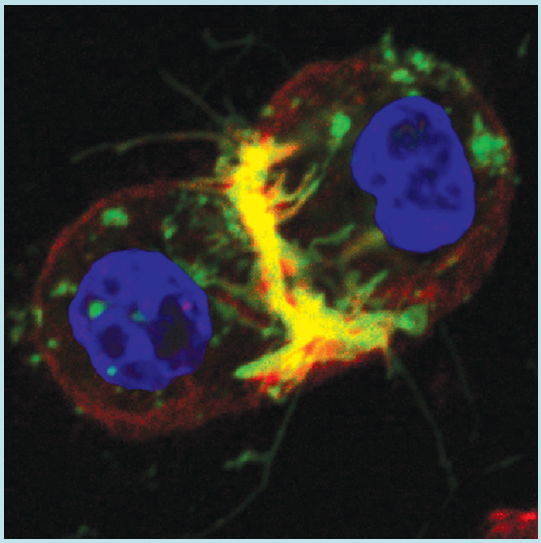
Spotlight

Prying at the Prions



Málaga-Trillo, E., *et al.* (2009) *PLoS Biol.* 2009, *7*, e55, DOI: 10.1371/journal.pbio.1000055.

Prion proteins (PrPs) have long been known to change in conformation and yield the telltale aggregates of such neurodegenerative diseases as Kuru and Creutzfeldt–Jacob diseases in humans or the infamous mad cow disease among our bovine counterparts. After two decades of research and a Nobel prize, the prion protein remains best described as a pathogen, but its normal role in the cell remains poorly understood. Proteins that exhibit PrP-like behavior appear in organisms spanning fungi to humans, so evolutionary selection predicts that these PrPs must do more than cause disease states. Now, a new study sheds light on the biological role of PrP proteins in two different vertebrate organisms.

Málaga-Trillo *et al.* (*PLoS Biol.* 2009, *7*, e55, DOI: 10.1371/journal.pbio.1000055) began their work in the zebrafish, a vertebrate with two distinct PrP genes that appear to have different timing. Knockdown of the early gene, PrP-1, resulted in embryos that failed to undergo gastrulation, while PrP-2 knockdown embryos were competent for gastrulation but showed brain development issues. But what role might a prion protein be playing in development? Turning to the mouse, where PrP is also expressed in the brain, the authors used a cell culture system to show that PrP proteins localize to cell–cell contacts. The proteins seem to mediate homophilic interactions as cells adhere to one another, an observation extended from the mouse to zebrafish and even into Drosophila S2 cells. While these direct interactions appeared independent of calcium ions, a secondary pathway influencing calcium-dependent interactions was also uncovered. The PrP proteins at the cell surface act in a signal transduction pathway as well to promote the processing and trafficking of E-cadherin, an important calcium-dependent cell adhesion molecule. An additional signaling role was shown to span the model systems as well. Members of the Src-family of kinases, which act as key regulators of calcium levels and the actin cytoskeletal dynamics, were activated by Prp-mediated signaling. Thus, PrP proteins in both mice and fish seem to have roles in cell–cell contacts and calcium signaling, but what might this mean for prion biology in disease? These model systems point the prion community in a new direction, pursuing how cellular crosstalk mechanisms function in the mammalian nervous system during PrP's dangerous aggregation. **Jason G. Underwood, Ph.D.**

NMR Comes Alive!

Nuclear magnetic resonance (NMR) has made invaluable contributions to the investigation of protein structure and function. Generally, the proteins used for NMR studies have either been plucked from their natural environment or recreated in order to effectively perform the experiments. Recent advances, however, have led to the development of in-cell NMR, an exciting extension of the technology that has enabled examination of the dynamics, structural characteristics, and ligand binding of proteins in an intracellular environment. Though in-cell NMR has been accomplished in both prokaryotic and a limited set of eukaryotic cells, application to complex structure determination and in a wide variety of cell types still faces various challenges. Two new reports (Inomata *et al.* (*Nature* 2009, *458*, 106–109) and Sakakibara *et al.* (*Nature* 2009, *458*, 102–106)) now describe advances in in-cell NMR, enabling generation of high-resolution two-dimensional (2D) NMR spectra of proteins inside live human cells and three-dimensional (3D) structure determination of proteins expressed in bacteria.

