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Hedgehog Signaling: Cooking with Gas1

Jong-Sun Kang, Wei Zhang, Robert S. Krauss

Abstract

During embryonic development, signaling proteins secreted by localized sources can function as morphogens, specifying distinct fates for cells within a target field in a concentration-dependent manner (1). The mechanisms by which morphogen concentration gradients are interpreted by such cells are of fundamental interest, but are complex and not well understood. Hedgehog (Hh) proteins regulate growth and patterning of a wide variety of mammalian body structures, including the central nervous system, limbs, axial skeleton, and face. One family member, Sonic hedgehog (Shh), often functions as a morphogen (2, 3). Hh proteins activate a signal transduction pathway in which the 12-pass transmembrane protein Patched1 (Ptch1) inhibits the activity of a second transmembrane protein, Smoothened (Smo). Binding of Hh ligands to Ptch1 relieves this inhibition, triggering a cascade that results in increased expression of target genes through the Gli family of transcription factors (4, 5). An important mechanism by which the strength and duration of Hh signaling (and therefore its morphogen activity) are regulated is through a complex feedback network that acts at the level of ligand binding. The expression of genes encoding Ptch1 and an unrelated cell surface protein, Hedgehog-interacting protein (Hhip1), is increased in response to Hh signaling; each protein directly binds and sequesters Hh ligands, and consequently they limit the magnitude and range of Hh signaling (6) (this role of Ptch1 is distinct from its role in inhibiting Smo). Two cell surface immunoglobulin superfamily proteins, Cdo and Boc, display an opposite pattern: They bind Shh and enhance signaling, but their expression is negatively regulated by Hh pathway activity (7, 8). Thus, Shh signaling strength within subregions of a target field is regulated by feedback mechanisms whereby the abundance of several distinct ligand-binding factors is controlled by Shh signaling itself. Studies by Allen et al. (9) and Martinelli and Fan (10) now add detailed information about another component of this feedback network, Gas1.

Gas1 Promotes Shh Signaling

Gas1 was originally identified as a gene whose expression is linked to arrested proliferation of cultured fibroblasts and that encodes a member of the glial cell–derived neurotrophic factor receptor α (GFRα) family of glycosyl phosphatidylinositol-anchored cell surface proteins (11–13). It was subsequently identified as a Shh-binding factor that antagonizes Shh signaling when overexpressed (14); however, the most recent work clearly implicates Gas1 as a positive regulator of the Hh pathway.

During the development of the central nervous system, Shh produced by the notochord induces formation of the floor plate (the ventralmost cells of the neural tube), which also expresses Shh. Shh from these sources specifies all ventral cell types of the neural tube, in a concentration-dependent manner (15, 16). Gas1 is initially expressed in the notochord and throughout the neural tube, but over time its expression becomes progressively restricted to more dorsal regions. This dorsalization of expression is lost in Shh−/− and Smo−/− embryos, indicating that expression of Gas1 is repressed by Shh signaling (9). However, Gas1−/− embryos display neural tube phenotypes consistent with a reduction of Shh signaling: Specification of the floor plate is partially compromised, as are specification, maintenance, and/or positioning of specific ventral neural tube cell types. Furthermore, Gas1 and Shh interact genetically. Reduction of Shh dosage in Gas1−/− embryos (that is, in Gas1−/−;Shh−/− embryos) leads to a further loss of ventral cell identities in the neural tube (9, 10). Gas1−/− embryos also display defects in digit patterning and craniofacial development that are consistent with reduced Shh signaling, and these phenotypes are also worsened by removal of one copy of Shh (9, 10). Gas1−/−;Shh−/− embryos display a more severe phenotype than Shh−/− embryos and are similar to embryos that lack both Shh and Indian hedgehog (Ihh) or to those that lack Smo. This result implies that Gas1 may regulate both Shh and Ihh function in the early mouse embryo (10).

These studies were complemented by gain-of-function experiments in which Gas1, or a Gas1 mutant unable to bind Shh, was expressed ectopically in the chick neural tube (9, 10). Ectopically expressed Gas1 promoted Shh-dependent cell-autonomous promotion of ventral cell fates, and this activity was dependent on the ability of Gas1 to bind Shh. Furthermore, RNA interference (RNAi)-mediated reduction of Gas1 reduced Shh-stimulated activation of a Gli-dependent reporter construct in cultured cells (10). These results are reminiscent of studies on the structurally unrelated Shh binding protein, Cdo (7, 8). The Drosophila orthologs of Cdo (Ihog and Boi) act synergistically with Drosophila Patched to confer cell surface binding of recombinant Hh (17). Martinelli and Fan now demonstrate that Gas1 and Cdo each display a similar synergism with Ptch1 in Shh binding. In contrast, Gas1 and Cdo do not synergize with each other for Shh binding in the absence of coexpressed Ptch1 (10). Taken together, the recent studies indicate that Gas1 functions by binding Shh, likely in conjunction with Ptch1, to promote Shh signaling.

