Mini-Symposium

The Elegance of Sonic Hedgehog: Emerging Novel Functions for a Classic Morphogen

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Sonic Hedgehog (SHH) signaling has been most widely known for its role in specifying region and cell-type identity during embryonic morphogenesis. This mini-review accompanies a 2018 SFN mini-symposium that addresses an emerging body of research focused on understanding the diverse roles for Shh signaling in a wide range of contexts in neurodevelopment and, more recently, in the mature CNS. Such research shows that Shh affects the function of brain circuits, including the production and maintenance of diverse cell types and the establishment of wiring specificity. Here, we review these novel and unexpected functions and the unanswered questions regarding the role of SHH and its signaling pathway members in these cases.

Introduction

Sonic Hedgehog (SHH) is a secreted factor; the protein is heavily modified through cleavage and posttranslational modification and its release from producing cells can also be modulated by interactions with Dispatched, Scube2, and heparan sulfate proteoglycan (Burke et al., 1999; Rubin et al., 2002; Tukachinsky et al., 2016). A growing list of genes encoding Shh receptor and coreceptor proteins, including BOC, CDON, GAS1, and glypicans, are expressed in cell-type- and developmental-stage-specific patterns in the nervous system and this variety likely contributes to the diversity of cellular responses to Shh.

The core components of the canonical Shh signaling pathway include Patched1 (PTCH1), the 12-pass transmembrane receptor that binds SHH directly; Smoothened (SMO), an obligate coreceptor the repression of which by PTCH1 is relieved upon ligand binding; and the GLI family of transcription factors, which includes GLI1, GLI2, and GLI3. Binding of PTCH1 by the SHH ligand releases the repression of SMO, enabling the subsequent modulation of the cleavage and stability of GLI2 and GLI3. Ultimately, this shift in processing of GLI2/3 results in a shift from transcriptional repression mediated through GLI3R to transcriptional activation and, at the highest levels of activity, GLI2-mediated transcription of the constitutive activator GLI1. In many, but not all cases, the interactions among PTCH1, SMO, and modulation of GLI proteins appear to be localized to the primary cilium, a microtubule-based organelle that functions as a signaling center within the cell. Developmental disorders affecting the primary cilium share many features with disorders in which Shh pathway components are disrupted.

The novel roles reviewed here require various components of this multifaceted pathway, with some events requiring "canonical" induction of transcriptional programs and others acting through signal transduction that is independent of the GLI factors. Recent investigations have identified functions for Shh pathway members in the regulation of early and late stem and progenitor cells, in the establishment of circuitry, and in communication between neurons and astrocytes within mature circuitry.

Shh signaling in neocortical progenitor expansion

Shh signaling has a long-known role in the regulation of neural progenitors (NPs) of the rostral neural tube, where it is required to establish distinct brain regions and to balance the number and

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type of neurons and glial cells produced (Fuccillo et al., 2006). In the developing neocortex, the primary NPs are the ventricular radial glia (vRG, also called apical RG), which produce neurons directly or indirectly via the outer RG (oRG, also called basal RG) and/or intermediate progenitors (IPs). Neocortical expansion and folding requires two coordinated processes that depend on NPs: the increased production and the tangential dispersion of new neurons. Recent findings show that Shh signaling is central to the mechanisms that promote growth and folding of the neocortex.

In mice, perturbations in Shh signaling cause defective proliferation of IPs and microcephaly (Komada et al., 2008). Furthermore, a recent study showed that Shh signaling is sufficient for both IP and oRG expansion and neocortical growth (Wang et al., 2016). Remarkably, elevated Shh signaling increases upper layer neuron production from mid-corticogenesis at embryonic day 13.5 (E13.5) on, leading to neocortical growth and folding in the otherwise-smooth mouse neocortex. Ectopic activation of high Shh signaling, via induction of the *SMOM2* allele, which encodes a tumor-derived constitutively active SMO protein, elicits two developmental characteristics that are absent in mouse but have been proposed to be necessary and sufficient for the evolution of an expanded and folded neocortex: oRG expansion and recurrent self-amplifying IP divisions (Lewitus et al., 2014). Elevated Shh signaling in early corticogenesis (E9–E10) misspecifies cortical NPs, and decreases IPs (Dave et al., 2011; Shikata et al., 2011; Wang et al., 2011; Yabut et al., 2015), demonstrating developmental stage-specific functions of Shh signaling.

