ena, no matter how isolated, to fit into some global prescription that accounts for all the time machines in the universe. Such a law might arise naturally from the existing principles underpinning physics (*7*). Other researchers, including Hawking, assert that the paradoxes associated with time travel are so unacceptable that the laws of nature must somehow conspire to prevent it (*8*). If this "chronology protection conjecture" is correct, then it must also rule out the warp drive, which now comes complete with time travel, at no additional charge.

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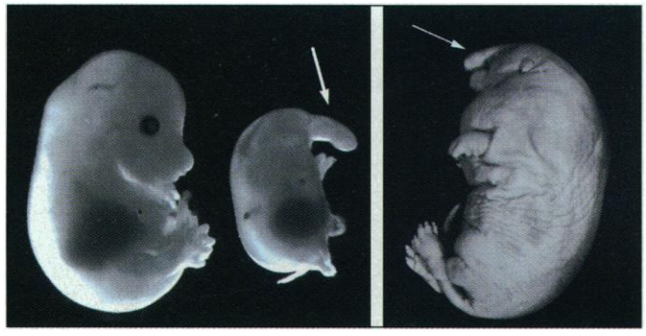
Pass the Butter . . .

Gail Martin

After all the bad press that cholesterol has received for its contribution to human atherosclerotic disease, butter lovers everywhere can take some comfort in the findings of a report on page 261 of this issue. Cholesterol has a new—and vital—function: It is required for the normal action of a key patterning molecule in embryogenesis. Porter *et al.* (*1*) describe a remarkable autoprocessing mechanism that covalently links cholesterol to the secreted signaling molecule Hedgehog (Hh). This finding, together with data from another study by these authors (*2*), suggests an unprecedented mechanism for concentrating Hh and associating it with the cell surface, a key step in modulating its activity as a regulator of embryonic tissue patterning.

The story begins with the identification of the *hedgehog* gene in a screen for mutations in *Drosophila* that affect the establishment of the basic body plan (*3*). Once the *Hh* gene had been isolated, rapid progress was made in characterizing its roles in patterning the embryonic segments and larval imaginal discs (*4*). The story took on broader significance when *Hh* homologs were identified in zebrafish, chick, mouse, human, and other vertebrate species (*5–9*). Among the vertebrate *Hh* family members, *Sonic hedgehog* (*Shh*) has received the most attention because there is considerable experimental evidence that it is a potent regulator of tissue patterning in a number of developmental settings. For example, Shh protein can function as (i) the morphogen produced in the zone of polarizing activity that patterns the developing limb (*10*), (ii) the signal produced by the notochord and floor plate that induces somitic cells to adopt a sclerotomal fate (*11*), and (iii) the molecule that patterns the ventral neural tube and its derivatives (*12, 13*).

Genetic evidence that Shh performs these functions in the normal embryo has come from a study published last week in *Nature*, in which Chiang *et al.* (*14*) describe the phenotype of mice that lack a functional *Shh* gene. As expected, limb, axial skeleton, and ventral neural tube development are severely compromised in Shh-deficient embryos. However, the most striking external features of the mutant embryos are cyclopia and the presence of a single tubelike proboscis above the eye (see figure). These features, together with other midline facial abnormalities, are reminiscent of a spectrum of human congenital malformations collectively known as holoprosencephaly (*15*). They are indicative of a failure of the process of midline development that normally bisects the prospective forebrain, eye, and nasal territories, enabling them to develop into bilateral structures.



Cholesterol's new job. Abnormal head development (holoprosencephaly) occurs both in mice lacking the signaling molecule Sonic hedgehog (left panel, arrow; a control embryo is at left) and in rat embryos developing in females treated with inhibitors of cholesterol biosynthesis (right panel). This congruence is explained by the new finding in this issue, in which the lipid adduct of Sonic hedgehog, thought to be necessary for proper function, is shown to be cholesterol. [Left panel reprinted with permission from *Nature* (*14*); copyright (1996) Macmillian Magazine Ltd; right panel courtesy of C. Roux]

One of the most intriguing features of Hh family proteins is that they act as both short- and long-range patterning signals. For example, in vertebrates, floor-plate induction by Shh involves short-range signaling that depends on contact between the target neural plate and *Shh*-expressing cells (*8*). In contrast, motor neuron and sclerotome induction by Shh does not require cell contact and can occur over relatively long distances (several hundred micrometers) (*16, 17*). Likewise, in *Drosophila*, Hh can act as either a short- or a long-range patterning signal (*4*). One explanation for this duality in Shh's mode of action is that different concentrations of Shh have different effects on cells: High concentrations induce neural plate explants to form floor plate, whereas low concentrations promote motor neuron formation (*13*). The need for cell-cell contact likely reflects a requirement for a very high concentration of Shh protein, localized in or near the cells that produce it, whereas long-range signaling may be achieved by lower concentrations of protein that has diffused away from the producing cells. [However, in some instances, the long-range effects of Hh proteins are clearly due to induction of a secondary signaling molecule (*4*).] The mechanism by which Hh protein is concentrated and associated with the cell surface is of critical importance, because a cell that fails to retain Hh at sufficiently high concentrations presumably cannot participate in short-range signaling. Recently, considerable progress has been made toward understanding this mechanism.

