

## This Month in Genetics

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### Disruption of Golgi Leads to a Skeletal Dysplasia

How does a Golgi-assembly protein with widespread expression lead to what appears to be a tissue-specific phenotype? This question arose during the research of Smits et al., who used a forward genetic approach to find the gene responsible for a neonatal lethal skeletal dysplasia in mice. Loss-of-function mutations in the human homolog of this gene, *TRIP11*, lead to a highly similar phenotype—Achondrogenesis type 1A—which is a neonatal lethal disorder that includes a lack of vertebral-body and skull ossification. The *Trip11* mutation ablates expression of the golgin protein GMAP-210, a protein known to have a role in assembly and maintenance of the Golgi apparatus. What Smits et al. discover is that the Golgi structure is disrupted in some, but not all, cell types in these mutant mice. At a protein level, there is also specificity to GMAP-210 activity; *Trip11* mutations disrupt glycosylation and transport of certain proteins expressed in chondrocytes. The particular sensitivity of chondrocytes to this defect leads to abnormal differentiation of these cells and increased apoptosis. Why are these cells particularly sensitive to this defect? Perhaps because they put more of a demand on the Golgi apparatus for secretion of proteins to the extracellular matrix and perhaps as a result of the specific proteins that GMAP-210 selects as cargo in these cells.

Smits et al. (2010). *N. Engl. J. Med.* 362, 206–216.

### Histone Modifications Regulate Alternative Splicing

Different cell types sometimes splice identical transcripts in different ways, a property that increases the diversity of gene products in the body. We don't have a full understanding of the mechanism by which each cell type recognizes the exons that should be included in an alternatively spliced transcript, although alternative splicing is influenced by the elongation rate of RNA polymerase II and by chromatin remodelers. Luco et al. propose a much more specific alternative-splicing model in which epigenetic modifications are a cue for alternative splicing. They base this model on their finding that local histone modifications influence the recruitment of splice regulator proteins, which themselves determine splicing. Luco et al. used *FGFR2* as a system to study alternative splicing because it includes two mutually exclusive exons that are chosen in a tissue-specific manner. Through comparisons of two cell types that use different *FGFR2* exons, they iden-

tified histone modifications that were enriched in one cell type compared to the other and that correlated with exon selection. For example, H3-K36me3 at *FGFR2* was enriched in cells that repress use of exonIIIb, and inclusion of this exon in spliced transcripts could be modulated by regulation of the expression levels of H3-K36 methyltransferases. Additional experiments by Luco et al. support a model in which the chromatin binding protein MRG15 binds to H3-K36me3, and this in turn recruits the splice regulator PTB to *FGFR2*. The same histone modifications and binding proteins influence alternative splicing at other genes but are not the universal regulators of splicing. However, it seems plausible that the same type of system could be used for regulating splicing at other genes, albeit with different histone modifications recognized by another chromatin binding protein.

Luco et al. (2010). *Scienceexpress*. Published online February 2, 2010. 10.1126/science.1184208.

### Hsp70: Two for the Price of One

How's this for translational research taking an unexpected direction? Kirkegaard et al. were interested in the way in which heat shock protein 70 (Hsp70) protects cells from stress-induced death by blocking lysosome permeabilization. Because this can promote survival of cancer cells, an understanding of this function of Hsp70 has clinical relevance. Although Hsps are classically thought to help proteins fold, some are also known to interact with cellular membranes. Kirkegaard et al. used an osteosarcoma cell line to show that under the acidic conditions of the lysosome, Hsp70 mediates its protective effect via an interaction with the anionic lipid bis(monoacylglycerol) phosphate (BMP). BMP interacts with and stimulates the acid sphingomyelinase (ASM), whose sphingomyelin-hydrolyzing activity is required for the stabilizing effect of Hsp70. Here's where the unexpected translational application of the research comes in. In what cells is ASM activity defective? Those from people with Niemann-Pick disease (NPD). In fact, these cells turn out to be particularly sensitive to stress-induced lysosomal permeabilization, but this effect can be rescued by recombinant Hsp70. More importantly for NPD, addition of Hsp70 normalizes lysosomal volume, which in untreated cells is markedly increased as a result of sphingomyelin storage. Thus, although they started out studying a target for cancer therapies, Kirkegaard et al. uncovered a potential therapeutic

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approach for a lysosomal-storage disorder. That adds up to two potential translational applications for the price of one basic research study.

