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SPOTLIGHT

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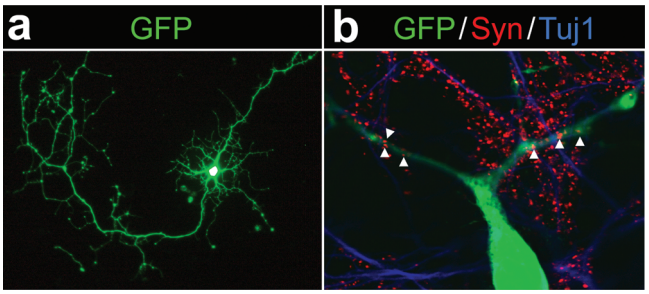
One-Step Conversion from Fibroblast to Neuron

Since the development of reprogramming technology that allows researchers to generate pluripotent cells from mature cells, scientists have attempted to derive specific cell types that serve as laboratory models of disease or could lead to treatments from patient-specific cells. This ability to convert cells back to a pluripotent state has also spurred renewed interest in direct reprogramming, the one-step conversion of one mature cell type into another mature cell type. In a new study, Pang *et al.* (*Nature*, advance online publication 26 May 2011, DOI: 10.1038/nature10202) have directly converted human fibroblasts into induced neuronal (iN) cells.



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The research follows up on prior work by the same laboratory that coaxed mouse fibroblasts to become induced neuronal (iN) cells. That direct reprogramming relies on the use of drug-inducible lentiviral vectors that encode genes for three transcription factors, Brn2, Ascl1, and Myt1l. Addition of the drug doxycycline forces the transcription of these genes and induces some of the fibroblast cells to take on the shape and function of neuronal cells.

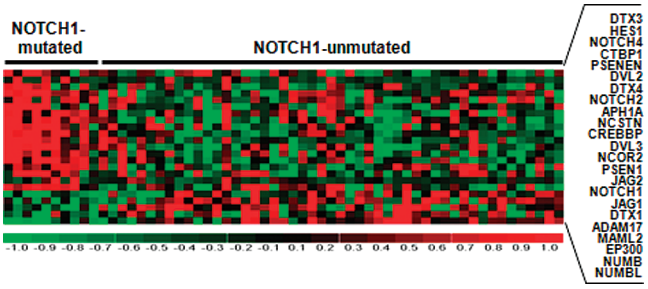
The factors that reprogram cells do not always translate across species, so the researchers first examined whether the conditions that worked in mice could accomplish one portion of the conversion, the differentiation of pluripotent cells, human embryonic stem cells, into neurons. When those cells produced functional iN cells, Pang *et al.* tried the same strategy with fetal fibroblast cells. The resulting cells were shaped like immature neurons but did not carry out neuronal functions such as generating action potentials.

Pang *et al.* then screened among 20 additional transcription factors for another ingredient for the transdifferentiation process. The combination of NeuroD1 and the three original transcription factors produced the greatest number of mature neuronal cells. The researchers also tested this combination of transcription factors in more mature fibroblasts. The resulting neurons were functional: they produced action potentials, expressed neurotransmitter receptors, formed synapses, and integrated into existing networks of neurons.

Although the researchers note that further research will be needed to optimize the conditions for producing these iN cells, access to these cells presents myriad options for studying the development of neurons and related diseases. **Sarah A. Webb, Ph.D.**

Sequencing Leukemia

Approximately one-third of all leukemia cases are chronic lymphocytic leukemia (CLL), which in 2010 was diagnosed in 15,000 individuals and caused over 4000 deaths. The heterogeneity of CLL at the molecular level underscores the need for detailed genomic characterization of the disease in order to develop more effective treatments. To this end, Puente *et al.* (*Nature*, advance online publication 5 June 2011, DOI: 10.1038/nature10113) perform whole-genome sequencing on four individual cases of CLL and explore the relevance of nearly 50 key mutations in over 300 clinical CLL cases.



