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# Gene Expression in Embryonic Neural Development and Stem Cell Differentiation

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## 1. Introduction

Since all cells ultimately are derived from a single cell - the fertilized egg - a complete overview on the neuron development should peruse through initial steps of neural induction in the ectoderm of the blastula embryo, and the sequential activation of the neurogenic program in the neural tube through neurite outgrowth during final differentiation step.

The concept of neural induction, i.e. the definition of the neural plate domain in the ectoderm, was first proposed by Spemann and Mangold after the classic experiment in which transplantation of the frog embryo's dorsal blastopore lip induced a complete neural axis from the acceptor embryo's ectoderm. Since then, much effort has been made aimed at identify the signals that confer the neural bias to the ectoderm. The resulting picture clearly indicates that neural induction is a multi-step process that requires the interplay of various pathways. The result of neural induction is the definition of a neural plate composed by proliferating neuroepithelial cells expressing pan-neural genes.

However, acquisition of neural bias is not sufficient to propel the neuroepithelial cell towards terminal neural differentiation path. However, acquisition of neural bias is not sufficient to propel the neuroepithelial cell towards A terminal neural differentiation path. Cell fate plasticity remains high and demands continuous reinforcement to proceed towards a specific differentiation path. The transition from proliferating precursor cell to post-mitotic state is also a highly regulated step. Thus, proneural genes have an important role, regulating both cell cycle arrest and initiation of neural differentiation.

In recent years, the potential and promise held by embryonic stem cells as a source for new cell-reposition therapies have attracted the attention of the scientific and lay community. Stem cells are, by definition, self-propagating cells that are extremely plastic and can potentially differentiate into multiple types of cells. However, the same plasticity that holds the promise of generation of multiple tissues from a single cell line is also the characteristic

that makes stem cell differentiation difficult to control. This has led to intense research aimed at understanding the process of cell differentiation. More often than not, stem cell biologists have approached differentiation from a developmental biology perspective. After all, the newly-fertilized egg is a single cell at its most undifferentiated and uncommitted state, and is exposed to all the signalling events necessary for generating all the differentiated tissues of a complete organism.

In support of this, several of the embryonic proneural genes and signalling pathways are also present during induction of ES-cell neural differentiation. An example of a protein that is active both in normal development and ES-cell differentiation is Ndel1. Ndel1 is a microtubule associated neuronal protein, which has been shown to be essential for neuronal differentiation and cell migration during the central nervous system development. Albeit the abundant literature on its functional role, expression modulation and protein positioning during the neuronal differentiation process, marginal attention has been paid for its localization and function in early neuronal development step. More recently, we have also demonstrated that its enzymatic activity plays an essential role in neurite outgrowth in differentiating PC12 cells. The Ndel1 gene expression modulation during the neuronal differentiation has been intensively studied and its expression in pluripotent ES cells undergoing neuronal differentiation has also been explored. Taken together, all these data strongly suggests that Ndel1 is a relevant component in the embryogenesis of the nervous system and in the differentiation of cells to neuronal phenotype.

## 2. Embryonic neural induction

The neural lineage derives from the ectodermal germ layer, which in turn originates through gastrulation from the epiblast. The ectoderm also gives rise to the epidermal lineage, and one of the first events that define the neural lineage is the choice between these two cell fates: neural or epidermal. Both lineages must be delimited both molecularly and anatomically. The earliest time point when we can detect this segregation is at the pre-gastrula epiblast. The epiblast receives signals that will generate a neural bias. Thereafter, this bias is progressively stabilized during neural specification and finally, the neural region is patterned in the three axes. Thus, neural induction can be subdivided into the response of the epiblast to neuralizing signals by adopting a neural bias at its central region, and the progressive stabilization of this bias through additional signals. Much of what we have learned about these events was gathered from experiments in the chick and amphibian embryo.

The precise stage at which the epiblast first demonstrates that it is competent to follow neural fate has been progressively pushed back as more molecular markers have become available. For instance, the early neural marker Sox3 and late marker Sox2 have been used as standard indicators of neural bias and specification (Fernandez-Garre et al., 2002; Rex et al., 1997; Streit et al., 2000, 1997; Uchikawa, 2003; Wood & Episkopou, 1999). These two genes have slightly different temporal expression pattern with an overlap at the neural induction stage. Sox3 is detected throughout the epiblast before neural induction in pre-gastrula embryos and becomes restricted to the future neuroepithelium as development progresses. Sox2 is first detected around the time when neural induction is believed to occur and thereafter its expression is limited to the neuroepithelium (Muhr et al., 1999; Rex et al., 1997).

