REVIEW

Positive and negative regulation of Shh signalling in vertebrate retinal development [version 1; referees: 3 approved]

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Abstract
Cell-to-cell communication is fundamental for embryo development and subsequent tissue homeostasis. This communication is often mediated by a small number of signaling pathways in which a secreted ligand binds to the surface of a target cell, thereby activating signal transduction. In vertebrate neural development, these signaling mechanisms are repeatedly used to obtain different and context-dependent outcomes. Part of the versatility of these communication mechanisms depends on their finely tuned regulation that controls timing, spatial localization, and duration of the signaling. The existence of secreted antagonists, which prevent ligand–receptor interaction, is an efficient mechanism to regulate some of these pathways. The Hedgehog family of signaling proteins, however, activates a pathway that is controlled largely by the positive or negative activity of membrane-bound proteins such as Cdon, Boc, Gas1, or Megalin/LRP2. In this review, we will use the development of the vertebrate retina, from its early specification to neurogenesis, to discuss whether there is an advantage to the use of such regulators, pointing to unresolved or controversial issues.

Keywords
Cell cell communication, Shh Signalling, retina, development, eye, patterning, regulation

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Introduction

The highest functions of the nervous system are based on communication among the huge variety of cells that compose the vertebrate brain. Communication among cells is also fundamental for correct development of the nervous system. Although there are several ways in which neural cells (and cells in general) exchange information, communication mediated by families of signaling molecules such as Wnt, bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and Hedgehog (Hh) is one of the most common. These molecules activate specific signaling pathways that share grossly similar designs, although individual molecular components are specific to each one of the pathways. Ligands are secreted from restricted cellular sources and bind to receptor complexes on the receiving cells. Ligand–receptor binding activates a signaling cascade that ultimately leads to transcriptional regulation of target genes or, less often, to alternative non-transcriptional pathways when more immediate responses are needed. These signaling pathways are used over and over in development to regulate events as diverse as cell specification, proliferation, migration, and differentiation. It follows that their activity needs to be exquisitely controlled, ensuring that information among cells is activated where required and switched off at, or prolonged for, the appropriate time in order to obtain the required context-dependent output. There are different levels of regulation for these signaling molecules. Perhaps the most direct is the existence of classes of secreted proteins that interact with the ligand in the extracellular space, thereby preventing binding to their receptor. This occurs, for example, in the case of BMPs or Wnts, for both of which a large number of secreted antagonists exist[1,2]. Signaling enhancement also depends on secreted proteins that in some cases promote ligand diffusion as described for Wnt proteins[3–6]. In contrast, the currently known ligand-binding modulators of the Hh pathway are membrane-bound proteins, prompting the question of whether there is an advantage to such an organization.

Sonic hedgehog (Shh) is the most prominent member of the Hh family in vertebrates and one of the best examples of a classic morphogen[7,8], as it induces the acquisition of specific identities in the receiving cells according to the levels and the duration of its signaling[9]. Shh activates signaling with a mechanism that has been recently defined as "double-negative"[10]. Indeed, in the absence of the ligand, its 12-pass transmembrane receptor Patched (Ptch) inhibits the seven-pass transmembrane GPCR (G-protein-coupled receptor)-like signal transducer Smoothened (Smo). In the absence of this inhibition, Smo would constitutively maintain the pathway active with the consequent transcription of Shh target genes, mediated by the family of Gli transcription factors. Shh binding to Ptch releases this inhibition and allows the expression of Gli-targeted genes. Gli targets include Ptch itself, thereby establishing a negative feedback loop, important also for limiting ligand dispersion[9]. Thus, Ptch represses Shh pathway activation by controlling both ligand dispersion and the activity of the signal transducer. *In vitro* and *in silico* models have demonstrated that this organization confers robustness to the signaling gradient[10] and thus to Shh activity as a morphogen and likely to the additional functions that Shh exerts. So, in principle, there is an advantage to such an organization (see 11 for further discussion). However, activation of Shh signaling is modulated by other surface molecules that either contribute to Shh release from the producing cells, such as Disp (Dispatched)[12], or, on the receiving cells, interact with Ptch or Shh or both. The latter include Cdon (cell adhesion molecule-related, downregulated by oncogenes), Boc (Brother of Cdon), Gas1 (growth arrest protein 1)[13,14], and Megalin/LRP2 (Megalin/low-density lipoprotein receptor-related protein 2)[15]. The regulation of the membrane availability of Smo by the tetraspanin Atthog/Mosmo (modulator of Smo) is a recently described additional mechanism of Shh regulation[16]. Is the presence of these membrane modulators also an advantage?

So far, no studies have formally addressed this question. Nevertheless, in this review, we will use the progressive formation of the vertebrate retina to discuss Shh functions in which some of these regulators have been implicated, pointing to potential advantages and unresolved or controversial issues.

Cdon, Boc, Gas1, and LRP2 enhance Shh signaling during optic vesicles' bilateralization

Shh is expressed along the entire axial mesoderm – anterior prechordal plate and posterior notochord – and the ventral midline of the vertebrate neural tube. This distribution prompted the use of the spinal cord as a primary model to understand the mechanism of Shh action[17]. However, the progressive formation of the vertebrate retina offers an experimental paradigm with which to study how Shh is repurposed to shape multiple developmental aspects of the same structure, from early specification to connectivity.

