cholesterol is then esterified and delivered to the liver, either directly (still in HDL) or indirectly (after exchange into other lipoprotein fractions). This process, reverse cholesterol transport, is necessary because all tissues take up LDL at some rate but most cannot degrade excess cholesterol. Although the limited tissue distribution of SR-BI suggests that it is not involved in HDL uptake of free cholesterol from peripheral tissues, liver SR-BI could facilitate the ultimate delivery of cholesterol from the periphery to the hepatocyte.

Despite an extensive search, no fully characterized HDL receptor for selective cholesterol uptake or reverse cholesterol transport has been convincingly demonstrated. The identification of SR-BI as a specific, cloned protein involved in the selective cholesterol ester transfer pathway is therefore an important advance. For example, the strong negative epidemiologic correlation between plasma HDL concentration and the risk of atherosclerosis may be a result of HDL's role in reverse cholesterol transport, but the inability to quantify reverse cholesterol transport or to modify it in vivo has hampered testing of this hypothesis. It may now be possible to verify whether SR-BI participates in hepatic uptake of cholesterol esters in mice by gene-targeting techniques and, if it does, then to test whether an SR-BI knockout mouse (without SR-BI) is more susceptible to cholesterol-induced atherogenesis. The importance of selective cholesterol ester uptake for steroidogenesis can also be tested with gene targeting. Finally, the availability of a well-defined protein receptor that can mediate selective cholesterol ester uptake will make it easier to elucidate the intimate molecular mechanisms by which cholesterol ester is moved from HDL across the plasma membrane and into the cell.

The SR-BI molecule was originally placed in the scavenger receptor family on the basis of its ability to bind modified forms of LDL and because of its homology to CD36, a receptor that binds oxidized LDL (*10*) and participates in the recognition and uptake of apoptotic cells (*11*). At least some of the receptors that mediate recognition and phagocytosis of damaged or apoptotic cells are also receptors for oxidized LDL (*12, 13*). Thus, at first glance SR-BI seems an ideal scavenger receptor. However, unlike other scavenger receptors, SR-BI binds native LDL, and its binding of modified forms of LDL is not competitively inhibited by polyanions such as polyguanosinic acid. Furthermore, if the tissue distribution of SR-BI is limited to steroidogenic tissues, as appears to be the case, and if SR-BI is not expressed on macrophages, can it even function as a scavenger receptor in vivo? Its homology with CD36 might imply that it recognizes apoptotic cells, but the homology is only about 30%. Is it possible that this receptor, although clearly related structurally to CD36, has evolved to carry out a quite different function, that is, facilitation of selective uptake of cholesterol esters?

Cells undergoing apoptosis in the absence of inflammation are presumably phagocytosed not by macrophages but by neighboring cells like themselves. Can these nonprofessional macrophages turn on expression of SR-BI just as smooth muscle cells and fibroblasts can be induced to express the acetyl LDL receptor (scavenger receptor A) under specialized circumstances (*14*)? If so, SR-BI could conceivably act like its homolog CD36 in scavenging for dying cells. Whether SR-BI is a bona fide member of the scavenger receptor family or only a distant cousin remains to be determined. A final decision regarding its quantitative role in cholesterol transport awaits in vivo studies, but it would seem safe to place it (additionally or instead) in a family of HDL receptors. Recent in vivo findings of Plump *et al.* (*15*) support this conclusion: Gene targeting (knockout) of the mouse *apo A-I* gene, but not the *apo A-II* or the *apo E* genes, causes striking depletion of adrenal cholesterol ester stores and blunted steroidogenic responses.

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Gating by Cyclic AMP: Expanded Role for an Old Signaling Pathway

Ravi Iyengar

Cells recognize and respond to external signals by means of their signaling pathways. The first of these to be identified was the hormone-stimulated adenylyl cyclase pathway, which uses the intracellular messenger cyclic adenosine monophosphate (cAMP) to transmit signals. This pathway was paradigmatical for the concepts of second messengers, protein phosphorylation, and signal transducers such as heterotrimeric G proteins (*1*). Recent studies indicate that the cAMP pathway may have yet another concept to reveal: gating as a means of regulating information flow within the cell.

