The contribution to human diseases of the genetic mechanism called imprinting has been discovered only during the past decade. Imprinting is not consistent with our previous understanding of how genes are expressed. Prior belief was that each gene in a cell had 2 alleles, one on the maternal and the other on the paternal chromosome. When a gene was turned on, both alleles were transcribed equally and functional protein was produced from both chromosomes.

For the majority of genes, this is exactly what happens. However, when there is genetic imprinting, just 1 of the 2 alleles is expressed, while the other is silenced or "imprinted." Whether the allele is transcribed or not depends on whether it lies on the chromosome derived from the father or that derived from the mother. Angelman and Prader-Willi syndromes are 2 illnesses that exemplify this mechanism. The molecular basis for Prader-Willi was discussed in the last column. The molecular basis for Angelman will serve as the focus for the present column. I will review the clinical symptoms before turning to a discussion of the genetic basis for the disorder.

Individuals with Angelman syndrome (AS) have severe motor and intellectual retardation. They are often hypotonic at birth, develop epilepsy soon thereafter, and rarely develop speech. They have unusual facies characterized by a large mandible and an open-mouthed expression. Additional features include an abnormal gait and puppet-like movements of their limbs. They are often described as "happy" children because of their frequent smiles and laughter. Several additional features include a facility for protruding their tongues, abnormal skin pigmentation, and a characteristic abnormal EEG discharge pattern.

Approximately 10 years ago, high-resolution cyogenetic studies of patients with Angelman and Prader-Willi syndromes were performed. The initial studies suggested that the same chromosomal band was deleted in both disorders (15q11.2). The deletions appeared indistinguishable by cytogenetic or...
molecular genetic methods. This was surprising as the 2 disorders have very distinct symptoms. Closer inspection, however, revealed that the deletions in patients with AS were somewhat larger, were more variable in size, and sometimes included bands 15q12 and part of 15q13.

It was then discovered that the deletions found in individuals with AS are usually derived from the maternal chromosome. This is in contrast to Prader-Willi syndrome, in which the deletions derive from the paternal chromosome. More recently, it has been demonstrated that although the deleted regions are very close to each other, they are in fact clearly separated. This separation of the affected chromosomal segments suggested a mechanism by which the paradox could be resolved. It was proposed that on chromosomes that came from one's father, a segment of the DNA is imprinted whereas a nearby region is not. The reverse is true on the chromosome that came from one's mother, with the first segment of DNA being transcribed while the nearby region is silenced.

This is indeed the case. In AS, the relevant region on chromosome 15q is normally imprinted on the paternal chromosome while it is expressed on the maternally derived chromosome. The normally expressed maternal copy is often deleted, and one might expect the allele on the paternal chromosome to compensate and produce functional protein. However, the paternal copy is unable to do so as it remains imprinted.

In Prader-Willi syndrome, a segment of DNA very near to the Angelman region is imprinted, but in the opposite direction. In this case, a group of genes on chromosome 15q are imprinted on the maternally derived chromosome and are expressed on the paternally derived chromosome. When a deletion occurs on the paternal chromosome, the relevant maternal genes are unable to compensate. The genes for these 2 disorders lie very close to each other, and it turns out that relatively large deletions occur that span both regions. This disorder occurs on whether the deletion lies on the paternal or maternal chromosome.

For the majority of cases with AS, the deletion of the actively transcribed segment on the maternal chromosome 15q12 is responsible for the disorder. However, several other genetic mechanisms have recently been discovered. Very rarely, both copies of a chromosome come from a single parent, a condition known as uniparental disomy. This genetic abnormality, in combination with imprinting, will lead to the disorder. Thus, AS sometimes occurs as a result of uniparental disomy of the father's chromosomes. When paternal disomy occurs, the affected individual still has 2 alleles of all genes. Both derive from the father, however, and remain silenced with no production of functional protein.

A third mechanism that has been discovered in AS is due to a mutation within the "imprinting center." This region on chromosome 15 regulates which segments of DNA are imprinted. It appears that there is one center that imprints both genes within the Angelman region and within the Prader-Willi region. Occasionally, mutations or structural abnormalities occur within this imprinting center. When they are present, the imprinting center is unable to function properly, and 1 of the 2 syndromes will result, depending on whether the maternal or paternal chromosome has been affected.

