



IN THE WOMB:

Our bodies respond to our environment // By flicking some genes on, others off // Then, when we reproduce, it seems we don't just pass on genes // But our on-off patterns too.

The New Heredity

■ BY RACHAEL MOELLER GORMAN // ILLUSTRATIONS BY DAVID M. BRINLEY

For hundreds of years, people in the tiny parish of Överkalix, in northern Sweden, have endured bad times and celebrated good ones with little connection to the outside world. To the north and west are Lapps, and to the east, Finns. Though they technically speak Swedish, residents of Överkalix use a dialect that makes them virtually unintelligible to fellow Swedes.

But since the sixteenth century, the people of Överkalix have kept impeccable records of their lives. Clergy logged births, causes of deaths, and land ownership; other historical records noted harvests and crop prices. When epidemiologist Gunnar Kaati arrived 20 years ago, he found an extensive set of meticulous data for this isolated, homogeneous population—a perfect foundation for the large, multigenerational study he hoped to conduct. Kaati wanted to use the data to probe a new idea in clinical medicine—that exposure to certain environments during crucial points in development might determine whether a child would suffer disease years later.

We're familiar with the notion that the environment is linked to disease—that a diet high in saturated fat may clog arteries and cause heart disease or that radiation mutates DNA and can lead to cancer. But in the emerging field of the fetal and developmental origins of adult disease, more subtle factors such as

the amount of food a mother ate during pregnancy or the type of mothering she provided directly after birth may determine whether her child will develop cardiovascular disease or be left neurologically susceptible to overstress years later.

These effects, some researchers believe, have nothing to do with mutations in the DNA code. Rather, they seem to involve what are known as epigenetic changes: structural alterations to the DNA double helix. The notion is that we experience periods in development when our bodies are programmed to collect information about our environment, then readjust our growth depending on what we find. To make this readjustment, our bodies flick genes on or off, sending us on an irreversible trajectory. For example, if a mother doesn't eat much during pregnancy, that may signal to her fetus that he is about to emerge into a food-poor environment, and he may be born smaller, with a slower metabolism, than if his mother had eaten heartily. Epigenetic changes can lead to, say, type 2 diabetes years later if the world the adult finds—such as a world full of food—is different from that forecast by the fetus.

Kaati took this idea a step further. He wanted to know not just whether a child's own early environment caused common diseases later in life but whether the environment a child's parents or even grandparents encountered had an impact. Animal studies suggest that such effects may persist in DNA for

generations, and Kaati's work, still at an early stage, hints that the same thing may happen in humans. Genes might "remember" what our ancestors ate, felt and experienced, altering our own lives generations later.

For many students of biology and evolution, such ideas immediately bring to mind Jean-Baptiste Lamarck, who theorized that traits acquired by an organism during its life can be passed on to its offspring. The classic example is the giraffe that stretches its neck to reach a tree's top leaves and then gives birth to longer-necked young. Lamarck died 30 years before the 1859 publication of Charles Darwin's *Origin of Species*, which detailed evolution as we now know it—a process by which chance differences (later recognized as mutations) improved an individual's chance of survival and thus ensured the propagation of those traits. Each man proposed a similar result, but by very different mechanisms; in Lamarck's

Lamarck theorized that a giraffe that stretched its neck to reach a tree's top leaves would give birth to longer-necked young. He may have been onto something.

view, alterations in a species were more immediately driven by environmental change, whereas Darwin saw a longer process of passive natural selection. The subsequent discovery of genes—the primary unit of natural selection—added credence to Darwin's theory, and Lamarck's was shelved, seemingly laughable compared with what had been learned about the body's sophisticated mode of transferring traits.

Yet advances in epigenetic research suggest that Lamarck may have been onto something. As with the giraffe's tall tree, environmental factors such as lack of food or inattentive mothering appear to alter our epigenomes and sometimes even those of our offspring. (Some researchers think epigenetic changes have helped speed evolution, causing more rapid alterations than could be explained by mutations alone.)

