NANOTECHNOLOGY

DNA nanostructures go 'live'

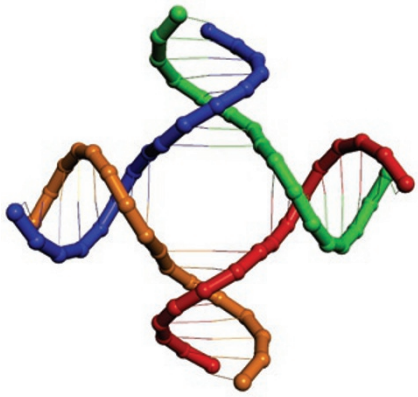
To scale up the production and complexity of DNA nanostructures, researchers enlist the help of *Escherichia coli* to replicate and assemble them *in vivo*.

"Let the cell do the work" was the mantra behind recent efforts to express complex DNA nanostructures in bacterial cells, led by Hao Yan of Arizona State University and Nadrian Seeman of New York University.

Two features of DNA make it an ideal molecule for the assembly of complex structures: its code of only four nucleotides is easy to program, and the resulting sequence is structurally predictable and should yield two-, even three-dimensional constructs for many different applications. The current bottleneck to actually achieve a higher-level production of DNA nanostructures is a limit to the scale at which long single-stranded DNA molecules of complex secondary structure can be produced.

Yan explains why it is important to scale-up production: "We want to build conformational information into the DNA nanostructure and allow the DNA to self-assemble into an algorithmic pattern." Sensing that this rather technical outlook may fail to excite outsiders to the field, he adds: "Look at the growth of a tree, it stops at the tip so it must have an algorithm of growth built in. DNA can potentially do the same thing; the big question is how to scale-up self-assembly to larger domains that contain more information."

The current state of the art is still very far removed from this 'DNA tree' image. Yan and his colleagues have assembled DNA nanostructures *in vitro* but could not easily exceed a length of ~120 bases of single-stranded DNA. To get longer pieces at higher yield, the researchers shifted the workload from the test tube to a bacterial cell. Yan's first question was, "Will nature's molecular machinery tolerate the complex structure?"



A complex DNA nanostructure is amplified and folded in bacterial cells. Image courtesy of H. Yan.

The answer was an unequivocal yes. Not only did *E. coli* produce long DNA strands in large quantities, it also tolerated their assembly inside the cell. Yan and his colleagues demonstrated that the clover-like

CHEMICAL BIOLOGY

NO SUNSHINE IN A SPOTLESS MIND?

Chemical-genetic manipulation of enzyme activity allows specific memory erasure in the mouse.

To study the role of a gene in a complex phenomenon such as memory processing, in particular when that gene belongs to a large superfamily, is no easy task.

Joe Z. Tsien, now at the Medical College of Georgia, Augusta, has been studying the molecular basis of memory for more than a decade. He has used Cre-*loxP*–mediated conditional gene knockouts in mice, as well as inducible, reversible and cell type–specific variations on this theme, to tease out how memories are acquired, consolidated, stored and retrieved in the mammalian brain.

But the problems with gene knockouts, in their potential lack of specificity for the process under study, are legion. And even inducible knockouts are not ideal, as it can often take at least several days before the pre-existing protein encoded by a gene of interest is entirely degraded. Five years ago, Tsien and colleagues developed a 'protein knockout' for calcium calmodulin-dependent kinase II (α-CAMK-II), an enzyme they considered likely to be involved in memory processing. They engineered a cavity into the ATP-binding domain of the enzyme and synthesized a bulky inhibitor that can fit precisely into this cavity. Because of its bulk, the inhibitor targets α-CAMK-II specifically and leaves other members of the CAMK superfamily unaffected.

Tsien and colleagues have used this technology to study several aspects of memory processing. "We have found many different temporal roles in memory for this enzyme using our system," he says. "I think it gives deeper molecular insights than more traditional approaches." Most recently, Tsien and his group have turned their attention to studying memory recall, a process that has typically been difficult to tackle.

Using multiple behavioral tests that examine both pleasant and unpleasant memories, to the extent that this can be defined for a mouse, the researchers found that mice transgenic for the engineered α-CAMK-II, overexpressed in the forebrain using its endogenous promoter, had impairments in both short- and long-term memory. And by administering the specific inhibitor to the mice at various stages of the experiment, which returned the total enzyme activity to normal levels within a few minutes, the researchers pinpointed this impairment to the recall step of the process.

"When you have memory impairment during recall," says Tsien, "a lot of times you cannot distinguish if there is a block

structure programmed into one DNA molecule was present inside the living cell.

Excited by this finding, Yan wants to fully exploit the possibilities of an *in vivo* production system and apply selective pressure so that the nanostructure can evolve. His end goal is a functional, not a static, structure with a myriad of applications, ranging from single-cell imaging, where a tweezers-like nanostructure can bring together two fluorescent proteins for energy transfer, to protein detection with a flexible structure containing an induced fit for a certain protein, to an aptamer that would recognize and neutralize a bacterial or viral intruder in the cell.

