CHEMICAL BIOLOGY

A trinucleotide repeat biosensor

A small-molecule ligand that binds to a $(CAG)_n$ hairpin repeat is the basis of a biosensor to detect repeat length, which may help diagnose trinucleotide repeat disease severity.

DNA trinucleotide repeat expansions are known to be associated with several hereditary neurodegenerative disorders, including Huntington disease, Fragile X syndrome and spinocerebellar ataxia. Unfortunately, because little is known about the mechanisms of pathogenesis, there are no effective therapies to treat these disabling diseases.

The presence of a $(CAG)_n$ repeat results in the inclusion of long tracts of polyglutamine in certain proteins, such as in the protein huntingtin, responsible for Huntington disease. The mutant huntingtin is thought to take on a new function that leads to cell death and debilitating symptoms. The causes of trinucleotide repeat expansions are not well-understood, but it has been proposed that these repeats are a result of DNA strand slippage mediated by the formation of a hairpin structure during DNA synthesis. The stability of the hairpin increases as the length of the repeat increases, as does the severity of the disease.

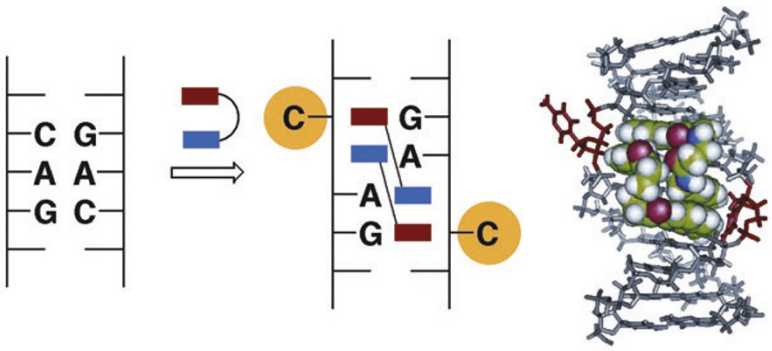

Figure 1 | Binding of NA to the CAG-CAG triad. The CAG-CAG triad results in an A-A mismatch (left). The small molecule naphthyridine-azaquinolone (NA) intercalates into the DNA helix, with the naphthyridine moiety (red) presenting complementary hydrogen bonds to guanine, and azaquinolone (blue) presenting complementary hydrogen bonds to adenine (center). Binding of two NA molecules causes extrusion of the two cytidines. The NMR structure of the NA-CAG-CAG complex with cytidines highlighted in red (right). Reprinted with permission from *Nature Chemical Biology*.

Recently, a collaborative effort from several institutions in Japan resulted in development of a small-molecule biosensor capable of determining $(CAG)_n$ hairpin repeat length. Formation of the $(CAG)_n$ repeat hairpin results in C–G pairs and A-A mismatches (**Fig. 1**, left). The researchers discovered a small-molecule ligand, naphthyridine-azaquinolone (NA), which recognizes

STEM CELLS

STEM CELL MATCH-MAKING

Korean researchers, in collaboration with American scientists, have generated the world's first patient-specific human embryonic stem cells (hESCs), with promising implications for future therapeutic strategies.

It was big news when, in 2004, researchers led by Woo Suk Hwang and Shin Yong Moon of the Seoul National University announced the successful cloning of an hESC line from a human embryo using a technique known as somatic cell nuclear transfer (SCNT; Hwang *et al.*, 2004). Unsurprisingly, the cameras were immediately followed by considerable debate over the ramifications of this research, even as many biologists eagerly sought more information about these findings.

A major objective in cloning hESCs is for therapeutic transplantation into patients suffering from degenerative disease or severe injury. One major obstacle to this process is developing cells that will not trigger a potentially fatal host immune response. The ideal would be to develop stem cells with cellular histocompatibility markers identical to those of the recipient, cells that will be recognized as 'self' in their new environment.

Gerald Schatten, a stem cell specialist at the University of Pittsburgh School of Medicine, first came into contact with the Korean team in late 2003, not long before the storm broke, hoping to learn more about their work for his own research with rhesus monkeys. The two groups became fast friends—"We are sort of kindred spirits," says Schatten—and the strength of this bond formed the foundation for a highly effective collaboration. The Korean team recruited a variety of somatic cell donors in Korea—several with pre-existing, heritable medical conditions such as juvenile diabetes—and isolated the nuclei from their somatic cells. These nuclei were injected into donated oocytes, generating 'patient-specific' blastocysts consisting of hESCs with the nuclear genetic profile of the somatic donor (Hwang *et al.*, 2005). One line was even derived in which both the nucleus and the oocyte came from the same donor.

The cells seem to behave as expected, with apparently complete pluripotency and full histocompatibility with their donors, although much more work needs to be done. Among other things, there are still concerns regarding the impact of potential maternal DNA (that is, mitochondrial DNA) mismatches in stem cell preparations in which the nucleus and oocyte donor are not identical. Nonetheless, the work

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and binds to the CAG-CAG triad. The 2-amino-1,8-naphthyridine moiety was designed to present hydrogen bonds complementary to guanine, and 8-azaquinolone to present hydrogen bonds complementary to adenine; the two groups are connected by a short, flexible linker. Solution of the NMR structure of the CAG-CAG triad revealed that two molecules of NA intercalate into the DNA helix, presenting hydrogen bonds to correct the A-A mismatch yet disrupting the C–G pair, causing the two cytidine nucleotides to 'flip' to the outside of the helix (**Fig. 1**, center and right).