Though Lamarck's work may have prefigured modern

epigenetics, the term itself wasn't coined until 1942, by a developmental biologist named Conrad Waddington. In Waddington's view, epigenetics was what we now call developmental biology—the study of how, during development, our genes give rise to our phenotype, the way we look and behave. By the 1990s a new definition had emerged, and today we consider epigenetics the study of changes in gene expression attributable not to alterations in DNA sequence (mutations that change the protein made by a gene) but in DNA structure (alterations to the scaffolding that carries the code, which can turn entire genes off so they make no protein at all).

Though there are several ways these structural changes can happen, the best known—and the focus of most epigenetic research—is DNA methylation, which occurs when a small chemical compound called a methyl group attaches to a cytosine, one of the four nucleotides in the DNA code. Methylation turns off nearby genes in two ways: by blocking transcription factors from attaching to the gene (and thus keeping those factors from translating the gene's code into a protein) and by altering the configuration of the DNA itself to make the gene less physically available for transcription. (In addition, some recent studies have suggested that methylation may sometimes alter the configuration to turn genes on.) When a cell divides and copies its DNA, it also copies the methyl group, so the same genes remain shut down in the replicated cells.

As an organism develops from a single cell into its final form,

epigenetic mechanisms help cells become distinct tissues. So while every cell contains the same DNA code, each type of tissue—hair, heart, brain—differentiates itself through a unique combination of gene expressions. Epigenetic mechanisms turn off the genes that aren't needed for a particular tissue type and help determine which proteins are expressed.

In recent years research has hinted that epigenetic mechanisms may be responsible for much more than just normal development. Development is inherently plastic, with organisms able to take a number of different paths depending upon the environment into which a fetus was introduced. But once certain developmental decisions are made, they are irreversible. David Barker, a professor of clinical epidemiology at the University of Southampton in England, has studied this idea in humans since the 1990s. In multiple studies he and others



occurred in genes that regulate sugar absorption and metabolism. Other studies have linked a baby's environment to kidney problems, asthma, osteoporosis and mental illness as an adult.

All these studies are merely correlational, with researchers noting that certain populations, having undergone a particular environmental stress early in life, have sometimes fallen ill years later. That raises questions of exactly how this may occur, whether epigenetics is the true mechanism and if there is anything to be done about it. While that has yet to be answered conclusively in humans, animal studies may be pointing the way.

For the purposes of epigenetics research, the agouti mouse is particularly apt. Its fur color is determined by the level of methylation on a piece of DNA found near the agouti gene. As a result, genetically identical offspring may look completely different from one another. One mouse might be yellow (indicating little methylation), another brown (a lot of methylation) and a third mottled (some cells with methylated genes, some not).

Randy Jirtle, an epigenetics researcher at Duke University, was intrigued by those tendencies and wanted to know whether early environmental influences could change the mouse's levels of methylation. In a 2003 experiment, he fed agouti mothers folic acid, vitamin B₁₂, choline and betaine—all methyl supplementers—during pregnancy. This not only increased the babies' DNA methylation near the agouti gene but also boosted the likelihood that they would be brown, establishing that changes in DNA methylation

are the mechanism that connects a mother's diet to her offspring's gene expression.

Then, in a 2006 study, Jirtle fed the mothers genistein, a component of soy, and found that it too increased methylation, making the offspring more likely to be brown. Next, he tracked the offspring's adult weight and found that they were less likely to be obese. That's because the agouti gene also governs the part of the brain that affects satiation. "The big question is how something that happens early, as a result of benign environmental influences, is linked to susceptibility to common diseases 20 or 30 years later," says Jirtle. "At least for the agouti mouse, that link is DNA methylation."

At about the time Jirtle was doing his mice experiments, Michael Meaney, a neuroendocrinologist at McGill University in Montreal, was working with rats, testing the methylation of

have found that babies with birth weights on the lower end of normal who grow up in affluent societies are much more likely to develop coronary heart disease, type 2 diabetes and hypertension as adults than are heavier babies. Barker has theorized that smaller babies are prepared for a diet low in carbohydrates and fat, and when they encounter just the opposite in the real world, they are predisposed to metabolic illnesses.

