

The study of the epigenome—the suite of biochemical signals that determine which genes in an individual's DNA can be turned on or off—is shedding light on human disease. Bone cancer, made visible here by a radioactive tracer, is one of many ailments wrought by epigenetic changes.

DNA IS NOT DESTINY

The new science of epigenetics rewrites the rules of disease, heredity, and identity

By Ethan Watters

Back in 2000, Randy Jirtle, a professor of radiation oncology at Duke University, and his postdoctoral student Robert Waterland designed a groundbreaking genetic experiment that was simplicity itself. They started with pairs of fat yellow mice known to scientists as agouti mice, so called because they carry a particular gene—the agouti gene—that in addition to making the rodents ravenous and yellow renders them prone to cancer and diabetes. Jirtle and Waterland set about to see if they could change the unfortunate genetic legacy of these little creatures.

Typically, when agouti mice breed, most of the offspring are identical to the parents: just as yellow, fat as pincushions, and susceptible to life-shortening disease. The parent mice in Jirtle and Waterland's experiment, however, produced a majority of offspring that looked altogether different. These young mice were slender and mousy brown. Moreover, they did not display their parents' susceptibility to cancer and diabetes and lived to a spry old age. The effects of the agouti gene had been virtually erased.

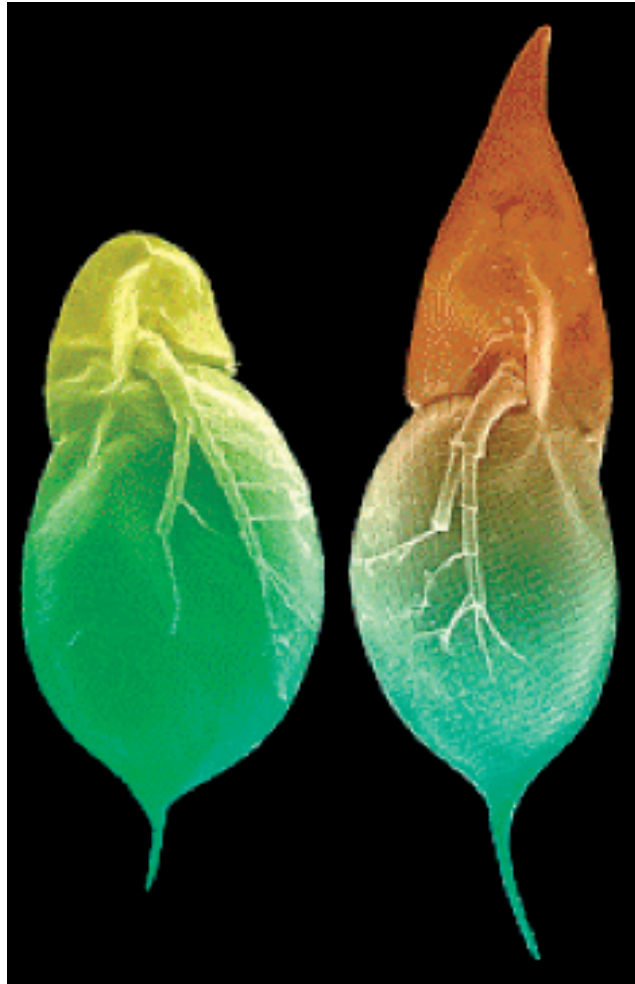
Remarkably, the researchers effected this transformation without altering a single letter of the mouse's DNA. Their approach instead was radically straightforward—they changed the moms' diet. Starting just before conception, Jirtle and Waterland fed a test group of mother mice a diet rich in methyl donors, small chemical clusters that can attach to a gene and turn it off. These molecules are common in the environment and are found in many foods, in-

cluding onions, garlic, beets, and in the food supplements often given to pregnant women. After being consumed by the mothers, the methyl donors worked their way into the developing embryos' chromosomes and onto the critical agouti gene. The mothers passed along the agouti gene to their children intact, but thanks to their methyl-rich pregnancy diet, they had added to the gene a chemical switch that dimmed the gene's deleterious effects.

