METHYL MADNESS: ROAD TO THE FINAL PHENOTYPE

One-third of American children are overweight or obese—that's 25 million kids. In the last thirty years, the percentage of obese two-to five-year-olds has doubled—and the percentage of obese six-to eleven-year-olds has tripled. A baby girl born in 2000 now has a *40 percent chance*—almost a coin toss—of developing of Type 2 diabetes, and that's directly related to the huge surge in heavy kids.

What's even sadder is that many of these children are showing symptoms of obesity-related illness while they're still kids. One recent study showed that about 60 percent of obese five-to ten-year-olds already exhibited at least one major risk factor for heart disease—high cholesterol, high blood pressure, high triglycerides, or high sugar levels. Of those kids, 25 percent had more than one risk factor. A 2005 report in *The New En gland Journal of Medicine* said that the epidemic of childhood obesity is the critical element in a gathering storm that could produce the first modern *decline* in American life expectancy—dropping life expectancy as much as five years.

There's no question that gallons of sugary soda, baskets of fatty fries, and too many hours watching television and playing video games instead of after-school sports is a fattening combo. But new research suggests that may not be the whole story.

There is emerging evidence that the dietary habits of parents, especially women in the earliest stages of pregnancy, may have an impact on the metabolism of their children. In other words, if you're trying to get pregnant, you really should think twice before you bite that Big Mac—once for your own waistline, and once for your potential child's.

Before you get the wrong idea, this isn't to suggest some strictly Larmarckian idea that a fat parent is going to have a fat child because the child will *inherit* the weight problem his or her parent *acquired.* But this *is* to say that new research is rapidly changing our understanding of how, when, and whether genes express themselves—that is, how, when, and whether the instructions in a gene are carried out. A series of groundbreaking research over the last five years has shown that certain compounds can attach themselves to specific genes and suppress their expression. These compounds act like a genetic light switch, essentially turning off the genes they attach to. And—here's where it gets really interesting—the research shows that environmental factors, like the food we eat or the cigarettes we smoke, can flick the switch on or off.

This research is changing the whole field of genetics—it's even launched a subdiscipline called epigenetics. Epigenetics is concerned with

the study of how children can inherit and express seemingly new traits from their parents *without* changes in the underlying DNA. In other words, the instructions are the same, but something else overrides them.

Being a gene isn't all that it was cracked up to be anymore.

THE TERM *EPIGENETICS* was coined in the 1940s, but the modern discipline is much younger, barely out of diapers. The first big breakthrough actually occurred in 2003—in the form of a skinny brown mouse.

The shocking thing about this skinny brown mouse is that its parents were both fat yellow mice. Actually, they were fat yellow mice from a long line of fat yellow mice. These mice were specifically bred to carry a gene called *agouti,* which gives them their characteristic pale coat and tendency toward obesity. When a male *agouti* mouse mates with a female *agouti* mouse, they have little *agouti* mouse babies time after time—fat and yellow. Or they did until they went to Duke, anyway.

A team of scientists at Duke University separated a gang of *agouti* mice into two groups—a control group and a pregnant group. They didn't do anything special with the control group. They fed it a normal diet and let fat yellow Mickeys mate with fat yellow Minnies, who gave birth to fat yellow babies. No surprise there.

The mice in the experimental group mated as well, but the expectant mothers in this group got slightly better prenatal care—in addition to their normal diet, they were given vitamin supplements. In fact, they were given a combination of compounds that is a variation on the prenatal vitamins given to pregnant women today—vitamin B_{12}, folic acid, betaine, and choline.

The results rocked the genetic world. Fat yellow female mice that had mated with fat yellow male mice had thin brown babies. That seemed to throw everything the scientific community understood about heredity up in the air. A genetic examination of the brown baby mice only added to the mystery. Their genes were the same as their parents'. The *agouti* gene in the thin brown mice was right where it was supposed to be, ready to send out instructions to make them fat and yellow. So what happened?

Essentially, one or more of the compounds in the vitamin supplements fed to the expectant mothers reached down into the mouse embryos and flicked the *agouti* gene into the "off" position. When the baby mice were born, their DNA still contained the *agouti* gene, but it wasn't expressed—chemicals had attached to the gene and suppressed its instructions.

This process of genetic suppression is called DNA methylation. Methylation occurs when a compound called a methyl group binds to a gene and changes the way that gene expresses itself, without actually changing the DNA. The compounds in the vitamin supplements include methyl donors—

molecules that form the methyl groups that become these genetic stop signs.

