BIOSENSORS

Hybridization chain reaction

A new technique, based on a chain reaction of recognition and hybridization events between two sets of DNA hairpin molecules, offers an enzyme-free alternative for the rapid detection of specific DNA sequences.

The processes that mediate target recognition and signal transduction in biosensors generally operate through distinct mechanisms. In the polymerase chain reaction (PCR), for example, recognition is provided by sequence-specific hybridization of short oligonucleotides to a larger target, whereas signal amplification requires the use of a polymerase coupled with externally driven melting and rehybridization. However, in the October 26th issue of the *Proceedings of the National Academy of Sciences*, Niles Pierce and graduate student Robert Dirks demonstrate how binding of DNA to a substrate can accomplish the roles of recognition and signal amplification without any external inputs. This is accomplished by the triggered self-assembly of DNA nanostructures in a novel process they term hybridization chain reaction (HCR).

According to Pierce, HCR came about not from a desire to compete with PCR but as a byproduct of engineering DNA hairpins that could be used as fuel packets to power DNA mechanical machines. By eliminating the machines from the design scenario, they found that the hairpins could be triggered to undergo a chain reaction. "The realization that got us excited was the idea that this could be used as an amplifying signal transducer," says Pierce.

The key to HCR in its simplest form is the storage of potential energy in two hairpin species. When a single-stranded DNA initiator is added to this previously stable mixture, it opens a hairpin of one species, exposing a new single-stranded region that opens a hairpin of the other species (**Fig. 1**). This process, in turn, exposes a single-stranded region identical

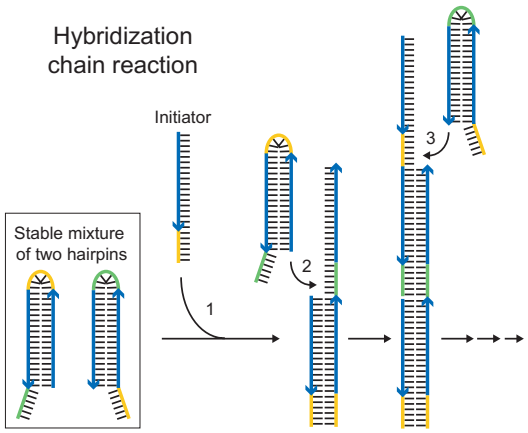

Figure 1 | Schematic of the basic hybridization chain reaction. Addition of an initiator strand of DNA to the stable mixture of two hairpin species triggers a chain reaction of hybridization events between the hairpins.

DRUG DISCOVERY

GETTING PAST THE PUMP

An assay integrating chemical screening and suppressor gene determination promises to accelerate the identification of potent antibacterial compounds and their target proteins and to clarify the key features of successful drugs.

In antibacterial drug design, developing compounds with the mechanistic potential to kill bacteria is only part of the job. The drug also needs to get into the cell and stay there long enough to kill it, as many bacteria come equipped with powerful pumps for the active removal of a wide variety of compounds, including many would-be antibiotics. The 'reverse genetics' approach, in which chemical libraries are screened against a target bacterial protein to identify putative inhibitors, has been a prevailing paradigm in antibiotic design. However, according to McMaster University researcher Eric Brown, "that's failed miserably in antibacterial drug discovery... [and] I think that the key failure is that we don't understand the rules for getting things into cells."

Brown and his colleagues were interested in developing a new, phenotype-based approach, which could first identify promising drug candidates and then reveal their targets. They began with a growth inhibition screen, using a diverse library of 8,640 commercially available compounds to identify chemicals that inhibited growth of the hyperpermeable *Escherichia Coli* strain MC1061, first in liquid culture and then on solid media.

These screens winnowed the field to 49 lead compounds, which were tested in a multicopy suppression assay, an approach that has a history of successful use for the identification of antibiotic targets. Such assays involve the identification of gene products from a bacterially expressed genomic library that, when overexpressed, can suppress the growth-inhibitory properties a given compound. By this approach, Brown's team identified suppressors for 33 of their 49 lead compounds.

Nearly all of the compounds were suppressed by the same gene, *acrB*, which encodes the inner membrane component of an important drug efflux pump (also notorious for making the *Pseudomonas aeruginosa* bacterium the drug-resistant scourge of hospital wards worldwide). This finding is unsurprising, according to Brown: "I think that, more and more, it's being appreciated that permeability is not so much about structural integrity of the cell, but more about molecules being pumped out... [and] very little is understood about what makes something a substrate for efflux." The group also found that two of the compounds, 1a and 2a, appeared to target a second gene,

to the original initiator. The resulting chain reaction leads to the formation of a nicked double helix that grows until the hairpin supply is exhausted. Detection of the resulting products does not require any specialized detection equipment. As Pierce remarks, "All you need is a gel apparatus, which can be found in any wet lab. We are also trying to make a nanogold-based colorimetric assay that will enable detection by eye alone."

