A Parent's Sex May Affect Gene Expression

Researchers are beginning to understand why genes that are inherited from mother may behave differently than the same genes inherited from father

A frog does not necessarily have to have a father. If an unfertilized frog egg is stimulated to divide by pricking it with a needle, it will develop as a normal embryo does. No one has ever been able to achieve a comparable result with mammalian eggs, however, because normal mammalian development requires contributions from both a maternal and paternal genome. Even though the maternal and paternal genes carry equivalent information, they are not, it seems, functionally equivalent. "Mendel's laws were not quite right," says Philip Leder of Harvard Medical School. "Specific genes must be inherited either from the mother or the father."

The question is what biochemical alterations might "imprint" a parent's gene and thereby influence its ability to work in a developing embryo and later in life. Several groups have now obtained evidence that imprinting may be the result of the different patterns of methylation that may be imposed on maternal and paternal genes. They have found that genes inherited from one parent, almost always the mother, may carry more methyl groups than the same genes inherited from the other parent.

Moreover, two of the groups have shown that methylation imprinting is related to gene activity, with the less methylated, paternal form of a gene being expressed while the more highly methylated maternal form is silent. The results provide further evidence for the hypothesis that methylation is important in regulating gene activity, both in development and in general.

The investigators who are studying the influences of parental methylation on gene expression came to the work by different routes. In the case of M. Azim Surani, Sheila Barton, and their colleagues at the Institute of Animal Physiology and Genetics Research in Cambridge, England, the methylation studies are an outgrowth of a series of experiments in which they have shown that both maternal and paternal genomes are necessary for the normal development of mouse embryos.

Over the past several years these researchers have found, for example, that fertilized eggs that were manipulated to have either two female or two male nuclei would not develop to full term. "The embryos went to the mid-gestation stage, but wouldn't go any further," Surani says. "The parental genomes have different functions during development." Davor Solter and his colleagues at the Wistar Institute in Philadelphia have performed similar egg experiments and come to the same conclusion.

In particular, Surani and his colleagues find that the formation of the placenta and the membranes surrounding the embryo depends on the presence of a male genome, whereas the development of the embryo itself requires the female genome. Human embryo development may have similar requirements.

The abnormal pregnancies known as hydatidiform moles are essentially accidents of nature in which a fertilized egg ends up with two paternal nuclei and none of maternal origin. In such pregnancies, which usually end spontaneously after about 2 months, there is excessive growth of extraembryonic tissues, whereas remains of the embryo proper are rarely found.

To explore further the nature of parental imprinting of genes, Surani, Wolf Reik, who is also at Cambridge, and their colleagues turned to "transgenes," foreign genes that are introduced into living mice by injecting them into newly fertilized mouse eggs. Some of the mice that develop from the injected eggs carry the foreign gene in intact form in their genomes and are able to transmit it to their progeny. The fate of the foreign gene can then be easily tracked in the animals' tissues. A transgene that inserts in a region of the genome that is parentally imprinted should undergo the same modifications as the indigenous genes.

Surani and his colleagues produced seven lines of transgenic mice, each of which had the foreign gene integrated at a different chromosomal location, and went on to trace the methylation patterns of the transgene in the various lines. The researchers found that the degree of methylation of the transgene in the mice of one line depended on whether the animals inherited it from the maternal or paternal parent. If the gene came from the mother it was always more methylated than if it came from the father.

Moreover, the methylation pattern would change from generation to generation according to the sex of the animal transmitting it. For example, if a male mouse inherited the transgene from his mother, it would be in the highly methylated form. But in his progeny, both male and female, the gene would be in the low-methylated form that is characteristic of male germline transmission. This mouse's daughters would in turn transmit the gene to their progeny in the high-methylated form. These methylation patterns are exactly what would be expected of a gene that is undergoing parental imprinting.