Typically, intracellular signaling pathways function as "bucket brigades," with each component handing the signal to the next until the final targets produce a response. These targets can be metabolic enzymes, transcription factors, or ion channels. Messages travel along the pathways by various means: protein-protein interactions, sequential protein phosphorylation, and generation of diffusible intracellular messengers (for example, cAMP). All signaling pathways at some stage use protein-protein interactions or protein phosphorylation to transmit signals. Some pathways, notably those that have G proteins as signal transducers, also use intracellular second messengers to transmit signals. Variable details notwithstanding, two features characterize an effective signaling pathway: (i) direct activation of downstream components produces the same response as the extracellular signal and (ii) inhibition of downstream components blocks the response evoked by the extracellular signal. Most pathways exhibit these two features: Glucagon receptor activation of glucose production in hepatocytes and luteinizing hormone receptor activation of steroid production in the ovary can be mimicked by cAMP analogs or activation of cAMP-dependent protein kinase (PKA) and blocked by inhibition of PKA (*2*). Similarly, growth factor activation of

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mitogen-activated protein (MAP) kinases and proliferation can be mimicked by direct activation of intermediate components such as the guanosine triphosphate (GTP)–binding protein Ras or the Ser-Thr kinase Raf, which are downstream of the receptor but upstream of MAP kinases, and can be blocked by dominant-negative Ras or Raf (*3*). Many signaling pathways have been mapped with the use of downstream activation to mimic the receptor effect and by the selective blockade of the receptor effect by inhibition of downstream components.

The cAMP pathway can also regulate signal flow through other pathways, that is, it can function as a gate (*4*). An early indication of gating by the cAMP pathway came from studies on the transformation of fibroblasts by components of the growth factor signaling pathway. Here PKA phosphorylates and inhibits Raf and thus blocks signal transmission from growth factor to MAP kinase (*5*). The elevation of cAMP concentration and the activation of PKA does not affect proliferation of NIH 3T3 cells, but they can inhibit Ras-stimulated MAP kinase activity and block Ras-triggered transformation (*6*). Activation of MAP kinase is necessary and sufficient to transform NIH 3T3 cells (*7*). This purely regulatory role of cAMP in the transformation of NIH 3T3 cells contrasts with its ability to stimulate steroidogenesis in ovary or glucose mobilization in liver and its function as a coincidence signal in the siphon withdrawal reflex of *Aplysia* (*4*).

Another cAMP-gated process is seen in the development of the mouse embryo. Long-range patterning by the diffusible morphogen Sonic Hedgehog is blocked by cAMP increases, although elevation of cAMP alone does not affect differentiation (*8*). Cyclic AMP gating of development may be evolutionarily conserved. In *Drosophila*, Hedgehog-induced limb development is regulated by PKA (*9*).

Cyclic AMP also functions as a gate for synaptic plasticity in the rat hippocampal CA1 region. Long-term potentiation (LTP) of synaptic responses evoked by three widely spaced stimuli occurs in two phases: an early phase independent of protein synthesis and a later, protein synthesis–dependent phase (*10*). The early phase requires the cAMP pathway in the postsynaptic cell, but postsynaptically this pathway by itself does not enhance synaptic response (*11*). Similarly, neurotrophin-dependent survival and growth of neurons of the central nervous system requires elevation of cAMP, although cAMP by itself does not promote growth or survival (*12*).

The evidence for the gating function of the cAMP pathway in these diverse biological processes comes from independent studies of the signaling pathways that regulate these processes. Activation of the Ras-Raf-MAPKK–MAP kinase pathway is often sufficient to trigger proliferation and transformation (*7*). PKA closes the gate on signals from this pathway. The pathway used by the Hedgehog family of morphogens to induce differentiation is not known, but genetic analysis does not favor PKA as part of the Hedgehog signaling system (*9*). Also, there may not be an extracellular trigger for cAMP increases during early development. An embryonic cell may need to express adenylyl cyclases with high basal activity (*13*) to block its response to morphogens or have adenylyl cyclases with low basal activities to differentiate. It is not known whether this occurs, although the basal activities of adenylyl cyclases can vary by nearly 30-fold (*13*), and cAMP can regulate differentiation.