"It was a little eerie and a little scary to see how something as subtle as a nutritional change in the pregnant mother rat could have such a dramatic impact on the gene expression of the baby," Jirtle says. "The results showed how important epigenetic changes could be."

Our DNA—specifically the 25,000 genes identified by the Human Genome Project—is now widely regarded as the instruction book for the human body. But genes themselves need instructions for what to do, and where and when to do it. A human liver cell contains the same DNA as a brain cell, yet somehow it knows to code only those proteins needed for the functioning of the liver. Those instructions are found not in the letters of the DNA itself but on it, in an array of chemical markers and switches, known collectively as the epigenome, that lie along the length of the double helix. These epigenetic switches and markers in turn help switch on or off the expression of particular genes. Think of the epigenome as a complex software code, capable of inducing the DNA hardware to manufac-

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To the surprise of scientists, many environmentally induced changes turn out to be heritable. When exposed to predators, *Daphnia* water fleas grow defensive spines (right). The effect can last for several generations.

ture an impressive variety of proteins, cell types, and individuals.

In recent years, epigenetics researchers have made great strides in understanding the many molecular sequences and patterns that determine which genes can be turned on and off. Their work has made it increasingly clear that for all the popular attention devoted to genome-sequencing projects, the epigenome is just as critical as DNA to the healthy development of organisms, humans included. Jirtle and Waterland's experiment was a benchmark demonstration that the epigenome is sensitive to cues from the environment. More and more, researchers are finding that an extra bit of a vitamin, a brief exposure to a toxin, even an added dose of mothering can tweak the epigenome—and thereby alter the software of our genes—in ways that affect an individual's body and brain for life.

The even greater surprise is the recent discovery that epigenetic signals from the environment can be passed on from one generation to the next, sometimes for several generations, without changing a single gene sequence. It's well established, of course, that environmental effects like radiation, which alter the genetic sequences in a sex cell's DNA, can leave a mark on subsequent generations.

Likewise, it's known that the environment in a mother's womb can alter the development of a fetus. What's eye-opening is a growing body of evidence suggesting that the epigenetic changes wrought by one's diet, behavior, or surroundings can work their way into the germ line and echo far into the future. Put simply, and as bizarre as it may sound, what you eat or smoke today could affect the health and behavior of your great-grandchildren.

All of these discoveries are shaking the modern biological and social certainties about genetics and identity. We commonly accept the notion that through our DNA we are destined to have particular body shapes, personalities, and diseases. Some scholars even contend that the genetic code predetermines intelligence and is the root cause of many social ills, including poverty, crime, and violence. "Gene as fate" has become conventional wisdom. Through the study of epigenetics, that notion at last may be proved outdated. Suddenly, for better or worse, we appear to have a measure of control over our genetic legacy.

"Epigenetics is proving we have some responsibility for the integrity of our genome," Jirtle says. "Before, genes predetermined outcomes. Now everything we do—everything we eat or smoke—can affect our gene expression and that of future generations. Epigenetics introduces the concept of free will into our idea of genetics."

Scientists are still coming to understand the many ways that epigenetic changes unfold at the biochemical level. One form of epigenetic change physically blocks access to the genes by altering what is called the histone code. The DNA in every cell is tightly wound around proteins known as histones and must be unwound to be transcribed. Alterations to this packaging cause certain genes to be more or less available to the cell's chemical machinery and so determine whether those genes are expressed or silenced. A second, well-understood form of epigenetic signaling, called DNA methylation, involves the addition of a methyl group—a carbon atom plus three hydrogen atoms—to particular bases in the DNA sequence. This interferes with the chemical signals that would put the gene into action and thus effectively silences the gene.

