field does not conflict with any fundamental principles. Indeed, in his classic paper on irreversible processes, Onsager (*13*) recognized that microscopic reversibility does not apply when external magnetic fields are present. However, Onsager's prescription of reversing *B* along with the motions of the interacting particles does not restore microscopic reversibility when *B* is a component of a falsely chiral influence; but even then, there will only be observable consequences if the particles are chiral. This is because, if the particles are achiral, the *P*-enantiomers are indistinguishable from the original so that M = M′ and the barriers to the left and right of R in the figure must coalesce, which is only possible if the forward and reverse barriers shown for production of a particular enantiomer become identical; but if M is not equal to M′, they can, in general, remain distinct.

A basic requirement for the generation of the velocity-dependent contributions that must be added to the usual adiabatic potential energy surface in the presence of collinear electric and magnetic fields is a circular motion of charge in a plane perpendicular to the magnetic field direction as the chiral reaction intermediate evolves (*7*). The function of the electric field is to partially align the dipolar molecules in the fluid so that one sense of circulation is preferred over the other for a particular enantiomeric intermediate in a particular orientation; it is therefore not required if the molecules are already aligned (*8*, *9*, *14*). Thus, a magnetic field alone might induce asymmetric synthesis if the prochiral reactant molecules are prealigned, as in a crystal, on a surface, or at an interface, and the reaction is far from equilibrium.

Curiously, although discredited, the results of Zadel *et al.* (*1*) prompt the notion that these conditions might still allow a magnetic field alone to induce an enantiomeric excess even if the prochiral reactant molecules are randomly oriented as in a bulk fluid. If the dipole axis of a particular prochiral molecule happened to be aligned parallel or antiparallel with the magnetic field at the instant it started to react, the "ratchet effect" of a breakdown of microscopic reversibility in conjunction with a chiral autocatalytic process might rapidly generate a large excess of one or other of the two possible enantiomeric products. This type of unlikely sounding process is "grist to the mill" for discussions of the origin of biological homochirality based on bifurcation theory (*15*), where dramatic bulk chiral symmetry-breaking effects are claimed to be possible from influences as tiny as the parity-violating weak neutral current (*16*, *17*).

Careful experiments with collinear electric and magnetic fields will be needed to see if the parallel and antiparallel arrangements will steer asymmetric reactions toward one or other enantiomeric product. A positive result, no matter how tiny the enantiomeric excess (provided it was routinely reproducible), would prove unequivocally that a breakdown of microscopic reversibility has been induced and would thereby initiate a new era in the study of reaction, transport, and phase transition processes involving chiral species, and of the origin and role of optical activity in nature.

References and Notes

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The Two Faces of Hedgehog

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From an obscure start as one of many genes regulating *Drosophila* development, *hedgehog* has made a meteoric rise to prominence. The Hedgehog family of cell-to-cell signals contains the best candidates for several of the most sought after factors in vertebrate embryology. Now, as reported in this issue (p. 1528), Hedgehog is teaching us lessons that extend beyond embryology to new principles of cell biology (*1*). Lee and co-workers (*1*) provide evidence that *hedgehog* has quite an unusual activity—it encodes not only a mature signaling molecule, but also a protease required for its own processing.

The Hedgehog story began in the late 1970s when it was identified by Eric Wieschaus and Christiane Nüsslein-Volhard in their screen for embryonic lethal mutations that affect the *Drosophila* embryonic body plan (*2*). From this screen emerged many molecules now recognized as key developmental regulators in many animals—cell-to-cell signals like Wingless (progenitor of the vertebrate Wnt family), receptors including DER [the *Drosophila* epidermal growth factor (EGF) receptor], and transcription factors such as Paired (progenitor of the vertebrate Pax family). Genetic and molecular analysis of this treasure trove of genes provided a detailed outline of how cell fates are established in *Drosophila* embryos and also unexpected insight into cellular processes. During *Drosophila* embryogenesis, an initial phase dominated by interplay among transcription factors is followed by a set of critical cell-cell interactions. Segment polarity gene products like Hedgehog act in the second phase, affecting cell fate choices within each embryonic segment. Most segment polarity genes encode protein components of two different cell-cell signaling pathways (*3*).

Until the past year, most attention focused on the pathway in which the cell-cell signal is encoded by *wingless*. However, early in the analysis of Wingless signaling it became clear that a second signaling pathway is initiated by cells neighboring those expressing the Wingless protein (*4*). In a clever series of genetic experiments, Ingham, Mohler, and others assembled evidence that Hedgehog has properties expected of this second signal (*5*, *6*), a prediction since confirmed by molecular analysis (*7*). Similar genetic analysis led to a tentative outline of the Hedgehog signaling pathway (*8*). Of particular interest is the Hedgehog receptor, which remains unidentified. Genetic evidence prompted the suggestion that the transmembrane Patched protein might be the Hedgehog receptor (*6*), but Patched also has Hedgehog-independent roles (*9*). Perhaps Patched is an accessory component for reception of Hedgehog as well as other signals.