*Kirkegaard et al. (2010). Nature 463, 549–553. 10.1038/nature08710.*

### Two Hit Models: Not Just for Cancer Anymore

Our ability to find copy-number changes in the human genome has gotten so good over the past few years that defining new microdeletion and microduplication syndromes seems to be pretty clear if you've got a good patient population and you're observant. The work of Girirajan et al. suggests that things might not always be so straightforward. They report a new recurrent microdeletion at chromosome 16p12.1 that is associated with developmental delay but has a variable phenotype that can include congenital heart defects, seizure disorders, growth abnormalities, and psychiatric disorders. Further evaluation of these individuals found that many of them had an additional large CNV at a different genomic location. In fact, the rate of second large hits in probands with the 16p12.1 deletion was six times that in a set of normal control individuals with at least one large CNV. Although the authors believe that the 16p12.1 microdeletion is an independent risk factor for intellectual disability and developmental delay, they propose a two-hit model for the phenotype whereby a second CNV, a mutation in a related gene, or an environmental exposure influences the severity of the phenotype. The second-hit model is not unique to the 16p12.1 microdeletion-associated

phenotypes; the authors believe that it might play a role in the expression of other genomic disorders with variability penetrance or expressivity. Such disorders might include the chromosome 1q21.1 microdeletion syndrome, which is also frequently associated with a second large CNV.

*Girirajan et al. (2010). Nature Genetics. Published February 14, 2010. 10.1038/ng.534.*

### Guilt by Association with FMRP

Loss of the Fragile X Mental Retardation Protein (FMRP) leads to intellectual disability, a key feature of the fragile X syndrome. On a functional level, FMRP interacts with microRNAs (miRNAs) and regulates protein synthesis of specific mRNAs, and on a cellular level, loss of FMRP function causes synaptic abnormalities and alters synaptic plasticity. A gap exists in our ability to connect these observations, but Edbauer et al. figured that the identification of FMRP-associated miRNAs could close it. They identified miR-125b and miR-132 as two FMRP-associated miRNAs that influence dendritic-spine structure. A predicted target of miR-125b regulation is NR2A, the regulatory subunit of NMDA receptors. Indeed, in cultured neurons, miR-125b and FMRP work together to negatively regulate NR2A through its 3' untranslated region, and manipulation of miR-125b levels influences the synaptic morphology and function of NMDAR channels.

*Edbauer et al. (2010). Neuron 65, 373–384. 10.1016/j.neuron.2010.01.005.*

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## This Month in Our Sister Journals

### An Approach to Population Screening for Fragile X Syndrome

Although the population frequency of fragile X syndrome pre- and full mutations is high enough to support the idea of population-based Fragile X screening, technical limitations have hampered the consideration of this idea. The basic problem is this: unless multiple genotyping techniques are used, the size range of the *FMR1* CGG repeat and its tendency to form secondary structure in a PCR reaction can lead to false negative results, particularly in females. Hantash et al. developed a PCR-based screening procedure that requires a single tube. The method is based on triplet-primed PCR with a set of primers that includes a CGG repeat. To reduce the formation of secondary structure by single-stranded stretches of DNA that include this repeat, they use the nucleotide analog 7-deaza-2-deoxyGTP, which reduces the potential hydrogen bonds that can form between the G's and C's and within higher-order structures. The reaction products are screened by capillary electrophoresis to allow qualitative identification of pre-mutation and full mutation carriers from a population. For

a set of more than 1300 samples, the results of this new method were fully concordant with the results from this group's currently used and more labor-intensive platform for Fragile X analysis. This screen is not diagnostic; follow-up will be required to accurately determine the CGG repeat size and methylation status of positive samples. However, compared to other screening methodologies, this method reduces the number of follow-up analyses and has increased sensitivity for full mutations and in mosaic samples. Now that this and other methods are getting around the tricky technical barriers to population screening, we have to face the perhaps even trickier social and ethical issues surrounding Fragile X screening.

*Hantash et al. (2010). Genetics in Medicine. 10.1097/GIM0b013e3181d0d40e.*

### Using both Linkage and Linkage Disequilibrium Information for Genotype Phasing

When you genotype a diploid organism, you get two alleles for each autosomal locus, but the raw data don't tell you which allele is on which chromosome of the

pair. Determining the likely combinations of alleles on a chromosome—in other words, phasing the genotypes—can be accomplished with population-based information or family-based information. Druet and Georges have developed a method for phasing high-density SNP genotypes that uses both types of data, and their approach improves the accuracy of phase reconstruction and the

imputation of missing genotypes. The authors test their approach on real and simulated datasets of dairy cattle. The reconstructed haplotypes can be clustered into related chromosomes that can be used for fine mapping of quantitative trait loci.

*Druet and Georges. (2010). Genetics. Published online February 14, 2010. 10.1534/genetics.109.108431.*