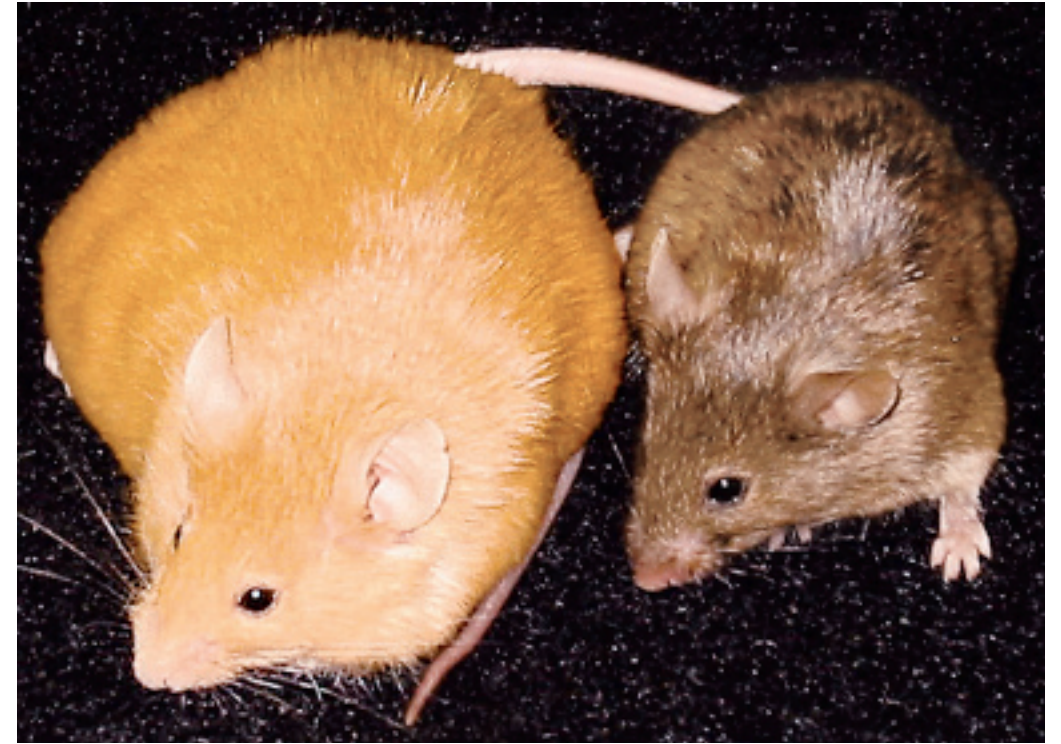
Until recently, the pattern of an individual's epigenome was thought to be firmly established during early fetal development. Although that is still seen as a critical period, scientists have lately discovered that the epigenome can change in response to the environment throughout an individual's lifetime.

"People used to think that once your epigenetic code was laid down in early development, that was it for life," says Moshe Szyf, a pharmacologist with a bustling lab at McGill University in Montreal. "But life is changing all the time, and the epigenetic code that controls your DNA is turning out to be the mechanism through which we change along with it. Epigenetics tells us that little things in life can have an effect of great magnitude."

Szyf has been a pioneer in linking epigenetic changes to the development of diseases. He long ago championed the idea that epigenetic patterns can shift through life and that those changes are important in the establishment and spread of cancer. For 15 years, however, he had little luck convincing his colleagues. One of his papers was dismissed by a reviewer as a "misguided attempt at scientific humor." On another occasion, a prominent scientist took him aside and told him bluntly, "Let me be clear: Cancer is genetic in origin, not epigenetic."

Despite such opposition, Szyf and other researchers have persevered. Through numerous studies, Szyf has found that common

With no more than a change in diet, laboratory agouti mice (left) were prompted to give birth to young (right) that differed markedly in appearance and disease susceptibility. Recently, researchers showed that an epigenetic change in nematode worms can be inherited for 80 generations.



signaling pathways known to lead to cancerous tumors also activate the DNA-methylation machinery; knocking out one of the enzymes in that pathway prevents the tumors from developing. When genes that typically act to suppress tumors are methylated, the tumors metastasize. Likewise, when genes that typically promote tumor growth are demethylated—that is, the dimmer switches that are normally present are removed—those genes kick into action and cause tumors to grow.

Szyf is now far from alone in the field. Other researchers have identified dozens of genes, all related to the growth and spread of cancer, that become over- or undermethylated when the disease gets under way. The bacteria *Helicobacter*, believed to be a cause of stomach cancer, has been shown to trigger potentially cancer-inducing epigenetic changes in gut cells. Abnormal methylation patterns have been found in many cancers of the colon, stomach, cervix, prostate, thyroid, and breast.