The eyes are bilateral structures. Their neural component, the retina, originates from a group of cells, known as the retinal field, in the anterior neural plate. As the neural plate folds, cells of the retinal field become displaced laterally, forming two balloon-shaped optic vesicles at the side of the forming neural tube. *Shh* expression at the prechordal plate is critical for this initial morphogenesis: in the absence of Shh, optic vesicle bilateralism is lost and embryos form, in the most severe case, a single cyclopic eye or, in the milder cases, smaller eyes that are closer together. This phenotype, observed from humans to zebrafish[18], is part of a developmental anomaly known as holoprosencephaly (HPE), in which the ventral forebrain is not specified and the dorsal forebrain hemisphere tends to fuse together[19,20]. In amniotes, there are two concomitant events that contribute to optic vesicle lateralization. The first one is the Shh-dependent specification of the neural plate overlying the prechordal plate into the hypothalamic primordium, which therefore intervenes the two vesicles[19]. The second is the patterning of the optic vesicles along their proximal–distal axis, which involves the Shh-mediated specification of the proximal/optic stalk domain (reviewed in 17). In teleost fishes, the Shh-mediated posterior-to-anterior migration of medial cells that intercalate into the retinal field is an additional factor[21]. Genetic inactivation of basic components of the Shh pathway in mouse or zebrafish and mutational screening in patients with HPE confirmed the importance of Shh signaling in ventral central nervous system (CNS) patterning and

thus in the proper positioning and growth of the optic vesicles[18,22]. Similar studies have also shown that *Cdon*, *Boc*, *Gas1*, and *LRP2* participate in these developmental events[18,23–26].

Cdon and Boc are closely related cell adhesion molecules that can form homophilic and heterophilic complexes and interact with both Shh and Ptch (reviewed in 27). Cdon/Boc interaction with Ptch increases high-affinity ligand binding, indicating their function as Ptch co-receptors and thus as positive signaling regulators[14,23,28–30]. The two genes are expressed with largely overlapping patterns that include the entire dorsal neural tube and the developing eye and ear and the olfactory system[31,32]. This distribution often coincides with that of *Gas1*[33], encoding a GPI (glycosylphosphatidylinositol)-linked protein that also interacts with Shh and Ptch[34,35] (Figure 1A). Mouse embryos lacking *Cdon*, *Boc*, and *Gas1* show a phenotype that mimics *Shh* loss of function[19], which leads to the absence of the entire ventral neural tube resulting in severe HPE and early embryonic

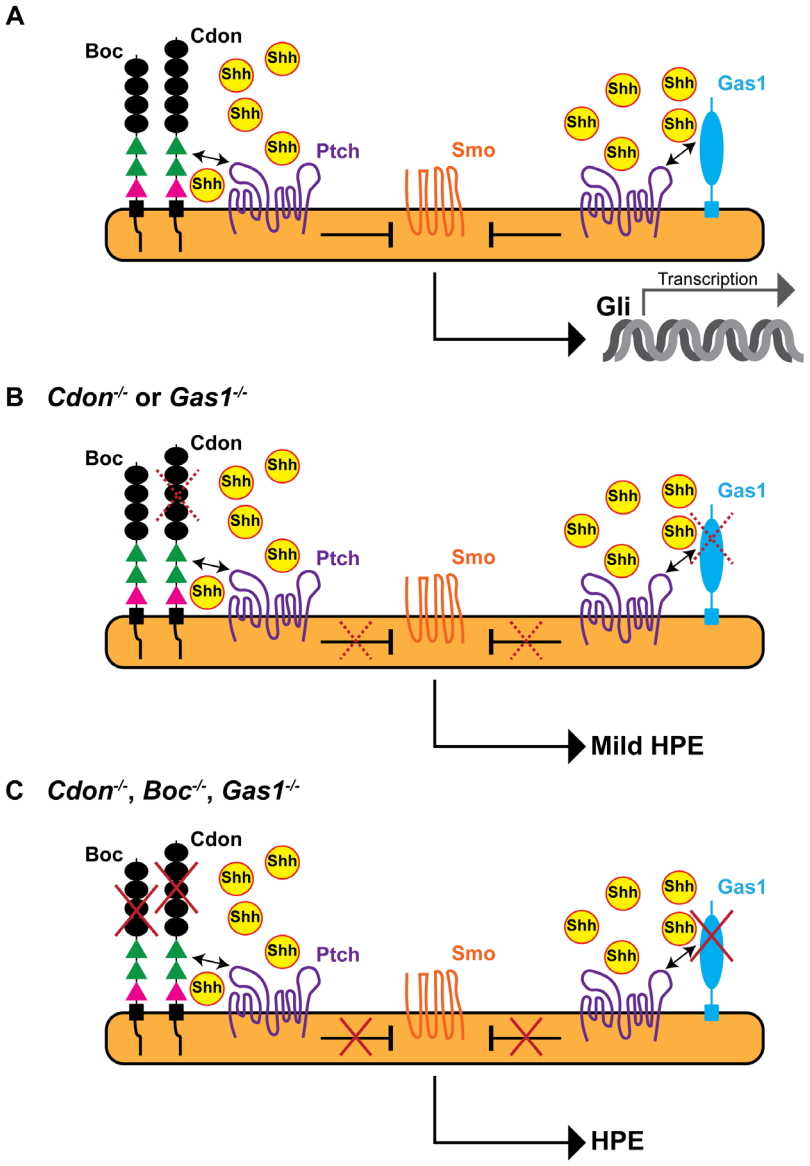

Figure 1. Cdon, Boc, and Gas1 act as positive regulators of Shh signaling during optic vesicle formation. The diagrams represent the interaction of Cdon, Boc, and Gas1 with Ptch and Shh during Shh-mediated patterning of the ventral neural tube in wild-type embryos (**A**) or in embryos with genetic inactivation of either *Cdon* or *Gas1* function (**B**) or lacking *Cdon*, *Boc*, and *Gas1* (**C**). The three co-receptors interact with Ptch and the complex binds Shh with high affinity. In the presence of Shh, Smo is de-repressed (red crosses) and activates a signal transduction cascade that culminates with Gli-mediated transcription of Shh target genes. The Cdon/Ptch and Boc/Ptch interactions are mediated by the FnIIIa and FnIIIb domains (green) of Cdon and Boc, respectively. Binding of Shh to Cdon or Boc is mediated by the FnIIIc domain (pink). (**B**) In the absence of either *Cdon* or *Gas1*, Shh is less activated (dotted red crosses), resulting in mild craniofacial defects. (**C**) Loss of all three co-receptors prevents pathway activation, resulting in severe HPE, a phenotype that mimics *Shh* loss of function. Boc, Brother of Cdon; Cdon, cell adhesion molecule-related, downregulated by oncogenes; Gas1, growth arrest protein 1; HPE, holoprosencephaly; Ptch, patched; Shh, sonic hedgehog; Smo, smoothened.

