Int. J. Dev. Biol. 57: 341-350 (2013)
doi: 10.1387/ijdb.120259jf

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Lateral inhibition and neurogenesis: novel aspects in motion

PAU FORMOSA-JORDAN[1], MARTA IBAÑES[1], SAÚL ARES[2,3] and JOSÉ-MARÍA FRADE*[,4]

[1]Department of Estructura i Constituents de la Matèria, Facultat de Física, Universitat de Barcelona, Barcelona, [2]Logic of Genomic Systems Laboratory, [3]Grupo Interdisciplinar de Sistemas Complejos (GISC), Spanish National Biotechnology Centre CNB-CSIC, Madrid and [4]Department of Molecular, Cellular and Developmental Neurobiology, Cajal Institute, IC-CSIC, Madrid, Spain.

ABSTRACT Neuronal production in metazoans is tightly controlled by Delta/Notch-dependent signals regulating lateral inhibition. It is currently thought that lateral inhibition takes place in clusters of precursors with equal capacity to trigger and receive Notch-dependent inhibitory signals. However, this view neglects crucial dynamical aspects of the process. In this review, we discuss two of these dynamic factors, whose alterations yield dysfunctions in neurogenesis. First, precursors show variable neurogenic capacity as they go through the cell cycle. Second, differentiating precursors are in direct contact with non-neurogenic cells at the wavefront of expanding neurogenic domains. We discuss the mechanisms adopted by Metazoa to prevent these dysfunctions in the lateral inhibitory process, which include cell cycle synchronization occurring in the invertebrate neural epithelium and during primary neurogenesis in anamniotes, interkinetic nuclear movement in the vertebrate neuroepithelium and generalized Delta expression ahead of the neurogenic wavefront. The emerging concept is that lateral inhibition during neurogenesis occurs in dynamic clusters of precursors and requires specific mechanisms to avoid distortions resulting from the interaction between neurogenic and non-neurogenic precursors. The advance in visualizing Notch dynamics with real-time imaging at cellular and subcellular levels will notably contribute to our understanding of these novel "aspects of motion" in neurogenesis.

KEY WORDS: *notch, delta, cell cycle, interkinetic nuclear movement, neurogenic wavefront*

Introduction

During the development of the nervous system, neural precursors proliferate and give rise to neurons through a process known as neurogenesis. Although the cellular mechanism of neurogenesis may vary between vertebrates and invertebrates, remarkably this process has a common molecular mechanism in all metazoans, based on the expression of a set of proteins that belong to the bHLH family of transcription factors encoded by proneural genes (Bertrand *et al.*, 2002). The expression of proneural genes is controlled through a mechanism referred to as lateral inhibition with feedback (Collier *et al.*, 1996), dependent on the signaling of the neurogenic receptor Notch upon activation by its neurogenic Dl ligand (Fig. 1A,B). Precursors committed to differentiate express high levels of Dl, subsequently delivering strong Notch-dependent signals to the surrounding precursors. The activation of Notch in these latter cells represses the expression of proneural genes and Dl itself, thereby preventing neuronal differentiation and the subsequent delivery of lateral inhibitory signals to differentiating precursors (Fig. 1A,B). Through this mechanism, commitment to differentiation is reinforced in differentiating precursors while it becomes abolished in their surrounding cells (Fig. 1C). The classical view, derived from early studies in *Drosophila*, proposes that lateral inhibition takes place in groups of adjacent equivalent cells called proneural clusters, having all equal capacity to express neurogenic and proneural genes and to differentiate (Simpson, 1990). In this context, lateral inhibition with feedback only enables the selection of a few precursors for differentiation (Fig. 1C) (Collier *et al.*, 1996), thus facilitating the maintenance of a pool of precursor cells for successive rounds of neurogenesis (Murciano *et al.*, 2002). Along

Abbreviations used in this paper: ac, achaete; ato, atonal; bHLH, basic helix-loop-helix; CNS, central nervous system; Dl, Delta; Dll1, Delta-like-1; emc, extra macrochaetae; h, hairy; HLH, helix-loop-helix; hh, hedgehog; hpf, hours postfertilization; lFng, lunatic fringe; INM, interkinetic nuclear movement; MF, morphogenetic furrow; NICD, Notch intracellular domain; sc, scute; PNS, peripheral nervous system; Shh, Sonic hedgehog; stg, string.

***Address correspondence to:** José María Frade. Cajal Institute, IC-CSIC, Avenida Doctor Arce 37, E-28002 Madrid, Spain. Tel.: +34 91 585 4740. Fax: +34 91 585 4754. E-mail: frade@cajal.csic.es - web: http://www.cajal.csic.es/ingles/departamentos/frade-lopez/frade-lopez.html

Accepted: 11 April 2013. *Final, author-corrected PDF published online:* 10 July 2013.

ISSN: Online 1696-3547, Print 0214-6282

these lines we discuss two dynamic processes which can drive non-equivalent cells with different neurogenic capacity to be in direct contact with each other. One case derives from the temporal variation of neurogenic capacity, i.e., capacity to express proneural and neurogenic genes, observed in neuronal precursors as they go through the cell cycle (Fig. 2A). In this case, adjacent cells in different phases of the cell cycle are expected to have different neurogenic capacity and hence a disrupted mutual lateral inhibition, thus leading to an exacerbation of neuronal production (Murciano *et al.*, 2002). An additional dynamic process by which cells with different neurogenic capacity can become in contact arises at the border of neurogenic regions expanding through areas constituted by non-neurogenic cells (Fig. 2C). In this case, non-neurogenic cells ahead of the growing neurogenic wavefront are expected to alter the neurogenic process since lateral inhibition with feedback is disrupted at the wavefront (Formosa-Jordan *et al.*, 2012).

In this review we first describe evidences for the acquisition of neurogenic capacity at specific cell cycle stages in precursor cells from different organisms and its relevance for neurogenesis. Based on the reported cell cycle dynamics between adjacent cells and the effects when these dynamics are altered, we propose two mechanisms used to prevent alterations arising from this variation of neurogenic capacity along the cell cycle (Fig. 2B). Finally, we review the inhibitory potential of non-neurogenic cells at the wavefront, proposing a mechanism to prevent potential distortions of the neurogenic process (Fig. 2D).

The neurogenic capacity of neural precursors is linked to specific stages of the cell cycle

Several studies in metazoans indicate that the capacity of neural precursors to express neurogenic and proneural genes becomes enhanced during G2, mitosis, and/or early G1. One example of a proneural gene whose expression becomes enhanced during G1 is *ato*, a gene required for photoreceptor differentiation in the eye imaginal disc of *Drosophila*. In this structure, *ato* is initially expressed by retinal precursors undergoing G1 and then it remains expressed in the differentiating photoreceptors (Jarman *et al.*, 1994). Proneural gene expression in a specific cell cycle window can also be observed in the wing disc of *Drosophila*, which gives rise to several structures, among them the sensory bristles of the adult wing margin. This is the case of *ac* and *sc*, two proneural genes that specify the sensory bristles of the adult wing margin. *ac* and *sc* become expressed by bristle precursor cells in G2 (Johnston and Edgar, 1998), and their expression becomes extinguished just before reentry into the cell cycle (Romani *et al.*, 1989). In this case, premature entrance into the cell cycle of the arrested sensory precursors is associated to a loss of sensory precursor cells, following a transcriptional downregulation of the proneural *ac/sc* genes (Nègre *et al.*, 2003). Another example of the association of neurogenesis with a particular cell cycle stage in *Drosophila* can be found in the neural precursors from the outer optic anlagen. This structure gives rise to the outer part of the medulla and lamina of the optic lobes, and their constituting neuroepithelial cells require to be in G1 to progress from neuroepithelial cells to neuroblasts (Reddy *et al.*, 2010), concomitant with an increase of Notch activity that facilitates the neuroblast fate selection (Weng *et al.*, 2012). Forcing cell cycle progression in the neuroepithelial cells with cell cycle regulators such as cyclin D, cdk4, E2F1 and DP has been shown to lead to a differentiation delay with a concomitant prevention of Dl expression (Reddy *et al.*, 2010).

