where reachability depends on people assigning nodes to "identity categories" [compare (*4*)], but is not good evidence that these cues are the most salient in everyday searches, where different, more complex and subtle information may be available.

We should also interpret with much caution the conclusion of Dodds *et al.* that hubs (nodes with many links to others) are less relevant to social searches than has been suggested by Barabasi (*5*) and others. This conclusion relies on identifying hubs by respondents' reports of whether they chose a particular acquaintance to send on the message because that person had many friends. But when giving a single response justifying one's choice of contact, location and occupation may seem more salient than number of ties. Moreover, peoples' information about how many contacts one's friends have is generally poor. Yet, it is likely that the choices respondents made were typically for individuals with a greater than average number of ties, because such individuals are considerably more likely than others to be chosen in network relations (*6*). Thus, the data may seriously understate the number of hubs, making it difficult to assess the part they play in social searches. More generally, close analysis of the characteristics of the *specific* nodes that ended failed chains, in comparison to specific nodes that reached intended targets, might tell us something beyond what can be learned by treating all nodes in all chains equally.

"Small-world studies offer tantalizing leads about connectivity and processes in natural networks..."

The broadest issue this research raises is what chain-length estimation experiments tell us about natural social processes. They suggest the need to extend our study to a wide range of situations where network chain length actually matters. This requires far more information than we now have on what people know about their networks, how they use this knowledge during searches, and how chain length impacts individuals *even when the search is absent*, as is common. Studies of how people find jobs through social networks show that while chain length is important, information reaches prospects who did not seek it in about one out of three cases—more for better jobs (*3*). Though people contract diseases when network distance to the already-infected is short, few searched their networks to achieve this outcome. Chain length and knowledge are critical, but the search complicated, when chain endpoints have opposing goals, as when searches are actively resisted by criminals, mob informers, missing deposed dictators or terrorist icons. Small-world studies offer tantalizing leads about connectivity and processes in natural networks, but then need confirmation and enrichment by studies that emerge from the laboratory to track actual networks. As in other branches of science, progress in understanding requires that tightly controlled experiment and real-world complexity regularly and systematically inform one another.

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DEVELOPMENT

Longing for Ligand: Hedgehog, Patched, and Cell Death

Isabel Guerrero and Ariel Ruiz i Altaba

Dependence on developmental signals, like dependence on love, can have catastrophic effects. Being unloved or lost in a multicellular organism can lead to self-inflicted death that people call suicide and cells call apoptosis. But how do lost cells recognize that they lack direction from developmental signals? That job may belong to ligand dependence receptors that induce cells bearing them to undergo apoptosis if the receptor remains unoccupied by ligand. These receptors are thought to ensure the survival of cells that remain close to the source of the appropriate developmental signal (the ligand), and the death of those cells that do not. On page 843 of this issue, Thibert *et al.* (*1*) provide evidence suggesting that the Patched1 (Ptc1) receptor is a dependence receptor that induces programmed cell death during chick neural tube development in the absence of its ligand, the signaling molecule Sonic hedgehog (Shh).

During neural development in vertebrates, Shh is produced first by the notochord and later by the floor plate (see the figure). A ventrodorsal gradient of Shh directs ventral patterning and cell differentiation [reviewed in (*2*)]. The Thibert *et al.* results provide insight into how the neural tube is shaped during development. In multicellular organisms, cells that are poorly positioned as a result of developmental errors can be eliminated because of failure to receive cues instructing them that they are in the correct location [reviewed in (*3*)]. Given the widespread importance of hedgehog (Hh) signaling during development, the results of Thibert *et al*. suggest how Ptc1 and Shh signaling may control tissue sculpting through selective cell death and survival.

To initiate the cell death program, proapoptotic dependence receptors require preliminary cleavage of their intracellular domain (at the DXXD site) by caspase enzymes. Thibert *et al.* present several lines of evidence indicating that Ptc1 is a proapoptotic dependence receptor. They show that overexpression of Ptc1 in cultured cells induces apoptosis, which is blocked by addition of Shh. In the developing chick neural tube, removal of the ventral source of Shh causes massive cell death, which is rescued by expression of a dominant-negative form of Ptc1 that interferes with the proposed function of wild-type Ptc1 in apoptosis. Cleavage of Ptc1 by caspase-3 exposes a carboxyl-terminal apoptotic domain. Transfecting cultured cells with the carboxyl-terminal region of Ptc1 is sufficient to induce cell death. In this region, there is a conserved aspartic acid residue in human, mouse, and chicken; mutation of this site (D1392N) in mouse Ptc1 prevents apoptosis when this receptor is unoccupied by ligand. Transfection of cultured cells with Ptc1 truncated at the caspase-cleavage site induces apoptosis that cannot be rescued by addition of Shh.