Th in and brown weren't the only benefits the mice gained through methylation. The *agouti* gene in mice is linked to higher rates of diabetes and cancer. The mice with the switched-off *agouti* genes had significantly lower rates of cancer and diabetes than their parents.

Of course, we've long understood the basic idea that good nutrition in an expectant mother is important for infant health. And we've also known that the connection goes beyond the obvious—sufficient nutrition, healthy birth weight, and so forth—to reduce the likelihood of certain diseases later in life. But until the Duke study, the "how" was very unclear. As Dr. Randy Jirtle, one of the leaders of the study, said:

> We have long known that maternal nutrition profoundly impacts disease susceptibility in their off spring, but we never understood the cause-and-effect link. For the first time ever, we have shown precisely how nutritional supplementation to the mother can permanently alter gene expression in her off spring without altering the genes themselves.

The impact of the Duke study was enormous, and the study of epigenetics has exploded since it was published. You can imagine why.

First, epigenetics erased the conviction that genetic blueprints are written in indelible ink. Suddenly, science had to take into account the notion that a given set of genes is *not* an immutable set of blueprints or instructions. The exact same set of genes can produce different outcomes depending on which genes have undergone methylation and which have not. There was a whole new layer to consider—a set of reactions that acted outside and above the genetic code, changing its result without changing the code itself. (That outside and above is where epigenetics gets its name—from the Greek prefix *epi,* meaning upon, after, or in addition.) This shouldn't have been a *complete* surprise—for fifty years, some researchers have pointed out that the same genes don't always produce the same results: identical twins (who share identical DNA) don't get the same diseases or fingerprints, just similar ones.

Second, the Duke study snuggled right up to the ghost of Lamarck. Environmental factors in the life of the mother were shown to affect the inheritance of traits in her off spring. These factors didn't change the DNA the baby mice inherited, but in changing the way the DNA was expressed, they changed heredity.

After those first mice experiments, other scientists at Duke showed that they could supercharge the brains of mice simply by adding a touch of choline to a pregnant mouse's diet. The choline triggered a methylation

pattern that turned off the gene that normally acted to limit cell division in the memory center of the brain. With the cell division governor turned off, these mice started producing memory cells in high gear—and sure enough, they developed mighty mouse memories. Their neurons fired more rapidly and could fire more often. As adults, these megabrain mice broke all the records in all the mazes.

RESEARCHERS WHO STUDY all kinds of animals—from mammals to reptiles to insects—have long noted the ability of some organisms to produce off spring that seem to be custom-tailored on the basis of the mother's experiences during pregnancy. They noted this ability—but they couldn't really explain it. Once scientists understood the possibility of epigenetic influence on heredity, it all made a lot more sense.

The vole is a furry little rodent that looks something like a fat mouse. Depending upon the time of year its mother is due to give birth, baby voles are born with either a thick coat or a thin coat. The gene for a thick coat is always there—it's just turned on or off depending on the level of light the mother senses in her environment around the time of conception. The developing genome basically gets a weather forecast before it has to go out into the world, so it knows what kind of coat it should grow.

The mother of the tiny freshwater flea *Daphnia* (which isn't really a flea at all; it's actually a crustacean) will produce off spring with a larger helmet and spines if it's going to give birth in an environment crowded with predators.

The desert locust lives in two remarkably different styles depending on the availability of food sources and the density of the local locust population. When food is scarce, as it usually is in their native desert habitat, locusts are born with coloring designed for camouflage and lead solitary lives. When rare periods of significant rain produce major vegetation growth, everything changes. At first, the locusts continue to be loners, just feasting off the abundant food supply. But as the extra vegetation starts to die off, the locusts find themselves crowded together. Suddenly, baby locusts are born with bright colors and a hankering for company. Instead of avoiding one another and hiding from predators through camouflage and inactivity, these locusts gather in swarms, feed together, and overwhelm their predators through sheer numbers.

One species of lizard is born with a long tail and large body or a small tail and small body depending on one thing only—whether their mother smelled a lizard-eating snake while pregnant. When her babies are entering a snake-filled world, they are born with a long tail and big body, making them less likely to be snake food.

