

EPIGENETICS

It's what turns you on...and off

BY DAVID SCHARDT

Why don't identical twins always have the same personality and the same risk of disease, even though they have identical genes? How does a brain cell know to make only more brain cells and not heart or kidney cells? Could a woman's diet or weight while she's pregnant influence whether her child has a higher risk of illness decades later?

The answers may lie in how our cells turn our genes on and off. If scientists can better understand that process, they may be able to prescribe specific foods or drugs that can slash our risk of obesity, cancer, diabetes, and more.

Making your Mark

When all the genes in a human being were recorded for the first time in 2000, "there was a lot of hope that we would then have a complete understanding of human disease," says Rob Waterland, an associate professor of pediatrics and molecular and human genetics at the Baylor College of Medicine in Houston. "But that certainly hasn't happened."

"We know that heredity plays a role in a large number of diseases like diabetes,

Alzheimer's, and cancer," notes geneticist Evan Rosen of Harvard University's Beth Israel Deaconess Medical Center in Boston. "Yet mutations in genes account for a minuscule portion of the inherited risks."

"We're finding that the impact of genetics on health is much more complicated than just the DNA sequence in an individual's genes," explains Waterland.

Genes consist of strings of DNA that serve as blueprints for synthesizing insulin, heart muscle, antibodies, and the other proteins that make life possible. Some

of our 20,000 genes are always active—producing their proteins—while others get switched on and off at various times.

What flips the switch? Things called epigenetic marks.

A common example is a *methyl* group (which consists of a carbon atom bonded to three hydrogen atoms). When enough methyl groups become attached to a gene, they can turn the gene off (see "Flipping the Gene Switch"). The same can happen when methyl groups become attached to the histone proteins that DNA strands wrap themselves around. But if enough *acetyl* groups—each consists of two carbons, one oxygen, and three hydrogen atoms—become attached to a histone, the gene may get switched *on*.

Some epigenetic marks—ones that tell the genes in brain cells to make more brain cells but not liver cells, for example—appear soon after conception and last a lifetime. Others can appear and disappear at any time, in response to diet, weight, stress, and exposure to tobacco or chemicals like DDT.

"What's particularly exciting about epigenetic marks is that we may be able to alter them with our diet," says Trygve Tollefsbol, a professor of epigenetics and gene regulation in cancer and aging at the University of Alabama at Birmingham.

May. So far, the evidence is limited to test tubes and animals.

Flipping the Gene Switch

When enough *methyl* groups become attached to a gene (a section of DNA) or a histone (a protein that DNA strands can wrap themselves around), the gene can become less active or turn off. When enough *acetyl* groups become attached to a histone, the gene can turn on or become more active. Both may affect our risk of obesity or disease decades later.

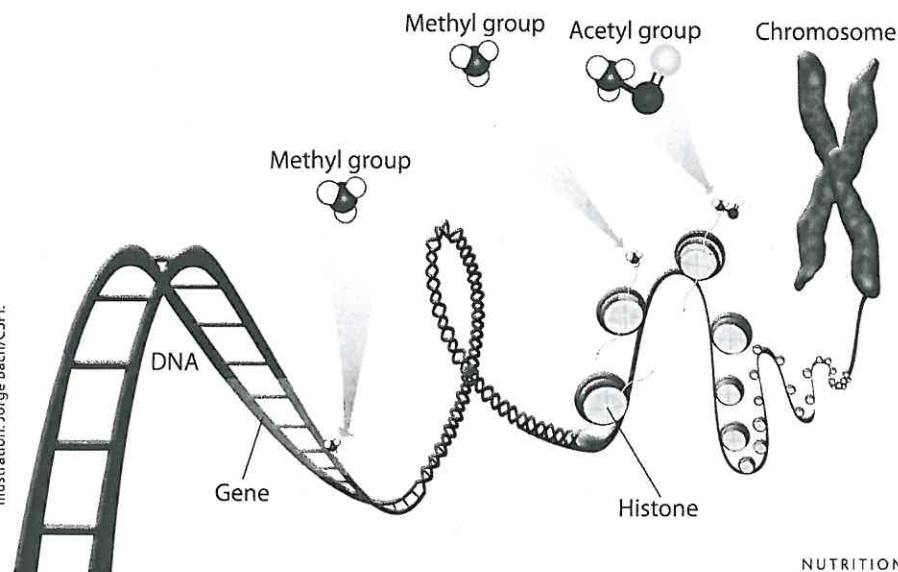


Illustration: Jorge Bach/CSPI.