These intriguing applications in mind—some of which are, as Yan acknowledges, still closer to fiction than to science—the researchers now seek to lay the necessary groundwork. For example, longer DNA molecules result in an increased error rate, consequently proofreading mechanisms are needed. The vector and promoters used for the *in vivo* replication must be carefully chosen; Yan cautions that with larger molecules one must make sure that the sequence of the vector does not interfere with the sequence of the nanostructure. So far the team has only tested the replication of nanostructures in *E. coli*; it is still an open question whether eukaryotic cells will support production and assembly.

The field of DNA nanotechnology, comprised of about ten research groups, is relatively small, but their dreams, and the implications for society, certainly are not.

Nicole Rusk

RESEARCH PAPERS

Lin, C. *et al. In vivo* cloning of artificial DNA nanostructure. *Proc. Nat. Acad. Sci. USA*, published online 16 October 2008.

of access to a well-stored memory, or if basically the memory is no longer there. But we are able to distinguish that." The scientists carried out sequential memory retrieval tests, first in the absence and then in the presence of the α-CAMK-II inhibitor, and determined that what causes the impairment is a recall-mediated erasure of memory. The erasure occurs within minutes and, remarkably, is selective for the particular memory being recalled. For example, in the case of fear memories, Tsien explains, "We can [get the animal to] link the fear to the room or to link it to a tone. And we can erase one type of memory and the other remains unaffected."

Tsien and colleagues have thus demonstrated that it is possible in theory to achieve selective memory erasure in the mammalian brain. Whether or not this will have implications for human beings suffering from traumatic memories will depend on a number of factors, in particular on whether the mechanisms described here apply to the more complex human brain as well. And, as Tsien emphasizes, "all memories, including painful ones, have their purpose. They can help us avoid making the same kinds of mistakes. That is very important."

A point, one might say, that is worth remembering.

Natalie de Souza

RESEARCH PAPERS

Cao, X. *et al.* Inducible and selective erasure of memories in the mouse brain via chemical-genetic manipulation. *Neuron* **60**, 353–366 (2008).

NEWS IN BRIEF

GENE TRANSFER

Single-copy transgene insertion in worms

Methods for making single-copy insertions of transgenes at specific sites in the *Caenorhabditis elegans* genome have been lacking. Now, Frøkjær-Jensen *et al.* adapt the *Drosophila melanogaster* Mos1 transposon for this purpose. They identify genetically neutral intergenic Mos1 insertion sites in the worm genome, and show that mobilization of the transposon and repair of the resulting double-stranded break can be used to create a single-copy insertion of a transgene into the chromosomal site.

Frøkjær-Jensen, C. *et al. Nat. Genet.* **40**, 1375–1383 (2008).

MICROARRAYS

Improving protein detection with Raman

Protein microarrays provide a high-throughput protein identification platform. Typically, fluorescence detection is used as the readout, but fluorescence is prone to background interference and autofluorescence, limiting the sensitivity of microarrays. Chen *et al.* describe a new, highly sensitive detection method, which uses functionalized single-walled carbon nanotubes as multicolor Raman scattering labels. The Raman-based detection system improves sensitivity by 1,000-fold over fluorescence, facilitating new research and clinical applications of protein microarrays.

Chen, Z. *et al. Nat. Biotechnol.*, advance online publication 26 October 2008.

SYSTEMS BIOLOGY

Digital zebrafish embryos

The global movements of cells during embryogenesis have not as yet been tracked in a vertebrate model. Using their newly developed digital scanned laser light sheet fluorescence microscopy, in which a thin beam of light is rapidly scanned through a specimen, Keller *et al.* track the movement of all cells over a 24-hour period of zebrafish development. The resulting digital embryos will constitute a useful resource for zebrafish biologists.

Keller, P.J., *et al. Science*, published online October 9, 2008.

METABOLOMICS

Profiling protein-metabolite interactions

The small molecules of the 'metabolome' have important roles in cellular processes by regulating protein function. Tagore *et al.* describe an approach to identify protein-metabolite interactions. They immobilize the protein of interest on a solid support and incubate it with a cellular metabolite mixture. Metabolites that bind to the protein are eluted and analyzed by a global liquid chromatography–mass spectrometry platform for identification.

Tagore, R. *et al. J. Am. Chem. Soc.* **130**, 14111–14113 (2008).

SPECTROSCOPY

Taking temperature with MRI

Something as basic as temperature is actually quite difficult to determine *in vivo*. Existing magnetic resonance imaging–based methods are subject to inaccuracies caused by inhomogeneous magnetic fields in tissues. Galiana *et al.* now describe a new magnetic resonance method for highly accurate *in vivo* temperature imaging, based on new pulse sequences for intermolecular multiple quantum coherence detection that reduce the effects of physiological noise.

Galiana, G. *et al. Science* **322**, 421–424 (2008).