To see a mismatch between a baby's real and predicted environment, consider the Dutch famine of 1944–45 and its legacy. When German forces cut off food supplies to parts of the Netherlands for six months, expectant mothers who starved during the final trimester were more likely to have babies who later developed type 2 diabetes. Programmed to expect hard times, these children grew up in an improving postwar environment. Researchers think epigenetic changes might have

a gene important to the stress response—a glucocorticoid receptor gene in the brain. It turns out it's not just what a mother eats but also how she treats her babies that affects their epigenome—the pattern of epigenetic marks that accumulates throughout development. Some rat mothers are particularly attentive to their pups, excessively licking and nursing during the first week after delivery. Studies have shown that the pups of these mothers are less fearful as adults and less fazed by stressful situations.

Meaney found striking differences in methylation patterns between pups with highly attentive mothers and those with neglectful mothers. Less attentive mothering resulted in more methylation near the glucocorticoid receptor gene, turning it off; better mothering kept it on, producing more receptors and

better regulation of the rats' stress response. To confirm his findings, Meaney transferred pups born to neglectful mothers to highly attentive ones immediately after birth; the methylation patterns of these adoptees were almost indistinguishable from those of the attentive mothers' natural offspring, and the adopted pups grew up to be as fearless as the natural pups.

Although these epigenetic changes happened only during one crucial period—the first week after delivery—their impact persisted into adulthood. Yet when Meaney injected a compound into adult rats that demethylated key genes, neglected animals became less fearful. His work provides the first evidence that the way a mother takes care of offspring might change them forever by altering the epigenome.

These studies demonstrate how a less than ideal environment during a critical developmental period may have long-lasting effects. Now, Michael Skinner, a molecular bioscientist at Washington State University in Pullman, is going further, showing that such exposure may change the lives of the altered animals' descendants too.

Skinner exposed pregnant rats to the toxin vinclozolin, a hormonelike compound known as an endocrine disrupter, during days eight through 15 of their embryos' development—when the cells that will become sperm are particularly open to epigenetic changes. He found that almost all males in four subsequent generations descended from the vinclozolin-exposed rats had far fewer and less vigorous sperm than normal and were also more likely to be infertile. Moreover, these effects appeared to relate to patterns of DNA methylation.

“The exposure to vinclozolin apparently reprogrammed the remethylation in the male germ line permanently,” Skinner says. In a later study, he found that vinclozolin exposure during the same period not only caused reproductive defects but also led to a number of adult diseases, including prostate disease, kidney disease and tumor development. It even dampened the rats' chances of finding a mate.

Skinner was the first to show that epigenetics propagates the effects of the environmental exposure of one generation to multiple subsequent generations. “We have a clearly trans-generational effect for four generations and a very high frequency of disease,” he says.

During his years working with the Överkalix data, Kaati has tried to link environmental developments in the parish with possible epigenetic changes in residents. He has followed the lives of people born in 1890, 1905 and

What's the Difference? //

TWO MECHANISMS CAN MAKE A LASTING IMPACT ON OUR GENES, BUT THEY GO ABOUT CHANGE IN DISTINCT WAYS.

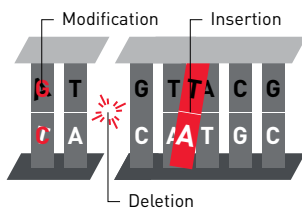
A MUTATION...

Modifies the genome—the set of genes each individual possesses

Can occur at any point in life

Could have a pathological effect; it's the result of damage or a molecular mistake

Changes the DNA sequence. Mutations include the insertion or deletion of a base pair or a change in a base pair:



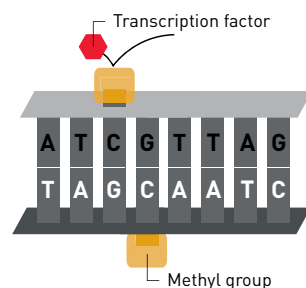
AN EPIGENETIC CHANGE...