In transformation and early differentiation, the active cAMP gate is in the closed mode, blocking signal flow through the transmittal pathway that evokes the biological response. In neuronal plasticity, the active gate is open, enhancing signal flow through the transmittal pathway. Activated Ca^{2+}-calmodulin–dependent protein (CaM) kinase in the postsynaptic neuron can enhance synaptic responses and occlude further increases in synaptic responses to external stimuli (*14*), suggesting that postsynaptic CaM kinase transmits the signals for early LTP. However, when LTP is evoked by external stimuli, postsynaptic cAMP is also required, although cAMP does not enhance synaptic responses as does activated CaM kinase. The cAMP pathway is thought to regulate LTP by inhibiting protein phosphatases, thus opening a gate that allows signals for LTP to be transmitted and enhance synaptic responses to subsequent stimuli (*11*). Similarly, for the survival of neurons, gating by cAMP allows the neurotrophin signals to be effective (*12*). Because neurotrophin signaling requires protein kinase cascades, cAMP regulation of phosphatases is also likely important for neurotrophin stimulation.

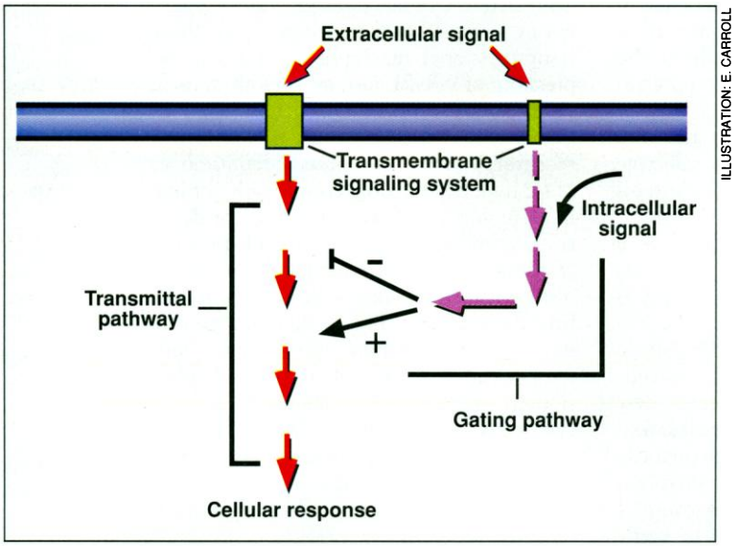

ILLUSTRATION: E. CARROLL

How a signaling pathway and a gating pathway may interact. The transmittal pathway receives information at the cell surface and transmits it through the pathway to evoke a response. A gating pathway can regulate information flow through the transmittal pathway positively (+) or negatively (–) at any point and may be constitutively active or activated by intracellular or extracellular signals.

The variable construction of the cAMP gate in these four systems indicates its flexibility. Nevertheless, a general scheme of interactions between the transmittal and gating pathways can be extracted (see figure) (*15*).

How does a signaling pathway function as a gate? The molecular mechanisms used for signal transmission can also be used for gating. In proliferative signaling, PKA phosphorylation of Raf inhibits Raf function (*5*). Hierarchical phosphorylation, in which phosphorylation at one site on a protein influences further phosphorylation at another site, can also be used for gating (*16*): The protein kinase regulated by the gating pathway would phosphorylate a component of the transmittal pathway, but such phosphorylation would be ineffective unless the component was being used during signal transmission. This mechanism allows for both opening and closing of gates. Alternatively, the gating pathway could regulate protein phosphatases, either directly or through protein kinases. For the cAMP pathway, PKA regulates protein phosphatase-1 activity by phosphorylating inhibitor-1. Inhibitor-1, when phosphorylated, associates with and inhibits protein phosphatase-1 (*17*). This mechanism has been proposed to function in LTP (*11*). Protein phosphatases can also be regulated without the intervention of protein kinases. The intracellular Ca^{2+} sensor calmodulin activates the protein phosphatase calcineurin and thus, at least in theory, allows the Ca^{2+} signaling pathway to function as a gate.