Shh signaling is also important for neocortical growth in gyrencephalic species. *GLI1*, the expression of which provides a faithful readout of Shh signaling, is highly expressed in human vRG (Wang et al., 2016) and mutations disrupting Shh signaling cause microcephaly in humans (Heussler et al., 2002; Derwińska et al., 2009; Izumi et al., 2011). In ferrets, *Gli1* expression is significantly higher in the ventricular zone (VZ) area that generates a thick subventricular zone (SVZ) containing many oRG, than in the VZ area that gives rise to a thin SVZ containing fewer oRG (de Juan Romero et al., 2015). Consistent with this, in human cerebral organoids (Lancaster et al., 2013), blocking SHH signaling decreases the number of oRG detected in human cerebral organoids (Wang et al., 2016). *GLI1* transcription is significantly higher in human fetal neocortex than in mouse embryonic cortex (Wang et al., 2016), consistent with more abundant oRG and IPs in human than mouse fetal cortex. Understanding underlying mechanisms for this differential Shh signaling activity, as well as molecular mechanisms by which Shh signaling expands oRG and IPs, will provide fundamental insights into the development and evolution of mammalian brains and the etiology of neurodevelopmental diseases.

Beyond its multifaceted activity in the neocortex, Shh has a well established role in the patterning of the subpallium, including the medial ganglionic eminence and the more caudal neural tube, where it is a major ventralizing factor (Echelard et al., 1993; Riddle et al., 1993; Ericson et al., 1995; Kohtz et al., 1998). More recent studies of Shh signaling in late embryogenesis have also revealed a requirement for cortical neuron- and interneuron-derived SHH ligand in the production of oligodendrocytes (Winkler et al., 2018). In postnatal brain, the persistent stem cell niches of both the ventricular subventricular zone (V-SVZ) and dentate gyrus (DG) are sites of continued Shh signaling, with multiple effects on neural versus glial fate selection and stem cell activity.

Shh regulates positional identity in the postnatal neural stem cell (NSC) niche

After birth, *Gli1* transcript displays a localized pattern within the V-SVZ. This niche of glial-like progenitors is organized into distinct subdomains committed to specific progeny fates, with ventral stem cells primarily generating immature neuroblasts, which then integrate into the olfactory bulb as deep granule interneurons. Examination of reporter alleles driven by *Gli1* (Ahn and Joyner, 2004, 2005) and lineage tracing at distinct postnatal time points in the mouse revealed that, whereas SMO is broadly expressed, high SHH activity and *Gli1* expression are predominantly ventral, where they are necessary and sufficient to generate deep granule interneurons in the olfactory bulb (Ihrie et al., 2011). However, dorsal V-SVZ *Gli1* is also observed during a wave of early postnatal oligodendrogenesis (Tong et al., 2015). Forced ectopic activation of high Shh signaling via induction of the *SMOM2* allele is sufficient to drive *Gli1* expression and to shift cell fate to oligodendroglial and deep granule cell fates, indicating that high SHH signaling can drive the adoption of ventral identities in this postnatal niche. Removal of inhibition of this pathway (via deletion of the transcriptional repressor GLI3) in dorsal cells, transplantation of dorsal cells to a ventral location, or the combination of these two manipulations does not result in a change of identity, suggesting that the induction of GLI1 is tightly regulated and might not be overcome in all experimental conditions (Petrova et al., 2013) (R.A.I., unpublished data).