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The four cases subjected to whole-genome sequencing were representative of two forms of the disease: a subtype that exhibits mutations in the immunoglobulin genes, and a distinct subtype that does not. Analysis of the whole-genome sequencing data revealed the presence of approximately 1000 somatic mutations in each tumor, 46 of which caused changes in protein-coding sequences. When these 46 mutations were analyzed in tumor samples from numerous CLL patients, four genes were identified as having at least one additional mutation: *NOTCH1*, *MYD88*, *XPO1*, and *KLHL6*. The mutations associated with *NOTCH1*, which has been implicated in promoting the survival of CLL cells, and *MYD88*, which regulates cell signaling pathways during the immune response, suggest that the resulting proteins are hyperactive. Furthermore, clinical observations indicate that CLL subtypes with these mutations manifest as more aggressive forms of the disease. Analysis of the mutations associated with *XPO1*, which encodes a protein involved in nuclear export of proteins and mRNAs, also suggests that the activity of the encoded protein is affected. Finally, *KLHL6*, which is involved in the B cell maturation process, appears to be a target of somatic hypermutation, a process implicated in the development of other lymphomas. The identification of these recurrently mutated genes in CLL offers key insights into the mechanisms underlying the progression of the disease and points to exciting new targets for therapeutic intervention. **Eva J. Gordon, Ph.D.**

Minimizing MicroRNA Immunostimulation

MicroRNAs (miRNAs) are short, single-stranded RNA molecules that posttranscriptionally regulate gene expression by

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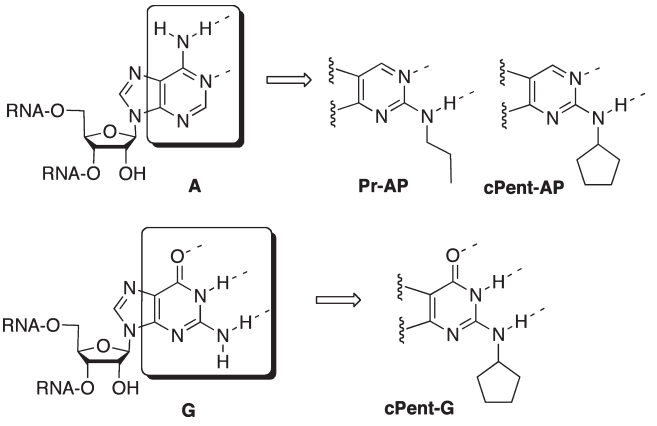
binding to mRNAs. Several of these ~22 nucleotide RNAs are thought to have promising therapeutic properties, including microRNA-122 (miR-122) whose expression is substantially downregulated in liver cancer cells. One ongoing challenge with miRNA therapies is their tendency to trigger the immune response, resulting in undesired and damaging side effects. Toward development of miRNAs that lack such immune activation properties, Peacock *et al.* (*J. Am. Chem. Soc.* published online May 25, 2011; DOI: 10.1021/ja202492e) now present the synthesis and biological activity of miR-122 duplex analogues that incorporate novel nucleobases.



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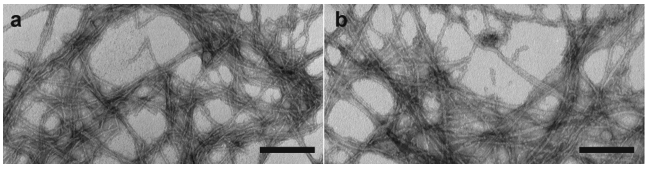
Building on previous studies demonstrating that modification of the ribose 2′-position of miRNAs inhibits their immunostimulatory properties while major groove modifications do not, the authors explore base modifications that affect the minor groove. Specifically, based on the hypothesis that base recognition within GU-rich sequence motifs contributes to immune stimulation, adenosine and guanosine analogs containing cyclopentyl and propyl minor-groove projections were synthesized and used to replace the native nucleosides at various positions along the duplex. Several of the analogs effectively knocked down miR-122 target gene expression and exhibited significantly reduced immune stimulatory activity, as measured by the expression of TNFα in white blood cells. Notably, the most effective miRNA in the series in terms of knockdown ability with little immune stimulation was a duplex containing one modified nucleoside on each strand. Intriguingly, analysis of the positions most effective at quashing immune activity suggested that the GU-rich motifs do not define the immunostimulatory regions; rather, certain single positions may act as immunostimulatory hotspots. This study illuminates an appealing new strategy for optimizing the therapeutic potential of miRNAs, which notably can be extended to other approaches within the field of RNA interference (RNAi) including small interfering RNAs (siRNAs). **Eva J. Gordon, Ph.D.**