A Feedback Network of Shh Binding Factors

These results must be viewed in the context of the regulation of expression of Gas1 and other Shh binding factors by Shh signaling itself. The papers present a model in which Cdo or Gas1, or both, are initially expressed with Ptch1 on naïve cells near a Shh source, ensuring that such cells are sensitive even to low concentrations of Shh (Fig. 1). As Shh concentrations and signal strength increase, expression of Ptch1 and Hhip1 is induced, whereas expression of Gas1 and Cdo is repressed. This results in high concentrations of inhibitory ligand-binding proteins and low concentrations of stimulatory ligand-binding proteins, thereby modulating further response. Cells at the fringes of a target field...
maintain Cdo or Gas1 expression because of the lower Shh concentration they experience, thus sensitizing such cells to low concentrations of ligand. Coordinate expression of positively and negatively acting Shh binding proteins in response to various amounts of pathway signaling activity should result in modulation of spatial and temporal gradients of Shh signaling, both of which are required for morphogenesis (1, 9, 18, 19).

Although Gas1 and Cdo expression show a similar, generally negative response to Shh signaling, and mice lacking these factors individually display similar, though not identical, phenotypes in the neural tube, patterns of expression of the two genes actually show only limited overlap. Each is expressed weakly in the notochord, but Cdo expression in the neural tube is restricted to low amounts in the floor plate. Unlike Gas1, Cdo is not detectably expressed in other ventral neural tube domains (7, 8, 20). It was therefore of interest to assess the phenotype of mice lacking both Gas1 and Cdo. Allen et al. (9) demonstrated that such animals have much more severe central nervous system and craniofacial phenotypes than do the single mutants. Gas1−/−;Cdo−/− embryos showed a decreased abundance of Shh in the notochord, and loss of markers of floor plate and several other ventral neural tube cell types. Shh is required for maintenance (but not formation) of the notochord (21). However, the notochord was intact in Gas1−/−;Cdo−/− embryos, as assessed by expression of a notochord-specific marker at embryonic day 9.5. Cdo and Gas1, therefore, appear to act in redundant or compensatory fashion to support Shh expression in the notochord. Because Shh can induce its own expression (for example, Shh from the notochord induces Shh expression in the floor plate), and Gas1 and Cdo positively regulate Shh signaling, a logical possibility is that Gas1 and Cdo are required for Shh to maintain its own expression in the notochord. However, the mechanisms by

![Fig. 1. Model for regulation of Shh signaling in a target field by controlled expression of Shh binding proteins. (A) Cells both near and distant to a source of Shh (the cells on the left and right, respectively) express Gas1 and Ptch1. These factors synergize to bind Shh, and the cells near the Shh source transduce a strong signal (gray arrow), whereas cells distant from the source do not yet experience a concentration of Shh sufficient for signaling. (B) Over time, strong Shh signaling in the cell near the Shh source leads to loss of Gas1 expression and increased expression of Ptch1 and Hhip1, both of which sequester Shh and restrain further signaling (hatched arrow). In contrast, Gas1 expression is maintained in the cell distant from the Shh source, allowing these cells to respond to the low concentration of Shh achieved in this region of the target field (light gray arrow, right). Signaling within the target field will also be regulated by expression of Cdo or Boc, or both. Cdo and Boc display a pattern generally similar to that of Gas1: They bind Shh to promote signaling but their expression is generally negatively regulated by Shh pathway activity (not shown).](stke.sciencemag.org/cgi/content/full/2007/403/pe50)
which Shh expression is induced and maintained in this structure, and how it may be influenced by Shh itself, are not well understood. It is conceivable that regulation of Shh expression in the notochord by Gas1 and Cdo may occur, at least in part, by a Shh-independent mechanism. In contrast to its effect in the neural tube, loss of Cdo did not alter the mild loss-of-Shh-function phenotype seen in the digits of Gas1−/− mice (9); therefore, there is regional specificity to the cooperation between Gas1 and Cdo. The lack of cooperation in digit patterning could reflect compensation by the Cdo-related factor Boc, which is expressed in the limb bud with Gas1 and Cdo but not in the ventral midline (7, 22, 23), or to a lack of direct involvement of Cdo in limb and digit patterning.

**Perspectives**

Although Gas1 and Cdo both directly bind Shh, synergize with Ptc1 in binding, and interact genetically to regulate Shh signaling in specific regions of the embryo, they are structurally unrelated proteins. Furthermore, each can participate in other signaling processes. Cdo promotes skeletal myogenesis in vivo and in vitro expressing in specific regions of the embryo, they are structurally unrelated, Ptch1 in binding, and interact genetically to regulate Shh signaling. Although Gas1 and Cdo both directly bind Shh, synergize with Ptc1 in binding, and interact genetically to regulate Shh signaling.


References
