# How Parents Make Their Mark on Genes

*The study of gene "imprinting" may help researchers understand why genes inherited from the mother don't always behave the same as those coming from the father.*

IMAGINE LEARNING THAT CERTAIN ATOMS can't be split, or that some varieties of fruit "fall" skyward. Then you can understand the surprise geneticists felt a few years ago when they discovered an axiom-smashing phenomenon called genomic imprinting.

"We all learned genetics the same way—Mendel's genetics," says Judith Hall, a human geneticist at University Hospital in Vancouver, British Columbia. "We were taught that there's no difference between mother's and father's genes." That was, until imprinting was discovered. It showed that some genes function properly only when they are donated by the father, while others function properly only when they come from the mother—a decidedly non-Mendelian way of behaving.

One implication of imprinting was immediately apparent: Both a mother and a father are necessary to produce normal mammalian offspring. Imprinting, says Philip Leder of Harvard Medical School, "seals us forever in a mode of sexual reproduction. And it justifies the existence of an entire gender—the males."

Other ramifications of imprinting were less obvious, however. In particular, researchers want to know what the chemical nature of the imprint is, and imprinting's role in embryonic development. Until recently, those questions could only be studied with foreign "transgenes" introduced into mouse embryos, which might not reflect what happens during natural imprinting. But in the past 6 months, the first three naturally imprinted genes have been identified, giving researchers a starting point for getting a more accurate picture of what is going on. Already, results with those genes suggest that some of the conclusions drawn from transgene imprinting may have been misleading.

Moreover, the stakes have also gone up during the past 2 or 3 years, as geneticists suspect a link between imprinting and the odd inheritance patterns of some human diseases, including certain embryonic tumors and several hereditary forms of mental retardation—fragile X, Prader-Willi, and Angelman syndromes.

Although the discovery of imprinting dates back to research done in insects in the 1960s by Helen Crouse of Columbia University, interest in the phenomenon really took off in the mid-1980s, when work in several labs showed that it occurs in mammals, too. For example, Bruce Cattanach and Anthony Searle of the Molecular Research Council Radiology Unit in Harwell, England, showed that mouse embryos in which both copies of certain chromosomes came from just one parent died during development.

Added to that, studies done independently by Davor Solter, then at the Wistar Institute in Philadelphia, and Azim Surani at the AFRC Institute in Cambridge, England, demonstrated that paternal and maternal genomes contribute in different ways to developing embryos. If all the genetic material was derived from the mother, the embryos developed almost normally, but the placental tissue was barely present. In contrast, embryos with paternally derived genomes showed the reverse effects.

These results, recalls Surani, could have been explained by a number of mechanisms. Imprinting seemed a long shot in the absence of molecular evidence for its existence. Nevertheless, he and his colleagues attempted what he now calls a "high-risk" experiment. They went looking for some molecular difference between maternal and paternal genes—and that's when they made the key decision of looking for differential methylation patterns in the genes. Methylation could be easily assayed, Surani says, and there was a biochemical reason as well for suspecting its involvement in gene imprinting. "We knew there that the more methylated a gene is the less likely it is to be expressed—so it wasn't a bad choice," says Surani.

As it turns out, it was an excellent choice. To make it easier to follow the methylation patterns, Surani and his colleagues measured the methylation over several generations of a foreign transgene introduced into mice. The result: The methylation pattern differed depending on which parent donated the gene. When it came from the mother it was more highly methylated than when it came from the father. Furthermore, the methylation pattern changed as the gene passed from females to males and vice versa. The highly methylated maternal gene, for example, would become demethylated in the mother's sons, who would then pass the gene along to their offspring with the male pattern. It appears that the female "imprint" was erased and the male "imprint" stamped on the gene in its stead.