Why have a gate? A gate allows the cell to integrate contextual information with the responses to external signals. Such in-

formation may be generated within the cell by internal processes or obtained from external signals. Storing information in a signaling pathway that functions as a gate allows the information to be used in a conditional manner. Information storage within a transmittal pathway requires sustained activation of components of the pathway, a process that is almost always deleterious. For example, continuous activation of Ras or Raf contributes to malignant transformation. Continuous activation of the gating pathway, however, would not cause potentially harmful overstimulation but would only modify the response generated by the signaling pathway upon receipt of external signals. Thus interactions between signaling and gating pathways could provide a biochemical basis for information storage and processing within the cell.

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BMP-1: Resurrection As Procollagen C-Proteinase

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Bone morphogenetic proteins (BMPs) are bone-inducing molecules that have been isolated and cloned from the extracellular matrix (ECM). Unlike the other bone morphogens, BMP-1 is not a member of the transforming growth factor–β (TGF-β) family, but rather looks more like a protease—some researchers have suggested that it cleaves and activates TGF-β. In last week's issue of *Science*, Kessler *et al.* (*1*) reported that BMP-1 is indeed a protease, but that its substrate is procollagen, not TGF-β. BMP-1 is identical to procollagen C–proteinase (PCP), an enzyme essential for the proper assembly of collagen within the ECM.

The ECM is a supramolecular assembly of collagens, proteoglycans, and glycoproteins (*2*) that holds cells together. Its appearance in evolution coincided with that of multicellular Metazoa. The ECM and its interaction with cells allowed the organization of cells into tissues. The ECM has an intimate relation with cells. It is secreted as a cellular product, but can itself act upon cells and tissues. For example, implantation of demineralized ECM derived from bone results in the formation of new bone (*3*).

The active factors in the ECM that mediate this morphogenetic effect comprise a family of proteins—the BMPs. A simple bioassay facilitated their cloning (*4*, *5*). Reconstitution of a soluble extract of the matrix with insoluble collagen allows bone formation (*6*). Almost all of the dozen or so members of the BMP family are members of the TGF-β superfamily, the one exception being BMP-1. The black sheep status of BMP-1 may be a result of flaws in the original bioassays for osteogenesis (*4*). From the photographs in the published report (*4*), the cartilage observed in the bioassay actually appears to be growth plate cartilage contaminating the insoluble bone matrix. Thus, old cartilage may have been misidentified as newly formed tissue.

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The deduced amino acid sequence of the human BMP-1 protein reveals a domain structure of a metalloprotease from the astacin family, an epidermal growth factor (EGF)–like domain, and three domains with considerable sequence similarity. Thus, BMP-1 is related to the *Drosophila* gene *tolloid*, which is implicated in the patterning controlled by the *decapentapeptide* gene by virtue of its ability to activate TGF-β–like morphogens.

If BMP-1 is not a true TGF-β family member, how does it function? Is it actually a protease that activates TGF-β, as its homology to *tolloid* would suggest? The incisive work of Kessler *et al.* (*1*) shows unexpected similarities between BMP-1 and a protease they have been studying (PCP). These investigators expressed a recombinant BMP-1 in a baculovirus system and purified the protein. The recombinant BMP-1 and purified mouse PCP yielded similar COOH-terminal procollagen peptides.

Morphogenesis is the culmination of the cascade of pattern formation, body plan establishment, and attainment of adult form. An integral part of the morphogenetic cascade is the assembly of the ECM. The supramolecular self assembly of triple-helical collagen is triggered by the processing of COOH-terminal procollagen peptide by the newly discovered function of BMP-1. The recent work by Kessler *et al.* presents a new solution to the old riddle of the biological function of BMP-1 and places it directly at an essential control point of morphogenesis.

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