The researchers used NA to create a surface plasmon resonance (SPR) biosensor that was sensitive to $(CAG)_n$ repeat length. "A longer $(CAG)_n$ repeat sequence bound to immobilized NA on the sensor more efficiently than the shorter $(CAG)_n$ repeat, and so we could see the difference in binding affinity by the SPR signal intensity," reports Kazuhiko Nakatani, principal investigator of this study. The NA-SPR sensor could be calibrated and used to determine $(CAG)_n$ repeat lengths, and therefore the severity of the disease state.

In addition to diagnosing $(CAG)_n$ repeat disorders, the discovery of NA may be an important step toward understanding the mechanism of trinucleotide repeat pathogenesis, and potentially, a cure. "One therapy may be to use NA to stop the translation of the $(CAG)_n$ repeat to the protein," says Nakatani. Nakatani and other researchers in this area are certainly hopeful that the discovery of new small-molecule ligands may one day open the door to effective therapies for this and other trinucleotide repeat expansion disorders.

Allison Doerr

RESEARCH PAPERS

Nakatani, K. *et al.* Small-molecule ligand induces nucleotide flipping in $(CAG)_n$ trinucleotide repeats. *Nat. Chem. Biol.* **1**, 39–43 (2005).

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remains a promising breakthrough, both for transplantation work and for the generation of disease-specific cells for research purposes.

Schatten points out that newly passed Korean legislation, which allows but closely regulates therapeutic stem cell cloning, has been a tremendous asset: "As an American sitting in Pennsylvania, one of a handful of states that still makes human embryonic stem cell derivation a criminal felony, I would have to say that more important than having the scientific skills, more important than having the research resources, is having a clear and enabling institutional state and national policy."

Indeed, these are trying times for American stem cell researchers, but Schatten remains positive, preferring instead to focus on gains like the recent Congressional vote to expand therapeutic stem cell work—even in the face of a veto threat. Above all, he is grateful for the chance to collaborate on this work with his international partners: "Without them, this type of research might have been delayed for decades… I just think we all owe them an immense debt of gratitude."

Michael Eisenstein

RESEARCH PAPERS

Hwang, W.S. *et al.* Patient-specific embryonic stem cells derived from human SCNT blastocysts. *Science*; published online 19 May 2005.

Hwang, W.S. *et al.* Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science* **303**, 1669–1674 (2004).

NEWS IN BRIEF

CHEMICAL TOOLS

Phosphorylation-driven protein-protein interactions: a protein kinase sensing system

Fluorescent probes are promising tools for the monitoring of kinase activity, but there remains a need for generalized strategies that can be applied to generate indicators for virtually any specific kinase. Wang and Lawrence describe such a strategy for probe design, an approach they believe could be used to prepare orthogonal probes for the simultaneous monitoring of several different kinases.

Wang, Q. & Lawrence, D.S. *J. Am. Chem. Soc.* **127**, 7684–7685 (2005).

PROTEIN BIOCHEMISTRY

Dissociation of ligand-receptor complexes using magnetic tweezers

Danilowicz *et al.* describe an inventive way to measure dissociation constants for ligand-receptor pairs. Receptors immobilized onto superparamagnetic beads are allowed to bind to their counterpart ligands, which have been adsorbed to a flat surface. Measurement of the force needed to magnetically separate the pairs permits accurate determination of dissociation kinetics.

Danilowicz, C. *et al. Anal. Chem.* **77**, 3023–3028 (2005).

PROTEOMICS

Global topology analysis of the *Escherichia coli* inner membrane proteome

Membrane proteins comprise a large percentage of the proteome but remain notoriously difficult to characterize. Daley *et al.* used two specialized reporter proteins to reveal the localization of the C termini for over 600 *E. coli* inner membrane proteins; with further computational analysis, this data allowed them to confidently determine the topology for each of these proteins.

Daley, D.O. *et al. Science* **308**, 1321–1323 (2005).

GENOMICS

Clustering and conservation patterns of human microRNAs

Altuvia *et al*. performed a detailed clustering analysis of more than 200 known human microRNAs, and found that miRNA genes tend to have significantly greater clustering than would be expected at random. Via sequence analysis, the group was then able to identify and confirm the existence of 18 additional, new human miRNA genes within or near these clusters.

Altuvia, Y. *et al. Nucleic Acids Res.* **33**, 2697–2706 (2005).

RNA INTERFERENCE

Antibody-mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors

A primary obstacle to the therapeutic application of RNAi is in actually getting small inhibitory RNAs (siRNAs) into target cells. Song *et al.* show that by conjugating siRNAs to protamine-fused antibody Fab fragments, one can achieve efficient targeted gene inhibition in HIV-infected or cancer cells.

Song, E. *et al*. *Nat. Biotechnol.*; published online 22 May 2005.