Intriguingly, modulations of other Shh pathway members indicate that lower levels of pathway activity, which would result in relief of GLI2/3-mediated repression but not expression of GLI1, likely have additional, broader functions in regulating the full dorsoventral stem cell niche. Ablation of the inhibitory coreceptor PTCH1 results in an accumulation of quiescent NSCs at the expense of more proliferative and differentiated progeny, indicating that inhibition of Shh signaling is required to maintain the balance between NSC quiescence and activation (Daynac et al., 2016). Ablation of GLI3 throughout the V-SVZ niche is sufficient to rescue many of the broad neurogenesis phenotypes seen when Smoothened is lost, suggesting that relief from GLI3-mediated repression by low Shh signaling is required for normal stem cell activity (Balordi and Fishell, 2007a,b; Petrova et al., 2013). Similarly, in the DG, detection of transcript and study of reporter alleles indicates that GLI1-positive cells are present within the stem cell pool and that SMO ablation (with a consequent increase in GLI3-mediated repression) significantly disrupts neurogenic activity (Ahn and Joyner, 2005; Han et al., 2008; Shin et al., 2015). In contrast to the V-SVZ, which is spread over a large spatial area and generates migratory progeny that integrate far from the niche, the DG is contained within a smaller area and generates neurons that integrate locally. As a consequence, potential heterogeneity in the function or localization of specific NSC subsets is not yet fully delineated in this niche.

The multiple requirements for Shh pathway members in the postnatal stem cell niches of the V-SVZ and DG raise several questions for future research. Intriguingly, studies of GLI1 expression in models of demyelinating lesions indicate that, whereas GLI1-expressing cells are mobilized to generate oligodendrocytes after damage, inhibition of GLI1 enhances the maturation of these oligodendrocytes and recovery after injury (Samanta et al., 2015). It will be of interest to test whether this production of oligodendrocytes after injury recapitulates the earlier postnatal oligodendrogenesis program. Similarly, it is not yet clear whether the pattern of Shh activity in the stem cell niche is

altered after other injuries or after repeated antimitotic challenge. Finally, whereas both the choroid plexus and local neurons in the basal forebrain and hilus have been identified as potential sources of SHH ligand, how this ligand is dynamically conveyed to GLI1-expressing cells and the broader V-SVZ and DG niches is not yet clear (Lai et al., 2003; Machold et al., 2003). Recent findings using live-tissue imaging in embryonic tissue indicate that, in some cases, SHH ligand may also be transported over longer distances via specialized signaling filopodia known as cytonemes or other dynamic projections that are not evident in fixed specimens (Sanders et al., 2013). However, whether such projections occur in the more restrictive tissue environment of the mature, myelinated brain remains to be investigated.

Shh signaling during circuit wiring

As secreted molecules that act over long distances, morphogens such as SHH are ideally situated to guide axonal projections as they innervate target regions and establish connectivity. Indeed, multiple morphogens, including WNTs, BMPs, and SHH, are repurposed to mediate axon guidance in multiple regions of the developing nervous system (Yam and Charron, 2013; Zuñiga and Stoeckli, 2017). The ability of SHH to function as an axon guidance molecule was first demonstrated in RGCs (Trousse et al., 2001). Recent work has elucidated the molecular underpinnings of Shh-mediated guidance in two critical processes: proper crossing of commissural axons at the spinal cord midline commissure and sorting of contralaterally and ipsilaterally projecting retinal ganglion cells (contra- and ipsi-RGCs) at the optic chiasm. In the mouse visual system, ipsi-RGCs originating from the ventrotemporal retina do not cross the optic chiasm, facilitating coherent processing of the contralateral visual hemifield. Disruption of Shh signaling through function-blocking antibodies or *Boc* deletion disrupts axon organization at the chiasm, ipsi-RGC guidance, and eye-specific segregation in the retinorecipient dorsal lateral geniculate nucleus (Sánchez-Camacho and Bovolenta, 2008; Fabre et al., 2010; Sánchez-Arrones et al., 2013). *Shh* is expressed exclusively by contra-RGCs, whereas *Boc* and *Ptc2* are expressed by ipsi-RGCs (Sánchez-Camacho and Bovolenta, 2008). Furthermore, *Boc* expression in contra-RGCs is sufficient to misroute them ipsilaterally (Fabre et al., 2010). *Shh* is not expressed by cells at the optic chiasm, suggesting a mechanism for guidance distinct from that used by spinal commissural axons. A recent, elegant study demonstrated that *Shh* expressed by contra-RGCs is trafficked to the chiasm, where it is enriched and repels ipsi-RGCs (Peng et al., 2018). This type of *trans*-axonal signaling represents a novel mechanism to ensure proper sorting at the chiasm. Intriguingly, recent data suggest that the Shh signaling component (Hedgehog interacting protein (HHIP) is also present in retinorecipient regions (U. Javed and J.W.T., unpublished observations), raising the possibility that this multifunctional molecule may serve yet another purpose in the assembly of visual system circuitry.