In each of these cases—the vole, the water flea, the locust, and the lizard—the characteristics of offspring are controlled by epigenetic effects that occur during fetal development. The DNA doesn't change—but the way it's expressed does. This phenomenon—the mother's experiences influencing gene expression in her offspring—is called a predictive adaptive response or maternal effect.

IMAGINE THE IMPLICATIONS of this for humans. By sending the right epigenetic signals, we can have healthier, smarter, better-adapted babies. As we learn more, we may be able to suppress the genes that express themselves in harmful ways even after birth—or turn helpful genes back on after they have been turned off. Epigenetics has the potential to give us a whole new measure of control over our health. DNA is destiny—until you get out the old methyl Magic Marker and start rewriting it.

The current focus in human epigenetics is on fetal development. It's now clear that the first few days after conception—when a mother may not even know she's pregnant—are even more critical than we've understood. That's when many important genes are switched on or off. And the earlier that epigenetic signals are transmitted, the more significant the potential changes are in the fetus. (In some ways, the womb may be like a tiny evolutionary laboratory, examining new traits to see whether they'll help the fetus survive and thrive; if they won't, the mother miscarries. Researchers have certainly noted that many miscarried fetuses have genetic abnormalities.)

Here's how epigenetics may be partially responsible for the epidemic of childhood obesity. The junk food that fills so many American diets is high in calories and fats, but often very low in nutrients, especially those that are important to a developing embryo. If a newly pregnant mother spends the first weeks of her pregnancy eating a typical junk-food-laden diet, the embryo may receive signals that it's going to be born into a harsh environment where critical types of food are scarce. Through a combination of epigenetic effects, various genes are turned on and off and the baby is born small, so it needs less food to survive.

But that's only half the story. Almost twenty years ago, a British medical professor named David Barker (who won the Danone International Prize for nutrition in 2005) first suggested a link between poor fetal nutrition and later obesity. His theory, known as the Barker Hypothesis or the thrifty phenotype hypothesis, has been gaining ground ever since. (Phenotype is the physical expression of your genotype; in other words, if you have one parent with attached earlobes and the other parent with detached earlobes, you will have detached earlobes, because that trait is dominant—detached earlobes

would be part of your phenotype. Epigenetic effects influence your phenotype without changing your genotype. So, in this hypothetical example, if a methyl marker turned off your gene for detached earlobes, your *phenotype* would change—you'd have attached earlobes—but your genotype would remain the same. You'd still have the gene for detached earlobes to pass on to your children in either the on or off state; it would just be deactivated in you.) According to the thrifty phenotype hypothesis, fetuses that experience poor nutrition develop "thrifty" metabolisms that are much more efficient at hoarding energy. When a baby with a thrifty phenotype was born 10,000 years ago during a time of relative famine, its conservationist metabolism helped it survive. When a baby with a thrifty metabolism is born in the twenty-first century surrounded by abundant food (that is also often nutritionally poor but calorie rich), it gets fat.

Epigenetics makes the thrifty phenotype hypothesis even more compelling, because it helps us to understand how a mother's eating habits could affect the metabolic makeup of her children. If you're thinking about having a baby, you're probably already asking yourself what you should eat and when during your pregnancy. We don't know enough yet to understand exactly when human fetuses reach epigenetic trigger points. But animal studies suggest the process starts very early.

One recent study of rats showed that when pregnant rats were fed a low-protein diet for just the *first four days* of pregnancy—before the embryo had even implanted in the uterus—their babies were prone to high blood pressure. Experiments with sheep showed similar maternal effects. Pregnant sheep that were underfed during the early days of pregnancy—again, even before the embryo implanted in the mother's uterus—gave birth to offspring that rapidly developed thickened arteries because their slower metabolisms stored more food as fat.

How do we know these are adaptive responses, as opposed to birth defects resulting from the mother's poor nutrition? Because the health problems—thickened arteries and increased weight—only occurred when the baby sheep were provided with normal diets. Baby sheep whose mothers were undernourished while pregnant showed no sign of arterial thickening when they were also undernourished as toddlers.

