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Spotlight

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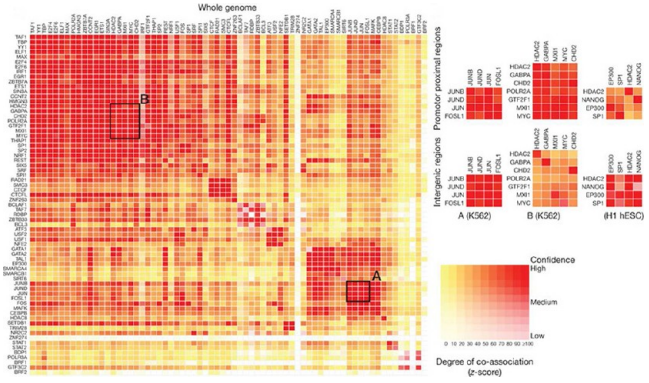
CODING, NON-CODING, ENCODEING

Five years ago, a large consortium announced the initial results of a bold project dubbed ENCODE, or Encyclopedia of DNA Elements. At the time, this impressive collection of data peered into 1% of the human genome to identify coding and noncoding RNAs, functional DNA elements and methylation states, transcription factor binding sites, and histone marks. This giant data set became publicly available and the nearly boundless opportunity for cross-comparison and correlation spawned hundreds of research projects. The years that followed the pilot study saw rapidly decreasing sequencing costs and many new discoveries that proved that the human genome was still ripe with secrets beyond the nucleotide code. Recently, the ENCODE team announced the completion of the project with this rich data set of the entire human genome now available on the web and published as a collection in a special issue (*Nature* 2012, *489*, 57−74).



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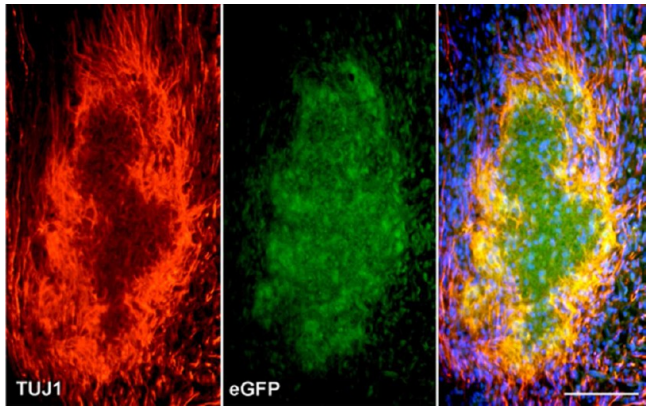
Though the notion that the human genome is full of "junk DNA" was debunked years earlier, the complete look offers finer classification of the vast regions that do not encode proteins. Drawing from parallel data sets on 147 normal and tumor cell types, the study estimates that 80% of the genome can be assigned a biochemical function in at least one cell type. A biochemical function was defined as an RNA or chromatin-associated event in that portion of the genome. In addition, much of the remaining DNA lies close to a regulatory event with 99% of the genome being within 1,700 nucleotides of a biochemical event characterized and measured by ENCODE. Interestingly, most of the SNPs, or single nucleotide polymorphisms, that were previously associated with a disease lie in or near ENCODE-defined functional noncoding elements and not in protein coding sequence. The cross-comparison between chromatin states, transcription factor binding and RNA production shows a strong correlation in most cases. This new encyclopedia stands as a gold standard data resource that will both assist basic scientists to better assign how the cell's rules are written and medical scientists to better understand what goes wrong in a cancer cell. As generating these type of data becomes even less costly, new cell types or cancer lines will be mined for their secrets and, just as with the first complete human genome sequence, the ENCODE data will be the critical resource against which all others are compared. **Jason G. Underwood, Ph.D.**

TURNING UP THE VOLUME OF STEM CELL THERAPY

One cause of deafness is a condition called auditory neuropathy, in which damage to special nerve cells in the inner ear called spiral ganglion neurons prevents proper transmission of sound information to the brain. Cochlear implants, which can treat deafness caused by loss of sensory hair cells, are not an appropriate therapy for this type of deafness, so other treatment options are needed. To this end, Chen *et al.* (*Nature*, advance online publication September 12, 2012; DOI: 10.1038/nature11415) report a method for generating auditory neurons from human embryonic stem cells (HESCs), carving a path for stem cell therapy of this perplexing condition.



