

A New Kind of Inheritance

Harmful chemicals, stress and other influences can permanently alter which genes are turned on without changing any of the genes' code. Now, it appears, some of these "epigenetic" changes are passed down to—and may cause disease in—future generations

By Michael K. Skinner

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WHEN MY KIDS WERE BORN, ABOUT 30 YEARS AGO, I KNEW THEY HAD INHERITED ABOUT half their DNA from me. At the time, the transfer of DNA from sperm or egg to an embryo was thought to be the only way that heritable information could flow from parents to children, at least in humans and other mammals.

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Of course, I understood that DNA is not destiny. Yes, many characteristics of a child may be written into his or her DNA and specifically into protein-coding genes—the sequences of DNA code that dictate the shapes and functions of proteins, the workhorses of the cell. But nurture matters, too. Many of the contingencies of life—what we eat, what pollutants are in our environment, how often we are stressed—affect how the genes operate. Social and environmental influences are often invoked, for instance, to explain why identical twins can end up with different diseases despite having highly similar complements of genes.

But we did not know back then that our biological bequest to our children includes more than just our DNA sequences—that, indeed, not just our kids but our grandchildren and great-grandchildren might inherit what is known as epigenetic information. Like DNA, epigenetic information resides in our chromosomes (which house our genes) and regulates cellular functions. But it is distinct from the DNA sequence and responds to the environment. It can take various forms, including small molecules that attach chemically to the DNA and to proteins in chromosomes.

Research at my laboratory and others, mainly on rats and mice, has found that certain pollutants—including agricultural chemicals, jet fuel and even some common plastics—can induce epigenetic modifications that cause disease and reproductive problems, all without changing the sequence of the animals' DNA. More startlingly, when such epimutations occur in the cells that give rise to eggs and sperm, they can apparently become fixed in place and then transmitted—along with any resulting health risks—to later generations.

The science in this area is evolving rapidly, and long-term studies in people now hint that epimutations may pass from generation to generation in humans as well. Given the many aspects of biology that we share with other mammals, it seems reasonable to expect that such epigenetic transgenerational inheritance does occur in people. If so, the implications for public health

could be profound. Some part of the increases in obesity, diabetes and other fast-rising diseases among baby boomers and more recent generations might have originated with their parents' and grandparents' exposure to pollutants such as DDT and dioxin.

THE DARK MATTER OF THE GENOME

EPIGENETIC EFFECTS ON CELLS have been recognized for some time, but the extent of their involvement has become clear only recently. Decades ago biologists noticed that lots of places in mammalian DNA have a methyl (CH_3) radical attached to them [see box on pages 48 and 49]. In humans, this epigenetic mark often occurs where a cytosine (C) code letter precedes a guanine (G) in the DNA sequence, which happens at about 28 million spots along the chromosomes. Scientists first thought that the main function of DNA methylation was to shut down transposons—dangerous stretches of DNA that can move themselves from their original positions on the chromosomes to other parts of the genome, sometimes in ways that cause disease. We now know that methylation also helps regulate the activity of normal genes and that it goes awry in many cancers and other disorders.

In the 1990s researchers began to work out the operation of more kinds of epigenetic marks. They found that methyl, acetyl and several other chemical modifications can tag beadlike structures composed of a group of proteins called histones. The DNA in chromosomes wraps around each histone bead. By controlling how tightly the DNA loops around the histone groups and whether adjacent beads spread apart or bunch up, the histone marks can effectively turn entire sets of genes on and off. Genes in tightly wound areas, for instance, get hidden from proteins that switch on gene activity.

Other epigenetic actors have since emerged, including the ever-shifting, three-dimensional structure of the DNA and chromosomes and a number of RNA varieties known as noncoding RNAs. Some of these RNAs interact with the epigenetic marks that sit on DNA and the histones. (They are called noncoding to distinguish

IN BRIEF

The actions of genes can be regulated by “epigenetic” factors, such as chemicals that attach to DNA and proteins in chromosomes and that encode information independently from the DNA sequence. Most epigenetic marks reset shortly after conception.

Pollutants, stress, diet and other environmental factors can cause persistent changes in the mix of epigenetic marks in chromosomes and, in that way, can alter how cells and tissue behave. Surprisingly, some acquired changes can be passed on to descendants.