Most of the epigenetic effects currently under study involve mothers, not fathers. In part, that's because an embryo or fetus never interacts with its father's environment, so many scientists believed epigenetic modifications only occurred after conception, in response to information the fetus received about the mother's environment. However, there is new and intriguing evidence that fathers can pass information to their offspring as well. A British study found that men who started to smoke before puberty had sons who were significantly fatter than normal by the time they were nine; this correlation was found only in sons, so scientists think these epigenetic

markers are passed on the Y chromosome. (Intuitively, you might expect the children of smoking fathers to be *smaller,* not fatter. It's possible that this effect is analogous to the thrifty phenotype, in which poor maternal nutrition in the early stages of pregnancy leads to the birth of small babies with thrifty metabolisms who have a high tendency to become fat. In this case, there may be an epigenetic change in the father's sperm triggered by the toxins in the smoke the father is inhaling. Those toxins would indicate a difficult environment, so the sperm is ready to create a baby with a thrifty metabolism. And when that thrifty metabolism is combined with a typical Western diet, the likelihood of that baby growing up to be a fat child dramatically increases.)

The lead scientist on the study, Marcus Pembrey, a British geneticist, believes this proves the existence of paternal effects in addition to maternal effects. He called this "proof of principle. The sperm have captured information about the ancestral environment, and this is modifying the development and health of subsequent generations."

This lends a whole new meaning to sons paying for the sins of their fathers.

MOM AND DAD may not be the only epigenetic influences in your life. Grandpa and Grandma may be reaching down from their perch above you in the family tree, leaving their own marks. That's certainly what many of the most prominent epigenetic researchers—from the authors of the fat yellow mice study at Duke to the researchers behind the smoking fathers report in London—think. They all believe that epigenetic changes can be passed through the germ line for many generations.

In the case of maternal inheritance, the opportunity for your ultimate genotype to get a methyl markup in your grandmother is actually very direct. When a human female is born, she already has the complete set of eggs she will have for life in her baby ovaries. As strange as it sounds, that means that the egg you developed from, with half of your chromosomes, was created in your mother's ovaries while she was still in your grandmother's womb. And new research demonstrates that when your grandmother passed epigenetic signals to your mother, she was also passing those signals to the egg that would eventually provide half of your DNA.

Just as epigenetics has helped to unlock the mystery of thin-coated voles and sociable locusts, it's now helping to explain a series of confusing correlations researchers have gathered over the last century. A group of researchers in Los Angeles found that children whose grandmothers smoked while pregnant were more likely to have asthma than children whose mothers smoked while pregnant. Before we started to crack the epigenetic code, this

correlation was impossible to explain. Now, scientists realize that the smoking grandmother triggered an epigenetic effect in her fetal daughter's supply of eggs. (Incidentally, if you're puzzled as to why the grandmothers' smoking habits affected their eggs more than their fetuses, you're not alone; scientists haven't figured that out yet.)

A harsh winter and a cruel embargo imposed by the Nazis combined to cause the Dutch famine of 1944 and 1945. Thirty thousand people died during the "Hunger Winter," or Hongerwinter, as the Dutch call it. An examination of birth records following the famine is one of the ways Barker confiremd his thrifty phenotype hypothesis. Women who were in the first six months of pregnancy during the Hongerwinter gave birth to small babies who grew up to be more prone to obesity, coronary disease, and a variety of cancers.

Although the results are still controversial, researchers reported an even bigger surprise around twenty years later when their studies indicated that the *grandchildren* of those women were also born with low birth weights. Is it possible that the methyl markers triggered by poor nutrition during the famine were passed on to the next generation? That's not known yet, but the effects of methylation, it seems, are real.

Many leading epigenetic scholars think epigenetic changes represent evolution's subtle effort to tweak an existing genome, although that's still quite contentious. The scientists at Duke who published the mouse study wrote:

> Our findings show that early nutrition can influence the establishment of epigenetic marks…[that] affect all tissues, including, presumably, the germ line. Hence, incomplete erasure of nutritionally induced epigenetic alterations…provides a plausible mechanism by which adaptive evolution may occur in mammals.

In other words, when methyl markers aren't erased, they can be passed on generation after generation, ultimately leading to evolution. Or in *other* other words, traits *acquired* by a parent or grandparent can ultimately be *inherited* by his or her descendants. Lamarck must be turning in his grave. The theory that he didn't come up with is on the verge of becoming all the rage. Marcus Pembrey, the scientist behind the parental smoking study, calls himself a "neo-Lamarckian." And Douglas Ruden, a researcher at the University of Alabama, told a reporter from *The Scientist,* "Epigenetics has always been Lamarckian. I really don't think there's any controversy."

MOST OF THE methyl effects we've talked about so far involve changes that take place before birth. But epigenetic changes occur throughout life, as the placement of methyl markers turns some genes off and the removal of methyl markers turns other genes back on.