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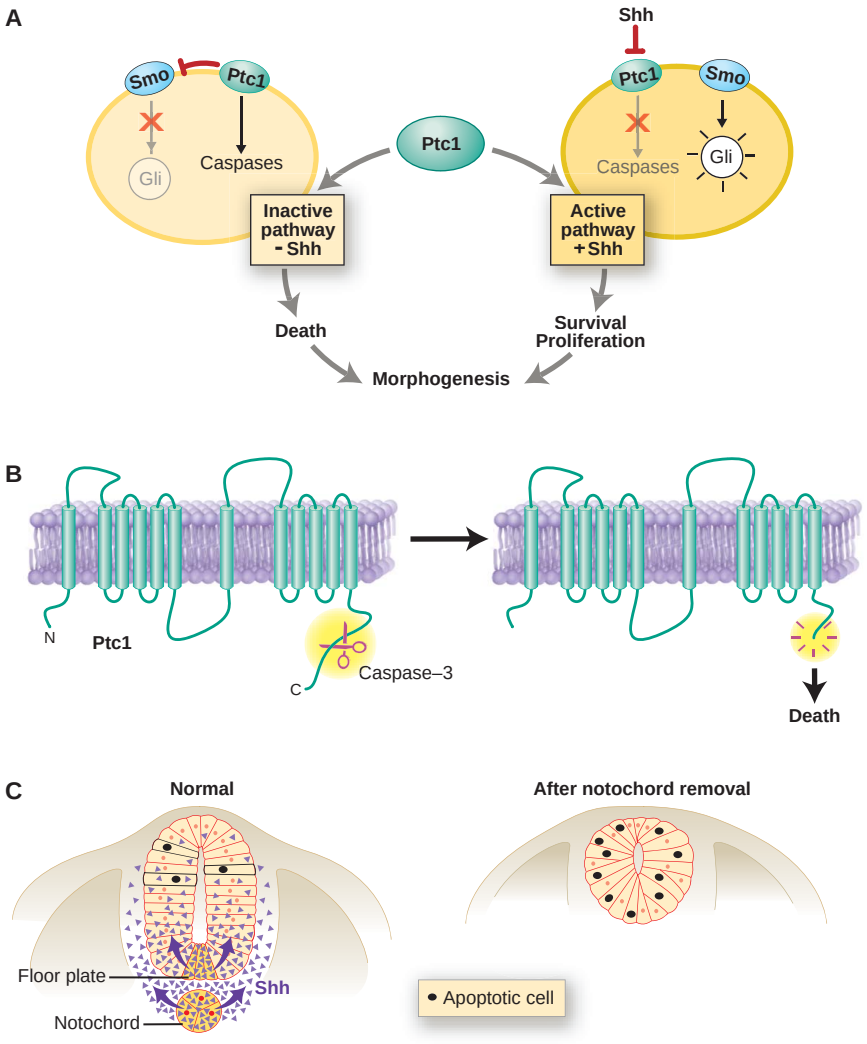

Tissue sculpting through cell death and survival. (**A**) The action of Ptc1 in the Shh signaling pathway. (Left) In the absence of Shh, Ptc1 may act as a proapoptotic dependence receptor inducing programmed cell death. It is not clear how the apoptotic activity of Ptc1 is regulated, apart from the need for Shh to be absent. (Right) In the presence of Shh, Ptc1 no longer represses Smoothened (Smo), leading to activation of the Gli transcription factor, cell survival, and proliferation. (**B**) Cleavage of part of the carboxyl-terminal region of membrane-bound Ptc1 by caspase-3 exposes an apoptotic domain. (**C**) Neural tube development in a generic vertebrate embryo in which Shh is secreted by the notochord and floor plate, inducing patterning of the ventral region. (Left) Note that there is a ventral-to-dorsal gradient of Shh ligand and Ptc1 expression. (Right) In the absence of Shh, for example through early removal of the notochord, the neural tube forms but lacks ventral cell types and shows a high degree of Ptc1-mediated apoptosis (*1*).