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Building on studies of the signaling pathways involved in auditory function in mice, the authors hypothesized that exposure of HESCs to fibroblast growth factors (FGFs) 3 and 10 could coax the cells to become otic progenitors, or cells capable of differentiating into sensory hair cells and auditory neurons. Indeed, gene expression and protein immunostaining analyses indicated that when HESCs were treated with FGF 3 and 10, they differentiated into two populations of otic progenitors, referred to as otic epithelial progenitors (OEPs) and otic neural progenitors (ONPs). When the progenitor cells were subjected to special culture conditions, the OEPs produced hair-cell-like cells, and the ONPs became neurons. To investigate the ability of these cells to restore hearing, ONPs were transplanted directly into the damaged site of a rodent model of auditory neuropathy. This treatment resulted in the cells implanting in the organ, differentiating to a more mature and functional auditory neuron, and improving auditory function in the hearing impaired animals. In addition to its therapeutic potential for auditory neuropathies, this exciting approach could also be developed for use in combination with

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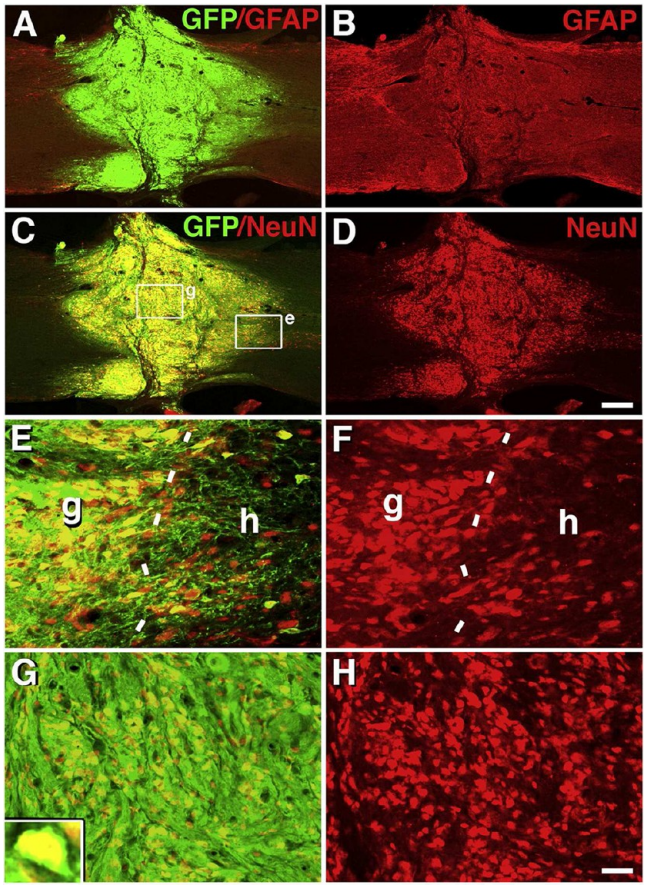
cochlear implants for treatment of a range of hearing disorders. **Eva J. Gordon, Ph.D.**

■ ILLUMINATING AXONAL REGENERATION

A significant challenge in the treatment of spinal cord injuries is overcoming the failure of axons, the thread-like projections of neurons that transmit information through the nervous system, to regenerate after they are damaged. Stem cell therapy holds much promise for treating spinal cord injuries, but the extent to which neurons are intrinsically capable of circumventing the inherently inhibitory environment of the adult central nervous system is not well understood. Lu *et al.* (*Cell* 2012, *150*, 1264–1273) now report that a variety of neural progenitor cells can, when transplanted to sites of injury in rat spinal cords, thrive, extend axonal projections over remarkably long distances, and restore function.



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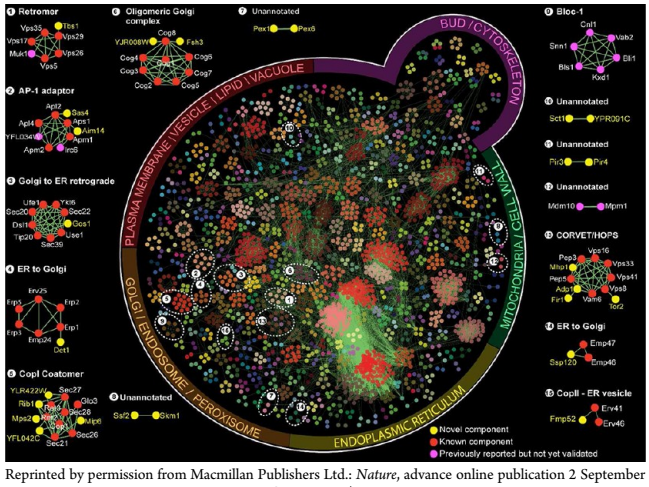
To monitor the behavior of transplanted neurons, the authors used neural stem cells from either rat embryos or one of two lines of cultured human stem cells, each of which was cleverly engineered to express green fluorescent protein. Two weeks after spinal cord injury in rats, the GFP-expressing stem cells were grafted to the injured sites. Notably, the cells were first embedded into fibrin matrices containing growth factor cocktails, which both enhanced the survival of the cells and promoted adequate filling of the injured site. Seven weeks later, the transplanted cells had differentiated into neurons and other types of spinal cord cells, extended large numbers of axons impressively long distances into various regions of the spinal cord, and expressed numerous protein markers characteristic of mature neurons. In addition, rats receiving the neural stem cell implants regained movement in their hindlimbs beginning at 3 weeks after treatment, in contrast to untreated rats who experienced minimal or no movement. The insight into axonal regeneration provided by this study is an exciting contribution toward the development of stem cell therapies for spinal cord injuries. **Eva J. Gordon, Ph.D.**