Both Hedgehog and Wingless participate in a variety of developmental decisions in *Drosophila* (*3*). Some of these, such as the interactions between Wingless-expressing and Hedgehog-expressing cells in the embryonic ectoderm, involve signaling

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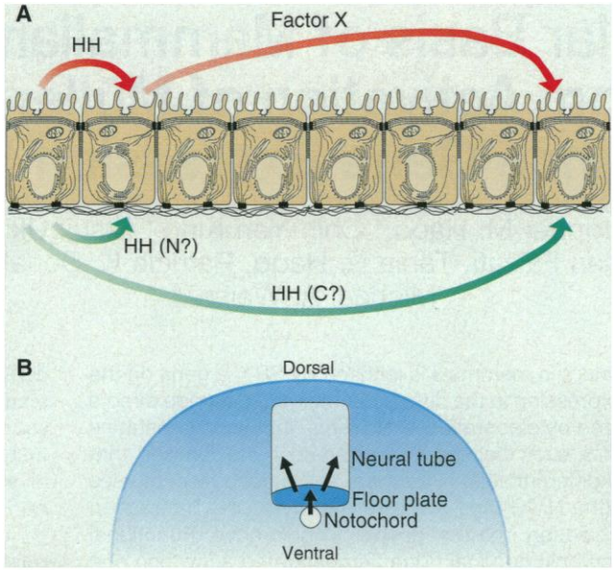
between cells in intimate contact or close proximity (*4*). Other events, like patterning the dorsal epidermis, require signaling across several cell diameters (*10*), or, as in the patterning of the *Drosophila* adult eye and appendage progenitors, may require signaling across hundreds of cells (*11*). How Hedgehog and Wingless contribute to these short- and long-distance processes remains a matter of contention. One particularly critical issue is whether Hedgehog and Wingless act exclusively as short-range signals, which then have effects over long distances by inducing other longer-range signaling molecules (see figure, part A, red arrows), or whether Hedgehog and Wingless themselves act at a distance (see figure, part A, green arrows). It is also not clear if Hedgehog and Wingless act as on-or-off signals or in a concentration-dependent fashion as morphogens. The current evidence is ambiguous: Under certain circumstances Hedgehog seems to be a diffusible morphogen (*10*), while in other cases it induces expression of a second signaling molecule (*11*).



Hedgehog signaling: By touch and by diffusion. (**A**) Hedgehog (HH) itself could act both locally and at a distance (green arrows). Hedgehog may act only locally, but its action may induce other longer range signals (red arrows). (**B**) The vertebrate nervous system uses both short- and long-range signals. Notochord induces the adjacent neural tube to form the floor plate; this signal is contact-dependent. Floor plate then sends a diffusible signal to the rest of the neural tube, inducing ventral cell fates.

Whatever the cell biological mechanisms, Hedgehog has risen to prominence on the basis of its importance in vertebrate development. Several groups independently cloned vertebrate Hedgehog homologs; without question they struck gold (*12–15*). At least four distinct vertebrate Hedgehog homologs exist. The expression and possible functions of one, *sonic hedgehog*, were analyzed in detail, revealing that the Hedgehog family can and likely does mediate several key developmental decisions in all vertebrate embryos.

To understand the functions of the Hedgehog family in vertebrates, we must examine two seemingly quite different developmental processes. The first is dorsal-ventral patterning of the neural tube (see figure, part B): A two-step inductive process occurs, in which the notochord induces the adjacent neural tube to form the floor plate, and then the floor plate further induces development of ventral neural elements (*16*). Expression analysis and ectopic expression studies implicated Sonic Hedgehog as a potential signal in both of these sequential inductive events (*12, 15*). The notochord signal is contact dependent, while the floor plate signal is diffusible; this raises the same questions about the range of Sonic Hedgehog action as those posed by the *Drosophila* data.

A second key developmental process likely requiring Sonic Hedgehog is vertebrate limb patterning. Classic developmental biology experiments implicated cells of the zone of polarizing activity (ZPA) as the agents directing digit specification (*16*). Sonic Hedgehog is expressed by cells of the ZPA, and transplantation of cells transfected with Sonic Hedgehog to an inappropriate location leads to mirror-image digit duplication (*14*). Consistent with a role for Hedgehog in both neural tube patterning and limb development, the notochord can also induce digit specification. Limb development requires a long-range signal, which could be either Sonic Hedgehog itself or another signaling molecule induced by Sonic Hedgehog.

The new experiments of Lee and co-workers may help clarify the situation (*1*). They found that Hedgehog is processed into several major and minor protein species. This in itself is not unusual—many signaling proteins are processed from longer precursors by proteases in the secretory pathway or outside the cell, and processing can regulate activity. The surprise came in looking for the processing protease. They did not need to look far; Hedgehog is its own processing protease. Although neither autocatalytic proteases nor signaling molecules are unusual, their combination in a single protein is a remarkable example of parsimony. Hedgehog is not the only protease that also acts as a signal; plasminogen, one of the proteases of the coagulation pathway, may regulate angiogenesis and endothelial cell growth by a pathway that does not require its proteolytic activity (*17*).

Not satisfied with providing us with this remarkable chimeric protein, Lee and co-workers (*1*) also present evidence that Hedgehog processing is critical for its biological activity and that the two predominant protein products have different biochemical properties. They then suggest a provocative model in which the amino- and carboxyl-terminal portions of Hedgehog serve its short-range and its long-range signaling roles, respectively (see figure, part A, green arrows).

This model makes explicit predictions that can now be tested. First, the two products should differ in diffusibility in the embryo; Lee and co-workers present tentative evidence for this that needs further exploration. Second, if two distinct signaling molecules are encoded by *hedgehog*, genetic dissection may allow separation of these two functions. Beyond these immediate goals, genetic analysis of vertebrate *hedgehog* genes will test the provocative suggestions about their roles in development. Finally, genetic and biochemical analysis will help identify the Hedgehog receptor and other components of its signal transduction pathway. Hedgehog has already provided both developmental and cell biologists with a great deal of entertainment; undoubtedly other equally surprising revelations remain to be uncovered.

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