Neurons located in the developing dorsal spinal cord extend axons toward the ventral floor plate, where they cross to the contralateral side and turn anteriorly (Evans and Bashaw, 2010). Similar to other guidance cues, SHH plays a dual role in this system, acting as both a chemoattractant and a chemorepellant. In combination with Netrin1, SHH is secreted from the floor plate, establishing a high-ventral to low-dorsal gradient of attractants for dorsal neurons (Charron et al., 2003; Kennedy et al., 2006). Shh-mediated attraction requires SMO (Charron et al., 2003), as well as the coreceptor BOC (Okada et al., 2006). Unlike its morphogenic effects, Shh-induced attraction is not transcriptionally mediated, but it does require new protein translation. Through a noncanonical pathway, SHH activates Src family kinases (SFKs) (Yam et al., 2009), well known regulators of the cytoskeleton during axon guidance (Robles et al., 2005). SFK-phosphorylated zipcode binding protein 1 (ZBP1) then induces local translation of β-actin in the growth cone, driving axon attraction to the floor plate (Lepelletier et al., 2017). More recently, it has been shown that the guanine nucleotide exchange factors DOCK 3 and 4 and their binding partners ELMO1 and 2 are required for cytoskeletal remodeling during axon attraction. After axons have crossed the midline, temporally regulated expression of 14-3-3 adapter protein isoforms, which reduce protein kinase A activity, function to switch SHH responsiveness from attraction to repulsion (Yam et al., 2012). After midline crossing, axons are repelled toward the anterior by a posterior-high gradient of SHH along the neural tube.

HHIP is a SHH receptor that has also been implicated in axon repulsion. In the developing chick spinal cord, *Hhip* is transiently expressed at the time when axons of commissural neurons cross the midline (Bourikas et al., 2005). *Hhip* upregulation is triggered by SHH binding to Glypican1 (Gpc1), a membrane-tethered glycoprotein (Wilson and Stoeckli, 2013). How Gpc1 signals to activate HHIP expression is unclear, as is the mechanism by which HHIP, which lacks an intracellular domain, mediates repulsion from the midline. One mechanism by which HHIP could influence axon guidance, however, is to sequester SHH and thus inactivate signaling (Chuang and McMahon, 1999). $Hhip^{-/-}$ embryos did not display guidance defects, raising the possibility that HHIP may function redundantly, as has been observed with other SHH receptors (Allen et al., 2011; Yam et al., 2012). Alternatively, HHIP may have essential functions in other aspects of circuit development independent of axon repulsion.

Shh signaling during synapse formation

After an axon reaches its target region, it is surrounded by a sea of potential synaptic partners, of which only a subset are appropriate targets. Although tremendous progress has been made in characterizing many of the long-range cues required to guide axons into their target region, the cellular mechanism by which neurons choose synaptic partners is still not fully understood (Charron et al., 2003; Yam and Charron, 2013; Belgacem et al., 2016; Zuñiga and Stoeckli, 2017). Several lines of evidence in both invertebrates and vertebrates suggest that Shh signaling has prominent functional roles during synapse formation and circuit assembly. In the *Drosophila* visual system, SHH derived from the axons of photoreceptor neurons signals to synaptic target neurons to promote differentiation and synapse formation (Chu et al., 2006; Belgacem et al., 2016). Recent studies in the mammalian cortex and hippocampus have likewise found that numerous components of the Shh signaling pathway are expressed in specific neural cell types. Both SMO and PTCH1 have been observed in the somatodendritic compartment of rat hippocampal neurons (Masdeu et al., 2007; Petralia et al., 2011). Moreover, treatment of cultured hippocampal neurons with exogenous SHH or Shh agonist has been shown to induce presynaptic differentiation and increase the size and number of presynaptic terminals (Mitchell et al., 2012). SHH itself is expressed in a variety of postmitotic neuronal cell types, including Purkinje cells (PCs) in the cerebellum and CA1 and CA3 pyramidal neurons in the hippocampus (Traiffort et al., 1999; Wechsler-Reya and Scott, 1999; Garcia et al., 2010; Harwell et al., 2012). In the cortex, *SHH* is expressed by layer V subcortical projection neurons, whereas BOC is expressed by layer II/III intracortical projection neurons

