CHEMICAL BIOLOGY

Alternatives to a horse

Synthetic receptors give cells new abilities to take up foreign molecules.

When the ancient Greeks were trying to conquer Troy, their main challenge was to get soldiers into the heavily fortified city. The solution, as everybody knows, was a giant wooden horse. Cell biologists today are facing a similar problem: they want to get foreign molecules—such as toxic drugs—into cancer cells. One way to do this is to design compounds mimicking natural ligands that a cell normally takes up without suspicion; however, once inside, these compounds reveal their toxic potential. Blake Peterson, a chemist at The Pennsylvania State University, decided to break with this tried-and-tested method and chose another path to penetrate a cell. In a recent article in the *Journal of the American Chemical Society,* he lays out an alternative strategy to give cells the capability of taking up foreign cargo (Boonyarattanakalin S. *et al.*, 2004).

"We became interested in the idea of trying to mimic receptors found on the cell surface using synthetic molecules," explains Peterson. His group set out to engineer receptors that insert into the cell membrane and, after delivering their cargo to intracellular vesicles, recycle back to the plasma membrane for the next round of transport (**Fig. 1**). It was this last aspect that posed the greatest challenge to the Peterson team, until they found that an amine derivative of cholesterol rapidly cycled between the cell surface and intracellular vesicles. Having settled on cholesterylamine for a membrane anchor, the researchers constructed a functional receptor by adding a linker and a cargo-binding domain. Surprisingly, Peterson found that it was the structure and length of this linker that determined the efficiency of the recycling mechanism. In general, the lon-

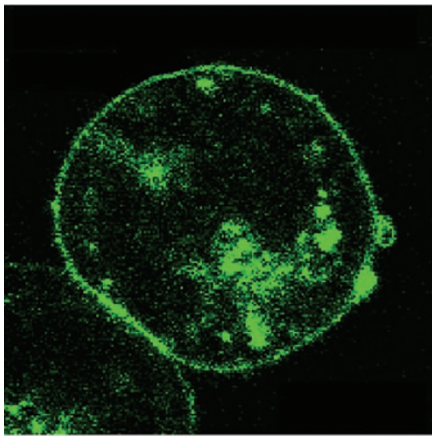

Figure 1 | Synthetic receptors cycling from the plasma membrane to intracellular vesicles and back to the plasma membrane.

MICROARRAYS

MEASURING A BACTERIA'S SWEET TOOTH

After DNA and protein chips, here come the carbohydrate microarrays capable of detecting and classifying bacteria based on their sugar binding affinities. From detection to design of infection inhibitors, these arrays could have wide-ranging applications.

Microarrays are widely used for high-throughput analysis of gene expression and more recently for screening protein-protein interactions. However, the technology is still in its early stages of use, and new applications are making an entrance. In a paper in *Chemistry and Biology*, Disney and Seeberger report the creation and use of high-density microarrays capable of measuring bacterial binding affinity for carbohydrates, thus providing a powerful technique to investigate these often-underappreciated interactions (Disney and Seeberger, 2004).

Bacteria have specialized proteins on their surface that enable them to bind simple sugars such as mannose as well as more complex carbohydrates. This bacterial sweet tooth mediates several biological functions and is involved in bacterial pathogenicity by helping bacteria bind to carbohydrates displayed on the surface of cells that are being infected. Furthermore, different bacteria have different tastes for sugar, expressed in various binding affinities for simple carbohydrates. For example, the virulence of different pathogens has been found to correlate with their binding affinity for the simple sugar mannose.

These differences in carbohydrate binding among different strains of bacteria could be used to find a resultant 'fingerprint' for each bacterial strain. However, such measurements require a high-throughput technology capable of detecting the binding of bacteria to various concentrations of a range of different carbohydrates. Furthermore, working with bacteria is complicated by their tendency to stick to the surface of experimental equipment. Disney and Seeberger were able to adopt methods developed by others to prevent cells from sticking and integrated them into commonly available microarray technology. According to Seeberger, "it took a couple of different linkers and a couple of different surface chemistries" before they arrived at the final working system.

An earlier report by another group demonstrated the potential for carbohydrate array technology in mammalian biology by examining the adhesion of hepatocytes and T cells to manually spotted carbohydrate arrays (Nimrichter *et al.*, 2004). The method reported by Disney and Seeberger, using readily available robotic microarray spotting technology, greatly simplifies the creation of dense high-throughput arrays of carbohydrates. According to Seeberger, "This allows anyone to look at carbohydrate-protein interactions and screen small-molecule libraries to look for molecules to block such interactions".

"The other goal of this work is to correlate bacterial strains with the carbohydrates they bind to, and this information

ger the linker, the better the receptors recycled. Even though this observation is intriguing and could shed light on basic trafficking mechanisms, the main application of these synthetic receptors is the transport of molecules into cells. This synthetic chemistry approach for accessing the cellular interior offers great versatility. Chemists may be able to engineer artificial receptors with different binding motifs that facilitate the uptake of virtually anything, to the benefit or destruction of cells. For example, in drug delivery, tumor cells carrying a specific synthetic receptor might be made more susceptible to toxic drugs.