Szyf views the link between epigenetics and cancer with a hopeful eye. Unlike genetic mutations, epigenetic changes are potentially reversible. A mutated gene is unlikely to mutate back to normal; the only recourse is to kill or cut out all the cells carrying the defective code. But a gene with a defective methylation pattern might very well be encouraged to reestablish a healthy pattern and continue to function. Already one epigenetic drug, 5-azacytidine, has been approved by the Food and Drug Administration for use against myelodysplastic syndrome, also known as preleukemia or smoldering leukemia. At least eight other epigenetic drugs are currently in different stages of development or human trials.

Methylation patterns also hold promise as diagnostic tools, potentially yielding critical information about the odds that a cancer will respond to treatment. A Berlin-based company called Epigenomics, in partnership with Roche Pharmaceuticals, expects to bring an epigenetic screening test for colon cancer to market by 2008. They are

working on similar diagnostic tools for breast cancer and prostate cancer. Szyf has cofounded a company, MethylGene, that so far has developed two epigenetic cancer drugs with promising results in human trials. Others have published data on animal subjects suggesting an epigenetic component to inflammatory diseases like rheumatoid arthritis, neurodegenerative diseases, and diabetes.

Other researchers are focusing on how people might maintain the integrity of their epigenomes through diet. Baylor College of Medicine obstetrician and geneticist Ignatia Van den Veyver suggests that once we understand the connection between our epigenome and diseases like cancer, lifelong "methylation diets" may be the trick to staying healthy. Such diets, she says, could be tailored to an individual's genetic makeup, as well as to their exposure to toxins or cancer-causing agents.

In 2003 biologist Ming Zhu Fang and her colleagues at Rutgers University published a paper in the journal *Cancer Research* on the epigenetic effects of green tea. In animal studies, green tea prevented the growth of cancers in several organs. Fang found that epigallocatechin-3-gallate (EGCG), the major polyphenol from green tea, can prevent deleterious methylation dimmer switches from landing on (and shutting down) certain cancer-fighting genes. The researchers described the study as the first to demonstrate that a consumer product can inhibit DNA methylation. Fang and her colleagues have since gone on to show that genistein and other compounds in soy show similar epigenetic effects.

Meanwhile, epigenetic researchers around the globe are rallying behind the idea of a human epigenome project, which would aim to map our entire epigenome. The Human Genome Project, which sequenced the 3 billion pairs of nucleotide bases in human DNA, was a piece of cake in comparison: Epigenetic markers and patterns are different in every tissue type in the human body and also change over time. "The epigenome project is much more difficult than the

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Human Genome Project,” Jirtle says. “A single individual doesn’t have one epigenome but a multitude of them.”

Research centers in Japan, Europe, and the United States have all begun individual pilot studies to assess the difficulty of such a project. The early signs are encouraging. In June, the European Human Epigenome Project released its data on epigenetic patterns of three human chromosomes. A recent flurry of conferences have forwarded the idea of creating an international epigenome project that could centralize the data, set goals for different groups, and standardize the technology for decoding epigenetic patterns.

Until recently, the idea that your environment might change your heredity without changing a gene sequence was scientific heresy. Everyday influences—the weights Dad lifts to make himself muscle-bound, the diet regimen Mom follows to lose pounds—don’t produce stronger or slimmer progeny, because those changes don’t affect the germ cells involved in making children. Even after the principles of epigenetics came to light, it was believed that methylation marks and other epigenetic changes to a parent’s DNA were lost during the process of cell division that generates eggs and sperm and that only the gene sequence remained. In effect, it was thought, germ cells wiped the slate clean for the next generation.

That turns out not to be the case. In 1999 biologist Emma Whitelaw, now at the Queensland Institute of Medical Research in Australia, demonstrated that epigenetic marks could be passed from one generation of mammals to the next. (The phenomenon had already been demonstrated in plants and yeast.) Like Jirtle and Waterland in 2003, Whitelaw focused on the agouti gene in mice, but the implications of her experiment span the animal kingdoms.