In 2004, Michael Meaney, a professor at McGill University in Canada, published a report that caused nearly as big a sensation as the Duke report about yellow and brown mice. Meaney's study showed that the interaction between mothers and their offspring *after* birth provoked the placement of methyl markers that caused significant epigenetic changes.

Meaney studied the behavior of rats that received different levels of attention from their mothers in the first few hours after birth. Pups that were gently licked by their mothers grew into confident rat babies that were relatively relaxed and could handle stressful situations. But rats that were ignored by their mothers grew to be nervous wrecks.

Now, this sounds like an experiment ripe for a nature versus nurture debate, doesn't it? Those on the nature side would argue that rat moms with bad social skills passed on their emotionally troubled genes to rat babies that grew up to have bad social skills, while the well-adjusted rats gave their babies well-adjusted genes. That makes sense as far as it goes—except that Meaney and his colleagues pulled a mate-and-switch. They gave babies from standoffish mothers to loving mothers, and vice versa. Pups that were fawned over grew to be calm regardless of their natural mother's behavior.

Are all you nurture advocates out there smelling victory? If rats that were treated well turned out well regardless of their genetic makeup, then that means their personalities developed in response to their parenting. Score one for Mother Nurture.

Not so fast.

An analysis of the rats' genes showed striking differences in methylation patterns between the two sets of rats. Rat pups that were attentively groomed by their mothers (biological or adopted) showed a *decrease* in methyl markers around the genes involved with brain development. The mothers' gentle attention somehow triggered the removal of methyl markers that would otherwise have blocked or impeded the development of a part of their babies' brains—almost as if they were licking them off. The part of the brain that dampened the stress response was more developed in those babies. This wasn't nature *versus* nurture; this was nature *and* nurture.

Meaney's paper was another epigenetic blockbuster. Something as simple as parental grooming was *changing the expression of a living animal's genetic code.* The notion was so shocking that some people had a hard time accepting it. One reviewer at a prominent journal actually went so far as to write that, despite the researchers' carefully marshaled evidence, he refused to believe it could be true. It just wasn't supposed to happen like that.

But it does.

WE DON'T REALLY know for sure whether parental care for human infants has the same kind of effect on the development of human brains. In one sense, though, it doesn't matter—because we already know that parent-child bonds from birth through early childhood have a profound impact on emotional development. We know that the emotional state of loving, responsive parents gets passed on to their children in a kind of mental methylation—and so does anything that increases a parent's anxiety. Everything from a dissolving marriage to health problems to financial trouble can raise the stress of a new parent and interfere with the child-parent relationship. Children whose parents are overly stressed are more prone to depression and have less self-control. Children whose parents are relaxed and available tend to be happier and healthier.

And while we don't know whether neonatal parenting is actually changing brain development, scientists who study this epigenetic connection in animals believe it's very unlikely that humans don't share it. In fact, the total picture suggests humans should be *more* prone to epigenetic effects in infancy. After all, cognitive development and physical development after birth in humans are much more significant than they are in most other mammals.

LIKE MUTATION, METHYLATION is neither good nor bad on its own—it all depends on what genes are being turned on and what genes are being turned off and for what reason. Good nutrition in pregnant mice led to the *addition* of methyl markers on the *agouti* gene that freed a generation of baby mice from a fat yellow future. Parental grooming in rats provoked the *removal* of methyl markers around genes responsible for brain development. The same thing is true in humans. Some genes are better turned off, and there are other genes that we want on duty 24/7. Methylation also doesn't always just turn a gene completely off. Genes can be partially methylated, and the degree of methylation correlates to how active the gene remains—the less methylation, the more active it is.

One set of genes that we want always on guard are those that suppress tumors and repair DNA. Those genes are the storm troopers and flight surgeons of the anticancer corps. Scientists have identified dozens of these genetic guardians—when they're shut down, cancerous cells have free rein.

A recent article in *Science News* told the story of two identical twins, Elizabeth and Eleanor (not their real names), who were born on November

19, 1939. From the moment the twins were born, they were treated the same because their mother never wanted either girl to feel she was more—or less—favored. Elizabeth said, "We were treated like a unit—more like one person instead of two separate individuals." They moved apart more than forty years ago, in their early twenties, but they're still very similar. From the way they look to the things they care about, it's clear that they're identical twins. With one big exception—seven years ago, Eleanor was diagnosed with breast cancer. Elizabeth has never been.