Hh is synthesized as a precursor that undergoes autoproteolysis into an NH_2-terminal domain (Hh-N) with signaling activity (*18, 19*), and a COOH-terminal domain (Hh-C) that contains the determinants for autoprocessing of the precursor (*19, 20*). Recently, Porter *et al.* (*2*) showed that in addition to peptide bond cleavage, the processing reaction includes covalent linkage of a lipid moiety to Hh-N. The significance of the lipid modification is that it increases the hydrophobic character of Hh-N, thereby influencing its spatial and subcellular distribution. Consistent with the hypothesis that such processing is critical for Hh signaling,

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Porter *et al.* (*2*) also found that Hh-N that is not modified diffuses more readily from cells, and expression of the unmodified form in flies causes long-range effects on patterning that are not detected when the processed form is similarly expressed. Thus, an interesting possibility is that Hh signaling by an individual cell might be regulated by modulating the amount of the lipid-linked and unmodified Hh-N it produces.

In a report in this issue, Porter *et al.* identify the lipid moiety added to both *Drosophila* Hh-N and mouse Shh-N as cholesterol (*1*). Previously, cholesterol has been known as a precursor in steroid hormone and bile component biosynthesis and as an important structural component of biological membranes in animals. The finding that it can be covalently linked to Hh family members, and that it appears to modulate their spatial and subcellular distribution, thereby affecting their patterning activities, suggests a brand-new function for cholesterol in animals. It also provides an unanticipated explanation for the observations of Roux (*21*, *22*), published more than 30 years ago, that rat embryos developing in females treated with inhibitors of cholesterol biosynthesis display manifestations of holoprosencephaly. Indeed, the rat embryo malformations are remarkably similar to those seen in mouse embryos homozygous for a null allele of Shh (*14*) (see figure). The model described above predicts that the ability of those drugs to phenocopy the *Shh* mutation is due, at least in part, to a failure of embryonic cells to carry out short-range Hh signaling, because they are unable to process Hh correctly as a result of the drug-induced perturbations in cholesterol biosynthesis.

Two additional findings (*2*) make this story even more exciting; these results suggest that the mechanism by which cholesterol is covalently linked to a secreted protein may not be limited to members of the Hh family. First, Hh-C can initiate the full autoprocessing reaction, including hydrophobic modification, even when it is attached to sequences unrelated to Hh. Second, six proteins from *Caenorhabditis elegans* contain domains related to Hh-C (but not Hh-N), and at least one of them undergoes in vivo cleavage at a site similar to the cleavage site in Hh. It remains to be determined whether these C. *elegans* proteins, or other proteins yet to be identified, also mediate intramolecular, covalent linkage of cholesterol to their NH_2-terminal sequences. If so, then the mechanism described by Porter *et al.* (*1*) may be a general one for regulating intercellular signaling.

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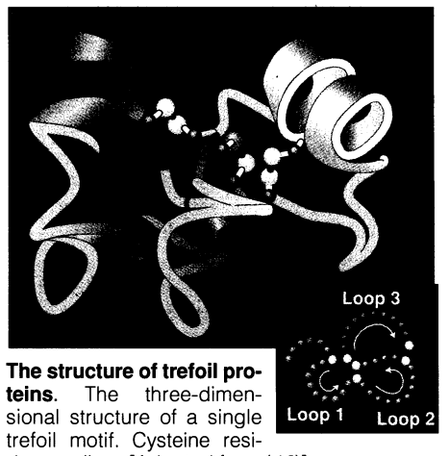
Trefoil Peptides: Less Clandestine in the Intestine

Rebecca Chinery and Robert J. Coffey

Trefoil peptides—small, highly stable molecules secreted principally by the mammalian gastrointestinal tract (*1*)—are highly conserved and abundant, but their function and mode of action have been nevertheless obscure and often contradictory (*2*, *3*). Now two studies (*4*, *5*) on knockout mice in this issue of *Science* confirm suspicions that trefoil peptides function in tissue repair (*2*, *3*, 6–8), but they also offer a surprise—the possibility that these peptides inhibit cellular proliferation.

The name trefoil (three leaf) derives from the three intrachain loops predicted to arise from the distinctive pairing of six cysteine residues (*9*, *10*), although the trefoil structure is not very apparent in the high-resolution x-ray or nuclear magnetic resonance structures (see figure). To date, three human trefoil peptides have been localized to mucus-secreting epithelia in the gut: pS2, intestinal trefoil factor (ITF), and spasmolytic polypeptide (SP). ITF is restricted to the normal intestine, whereas pS2 and SP are found mainly in the gastric fundus and antrum, respectively. All three peptides are secreted into the lumen, where their compact structure confers resistance to the harsh conditions of the gut (acid degradation and proteolytic digestion).

In the new studies, the genes for trefoil peptides have been deleted by gene-targeting techniques. The ITF-deficient mice described by Mashimo *et al.* (*5*) are less able to withstand injury to the intestine, confirming a role for ITF in the maintenance of the intestinal barrier. This endpoint was not measured in the pS2-deficient mice of Lefebvre *et al.* (*4*), but the pS2 null mice did exhibit an unexpected phenotype: At 5 months of age the mice showed adenomatous changes in the gut, and 30% showed multifocal carcinoma. The authors suggest that pS2 may be a tumor suppressor [although pS2 expression is increased in breast cancer (*11*)]. Both results raise the intriguing possibility that trefoil peptides alter normal cell maturation or turnover in the gut. The differing effects of pS2 and ITF disruption on mucus levels will fuel the debate regarding the putative importance of trefoil peptide–mucin colocalization.



The structure of trefoil proteins. The three-dimensional structure of a single trefoil motif. Cysteine residues, yellow. [Adapted from (*12*)]

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