For more diverse biosensing applications, DNA and RNA aptamers selected to bind specific molecules hold promise for the development of HCR triggers that will initiate the chain reaction only in the presence of the target molecule. The authors have used this aptamer trigger concept to specifically discriminate ATP from GTP. Pierce remarks, "If we succeed in developing a general aptamer triggering mechanism, then HCR amplification could be incorporated in sensors for a wide range of small molecules."

Unlike PCR, which provides exponential amplification, the current form of HCR provides linear amplification. "We are now developing nonlinear versions of HCR that provide quadratic, cubic or exponential growth after being triggered by the initiator," says Pierce. "However, false positives become a much bigger problem, as spurious initiation events are also amplified nonlinearly." Successful development of an exponential HCR amplification system would increase the sensitivity to target molecules at very low concentrations. This would open up the possibility of attaining a PCR-like level of sensitivity for a variety of small molecules without the need for any expensive equipment or reagents.

Daniel Evanko

RESEARCH PAPERS
Dirks, R.M. & Pierce, N.A. Triggered amplification by hybridization chain reaction. *Proc. Natl. Acad. Sci. USA* **101**, 15275–15278 (2004).

folA, encoding dihydrofolate reductase, a known drug target. Follow-up experiments confirmed that elevated bacterial expression of *folA* increased the amount of 1a or 2a needed to inhibit growth.

Brown is slightly disappointed by the extent to which efflux pumps appear to drown out target identification, but he also sees important benefits for future research. "One of the things that comes out of this paper, I think, is a way to better understand what is the substrate specificity of an efflux pump," says Brown, who indicates that his team has already learned quite a bit about how properties such as the extent of hydrophobicity might lead to increased drug efflux. His team is seeking ways to potentially identify additional targets that might be lost amid the 'noise' generated by efflux pump suppressor genes, but Brown believes that their system already has a lot to offer: "[From] eight and a half thousand molecules, we pulled out two compound-target pairs, and that's not really such a bad success rate. I think that as is, you could take this forward with much higher throughput, and I daresay that there are companies that would have the resources to do that."

Michael Eisenstein

RESEARCH PAPERS
Li, X. *et al*. Multicopy suppressors for novel antibacterial compounds reveal targets and drug efflux susceptibility. *Chem. Biol.* **11**, 1423–1430 (2004).

NEWS IN BRIEF

VIROLOGY

A novel approach for producing lentiviruses that are limited to a single round of infection

The development of lentiviral strains capable of only one round of infection is of great interest to researchers investigating the dynamics and pathogenesis of HIV and SIV. Evans *et al.* present such an SIV strain, created by mutating the ribosomal frameshifting site between the *gag* and *pol* reading frames, observing successful restriction *in vitro* and *in vivo*.

Evans, D.T. *et al. J. Virol.* **78**, 11715–11725 (2004).

MICROSCOPY

Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure

Block-face imaging offers an effective means for the generation of serial microscopic images. Denk and Horstmann apply this system in the context of environmental scanning electron microscopy, obtaining serial data that enable the high-resolution three-dimensional reconstruction of neural circuits and other nanostructures.

Denk, W. & Horstmann, H. *PLoS Biol.*, published online 19 October 2004.

CELL BIOLOGY

Mapping the dynamic organization of the nuclear pore complex inside single living cells

About 30 different nucleoporin proteins compose the nuclear pore complex, mediating transport of molecules between cytoplasm and nucleus. Rabut *et al.* have created and analyzed a series of cell lines expressing many of these proteins as GFP fusions, in an effort to better understand the *in vivo* dynamics of each different pore-complex component.

Rabut, G. *et al. Nat. Cell Biol.*, **6**, 1114–1121 (2004).

MICROARRAYS

Distinct effects on gene expression of chemical and genetic manipulation of the cancer epigenome revealed by a multimodality approach

Gius *et al.* apply a microarray strategy to characterize the impact on gene expression patterns resulting from different methods of modulating DNA methylation and find that the effects of genetic modification to eliminate DNA methyltransferase expression unexpectedly differ from those induced by drugs altering methylation or histone acetylation.

Gius, D. *et al. Cancer Cell* **6**, 361–371 (2004).

IMMUNOCHEMISTRY

Targeted gene alteration in *Caenorhabditis elegans* by gene conversion

Transposon-based mutagenesis is a popular method for *C. elegans* genetic studies, but it requires the screening of large numbers of worms, and researchers have little control over the introduced changes. Barrett *et al.* describe a combined approach using transposons and modified transgenes to efficiently introduce targeted replacements, deletions and insertions in a mutator worm strain.

Barrett, P.L. *et al. Nat. Genet.* **36**, 1231–1237 (2004).