Conceivably, the health of both you and your children may be affected by what your great-grandmother was exposed to during pregnancy. Epigenetic inheritance might play a role in health problems such as obesity and diabetes, as well as in the evolution of species.

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them from the RNA strands that get copied from DNA to serve as templates for making the proteins encoded by genes.)

Together these epigenetic actors influence gene activity in complex ways that are independent of the DNA sequence. The interplay between the genes and the epigenome is dynamic and still rather mysterious. We do know, however, that each time a cell replicates, the epigenetic marks in its chromosomes get copied into the chromosomes of the daughter cells. Epigenetic events early in life can thus alter how cells behave later on.

We also know that whereas cells work hard to protect the sequence of DNA in the chromosomes from any alterations, they revise the patterns of epigenetic marks during an organism's development and aging. These changes help to determine how cells specialize to become, for example, a skin cell or a brain cell; subtle shifts in epigenetic information modify which genes are

As the great-great-grandpups matured, the males suffered problems similar to those of their distant ancestors. All from a fleeting dose of widely used agricultural chemicals.

active in each part of the body. Harmful chemicals, nutrient deficiencies and other stresses can also cause epigenetic marks to be added or removed in ways that affect gene activity.

Today no one doubts that epigenetic effects play a crucial role in development, aging and even cancer. But biologists debate whether epimutations—abnormal epigenetic changes—can be passed down through many generations in mammals. Evidence from a rapidly growing number of experiments, by my group and many others, has convinced me that they can.

ACCIDENTAL INHERITANCE

MY FIRST GLIMPSE of multigenerational epimutations was a product of serendipity. About 13 years ago Andrea Cupp and I, with a few of our colleagues at Washington State University, were using rats to study the reproductive effects of two chemicals widely applied in farming—the pesticide methoxychlor and the fungicide vinclozolin. Like many agricultural chemicals, they are endocrine disruptors: they interfere with the hormonal signals that help to direct the formation and operation of the reproductive system. We had injected the chemicals into pregnant rats during the second week of gestation—when the embryo's gonads develop—and saw that nearly all male offspring grew up to have abnormal testes that make weak sperm and too few of them.

We were not thinking about epigenetics at the time, and it never occurred to us that these defects might be heritable, so we had no plan to breed the rats that had been exposed to methoxy-

chlor or vinclozolin while in the womb. But one day Cupp came into my office to apologize: by mistake, she had mated unrelated male and female pups from that experiment.

I told her to check the grandchildren of the exposed dams for defects, although I did not expect she would find any. To our amazement, more than 90 percent of the males in these litters showed the same testicular abnormalities as their fathers, even though their parents were just pinhead-sized fetuses when they and their grandmothers were briefly exposed.

This result was surprising because many toxicology studies had looked for evidence that environmental chemicals such as vinclozolin cause DNA mutations but had found none. We confirmed ourselves that the frequency of genetic mutations was not elevated in the rats exposed to the agents. Moreover, classical genetics could not explain a new trait that appears with 90 percent frequency in different families.

I knew, however, that the minuscule fetus contains primordial germ cells, which are the progenitor cells that give rise to sperm or eggs. Most likely, I thought, the chemical had directly influenced these progenitor cells, and this effect simply persisted as the cells divided into sperm or eggs—and eventually into grandchildren. If this were the case, then the brief chemical exposure caused the grandpups' testicular problems directly, and future generations should be perfectly normal.

There was one sure test to find out whether direct influence was to blame. We bred a fourth generation and then a fifth, each time mating unrelated descendants of the original exposed rats to avoid diluting the trait. As the great-grandchildren—and later the great-great-grandchildren—matured, the males of each generation suffered problems similar to those of their ancestors. All these changes stemmed from a fleeting (but unnaturally high) dose of agricultural chemicals that for decades were sprayed on fruits, vegetables, vineyards and golf courses.

I was shocked by these results. Over several years we repeated the experiments multiple times to confirm them and collect additional evidence. The most plausible explanation, we concluded, was that the exposure causes an epimutation that interferes with gonad development in male embryos—and this epimutation passes from sperm to the cells of a developing embryo, including to primordial germ cells, and so on for generations. In 2005 we published these results in *Science*, along with our epimutation hypothesis and tantalizing but preliminary supportive evidence that exposure to the fungicide had altered methylation at several important spots in the DNA of the descendants' sperm.