These provocative results raise several questions about the in vivo mode of action of Ptc1 in apoptosis. For example, it remains unclear when Ptc1-induced apoptosis takes place during neural tube formation and which cells are selectively killed. Indeed, Ptc1 is widely expressed in regions of the brain that are located far from sources of Shh. In addition, in the developing neural tube, some cells derived from Shh-responsive precursors such as spinal oligodendrocytes (*4*) happily migrate away from the local environment where Shh acts. This suggests that the proapoptotic activity of Ptc1 must be tightly regulated; for example, a critical threshold level of Ptc1 may be required to completely inhibit the Shh pathway and initiate apoptosis. Also, there could be other Ptc1 ligands in addition to Shh whose absence leads to Ptc1-mediated apoptosis.

In a previous study in the chick (*5*), a Ptc1 mutant protein was engineered to lack the second extracellular loop and so was unable to bind to Shh. Introducing this mutant Ptc1 (which had an intact carboxyl terminus) into the chick neural tube resulted in changes in cell type specification without obvious changes in cell number (although apoptosis per se was not measured) (*5*). Reconciling these findings with those of Thibert *et al.*—who show that Ptc1 with an intact carboxyl terminus induces apoptosis in the chick neural tube—will require both groups to perform cell death and cell differentiation assays. It remains possible, however, that the second extracellular loop of Ptc1 not only controls Shh binding, but also affects carboxyl-terminal cleavage and exposure of the proapoptotic domain. But this seems inconsistent with the Thibert *et al.* finding that expression of the entire last intracellular domain of Ptc1 is sufficient to induce cell death (*1*). It is possible that the ectopic expression of mutant Ptc1 lacking the second extracellular loop still permits a low level of Shh signaling that is sufficient to prevent apoptosis.

Ptc1 may be a dependence receptor that controls the size and shape of the chick neural tube by inducing some ventral cells to undergo apoptosis (*6*). Indeed, its ligand, Shh, is critical for regulating precursor cell numbers in different regions of the central nervous system, such as the cerebellum [reviewed in (*7*)]. However, as Thibert *et al.* show in Shh-deprived embryos, blocking Ptc1-induced apoptosis with a dominant-negative Ptc1 protein lacking proapoptotic activity does not fully rescue the loss-of-Shh phenotype; the neural tube is still small and poorly shaped. Therefore, the overall action of Shh signaling in ventral neural tube patterning could be a combination of mitogenic, morphogenetic, and cell survival activities.

The relationship of Ptc1-induced apoptosis to the Shh signaling pathway remains to be clarified. Expression of a Ptc1 mutant lacking part of its carboxyl-terminal domain still induces cell death even when Shh is added to the cells (*1*). Because there is no evidence that cells in which the Shh signaling pathway is active die, these results suggest that expression of a truncated Ptc1 receptor does not activate the Shh pathway. However, several PTCH1 mutations that appear to cause inappropriate activation of the SHH pathway in tumors do map to the carboxyl terminus [reviewed in (*8*)]. Moreover, in the fruit fly *Drosophila*, removal of the carboxyl-terminal region of the Ptc1 receptor induces activation of the Hh pathway (*9*). These results raise interesting questions. Do fly Ptc and vertebrate Ptc1 behave similarly? Does the related Ptc2 receptor behave like Ptc1? What is the relationship between Ptc1 and Gli3 (*10*), a downstream transcription factor in the Hh pathway that is known to affect apoptosis? Direct in vivo testing of the activation of Shh-Gli targets under various conditions, including rescue experiments with the dominant-negative form of Ptc1, should help to clarify these issues.

Most of our knowledge of the Hh signaling pathway originally came from studies in flies, where amazing conservation of Hh function enabled extrapolation of results to

larger organisms. In flies, however, there is no evidence that Ptc acts as a dependence receptor. Cells that cease to receive an Hh signal, for example, in *smoothened* (*Smo*) mutant clones (*11*), or that express high levels of Ptc (*5*, *12*) in areas outside the organizing region of the wing imaginal disc (anterior-posterior compartment border), survive and proliferate. In the organizing region, cells that do not receive an Hh signal do not die, but a complete block in Hh signaling in the wing primordium prevents the activation of other morphogenetic signals such as Decapentaplegic (Dpp) (*13*), leading to inhibition of wing development (*14*). In the *Drosophila* Ptc receptor, the consensus caspase recognition site is missing. This is not entirely unexpected because in both nematodes and flies, extrinsic death receptor activation of caspases seems to be absent (*15*). It is possible that Ptc-induced apoptosis could be a late evolutionary acquisition that was never present in insects, or that insects eliminated this function over the course of evolution.