Identical twins share the same exact DNA—but DNA isn't fate. And one of the reasons is methylation. It's possible that forty-plus years of exposure to a different environment produced a different methylation pattern around Eleanor's genes, a pattern that unfortunately may have led to breast cancer.

In 2005, Manel Esteller of the Spanish National Cancer Center, along with colleagues, issued a report showing that identical twins shared almost identical methylation patterns at birth that diverged as they grew older. And the report indicated that those patterns diverged much more dramatically when the twins lived apart for most of their lives, just as Eleanor and Elizabeth have. Esteller said:

> We believe these different epigenetic patterns in twins depend many times on the environment, whether it's exposure to different chemical agents, diets, smoke, or whether people live in a big city or the countryside.

There's more evidence coming in to support the idea that methylation of specific genes is tightly connected to cancer. In Germany, scientists at a company called Epigenomics have reported an overwhelming connection between breast cancer recurrence and the amount of methylation of a gene called *PITX2*. Ninety percent of the women with low methylation of the *PITX2* gene were cancer-free after ten years, while only 65 percent of the women with high methylation were as lucky. Ultimately, this kind of information will help doctors to custom tailor cancer treatments—the more help they can get from the body's natural cancer fighters, the less aggressive they may need to be in terms of chemotherapy and radiation. The data from Epigenomics is already being used to help women who have low methylation of *PITX2* decide if chemotherapy is necessary after their tumor is removed.

Scientists are establishing clear links between methylation of cancer-fighting genes and cancer-causing behavior. Over time, habits like smoking can cause a massive buildup of methyl markers around these genes. Scientists call this hypermethylation. People who smoke exhibit

hypermethylation around genes that would otherwise combat lung cancer. Genes that are supposed to fight prostate cancer are hypermethylated in smokers, too.

In part because of the hypermethylating effect of potentially carcinogenic habits, methylation patterns can also be an early warning signal. In India, millions of people are addicted to betel nuts, a peppery seed that stains the teeth and gums red when it's chewed and, like nicotine, is mildly intoxicating, highly addictive, and seriously carcinogenic. Because of betel nut chewing, oral cancer is the most common cancer in Indian men. And because oral cancer often doesn't manifest any symptoms for a long time, it's often fatal—70 percent of the people diagnosed with oral cancer in India eventually die of it. A lifetime of betel nut chewing can lead to hypermethylation of three cancer-fighting genes—one that suppresses tumors, one that repairs DNA, and one that hunts out lone cancer cells and gets them to self-destruct. Reliance Life Sciences, the Indian company that established this link, has developed a test to measure the degree of methylation in these genes. "We'd like to use the degree of methylation at sites near these three genes as a predictive marker to qualitatively say how far a person is from developing oral cancer," said Dr. Dhananjaya Saranath, one of the scientists at Reliance Life Sciences. Ultimately, tests like this could be an enormous tool in measuring cancer risk, leading to much earlier diagnosis and much higher survival rates.

RIGHT NOW EPIGENETICS is in a bit of a the-more-we-know-the-less-we-understand phase. One thing is clear—it seems pretty certain that things we know to be bad for us can end up being bad for our descendants, as epigenetic markers get passed on from generation to generation. So smoking two packs a day and living a Super-Sized life may actually make your children—and even their children—more prone to disease.

But what about using methyl markers to have a positive influence on our kids? Folic acid and B_{12} worked for mice—will it work for humans? If your family's had a bit of a weight problem as far back as you can remember, can a few methyl markers prevent that heritage from weighing your baby down? The truth is, we just don't know—and we don't even know everything we don't know yet.

Here's the first thing we don't know—we don't have anywhere near a complete understanding of which genes are turned off or turned down by which methyl donors. For example, methylation of a gene that influences hair color might lead to a harmless change—but the same process that triggered methylation of the hair color gene may also be suppressing a tumor suppressor. To complicate things further, methyl stop signs often land near

transposons—those jumping genes. When that transposon inserts itself somewhere else in the genome, it may carry methyl markers with it where they may attach themselves to another gene, muting its expression or at least turning down the volume.

In fact, the authors of the Duke study were so impressed by the enormous range of potential epigenetic effects that they issued a word of caution to anyone interested in applying the results of their research to humans:

> These findings suggest that dietary supplementation, long presumed to be purely beneficial, may have unintended deleterious influences on the establishment of epigenetic gene regulation in humans.

