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Review

Modeling the spatio-temporal network that drives patterning in the vertebrate central nervous system

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ABSTRACT

In this review, we discuss the gene regulatory network underlying the patterning of the ventral neural tube during vertebrate embryogenesis. The neural tube is partitioned into domains of distinct cell fates by inductive signals along both anterior–posterior and dorsal-ventral axes. A defining feature of the dorsal-ventral patterning is the graded distribution of Sonic hedgehog (Shh), which acts as a morphogen to specify several classes of ventral neurons in a concentration-dependent fashion. These inductive signals translate into patterned expressions of transcription factors that define different neural progenitor subtypes. Progenitor boundaries are sharpened by repressive interactions between these transcription factors. The progenitor-expressed transcription factors induce another set of transcription factors that are thought to contribute to neural identities in post-mitotic neural precursors. Thus, the gene regulatory network of the ventral neural tube patterning is characterized by hierarchal expression [inductive signal → progenitor specifying factors (mitotic) → precursor specifying factors (post mitotic) → differentiated neural markers] and cross-repression between progenitor-expressed regulatory factors. Although a number of transcriptional regulators have been identified at each hierarchal level, their precise regulatory relationships are not clear. Here we discuss approaches aimed at clarifying and extending our understanding of the formation and propagation of this network.

1. Overview of neural tube patterning

The neural tube is the embryonic precursor to the central nervous system in vertebrates. It spans nearly the entire length of an embryo forming brain structures anteriorly and spinal cord at more posterior levels. Though many of the molecular genetic programs governing neural tube development appear to be shared over a wide range of anterior–posterior (AP) axial levels, this review will focus on the presumptive spinal cord, the most widely studied component. Specifically, it will examine the specification of the ventral component, the target of Sonic hedgehog morphogen action.

In transverse sections, the neural tube appears as an oval-shaped mass of neural progenitor cells. At the dorsal and ventral poles of the neural tube are thin layers of two, distinct non-neuronal cell populations, the roof plate and floor plate, respectively; each is critical for patterning the organ (Fig. 1). Surrounding the lumen is a pseudostratified epithelium of proliferating neural progenitors within the ventricular zone (Fig. 1). As neural progenitors differentiate to post-mitotic neural precursors, they migrate out of the ventricular zone to form an outer mantle zone. Depending on the specific precursor, an additional migrating phase may take place to establish the appropriate spatial organization.

Fate mapping and molecular marker analyses of the neural tube at spinal cord levels predict 11 neural progenitor domains organized along the dorsal-ventral (DV) axis (the ventral 5 domains are described in Figs. 2 and 3; for review see [1]). Likewise, the mantle zone is divided into 11 domains, each of which derives from the adjacent progenitor domain in the ventricular zone. These 11 domains will produce distinct subtypes of sensory-, inter-, and motor-neurons [1]. The identity and position of progenitor types are specified by extracellular cues including Sonic Hedgehog (Shh), Wnt, TGF-β family members [eg. BMPs and GDFs], Fibroblast Growth Factor (FGF), and Retinoic acid (RA). In particular, Shh forms an activity gradient along the DV axis and plays a central role as a morphogen to pattern the ventral neural tube [2–5]. A Shh activity gradient is translated into a

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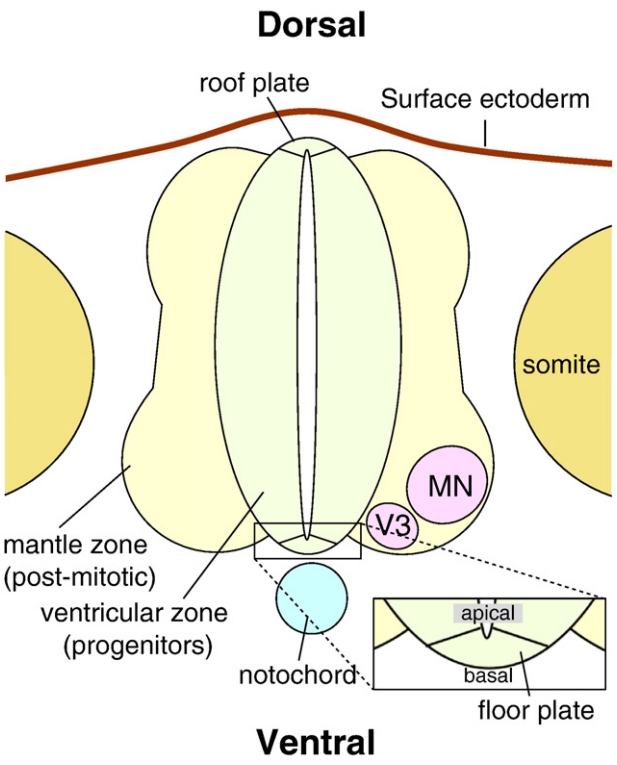

Fig. 1. Cartoon of a transverse section of the neural tube. The neural tube overlies the notochord at the midline and is flanked by somites on either side. The roof and floor plate are located at the dorsal and ventral pole, respectively. The apical–basal orientation of the ventricular zone is marked. MN: motor neurons, V3: V3 interneurons.

gradient of Gli transcription factor activity, which in turn directs the expression of key transcriptional regulators in neural progenitors in a stereotyped spatio-temporal order.