■ MAPPING MEMBRANE PROTEIN COMPLEXES

Membrane proteins have earned their reputation for being challenging to study in large part due to the hydrophobic nature of their transmembrane domain, which makes them difficult to purify. Consequently, the creation of interaction databases delineating membrane protein complexes has lagged considerably behind those for their soluble, easily purified counterparts. Toward gaining a better understanding of these vitally important membrane protein interactions, Babu *et al.* (*Nature*, advance online publication September 2, 2012; DOI:10.1038/nature11354) develop an improved method for purifying membrane protein complexes which, with the help of mass spectrometry, they exploit to create a physical interaction map of 1726 membrane protein interactions encompassing 905 membrane proteins in yeast. Combining this data with similar data for soluble yeast proteins yielded an overall network of 13,343 associations among 2875 yeast proteins.



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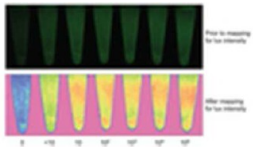
Key to the success of their strategy was the use of a tandem affinity purification method incorporating one of three mild, nondenaturing detergents to isolate tagged membrane proteins. Using this technique, 1590 out of 2141 known membrane proteins were processed, and of these, 1228 were successfully purified. The utility of this interaction network was exemplified by the identification of binding partners for 20 plasma membrane proteins with previously unreported interactions, 8 of which are orthologs of human therapeutic targets. The authors also used high-content fluorescence screening of mutant strains to investigate whether manipulation of the individual components of a complex resulted in similar phenotypes, and 20 of 26 tested did. Moreover, they discovered 321 functionally uncharacterized proteins associated with membrane proteins, providing clues to their function. Finally, by exploring the broader evolutionary conservation of membrane protein complexes, they found that one-third were present among 90% of eukaryotic genomes, and only 10% were restricted to fungi. The interaction map created in this study

provides incredibly insightful details into the membrane biology of yeast and other eukaryotes. **Eva J. Gordon, Ph.D.**

A SNAPSHOT OF TUBERCULOSIS

Second only to HIV as the most deadly infectious agent in the world, *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis, kills over 1 million people each year. Transmission of this highly contagious bacteria can be reduced greatly if detected early, but current methods are limited by high cost, long turn-around time, or low sensitivity. Thus development of inexpensive, accurate, and sensitive new tests that can detect *Mtb* is imperative, especially in developing countries lacking more technologically advanced diagnostic methods. An attractive target for detection of the bacteria is an enzyme secreted by *Mtb* called BlaC, which is a member of the *β*-lactamase family. Now, Xie *et al.* (*Nat. Chem.* 2012, *4*, 802−809) report the creation of several fluorescent BlaC substrates that could serve as rapid, low-cost, and highly sensitive *Mtb* detection agents.



Examination of the structure of BlaC guided the design of a series of specific, cephalosporin-derived BlaC substrates that become fluorescent upon reaction with the enzyme. In particular, the authors exploited a flexible loop in the substrate binding site by incorporating a bulky substituent on the lactam moiety of the cephalosporin. Structural, kinetic, and biochemical analysis of the synthetic substrates led to the identification of a compound referred to as CDG-OMe as especially promising for further testing. Indeed, CDG-OMe showed remarkable sensitivity and selectivity for *Mtb* in human sputum. To demonstrate the applicability of this detection strategy as a rapid and low cost test for *Mtb*, a simple device was constructed from a handmade box, an LED light source, excitation and emission filters, and a cell phone. Using this device, as little as 10 colony forming units of the bacteria in 200 *μ*L of human sputum was readily detected and imaged using the camera of the cell phone. These exciting findings support the development of this strategy for the early detection of *Mtb*, especially in developing regions. **Eva J. Gordon, Ph.D.**

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