In other words, we don't really know everything that's going on here, folks.

To be clear, if you're getting ready to have a baby, this isn't to suggest that you throw out the container of vitamins your doctor prescribed. These vitamins have a lot to recommend them—as we mentioned a few chapters ago, folic acid is very important during pregnancy. Study after study has shown that folic acid supplements reduce birth defects that can cause damage to a developing brain or spinal cord. The connection is so strong that the government required grains to be fortified with folic acid much as drinking water is fortified with fluoride. And there's been a corresponding decrease in diseases, such as spina bifida, that are related to folic acid deficiency in pregnant women.

That's a wonderful thing—but it may not be the whole story. Our understanding of epigenetics is so immature we have to be wary about unintended consequences. We just don't know what other genes may be influenced by pumping methyl donors into the food supply, and we probably won't know for years.

When doctors expect a pregnant woman to give birth prematurely, she is often injected with a drug, usually betamethasone, to help speed up the development of her fetus's lungs, dramatically improving its chance of survival. Now, there are signs that children whose mothers received multiple doses of betamethasone have increased levels of hyperactivity and slower than normal overall growth. A recent University of Toronto study demonstrated that these effects may continue for multiple generations. The leader of the study believes the betamethasone causes epigenetic changes in the fetus that are passed on to its own offspring in turn. One doctor who specializes in treating premature babies said the study was "terrifying beyond comprehension."

Vitamins and drugs that cause methylation in addition to fulfilling their primary purpose are just the beginning. Now we're starting to see drugs actually designed to affect methylation patterns. The first of these drugs was approved by the Food and Drug Administration in 2004. Called azacitidine in its generic form, it was hailed as a breakthrough for the treatment of myelodysplastic syndrome, or MDS. MDS is a collection of blood disorders that is very difficult to treat and often leads to potentially deadly leukemia—a new drug for MDS would be a significant advance. Azacitidine inhibits the methylation of certain genes in blood cells, helping to restore proper DNA function and reducing the risk that MDS will develop into leukemia. Azacitidine was met with tremendous excitement at its introduction. Peter Jones, a professor of biochemistry and molecular biology at the University of Southern California, said:

> This is the first approved drug in a new kind of therapy—epigenetic therapy. That gives it tremendous potential importance not just in this disease, but in a host of others as well.

Of course, in a report by Dr. Jones and some colleagues, he also noted:

> It is apparent that we are just at the beginning of understanding the substantial contribution of epigenetics to human disease and there are probably many surprises ahead.

"Many surprises ahead." Well, he was right. Six months after azacitidine was approved, researchers at Johns Hopkins published a report of their investigation into the epigenetic effects of two drugs, one of them a close chemical relative of azacitidine. These drugs were all but spray painting the genome with new methylation patterns, turning off as many genes as they were turning on—hundreds of each.

Don't get me wrong—epigenetics has unbelievable potential to have a positive impact on human health. A Rutgers University professor named Ming Zhu Fang has studied the effect of green tea on human cell lines. He's found that compounds in green tea inhibit the placement of methyl markers on genes that help to fight colon, prostate, and esophageal cancer. Methylation of those genes would take them out of the cancer suppression business—by inhibiting their methylation, green tea keeps them in the anticancer fight.

The same Duke team responsible for the original study of vitamin-triggered methylation in *agouti* mice has demonstrated a similar methylating effect from genistein, the estrogenlike compound found in soy. They've

speculated that genistein may also help to reduce the risk of obesity in humans, perhaps even helping to explain why Asian rates of obesity are comparatively low. But again, their speculation is tempered with a note of caution. Dana Dolinoy, one of the study's authors, said:

> What is good in small amounts could be harmful in large amounts. We simply don't know the effects of literally hundreds of compounds that we intentionally or inadvertently ingest or encounter each day.

There are 3 billion base pairs of nucleotides in the human genome engaged in a vast and complex dance that makes us who we are. We need to be awfully careful when we start to change the choreography, especially given our current lack of precision. When you try to move one dancer with a bulldozer, you're pretty darn certain to scoop up more than one Rockette.

IF THAT'S NOT complicated enough, methyl markers aren't the only way genes are turned on or off. There is a whole system of promoters and repressors that govern how much a given gene expresses itself by transcribing into mRNA and then translating into a protein. This system amounts to an internal regulator that can turn on, turn off, or even crank up production of specific proteins in response to the body's changing needs.