Although such applications hold promise, there are still big hurdles to overcome. One is to show that these synthetic receptors, which have primarily been tested on cells in culture, also work in animals. Peterson is cautiously optimistic; his team has preliminary results from a vertebrate model in which the receptors are incorporated into cellular membranes. Whether they still work as receptors and how toxic they are in an animal over a longer time span is now being tested.

Nonetheless, this approach offers an innovative solution to an old problem. If Odysseus had met Blake Peterson, maybe the Greeks would have dismissed the idea of a horse and instead presented the Trojans with a city gate containing a clandestine portal.

Nicole Rusk

RESEARCH PAPERS

Boonyarattanakalin, S. *et al.* Synthetic mimics of small mammalian cell surface receptors. *J. Am. Chem. Soc.* **126**, 16379–16386 (2004).

can be utilized for detection," says Seeberger. As the next step to this goal, the Seeberger lab is using the array technology to screen bacterial strains in an attempt to define a unique carbohydrate-binding 'fingerprint' for each strain. Intriguingly, if the strength of binding to specific carbohydrates is found to correlate with bacterial pathogenicity, the method could be used to quickly estimate the danger associated with a bacterial infection.

Detection of bacteria is just one possible use for this promising technology. Of more interest to researchers is the possibility of using carbohydrate arrays to examine carbohydrate-protein interactions. Carbohydrates are displayed on the surface of all cells and are involved in important cellular functions such as adhesion, recognition and signaling, in addition to being a route for bacterial infection. It has also been suggested that surface carbohydrates could serve as markers for cancer progression. Although they have yet to publish their results, Seeberger expects these new arrays to work on mammalian cells in addition to bacteria. These new technologies should make it much easier for researchers to exploit the many promising avenues of research embodied in carbohydrate mediated interactions.

Daniel Evanko

RESEARCH PAPERS

Disney, M.D. & Seeberger, P.H. The use of carbohydrate microarrays to study carbohydrate-cell interactions and to detect pathogens. *Chem. Biol.* **11**, 1701–1707 (2004).

Nimrichter, L. *et al.* Intact cell adhesion to glycan microarrays. *Glycobiology* **14**, 197–203 (2004).

NEWS IN BRIEF

GENE REGULATION

Substrate-induced gene expression screening of environmental metagenome libraries for isolation of catabolic genes

Uchiyama *et al.* present a new technique for the identification of genes induced by a catabolite of interest: substrate-induced gene expression screening (SIGEX). Genomic fragments from an organism of interest are cloned into an operon-trap GFP expression vector, and FACS is used to separate clones in which the presence of a particular catabolite induces expression.

Uchiyama, T. *et al. Nat. Biotechnol.*, **23**, 88–93 (2005).

VIROLOGY

Synchronized infection of cell cultures by magnetically controlled virus

Current strategies for the retroviral infection of cultured cells rely on the diffusion of viral particles. By coating lentivirus with iron oxide nanoparticles and using a magnetic field to draw the coated viruses to the cell monolayer, Haim *et al.* demonstrate a dramatically enhanced rate of association, suggesting the possibility of tightly synchronized studies of the infection process.

Haim, H. *et al. J. Virol.* **79**, 622–625 (2004).

IMAGING AND VISUALIZATION

Tumor imaging by proteolytic activation of cell-penetrating peptides

Previous studies have demonstrated that relatively simple cell-penetrating peptides (CPPs) can facilitate passage through cell membranes by an associated 'cargo' molecule. Jiang *et al.* further enhance this technology, creating CPPs that rely on proteolytic cleavage for activation and demonstrating their capacity to label tumor cells.

Jiang, T. *et al. Proc. Natl. Acad. Sci. USA* **51**, 17867–17872 (2004).

DNA CLONING AND AMPLIFICATION

Accurate multiplex gene synthesis from programmable DNA microchips

Oligonucleotide synthesis can be a costly and error-prone process. Tian *et al.* present a microarray-based strategy for the rapid and affordable synthesis, amplification and purification of oligonucleotides. They demonstrate the rapid production of highly accurate oligonucleotides and their application to the synthesis of a variety of full-length genes.

Tian, J. *et al. Nature* **432**, 1050–1054 (2004).

GENOMICS

Global identification of human transcribed sequences with genome tiling arrays

Bertone *et al.* demonstrate the use of a human genome tiling array for the large-scale investigation of transcriptional activity. cDNA derived from liver mRNA was hybridized against arrays representing roughly 1.5 Gb of nonrepetitive genomic sequence, confirming numerous known and predicted genes, and revealing over 10,000 previously unidentified transcribed sequences.

Bertone, P. *et al. Science* **306**, 2242–2246 (2004).