In the report by Shirakawa and co-workers, use of in-cell NMR in eurkayotic cells is expanded from the very limited application in *Xenopus laevis* oocytes to human cells. In-cell NMR requires delivery of isotope-labeled proteins into cells, which has been difficult to achieve efficiently in human cells. Conjugation of proteins of interest to a cell-penetrating peptide (CPP) derived from the HIV-1 protein Tat, followed by isotopic labeling, enabled direct translocation of the CPP-linked proteins into the cytosol of human cells. The con-

Published online May 15, 2009 • 10.1021/cb900102d CCC: $40.75

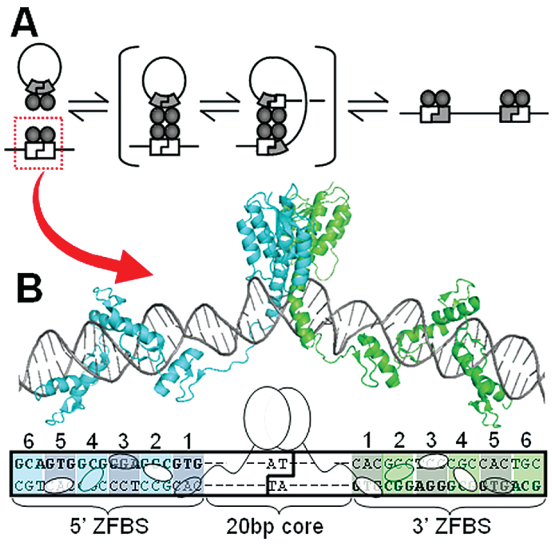
jugations were designed such that the CPP was cleaved on entering the cytosol, leaving the protein of interest to interact with its intracellular surroundings. Cytosolic delivery of three distinct proteins demonstrated the utility of this method. First, a ubiquitin derivative-CPP conjugate was delivered to a human epithelial cell line. Analysis of the 2D spectrum revealed well-resolved cross-peaks, suggesting the presence of a stably folded and homogeneously dispersed protein. Notably, cleavage of the CPP, presumably by deubiquitinating enzymes, was found to be essential for observing such well-resolved spectra. Next, the B1 domain of streptococcal protein G, conjugated to CPP via disulfide bonds, was delivered to cells. The CPP was likely removed under the reducing environment of the cytosol, and well-resolved signals that matched signals obtained from the purified protein were observed. Finally, the in-cell NMR spectrum of the protein FKBP12 was measured, alone and in the presence of its small molecule ligands, the immunosuppressive drugs FK506 and rapamycin. Examination and comparison of the spectra showed good correlation with the protein *in vitro*, as well as clear indication that specific protein−ligand complexes had formed.

Taking in-cell NMR to another dimension, Ito and co-workers describe the elucidation of the 3D structure of a protein overexpressed in bacterial cells. Despite the progress in in-cell NMR technology, low sensitivity and short sample lifetime have precluded determination of three-dimensional protein structures thus far. Ito and co-workers overcame these challenges using a few clever time-savings tricks during acquisition of the spectra, including applying a nonlinear sampling scheme for indirectly acquired dimensions. TTHA1718, a putative heavy-metal binding protein from the bacterium *Thermus thermophilus*, was overexpressed in *Escherichia coli*, and acquisition of 3D NMR spectra revealed a high quality structure similar to that obtained *in vitro*. The success of this approach was also greatly facilitated by selective protonation at methyl groups of alanine, leucine, and valine, which enabled identification of long-range interactions within the structure.

The increased versatility and applicability of in-cell NMR demonstrated in these reports brings to light an exciting and powerful new perspective for the exploration of protein structure and function in a cellular environment. **Eva J.Gordon, Ph.D.**

Rewriting the Genomic Script

One of the many exciting consequences of sequencing the human genome is the opportunity to rewrite parts of the three billion-base-pair sequence and, in turn, to alter genes, gene expression, and epigenetic state. Current methods that attempt to modify targeted sites within the genome suffer from low specificity, which can result in a variety of adverse consequences including sequence deletion and cytotoxicity. Exploiting the targeting specificity of serine recombinases and the sequence specificity of zinc-finger DNA-binding proteins, Gordley *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2009, *106*, 5053−5058) now present a novel strategy for specifically and efficiently targeting a single site within the genome.



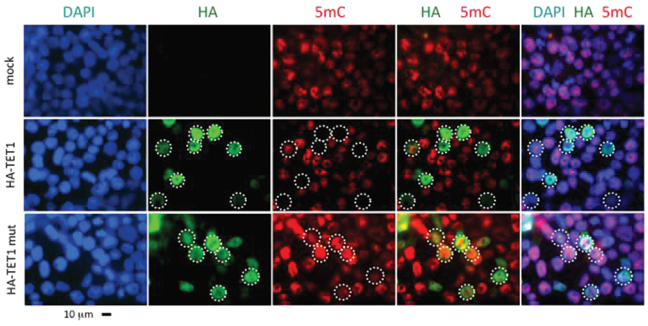
Gordley, R. M., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *106*, 5053−5058. Copyright 2009 National Academy of Sciences, U.S.A.