TROUBLING IMPLICATIONS

A TEMPEST OF DEBATE ensued. One reason was that researchers at companies that sell vinclozolin, as well as a nonindustry study, reported difficulty reproducing some of our results—probably because they used different experimental methods, such as giving the chemical orally, using inbred strains of rats, or breeding the affected males with those from an unexposed control lineage, a practice that depresses the trait substantially in subsequent generations.

In recent years, however, evidence has accumulated that epi-

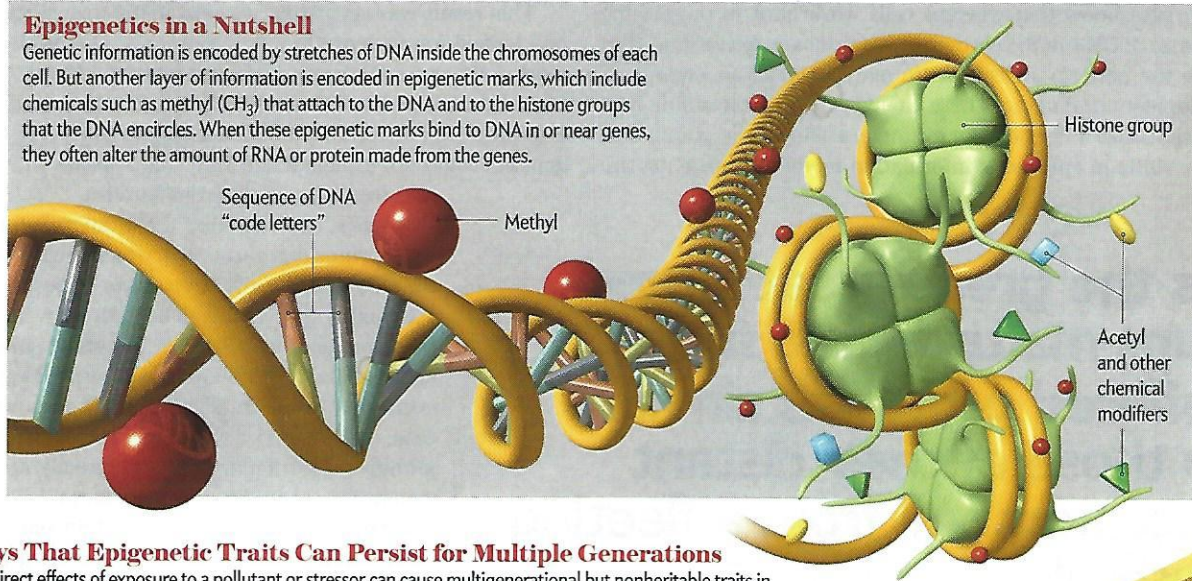
Inheritance outside the Genes

Biologists have discovered that the life experiences of animals and plants—such as exposure to certain pollutants or stressful events—can affect the health of their descendants without mutating their DNA. Such exposures can have multigenerational effects on children

and grandchildren through their direct actions on sperm, eggs and other reproductive cells. But transgenerational epigenetic inheritance, via heritable alterations to chemicals attached to the DNA inside these cells, can affect even more distant descendants.