that make synaptic connections onto layer V projection neurons (Harwell et al., 2012). Loss of *Boc* or *Shh* in cortical neurons disrupted functional connections between layer II/III neurons and their layer V synaptic targets, whereas layer II/III intralaminar connectivity was preserved. These studies demonstrate a specific requirement for Shh signaling during the final steps of synaptic partner selection in addition to its established role in guiding axons to target regions.

The precise molecular mechanism by which SHH directs synapse formation and specificity has yet to be addressed. It is currently unknown whether SHH functions as a permissive cue, signaling to incoming axons to begin synapse formation in their target layer, or as an instructive cue bound to the membrane of specific subsets of SHH-expressing neurons. It has been shown that *Shh*-dependent synapse formation between subsets of neurons in the cortex requires *Boc* expression, which is necessary for noncanonical *Shh* signaling. However, whether the same cytoskeletal regulatory pathways involved in *Boc*-dependent axon guidance are also involved in synapse formation has yet to be investigated.

Shh signaling in astroglial cells of the CNS

The conventional model of Shh signaling has focused predominantly on its roles in a wide range of neurodevelopmental processes that largely affect the development of neurons and oligodendrocytes. Less well understood is the role of Shh signaling in astroglial cells, the principal cells in the postnatal and mature brain where Shh activity persists (Garcia et al., 2010; Farmer et al., 2016). Astrocytes play key roles in a broad array of nervous system processes, including the nervous system response to injury and disease, maintenance of the blood–brain barrier (BBB), as well as synapse formation and function (Sofroniew and Vinters, 2010). The molecular mechanisms that mediate and regulate these processes are not fully understood, but an emerging body of evidence suggests a role for Shh.

Astrocytes are enriched for *Smo*, *Ptch1*, and *Gli1* (Cahoy et al., 2008; Garcia et al., 2010; Ihrie et al., 2011; Farmer et al., 2016). Gli1 expression is restricted to discrete subpopulations of astrocytes throughout the brain (Garcia et al., 2010; Farmer et al., 2016), suggesting that Shh signaling may drive distinct molecular and functional programs. Indeed, in the cerebellum, two classes of astroglial cells possess unique molecular profiles that are dependent on Shh signaling (Farmer and Murai, 2017). Bergmann glia (BG) and velate astrocytes (VAs) were recognized as distinct cell types >100 years ago by Santiago Ramón y Cajal (Ramón y Cajal, 1911). BG are one of the few glial cells that retain a radial morphology within the adult brain. Their somata reside adjacent to PCs, where they project multiple radial processes across the molecular layer to form end feet on the basal lamina of the pial surface of the cerebellar cortex. This intimate association with PCs places BG as critical players in cerebellar physiology (Takayasu et al., 2006; Bellamy, 2007; Saab et al., 2012; Wang et al., 2012). Although BG have been extensively studied, very little is known about VAs outside of their morphology and spontaneous calcium transients (Chan-Palay and Palay, 1972; Hoogland and Kuhn, 2010). VAs are nonpolarized cells that tile the granule cell layer, where they project veil-like processes to envelope granule neuron somata and mossy fiber terminals. Given their vastly different environments, it is not surprising that BG and VAs display distinct gene expression profiles. BG express a defining array of transporters, channels, and receptors, including high levels of the glutamate transporter GLAST, the inward-rectifying potassium channel Kir4.1, and the ionotropic AMPA receptors GluA1 and GluA4 (Rothstein et al., 1994; Iino et al., 2001; Saab et al., 2012; Farmer et al., 2016). Conversely, VAs express low levels of GLAST, Kir4.1, GluA1, and GluA4 while expressing high levels of the water channel AQP4 (Farmer et al., 2016).