Proteins Recycle, Too

Alzheimer's disease, the neurodegenerative disorder characterized by the decline of numerous cognitive abilities, most notably memory, affects approximately 18 million people worldwide. The cause of this devastating disease is not fully understood but is undeniably linked to the aberrant aggregation of the amyloid-β (Aβ) protein, which results in the deposition of amyloid fibrils in the brain. Recent evidence suggests that it is not the fibrils themselves but Aβ oligomers that are the toxic species, though the fibrils may serve as a reservoir for oligomer formation. Sánchez *et al.* (*J. Am. Chem. Soc.* 2011, *133*, 6505−6508) now delve into the kinetics of interconversion between different species present during Aβ aggregation.



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The authors use a variety of methods to investigate the dynamic behavior of Aβ amyloid fibrils including electron microscopy, hydrogen−deuterium exchange experiments, and molecular dynamics simulations. The authors study fibrils of Aβ40 and Aβ42, variants of 40 and 42 residues long. Monitoring the hydrogen/deuterium exchange in the fibrils revealed that Aβ molecules continuously dissociate and reassociate. Moreover, this molecular recycling process was found to occur to a much greater extent in Aβ40 than in Aβ42, which is significant because though Aβ40 is the more abundant species, Aβ42 is thought to be more toxic. With the help of molecular simulations, the authors show that the rate constant for dissociation of molecules from the fibril is greater for Aβ40 than for Aβ42, offering an explanation for the differing kinetic properties of the two species. These intriguing characterizations of Aβ have implications for delineating the cause of AD and for the design of new therapeutics targeting Aβ formation and toxicity. **Eva J. Gordon, Ph.D.**

Improving Human Growth Hormone Therapy

The introduction of non-natural amino acids into proteins is a new and efficient way of engineering proteins with new functionalities. Ribosomal incorporation of these amino acids at site-specific locations on peptide chains is accomplished using an amber nonsense codon, TAG, and an orthogonal aminoacyl-tRNA synthetase/amber suppressor tRNA pair. Now, Cho *et al.* (*Proc. Natl. Acad. Sci.* 2011, *108*, 9060−9065) use this technology to engineer and optimize human growth hormone (hGH), a therapeutic protein used to treat growth abnormalities.

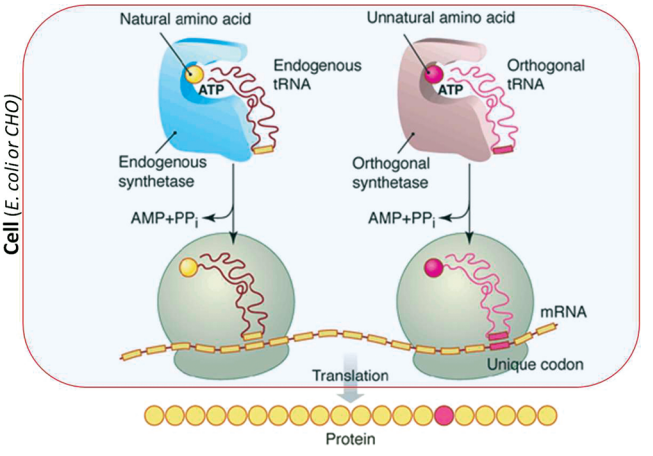
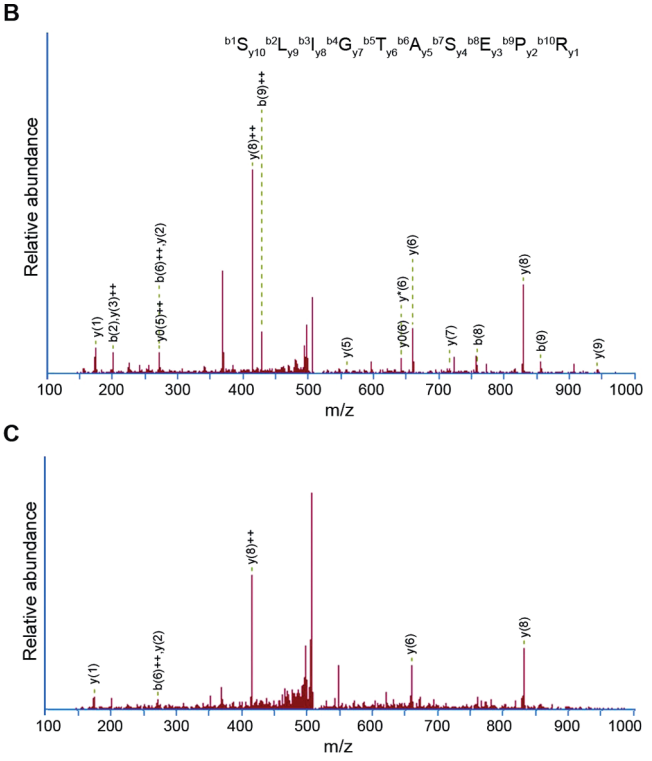