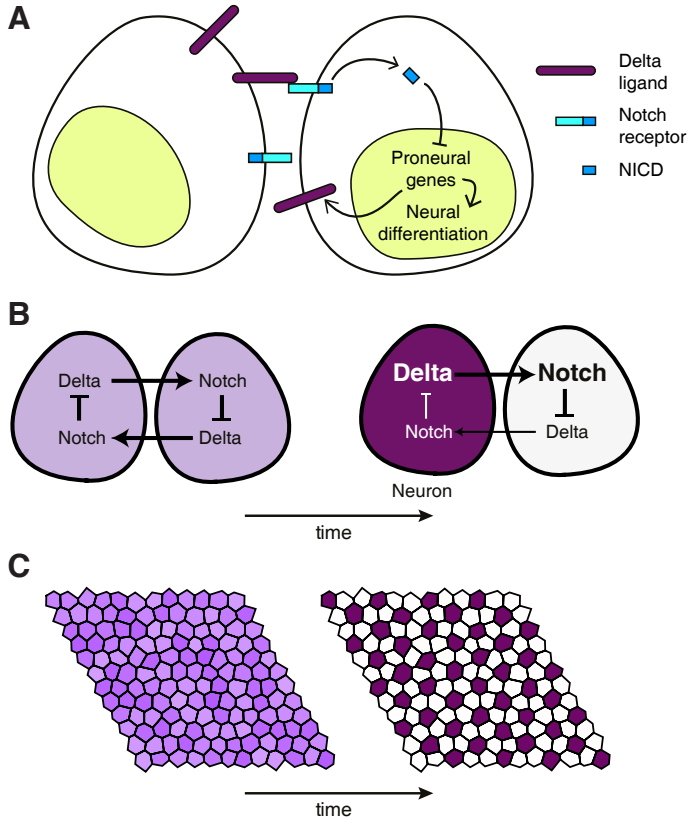

Fig. 1. Neurogenic selection through lateral inhibition. (A) *The Notch signaling pathway mediates lateral inhibition. The neurogenic receptor Notch is activated upon the binding to the Delta ligand that is anchored on the membrane of a neighboring cell. This triggers the translocation of the Notch intracellular domain (NICD) to the nucleus, which ultimately inhibits the proneural genes. Proneural genes drive the expression of Delta and also enable neural differentiation. Hence, a cell expressing the Delta ligand inhibits the production of the ligand in its neighboring cell.* **(B)** *Mutual lateral inhibition between neighboring cells drives an intercellular positive feedback loop. The positive feedback amplifies small differences between cells that are initially equivalent (left) leading to two mutually exclusive fates (right). Normal and blunt arrows within and between cells denote activation and inhibition respectively. The size of the arrows and the Delta/Notch labeling indicates the importance of such elements.* **(C)** *The classical view of lateral inhibition proposes that mutual inactivation between cells occurs in a tissue of equivalent precursors with the same neurogenic potential (left), leading to an ordered salt-and-pepper pattern of two cell fates. This feedback between equivalent neurogenic precursors enables the proper neurogenic selection of cells (purple cells) maintaining a pool of neurogenic precursors (white cells) for successive rounds of neurogenesis.*

In vertebrates, the expression of proneural and neurogenic genes has also been shown to vary as the neuronal precursors proceed throughout the cell cycle (for a comprehensive review see Latasa *et al.*, 2009). In this regard, *Notch1* expression is much higher in neural progenitors undergoing G2/M/early G1 than in those in S-phase in different neural tissues from vertebrates including chick (Murciano *et al.*, 2002; Cisneros *et al.*, 2008), mouse (Cisneros *et al.*, 2008), and zebrafish (Del Bene *et al.*, 2008). Moreover, *Dll1* and the proneural gene *Neurogenin 2* (*Ngn2*) have both been shown

to initiate their expression during G2 in chick and mouse progenitors fated to differentiate (Murciano *et al.*, 2002; Cisneros *et al.*, 2008). In vertebrates, the capacity to express the neurogenic genes *Notch1* and *Dll1* in a cell cycle-dependent manner is dependent on the stability of their transcripts (Cisneros *et al.*, 2008). Indeed, the RNA binding protein Elavl1/HuR, which is expressed by the neuronal precursors in G2/M/early G1, has been shown to interact with the 3' untranslated region of *Dll1* mRNA and stabilize this transcript (García-Domínguez *et al.*, 2011). So far, the mechanism that increases proneural gene expression in neural precursors undergoing G2/M/early G1 remains unknown.

Evidence discussed above indicates that the "neurogenic capacity" of neuronal precursors, as indicated by the capacity to express proneural and neurogenic genes, seems to be restricted to particular stages of the cell cycle. This suggests that, in the absence of mechanisms preventing mutual interaction of precursors with different neurogenic potential, the normal process of lateral inhibition would be disrupted, resulting in an increase of the rate of neurogenesis (Murciano *et al.*, 2002). We propose that metazoans have adopted two different strategies to avoid this possibility (Fig. 2B). On the one hand, neural precursors from monostratified neuroepithelia show waves of cell cycle synchronization that create clusters of precursors with neurogenic capacity when they enter into G2/M (Fig. 2B top). This strategy can be observed in the developing nervous system of invertebrates as well as during primary neurogenesis in anamniotes (see below). On the other hand, vertebrates have adopted an alternative mechanism derived from the increase of cellular density that occurs in the vertebrate neuroepithelium, which is crucial for the generation of the enormous amount of neurons constituting the vertebrate brain. Vertebrate neural precursors divide vigorously in an unsynchronized manner, creating a highly packed, pseudostratified neuroepithelium characterized by the position of the nuclei at different levels, depending on their stage in the cell cycle (Fig. 2B bottom). Nuclei, therefore, move from basal to apical positions during the S/G2 transition to undergo mitosis apically, while they move backwards as they progress from G1 to S-phase. This to-and-fro displacement of the nuclei, referred to as interkinetic nuclear movement (INM) (Frade, 2002), creates an apical neurogenic region in the neuroepithelium, equivalent to a proneural cluster, that allows lateral inhibition to take place in precursors undergoing G2/M/early G1 (Murciano *et al.*, 2002; Del Bene *et al.*, 2008; Latasa *et al.*, 2009). Therefore, the vertebrate neuroepithelium is characterized by its capacity to continuously produce neurons while maintaining a local synchronization of precursors showing neurogenic capacity in G2/M/earlyG1. This strategy likely facilitates an uninterrupted production of neurons, necessary for the creation of large vertebrate brains.

Waves of cell cycle synchronization facilitate the creation of proneural clusters constituted by precursors with equivalent neurogenic capacity

The *Drosophila* embryo contains at least twenty-five clusters of cells undergoing locally synchronous mitosis (i.e. "mitotic domains") (Foe, 1989; Hartenstein *et al.*, 1994), which progress throughout the cell cycle according to an invariant spatiotemporal pattern (Edgar *et al.*, 1994). Within each cluster, mitosis starts in a single or a small number of cells and then it spreads wave-like in all directions until it stops at the boundary of the cluster (Foe, 1989). During postblastoderm embryogenesis, a number of structures are generated out from these mitotic domains, including the CNS and the sensory organs of the larva (Namba and Minden, 1999; Minden, 2008). Neurogenesis in these structures is regulated by genes of the *ac/sc* complex (Jiménez and Campos-Ortega, 1987),