Of Mice and Humans

An agouti mouse can grow up fat and sick or lean and healthy. It depends on what happens to one particular gene during the animal's time in the womb.

If that gene is over-methylated—and therefore dimmed or silenced—the mouse grows up with a darker coat and is lean and healthy. If the gene isn't methylated—and therefore remains turned on—the mouse grows a lighter yellow coat, becomes obese, and is prone to cancer and diabetes later in life.

In 2003, when Waterland was at Duke University, he and Randy Jirtle altered the diet of yellow-coated mother agouti mice during their pregnancy.

"We fed the mothers extra folic acid, vitamin B-12, betaine, and choline," says Waterland. "Those nutrients provided methyl groups that increased the methylation of the *agouti* gene."

> > > >

That silenced the gene, so that the mothers gave birth to leaner, healthier offspring.¹ The same happened when the researchers fed the mothers genistein, an estrogen mimic that occurs naturally in soybeans.² And the offspring of those mothers were less likely to grow up to be obese adults than mice whose mothers got no genistein.

While other studies have found that darker-coated agouti mice are less likely to develop diabetes or cancer, researchers haven't tested whether B vitamins or genistein lowers their risk of those diseases.

What about humans? If scientists could identify epigenetic marks in people, then change them with diet or drugs, they could, at least in theory, silence cancer, obesity, or diabetes.

That's the tantalizing promise. But researchers have a long way to go.

Cancer

"We used to think that cancer was caused mainly by mutations of genes, but we now believe that epigenetic aberrations are responsible for more than half of cancer cases," says Trygve Tollefsbol, who is senior scientist at the University of Alabama at Birmingham's Comprehensive Cancer Center.

"That's an important change because genetic mutations are very difficult, if not impossible, to correct, while epigenetic marks are potentially reversible," he explains.

"If you have a *BRCA1* gene mutation that increases your risk of breast cancer, you can't change that mutation," says Emily Ho, an epigenetics researcher at the Linus Pauling Institute at Oregon State University. But if an epigenetic mark turns that gene on or off, "that's potentially something you can change either with drugs or with diet." (That *BRCA1* mutation led actress Angelina Jolie to undergo a preventive double mastectomy in February.)

Our cells have families of "oncogenes" that can promote cancer by making cells proliferate. "Think of them as gas pedals that speed up cell growth," says Tollefsbol. We also have families of genes that suppress tumor growth. "Think of them as brakes," says Tollefsbol.

In healthy cells, the brake genes and the gas pedal genes are in balance. But if the oncogenes turn on (because they lack methyl groups) or the tumor-suppressing genes turn off (because they have too many methyl groups), "it's like a car

barreling down the road with a stuck gas pedal or no brakes headed for cancer," says Tollefsbol.

So far, says Ho, researchers know that certain compounds in foods can change epigenetic marks in cancer cells, at least in test tubes. For example, the curcumin in turmeric, the EGCG in green tea, the genistein in soybeans, the resveratrol in grapes, and the sulforaphane in cruciferous vegetables like broccoli can hinder the enzymes that help attach methyl groups or remove acetyl groups from genes.³

"We've shown that feeding sulforaphane to mice that are genetically susceptible to colon cancer reduced the number and size of intestinal polyps in the animals and also altered epigenetic marks in their polyps," says Ho.⁴ "But we're still not entirely sure whether these changes to epigenetic marks are a cause or a consequence of the lowered tumor growth."

In humans, there is only weak evidence linking foods rich in genistein, sulforaphane, curcumin, or EGCG to a lower risk of cancer.

"There's a lot we still don't know, such



Unless epigenetic changes silence a certain gene, Agouti mice grow up obese and unhealthy (left).

as whether people can absorb enough of these substances from food, whether the compounds they're metabolized into can get to the right cells, and how rapidly we clear, or remove, the compounds from our bodies," notes Ho.

Meanwhile, scientists are beginning to investigate how epigenetics could help identify people at high risk for cancer.

Researchers at the National Institute of Environmental Health Sciences studied 910 women who had a sister with breast cancer. Those who were diagnosed with breast cancer during five years of follow-up had a different DNA methylation pattern in blood samples taken when they entered the study

than those who remained cancer-free.⁵

"Since there was, on average, only 1.3 years from blood draw to diagnosis, we don't know whether the methylation pattern was a risk factor or a result of the cancer," says Regina Ziegler of the National Cancer Institute, who co-authored an editorial accompanying the study.⁶

"It could be an early and useful marker of disease that has not yet been clinically detected."

Obesity

"The nutritional status of a mother during pregnancy can have a profound, lifelong impact on whether her children become overweight or obese," says Baylor's Rob Waterland.