2. Shh patterns the ventral neural tube

2.1. Shh signaling and its morphogen action in the neural tube

The secreted signaling molecule Shh regulates many aspects of embryogenesis in vertebrates, and has been implicated in a number of forms of cancer [6]. Shh signaling occurs via a cell surface receptor-mediated process and results in transcriptional events executed by the Gli zinc finger transcription factors, vertebrate homologues of the *Drosophila* protein Cubitus interruptus (Ci) (reviewed in [7]).

In the neural tube, Shh protein is initially produced in the notochord and this secondarily induces Shh-expressing floor plate (FP) (Fig. 2) [3,8,9]. Importantly, notochord-derived Shh is sufficient for the initial induction of all Shh-dependent ventral neural progenitor types [10–12]. Embryological and genetic evidence suggest that Shh forms an activity gradient along the DV axis in the neural tube to specify ventral progenitor domains in a concentration-dependent manner (Fig. 2) [2–5]. Application of recombinant Shh protein to naïve neural plate explants results in the specific induction of ventral neuronal cell types with increasing concentrations inducing progressively more ventral neural identities [2,3,13]. Consistent with this data, gain-of function mutants in the Shh pathway have an expansion of ventral progenitor domains along the dorsal-ventral axis with neural identities that are normally restricted to a specific ventral position now ectopically induced at more dorsal positions [11,14]. Similarly, loss-of-function mutants exhibit a reduction or complete absence of ventral neural identities [11,15]. When Shh input is attenuated, the most ventral progenitor domains are the most sensitive to decreased signaling activity [11,15].

2.2. Interpretation of Shh activity gradient by Gli transcription factors

The responses to graded Shh signaling are thought to be achieved by a range of activities mediated by the Gli-family of transcriptional regulators [16–18]. In the complete absence of Hh signaling, the majority of Gli proteins present in the cell is post-translationally processed in a series of phosphorylation events, that result in a carboxy-terminal cleavage [19,20]. These truncated forms act as transcriptional repressors, leading to the down-regulation or silencing of Hh targets [21,22]. In contrast, the presence of Hh results in the generation of an activator form [23]. As discussed in the following sections, the three mammalian Gli proteins play overlapping and distinct molecular and genetic roles in the neural tube. However, they are thought to form an integrated Gli transcriptional activity gradient along the DV axis in response to the Shh gradient (Fig. 2). Where Shh concentration is highest in the ventral most progenitor domains, Gli activator levels are highest whereas Gli repressor activity is inversely correlated with Shh gradient and forms a gradient of decreasing repressor activity from dorsal to ventral (Fig. 2). Directly or indirectly, this Gli activity gradient will regulate patterned expression of progenitor-specifying transcription factors.

2.3. Gli activators specify ventral cell fates

In the neural tube, Gli1 and Gli2 are thought to act together in the two most ventral domains, floor plate and pV3, to specify these cell fates (Fig. 2) [10,24,25]. Gli1 null mice have no phenotype while Gli2 null embryos lack FP and contain reduced numbers of pV3 cells. Loss of Gli1, however, increases the severity of Gli2 mutants such that compound mutants fail to form pV3 progenitors [10,24,26]. Importantly, Gli1 expression is entirely dependent on prior Shh signaling in the developing spinal cord and is diminished in Gli2 mutants [26].

Gli1 and Gli2 are thought to function exclusively (Gli1) or predominantly (Gli2) as transcriptional activators in the neural tube [19,27,28]. Gli3 is primarily a repressor, but does appear to have a minor role as an activator [17]. Importantly, in FP and pV3, Gli1 activator can directly regulate the transcriptional activation of the key cell fate-determining transcription factors *FoxA2* (FP) and *Nkx2.2* (pV3) by binding to defined cis-regulatory domains that are critical for directing transcription [29–31] although in vivo functional and genetic studies indicate that Gli2 activator is the principle player [25,26].

2.4. Dorsoventral domains are patterned via Gli3 repressor activity

Gli3 acts predominantly as a transcriptional repressor with a key role in the more dorsally positioned ventral progenitor domains

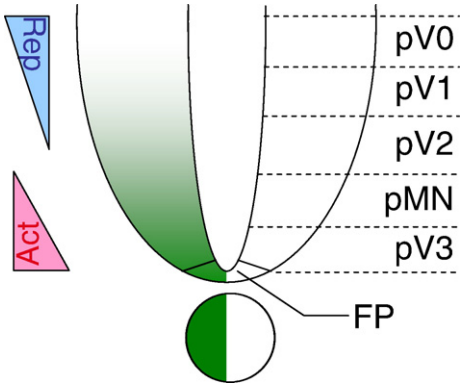

Fig. 2. Schematic view of Shh protein expression and Gli activity gradients (left) and progenitor domains (right) in the ventral neural tube. Shh (shown as green filling) is produced from the notochord initially and floor plate secondarily and forms a gradient along the DV axis. pV0–pV3 are distinct subtypes of interneuron progenitors. Motor neuron progenitor is indicated by pMN. FP indicates the floor plate.