mosphere and deep sea seem to have been permanently anoxic? Philosophically, should we expect to find a single process that overwhelms all others in regulating atmospheric oxygen levels, or is it more likely that multiple feedback loops are working in concert to prevent large oxygen fluctuations?

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A Docking Receptor for HDL Cholesterol Esters

Daniel Steinberg

Cholesterol and cholesterol esters, very hydrophobic molecules, are carried through the hydrophilic environment of the bloodstream in lipoproteins. Perhaps the most familiar, low density lipoprotein (LDL), delivers cholesterol and its metabolites to cells by binding to specific receptors on the cell surface. In this process of "holoparticle uptake," the entire LDL particle is bound, endocytosed, and ultimately delivered to lysosomes where degradation of both protein and lipid occurs (*1*). Although uptake of high density lipoprotein (HDL) into most tissues can probably occur by a similar mechanism, HDL also uses a more selective means of delivering its cargo: In certain cells, HDL attaches ("docks"), delivers some of its cholesterol esters (and perhaps other lipids), and then dissociates from the cell surface and continues to circulate in the blood, now as a partially lipid-depleted particle [(*2*); see figure]. A receptor for HDL that mediates this "selective cholesterol ester uptake" has been identified by Acton *et al.* (*3*) and is reported in this issue to be SR-BI, a previously reported cell-surface molecule (*4*).

Selective cholesterol ester uptake occurs both in vivo and in vitro (*5*–*9*). By labeling HDL with "trapped ligands" (molecules that cannot escape from the cells after endocytotic uptake and delivery to the lysosome), the selective uptake of cholesterol ester from HDL has been shown in the rat to occur primarily in liver, adrenal gland, and ovary (*5*–*7*). The rate of uptake of cholesterol ester in these tissues is two to seven times more rapid than the uptake of apoprotein A-I, the major HDL protein. In other tissues, the uptake rates of HDL and apoprotein A-I are equal (with the exception of the kidney, which in the rat filters lipid-unassociated apoprotein A-I into the glomerular fluid). Selective cholesterol uptake in vitro does not appear to depend strongly on the nature of the apoproteins in HDL (*8*). Furthermore, other nonpolar lipids in the lipid core of HDL can also be selectively transferred. Selective uptake does not require endocytosis (*9*), but the precise mechanism of transfer across the cell membrane into the cytoplasm is not known. Acton *et al.* (*3*) now show that murine SR-BI, originally cloned on the basis of its ability to bind modified lipoproteins (such as acetyl LDL and oxidized LDL) (*4*), also binds HDL and mediates selective cholesterol ester uptake in transfected Chinese hamster ovary cells. They show further that SR-BI in mouse is expressed almost exclusively in liver, adrenal gland, and ovary, precisely those tissues in which selective uptake of HDL cholesterol esters has been demonstrated in vivo. These findings strongly support the identification of SR-BI as an HDL receptor.

High density lipoprotein selectively delivers cholesterol esters to steroidogenic tissues, and SR-BI is almost certainly involved in this process. High density lipoprotein also serves another crucial purpose: It picks up excess free cholesterol from peripheral tissues, which do not have the capacity for HDL degradation or excretion. The free

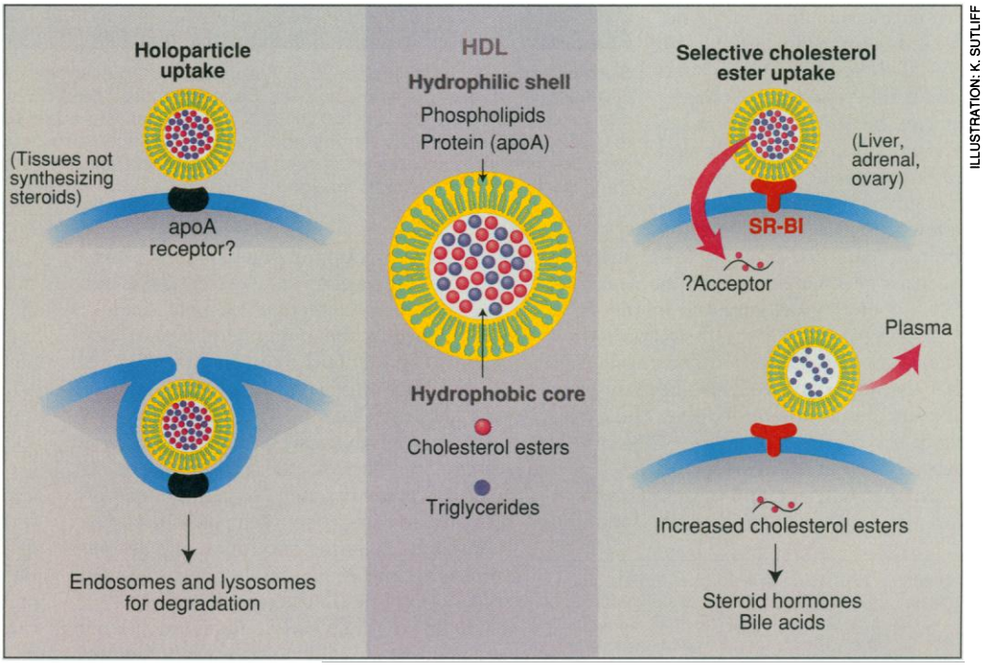

ILLUSTRATION: K. SUTLIFF

Two ways to get cholesterol into a cell.

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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

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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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