The different location, morphology, and gene expression profile of BG and VAs hints at different developmental trajectories; however, it has been difficult to pinpoint where the two cell types diverge during development (Buffo and Rossi, 2013). Surprisingly, it was recently revealed that the molecular and physiological properties of these astrocytes are determined, not by a developmental program, but rather by persistent signaling from PCs via the Shh pathway. Mature PCs secrete the SHH ligand, which signals to adjacent BG. Blocking Shh signaling in mature BG by Cre-Lox-mediated conditional KO of *Smo* results in the loss of GLAST, GluA1, GluA4, and Kir4.1 protein and increased expression of AQP4, an expression profile resembling that of VAs (Figure 1). In addition to altering gene expression, loss of Smo function in BG causes reduced AMPA-mediated currents indicating that the Shh pathway regulates intrinsic physiological properties of BG as well. Loss of the Shh ligand from discrete clusters of mature PCs phenocopies *Smo* loss-of-function in adjacent BG. Therefore, PC-derived Shh is responsible for maintaining the unique gene expression and physiology of BG. Interestingly, the profound changes in molecular phenotype caused by disrupting Shh signaling are not associated with gross alterations in BG morphology.

Whereas loss of Shh signaling in BG results in a VA-like molecular phenotype, activating the Shh pathway in mature VAs by the conditional expression of constitutively active Smoothened leads to the expression Glast, GluA1, and Kir4.1 and the reduction of AQP4. Therefore, mature VAs are not only responsive to Shh signaling, but also adopt a BG-like expression profile when the pathway is activated. As seen in BG, manipulating Shh signaling in VAs does not grossly affect morphology.

Together, these data show that the Shh pathway is both necessary and sufficient to maintain the BG-specific gene expression and physiology in cerebellar astrocytes. Therefore, persistent PC–BG signaling through Shh is a major determinant of cerebellar astrocyte phenotype (Farmer et al., 2016). Interestingly, Shh signaling does not regulate the expression of GLAST, GluA1, or GluA4 in the cerebral cortex or hippocampus, but Kir4.1 is induced in the forebrain and cerebellum in an Shh-dependent manner (Farmer et al., 2016), suggesting that astrocytes from different brain regions initiate distinct transcriptional programs in response to Shh pathway activation. Furthermore, the data suggest that, similar to the Gli1-expressing astrocyte-like adult stem cells, the specialized properties of astrocytes require persistent reinforcement by extrinsic cues, illustrating that cellular diversity in the brain is not determined exclusively during development, but rather continues to be refined into adulthood (Farmer et al., 2016).

The observation that Shh regulates expression of several synaptic-related proteins suggests that it may play a role in mediating astrocyte–synapse interactions. Indeed, a growing body of evidence now shows that astrocytes regulate the formation of specific synaptic connections in the developing cortex (Allen and Eroglu, 2017). An intriguing question for the future is whether neuron-derived SHH coordinates the activities of both *Boc*-expressing neurons and *Gli1*-expressing astrocytes during circuit assembly and how these activities compare with the effects of Shh signaling in other functions of astrocytes.

In contrast to the cerebellum, where *Gli1* expression identifies distinct classes of astroglial cells that are regionally and morpho-