The induction of neural fate in the ectoderm for a long time was claimed to be the 'default' fate, where the absence of additional extracellular signals is sufficient to drive towards neural bias. This model was mainly sustained on data obtained from dissociated amphibian ectodermal cell cultures (Wilson & Hemmati-Brivanlou, 1995). The mainstay of this model was that activation of ectodermal BMP signalling pathway conferred an epidermal bias. Thus, neural bias could be promoted by the absence signalling; i.e. inhibition of BMP signalling either through the addition of extracellular BMP inhibitors (e.g. Chordin, Noggin) or decrease of extracellular BMP concentration through dilution (Wilson & Hemmati-Brivanlou, 1995; reviewed in Almeida et al., 2010).

Lately, the default model has been modified by experiments done in whole avian and amphibian embryos. The current model sustains that ectopic expression of Sox2 and Sox3 and other neural bias markers is achieved when there is concomitant inhibition of BMP and stimulation of FGF signalling (Linker & Stern, 2004). In this revised model, FGF is an early neural inducer that acts by counteracting BMP signalling in the epiblast (Pera et al., 2003; Streit et al., 2000; Wilson et al., 2000, 2001). Thereafter, the presence of extracellular BMP inhibitors such as Chordin is required to maintain and stabilize the neuroepithelium's neural bias during gastrulation (Streit et al., 1998).

### 3. Cell cycle exit and neurogenic differentiation

The vertebrate neuroepithelium starts with a relatively small number of proliferative progenitor cells. At early developmental stages, progenitor cells proliferate rapidly through symmetric division and give rise only to additional progenitor cells, thus increasing the population of progenitor cells. Vertebrate neurons are generated in the ventricular zone, an epithelial layer that delimits the ventricles. Proliferation at the ventricular zone occurs in an unsynchronized fashion and is characterized by the process known as the interkinetic nuclear migration (Hayes & Nowakowski, 2000). This movement spans the apical-basal cell axis and positions the nucleus at the basal side during the G1 and S phase of mitosis and at the apical side during G2 and M phases (reviewed in Latasa et al. 2009). Once a certain critical mass is attained, the neuroepithelium produces neurons through asymmetric neurogenic divisions. In this scenario, one daughter cell remains proliferative and maintains the neuroblast pool, while the other arrests from the cell cycle and proceeds towards neurogenic differentiation to populate the central nervous system. The difference in fate is given by unequal distribution of proteins amongst the daughter cells, which will direct towards self-renewal or differentiation. The mechanism that controls this asymmetric distribution is still being investigated.

One of the hypotheses is that the choice between symmetric and asymmetric segregation depends on the position of the mitotic spindle. This proposal derives from results obtained in the ferret cortex. In this model system, asymmetrical cell division is determined by the position of the mitotic spindle relative to the apical surface of the neuroepithelium (Chenn & McConnell, 1995). When the cleavage plane is perpendicular, both daughter cells inherit equal portions of apical and basal membrane, thus generating proliferating progenitor cells symmetrically. Conversely, when the mitotic spindle is parallel, the unequal distribution of apical and basal membranes amongst the daughter cells leads to the birth of an apically-located proliferating progenitor and a basally-located postmitotic progenitor (Chenn & McConnell, 1995). However, in other vertebrates, the role of mitotic spindle positioning in

determining the balance between asymmetric and symmetric division has been controversial (Konno et al., 2008; reviewed in Shioi et al., 2009; Zigman et al., 2005). The discrepancies observed in the various reports could be attributed to technical difficulties in imaging the apical domain of the pseudostratified neuroepithelial cells of the mammalian embryo. Irrespective of the role of mitotic spindle in the control of symmetric and asymmetric cell division, it is a consensus that the distinct cell fates arise from the asymmetric distribution of cellular components. As such, the PAR polarity proteins have been recently associated with unequal segregation of the progenitor cell components (Bultje et al., 2009; Ossipova et al., 2009; Tabler et al., 2010).