At least three additional groups of investigators have shown that the methylation state of transgenes depends on the sex of the parent from which they are inherited. The groups are those of Carmen Sapienza of the Ludwig Institute for Cancer Research and Janet Rossant of the Mount Sinai Hospital Research Institute in Toronto; Judith Swain of Duke University Medical Center in Durham with Leder and Timothy Stewart of Harvard Medical School; and Christine Pourcel, Pierre Tiollais, and their colleagues at the Pasteur Institute and the Institut National de la Santé et de la Recherche Médicale in Paris.

Some of these investigators made the discovery more or less serendipitously. Swain, Stewart, and Leder, for example, were studying the expression of a *myc* gene that they had introduced into mice. (*myc* is one of perhaps 50 oncogenes that can cause cells to become cancerous when the genes are appropriately activated.)

The transgene behaved in a puzzling manner; it was expressed in some mice, but not in others. What was happening, the researchers soon learned, was that the gene was active only in animals that had inherited it from their fathers. Swain, Stewart, and Leder then noted that the paternally transmitted genes were less methylated than those transmitted maternally, a pattern like the one observed by the Cambridge group.

The other investigators have also found that paternal genes carry fewer methyl groups than maternal genes, although there is an apparent exception. In one line obtained by Sapienza and his colleagues, the opposite seemed to be true, with a transgenic male transmitting the more highly methylated form of the foreign gene.

This line died out before more thorough studies could be undertaken, however, and for now at least, it appears that transgenes transmitted through the female germline are almost always the ones that end up hyper-

methylated. What this means is unclear. Since both maternal and paternal genes are needed for normal embryonic development, it would seem that paternal genes might sometimes be the ones inactivated by methylation. "A priori, you would think that it [hypermethylation] should also be able to go the other way," Sapienza says, "but nobody finds that."

The Pasteur group picked up another variation. Unlike the other investigators, all of whom found that methylation patterns can switch back and forth between the male and female types from one generation to the next, the French workers found that once their transgene passes through a female it becomes irreversibly hypermethylated and cannot go back to the male pattern.

There are indications that methylation imprinting affects the ultimate ability of the transgenes to be expressed in the mice that inherit them. As already mentioned, Swain, Stewart, and Leder found that their transgenic mice expressed the foreign gene only when it was paternally inherited in the low-methylated form, and not when it was maternally inherited, and the same was true for the mice studied by Pasteur group.

These results agree with the general picture of how methylation influences gene expression. Addition of methyl groups to a gene usually, but not always, results in its inactivation, whereas their removal generally allows a gene to be turned on.

The imprinted transgenes did not always behave in the predicted, orderly fashion, however. Surani and his colleagues did not detect any expression of the transgene they are studying, no matter which parent it was inherited from. And Sapienza observed just the reverse with his transgene, which encodes a muscle protein. It is expressed in all the animals carrying it, even if they acquired it from their mothers and it was originally hypermethylated.

A great deal remains to be learned about methylation and gene imprinting. The methylation state of a gene is clearly only one of the factors that influences its expression. Others include control sequences that allow it to be activated in some tissues, but not others.