lethality (Figure 1C). This indicates that the three co-receptors play positive and overlapping roles in regulating Shh pathway activation[13,14]. Furthermore, Shh signaling represses *Cdon*, *Boc*, and *Gas1* expression[30,36]. This suggests that these co-receptors may serve as buffers to prevent possible defects due to abnormally low Shh signaling because, if Shh activity decreases, their upregulation could boost signaling again. However, genetic inactivation of the individual co-receptor genes reveals non-equivalent roles. *Gas1* null mouse embryos present ventral neural tube defects, mild HPE, and mis-specification of the ventral retinal pigmented epithelium into a neural retina-like tissue[33,36]. *Cdon* null embryos display a similar mild HPE (Figure 1B) with small eyes and coloboma (opened optic fissure)[23,37]. *Boc* null mice instead have none of these defects but, when crossed with either *Cdon* or *Gas1* mutants, enhance their respective HPE phenotype[24,25]. Whether Boc also modifies their respective specific eye phenotype remains to be studied. Somewhat in line with these differences, systematic genomic sequencing analysis of patients with HPE has identified causative mutations in the *CDON* gene[23,38] but only sequence variations suggestive of a modifier role for *BOC* and perhaps for *GAS1*[38]. Given that these co-receptors have all been shown to foster Shh signaling, it is not obvious why their loss of function causes these phenotypic differences, especially in the case of the closely related *Cdon* and *Boc*. One possibility is that Shh signaling exerts a differential negative regulation on their expression. Alternatively (or additionally), Cdon and Boc may employ distinct mechanisms to enhance Shh signaling, as recently suggested[29]. For example, the ectodomain of Boc, but not that of Cdon, can be proteolyzed[29]. If this proteolysis occurs *in vivo*, which is still a matter of speculation, Boc ectodomain could enhance Shh diffusion and at the same time terminate high-affinity binding of Shh to Ptch. The two effects may compensate one another, explaining the lack of HPE phenotype in *Boc* mutants. Variations in *Cdon*, *Boc*, and *Gas1* distribution may also underlie the observed differences in the mutants' phenotype. This differential expression may also offer an alternative explanation for how Cdon and Boc influence Shh signaling. Indeed, whereas the ventral neural tube and optic vesicle expression of *Gas1*[33] makes it easy to understand its Ptch co-receptor function, the predominant dorsal expression of *Cdon* and *Boc*[31,32] makes the same function less immediately understood. *Boc* and *Cdon* could be transiently expressed in the ventral neural tube right when needed for early patterning, as reported for the zebrafish *Boc* orthologue[39]. However, the expression of *Cdon*, but not *Boc*, in the axial midline of both mouse and zebrafish[30,40] suggests that Cdon could have the additional role of favoring Shh release from the producing cells. The *Drosophila* homologue of Cdon, interference Hh (*ihog*), has been reported to have such an activity[41], although motif differences between Shh and its *Drosophila* homologue Hh call for caution in applying directly to vertebrates what has been learned in the fly[42]. Nevertheless, the HPE phenotype of *Cdon* null embryos could easily be explained by an attenuated Shh release from the midline, a function in which *Boc* may not be implicated.

At the moment, this is only a hypothesis but it may be worth testing. It is equally unexplored whether LRP2 can functionally interact with Boc, Cdon, or Gas1 or with their possible different heterodimeric or trimeric complexes. LRP2 facilitates Shh/Ptch binding and promotes the internalization of the complex, which is required to relieve Smo inhibition. Thus, in the absence of LRP2, Shh signaling is impaired, leading to embryos with an HPE phenotype[43]. It remains an open question whether LRP2 promotes Cdon, Boc, and Gas1 internalization when bound to Ptch or instead competes with them for Ptch and Shh binding.

Cdon, Boc, and LRP2 can counteract Shh signaling during retinal development

The work we have discussed so far, independently of the still-puzzling aspects, supports a positive role of Cdon, Boc, Gas1, and LRP2 in Shh signaling and thus in the specification of the ventral CNS and eye separation. However, Cdon, Boc, and LRP2 have been shown to act as negative regulators of Shh signaling as retinal development progresses, although each one of them does so in different contexts (Figure 2).