Naturally, the question arises about the nature of the proteins that direct towards self-renewal or differentiation of the neural progenitor cells. The cell-surface transmembrane Notch receptor has an evolutionary conserved role in determining cell-fate specification (reviewed in Pierfelice et al., 2011). Overwhelming evidence has indicated that Notch signalling is one of the main players in regulating the choice between proliferation and differentiation in the vertebrate nervous system. Activation of the Notch pathway is regulated by cell-cell signalling. In brief, Notch receptors are activated by Delta-like or Jagged proteins expressed on the membranes of neighbouring cells. Receptor activation results in the cleavage of the intracellular domain of Notch, its translocation to the nucleus and transcription of target genes. Of these, the Hes family of basic helix-loop-helix (bHLH) transcription factors has been consistently associated with the repression of proneural transcription factors expression, and consequently of neural differentiation. Thus, cells whose Notch pathway is triggered will remain in mitosis at the ventricular zone (Akai et al., 2005; Hammerle & Tejedor, 2007; Kawaguchi et al., 2008; Latasa et al., 2009; Le Roux et al., 2003). Conversely, inhibition of Notch signalling removes progenitor cells from mitosis (Hammerle & Tejedor, 2007). In other words, the Notch signalling pathway is intimately related to the binary cell fate choice between proliferation and differentiation. Although inhibition of Notch signalling is required for cell cycle arrest (Kawaguchi et al., 2008), it is insufficient to drive differentiation. Overexpression of the truncated form of the Delta ligand or of the Notch receptor induces cell cycle arrest, but does not increase the proportion of cells expressing differentiation markers (Akai et al., 2005; Hammerle & Tejedor, 2007).

#### 4. The neurogenic transcriptional cascade

The transition from proliferative to postmitotic neuron is a highly-regulated multi-stepped process. Initiation of neurogenic differentiation requires expression of proneural bHLH transcription factors such as Neurogenin 1 and 2, which trigger a transcriptional cascade that culminates in the expression of terminal differentiation genes (Bertrand et al., 2002). Several of the vertebrate proneural genes were identified through homology with *Drosophila* achaete-scute (*asc*) and *atonal* (*ato*) family of genes. Overexpression of the orthologues of the *asc* (*Xash1*; Talikka et al., 2002) or *ato* (*XNgnr1*; Ma et al., 1996) induces ectopic neurogenesis and expression of downstream neurogenic bHLH transcription factors (Lin et al., 2004; Ma et al., 1996).

However, instead of generating multiple neural lineages, the overexpression of a single vertebrate proneural gene affects only specific neural subsets. For instance, *Mash1*<sup>-/-</sup> mice display severe defects in neurogenesis in the ventral telencephalon and the olfactory sensory epithelium (Casarosa et al., 1999; Horton et al., 1999). Similarly, Neurogenin1 (*Ngn1*) or

Neurogenin2 (Ngn2) single-mutant mice lack cranial sensory ganglia while Ngn1/2 double mutants also lack components of the peripheral nervous system (Fode et al., 1998; Ma et al., 1998, 1999). The complexity of the phenotypes generated confirm the diversity of existing genetic programs underlying the development of each neuronal subtype and implies that the importance of a single bHLH factor depends on the neural cell lineage (Powell & Jarman, 2008). This emphasizes the importance of using a wide array of marker genes to identify progression of neural differentiation, as a single proneural gene might not be involved in the differentiation of the neural lineage under investigation. Furthermore, it has important implications for experimental approaches that aim to direct the *in vitro* differentiation of stem cell lines.

Vertebrate proneural genes are first expressed, while precursor cells are still at the ventricular zone. Indeed, several of the above-mentioned proneural genes expression is regulated by the Delta-Notch pathway (Kageyama et al., 2008; Ma et al., 1996). However, neural differentiation does not occur in the ventricular zone. Rather, postmitotic neural precursors undergo migration towards outer layers of the neural tube. Proneural bHLH proteins are also involved in this migratory behaviour. Overexpression of Neurogenin1, Neurogenin2, NeuroD and Mash1 increases progenitor cell migration in the mouse cortex and regulates the expression of the cytoskeleton-regulating GTPases RhoA (Ge et al., 2006).

An anatomical consequence of this migratory behaviour coupled to differentiation is the organization of neural tube in distinct cell layers, compartmentalizing differentiation stages progressively in concentric layers, where internal layers harbour younger, more undifferentiated precursors and more external layers contain more mature neurons. This spatial organization facilitates the positioning of marker genes in the neurogenic programs hierarchy. For instance, Notch pathway receptor and ligand genes are expressed in the ventricular zone (Fig. 1; Le Roux et al., 2003; Myat et al., 1996) In contrast, consistent with