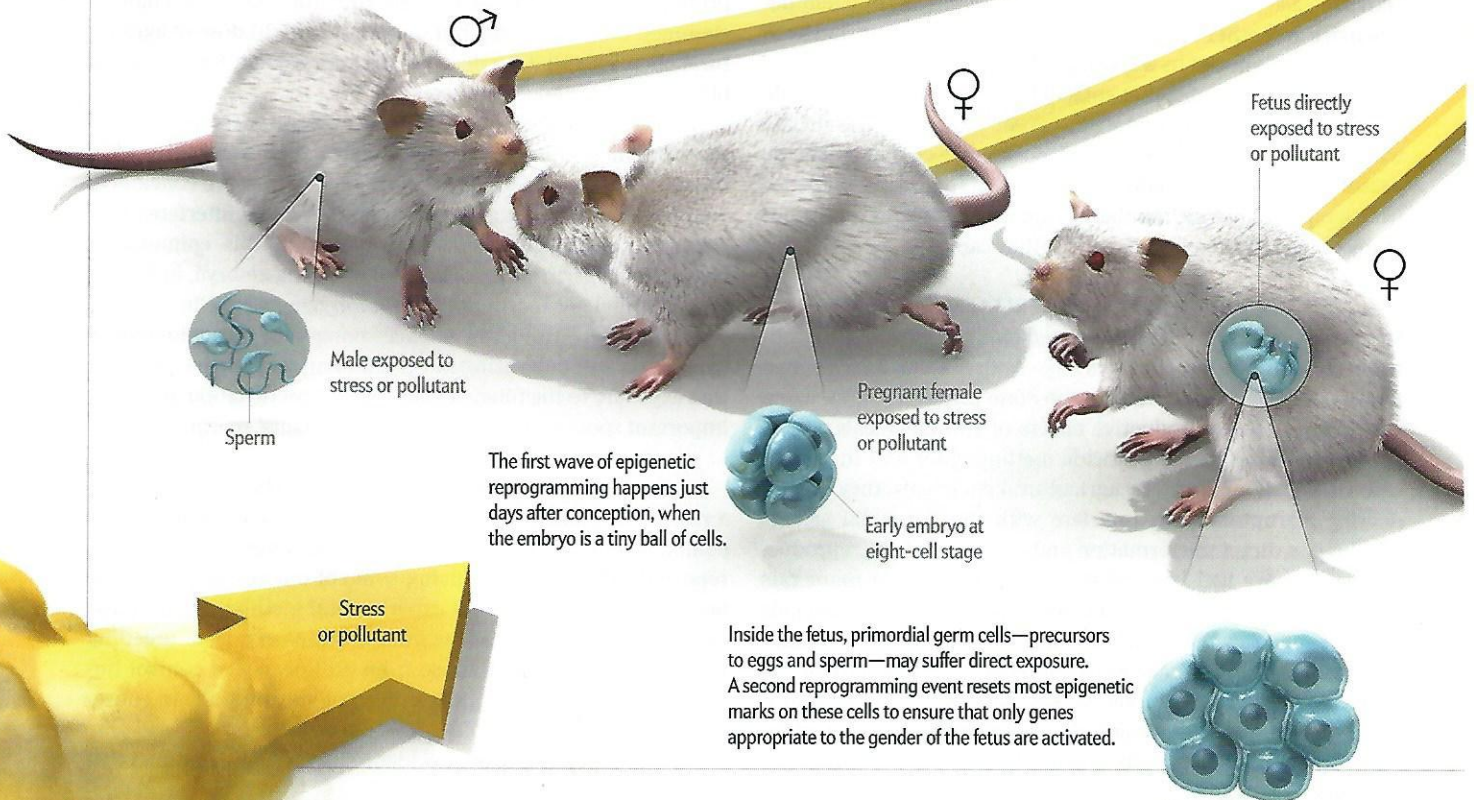
Epigenetics in a Nutshell

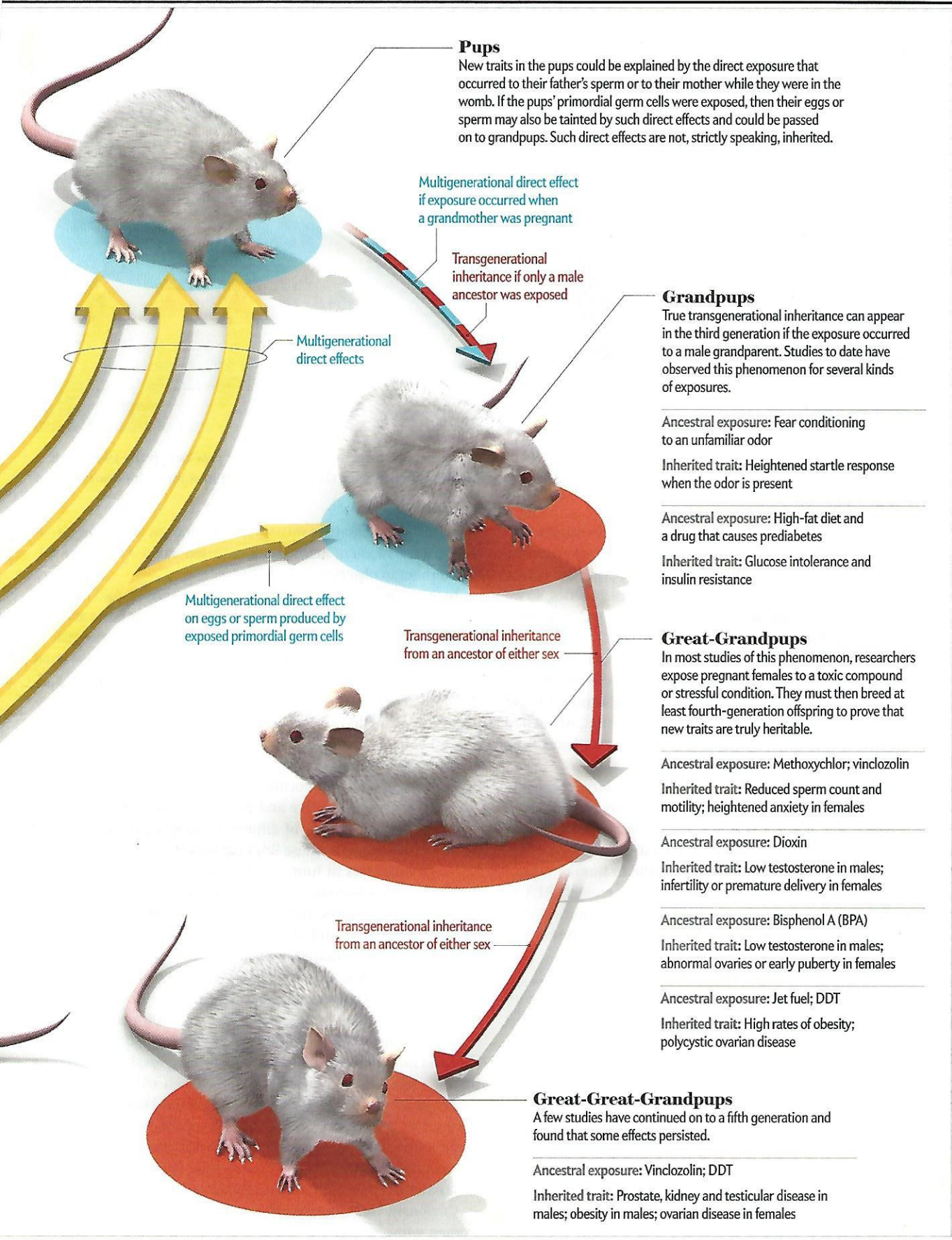
Genetic information is encoded by stretches of DNA inside the chromosomes of each cell. But another layer of information is encoded in epigenetic marks, which include chemicals such as methyl (CH_3) that attach to the DNA and to the histone groups that the DNA encircles. When these epigenetic marks bind to DNA in or near genes, they often alter the amount of RNA or protein made from the genes.



Ways That Epigenetic Traits Can Persist for Multiple Generations

The direct effects of exposure to a pollutant or stressor can cause multigenerational but nonheritable traits in two generations if the exposure occurs to a male and his sperm or in up to three generations if it occurs to a female at a particular stage of pregnancy (blue highlighting on opposite page). For an epigenetic trait to be inherited by subsequent generations (red), altered marking must survive two distinct waves of reprogramming (below) after conception. Both waves remove most epigenetic tags from the chromosomes and later rewrite them afresh. Rodent studies indicate that such persistence is possible.





mutations can persist for several generations. Follow-up studies at my lab have shown that the great-grandchildren of fungicide-treated rats have consistently altered patterns of methylation in their sperm, testes and ovaries, as well as abnormal gene activity in their primordial germ cells. We also found that fourth-generation offspring are prone to weight gain and anxiety; they even select mates differently. Meanwhile we and other scientists have added more pollutants and stressors to the list of factors that induce the effect, and transgenerational inheritance of acquired characteristics has been observed in a wide range of species, including plants, flies, worms, fish, rodents and pigs.