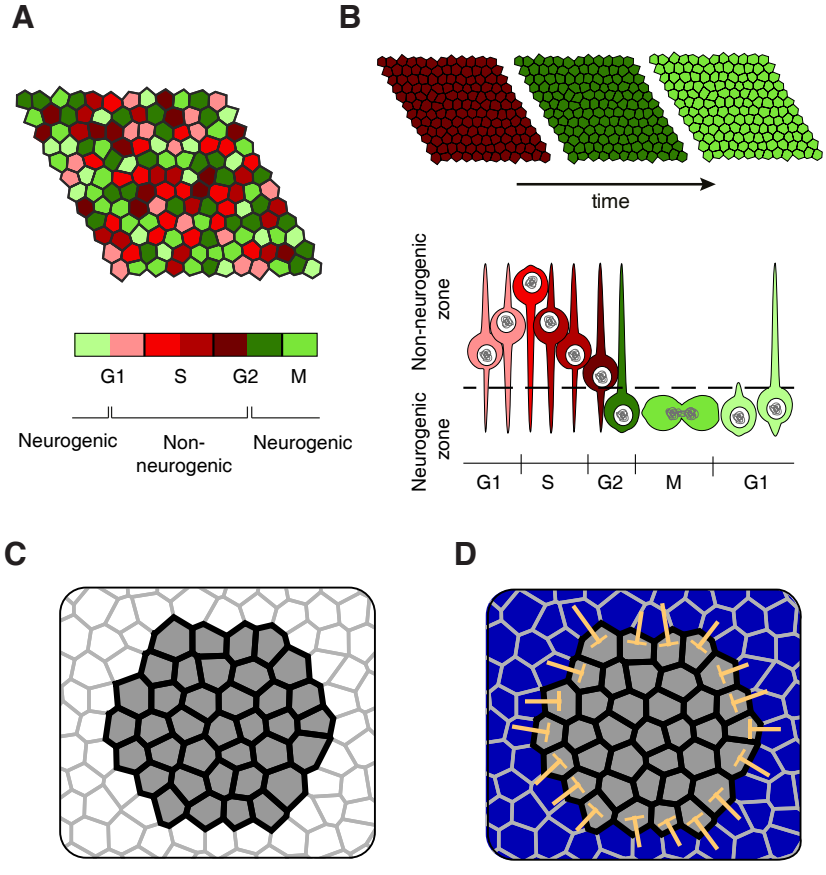

Fig. 2. Two examples in which cells with different neurogenic capacity are in contact, challenging the classical lateral inhibition model. (A) *Precursors at different cell cycle stages have different neurogenic capacity. A tissue with unsynchronized precursors would have intermingling of cells with different neurogenic capacity and disrupted mutual lateral inhibition between cells.* **(B)** *Two different strategies have been adopted to prevent interactions between unsynchronized precursors: (top) synchronization of neural precursors creates clusters of cells with equivalent neurogenic capacity; (bottom) INM in the vertebrate pseudostratified neuroepithelium allows lateral inhibition between apically located precursors (green cells) that are undergoing late G2/M/early G1 phase, while maintaining a pool of cells in the late G1/S/early G2 phases without neurogenic potential (red cells) in the basal part of the epithelium. In (A-B) panels, green refers to neurogenic, and red refers to non-neurogenic.* **(C)** *Neurogenic tissue (gray central cells with black boundaries) is often in contact with surrounding non-neurogenic tissue (white cells with gray boundaries) (white).* **(D)** *A strategy to avoid neurogenic perturbations at the border of neurogenic tissue would consist on a non-neurogenic tissue (blue cells) exerting inhibition (blunt arrows) on the neurogenic border.*

which are initially expressed in cellular clusters (Campuzano and Modolell, 1992). We propose that cell cycle synchronization in these neural epithelia facilitates that proneural clusters are constituted only by precursors with equivalent neurogenic capacity at the time of differentiation, thus avoiding interferences in the lateral inhibition process.

One example of a neural tissue in *Drosophila* showing cell cycle synchronization during the process of neuronal differentiation is the compound eye. The compound eye of *Drosophila* develops during larval life in a specialized retinal epithelium, the eye imaginal disc (Tomlinson and Ready, 1987). During the third larval instar, this tissue undergoes progressive transformation from a relatively amorphous epithelial sac into the complex arrangement of ommatidial facets that comprises the adult compound eye. At a certain point, ommatidia begin to be produced at one extreme, creating the MF, a dorsoventral constriction that sweeps anteriorly across the disc (Wolff and Ready 1991) (Fig. 3A,B). The posterior-to-anterior movement of the MF coordinates cell cycle progression with early events of pattern formation (Tomlinson and Ready, 1987). In this manner, retinal precursors anterior to the MF become synchronized in G2 as a result of the expression of *stg* (Mozer and Easwarachandran, 1999), the *Drosophila* homologue of the *cdc25* gene. These precursors proceed synchronously through mitosis and G1 (Mozer and Easwarachandran, 1999), prior to the start of neurogenesis (Firth and Baker, 2005), and concomitantly with a basal displacement of the nuclei that results in the MF. As cells enter the MF, *ato* expression and pattern formation begins (Jarman *et al.*, 1994; Thomas *et al.,* 1994), and failure to synchronize cells in G1 disrupts ommatidial patterning (Thomas *et al.,* 1994).

As indicated above, the wing disc gives rise to sensory bristles of the adult wing margin. In two subdomains of this structure, G2-arrested cells express the proneural genes *ac* and *sc* (Johnston and Edgar, 1998), which regulate the differentiation of the bristles. Interestingly, *ac*/*sc* expression is extinguished just before reentry into the cell cycle after pupariation (Romani *et al.*, 1989). Therefore, precursors are synchronized in G2 at the time when the decision to differentiate occurs. Consistently, premature entrance into the cell cycle of arrested precursors, induced by overexpression of *stg*, is associated to a loss of precursor cells, following transcriptional downregulation of the determinant proneural *ac*/*sc* genes (Nègre *et al.*, 2003). This is a clear example in which exit from the cell cycle in G2 is required for proper neural cell fate determination.

Another example of cell cycle synchronization required for neurogenesis can be found in the outer optic anlagen. In this region, the constituting neuroepithelial cells need to undergo a transient arrest of the cell cycle in G1 to progress from neuroepithelial cells to neuroblasts (Reddy *et al.*, 2010). In these cells, a differentiation delay can be elicited by overexpressing cell cycle regulators such as cyclin D, cdk4, E2F1 and Dp, with a concomitant delay in the expression of *Dl* (Reddy *et al.*, 2010).

In the pupa stage, delamination of microchaete precursors in the notum takes also place in regions containing synchronized precursor cells, immediately after they undergo mitosis (Hartenstein and Posakony, 1989). Subsequently, sensory organ precursors proliferate in synchrony before becoming differentiated (Hartenstein and Posakony, 1989).

Finally, a similar situation can be observed for the neuroblasts giving rise to the embryonic PNS. During the specification of the sensory organ precursors (sensory mother cells), they become arrested in G2 (Kimura *et al.*, 1997) with a concomitant upregulation of proneural genes followed by delamination out from synchronized cells shortly before mitosis (Hartenstein *et al.*, 1994).

In anamniotes, neurogenesis takes place in two phases. The first phase occurs soon after gastrulation giving rise to primary neurons, which are required for the early movements and responses of the larvae (Roberts, 2000). In contrast, secondary neurogenesis occurs later at the tadpole stage, and can be compared with neurogenesis in amniotes. Primary neurons are born within the neural plate before the pseudostratified neuroepithelium is completely structured, concomitantly with a wave of mitosis that sweeps over

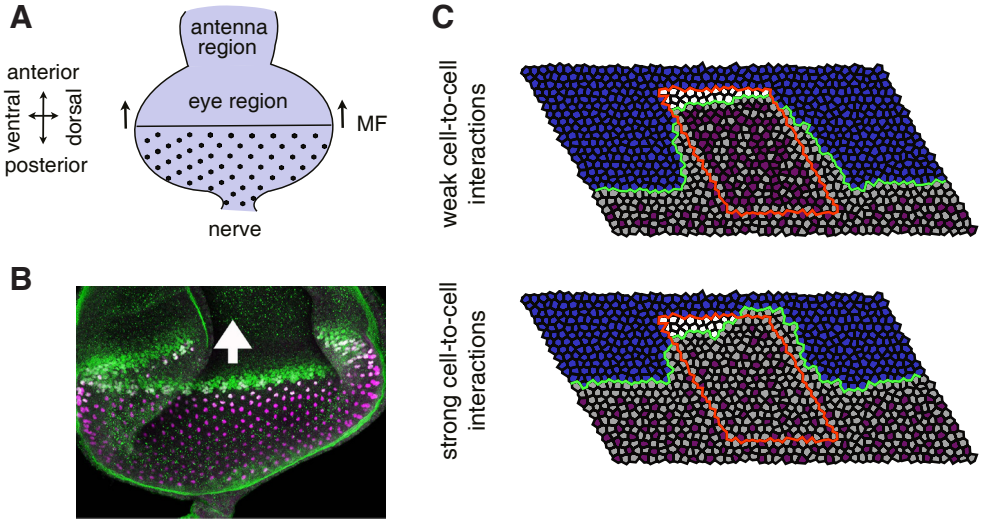

Fig. 3. Morphogenetic furrow (MF) progression in *Drosophila*. (A) *Cartoon of the antennal-eye disc region showing wavefront progression from posterior to anterior, leaving in its wake a fine grained pattern of photoreceptors (black spots). Black arrows indicate direction of the MF progression.* **(B)** *Pattern of R8 photoreceptors (magenta spots) in* Drosophila *eye. Panel adapted from Fig. 1A of Lubensky* et al., *(2011). Green corresponds to A to expression and magenta to stable Sens expression from R8 cells. White arrow indicates direction of the MF progression.* **(C)** In silico *results of the model presented in Formosa-Jordan* et al., *(2012) emulating MF progression (green line) when it crosses a clone of cells (region enclosed by the orange/red line) without Delta at the non-neurogenic state. Results for weaker (top) and stronger (bottom) cell-to-cell Notch mediated interaction rates are presented. MF progression is faster inside the clone than in the WT tissue. Moreover, at weaker cell-to-cell interactions (top) there is overproduction of R8 cells inside the clone, disrupting the lateral inhibition pattern. At stronger cell-to-cell interactions the morphology of the front is altered within the clone (bottom). Blue (white) cells are non-neurogenic cells with (without)* Dl. *Gray cells are neurogenic cells. Purple cells are neurogenic cells that have already been committed to the neural fate. Equivalent results for the avian wavefront are presented in Fig. 4. Simulation details can be found in Formosa-Jordan* et al., *(2012). Panel (A) and bottom image in panel (C) have been adapted from Formosa-Jordan* et al., *(2012).*