As mentioned before, the formation of two bilateral optic vesicles implies the compartmentalization of its neuroepithelium in different domains along the different axes. One of the first subdivisions occurs along the proximo–distal axis of the vesicle and originates the prospective optic stalk proximally and the prospective retina distally (Figure 2A). The establishment of the optic stalk and retinal domains is defined by the specific and respective expression of two paired- and homeobox-containing transcription factors: paired box protein Pax-2 (Pax2) and Pax6 (reviewed in 44). The two factors cross-repress each other and thus define a sharp border between the two territories[45] (Figure 2A). Shh signaling promotes *Pax2* expression, thereby imposing optic stalk identity. When Shh is reduced or absent, the optic stalk domain is smaller or absent and the two retinal domains tend to fuse together. Shh overexpression has the opposite effect with an excess of Pax2-positive optic stalk that overtakes the retinal domain by repressing *Pax6*[17,44]. This means that the right amount of Shh signaling is critical to form a precise boundary between the optic stalk and the retina. Recent studies have shown that, at least in zebrafish and chick embryos, Cdon participates in the establishment of this boundary[40]. In both species, *ptch* is expressed in the *pax2*-positive optic stalk, whereas *cdon*, but not *boc*, is strongly expressed in the presumptive neural retina overlapping with *pax6* distribution (Figure 2A)[40]. The complementarity between *ptch* and *cdon* expression advocates against a synergistic role. Indeed, morpholino-mediated knockdown of *cdon* allows for the expansion of the optic stalk, decreases eye size, and prevents optic fissure closure, indicating that Cdon counteracts Shh effect[40]. This phenotype depends on the ability of Cdon to bind Shh but not Ptch. Furthermore, it is a direct consequence of Cdon activity in the retina because targeted *cdon* overexpression in the zebrafish retina is sufficient to rescue the phenotype of *cdon* knockdown and spatiotemporal restricted interference with *Cdon* retinal expression in chick embryos mimics the zebrafish phenotype[40]. The precise mechanism by which this happens is still unrefined; however, when mis-expressed close to the optic recess midline (a Shh source), Cdon binds Shh with great efficacy and serves as a sink to limit ligand availability to the nearby cells[40]. This indicates that Cdon acts as a decoy receptor to protect the neural retina from Hh activity (Figure 2A).

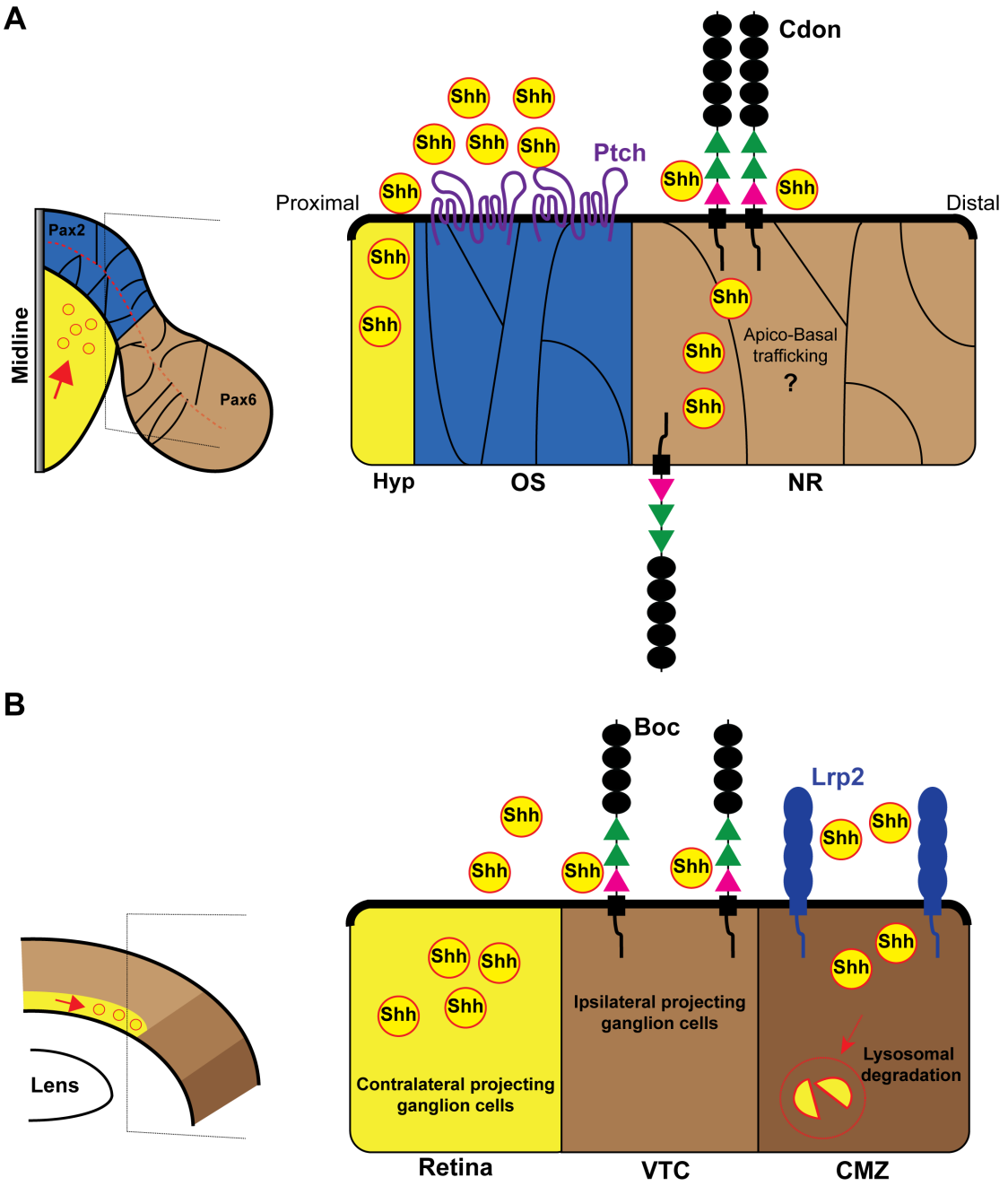

Figure 2. Cdon, Boc, and Lrp2 antagonize Shh activity during retina development. (**A**) Schematic dorsal view of the optic vesicle (left) and enlarged view of the optic stalk/neural retina border (right). The expression domains of Pax2 (blue) and Pax6 (brown) are indicated in the scheme. At the border of these two domains, Cdon binds Shh, serving as a decoy receptor to protect the neural retina from midline-derived Shh activity. Note that the Ptch receptor localizes only in the Pax6-positive neural retina domain. (**B**) Schematic frontal view of mature retina (left) and enlarged view of the retinal periphery (right). Contralateral RGCs produce and secrete Shh. Ipsilateral projecting RGCs express the co-receptor Boc that prevents Shh diffusion and thus signal activation. Low Shh signal allows for the specification of ipsilateral program specification in RGCs of the VTC. Lrp2/Megalin instead limits Shh proliferative activity by endocytic clearance of Shh at the CMZ. Boc, Brother of Cdon; Cdon, cell adhesion molecule-related, downregulated by oncogenes; CMZ, ciliary marginal zone; Hyp, hypothalamus; Lrp2, low-density lipoprotein receptor-related protein 2; NR, neural retina; OS, optic stalk; Pax2, paired box protein Pax-2; Pax6, paired box protein Pax-6; Ptch, patched; RGC, retinal ganglion cell; Shh, sonic hedgehog; VTC, ventrotemporal crescent.