Serine resolvases and invertases recombine target sites within the same DNA molecule with exquisite selectivity, and development of hyperactive mutants and bioengineering efforts have expanded their versatility. However, DNA-binding domain (DBD) of these proteins is not well suited for genomic recombination, as its recognition motifs are short and degenerate. Zinc finger DNA-binding proteins, on the other hand, specifically recognize target DNA sites of variable lengths. By replacing the DBD of the hyperactive serine invertase Gin with the zinc finger protein C5, a novel "zinc finger-recombinase" (RecZF), referred to as GinC5, was created. GinC5 was capable of catalyzing targeted integration of transgenes into the human genome with high accuracy. A similar strategy was used to create a second RecZF, this time using a hyperactive mutant catalytic domain from the serine resolvase Tn3 with the C5 zinc finger. As with GinC5, Tn3C5 mediated transgene integration with high specificity. The clever combination of specific functions employed by this strategy, coupled with directed evolution technology, opens the door to the creation of a vast collection of novel enzymes with which to rewrite the genome to our own specifications. **Eva J. Gordon, Ph.D.**

The Mysterious Case of the New Base

Though the genetic code is made up of just four nucleotides, enzymatic activities in the cell extend the code through covalent modifications of the nucleotides. In the RNA realm, nearly 100 modifications on tRNAs and other noncoding transcripts have been uncovered, and many play important biological roles in all king-

doms of life. In DNA, the number appears smaller, but the role of cytosine methylation has emerged as a critical epigenetic regulator and defense mechanism against parasitic DNA insertions. A new pair of studies extends the possibilities one step further by uncovering another naturally occurring DNA modification present in mammalian cells. The base, 5-hydroxymethylcytosine (hmC), is a further enzymatic modification of methyl-C. Both studies came upon this base in different ways, but the unified conclusion shows that some, but not all, mammalian cell types have hmC present within their genomic DNA. While its biological role remains speculative, the implications are grand for those interested in epigenetic regulation. Interestingly, known methyl-C binding proteins cannot recognize this modified base.



From Tahiliani, M., *et al.*, *Science*, 2009, DOI: 10.1126/science.117011. Reprinted with permission from AAAS.

The study by Tahiliani *et al.* (*Science* Published Online April 16, 2009; DOI: 10.1126/science.1170116) began with an unlikely clue, the hydroxylation of thymine observed in the protozoan Trypanosomes. Using the enzyme that performs this modification as bait, the human genome was queried for oxygenase domains of similar sequence and predicted fold. Three putative human genes were identified that contained residues consistent with 2-oxoglutarate and Fe(II)-dependent oxygenase activity. One candidate, TET1, was chosen for further characterization. Upon overexpression in human cells, the enzyme caused a concomitant decrease in methyl-C antibody staining. Upon complete digestion of the DNA and separation of the nucleotides, a new unknown spot appeared. Through mass spectrometry with an authentic standard, the mystery was solved. But is hmC present in normally growing mammalians cells? For this question, the authors turned to several cell lines and found that the answer is not universal. Most compelling, the studies in mouse embryonic stem (ES) cells showed that about one in every 3000 bases is hmC in undifferentiated cells, but this declines as differentiation progresses. Interestingly, the levels of TET1 protein went down during this same transition. Using RNA interference to knockdown TET1 mRNA, this gene was further implicated in hmC generation in ES cells. Finally, the authors discuss known disruptions or fusions of the TET genes in numerous cancers, adding to the likelihood that this new epigenetic mark plays a critical role in gene regulation.

The other group, Kriaucionis and Heintz (*Science* Published Online April 16, 2009; DOI: 10.1126/science.1169786), happened upon hmC from the angle of a completely different discipline, neuroscience. They were investigating methyl-C (mC) in various cell types in the mouse brain since this mark plays a role in gene regulation. Using a new method to isolate nuclei from rare cell types, an extra and quite abundant spot was consistently observed on chromatograms of cerebellar Purkinje cell DNA digests. Using mass spectrometry, they discovered that this extra spot was hmC. In these neurons with large, euchromatic nuclei, the authors show that hmC can be found on average as one in every 150 bases in the genome. But could Purkinje cells be an exception to the rule? After checking numerous cell lines and tissues, it appeared that hmC is not in all mammalian cells and varies substantially in those cells that do have this modification. Cells of the brain showed specific enrichment, with the cerebral cortex and brainstem harboring the most hmC bases.

With the sequences of many genomes now available, many groups are switching their focus to epigenomics, the effect of chromatin and DNA modifications on gene expression. This pair of studies demonstrates that this worthy pursuit is further complicated by yet another modification, and the intersection of TET and hmC with two hot areas of research, stem cell biology and neuroscience, will surely lead to exciting new directions. **Jason G. Underwood, Ph.D.**