This is how people build up their tolerance to drugs and alcohol, for example. When someone drinks alcohol, the genetic promoters in his or her liver cells crank up production of the enzyme (remember alcohol dehydrogenase?) that helps to break it down. The more you drink, the more your liver produces alcohol dehydrogenase—its biological anticipation of the next drink. And the reverse is also true—you might notice your tolerance drop after a period of sustained teetotaling, because your body slows down the production of alcohol dehydrogenase when it no longer senses the regular need for it.

There's a similar phenomenon with other drugs, from caffeine to many prescription drugs. Have you ever been prescribed a drug that gave you some unpleasant side effects only to have your doctor tell you just to wait a few weeks and they'll go away? If you have, and they've gone away, you've experienced another form of gene expression. Your body adapted to the presence of the drug by promoting or suppressing the expression of specific genes that helped you to process it.

IF YOU REALLY want to understand how *little* we understand about possible epigenetic and maternal effects, consider the following. In the months immediately after the terrorist attacks on New York and Washington on September 11, there was a dramatic spike in the number of late-term miscarriages—in California. It would be tempting to assume that there is an obvious, behavior-related explanation for this—higher stress made it harder for some expectant mothers to take care of themselves. It is tempting to accept this except for one thing—the rise in miscarriages only affected male fetuses.

In California, in October and November 2001, there was a 25 percent increase in the rate of male miscarriages. Something—and we don't know what—in the mother's epigenetic or genetic architecture sensed that she was carrying a boy and triggered a miscarriage.

We can speculate why this occurred, but we really don't know the truth. Males are both more demanding physiologically on the mother's body during pregnancy and less likely to survive if malnourished as children. Perhaps we have evolved a kind of automatic resource conservation system that is triggered in times of crisis—lots of females and a few strong males gives a population a better chance for survival than the other way around.

Whatever the evolutionary reason, it is clear that these pregnant women responded to a perceived environmental threat with a dramatic—and automatic—reaction. The fact that the actual attack occurred so far away only makes it more interesting. And this isn't the first time such a reaction has been documented. During the reunification of Germany in 1990, the birth rate in the former East Germany (where reunification was difficult, tumultuous, and anxiety-producing) skewed toward females. A study of births after the ten-day war in Slovenia during the Balkan conflicts of the 1990s and another study of births after the Hanshin earthquake of 1995 in Kobe, Japan, showed evidence of a similar pattern.

On the other side of the coin, there is evidence that in times after great conflict, the male birth rate goes up. That's what happened after World War I and World War II. A more recent study of six hundred mothers living in Gloucestershire, England, revealed that those who predicted that they would live well into old age were more likely to have male babies than those who predicted that they would die relatively young.

Somehow, an expectant mother's mental state can trigger physiological or epigenetic events that can affect her pregnancy and the relative viability of male or female fetuses. Good times mean more boys. Tough times mean more girls. And epigenetics means we've got more—much more—to learn.

THE FIRST BIG epigenetic breakthroughs were published just as other scientists were announcing the completion of the Human Genome Project—the mammoth ten-year effort to map out the sequence of all 3 billion nucleotide pairs that make up our DNA. When they were done, project organizers announced that they had effectively created "all the pages of a manual needed to make the human body."

And then epigenetics really rained on their parade. After ten years of painstaking work, the scientists came out of their labs to find out that their map was only a starting point. The scientific community might as well have said, "Thanks for the map. Now can you tell us which roads are open and which roads are closed so we can make some use of it?"

Of course, epigenetics doesn't really make the Human Genome Project worthless—to the contrary, a map of the epigenome has to begin with a map of the genome. And sure enough, work has begun to make one. In the fall of 2003, a group of European scientists announced the Human Epigenome Project. Their goal is to add an indicator to every spot where methyl markers can attach and change the expression of a given gene. As they say:

> The goal of the Human Epigenome Project is to identify all the chemical changes and relationships…that provide function to the DNA code, which will allow a fuller understanding of normal development, aging, abnormal gene control in cancer and other diseases, as well as the role of the environment on human health.

The money is slowly coming in, and they hope to have most of the epigenome mapped in the next few years, but it won't be easy.

Science never is.

From: Survival of the Sickest: The Surprising Connections Between Disease and Longevity, 2007, by Sharon Moalem and Jonathan Prince; Chapter 7.