“It changes the way we think about information transfer across generations,” Whitelaw says. “The mind-set at the moment is that the information we inherit from our parents is in the form of DNA. Our experiment demonstrates that it’s more than just DNA you inherit. In a sense that’s obvious, because what we inherit from our parents are chromosomes, and chromosomes are only 50 percent DNA. The other 50 percent is made up of protein molecules, and these proteins carry the epigenetic marks and information.”

Michael Meaney, a biologist at McGill University and a frequent collaborator with Szyf, has pursued an equally provocative notion:

“Epigenetics will have a dramatic impact on how we understand history, sociology, and political science.”

that some epigenetic changes can be induced after birth, through a mother’s physical behavior toward her newborn. For years, Meaney sought to explain some curious results he had observed involving the nurturing behavior of rats. Working with graduate student Ian Weaver, Meaney compared two types of mother rats: those that patiently licked their offspring after birth and those that neglected their newborns. The licked newborns grew up to be relatively brave and calm (for rats). The neglected newborns grew into the sort of rodents that nervously skitter into the darkest corner when placed in a new environment.

Traditionally, researchers might have offered an explanation on one side or the other of the nature-versus-nurture divide. Either the

newborns inherited a genetic propensity to be skittish or brave (nature), or they were learning the behavior from their mothers (nurture). Meaney and Weaver’s results didn’t fall neatly into either camp. After analyzing the brain tissue of both licked and nonlicked rats, the researchers found distinct differences in the DNA methylation patterns in the hippocampus cells of each group. Remarkably, the mother’s licking activity had the effect of removing dimmer switches on a gene that shapes stress receptors in the pup’s growing brain. The well-licked rats had better-developed hippocampi and released less of the stress hormone cortisol, making them calmer when startled. In contrast, the neglected pups released much more cortisol, had less-developed hippocampi, and reacted nervously when startled or in new surroundings. Through a simple maternal behavior, these mother rats were literally shaping the brains of their offspring.

How exactly does the mother’s behavior cause the epigenetic change in her pup? Licking and grooming release serotonin in the pup’s brain, which activates serotonin receptors in the hippocampus. These receptors send proteins called transcription factors to turn on the gene that inhibits stress responses. Meaney, Weaver, and Szyf think that the transcription factors, which normally regulate genes in passing, also carry methylation machinery that can alter gene expression permanently. In two subsequent studies, Meaney and his colleagues were even able to reverse the epigenetic signals by injecting the drug trichostatin A into the brains of adult rats. In effect, they were able to simulate the effect of good (and bad) parenting with a pharmaceutical intervention. Trichostatin, interestingly, is chemically similar to the drug valproate, which is used clinically in people as a mood stabilizer.

Meaney says the link between nurturing and brain development is more than just a curious cause and effect. He suggests that making postnatal changes to an offspring’s epigenome offers an adaptive advantage. Through such tweaking, mother rats have a last chance to mold their progeny to suit the environment they were born into. “These experiments emphasize the importance of context on the development of a creature,” Meaney says. “They challenge the overriding theories of both biology and psychology. Rudimentary adaptive responses are not innate or passively emerging from the genome but are molded by the environment.”

Meaney now aims to see whether similar epigenetic changes occur when human mothers caress and hold their infants. He notes that the genetic sequence silenced by attentive mother rats has a close parallel in the human genome, so he expects to find a similar epigenetic influence. “It’s just not going to make any sense if we don’t find this in humans as well. The story is going to be more complex than with the rats because we’ll have to take into account more social influences, but I’m convinced we’re going to find a connection.”

In an early study, which provided circumstantial evidence, Meaney examined magnetic resonance imaging brain scans of adults who began life as low-birth-weight babies. Those adults who reported in a questionnaire that they had a poor relationship with their mother were found to have hippocampi that were significantly smaller than average. Those adults who reported having had a close relationship with their mother, however, showed perfectly normal size hippocampi. Meaney acknowledges the unreliability of subjects reporting on their own parental relationships; nonetheless, he strongly suspects that the quality of parenting was responsible for the different shapes of the brains of these two groups.