A similar function has been postulated for Boc during mouse retinogenesis[46]. Retinal ganglion cells (RGCs) are the first neurons to be born in the retina of all vertebrates. Newly generated RGCs express Shh, and this expression promotes the propagation of RGC specification and differentiation, the proliferation of retinal precursors, and their differentiation toward other neuronal cell types (reviewed in 47,48). In the mouse, a small proportion of RGCs located in the ventrotemporal crescent of the retina do not express Shh[49] (Figure 2B). These neurons are special because, in contrast to all the Shh-positive RGCs, they project to the ipsilateral side of the brain, enabling the semi-binocular vision typical of rodents. These ipsilateral RGCs express Boc[49,50]. In these neurons, Boc is necessary to keep Shh signaling low, thereby enabling the expression of the transcription factor Zic2[46], a determinant of the ipsilateral program[51]. Thus, in *Boc* null mice, part of ipsilateral RGCs are mis-specified, acquiring a contralateral projecting phenotype with a consequent alteration of the retinal projections[46]. In an additional and not necessarily contrasting view, Boc, present on the membrane of ipsilateral RGC growth cones, mediates guidance information provided by Shh at the optic chiasm midline, forcing the axons to enter the ipsilateral optic tract[50]. Notably, Shh, transported along the axons of the contralaterally projecting RGCs[49,52], seems to be released at high concentrations and with a still-unknown mechanism (see 53 for discussion), right at the chiasm providing Boc-mediated repulsive information to ipsilateral axons[52]. Thus, in this case, Boc would act as a positive mediator of Shh. Whether the same molecule can have a double function in the same cell remains to be established, but, in a speculative view, Boc interactions at the perikaryon could be different from those existing at the growth cone.

Independently of this still-unanswered question, both Cdon and Boc can function as negative regulators of Shh activity, limiting ligand dispersion, a function that has been observed in *Drosophila* wing disc and ovary development[41,54,55]. Incidentally, both Cdon and Boc, when ectopically expressed close to a Shh source, localize predominantly at the basal side of the neuroepithelial cells, where they accumulate most of the bound Shh protein[40]. A recent study revisiting the function of Hhip (Hh-interacting protein)—initially defined as a membrane-bound negative regulator of HH signaling[56]—showed that Hhip is secreted and localizes to the neuroepithelial basal membrane[57]. The basal localization of both Cdon and Hhip is interesting because it may serve to clear the ligand from the apical surface of the neuroepithelial cells, where the primary cilium localizes. This organelle is fundamental for Shh signal transduction, as it hosts the main components of the transduction machinery of this pathway[58].

A negative regulation of Shh signaling, based on a different mechanism of ligand clearance, has also been proposed for LRP2. Though initially expressed in the whole optic cup, LRP2 expression becomes restricted to the peripheral margin as retina differentiation proceeds. This region, called the ciliary marginal zone (CMZ), is a source of progenitor cells in fish and amphibians[59] and likely also in the mammalian embryonic retina[60,61]. The CMZ is normally devoid of Shh activity. Deficiency of *Lrp2* in mice or zebrafish causes enlarged and exophthalmic eyes[62–65], a pathological condition known as buphthalmos and observed in patients carrying *LRP2* mutations[66]. Searching for an explanation for this phenotype, Christ *et al.*[65] found elevated transcript levels for GLI family zinc finger 1 (*Gli1*) and *Ptch1* genes, two Shh targets, suggesting that LRP2 protects the CMZ from the influence of RGC-derived Shh. In the absence of LRP2, Shh induces CMZ progenitor hyperproliferation, expanding the overall eye size. Mechanistically, LRP2 mediates lysosomal clearance of Shh alone, thereby maintaining the CMZ quiescent[65] (Figure 2B).

Although their local function has not been explored in detail, *Cdon*, *Boc*, and *Gas1* are also strongly expressed in the CMZ[36,40,49], making this structure an attractive model to study possible interaction among all these Shh-binding proteins. In a speculative view, they could all concur to make the CMZ a Shh-free zone given that Gas1 has also been initially proposed to work as a Shh sink[34].

In conclusion, going back to the original discussion point, "economy" could be the main advantage of controlling potent signaling molecules with membrane-bound proteins. Cdon, Boc, and Lrp2—in the specific case of Shh—seem to act as both positive and negative regulators of the signaling pathway depending on their additional interaction with other membrane-bound proteins (Ptch). In a speculative view, an economical way of changing the role for these regulators from a positive to a negative one might be their shuttling from the apical to the basal membrane. The destiny of Shh bound to Boc or Cdon when these proteins act as negative regulators is a matter of speculation. However, in the same economical view, unwanted Shh could be recycled back to the tissue where it is needed, such as from the neural retina to the optic stalk. The observed redundancy of regulatory molecules may not fall into the view of economy, although redundancy might be the best way of ensuring the needed levels of Shh during development and homeostasis.