My team reported in 2012 that exposure of pregnant rats to either the pollutant dioxin, jet fuel, insect repellent, or a combination of bisphenol A (BPA) and phthalates—chemical components of plastics in food containers and tooth fillings—induce a variety of heritable disorders in fourth-generation descendants, such as pubertal abnormalities, obesity, and diseases of the ovaries, kidneys and prostate. We have observed hundreds of exposure-specific changes in DNA methylation patterns in sperm. The effects do not follow the inheritance patterns of classical genetics, so we believe that epimutations, not mutations to the DNA sequence, are causing these disorders.

Kaylon Bruner-Tran and Kevin Osteen of the Vanderbilt University School of Medicine also studied dioxin's effects on mice and found that about half of the daughters of exposed dams were infertile; among those that could get pregnant, many had premature deliveries. These problems conceiving and gestating persisted for at least two more generations.

The chemical doses in these studies are much larger than one would typically receive from a contaminated environment, but research by Jennifer Wolstenholme and others at the University of Virginia School of Medicine reported transgenerational effects from doses that are more comparable to human experience. They found that when mice were given enough BPA in their food to produce blood levels similar to those measured in pregnant American women, their descendants out to the fifth generation spent less time exploring their cages and more time interacting with other mice. The researchers suspect that the personality shift was caused by altered activity of the genes for oxytocin and vasopressin, both of which are known to affect social behavior. Although it seems likely that, as in our study on BPA, the effects coincide with altered DNA methylation patterns, the evidence for this association is still indirect. Other kinds of epigenetic changes could also be involved.

Studies are now under way that may be able to determine whether epimutations affect multiple generations of people as they do rodents. One such investigation is following up on an unfortunate natural experiment. In 1976 an explosion at a chemical plant in Seveso, Italy, exposed nearby residents to the highest concentrations of dioxin ever recorded in a public release of



FOGGING WITH DDT, a common mosquito-control practice in the 1940s and 1950s, might have caused epimutations that persist even in some babies born today.

this chemical. Scientists measured the amount of dioxin circulating in the blood of nearly 1,000 affected women and have followed them to observe their health.

In 2010 the researchers reported that for each 10-fold increase in a woman's exposure to dioxin during the accident, the average time needed to get pregnant rose by 25 percent, and the risk of infertility doubled. The team also observed in 2013 that women who were younger than 13 years at the time of the accident had, as adults, double the normal risk of developing metabolic syndrome—a collection of conditions, such as elevated blood pressure and blood glucose, that together predispose people to diabetes and heart disease. They found that many granddaughters of exposed women have abnormal results on thyroid tests.

Given that reproductive and metabolic disorders seem to be the most common kinds of ailments transmitted via the epigenome in lab animals, these findings hint that dioxins may promote epimutations in humans. The suspicion will be strengthened if, in years to come, the children and grandchildren of the exposed women show higher rates of infertility, obesity and related conditions—and show abnormal methylation patterns.

Capitalizing on another natural experiment, Marcus Pembrey of University College London, Lars Olov Bygren of the Karolinska Institute in Stockholm and their colleagues have done an intriguing series of studies using data from about 300 people born in 1890, 1905 and 1920 in Överkalix, Sweden, as well as their parents and grandparents. The researchers compared death records for the study subjects against reconstructed estimates of food supply in the town, which went through several two-year periods during the 19th century when good harvests were followed by crop failures. It appears that women whose paternal grandmothers experienced one of these feast-famine swings as young children had markedly higher rates of fatal cardiovascular disease.

Curiously, the increased risk was not seen for men, nor for women whose maternal grandmother or grandfathers endured

a rapid dive into food scarcity. For various reasons, such an odd inheritance pattern strongly suggests that epigenetics are at work and, in particular, a phenomenon known as imprinting. Similar observations have been made in descendants of a Dutch population that experienced famine during World War II.

THE EPIGENETIC IMPRINT OF PARENTHOOD

DESPITE THE MOUNTING evidence, many biologists still recoil from the idea that environmentally induced epimutations can settle into the germ line. The hypothesis seems to contradict a long-established belief that nearly all epigenetic marks are erased from the DNA and then rewritten during the reproductive process—not just once but twice. These processes, the reasoning goes, should wipe clean any acquired epimutations before they can cause trouble in the next generation. This same logic is another reason that our 2005 findings met with such a firestorm. The erasures do occur, but just how thoroughly is an open question.