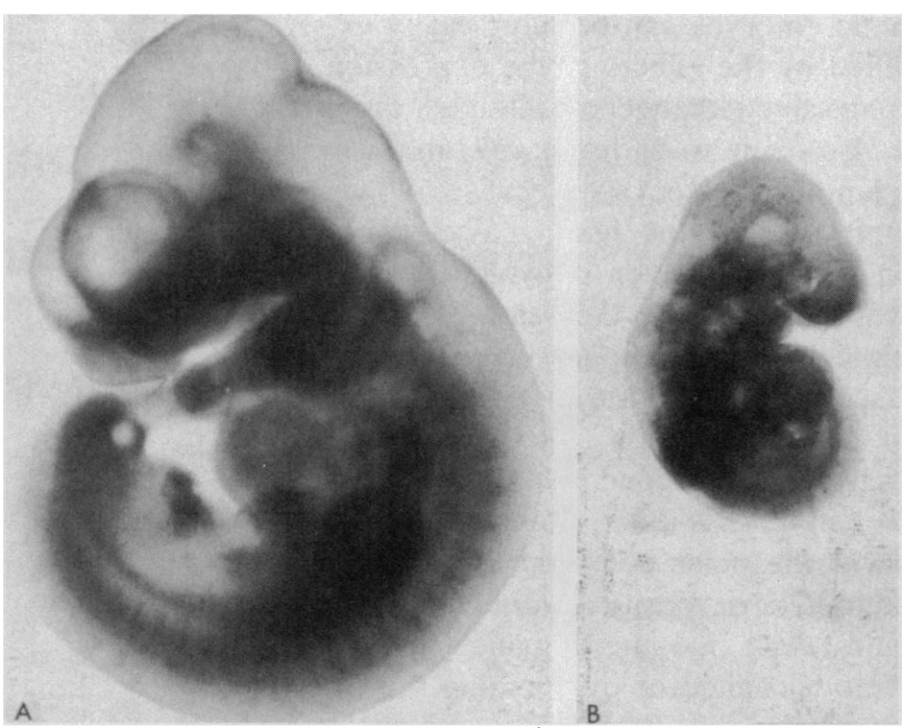
The transgenic mice studied by the Leder group provide an illustration. When the animals inherit the low-methylated paternal form of the gene, they carry it in all their tissues, but express it only in heart muscle. "It's reasonable to assume that there are several layers of regulation, one of which makes a gene eligible for the second level," Leder explains. In this case, a low methylation state apparently made the transgene eligible for expression, but tissue-specific regulatory factors also came into play.

Additionally, there may be control sequences that determine when or where methyl groups are added to or removed from genes. Not all the foreign genes that were introduced into mice in the current work underwent methylation imprinting. Parental imprinting may only affect genes located at certain specific chromosomal sites. This would be consistent with the findings of Bruce Cattanach and M. Kirk of the Medical Research Council's Radiobiology Unit in Harwell, England, who have shown that some chromosomes must be inherited from both parents, but that this requirement does not apply to others.

Another major issue concerns when and where parental imprinting of genes occurs. The most obvious choice would seem to be in the testis and ovary during the formation of sperm and eggs. Investigators have found, for example, that the DNA is relatively undermethylated in the testis, a result that suggests that methyl groups are being removed there. This is consistent with the observations that paternally inherited transgenes may contain fewer methyl groups than maternally inherited transgenes.

However, according to Sapienza, recent data from his laboratory on variations in the methylation patterns of maternally and paternally inherited genes would be best explained if the addition or removal of the methyl groups were occurring very early in development, perhaps only a few cell divisions after fertilization takes place. He notes that this agrees with findings by Rudolf Jaenisch and Philippe Soriano of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, that indicate that the cells of the germline are set aside very early in development.

Nevertheless, Surani points out that methylation may not be the primary imprinting signal. Some other signal may already be present at the time the methylation changes occur to distinguish the paternal and maternal gene variants. The primary signals are as yet unknown, as are the time and site at which they are applied. Although numerous questions remain concerning parental imprinting, the current work at least shows that transgenic mice can provide a system in which the questions may be addressed. ■ JEAN L. MARX



Sheila Barton and M. Azim Surani

Embryo without a paternal genome. *Both mouse embryos developed in the same female and were removed on day 11 of pregnancy. Embryo A is normal, whereas embryo B developed from an egg with two maternal nuclei.*

ADDITIONAL READING

M. Hadchouel, H. Farza, D. Simon, P. Tiollais, C. Pourcel, "Maternal inhibition of hepatitis B surface antigen gene expression in transgenic mice correlates with *de novo* methylation, *Nature (London)* **329**, 454 (1987).

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J. L. Swain, T. A. Stewart, P. Leder, "Parental legacy determines methylation and expression of an autosomal transgene: A molecular mechanism for parental imprinting," *Cell* **50**, 719 (1987).