enters the A site of the ribosome and forms an amide bond with the translated peptide. The result is a stable mRNA/DNA/peptide hybrid in which the translated peptide is physically attached to the RNA from which it was translated and can, in turn, be amplified by PCR between rounds of peptide selection.

Peptide-small molecule hybrid libraries could be conceived in two ways. One possibility is that the hybrid library centers on a small lead molecule which is combined with a very large number of peptide appendages. The alternative is that a large peptide library is produced containing combinations of the normal complement of 20 proteinogenic amino acids plus a noncoded residue/small molecule. Roberts previously reported the preparation of peptide libraries containing noncoded amino acids (the equivalent of the small molecule in the present report) that were prepared by suppression mutagenesis [6]. This technique requires a substantial synthetic effort in the preparation of the requisite suppressor tRNA, and furthermore, it is perhaps not generally recognized that the efficiency of the suppression itself, i.e., introduction of the noncoded amino acid via "reading" of a stop codon signal by the synthetic acylated tRNA, is highly variable. This element of capriciousness makes this route inefficient; thus, resorting to a more traditional method of chemical posttranslational modification of natural or introduced cysteine residues within a peptide as a means of attaching the small molecule in the hybrid library is likely to be easier and more reliable than suppression mutagenesis.

The molecular bridge connecting the small molecule and the biopolymer favored by Li and Roberts features well-established and robust crosslinking chemistry: a highly electrophilic bromoacetamide bearing the β-lactam core of penicillin is attacked by the nucleophilic free thiol of a cysteine in the peptide. The chemoselectivity of cysteine is unrivaled among the 20 naturally occurring amino acids and has been the basis for numerous schemes for covalent modification of proteins, from native chemical ligation to proximity probes. In their report, an elegant series of controls demonstrate the specificity of this chemistry for the targeted cysteine only. Using this strategy, a molecule with 100 times greater affinity than penicillin for the *Staphlococcus aureus* penicillin binding protein 2a (PBP2a) was obtained from a hybrid library of approximately a trillion different peptide-drug conjugates. In addition to providing significant technological advances over the phage display and standard mRNA display library protocols described, the hybrid library has generated inhibitors to PBP2a that could be useful for overcoming β-lactam resistance in methicillin-resistant *S. aureus*.

The results presented by Li and Roberts may be most memorable for their promise of harnessing the vast diversities of peptide/small molecules in a library format rather than for the specific achievement of tethering penicillin to a peptide library. By appending the peptide to a site on penicillin known to tolerate (and benefit from) additional functionality, the results of the experiment are perhaps not too surprising. The two researchers argue convincingly that the modest affinities achieved in their experiment are not indicative of the actual improvement gained from the tethered peptide, and that it is the 100-fold increase in affinity that demonstrates the value of their technique. Regardless, this experiment builds a bridge to a new frontier of peptide-small molecule hybrid libraries that holds far-reaching and exciting possibilities for rapidly optimizing small molecule binding affinities without the need to synthesize large libraries of small molecules.

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Selecting Selective Suppressors of Selective Uptake

Scavenger receptor BI (SR-BI) is a high-density lipoprotein (HDL) receptor that mediates the selective uptake of HDL cholesteryl ester (CE) and the bidirectional flux of free cholesterol (FC). The identification of selective uptake inhibitors holds promise for mechanistic studies of SR-BI and for discovery of pharmaceuticals useful in therapy of atherosclerosis.