Surani wasn't the only one to notice differential methylation in inherited transgenes. Similar observations were made about the same time by Leder's group and by Carmen Sapienza at the Ludwig Institute for Cancer Research in Montreal. Moreover, Leder and his colleagues went a step further, demonstrating that the changing methylation pattern correlated with gene expression in the way expected if methylation does indeed constitute the gene imprint. The highly methylated maternal gene was not expressed, the researchers found, whereas the undermethylated paternal gene was active in heart muscle. When the three groups published their results in 1987, the work "made the thing more real. It demonstrated the existence of imprinting machinery." Since then, Surani says, there's been a massive influx of researchers into the field.

One of the more recent outcomes of that influx is the discovery of the first naturally imprinted mouse genes. Denise Barlow at the Research Institute for Molecular Pathology in Vienna and her colleagues at the Max Planck Institute in Tübingen, Germany, and at Vanderbilt found that the gene for the receptor for insulin-like growth factor-2 was imprinted; Argiris Efstradiadis, Elizabeth Robertson, and their colleagues at Columbia University showed that the gene for insulin-like growth factor 2 is also imprinted; and Shirley Tilghman and her colleagues at Princeton found that the H19 gene, which encodes an RNA of unknown function, is imprinted as well. All three research groups knew they had evidence of imprinting because they found that either the maternal or the paternal gene was expressed, but not both of them.

Researchers hope that studies of these genes will help them clarify the biochemical changes that underlie imprinting. So far, however, the finding has confused rather than clarified the picture. It has challenged the notion that methylation is the important change in imprinting.

For one thing, the transgenes are almost always more heavily methylated and there-

fore less likely to be expressed when they are donated by the mother and not the father. Preliminary studies indicate that is not always the case for endogenous genes. "Maybe the imprinting mechanism for transgenes has nothing to do with the endogenous mechanism," says Tilghman.

And even Sapienza and Surani are beginning to question whether methylation is the primary change that causes imprinting. The Surani group, for example, detects not only parent-of-origin-dependent changes in methylation, but they have also seen alterations in the structure of the chromatin surrounding the transgenes they are studying. In particular, the chromatin may be more tightly wound on chromosomes known to contain imprinted genes. Surani suggests that chromatin structure may dictate the methylation pattern, which in turn can influence gene expression.

According to this notion, a gene might become imprinted if it sits near or in a tightly wound chromatin domain. Both groups are now seeking out proteins that influence chromatin winding and, as a consequence, gene expression. The hope is that the winding proteins are in fact doing the imprinting. This general model, notes Sapienza, is reminiscent of what's known to happen in the fruit fly, where winding proteins have been found to alter gene expression. But is it fair to compare insects and mammals? Yes, says Surani, "the basic mechanism could be the same."

Unfortunately, the newly discovered endogenous imprinted genes are no help at all when it comes to resolving the issue of whether it's methylation or position that's important for imprinting. The insulin-like growth factor and H19 genes lie close together, so if position is the key, then both genes should be imprinted, which they are. But both Tilghman and Efstradiadis point out that the genes are imprinted in different directions. That is, the paternal IGF2 gene is expressed, while for H19, the maternal gene is the one expressed. And that, they note, argues against position as the overriding factor in determining whether these genes become imprinted.

Efstradiadis has yet another proposition to explain what causes imprinting. He suggests that the cues to imprint lie within the gene structure itself, and he is now searching for an "imprinting box," a specific region of the gene that has to become modified, for example, by methylation in order for the gene to be imprinted.

Whatever the mechanism of imprinting, the new work helps support evidence accumulating from other studies—like the earlier work of Cattanach, Solter, and Surani—that suggests that imprinted genes function early in development. All three of the recently discovered endogenous genes are expressed during this time, and deletion of either the maternal gene for the receptor for insulin-like growth factor 2 or the maternal H19 gene causes mouse embryos to die by day 15 of development.