Image Courtesy of Ambrx, Inc.

The authors synthesized homogeneous variants of hGH by incorporating *p*-acetylphenylalanine (pAcF) at locations that minimally perturbed the conformation of the protein. The *p*-acetyl group of this unnatural amino acid allowed conjugation with poly(ethylene glycol) *via* oxime bond formation to enhance the molecular size and to reduce unwanted renal clearance. Using prior structural data, 19 amino acid positions that were adequately far removed from the hGH receptor-binding interface were selected for substitution with pAcF. The hGH variants were recombinantly expressed and isolated from *E. coli* and were conjugated with PEG. One of these PEGylated hGH variants showed improved rodent pharmacological properties by way of a dose-dependent increase in tibia bone length and body weight as compared to placebo control in growth hormone-deficient rats. Subsequent human clinical studies showed that this variant could be safely administered to growth-hormone deficient adults with increased potency and reduced injection frequency. Thus, this report demonstrates the power of using non-natural amino acids to synthesize better protein therapeutics. **Jitesh A. Soares, Ph.D.**

The Transcriptome Gets a Rewrite?

In eukaryotes, the transcriptome is far more than just a simple RNA copy of the genome. Alternative promoters, splicing, polyadenylation, and RNA editing each add their own flare to the genomic information, often yielding RNA transcripts that are tuned for a particular tissue or developmental stage. In recent years, high-throughput sequencing has helped scientists unlock more genomic information than in the previous decades combined. Last year, the landmark 1000 Genomes project mapped 1000 new human genomes to look for variations among modern humans. Now, a new study (*Science*, advance online publication 19 May 2011, DOI: 10.1126/science.1207018) made use of this data combined with transcriptome data to uncover a curious phenomenon. Li *et al.* found thousands of sites in the transcriptome which were different than the cognate DNA.



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The researchers began with immortalized B-cell lines from 27 different individuals sequenced by the HapMap and 1000 Genomes Projects. They then compared the mRNA data obtained for these cell lines to detect many RNA to DNA differences (RDD). As an additional filter, each RDD site had to happen in at least 2 individuals and the genotype reconfirmed by Sanger sequencing the loci of interest. Interestingly, all 12 types of nucleotide changes were observed and the changes were not evenly distributed across the genome. RDD sites were significantly enriched in genes with helicase activity or protein and nucleotide binding. In many cases, the DNA-encoded version of an mRNA was the prevalent form while the minor form was the RDD mRNA, with an average RDD level of 20%. In other cases, the RDD form of the transcript was the major form with nearly 100% of the RNAs possessing the difference. To further bolster the evidence, mass spectrometry was used to see which form was translated into cellular proteins. Many of the recovered peptides showed that both the genomic copy and the RDD form were translated into proteins. Next, the burden of proof will surely lie in the mechanism since many of the RDD sites are not easily explained by the biochemistry of canonical transcription or post-transcriptional processing. **Jason G. Underwood, Ph.D.**