The selective uptake of HDL CE is a major pathway by which plasma HDL cholesterol is delivered to the liver and steroidogenic cells [1–3]. In contrast to the LDL receptor pathway in which LDL particles are endocytosed and degraded in cells to release cholesterol [4],

HDL-selective uptake involves the transfer of HDL CE to the plasma membrane without HDL uptake and degradation [5, 6]. The discovery of SR-BI as the cell surface receptor that mediates HDL CE-selective uptake [7] was followed quickly by the finding that SR-BI also stimulates the bidirectional flux of FC between HDL and cells [8]. Studies with SR-BI knockout mice and mice engineered to overexpress SR-BI demonstrated that SR-BI is a key determinant of plasma HDL concentration and the hepatic uptake and transfer of HDL cholesterol to bile, the major pathway by which cholesterol is eliminated from the body [9–12]. Further studies in mice show that this HDL receptor is protective against the development of atherosclerosis [10, 13–16]. As a key player in the metabolism of the "good" cholesterol, SR-BI has been the subject of intense study in academic and pharmaceutical laboratories.

In a recent report, Nieland et al. used a high throughput screen based on the uptake of a fluorescent lipid from HDL to identify small molecule inhibitors of SR-BI [17]. Of the inhibitors, designated blocking lipid transport (BLT) 1–5, three appear structurally dissimilar from two others. All are hydrophobic and contain O and N atoms that could participate in hydrogen bonding with amino acid side chains. Structure-activity studies are warranted to define chemical features important for SR-BI inhibition.

One surprise from their results is that the BLTs do not disrupt SR-BI-mediated lipid transport by blocking HDL binding. In fact, the BLTs modestly increase the binding affinity of SR-BI for HDL, in part by decreasing the HDL dissociation rate. In addition to blocking selective lipid uptake, BLTs inhibit SR-BI-mediated efflux of FC from cells to HDL. This result may indicate a common pathway for transfer of CE and FC between cells and HDL. Early studies led to the hypothesis that SR-BI provides a hydrophobic channel along which CE molecules diffuse down a concentration gradient from the HDL particle to the plasma membrane [18]. SR-BI-mediated net transfer of FC to and from HDL also depends on the FC concentration gradient between the cell and the HDL particle [19, 20]. One way in which these inhibitors may act is by occupying the hydrophobic channel to impede the movement of FC and CE. Another possibility is that BLTs, although they do not inhibit HDL binding, may alter the proper alignment of the HDL/SR-BI complex. High-affinity binding of HDL to SR-BI is not in itself sufficient for efficient lipid transfer; presumably, apoA-I, the major HDL protein, must assume a correct conformation on the HDL surface to interact productively with SR-BI to promote efficient lipid transfer [21, 22]. A recently described system in which SR-BI is reconstituted into membrane vesicles should prove useful for testing whether BLTs bind directly to SR-BI [23].

The BLTs should be useful in a number of arenas. For example, we know a lot about HDL CE-selective uptake in the rodent but much less in other species, including man, that express cholesteryl ester transfer protein (CETP). CETP moves CE from HDL to VLDL and LDL, permitting CE removal from the plasma via the LDL receptor pathway [4]. CETP makes kinetic studies of HDL CE clearance from plasma difficult and has held back our understanding of the quantitative importance of SR-BI-mediated pathways in man. If BLTs are active in vivo in other species, it will permit a simpler assessment of SR-BI function in many species not amenable to the genetic targeting strategies used in mice. This is particularly true in nonhuman primates that provide the best models for human lipoprotein metabolism and coronary artery disease [24]. Information from such studies may be particularly useful in determining whether pharmaceutical elevation of SR-BI activity will be therapeutically useful in man. The BLTs may also accelerate studies in gene-targeted mice. Krieger and colleagues, for example, found that the SR-BI/apoE double knockout mouse shows unexpectedly accelerated and exacerbated atherosclerosis compared to the atherosclerosis-susceptible apoE knockout mouse [15], and even shows coronary artery lesions and myocardial infarctions [25], features not typically observed in mice. Such studies often reveal phenotypes due to unrecognized interactions between atherosclerosis susceptibility genes but require lengthy animal breeding to combine different targeted genes. BLTs may facilitate rapid evaluations of the importance of SR-BI in HDL metabolism and atherosclerosis in other gene-targeted models by chemically creating SR-BI deficiency.