Further evidence that imprinted genes function to regulate growth during development comes from work by Surani and his colleagues in which they implanted cells whose genetic material derives entirely from a male or female into early mouse embryos to see the effects. They found that female-derived genomes retard the growth of the whole embryo by 50%, while male-derived genomes enhance embryonic growth by the same amount. These findings gain further support from Efstradiadis' functional studies of the insulin-like growth factor gene, in which knocking out the paternally derived gene produces offspring that are approximately half of normal size.

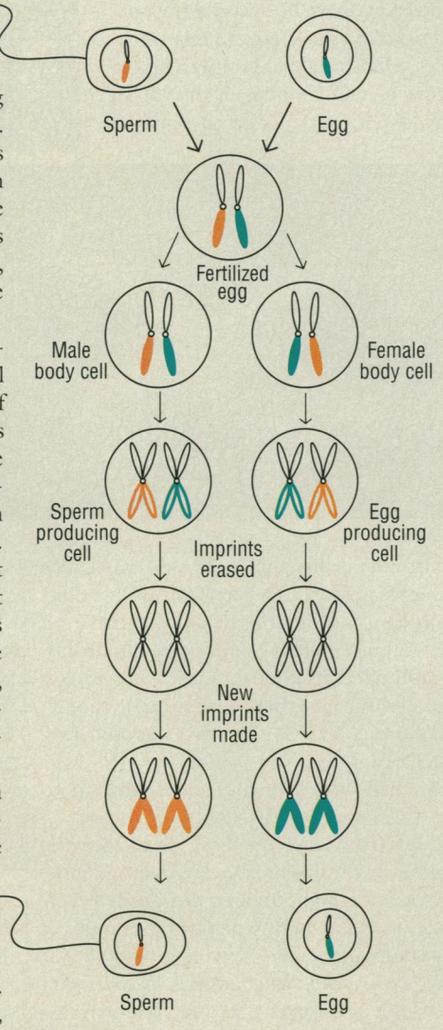
The Surani group has also traced the fate of the implanted cells, and the results suggest that male and female genomes contribute differentially to specific tissues. Paternally derived genomes have a marked effect on skeletal elements, Surani says. For example, developing ribs are enlarged. In contrast, maternally derived genomes contribute to the developing brain but make virtually no contribution to skeletal muscle. Tilghman also believes that imprinted genes may have their primary importance during development but suggests that they also function later in mammalian life. "Maybe the growth correlation is the only one known because it produces such an obvious phenotype. There may be other effects that are more subtle," she says.

An understanding of how imprinting works may shed light not just on normal development but on certain hereditary diseases in which imprinting now appears to play a role. These include fragile X syndrome (also see *Science,* 24 May, p. 1070), as well as certain embryonic tumors, in which loss or inactivation of a gene from one particular parent predisposes offspring to the condition. Especially suspect are diseases whose inheritance patterns have defied explanation by classical genetics—certain forms of diabetes, for example, where the disease is most likely to be manifest in children if they inherit a mutant gene from their fathers. The clearest evidence for possible imprinting in human disease, however, comes from two forms of mental retardation that are distinguishable mainly because their accompanying symptoms are almost the exact opposite of one another.

Patients with Angelman syndrome are hyperactive, while those with Prader-Willi syndrome are slow moving and overweight. Yet both are disorders of human chromosome 15. In Prader-Willi syndrome, two maternal copies of the chromosome are inherited, whereas in Angelman syndrome both copies come from the father.

Peter Goodfellow, a molecular biologist at the Imperial Research Cancer Fund believes the opposing phenotypes of the two conditions present "strong evidence that there might be imprinting involved." But he is cautious about invoking imprinting for several of the other conditions.

"Things go in fashions," he says. "At the moment every unusual genetic phenomenon is being explained by imprinting. Philosophically it is a 'god of the gaps.'" Perhaps, but with so many questions up in the air, what god will help to explain imprinting itself? ■ MICHELLE HOFFMAN




**Erase and switch.** *Imprints change as genes pass from one sex to the other.*