In an effort to solidify the connection, he and other researchers have launched an ambitious five-year multimillion-dollar study to examine the effects of early nurturing on hundreds of human babies. As a test group, he’s using severely depressed mothers who often have difficulty bonding and caring for their newborns and, as a result, tend to caress their babies less than mothers who don’t experience depression or anxiety. The question is whether the babies of depressed mothers show the distinct brain shapes and patterns indicative of epigenetic differences.

The science of epigenetics opens a window onto the inner workings of many human diseases. It also raises some provocative new questions. Even as we consider manipulating the human epigenome to benefit our health, some researchers are concerned that we may already be altering our epigenomes unintentionally, and perhaps not for the better. Jirtle notes that the prenatal vitamins that physicians commonly encourage pregnant women to take to reduce the incidence of birth defects in their infants include some of the same chemicals that Jirtle fed to his agouti mice. In effect, Jirtle wonders whether his mouse experiment is being carried out wholesale on American women.

“On top of the prenatal vitamins, every bit of grain product that we eat in the country is now fortified with folic acid,” Jirtle notes, and folic acid is a known methyl donor. “In addition, some women take multivitamins that also have these compounds. They’re getting a triple hit.”

While the prenatal supplements have an undisputed positive effect, Jirtle says, no one knows where else in the fetal genome those gene-silencing methyl donors might be landing. A methyl tag that has a positive effect on one gene might have a deleterious effect if it happens to fall somewhere else. “It’s the American way to think, ‘If a little is good, a lot is great.’ But that is not necessarily the case here. You might be overmethylating certain genes, which could potentially cause other things like autism and other negative outcomes.”

Szyf shares the concern. “Fueling the methylation machinery through dietary supplements is a dangerous experiment, because there is likely to be a plethora of effects throughout a lifetime.” In the future, he believes, epidemiologists will have their hands full looking for possible epigenetic consequences of these public-health choices. “Did this change in diet increase cancer risk? Did it increase depression? Did it increase schizophrenia? Did it increase dementia or Alzheimer’s? We don’t know yet. And it will take some time to sort it out.”

The implications of the epigenetic revolution are even more profound in light of recent evidence that epigenetic changes made in the parent generation can turn up not just one but several generations down the line, long after the original trigger for change has been removed. In 2004 Michael Skinner, a geneticist at Washington State University, accidentally discovered an epigenetic effect in rats that lasts at least four generations. Skinner was studying how a commonly used agricultural fungicide, when introduced to pregnant mother rats, affected the development of the testes of fetal rats. He was not surprised to discover that male rats exposed to high doses of the chemical while in utero had lower sperm counts later in life. The surprise came when he tested the male rats in subsequent generations—the grandsons of the exposed mothers. Although the pesticide had not changed one letter of their DNA, these second-

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LAMARCK’S LAST LAUGH

Fifty years before Charles Darwin (left), Jean-Baptiste Lamarck (right) proposed a fully formed, if inadequate, theory of evolution: Organisms evolve because they try to. Through what Lamarck called “the inheritance of acquired characteristics,” the environment changes an organism, and future generations inherit that change. Giraffes, he argued, have long necks because successive generations stretched to reach ever-higher leaves. In Lamarck’s world, Arnold Schwarzenegger’s children would be born buff thanks to their dad’s obsessive bodybuilding.

In contrast, Darwin’s theory of evolution by natural selection rested on three basic observations: that no two individuals are exactly alike, that children resemble their parents, and that some of the differences affect how many children an individual has. By Darwin’s reasoning, giraffes have long necks because longer-necked giraffes can reach more leaves, thrive, produce more offspring, and so increase the proportion of long-necked giraffes.

Although Lamarck’s ideas prompt laughter in biology classes today, Darwin himself took them seriously, even suggesting in *On the Origin of Species* that organs can grow and shrink through use or disuse and that those changes are heritable. However, an experiment in the late 19th century by biologist August Weismann demonstrated that sons do not inherit the sins, enlarged organs, workout programs, or leaf-eating aspirations of their fathers. Weismann cut off the tails of hundreds of mice, and not one of their children came out tailless.