the neural plate in a lateral to medial direction (Hartenstein, 1989). Most cells of the neural plate undergo a single division during this wave, and after this first division many cells leave the cell cycle and differentiate as primary neurons (Hartenstein, 1989). Therefore, like in *Drosophila*, cell cycle synchronization in monostratified neural epithelia in anamniotes seems to be required for the generation of neurons. Indeed, primary neurogenesis can be suppressed in part by expression of XBF-1, a Fox transcription factor that maintains active cell division via the inhibition of $p27^{Xic1}$ (Hardcastle and Papalopulu, 2000). Conversely, a number of genes have been identified which are expressed in the territories of primary neurogenesis and facilitate neuronal differentiation through the induction of cell cycle arrest, including *$p27^{Xic1}$*, the p21 activated kinase (*XPak3*), and *XGadd45-g* (De la Calle-Mustienes *et al*., 2002; Souopgui *et al*., 2002; Vernon *et al*., 2003). Moreover, cell cycle arrest seems to be a prerequisite for primary neurogenesis to occur since knocking-down of the *$p27^{Xic1}$*, *XPak3*, and *XGadd45-g* genes has been shown to prevent primary neurogenesis (De la Calle-Mustienes *et al*., 2002; Souopgui *et al*., 2002; Carruthers *et al*., 2003).

Caenorhabditis elegans vulval development is another example in which the cell cycle is coordinated with Notch signaling (Ambros, 1999; Nusser-Stein *et al*., 2012). In this case, a row of six multipotent vulval precursor cells ends up in a very robust pattern of three different fates, the 1^o, 2^o and 3^o, placed in the following order: $3^o3^o2^o1^o2^o3^o$. It has been shown that 1^o and 2^o cell fate specification occurs sequentially and is coupled to cell-cycle progression (Ambros, 1999). Recently, Nusser-Stein *et al.,* (2012) found that NICD degradation only occurs after entry to the G2 phase. In this study, the authors also propose that precursors should exhibit a certain degree of asynchrony to break their equivalence, a phenomenon that has been called bounded asynchrony (Fisher *et al*., 2008, Nusser-Stein *et al.,* 2012). Nusser-Stein *et al.,* (2012) proposed that NICD degradation at the G2 phase would keep precursors synchronized to a certain extent, i.e. keeping a bounded asynchrony, contributing to the temporal order of 1^o and 2^o cell fate specifications.

A dynamic proneural cluster at the apical portion of the neuroepithelium

As described above, a common aspect of nervous system development is that precursor cells normally arrest in the cell cycle window comprising G2/M/early G1 prior to neuronal differentiation, thus providing a quiescent stage to begin differentiation. This strategy is characterized by long periods of time during which neural precursors stay in interphase without neuronal production, which may be sufficient for the genesis of small brains like those of invertebrates, or primary neurons for basic functions in the larva of anamniotes. In contrast, the vertebrate brain is constituted by an enormous amount of neurons generated during a short period of embryonic development and, unlike what is observed in *Drosophila*, vertebrate neuroblasts are postmitotic at the time they abandon the neuroepithelium (Götz and Huttner, 2005). Therefore, vertebrates have adopted a strategy based on the continuous production of neurons, while precursor cells enrolled in neurogenesis maintain a close contact between them due to the INM (for reviews see Latasa *et al*., 2009, Kosodo, 2012).

The INM leads to the presence of cells with neurogenic capacity in the apical portion of the neuroepithelium, facilitating the restriction to this region of lateral inhibition. The apical domain of the neuroepithelium can therefore be considered as an actual proneural cluster with dynamic synchronization of precursors (Murciano *et al*., 2002). In the absence of INM, neuronal precursors lacking neurogenic capacity (undergoing S-phase) could freely interact with neurogenic precursors, thus resulting in an overproduction of neurons. This concept, initially proposed by Murciano *et al.,* (2002) based on computer simulations and pharmacological inhibition of either cell cycle progression or nuclear displacement, has been confirmed by several studies performed afterwards. Thus, Xie *et al.,* (2007) have shown that silencing of Cep120, a centrosomal protein expressed by neuronal precursors during cortical development, impairs INM and favors neuronal overproduction. Cep120 interacts with transforming acidic coiled-coil proteins (TACCs), a group of centrosome-associated proteins whose silencing causes defects in INM and neural progenitor cell self-renewal (Xie *et al*., 2007). In this regard, Yang *et al.* (2012) have recently shown that DOCK7, a member of the DOCK 180 family of proteins that antagonizes the microtubule growth-promoting function of TACC3, also influences cortical neurogenesis by controlling apically-directed INM of radial glial progenitor cells. Similarly, disruption *in vivo* of the interaction between Hook3 and PCM1, two pericentriolar proteins, impairs INM, thus resulting in the overproduction of neurons at the expense of the neural progenitor pool in the developing neocortex (Ge *et al*., 2010). Del Bene *et al.,* (2008) have provided additional genetic proof for the implication of INM in neurogenesis. These authors have shown that the retina of *mok* zebrafish mutants, in which INM is disrupted due to a mutation in the motor protein Dynactin-1, shows an increase of neurogenesis as well. In another study, Schenk *et al.,* (2009) have demonstrated that the disturbance of INM in a culture system of mouse embryonic telencephalon reproducing cortical development is able to change neural progenitor fate toward a more differentiated state. Further evidence for the involvement of INM in the regulation of neurogenesis comes from the medaka mutant *tab*, which shows an abnormal pattern of INM associated with basally mislocalized mitosis and accelerated neurogenesis in the neural tube (Tsuda *et al*., 2010). Overall, these studies based on both pharmacological and genetic approaches to prevent INM demonstrate the importance of INM in the process of neurogenesis. These studies indicate that perturbation of INM is able to cause enhanced neurogenesis at the expense of neural progenitor cells, likely due to the interaction of neurogenic precursors with neuroepithelial cells lacking capacity to trigger Notch-dependent signaling (Murciano *et al*., 2002). Surprisingly, Nishikawa *et al.,* (2011) have reported a reduction of newborn cortical neurons in embryos treated with an antagonist of the endothelin B receptor, a treatment that correlates with slowing down of the INM. One possible explanation for this observation derives from the known effect of endothelin on favoring enteric neurogenesis (Barlow *et al*., 2003). Therefore, inhibition of the endothelin pathway might result in a reduction of neurogenesis in the brain cortex even if INM is affected, provided that this neuromodulator is also required for neurogenesis in the CNS.