Modifies the epigenome, which determines which genes are expressed (turned on) or silenced (turned off)

Mainly occurs during crucial points in development, particularly in the womb and during the slow growth period, which precedes puberty

Is usually an adaptive measure to help a person survive in his or her environment

Changes the scaffolding that carries the DNA sequence. In one method, methylation, a chemical compound called a methyl group attaches to cytosine (C), barring nearby genes from being transcribed into a protein:





But because these studies are preliminary, researchers are cautious. “There’s almost a wish that epigenetic phenomena affect our lives—that when we change our diet, for example, it might change the way our genes are expressed,” says Adrian Bird, a molecular geneticist at the University of Edinburgh who specializes in methylation. “But we have a way to go before we can be sure.”

Several initiatives may boost this research. The National Institutes of Health names epigenetics one of four “grand challenges in biomedical health/research” that “can be uniquely addressed by NIH as a whole.” Therapies that could turn on important genes, especially for the treatment of cancer, are being developed, and

1920 and consulted crop data compiled during the lives of their parents and grandparents. His goal was to find how much food was available to people during one crucial stage of development: the slow growth period (SGP) before puberty begins (between ages eight and 10 for girls and nine and 12 for boys).

In a series of studies published since 2001, Kaati has shown that when food was scarce during a father’s SGP, his son was far less likely to die of cardiovascular disease. And if a paternal grandfather had plenty to eat during his SGP? His grandchild tended to have a shorter life, and his son had a quadrupled risk of dying of diabetes. (These findings may seem to contradict those of Barker and the Dutch famine researchers, yet the crucial difference may be that a child, during his SGP, requires little food, whereas a fetus requires a great deal.) But while these patterns suggest possible epigenetic links, researchers don’t know yet whether there’s a causal connection or which mechanism might be involved. Still, they have their suspicions. “The slow growth period is a time when sperm cells are maturing and during which information is imprinted on those cells,” says Kaati. “For our study’s next phase, we want to see whether these mechanisms extend beyond the three generations we have discovered.”

And if future studies confirm what Kaati and his fellow researchers suspect? “It might be dangerous to overeat during the slow growth period,” he says. “That’s what is happening now, with kids becoming fatter and fatter.” The resulting harm might conceivably persist for generations to come.

some drugs are believed to modify the epigenome for such diseases as epilepsy and bipolar disorder.

There’s also some concern that industrial chemicals may need to undergo testing to make sure they don’t alter the epigenome in a way that could lead to disease. “In the future, we’ll need to test compounds for their ability not only to mutate our DNA but also to alter the epigenome,” says Duke’s Jirtle.

The field of epigenetics may just be dawning, but it could someday change the way doctors approach medicine. “If you think of the genome as a computer’s hardware, then the epigenome is the software that tells the computer how to work,” says Jirtle. “I think we’ll discover that many diseases aren’t the result of hardware problems—mutations—at all. They’ll turn out to be due to software—epigenetic—problems.” ■

→ DOSSIER

1. “Environmental Epigenomics and Disease Susceptibility,” by Randy L. Jirtle and Michael K. Skinner, *Nature Reviews: Genetics*, April 2007. A thorough review of the environment’s effects on the epigenome, it uses vivid diagrams and photos to illustrate key points.
2. “Transgenerational Response to Nutrition, Early Life Circumstances and Longevity,” by Gunnar Kaati et al., *European Journal of Human Genetics*, April 2007. The latest in Kaati’s series of fascinating studies on health in an isolated Swedish village shows that food supply during childhood can alter disease risk generations later.
3. Epigenetics? [<http://epigenome.eu>] This European site tackles the tough field of epigenetics for the general public with in-depth feature stories, frequent updates from the laboratory and the latest news.