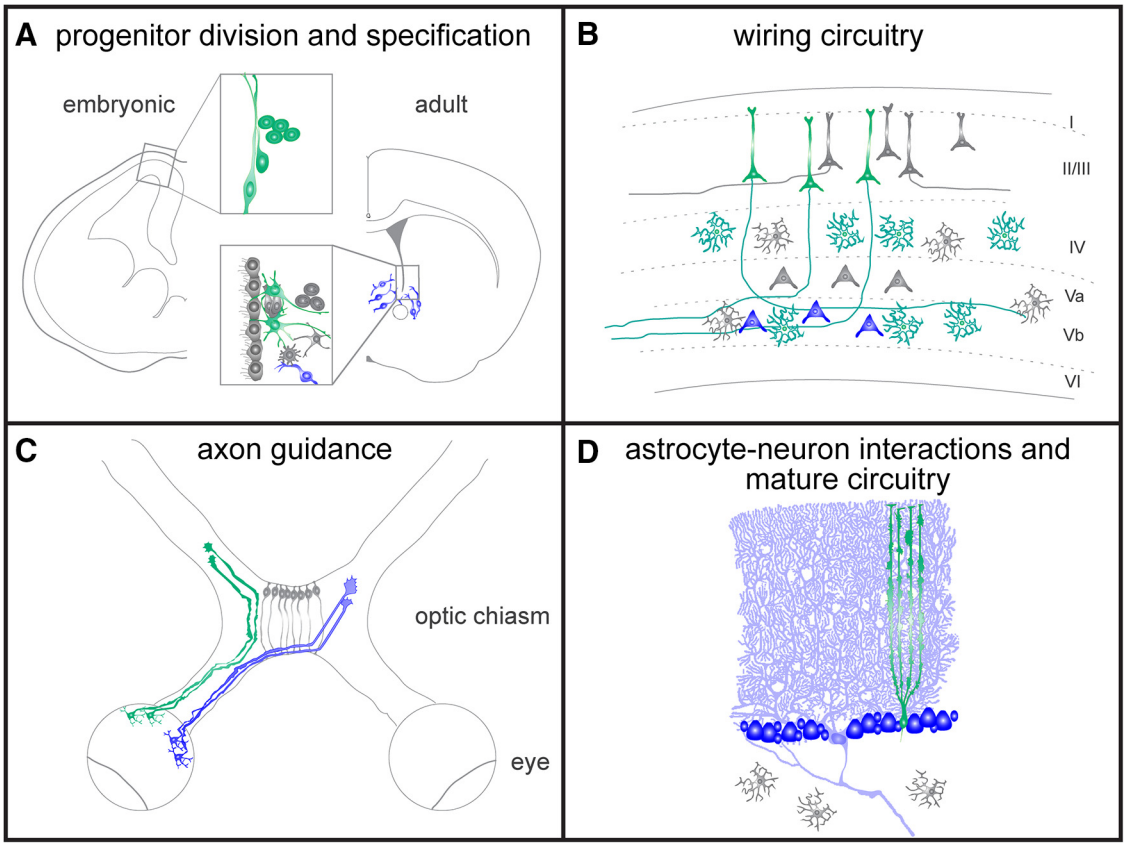

Figure 1. Simplified schematics of Shh signaling in the contexts discussed herein. Where known, the source of SHH ligand is indicated in blue and Shh-responsive cells are shown in green. ***A***, Shh signaling, primarily indicated by expression of *Gli1* in stem and progenitor cells, is critical for stem cell division and specification in both the embryonic and mature brain. In the embryonic neocortex, *Gli1* transcript is found in the VZ and the SVZ that contain radial glia and intermediate progenitors (all shown in green, inset) and elevation of Shh activity via expression of a constitutively active Smo (SmoM2) results in an expansion of oRG and IP populations. During development, many potential sources of SHH ligand exist, but the key source driving cortical signaling is not yet known. In the adult stem cell niche, *Gli1* is found in a ventrally located subset of stem/progenitor cells and is sufficient to impose a ventral identity when ectopically expressed. In the mature niche, one likely source of SHH ligand is local neurons in neighboring areas, as indicated by blue cell placement. ***B***, During circuit assembly in the postnatal cortex, *Shh* is expressed by deep layer subcortical projection neurons and functions to guide synapse formation with Boc expressing intracortical projection neurons within layer V. As the cortex matures, a subset of differentiated astrocytes expresses *Gli1*. ***C***, Axon guidance in the developing visual system, where *Shh* is expressed exclusively by contra-RGCs, whereas *Boc* and *Ptch2* are expressed by ipsi-RGCs. *Shh* from contra-RGCs repels ipsi-RGCs, facilitating proper axon sorting and eye-specific segregation. ***D***, Maintenance of specialized astrocyte phenotypes in mature circuitry of the cerebellum. BG adopt specific molecular programs in response to SHH produced by neighboring PCs.