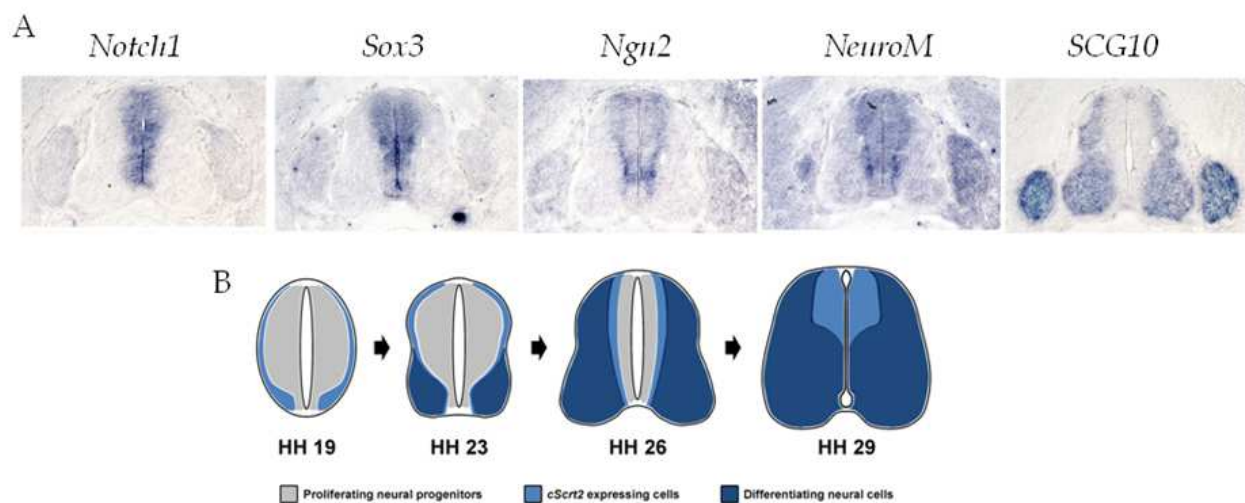


Fig. 1. Progression of neural differentiation is associated with more external layers of the neural tube. A) *In situ* hybridization for markers for proliferation (*Notch1*), transition between proliferation and cell cycle arrest (*Sox3* and *Ngn2*), postmitotic differentiation (*NeuroM*) and late differentiation (*SCG10*) in chick HH26 truncal neural tube. B) Diagram summarizing the anatomical changes in the different differentiation compartments.

their role in initiating differentiation, Sox3 and Neurogenin1 are expressed at the ventricular layer and slightly beyond the proliferative zone as well (Fig. 1; Bylund et al., 2003). Ath3/NeuroM is mainly expressed by post-mitotic neural precursors, and the expression domain borders the external perimeter of Neurogenin1 and Sox3 (Fig. 1; Roztocil et al., 1997). This domain is also known as the intermediate layer and contains neural progenitors in the early stages of differentiation. Other markers for this stage include the RNA-binding protein Hu and the RNA splicing factor NeuN/Fox3 (Dent et al., 2010; Kim et al., 2009; Wakamatsu & Weston, 1997). Finally, the late differentiation marker SCG10 is expressed by cells at the outer border (mantle layer) of the neural tube (Fig. 1; Stein et al., 1988).

SCG10 encodes a membrane-associated protein associated with the growth cones of developing neurons (Stein et al., 1988). Its presence in the developing neural tube correlates with the onset of late differentiation events such as neuritogenesis. An additional marker that is widely used to characterize post-mitotic differentiating neurons is beta III tubulin (recognized by the monoclonal antibody Tuj1; Lewis & Cowan, 1988; Lee et al., 1990; Menezes and Luskin, 1994).

In the chick embryo truncal neural tube, beta III tubulin presence is particularly strong at the developing ventral root, corresponding to axons emitted by the motor neurons in the ventral lateral regions of the tube (Fig. 2). Thereafter, its presence becomes increasingly prevalent and can be detected in the outer mantle layer and in the developing dorsal root ganglions (Fig. 2). Beta III tubulin has been associated with the emergence of stable microtubule cytoskeletal scaffolds in axons and dendrite, suggesting that beta III tubulin is required for neurite maintenance (Ferreira and Caceres, 1992). Indeed, neurons with decreased levels of beta III tubulin have shorter neurites (Tucker et al., 2008). However, Tuj-1 immunoreactivity is not limited to neurons undergoing neuritogenesis. Beta III tubulin is also present in cells that are migrating from the ventricular and subventricular zone (O'Rourke et al., 1997).