The observations described above are consistent with the activation of Notch at the apical portion of the normal neuroepithelium (Ochiai *et al*., 2009), an event recently confirmed *in vivo* with a reporter for Notch activity (Vilas-Boas *et al*., 2011). This latter study, which used a Notch reporter gene based on the expression of GFP under the control of the Hes5-1 promoter coupled

with multiple elements that confer instability to the molecule, has demonstrated that Notch is most frequently activated in neuronal precursors prior to mitosis (Vilas-Boas *et al.*, 2011). Since Notch1 signaling is triggered by the translocation of its intracellular domain to the nucleus upon ligand binding (Schroeter *et al.*, 1998), monitorization of Notch signaling in the neuroepithelium has also been performed with antibodies that recognize the NICD (Tokunaga *et al.*, 2004; Del Monte *et al.*, 2007). In this regard, Tokunaga *et al.,* (2004) have demonstrated that the neuroepithelial cells showing NICD immunoreactivity are located closer to the apical portion of the neuroepithelium than those expressing Notch1, thus indicating that Notch1 is activated in a cell cycle-dependent manner. Del Bene *et al.,* (2008) have also shown NICD-specific immunoreactivity in the nuclei of neural precursors located at the apical surface of the mouse embryonic retina. In another study, Del Monte *et al.,* (2007) have observed NICD-specific immunoreactivity throughout the developing CNS in a pattern resembling that of BrdU incorporation, but double labeling for NICD and BrdU to unambiguously demonstrate the area in which NICD localizes was lacking in this study. Another proof for the activity of Notch being maximal at the apical neuroepithelium is based on the analysis of the expression of the downstream effectors of Notch such as *HES5* and *her4*. For instance, *HES5* has been shown to be enriched in the apical neuroepithelium of chick (Cisneros *et al.*, 2008), and *her4* has been shown to be increased in neural precursors from zebrafish embryonic retina as they move from the basal to the apical end of the neuroepithelium (Del Bene *et al.*, 2008), further indicating that Notch signaling is activated during G2/M in both species. The apical location in the neuroepithelium of gene transcripts encoding for proteins involved in the regulation of Notch activity such as *lFng* (Cisneros *et al.*, 2008) is also consistent with a regionalization of the activity of Notch during G2/M/early G1. Finally, experiments in which the apical domain in vertebrate retinal neuroepithelia is manipulated to be expanded demonstrate an increased activity of Notch and reduced rates of neurogenesis (Clark *et al.*, 2012), as expected from the apically-located activity of this neurogenic receptor.

The increase of Notch activity in the apical portion of the neuroepithelium is also consistent with the observation that *Dll1* begins to be expressed as precursor cells move apically (Murciano *et al.*, 2002). This observation is in apparent contradiction with the report by Del Bene *et al.,* (2008) demonstrating that, in 26 hpf zebrafish embryos, *deltaB* and *deltaC* can be detected throughout the whole neuroepithelium, including retinal precursors located at the basal neuroepithelium, where S-phase takes place. In this regard, it is important to indicate that neuronal differentiation in zebrafish begins at 32 hpf (Schmitt and Dowling, 1999). Therefore, at this preneurogenic stage, *Dl* expression is generalized throughout the

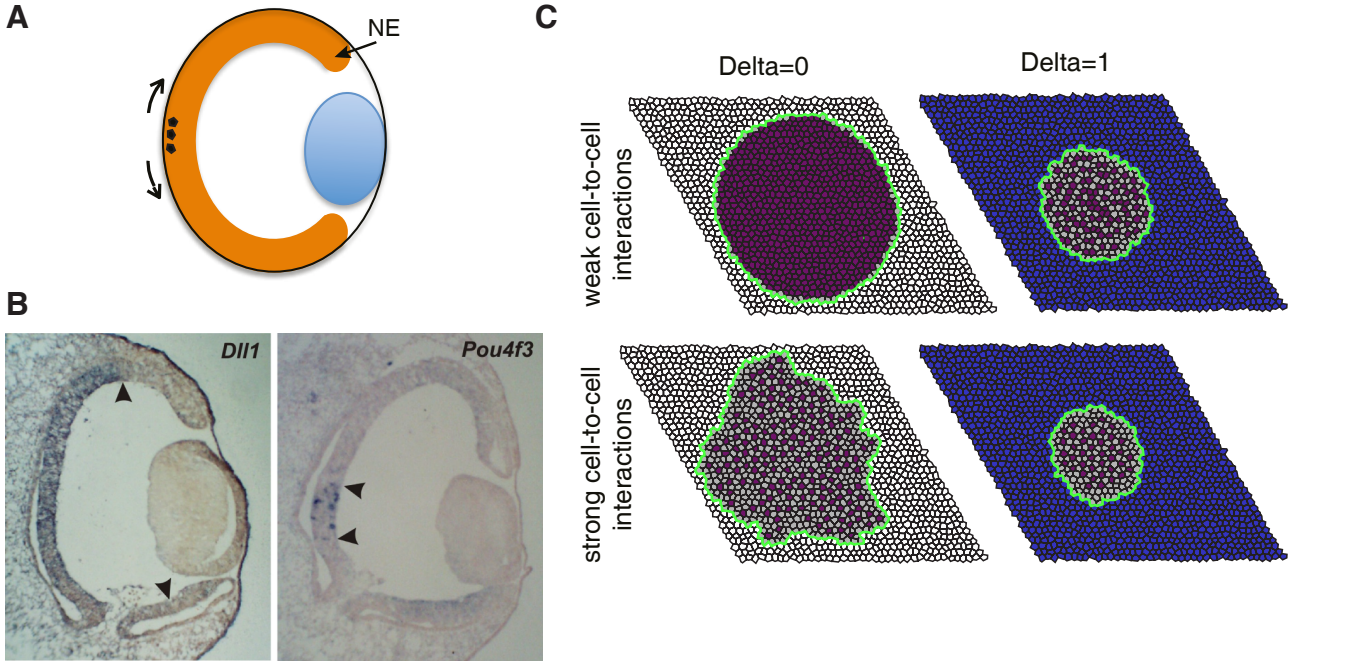

Fig. 4. Wavefront progression in the embryonic vertebrate retina. (A) *Schematic representation of an eye section. At the center of the neuroepithelium (NE, orange) neurogenesis starts (black spots) and spreads to more peripheral regions (arrows) through a self-regulated wavefront.* **(B)** *Cryostat sections in the chick retina at Hamburger-Hamilton stage 14 showing expression patterns of* Dl1 *and* Pou4f3 *obtained by* in situ *hybridizations.* Dl1 *is expressed in the whole central retina, while neurogenesis occurs in a more restricted area within this domain. The neural marker* Pou4f3 *is expressed in retinal ganglion cells, the first neurons differentiated in the chick retina.* **(C)** In silico *results of the model presented in Formosa-Jordan* et al., *(2012) emulating wavefront progression (green line) when* Dl1 *is absent ahead of the neurogenic wavefront (Delta=0 scenario, left column) and when* Dl1 *is expressed ahead of the neurogenic wavefront (Delta=1 scenario, right column) at weak (top) and strong (bottom) cell-to-cell Notch mediated interaction rates. Simulation snapshots at the same cell-to-cell interaction rates (i.e. panels in the same row) are shown for the same time point.* Dl1 *ahead of the wavefront enables the proper selection of neural-committed cells, slows down wavefront progression and maintains a regular wavefront morphology. Color codes as in Fig. 3C. Simulation details can be found in Formosa-Jordan* et al., *(2012). Panels (A-B) have been adapted from Formosa-Jordan* et al., *(2012).*

whole retina (Smithers *et al*., 2000), as it occurs in the chick and mouse retina (Rocha *et al*., 2009; Formosa-Jordan *et al*., 2012). *Dl* expression at these preneurogenic stages is likely to prevent alterations in the lateral inhibitory process as the neurogenic region begins within the central retina, as discussed in the next section.

Neurogenic wavefronts as areas of lateral inhibition instability

In the neural epithelium, neurogenesis often begins in restricted areas, surrounded by non-neurogenic cells (Fig. 2C). The neurogenic region expands as development proceeds, giving rise to a wavefront at the boundary with the non-neurogenic tissue. In *Drosophila*, the wave of photoreceptor differentiation in the eye imaginal disc (Fig. 3 A,B) occurs through the progression of the MF, driven by the diffusing morphogen *hh* (Domínguez and Hafen, 1997). Two HLH regulatory proteins known to prevent proneural gene expression and function respectively are expressed ahead of the MF: *h* and *emc* (Brown *et al*., 1995). Therefore the neurogenic region (behind and within the MF) expands over a non-neurogenic tissue (ahead the MF). The vertebrate retina represents another paradigmatic example of such expansion (Fig. 4 A,B). In this tissue, signals within a small cluster of cells in the central region drive the initiation of neurogenesis (Martinez-Morales *et al*., 2005), which then gradually spreads to the periphery through the release of the morphogen Shh (Neumann and Nuesslein-Volhard, 2000). Neuroepithelial cells located ahead of the neurogenic wavefront in the retina lack *Notch* and *lFng* expression (Formosa-Jordan *et al*., 2012), the latter encoding a glycosyl transferase crucial for Delta/Notch signaling (Moloney *et al*., 2000). The absence of the Notch receptor within the peripheral retina rules out the possibility that this region is constituted by neurogenic cells in a state of mutual inhibition.