So far it’s still true that losing a tail won’t amputate your offspring. But the burgeoning science of epigenetics suggests that Lamarck may have been at least a little right. The diet, behavior, and environmental surroundings of today’s parents can indeed reshape their hereditary legacy for generations to come. If mutations aren’t all that great-grandparents pass on, says Tel Aviv University philosopher of science Eva Jablonka, “we have to change basic concepts of heredity and of evolution.”

Jessica Ruvinsky

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generation offspring also had low sperm counts. The same was true of the next generation (the great-grandsons) and the next.

Such results hint at a seemingly anti-Darwinian aspect of heredity. Through epigenetic alterations, our genomes retain something like a memory of the environmental signals received during the lifetimes of our parents, grandparents, great-grandparents, and perhaps even more distant ancestors. So far, the definitive studies have involved only rodents. But researchers are turning up evidence suggesting that epigenetic inheritance may be at work in humans as well.

In November 2005, Marcus Pembrey, a clinical geneticist at the Institute of Child Health in London, attended a conference at Duke University to present intriguing data drawn from two centuries of records on crop yields and food prices in an isolated town in northern Sweden. Pembrey and Swedish researcher Lars Olov Bygren noted that fluctuations in the towns' food supply may have health effects spanning at least two generations. Grandfathers who lived their pre-teen years during times of plenty were more likely to have grandsons with diabetes—an ailment that doubled the grandsons' risk of early death. Equally notable was that the effects were sex specific. A grandfather's access to a plentiful food supply affected the mortality rates of his grandsons only, not those of his granddaughters, and a paternal grandmother's experience of feast affected the mortality rates of her granddaughters, not her grandsons.

This led Pembrey to suspect that genes on the sex-specific X and Y chromosomes were being affected by epigenetic signals. Further analysis supported his hunch and offered insight into the signaling process. It turned out that timing—the ages at which grandmothers and grandfathers experienced a food surplus—was critical to the intergenerational impact. The granddaughters most affected were those whose grandmothers experienced times of plenty while in utero or as infants, precisely the time when the grandmothers' eggs were forming. The grandsons most affected were those whose grandfathers experienced plenitude during the so-called slow growth period, just before adolescence, which is a key stage for the development of sperm.

The studies by Pembrey and other epigenetics researchers suggest that our diet, behavior, and environmental surroundings today could have a far greater impact than imagined on the health of our distant descendants. "Our study has shown a new area of research that could potentially make a major contribution to public health and have a big impact on the way we view our responsibilities toward future generations," Pembrey says.

The logic applies backward as well as forward: Some of the disease patterns prevalent today may have deep epigenetic roots. Pembrey and several other researchers, for instance, have wondered whether the current epidemic of obesity, commonly blamed on the excesses of the current generation, may partially reflect lifestyles adopted by our forebears two or more generations back.

Michael Meaney, who studies the impact of nurturing, likewise wonders what the implications of epigenetics are for social policy. He notes that early child-parent bonding is made more difficult by the effects of poverty, dislocation, and social strife. Those factors can certainly affect the cognitive development of the children directly involved. Might they also affect the development of future generations through epigenetic signaling?

"These ideas are likely to have profound consequences when you start to talk about how the structure of society influences cognitive development," Meaney says. "We're beginning to draw cause-and-effect arrows between social and economic macro-variables down to the level of the child's brain. That connection is potentially quite powerful."

Lawrence Harper, a psychologist at the University of California at Davis, suggests that a wide array of personality traits, including temperament and intelligence, may be affected by epigenetic inheritance. "If you have a generation of poor people who suffer from bad nutrition, it may take two or three generations for that population to recover from that hardship and reach its full potential," Harper says. "Because of epigenetic inheritance, it may take several generations to turn around the impact of poverty or war or dislocation on a population."

Historically, genetics has not meshed well with discussions of social policy; it's all too easy to view disadvantaged groups—criminals, the poor, the ethnically marginalized—as somehow fated by DNA to their condition. The advent of epigenetics offers a new twist and perhaps an opportunity to understand with more nuance how nature and nurture combine to shape the society we live in today and hope to live in tomorrow.

"Epigenetics will have a dramatic impact on how we understand history, sociology, and political science," says Szyf. "If environment has a role to play in changing your genome, then we've bridged the gap between social processes and biological processes. That will change the way we look at everything." ■



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