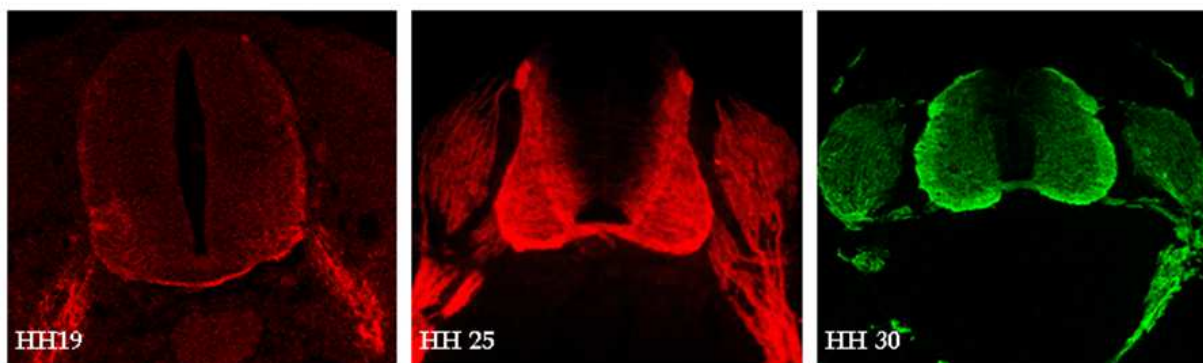


Fig. 2. Evolution of beta III tubulin expression in the chick truncal neural tube. Beta III tubulin was detected by immunohistochemistry with the monoclonal antibody Tuj1. The above staining shows that beta III tubulin is first detected in the motorneurons neurites that comprise the ventral root (HH19) and in some outer peripheral neurites. Thereafter, expression progresses so as to expand towards inner layers as well. At HH25, a strong immunofluorescent signal can be seen at the ventral motorneuron domain, ventral root and dorsal root ganglion. At HH30, beta III tubulin is clearly present in the axons of the outer mantle layer and is only excluded from the innermost layer that borders the ventricular region.

Consistent with the importance of microtubule cytoskeleton in the latter stages of neural differentiation, several microtubule-associated proteins such as MAP2 and Tau are also up-regulated. MAP2 and MAP1B double knockout mice have fiber tract malformations and retarded neuronal migration. Additionally, primary neuronal cultures derived from these mice display reduced neurite outgrowth (Teng et al., 2001).

## 5. Stem cell neural differentiation recapitulates embryogenesis

*In vitro* differentiation of embryonic stem cells into neural lineages aims to recapitulate the multistep process – from induction to terminal differentiation – of neural embryogenesis described above. Indeed as in embryonic epiblast induction, some cell lines, neural induction is more efficiently induced by the combination of fibroblast growth factor (FGF) signalling and bone morphogenetic protein (BMP) inhibition (LaVaute et al., 2009; Tropepe et al., 2001; Ying et al., 2003). In these reports, the endogenous production of BMP inhibitors was sufficient to avoid epidermal fate. However, conservation of embryogenic signalling is not a rule for all cell lines. Some iPSCs (induced Pluripotent Stem Cells) do not improve their neural differentiation rate with FGF signalling and/or BMP inhibition (Hu et al., 2010). Thus, the extent of recall of embryogenesis in these experimental paradigms is still an open question and begs for future analysis.

There are multiple protocols for *in vitro* neural induction and the depth of analysis regarding similarity with embryogenesis varies. Some groups provide a detailed comparison with embryogenesis. For instance, Abranches and collaborators report expression of Sox genes during the early phases of induced differentiation, interkinetic nuclear migration and Notch-signalling and subsequent expression of the postmitotic neural (Hu) and glial markers (GFAP) (Abranches et al., 2009). However, most reports concentrate on the detection of late developmental neural markers such as MAP2, Tau, NeuN and beta III tubulin, which have been generally accepted in the community as indication for stem cell neuronal differentiation (Tropepe et al., 2001).

For instance, Kerkis and collaborators detected the presence microtubule-associated proteins (MAPs), such as Lis1 and Ndel1, as neural markers at early stages of *in vitro* model for neuronal differentiation from pluripotent stem cells (Kerkis et al., 2011).

## 6. Lis1 and Ndel1: Microtubule associated proteins involved in neural development

The microtubule associated proteins (MAPs), Lis1 and Ndel1 are involved in neuronal differentiation and cell migration during the CNS development.

