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

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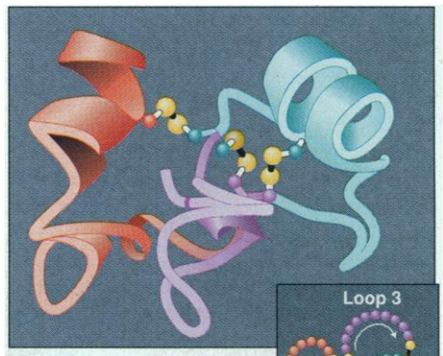
Trefoil peptides—small, highly stable molecules secreted principally by the mammalian gastrointestinal tract (*1*)—are highly conserved and abundant, but their function and mode of action have been nevertheless obscure and often contradictory (*2*, *3*). Now two studies (*4*, *5*) on knockout mice in 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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