logically defined, Gli1 astrocytes in the forebrain are distributed both between and within distinct regions. Gli1 astrocytes are absent from all white matter tracts, but are found with varying ratios to Gli1-negative astrocytes throughout the hypothalamus, thalamus, globus pallidus, septum, and cortex (Garcia et al., 2010). Whether Shh drives distinct molecular or functional properties of Gli1 astrocytes throughout these regions, as has been observed in the cerebellum, is an area of active research.

Shh signaling during brain injury and repair

Although the functional significance of Shh signaling to astrocytes remains largely unknown, current efforts aimed at understanding its role in astrocyte function have focused largely on its role in injury and neural repair. Astrocytes are central to the injury response in the pathological CNS, mitigating secondary damage through the production of the glial scar (Sofroniew, 2014). It has been widely reported that both SHH and GLI1 increase in response to various injury models, including stroke and stab wound and spinal cord injury (Bambakidis et al., 2003; Amankulor et al., 2009; Sims et al., 2009; Bambakidis et al., 2010; Pitter et al., 2014; Jin et al., 2015). Both pharmacological and genetic interruption of Shh signaling reduce proliferation of reactive astrocytes (Amankulor et al., 2009), which are required for the production of a glial scar (Wanner et al., 2013). This suggests that SHH may promote neural repair mechanisms through its actions as a mitogen. Indeed, Shh signaling may promote proliferation of local neural progenitors, as discussed above, and has even been suggested to promote the adoption of NSC properties by reactive astrocytes (Bambakidis et al., 2003; Bambakidis et al., 2010; Sirko et al., 2013).

Beyond their role in glial scar formation, astrocyte interactions with endothelial cells are critical for maintaining the BBB (Abbott et al., 2006). Shh signaling between astrocytes and endothelial cells restricts the permeability of the BBB, limiting the extravasation of blood-derived inflammatory molecules into the CNS (Alvarez et al., 2011). In addition, promoting Shh activity dramatically reduces the infiltration of blood-borne macrophages into the CNS after a stab wound injury (R.V. Allahyari and A.D.R.G., unpublished observations). This effect is abolished in conditional mutants in which *Smo* is selectively deleted in astrocytes, suggesting a requirement for Shh signaling. Together, these observations suggest that Shh signaling in astrocytes mitigates inflammation and may provide neuroprotective benefit to reorganization and restoration of homeostasis to tissues following injury.

In contrast to reports describing upregulation of Shh in reactive astrocytes, emerging studies have demonstrated a loss of Shh signaling after injury. Using genetic labeling strategies, Gli1 expression in astrocytes is downregulated after injury, suggesting that Shh signaling does not persist in reactive astrocytes (Mierzwa et al., 2014) (R.V. Allahyari and A.D.R.G., unpublished observations). A major distinction between these studies and those reporting an increase in SHH and GLI1 is the use of genetic tools to identify GLI1-expressing cells. Whereas earlier reports have relied primarily on antibody labeling to identify SHH and GLI1, studies reporting a reduction in Gli1 have relied on genetic mouse models in which a tamoxifen-inducible Cre is targeted to the *Gli1* locus, enabling cell- and temporally specific expression of reporter proteins (Ahn and Joyner, 2004). Resolving these apparently conflicting observations will be an important step toward gaining a better understanding of Shh signaling in astrocyte function.

Conclusion

As discussed herein, a growing body of research has demonstrated diverse functional requirements for Shh signaling in the developing and mature nervous system. Although the core components of this pathway recur in many of these cases, the diversity of coreceptors and downstream pathway components that can modulate *Ptc1*, *Smo*, and *Gli1* mean that this pathway can act in cell-type- and context-dependent patterns to execute a wide variety of cellular programs. Future research focused on the delivery and contextual interpretation of Shh signals by differing neural cell types will continue to enhance our understanding of this adaptable, multifunctional pathway.

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