Lis1, also known as platelet-activating factor acetylhydrolase (PAF-AH), regulates microtubule function and is essential for proper neuronal migration during cortical development (Arai, 2002). Mutations in Lis1 gene have been associated with neuronal migration defects and abnormal layering of the cortex (Reiner et al., 1995; Saillour et al., 2009; Youn et al., 2009). For instance, haploinsufficiency of Lis1 alone causes congenital malformation of brain folds and grooves, i.e. lissencephaly. Lis 1 microdeletion is also part of the genetic causes of Miller-Dieker syndrome (MDS; Miller, 1963; Dieker et al., 1969; Reiner et al., 1993). Besides lissencephaly, MDS patients also present hypoplastic corpus callosum. Together, these data underscore the importance of Lis1 in proper neuronal migration and axon formation.



In support of the importance of Lis1 in neural development, Lis1-binding protein Ndel1 (Nuclear-distribution Element like-1) also plays a relevant role in the proper establishment of the nervous system. Lis1 and Ndel1 co-localize predominantly in the centrosome in early neuroblasts, and later, redistribute to axons during neuronal development (Shu et al., 2004; Guo et al., 2006; Bradshaw et al., 2008; Hayashi et al., 2010). The direct association of Lis1 with the Ndel1 fungal homologue was first shown in 2000 (Kitagawa et al., 2000), and soon after the interaction with the mammalian homologue was also demonstrated (Sweeney et al., 2001).

Ndel1 is also known as endooligopeptidase A or EOPA and was first isolated due to its ability to inactivate bioactive peptides. Ndel1/EOPA, is a thiol-sensitive enzyme inactivates physiologically important peptides such as bradykinin and neurotensin, and also converts opioid oligopeptides into enkephalins (Camargo et al., 1973, 1983, 1987; Gomes et al., 1993; Hayashi et al., 2000, 2005). The contribution of bradykinin and neurotensin neuropeptides in neurite outgrowth was also previously described (Zhao et al., 2003; Tischler et al., 1991; Robson and Burgoyne, 1989; Tischler et al., 1984).

In normal cortical development Ndel1 is involved in microtubule organization, nuclear translocation and neuronal positioning (Shu et al., 2004; Youn et al., 2009; Bradshaw et al., 2011). Knockdown or ablation of cortical Ndel1 function also results in impaired migration of neocortical projection neurons (Sasaki et al., 2005; Youn et al., 2009). Deletion of Ndel1 by RNAi leads to deficits in neuronal positioning and uncouples the centrosome from the nucleus, resulting in aberrant neuronal migration (Shu et al., 2004). Ndel1 homozygous knockout mice have similar deficits in neuronal positioning (Sasaki et al., 2005; Youn et al., 2009).

## 7. Expression of Ndel1 in the developing CNS and ES cells

Consistent with its importance in the development of the nervous system and its association with the microtubule cytoskeleton, Ndel1 domain of expression in the developing embryonic neural tube coincides with that of beta III tubulin in the outer mantle zone (Fig. 3). As mentioned in previous sections, the expression of beta III tubulin in post-mitotic cells is associated with neurons that are migrating or emitting neurites. Thus, the co-localization shown here suggests that Ndel1 is involved in these processes as well in the chick developing neural tube.

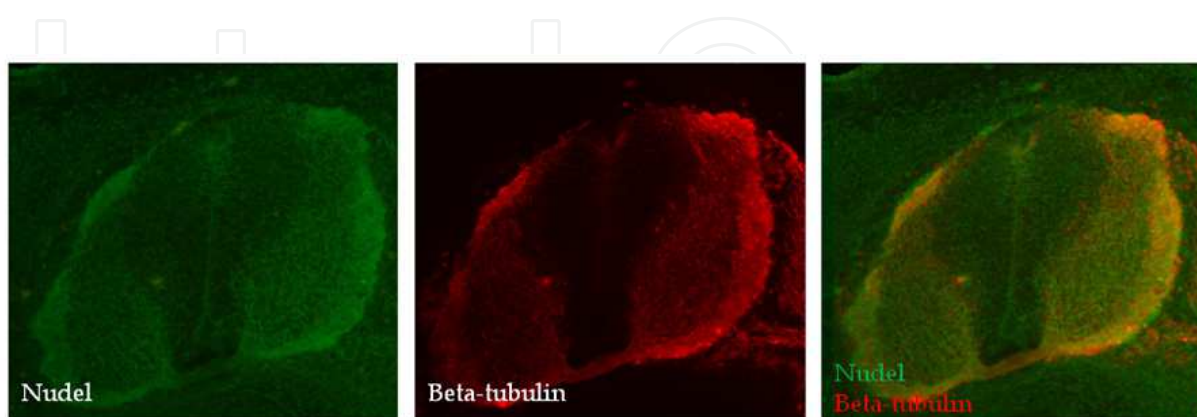


Fig. 3. Ndel1 expression co-localizes with beta III tubulin in the mantle zone of the embryonic neural tube. Immunofluorescence of adjacent slices of HH 30 embryos truncal neural tube with monoclonal anti-Ndel1 and anti beta III tubulin (Tuj1) antibodies. The overlay at the rightmost figure is provided for comparative purposes only.