Abbreviations

Bmp, bone morphogenetic protein; Boc, Brother of Cdon; Cdon, cell adhesion molecule-related, downregulated by oncogenes; CMZ, ciliary marginal zone; CNS, central nervous system; Gas1, growth arrest protein 1; Hh, hedgehog; Hhip, hedgehog-interacting protein; HPE, holoprosencephaly; LRP2, low-density lipoprotein receptor-related protein 2; Pax2, paired box protein Pax-2; Pax6, paired box protein Pax-6; Ptch, patched; RGC, retinal ganglion cell; Shh, sonic hedgehog; Smo, smoothened

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References

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1. Miyazono K: **Positive and negative regulation of TGF-beta signaling.** *J Cell Sci.* 2000; **113**(Pt 7): 1101–9.
PubMed Abstract

2. Bovolenta PG, Gorny AK, Esteve P, *et al.*: **Secreted Wnt Inhibitors or Modulators.** *Wnt Signaling in Development and Disease: Molecular Mechanisms and Biological Functions.* 2014; 177–93.
Publisher Full Text

3. Esteve P, Sandonìs A, Ibañez C, *et al.*: **Secreted frizzled-related proteins are required for Wnt/β-catenin signalling activation in the vertebrate optic cup** *Development.* 2011; **138**(19): 4179–84.
PubMed Abstract | Publisher Full Text

4. Holly VL, Widen SA, Famulski JK, *et al.*: **Sfrp1a and Sfrp5 function as positive regulators of Wnt and BMP signaling during early retinal development.** *Dev Biol.* 2014; **388**(2): 192–204.
PubMed Abstract | Publisher Full Text

5. Mii Y, Taira M: **Secreted Wnt "inhibitors" are not just inhibitors: Regulation of extracellular Wnt by secreted Frizzled-related proteins.** *Dev Growth Differ.* 2011; **53**(8): 911–23.
PubMed Abstract | Publisher Full Text

6. Sugiyama Y, Shelley EJ, Wen L, *et al.*: **Sfrp1 and Sfrp2 are not involved in Wnt/β-catenin signal silencing during lens induction but are required for maintenance of Wnt/β-catenin signaling in lens epithelial cells.** *Dev Biol.* 2013; **384**(2): 181–93.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Turing AM: **The chemical basis of morphogenesis. 1953.** *Bull Math Biol.* 1990; **52**(1–2): 153–97, discussion 119–52.
PubMed Abstract | Publisher Full Text

8. Wolpert L: **Positional information and patterning revisited.** *J Theor Biol.* 2011; **269**(1): 359–65.
PubMed Abstract | Publisher Full Text

9. Briscoe J, Small S: **Morphogen rules: design principles of gradient-mediated embryo patterning.** *Development.* 2015; **142**(23): 3996–4009.
PubMed Abstract | Publisher Full Text | Free Full Text

10. F Li P, Markson JS, Wang S, *et al.*: **Morphogen gradient reconstitution reveals Hedgehog pathway design principles.** *Science.* 2018; **360**(6388): 543–8.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

11. F Stapornwongkul KS, Salbreux G, Vincent JP: **Developmental Biology: Morphogen in a Dish.** *Curr Biol.* 2018; **28**(13): R755–R757.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

12. Tukachinsky H, Kuzmickas RP, Jao CY, *et al.*: **Dispatched and scube mediate the efficient secretion of the cholesterol-modified hedgehog ligand.** *Cell Rep.* 2012; **2**(2): 308–20.
PubMed Abstract | Publisher Full Text | Free Full Text

13. Allen BL, Song JY, Izzi L, *et al.*: **Overlapping roles and collective requirement for the coreceptors GAS1, CDO, and BOC in SHH pathway function.** *Dev Cell.* 2011; **20**(6): 775–87.
PubMed Abstract | Publisher Full Text | Free Full Text

14. Izzi L, Lévesque M, Morin S, *et al.*: **Boc and Gas1 each form distinct Shh receptor complexes with Ptch1 and are required for Shh-mediated cell proliferation.** *Dev Cell.* 2011; **20**(6): 788–801.
PubMed Abstract | Publisher Full Text | Free Full Text

15. F Christ A, Christa A, Kur E, *et al.*: **LRP2 is an auxiliary SHH receptor required to condition the forebrain ventral midline for inductive signals.** *Dev Cell.* 2012; **22**(2): 268–78.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

16. Pusapati GV, Kong JH, Patel BB, *et al.*: **CRISPR Screens Uncover Genes that Regulate Target Cell Sensitivity to the Morphogen Sonic Hedgehog.** *Dev Cell.* 2018; **44**(1): 113–129.e8.
PubMed Abstract | Publisher Full Text | Free Full Text

17. Martí E, Bovolenta P: **Sonic hedgehog in CNS development: one signal, multiple outputs.** *Trends Neurosci.* 2002; **25**(2): 89–96.
PubMed Abstract | Publisher Full Text

18. Petryk A, Graf D, Marcucio R: **Holoprosencephaly: signaling interactions between the brain and the face, the environment and the genes, and the phenotypic variability in animal models and humans.** *Wiley Interdiscip Rev Dev Biol.* 2015; **4**(1): 17–32.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Chiang C, Litingtung Y, Lee E, *et al.*: **Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function.** *Nature.* 1996; **383**(6599): 407–13.
PubMed Abstract | Publisher Full Text

20. Wallis DE, Muenke M: **Molecular mechanisms of holoprosencephaly.** *Mol Genet Metab.* 1999; **68**(2): 126–38.
PubMed Abstract | Publisher Full Text

21. Sinn R, Wittbrodt J: **An eye on eye development.** *Mech Dev.* 2013; **130**(6–8): 347–58.
PubMed Abstract | Publisher Full Text

22. Solomon BD, Mercier S, Vélez JI, *et al.*: **Analysis of genotype-phenotype correlations in human holoprosencephaly.** *Am J Med Genet C Semin Med Genet.* 2010; **154C**(1): 133–41.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Bae GU, Domené S, Roessler E, *et al.*: **Mutations in *CDON*, encoding a hedgehog receptor, result in holoprosencephaly and defective interactions with other hedgehog receptors.** *Am J Hum Genet.* 2011; **89**(2): 231–40.
PubMed Abstract | Publisher Full Text | Free Full Text