This dynamic pattern of neurogenesis has important implications for the process of neuronal differentiation. Specifically, precursors located at the neurogenic wavefront are in direct contact with non-neurogenic precursors that are not expected to sense or trigger differentiation inhibitory signals. As a result, these non-neurogenic cells cannot feedback to neurogenic ones, preventing the selection of new precursors. Therefore the conditions at the wavefront are expected to have relevant consequences for the final pattern of neuronal differentiation. Computer simulations using a mathematical model for the initiation and morphogen-dependent spreading of the neurogenic process, which restricts the dynamics of lateral inhibition to the neurogenic region, predict that alterations of neurogenesis can often arise in the presence of non-neurogenic precursors ahead of the wavefront (Formosa-Jordan *et al*., 2012). Examples of these alterations are massive neurogenesis (all cells become committed to differentiation) or neural overproduction, morphological irregularities of the wavefront itself, and faster progression of the wavefront through the neuroepithelium (Figs. 3C and 4C) (Formosa-Jordan *et al*., 2012).

The mechanism that prevents these alterations in the pattern of neurogenesis seems to be generalized *Dl* expression (i.e. not modulated by Notch) in non-neurogenic tissues surrounding neurogenic regions (Fig. 4B) (Formosa-Jordan *et al*., 2012). This expression can be detected at early developmental stages in a number of neural tissues of different species. In *Drosophila*, *Dl* expression has been described within eye imaginal discs on the surfaces of unpatterned cells ahead of the MF (Kooh *et al*., 1993). As in other neural structures, generalized *Dl* expression ahead of the MF seems to be independent of canonical Notch signaling (Kunisch *et al*., 1994) since the proneural repressors *h* and *emc* are both expressed in this region (Brown *et al*., 1995). In the early zebrafish embryo, strong *Dl* expression can be observed in all precursors, even those in S-phase, a few hours before the initiation of neurogenesis in this tissue (Haddon *et al*., 1998; Del Bene *et al*., 2008). In both the avian and murine retina, *Dll1* is expressed more peripherally than its homolog *Dll4*, being detected in a high proportion of mitotically-active progenitor cells (Rocha *et al*., 2009; Formosa-Jordan *et al*., 2012). As mentioned above, *Dl* expression in all these areas is often observed in most cells, suggesting that it is not a result of a purely lateral inhibitory process in which the already differentiated cells inhibit their surrounding cells.

Computer simulations predict that the presence of Delta ahead of the neurogenic wavefront results in increased robustness of the lateral inhibition process, thus avoiding massive neurogenesis and morphological distortions of the wavefront, while driving a slower regular expansion of the neurogenic region (Figs. 3C and 4C) (Formosa-Jordan *et al*., 2012). These predictions could explain observations made by Rocha *et al.* (2009) in the retina of mice where *Dll1* is conditionally mutated, while *Dll4*-dependent lateral inhibition remains within the neurogenic region. Also, these predictions could be consistent with the appearance of extra photoreceptors at the edges of Delta mutant patches in the adult eyes in *Drosophila*, although the absence of photoreceptors within such patches are explained because Delta is a proneural enhancer in this region (Baker and Yu, 1997).

Overall, these observations suggest that generalized *Dl* expression ahead of the neurogenic wavefront is required for the avoidance of disturbances in lateral inhibition during the neuronal differentiation process (Formosa-Jordan *et al*., 2012).

Perspectives

The selection of precursors to become neurons is driven through lateral inhibition with feedback, a dynamic process of mutual inhibition between cells. Moreover, there are two additional dynamic components of the process, whose relevance for proper neurogenic selection has been emphasized herein. These dynamic components are cell cycle progression and neurogenic wavefront expansion. Both of them involve the disruption of the lateral inhibition interaction between cells, since cells which lack the capacity to trigger and/or to receive inhibitory signals (non-neurogenic) could be potentially mixed within cells that do have this capacity (neurogenic). We have discussed mechanisms used to prevent such disruption. One mechanism avoids the mixing between neurogenic and non-neurogenic cells, either through synchronizing the cell cycle globally in the tissue or locally in a pseudostratified neuroepithelium. The other mechanism provides non-neurogenic cells with the capacity to trigger inhibitory signals, but not to receive them.

There are additional features of the Notch signaling pathway that can drive cells with different neurogenic capacity to interact. One example is cis-inhibition. In this case, Notch receptors can bind the ligand within the same cell (in cis) and trigger no signal. As a result, cis-interactions trap the receptor and the cell has reduced capacity to sense inhibitory signals (Miller *et al*., 2009; Sprinzak *et al*., 2010). Whether neurogenesis commonly involves the in-

termingling between cells with different amounts of cis-inhibition remains to be elucidated. If cis-inhibition occurs homogeneously in all precursors, lateral inhibition patterning is predicted to be facilitated (Sprinzak *et al.*, 2010) and with reduced errors (Barad *et al.,* 2010). What would occur if cis-inhibition is restricted to some precursors is yet to be evaluated.

Another open question is the relevance of the dynamic expansion of wavefronts and its self-regulation. Neurogenic expansion occurs through the release of morphogen, which enables cells to interact through lateral inhibition, by already differentiated cells, which in turn become unable to sense inhibition anymore. Such self-regulated progression ensures patterning is occurring in a small moving region, in between non-neurogenic tissue and already patterned tissue. From a theoretical point of view, moving wavefronts of lateral inhibition are receiving increasing attention (Owen, 2002; Plahte and Øyehaug, 2007; Pennington and Lubensky, 2010; Lubensky *et al.,* 2011; O'Dea and King, 2011; O'Dea and King, 2012; Formosa-Jordan *et al.,* 2012; Simakov and Pismen, 2013). The advantage of a moving wavefront versus a neurogenic region surrounded by a fixed boundary remains to be elucidated.

In silico experiments support that neurogenesis within a tissue with a fixed boundary surrounded by non-neurogenic cells also involves disrupted lateral inhibition at the boundary (unpublished observations). Therefore, generalized ligand levels in non-neurogenic cells could also be relevant in these cases, and in particular, for small non-expanding neurogenic clusters. In this regard, generalized Delta expression has been observed in other regions where neurogenesis occurs in restricted regions without the need of a neurogenic front. For instance, *Dl* expression has been shown to precede ac protein accumulation in microchaeta proneural stripes (Parks *et al.*, 1997).

The dynamical aspects of Notch signaling prompt for advancing in molecular techniques such as time-lapse fluorescence microscopy that could visualize such dynamics and quantify it (Sprinzak *et al.*, 2010). Additionally, the engineering of synthetic systems (Sprinzak *et al.*, 2010; Matsuda *et al.*, 2012) in which dynamics can be more easily assessed and the intermingling between cells with different neurogenic capacity can be controlled, could shed new light on the functioning of Notch signaling for neurogenic selection of precursors. Time-lapse microscopy in combination with synthetic biology techniques and a modeling approach has recently enabled a quantification of how cis-inhibition affects the Notch signaling pathway, revealing the existence of a molecular ultrasensitive switch due to the cis-interactions (Sprinzak *et al.*, 2010). Moreover, the design of new luciferase reporters enabled quantification at timescales of a few hours and even minutes (Imayoshi *et al.*, 2013), and represents a good starting point for quantifying the varying neurogenic capacity throughout the cell cycle.

The emerging concept is that lateral inhibition during neurogenesis occurs in dynamic clusters of precursors and requires specific mechanisms to avoid distortions resulting from the interaction between neurogenic and non-neurogenic precursors. The synergy of real-time imaging of Notch dynamics at cellular and subcellular levels combined with both Synthetic Biology and theoretical perspectives will notably contribute to our understanding of these novel aspects in motion of neurogenesis.

Acknowledgements

This study was supported by BFU2009-07671 and SAF2012-38316 (J.M.F.), FIS2009-13360-C03-01 and FIS2012-37655-C02-02 (P.F-J., M.I.); Generalitat de Catalunya 2009SGR14 (P.F-J., M.I.). P.F-J. acknowledges support from the MECD fellowship FPU-AP2008-03325; S.A. acknowledges a JAE-Doc contract (JAEDOC014, 2010 call) from the CSIC program "Junta para la Ampliación de Estudios" co-funded by the European Social Fund. We thank D. K. Lubensky for providing us with the image for panel B in Fig. 3.

References

AMBROS V (1999). Cell cycle-dependent sequencing of cell fate decisions in Caenorhabditis elegans vulva precursor cells. *Development* 126: 1947-1956.

BAKER N E, YU S Y (1997). Proneural function of neurogenic genes in the developing *Drosophila* eye. *Curr Biol* 7, 122-132.