Likewise, the dynamics of Ndel1 localization in stem cells during neural differentiation suggests that it is recruited for neurite extension. In undifferentiated ES cells, both Lis1 and Ndel1 show a perinuclear co-localization (Hayashi et al., 2011). In contrast, after the onset of neuronal differentiation, Lis1 presents a cytoplasmic and Ndel1 a perinuclear localization. Following differentiation, both Lis1 and Ndel1 co-localize in the outgrowing neurites (Kerkis et al., 2011).

The presence of Ndel1 persists in adult brains. Northern blot analysis confirmed its preferential expression in the rabbit and rodent CNS (Hayashi et al., 1996; 2000), although this could not be confirmed for humans (Guerreiro et al., 2005). Later, the presence of Ndel1 in the adult brain was confirmed by *in situ* hybridization studies, with higher expression in some regions, such as the hippocampus, cerebellum, and basal nucleus of Meynert (Hayashi et al., 2001). This study provided a basis for phenotypic identification of Ndel1-expressing neurons throughout the rat brain and showed a correlation between the distribution of Ndel1 neurons and systems responsible for motor, sensory, endocrine, and possibly for other functions. Together, these expression patterns argue in favour of a role for Ndel1 in neurite growth and maintenance.

### 8. *In vitro* assays for Ndel1 cellular function

As mentioned previously, clinical correlation data suggested strongly that Lis1 and Ndel1 are involved in neuronal migration during cortical layer formation. Lis1 and Ndel1 participate in nuclear and centrosomal transport in migrating neurons (Shu et al., 2004; Tsai et al., 2005). Additionally, they influence centrosome positioning in migrating non-neuronal cells (Dujardin et al., 2003; Stehman et al., 2007; Shen et al., 2008). Moreover, dominant negative overexpression of either the enzymatic active form of Ndel1 or its orthologue mNudE disrupted CNS lamination in *Xenopus laevis* embryos (Hayashi et al., 2004; Feng et al., 2000).

In an attempt to elucidate the exact role of Lis1 and Ndel1 in neuronal migration during cortical layer formation, we have used long-term adherent neurosphere cultures to mimic the development of cortical layers *in vitro* (Hayashi et al., 2011). In this experimental model, the neurospheres grow for two weeks without splitting and the resulting aggregates present an inner core that would correspond to the inner cortical layer where migrating neurons originate from, and an outer layer that harbors neurons that finished their migration. In this experimental paradigm, a significant variation in spatial distribution of Lis1 and Ndel1 proteins was observed (Kerkis et al., 2011). Lis1, but not Ndel1, was detected in the rosette cells localized at the inner part of the cellular aggregates. In contrast, co-localization of both Lis1 and Ndel1 was observed in the cells at the peripheral layer of the cellular aggregates (Kerkis et al., 2011). Although further analysis with other MAPs would provide a better picture of the role of Lis1 and Ndel1 in neuronal migration during establishment of cortical layers, these data nonetheless indicate that these two proteins play a differential role in the establishment and maintenance of neuronal layers.

The role of Ndel1 in neurite outgrowth has been better characterized. Knockdown of Ndel1 expression in rat pheochromocytoma PC12 cell line inhibits neurite outgrowth. This inhibition can be rescued by wild-type Ndel1 (Ndel1<sub>WT</sub>), but not by a mutant (Ndel1<sub>mut273</sub>), which does not have enzymatic activity (Hayashi et al., 2010). This result indicates that

Ndel1 enzymatic activity plays a crucial role in neurite outgrowth. In support to this, a significant increase of Ndel1 promoter activity during the period of maximal neurite outgrowth was observed (Hayashi et al., 2010).

Clearly, the expression of Ndel1 shows strong correlation with the onset of various aspects of embryonic neural development and ES cells and PC12 cells neural differentiation. Thus, we directed our attention towards cis-regulatory elements that could regulate neuro-specific Ndel1 expression in a variety of experimental models.