24. Seppala M, Xavier GM, Fan CM, *et al.*: ***Boc* modifies the spectrum of holoprosencephaly in the absence of *Gas1* function.** *Biol Open.* 2014; **3**(8): 728–40.
PubMed Abstract | Publisher Full Text | Free Full Text

25. Zhang W, Hong M, Bae GU, *et al.*: ***Boc* modifies the holoprosencephaly spectrum of *Cdo* mutant mice.** *Dis Model Mech.* 2011; **4**(3): 368–80.
PubMed Abstract | Publisher Full Text | Free Full Text

26. F Christ A, Herzog K, Willnow TE: **LRP2, an auxiliary receptor that controls sonic hedgehog signaling in development and disease.** *Dev Dyn.* 2016; **245**(5): 569–79.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

27. Sanchez-Arrones L, Cardozo M, Nieto-Lopez F, *et al.*: **Cdon and Boc: Two transmembrane proteins implicated in cell-cell communication.** *Int J Biochem Cell Biol.* 2012; **44**(5): 698–702.
PubMed Abstract | Publisher Full Text

28. Kang JS, Gao M, Feinleib JL, *et al.*: **CDO: An oncogene-, serum-, and anchorage-regulated member of the Ig/fibronectin type III repeat family.** *J Cell Biol.* 1997; **138**(1): 203–13.
PubMed Abstract | Publisher Full Text | Free Full Text

29. F Song JY, Holtz AM, Pinskey JM, *et al.*: **Distinct structural requirements for CDON and BOC in the promotion of Hedgehog signaling.** *Dev Biol.* 2015; **402**(2): 239–52.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

30. Tenzen T, Allen BL, Cole F, *et al.*: **The cell surface membrane proteins Cdo and Boc are components and targets of the Hedgehog signaling pathway and feedback network in mice.** *Dev Cell.* 2006; **10**(5): 647–56.
PubMed Abstract | Publisher Full Text

31. Mulieri PJ, Kang JS, Sassoon DA, *et al.*: **Expression of the *boc* gene during murine embryogenesis.** *Dev Dyn.* 2002; **223**(3): 379–88.
PubMed Abstract | Publisher Full Text

32. Mulieri PJ, Okada A, Sassoon DA, *et al.*: **Developmental expression pattern of the *cdo* gene.** *Dev Dyn.* 2000; **219**(1): 40–9.
PubMed Abstract | Publisher Full Text

33. Allen BL, Tenzen T, McMahon AP: **The Hedgehog-binding proteins Gas1 and Cdo cooperate to positively regulate Shh signaling during mouse development.** *Genes Dev.* 2007; **21**(10): 1244–57.
PubMed Abstract | Publisher Full Text | Free Full Text

34. Lee CS, Buttitta L, Fan CM: **Evidence that the WNT-inducible *growth arrest-specific gene 1* encodes an antagonist of sonic hedgehog signaling in the somite.** *Proc Natl Acad Sci U S A.* 2001; **98**(20): 11347–52.
PubMed Abstract | Publisher Full Text | Free Full Text

35. Martinelli DC, Fan CM: ***Gas1* extends the range of Hedgehog action by facilitating its signaling.** *Genes Dev.* 2007; **21**(10): 1231–43.
PubMed Abstract | Publisher Full Text | Free Full Text

36. Lee CS, May NR, Fan CM: **Transdifferentiation of the ventral retinal pigmented epithelium to neural retina in the *growth arrest specific gene 1* mutant.** *Dev Biol.* 2001; **236**(1): 17–29.
PubMed Abstract | Publisher Full Text

37. Zhang W, Mulieri PJ, Gaio U, *et al.*: **Ocular abnormalities in mice lacking the immunoglobulin superfamily member Cdo.** *FEBS J.* 2009; **276**(20): 5998–6010.
PubMed Abstract | Publisher Full Text

38. F Roessler E, Hu P, Marino J, *et al.*: **Common genetic causes of holoprosencephaly are limited to a small set of evolutionarily conserved driver genes of midline development coordinated by TGF-β, hedgehog, and FGF signaling.** *Hum Mutat.* 2018; **39**(10): 1416–27.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

39. F Bergeron SA, Tyurina OV, Miller E, *et al.*: ***Brother of cdo* (*umleitung*) is cell-autonomously required for Hedgehog-mediated ventral CNS patterning in the zebrafish.** *Development.* 2011; **138**(1): 75–85.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

40. F Cardozo MJ, Sánchez-Arrones L, Sandonis A, *et al.*: **Cdon acts as a Hedgehog decoy receptor during proximal-distal patterning of the optic vesicle.** *Nat Commun.* 2014; **5**: 4272.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

41. Bilioni A, Sánchez-Hernández D, Callejo A, *et al.*: **Balancing Hedgehog, a retention and release equilibrium given by Dally, Ihog, Boi and shifted/DmWif.** *Dev Biol.* 2013; **376**(2): 198–212.
PubMed Abstract | Publisher Full Text

42. F Roelink H: **Sonic Hedgehog Is a Member of the Hh/DD-Peptidase Family That Spans the Eukaryotic and Bacterial Domains of Life.** *J Dev Biol.* 2018; **6**(2): pii: E12.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

43. Spoelgen R, Hammes A, Anzenberger U, *et al.*: **LRP2/megalin is required for patterning of the ventral telencephalon.** *Development.* 2005; **132**(2): 405–14.
PubMed Abstract | Publisher Full Text

44. Macdonald R, Wilson SW: **Pax proteins and eye development.** *Curr Opin Neurobiol.* 1996; **6**(1): 49–56.
PubMed Abstract | Publisher Full Text