BARAD O, ROSIN D, HORNSTEIN E, BARKAI N (2010). Error minimization in lateral inhibition circuits. *Sci Signal* 3: ra51.

BARLOW A, DE GRAAFF E, PACHNIS V (2003). Enteric nervous system progenitors are coordinately controlled by the G protein-coupled receptor EDNRB and the receptor tyrosine kinase RET. *Neuron* 40: 905-916.

BERTRAND N, CASTRO D S, GUILLEMOT F (2002). Proneural genes and the specification of neural cell types. *Nat Rev Neurosci* 3: 517-530.

BROWN N L, SATTLER C A, PADDOCK S W, CARROLL S B (1995). Hairy and Emc negatively regulate morphogenetic furrow progression in the *Drosophila* eye. *Cell* 80: 879-887.

CAMPUZANO S, MODOLELL J (1992). Patterning of the *Drosophila* nervous system: the achaete-scute gene complex. *Trends Genet* 8: 202-208.

CARRUTHERS S, MASON J, PAPALOPULU N (2003). Depletion of the cell-cycle inhibitor $p27^{Xic1}$ impairs neuronal differentiation and increases the number of $ElrC^{+}$ progenitor cells in *Xenopus* tropicalis. *Mech Dev* 120: 607-616.

CISNEROS E, LATASA M J, GARCÍA-FLORES M, FRADE J M (2008). Instability of Notch1 and Delta1 mRNAs and reduced Notch activity in vertebrate neuroepithelial cells undergoing S-phase. *Mol Cell Neurosci* 37: 820-831.

CLARK B S, CUI S, MIESFELD J B, KLEZOVITCH O, VASIOUKHIN V, LINK B A (2012). Loss of Llgl1 in retinal neuroepithelia reveals links between apical domain size, Notch activity and neurogenesis. *Development* 139: 1599-1610.

COLLIER J R, MONK N A, MAINI P K, LEWIS J H (1996). Pattern formation by lateral inhibition with feedback: a mathematical model of Delta-Notch intercellular signalling. *J Theor Biol* 183: 429-446.

DE LA CALLE-MUSTIENES E, GLAVIC A, MODOLELL J, GÓMEZ-SKARMETA J L (2002). Xiro homeoproteins coordinate cell cycle exit and primary neuron formation by upregulating neuronal-fate repressors and downregulating the cell-cycle inhibitor XGadd45-gamma. *Mech Dev* 119: 69-80.

DEL BENE F, WEHMAN A M, LINK B A, BAIER H (2008). Regulation of neurogenesis by interkinetic nuclear migration through an apical-basal notch gradient. *Cell* 134: 1055-1065.

DEL MONTE G, GREGO-BESSA J, GONZÁLEZ-RAJAL A, BOLÓS V, DE LA POMPA J L (2007). Monitoring Notch1 activity in development: evidence for a feedback regulatory loop. *Dev Dyn* 236: 2594-2614.

DOMÍNGUEZ M, HAFEN E (1997). Hedgehog directly controls initiation and propagation of retinal differentiation in the *Drosophila* eye. *Genes Dev* 11: 3254-3264.

EDGAR B A, LEHMAN D A, O'FARRELL P H (1994). Transcriptional regulation of string (cdc25): a link between developmental programming and the cell cycle. *Development* 120: 3131-3143.

FIRTH L C, BAKER N E (2005). Extracellular signals responsible for spatially regulated proliferation in the differentiating *Drosophila* eye. *Dev Cell* 8: 541-551.

FISHER J, HENZINGER T A, MATEESCU M, PITERMAN N (2008). Bounded Asynchrony: Concurrency for Modeling Cell-Cell Interactions. FMSB 2008, LNBI 5054, pp. 17-32, 2008. Springer-Verlag, Berlin/Heidelberg.

FOE V E (1989). Mitotic domains reveal early commitment of cells in *Drosophila* embryos. *Development* 107: 1-22.

FORMOSA-JORDAN P, IBAÑES M, ARES S, FRADE J M (2012). Regulation of neuronal differentiation at the neurogenic wavefront. *Development* 139: 2321-2329.

FRADE J M (2002). Interkinetic nuclear movement in the vertebrate neuroepithelium: encounters with an old acquaintance. *Prog Brain Res* 136: 67-71.

GARCÍA-DOMÍNGUEZ D J, MORELLO D, CISNEROS E, KONTOYIANNIS D L,

FRADE J M (2011). Stabilization of Dll1 mRNA by Elavl1/HuR in neuroepithelial cells undergoing mitosis. *Mol Biol Cell* 22: 1227-1239.

GE X, FRANK C L, CALDERÓN DE ANDA F, TSAI L H (2010). Hook3 interacts with PCM1 to regulate pericentriolar material assembly and the timing of neurogenesis. *Neuron* 65: 191-203.

GÖTZ M, HUTTNER W B (2005). The cell biology of neurogenesis. *Nat Rev Mol Cell Biol* 6: 777-788.

HADDON C, SMITHERS L, SCHNEIDER-MAUNOURY S, COCHE T, HENRIQUE D, LEWIS J (1998). Multiple *delta* genes and lateral inhibition in zebrafish primary neurogenesis. *Development* 125: 359-370.

HARDCASTLE Z, PAPALOPULU N (2000). Distinct effects of XBF-1 in regulating the cell cycle inhibitor p27(XIC1) and imparting a neural fate. *Development* 127: 1303-1314.

HARTENSTEIN V (1989). Early neurogenesis in *Xenopus*: the spatio-temporal pattern of proliferation and cell lineages in the embryonic spinal cord. *Neuron* 3: 399-411.

HARTENSTEIN V, POSAKONY J W (1989). Development of adult sensilla on the wing and notum of *Drosophila Melanogaster*. *Development* 107: 389-405.

HARTENSTEIN V, YOUNOSSI-HARTENSTEIN A, LEKVEN A (1994). Delamination and division in the *Drosophila* neurectoderm: spatiotemporal pattern, cytoskeletal dynamics, and common control by neurogenic and segment polarity genes. *Dev Biol* 165: 480-499.

IMAYOSHI I, SHIMOJO H, SAKAMOTO M, OHTSUKA T, KAGEYAMA R (2013). Genetic visualization of notch signaling in mammalian neurogenesis. *Cell Mol Life Sci* 70: 2045-2057.

JARMAN A P, GRELL E H, ACKERMAN L, JAN L Y, JAN Y N (1994). Atonal is the proneural gene for *Drosophila* photoreceptors. *Nature* 369: 398-400.

JIMÉNEZ F, CAMPOS-ORTEGA J A (1987). Genes in subdivision 1B of the *Drosophila Melanogaster* X-chromosome and their influence on neural development. *J Neurogenet* 4: 179-200.

JOHNSTON L A, EDGAR B A (1998). Wingless and Notch regulate cell-cycle arrest in the developing *Drosophila* wing. *Nature* 394: 82-84.

KIMURA K I, USUI-ISHIHARA A, USUI K (1997). G2 arrest of cell cycle ensures a determination process of sensory mother cell formation in *Drosophila*. *Dev Genes Evol* 207: 199-202.

KOOH P J, FEHON R G, MUSKAVITCH M A (1993). Implications of dynamic patterns of Delta and Notch expression for cellular interactions during *Drosophila* development. *Development* 117: 493-507.

KOSODO Y (2012). Interkinetic nuclear migration: beyond a hallmark of neurogenesis. *Cell Mol Life Sci* 69: 2727-2738

KUNISCH M, HAENLIN M, CAMPOS-ORTEGA J A (1994). Lateral inhibition mediated by the *Drosophila* neurogenic gene delta is enhanced by proneural proteins. *Proc Natl Acad Sci USA* 91: 10139-10143.

LATASA M J, CISNEROS E, FRADE J M (2009). Cell cycle control of Notch signaling and the functional regionalization of the neuroepithelium during vertebrate neurogenesis. *Int J Dev Biol* 53: 895-908.

LUBENSKY D K, PENNINGTON M W, SHRAIMAN B I, BAKER N E (2011). A dynamical model of ommatidial crystal formation. *Proc Natl Acad Sci USA* 108: 11145-11150.

MARTINEZ-MORALES J R, DEL BENE F, NICA G, HAMMERSCHMIDT M, BOVOLENTA P, WITTBRODT J (2005). Differentiation of the vertebrate retina is coordinated by an FGF signaling center. *Dev Cell* 8: 565-574.

MATSUDA M, KOGA M, NISHIDA E, EBISUYA M (2012). Synthetic Signal Propagation Through Direct Cell-Cell Interaction. *Sci Signal* 5: ra31.