## 9. Control of Ndel1 expression

The promoter of both rabbit and human Ndel1 gene was analyzed by the group in cultured cell lines. Interestingly, the Ndel1 promoter activity was shown to be very different in neuronal and non-neuronal cells, with a stronger activity in NH15 neuronal compared to C6 glial cells for the rabbit full-length promoter, thus confirming the preferential neuronal expression of. However, such difference was not observed for the human full-length promoter under the same conditions (Guerreiro et al., 2005).

We've isolated the rabbit promoter fragment -888/-744 as the region responsible for determining the neuronal-specific expression. This DNA segment contains potential binding motifs for the CP2 and SRY (sex-determining region Y) transcription factors. SRY is the founding member of the Sox (Sry-related HMG box) gene family (Sekido, 2010). Moreover, strong negative regulator elements were found within positions -755/-450 and -314/-245 in both human and rabbit promoters. Of these, at least one common negative cis-regulating region seems to be acting in the control Ndel1 expression in both species (Guerreiro et al., 2005). During neural development, these elements may restrict Ndel1 promoter activity to a neuronal subtype or a specific period of differentiation.

In the human Ndel1 promoter, the critical regulatory domain lies between -314/-245. Within this region we also found a single putative binding site for a member of the Sox transcription factor family. It is tempting to speculate on the identity of members of the Sox family, which now number more than 20, that regulate Ndel1 expression (Lefebvre et al., 2007). There are certain members of the Sox family, which we could speculatively nominate as candidates to mediate the increased expression of Ndel1. For instance, accumulated evidence has shown that Sox 11 is relevant for neurite outgrowth. As Neuro2a cells undergo retinoic acid-induced neuronal differentiation, Sox 11 levels increase significantly. Conversely, RNAi knockdown of Sox 11 inhibits axon outgrowth in Neuro2a cells, Dorsal Root Ganglion neurons and regeneration in nerve injury models (Jankowski et al., 2006; Jankowski et al., 2009).

## 10. Conclusion

Microtubule-associated proteins (MAPs) are essential for neuronal differentiation and cell migration during the central nervous system (CNS) development and also in the adult nervous system. In particular the distribution and role of lissencephaly (Lis1) and nuclear distribution element-like (Ndel1) allows the comparison between neural differentiation in stem cells and during embryo development. They are very powerful tools not only due to

their putative role as expression markers of the differentiation process, but also due to their confirmed role in the cell maturation and migration processes. Furthermore, the study of cis-regulatory regions that confer neural-specificity to Ndel1 expression can increase our understanding of gene expression control during neural differentiation.

## 11. Acknowledgment

The authors are grateful for the continuous support by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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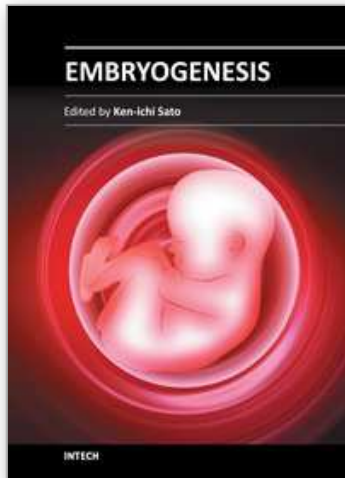
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## **Embryogenesis**

Edited by Dr. Ken-Ichi Sato

ISBN 978-953-51-0466-7

Hard cover, 652 pages

**Publisher** InTech

**Published online** 20, April, 2012

**Published in print edition** April, 2012

The book "Embryogenesis" is a compilation of cutting edge views of current trends in modern developmental biology, focusing on gametogenesis, fertilization, early and/or late embryogenesis in animals, plants, and some other small organisms. Each of 27 chapters contributed from the authorships of world-wide 20 countries provides an introduction as well as an in-depth review to classical as well as contemporary problems that challenge to understand how living organisms are born, grow, and reproduce at the levels from molecule and cell to individual.

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C. Y. Irene Yan, Felipe M. Vieceli, Tatiane Y. Kanno, José Antonio O. Turri and Mirian A. F. Hayashi (2012). Gene Expression in Embryonic Neural Development and Stem Cell Differentiation, Embryogenesis, Dr. Ken-Ichi Sato (Ed.), ISBN: 978-953-51-0466-7, InTech, Available from:  
<http://www.intechopen.com/books/embryogenesis/gene-expression-in-embryonic-neural-development-and-stem-cell-differentiation>

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