The BLT's should prove useful in studies of SR-BI effects on cellular cholesterol metabolism where SR-BI shows a variety of apparently distinct effects. SR-BI enhances FC efflux to nonphysiological cyclodextrin acceptors by increasing the size of the fast kinetic pool of membrane FC [26]. Additionally, SR-BI-mediated FC efflux to small unilamellar vesicles (SUV) composed of neutral or acidic phospholipids is equivalent [19] despite the fact that acidic SUV bind to SR-BI with high affinity and neutral SUV bind poorly if at all [27]. SR-BI also increases the fraction of plasma membrane FC that is sensitive to oxidation by exogenous cholesterol oxidase, suggesting an altered organization of FC in the membrane [19]. These data suggest that one component of SR-BI-mediated FC flux occurs independently of HDL and involves changes in the plasma membrane that facilitate FC desorption. Several findings also support a key role for HDL binding to SR-BI [21, 28]. Taken together, these data support a model of SR-BI-mediated FC efflux that involves two components. One, as exemplified by the SR-BI mutations [21, 28], requires HDL binding to SR-BI. The other, as exemplified by FC efflux to cyclodextrins and neutral SUV [19, 26], involves SR-BI-mediated changes in the plasma membrane. The BLTs may prove useful in understanding these components and their quantitative importance in overall FC flux between cells and HDL.

Perhaps the most striking feature of the study by Nieland and colleagues [17] is the proof of principle that a cell-based, high throughput screen is capable of identifying molecules that modulate a process important for the regulation of plasma cholesterol by the liver in vivo. Screening of additional chemical libraries may uncover agents that enhance SR-BI activity and have therapeutic potential to increase reverse cholesterol transport, the overall movement of cholesterol from peripheral cells to the liver. Similar screens could be used with the LDL receptor to identify agents that act in this pathway but at a different level than the popular statin drugs.

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Disarming the Invader

Type III secretion systems are used by many gram-negative bacterial pathogens of animals and plants to deliver essential virulence factors into targeted host cells. The identification of chemical compounds that block the function of these systems is the first step toward developing chemical attenuation as an effective method for the treatment of infectious disease.

Over the past decade it has become abundantly clear that despite vastly different disease outcomes caused by pathogenic bacteria, common mechanisms exist for targeting specific virulence factors to host sites. Type III secretion (TTS) systems are essential for virulence of many gram-negative pathogens of animals including species of *Bordetella, Chlamydia, Pseudomonas, Salmonella, Shigella*, and *Yersinia* [1]. In humans, these bacteria cause a variety of diseases such as whooping cough, plague, and several forms of gastroenteritis. Moreover, several plant diseases, which have had great economic impact, are caused by bacteria that utilize TTS systems such as *Erwinia* spp., *Pseudomonas syringae*, *Ralstonia solanacearum*, and *Xanthomonas campestris* [1]. TTS systems function in many cases only when the pathogen is intimately associated with a host cell. In this context, the physical interaction between the bacterium and the host cell induces the TTS system to deliver virulence proteins in a single step from the bacterial cytosol into the cytosol of the cell. This is a remarkable task when one considers that the delivery of proteins by gram-negative bacteria into a eukaryotic cell demands transport across three biological membranes. The particular set of proteins delivered by different pathogens is highly divergent, but the bacterial machinery composing the TTS systems is quite conserved. Thus, many gram-negative bacteria that cause disease have in common TTS systems, which can be targeted for the development of chemical compounds to block an essential virulence activity and effectively disarm this group of bacterial invaders.

While TTS systems are required for survival of bacteria during infection, they are dispensable for bacteria that have a free-living stage in their life cycle. Thus, a compound that blocks TTS will not necessarily inhibit bacterial growth. Traditionally, antibiotics are developed to interfere with an activity, such as synthesis of DNA, RNA, peptidoglycans, or proteins, which is essential for bacterial growth or survival [2]. This approach has been very productive and has changed the fate of humanity