MILLER A C, LYONS E L, HERMAN T H (2009). Cis-Inhibition of Notch by Endogenous Delta Biases the Outcome of Lateral Inhibition. *Curr Biol* 19: 1378-1383.

MINDEN J (2008). Dissection of the embryonic brain using photoactivated gene expression. *Adv Exp Med Biol* 628: 57-68.

MOLONEY D J, PANIN V M, JOHNSTON S H, CHEN J, SHAO L, WILSON R, WANG Y, STANLEY P, IRVINE K D, HALTIWANGER R S, VOGT T F (2000). Fringe is a glycosyltransferase that modifies Notch. *Nature* 406: 369-375.

MOZER B A, EASWARACHANDRAN K (1999). Pattern formation in the absence of cell proliferation: tissue-specific regulation of cell cycle progression by string (stg) during *Drosophila* eye development. *Dev Biol* 213: 54-69.

MURCIANO A, ZAMORA J, LÓPEZ-SÁNCHEZ J, FRADE J M (2002). Interkinetic nuclear movement may provide spatial clues to the regulation of neurogenesis. *Mol Cell Neurosci* 21: 285-300.

NAMBA R, MINDEN J S (1999). Fate mapping of *Drosophila* embryonic mitotic domain 20 reveals that the larval visual system is derived from a subdomain of a few cells. *Dev Biol* 212: 465-476.

NÈGRE N, GHYSEN A, MARTINEZ A M (2003). Mitotic G2-arrest is required for neural cell fate determination in *Drosophila*. *Mech Dev* 120: 253-265.

NEUMANN C J, NUESSLEIN-VOLHARD C (2000). Patterning of the zebrafish retina by a wave of sonic hedgehog activity. *Science* 289: 2137-2139.

NISHIKAWA K, AYUKAWA K, HARA Y, WADA K, AOKI S (2011). Endothelin/endothelin-B receptor signals regulate ventricle-directed interkinetic nuclear migration of cerebral cortical neural progenitors. *Neurochem Int* 58: 261-272.

NUSSER-STEIN S, BEYER A, RIMANN I, ADAMCZYK M, PITERMAN N, HAJNAL A, FISHER J (2012). Cell-cycle regulation of NOTCH signaling during C. elegans vulval development. *Mol Syst Biol* 8: 618.

OCHIAI W, NAKATANI S, TAKAHARA T, KAINUMA M, MASAOKA M, MINOBE S, NAMIHIRA M, NAKASHIMA K, SAKAKIBARA A, OGAWA M, MIYATA T (2009). Periventricular notch activation and asymmetric Ngn2 and Tbr2 expression in pair-generated neocortical daughtercells. *Mol Cell Neurosci* 40: 225-233.

O'DEA R D, KING J R (2011). Multiscale analysis of pattern formation via intercellular signalling. *Math Biosci* 231: 172–185.

O'DEA R D, KING J R (2012). Continuum limits of pattern formation in hexagonal-cell monolayers. *J Math Biol* 64: 579–610.

OWEN M R (2002). Waves and propagation failure in discrete space models with nonlinear coupling and feedback. *Physica D* 173: 59.

PARKS A L, HUPPERT S S, MUSKAVITCH M A (1997). The dynamics of neurogenic signaling underlying bristle development in *Drosophila Melanogaster*. *Mech Dev* 63: 61-74.

PENNINGTON M, LUBENSKY D (2010). Switch and template pattern formation in a discrete reaction diffusion system inspired by the drosophila eye. *Eur Phys J E* 33: 129-149.

PLAHTE E, ØYEHAUG L (2007). Pattern-generating travelling waves in a discrete multicellular system with lateral inhibition. *Physica D* 226: 117–128

REDDY B V, RAUSKOLB C, IRVINE K D (2010). Influence of fat-hippo and notch signaling on the proliferation and differentiation of *Drosophila* optic neuroepithelia. *Development* 137: 2397-2408.

ROBERTS A (2000). Early functional organization of spinal neurons in developing lower vertebrates. *Brain Res Bull* 53: 585-593.

ROCHA S F, LOPES S S, GOSSLER A, Henrique D (2009). Dll1 and Dll4 function sequentially in the retina and pV2 domain of the spinal cord to regulate neurogenesis and create cell diversity. *Dev Biol* 328: 54-65.

ROMANI S, CAMPUZANO S, MACAGNO E R, MODOLELL J (1989). Expression of achaete and scute genes in *Drosophila* imaginal discs and their function in sensory organ development. *Genes Dev* 3: 997-1007.

SCHENK J, WILSCH-BRÄUNINGER M, CALEGARI F, HUTTNER W B (2009). Myosin II is required for interkinetic nuclear migration of neural progenitors. *Proc Natl Acad Sci USA* 106: 16487-16492.

SCHMITT E A, DOWLING J E (1999). Early retinal development in the zebrafish, Danio rerio: light and electron microscopic analyses. *J Comp Neurol* 404: 515-536.

SCHROETER E H, KISSLINGER J A, KOPAN R (1998). Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature* 393: 382-386.

SIMAKOV D S A, PISMEN L M (2013). Discrete model of periodic pattern formation through a combined autocrine-juxtacrine cell signaling. *Phys Biol* 10: 046001.

SIMPSON P (1990). Lateral inhibition and the development of the sensory bristles of the adult peripheral nervous system of *Drosophila*. *Development* 109: 509-519.

SMITHERS L, HADDON C, JIANG Y J, LEWIS J (2000). Sequence and embryonic expression of deltaC in the zebrafish. *Mech Dev* 90: 119-123.

SOUOPGUI J, SÖLTER M, PIELER T (2002). XPak3 promotes cell cycle withdrawal during primary neurogenesis in *Xenopus laevis*. *EMBO J* 21: 6429-6439.

SPRINZAK D, LAKHANPAL A, LEBON L, SANTAT L A, FONTES M E, ANDERSON G A, GARCIA-OJALVO J, ELOWITZ M B (2010). Cis-interactions between Notch and Delta generate mutually exclusive signalling states. *Nature* 465: 86-90.

THOMAS B J, GUNNING D A, CHO J, ZIPURSKY L (1994). Cell cycle progression in the developing *Drosophila* eye: roughex encodes a novel protein required for the establishment of G1. *Cell* 77: 1003-1014.

TOKUNAGA A, KOHYAMA J, YOSHIDA T, NAKAO K, SAWAMOTO K, OKANO H

(2004). Mapping spatio-temporal activation of Notch signaling during neurogenesis and gliogenesis in the developing mouse brain. *J Neurochem* 90: 142-154.

TOMLINSON A, READY D F (1987). Neuronal differentiation in *Drosophila* ommatidium. *Dev Biol* 120: 366-376.

TSUDA S, KITAGAWA T, TAKASHIMA S, ASAKAWA S, SHIMIZU N, MITANI H, SHIMA A, TSUTSUMI M, HORI H, NARUSE K, ISHIKAWA Y, TAKEDA H (2010). FAK-mediated extracellular signals are essential for interkinetic nuclear migration and planar divisions in the neuroepithelium. *J Cell Sci* 123: 484-496.

VERNON A E, DEVINE C, PHILPOTT A (2003). The cdk inhibitor $p27^{Xic1}$ is required for differentiation of primary neurones in *Xenopus*. *Development* 130: 85-92.

VILAS-BOAS F, FIOR R, SWEDLOW J R, STOREY K G, HENRIQUE D (2011). A novel reporter of notch signalling indicates regulated and random Notch activation during vertebrate neurogenesis. *BMC Biol* 9: 58.

WENG M, HAENFLER J M, LEE C Y (2012). Changes in Notch signaling coordinates maintenance and differentiation of the *Drosophila* larval optic lobe neuroepithelia. *Dev Neurobiol* 72: 1376-1390.

WOLFF T, READY D F (1991). The beginning of pattern formation in the *Drosophila* compound eye: the morphogenetic furrow and the second mitotic wave. *Development* 113: 841-850.

XIE Z, MOY L Y, SANADA K, ZHOU Y, BUCHMAN J J, TSAI L H (2007). Cep120 and TACCs control interkinetic nuclear migration and the neural progenitor pool. *Neuron* 56: 79-93.

YANG Y T, WANG C L, VAN AELST L (2012). DOCK7 interacts with TACC3 to regulate interkinetic nuclear migration and cortical neurogenesis. *Nat Neurosci* 15: 1201-1210.