The first wave of removal happens within days after conception. Methyl marks are stripped from the chromosomes—a process that confers on the embryonic stem cells the ability to give rise to every kind of cell. The tags are then added back while the fetus starts to develop. As the cells divide and specialize, distinctive patterns of DNA methylation appear in each cell type and help to tailor the cells to fit their particular functions.

Something shields a few special genes from this first wave of epigenetic erasure, however. Biologists refer to these genes as maternally or paternally “imprinted” because the epigenetic marks are preserved and guarantee that only the mother’s copy or the father’s copy of the gene gets used for making a protein. For example, in my children the gene *IGF2*, which encodes a hormone important for fetal growth, is active only on the chromosome they inherited from me. The copy of the gene from their mother is shut down by the combined action of DNA methylation and a form of noncoding RNA.

The second wave of epigenetic erasure and reprogramming begins later, when a rat fetus is the size of a pinhead and a human fetus is the size of a pea. This is when primordial germ cells start to appear inside the embryo’s newly formed gonads—and when we administer vinclozolin or other pollutants to lab animals in our experiments on epigenetic inheritance. In rats, this period lasts about a week; in humans, it stretches from the sixth to the 18th week of pregnancy.

This second wave is thought to be essentially complete—methyl marks are stripped off even the imprinted genes in the precursor cells to eggs and sperm. Later, however, marks are added again to establish the sex-appropriate pattern: in females, the chromosomes that will end up in eggs get a maternal methylation pattern, whereas in males, the chromosomes that will end up in sperm get a paternal pattern. The process avoids any offspring receiving two inactivated or two activated copies of imprinted genes when what it needs is one active and one inactive copy.

The same mechanism that reestablishes tags on imprinted genes might be influenced by environmental insults to fix new epimutations into the germ line. If an exposure—whether to a pollutant, a hormonal imbalance brought on by stress, or a nutritional deficiency that affects methylation—hits the embryo when that second sweep is about to begin, it might alter which epigenetic tags are brushed away forever and which are spared the broom or reset at the end of the reprogramming phase.

Most epimutations probably have little consequence or get corrected in the next generation, but every rule has an exception. If an epimutation in a germ-line cell becomes protected from the reprogramming of the epigenome, in much the same way that an imprinted gene is, it may hang on to affect the next generation—and perhaps many generations down the line.

If this idea is correct, epigenetic inheritance could have important consequences for medicine. Some scientists are investigating whether “obesogens”—environmental chemicals that upset human metabolism in ways that induce weight gain—might increase the risk of obesity in heritable ways. Bruce Blumberg and his colleagues at the University of California, Irvine, showed last year that pregnant mice that drank water laced with tributyltin, widely used to keep barnacles off ship hulls, bore pups prone to developing extra fat cells and fatty livers. The changes persisted for two more generations, an effect that is most easily explained by an epimutation. Thus, although shifts in lifestyle and food availability no doubt account for much of the increase in obesity, diabetes and other “rich-country” diseases over the past 50 years, it is conceivable that ancestral exposures have increased our susceptibility to such diseases.

In countries like the U.S. where children in the 1940s and 1950s were exposed to DDT, for example, it might be significant that when we injected animals with DDT, we found that more than half of the fourth-generation great-grandpups developed obesity—even though the second-generation offspring were normal in size—and that epigenetics seemed to be at fault. In three generations since the 1950s, the obesity rate among American adults has risen dramatically and now exceeds 35 percent.

If the environment can sometimes directly produce long-term, transgenerational changes in gene activity without first altering the DNA coding sequence, then the classical view of evolution—as a slow product of random mutations that get “selected” because of the reproductive or survival advantage they offer—will have to be expanded. It is even conceivable that epigenetic inheritance could explain why new species emerge more often than one would expect, given the rarity of advantageous genetic mutations. Epigenetic changes appear to occur 1,000 times more frequently. The most important effect of epigenetic marks—maybe their reason for existing—might be to wildly expand the number of variant individuals in a population. Natural selection would then pick the best adapted among them to thrive and carry on—genome, epigenome, and all. ■

MORE TO EXPLORE

Epigenetic Transgenerational Actions of Environmental Factors in Disease

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FROM OUR ARCHIVES

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