Beyond normal development, the finding that Ptc1 may be a proapoptotic dependence receptor could have important implications for our understanding of human cancer. PTCH1 is a tumor suppressor protein that is mutated in patients with basal cell nevus syndrome (*16*, *17*) and in cells of various types of sporadic tumors, including those of the skin and brain [reviewed in (*18*)]. Mice carrying Ptc mutations also develop tumors of the cerebellum (medulloblastomas) (*19*). Mutations in PTCH1 may be one of the many ways in which the HH pathway is switched on, leading to activation of GLI transcription factors and the initiation of tumor formation [reviewed in (*18*)]. Indeed, expression of GLI1, a marker of HH pathway activation, is the hallmark of sporadic tumors such as basal cell carcinomas and medulloblastomas that arise from inappropriate HH pathway activity (*20*, *21*). Misexpression of GLI1 is sufficient to induce basal cell carcinoma–like tumors (*20*, *21*); and medulloblastomas and basal cell carcinomas require an active SHH-GLI pathway for maintained proliferation (*21*, *23*, *24*). In this context, the results of Thibert *et al.* suggest an additional twist. Is it possible that null mutations in PTCH1 leading to the absence of caspase-mediated cell death and pathway activation allow certain cells to survive and initiate tumorigenesis? Such a scenario could provide a possible reason for why PTCH1 loss-of-function mutations (versus those in other HH pathway components) are common in sporadic tumors: two effects for the price of one component. Nevertheless, given that the absence of Shh can induce PTCH1-mediated apoptosis, why does the activation of SHH pathway components downstream of PTCH1 [including SMOH (*25*, *26*)] in the apparent absence of SHH result in tumorigenesis? Can these components talk back to PTCH1 or prevent PTCH1-mediated apoptosis? Or is there a tonic level of SHH required to inhibit PTCH1-induced apoptosis and allow cancer growth?

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CLIMATE

Taking the Pulse of the Tropical Water Cycle

Georg Hoffmann

Oxygen isotope signals from high-altitude glaciers in the Andes (see the first figure) provide unique insights into past climate variability in the tropics (*1*–*3*). Some of these ice cores go back to the last glacial period and have proved that rapid climate variability such as during the Younger Dryas, a cold period at the end of the last glacial, affected the tropics at least as far south as 20°S.

In these tropical records, the isotopic shift from glacial to modern climate is about the same as in the better understood polar isotope records (5 to 6‰ in $\delta^{18}O$, where $\delta^{18}O$ is the ratio of ^{18}O to ^{16}O in the sample relative to a standard). The signals were therefore originally interpreted in terms of temperature, similar to those in Greenland or Antarctica (*2*).

In mid- and high latitudes, this interpretation is justified, because the isotopic composition of precipitation is largely controlled by temperature fluctuations on all time scales, from seasonal and interannual to glacial-interglacial. But in the tropics, things are more complicated. As well as temperature, factors such as amount of precipitation, intensity of water vapor recycling, and circulation changes affect $\delta^{18}O$. Several recent publications have taken a fresh look at how oxygen isotope records from the tropics should be interpreted.

Over the 20th century, ice cores can be dated relatively precisely, and direct observations are available. Vuille and co-workers (*4, 5*) analyzed the oxygen isotope variability in the tropical Americas from 1979 to 1998 with two different atmospheric general circulation models (GCMs) fitted with oxygen isotope tracers. They concluded that the isotopes are strongly influenced by precipitation anomalies caused by El Niño–Southern Oscillation (ENSO) on seasonal and interannual time scales.

During El Niño, rainfall amounts tend to be higher than normal west of the Andes and lower than normal on the Altiplano and in the Amazon basin (see the first figure). Although atmospheric temperatures are higher throughout the Central Pacific and the tropical Americas during El Niño, the oxygen isotopes are dominated by these precipitation changes. As a result, precipitation is isotopically depleted (low $\delta^{18}O$) over the tropical Pacific and enriched (high $\delta^{18}O$) on the Altiplano and in the Amazon basin.

Tropical South America varies between a warm/dry mode (El Niño) and a cold/wet mode (La Niña). Therefore precipitation and temperature act in concert to produce the Andean oxygen isotope signal (which is enriched during El Niño). This mechanism controls the oxygen isotopes in all available Andean ice core records (*1, 2, 6*). Throughout the 20th century, the records are closely correlated, even though the glaciers are situated in different climatic zones. The dominant variability in all records is decadal, unlike the typical ENSO variability of 2 to 5 years seen in most other tropical records. This difference may be a result of dating problems, smoothing of the snow after precipitation, or the variable influence of ENSO on precipitation in South America.

To aid comparison with other tropical records (*7*–*9*), an Andean Isotope Index

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