45. Schwarz M, Cecconi F, Bernier G, *et al.*: **Spatial specification of mammalian eye territories by reciprocal transcriptional repression of Pax2 and Pax6.** *Development.* 2000; **127**(20): 4325–34.
PubMed Abstract

46. Sánchez-Arrones L, Nieto-Lopez F, Sánchez-Camacho C, *et al.*: **Shh/Boc signaling is required for sustained generation of ipsilateral projecting ganglion cells in the mouse retina.** *J Neurosci.* 2013; **33**(20): 8596–607.
PubMed Abstract | Publisher Full Text | Free Full Text

47. Amato MA, Boy S, Perron M: **Hedgehog signaling in vertebrate eye development: a growing puzzle.** *Cell Mol Life Sci.* 2004; **61**(7–8): 899–910.
PubMed Abstract | Publisher Full Text

48. Esteve P, Bovolenta P: **Secreted inducers in vertebrate eye development: more functions for old morphogens.** *Curr Opin Neurobiol.* 2006; **16**(1): 13–9.
PubMed Abstract | Publisher Full Text

49. Sánchez-Camacho C, Bovolenta P: **Autonomous and non-autonomous Shh signalling mediate the *in vivo* growth and guidance of mouse retinal ganglion cell axons.** *Development.* 2008; **135**(21): 3531–41.
PubMed Abstract | Publisher Full Text

50. Fabre PJ, Shimogori T, Charron F: **Segregation of ipsilateral retinal ganglion cell axons at the optic chiasm requires the Shh receptor Boc.** *J Neurosci.* 2010; **30**(1): 266–75.
PubMed Abstract | Publisher Full Text

51. F Herrera E, Brown L, Aruga J, *et al.*: **Zic2 patterns binocular vision by specifying the uncrossed retinal projection.** *Cell.* 2003; **114**(5): 545–57.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

52. F Peng J, Fabre PJ, Dolique T, *et al.*: **Sonic Hedgehog Is a Remotely Produced Cue that Controls Axon Guidance Trans-axonally at a Midline Choice Point.** *Neuron.* 2018; **97**(2): 326–340.e4.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

53. Herrera E, Sitko AA, Bovolenta P: **Shh-ushing Midline Crossing through Remote Protein Transport.** *Neuron.* 2018; **97**(2): 256–8.
PubMed Abstract | Publisher Full Text

54. F Hartman TR, Zinshteyn D, Schofield HK, *et al.*: ***Drosophila* Boi limits Hedgehog levels to suppress follicle stem cell proliferation.** *J Cell Biol.* 2010; **191**(5): 943–52.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

55. Yan D, Wu Y, Yang Y, *et al.*: **The cell-surface proteins Dally-like and Ihog differentially regulate Hedgehog signaling strength and range during development.** *Development.* 2010; **137**(12): 2033–44.
PubMed Abstract | Publisher Full Text | Free Full Text

56. F Jeong J, McMahon AP: **Growth and pattern of the mammalian neural tube are governed by partially overlapping feedback activities of the hedgehog antagonists patched 1 and Hhip1.** *Development.* 2005; **132**(1): 143–54.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

57. F Holtz AM, Griffiths SC, Davis SJ, *et al.*: **Secreted HHIP1 interacts with heparan sulfate and regulates Hedgehog ligand localization and function.** *J Cell Biol.* 2015; **209**(5): 739–57.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

58. F Bangs F, Anderson KV: **Primary Cilia and Mammalian Hedgehog Signaling.** *Cold Spring Harb Perspect Biol.* 2017; **9**(5): pii: a028175.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

59. Perron M, Harris WA: **Retinal stem cells in vertebrates.** *Bioessays.* 2000; **22**(8): 685–8.
PubMed Abstract | Publisher Full Text

60. F Bélanger MC, Robert B, Cayouette M: **Msx1-Positive Progenitors in the Retinal Ciliary Margin Give Rise to Both Neural and Non-neural Progenies in Mammals.** *Dev Cell.* 2017; **40**(2): 137–50.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

61. F Marcucci F, Murcia-Belmonte V, Wang Q, *et al.*: **The Ciliary Margin Zone of the Mammalian Retina Generates Retinal Ganglion Cells.** *Cell Rep.* 2016; **17**(12): 3153–64.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

62. Veth KN, Willer JR, Collery RF, *et al.*: **Mutations in zebrafish *lrp2* result in adult-onset ocular pathogenesis that models myopia and other risk factors for glaucoma.** *PLoS Genet.* 2011; **7**(2): e1001310.
PubMed Abstract | Publisher Full Text | Free Full Text

63. Storm T, Heegaard S, Christensen EI, *et al.*: **Megalin-deficiency causes high myopia, retinal pigment epithelium-macromelanosomes and abnormal development of the ciliary body in mice.** *Cell Tissue Res.* 2014; **358**(1): 99–107.
PubMed Abstract | Publisher Full Text | Free Full Text

64. Cases O, Joseph A, Obry A, *et al.*: **Foxg1-Cre Mediated Lrp2 Inactivation in the Developing Mouse Neural Retina, Ciliary and Retinal Pigment Epithelia Models Congenital High Myopia.** *PLoS One.* 2015; **10**(6): e0129518.
PubMed Abstract | Publisher Full Text | Free Full Text

65. F Christ A, Christa A, Klippert J, *et al.*: **LRP2 Acts as SHH Clearance Receptor to Protect the Retinal Margin from Mitogenic Stimuli.** *Dev Cell.* 2015; **35**(1): 36–48.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

66. Pober BR, Longoni M, Noonan KM: **A review of Donnai-Barrow and facio-oculo-acoustico-renal (DB/FOAR) syndrome: clinical features and differential diagnosis.** *Birth Defects Res A Clin Mol Teratol.* 2009; **85**(1): 76–81.
PubMed Abstract | Publisher Full Text | Free Full Text

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