, Cayouette M: Numb is required for the production of terminal asymmetric cell divisions in the developing mouse retina. J Neurosci 2012, 32:17197-17210.

41. Karrn M, Elston TC, Blake WJ, Collins JJ: Stochasticity in gene expression: from theories to phenotypes. Nat Rev Genet 2005, 6:451-464.

42. Roorda A, Williams DR: The arrangement of the three cone classes in the living human eye. Nature 1999, 397:520-522.

43. Johnston RJ, Desplan C: Stochastic mechanisms of cell fate specification that yield random or robust outcomes. Annu Rev Cell Dev Biol 2010, 26:689-719.

44. Swain PS: Efficient attenuation of stochasticity in gene expression through post-transcriptional control. J Mol Biol 2004, 344:956-976.

45. Raser JM, O’Shea EK: Noise in gene expression: origins, consequences, and control. Science 2005, 309:2010-2013.

46. Hu XL, Wang Y, Shen Q: Epigenetic control on cell fate choice in neural stem cells. Protein Cell 2012, 3:278-290.

47. Austin CP, Feldman DE, Ida JA Jr, Cepko CL: Vertebrate retinal ganglion cells are selected from competent progenitors by the action of Notch. Development 1995, 121:3637-3650.

48. Dorsky RI, Rapaport DH, Harris WA: Xotch inhibits cell differentiation in the Xenopus retina. Neuron 1995, 14:487-496.

49. Dorsky RI, Chang WS, Rapaport DH, Harris WA: Regulation of neuronal diversity in the Xenopus retina by Delta signaling. Nature 1997, 385:67-70.

50. Shimojo H, Ohtsuka T, Kageyama R: Oscillations in notch signaling regulate maintenance of neural progenitors. Neuron 2008, 58:52-64.

51. Bonev B, Stanley P, Papalopulu N: MicroRNA-9 Modulates Hes1 ultradian oscillations by forming a double-negative feedback loop. Cell Rep 2012, 2:10-18.

52. Imayoshi I, Isomura A, Harima Y, Kawaguchi K, Kori H, Miyachi H, Fujiiwa T, Ishidate F, Kageyama R: Oscillatory control of factors determining multipotency and fate in mouse neural progenitors. Science 2013.

53. Del Bene F, Wehman AM, Link BA, Baier H: Regulation of neurogenesis by interkinetic nuclear migration through an apical–basal notch gradient. Cell 2008, 134:1055-1065.

54. Norden C, Young S, Link BA, Harris WA: Actomyosin is the main driver of interkinetic nuclear migration in the retina. Cell 2009, 138:1195-1208.