It was discovered recently that a fourth mechanism, a mutation in a single gene (UBE3A), can also lead to AS. This discovery underscores an important distinction between Angelman and Prader-Willi syndromes. Prader-Willi appears to result from abnormalities in several genes. No family has yet been discovered in which mutations of a single gene lead to the PWS phenotype.

To summarize, in about 70% of the cases, AS occurs because of a deletion occurring on the maternal chromosome at 15q11-q13. In about 2% of the cases, paternal uniparental disomy is the genetic mechanism, while in approximately 3% of cases a mutation in the imprinting center occurs. In the remaining 25% of cases, it appears that the molecular basis is mutations of the single gene, UBE3A.

What is the normal function of the UBE3A protein, and how does its absence lead to disease? Many proteins within cells are long-lived. Examples of such proteins are cytoskeletal protein required for the structural integrity of the cell. Other proteins, however, are short-lived and must be rapidly removed and degraded. Examples of such proteins are transcription factors, receptors, and proteins involved in signal transduction pathways. It is important to have a quick turnover of these proteins, which regulate many basic cellular functions. This is analogous to what happens at the synaptic cleft, where neurotransmitters must be quickly removed to allow for the rapid and repeated signals necessary for sustained synaptic transmissions.

In addition, it is important to remove cellular proteins that are damaged or not folded correctly, as these structural abnormalities may interfere with the normal functioning of biochemical pathways. Cells have evolved an intricate system for the removal of these proteins. Proteins that are to be targeted for degradation are covalently linked to a 76 amino acid protein called ubiquitin. Ubiquitin was originally named because it is expressed in most cells of our bodies as well as in most organisms. It is highly conserved from yeast and Drosophila to humans, and only a few amino acid changes exist between any 2 homologues.

Several steps must occur to ensure the orderly removal of proteins destined for destruction. The concerted actions of 3 enzymes (E1, E2, and E3) are required. In most organisms, there is a single E1, but many different E2 and E3 isoforms. In the first step of the process, the E1 enzyme activates ubiquitin. Activated ubiquitin is passed on to a ubiquitin-conjugating protein (E2). E3 is required for the last step in which ubiquitin is attached to the protein targeted for destruction. In many cases, long chains of ubiquitin are attached to the protein. It is the final step that involves the protein, UBE3A.

The addition of ubiquitin results in the rapid destruction of the targeted protein by a cellular organelle termed the protea-
some. The proteasome recognizes proteins that have stretches of ubiquitin attached and engulfs them. A series of proteases within the proteasome complex proteolytically cleaves the ubiquitinated proteins to single amino acids that are reused by the cell. In the absence of UBE3A, many unwanted proteins cannot be degraded and many cellular functions become compromised. Although there appear to be specific proteins that UBE3A targets, these are still unknown. It is likely that severe disturbances in normal cellular function occur within neurons, and these disturbances are then reflected in abnormal cortical functioning and severe mental retardation.

An added twist to the imprinting story was recently discovered. UBE3A is imprinted only within the brain. This means that throughout much of the rest of the body, both alleles are expressed whether they derive from the mother's or the father's chromosome. This discovery suggests yet another level of control over the expression pattern for various proteins. It is likely that as the molecular bases for other childhood disorders are discovered, more genes will be found to be imprinted within the CNS or perhaps within specific subregions of the brain.

A final point is worth mentioning. One of the genes that lies within the deleted region in affected individuals is a critical component of the receptor that binds to the inhibitory neurotransmitter, γ-aminobutyric acid (GABA). This gene encodes for a subunit of the GABA receptor and was initially thought to be the gene responsible for AS. It is indeed responsible for some of the symptoms. For example, these individuals often have significant EEG abnormalities and clinical epilepsy, and it is now thought that the GABA receptor is responsible for these symptoms. A mouse model that lacks this particular gene was recently developed that has some of the same abnormal discharge patterns seen in subjects with AS. However, the mutation does not explain the majority of symptoms. Moreover, the finding of point mutations in the UBE3A gene alone indicates that this gene is responsible for the core symptoms in AS, while additional symptoms may be caused by the additional mutations of nearby genes. This is another example of a contiguous gene syndrome in which several genes are deleted and cause the observed phenotype in affected individuals.

WEB SITES OF INTEREST
http://shell.idt.net/~julhyman/ASresources/organizations.htm

ADDITIONAL READINGS
DeLorey TM, Handforth A, Anagnostaras SG et al. (1998), Mice lacking the beta3 subunit of the GABA(A) receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. J Neurosci 18:8505–8514

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