That was shown dramatically among the survivors of the Dutch "Hunger Winter," a period of starvation in the Netherlands during the winter of 1944-1945, the final year of World War II. When the Germans set up a blockade to keep food and fuel from reaching the western part of the country, people there had fewer than 1,000 calories—and sometimes as few as 500 calories—of food to eat a day.

Fifty years later, those who had been conceived during the days when food was most scarce weighed an average of 14 more pounds, had waists that were an average of 1½ inches larger, and were three times more likely to have coronary heart disease than those whose mothers were in their second or third trimester at the time.⁷

What role could epigenetics have played? Researchers found that an important gene for growth during pregnancy (it's the blueprint for making insulin-like growth factor 2, or IGF2) was less methylated—more turned on—in people who had been conceived during the worst of the starvation than in those who were less than six months away from being born.⁸

The study was the first evidence "that early-life environmental conditions can cause epigenetic changes in humans that persist throughout life," said the authors.

Those changes may have altered the fetuses' metabolism so that they could get the most out of the limited amount of food available. But when food eventually became abundant again after the war, the epigenetic changes that resulted in a "thrifty" metabolism were never reversed, and the children were more likely to weigh more as adults.

It's not just too few calories that may lead to overweight offspring. It's also too many.

Consider two studies that looked at 162 obese Canadian mothers who had children before; and then after, weight-loss surgery. The children who were born after the surgery were half as likely to grow up overweight or obese as the children who were born before the surgery.^{9,10}

And the same researchers recently reported that 25 children born before their mothers lost an average of 103 pounds following gastric bypass surgery had different patterns of epigenetic marks than 25 of their siblings who were born after their mothers had the surgery. Genes that play a role in diabetes, inflammation, and cardiovascular disease were most affected.¹¹

"The weight-loss studies are evidence that epigenetic changes in response to the mother's health during pregnancy may play a role in whether someone grows up lean or overweight," says Waterland.

"But there could be other explanations," he adds. For example, weight-loss surgery could have changed the women's gut microflora or blood sugar levels, or how their bodies used insulin to get blood sugar into cells. And that could have accounted, at least in part, for changes in their children's weight.

It's not just mothers who may matter.

In a study of 79 newborns, Duke University researchers reported that the gene for IGF2 was less methylated—more turned on—in those born to obese fathers than in those born to normal-weight fathers.¹² That might increase those children's risk of becoming obese adults.

How much newborns eat soon after birth may also be critical. In a 2013 study, Rob Waterland and his colleagues found that mice that were overfed during their first few weeks of life showed subtle changes in the methylation of genes in their hypothalamus that persisted into adulthood. (The hypothalamus is a specialized region in the brain that helps regulate body weight.) The overfed mice grew up to become heavier and fatter adults.¹³

"Epigenetic changes can be induced by early over-nutrition and may have a major long-term impact on behavior and weight," says Waterland.

Beyond Cancer & Obesity

Scientists are looking at whether epigenetics plays a role in other diseases. Most of the research is in its infancy.

Alzheimer's disease and cognitive decline. "Epigenetic changes may be an important part of the chain of events

that leads to cognitive decline and to Alzheimer's disease," says Paul Coleman, director of the L.J. Roberts Center for Alzheimer's Research in Sun City, Arizona.

Coleman and his colleagues studied a pair of identical twins, one of whom developed Alzheimer's disease and one of whom didn't, even though they had identical genes.¹⁴

"We found that the brother with Alzheimer's had suffered a massive loss of DNA methylation in his brain cells," says Coleman. "The healthy brother hadn't."

That's consistent with a postmortem study that showed less methylation of genes in the brains of 20 Alzheimer's patients than in the brains of 20 people



The Sister Study suggests that epigenetic marks may be an early sign of breast cancer.

without Alzheimer's.¹⁵

But there's no way to know if the epigenetic changes caused the dementia or vice versa.

"However, we know from experiments with brain cells in test tubes that certain chemicals can cause epigenetic changes

The Bottom Line

■ Epigenetics may help explain how diet, body weight, physical activity, stress, or exposure to chemicals may increase or decrease our risk of heart disease, cancer, diabetes, and other diseases.

■ We can't change our genes, but we may some day be able to change our genes' epigenetic marks with food and drugs.

■ Not all epigenetic changes are beneficial, so until researchers learn more, don't try to alter your epigenetic marks with food or supplements.

that result in the formation of the plaques and tangles that we think cause Alzheimer's," notes Coleman.

"If we can identify these epigenetic changes early enough in people, we may eventually be able to intervene and postpone or prevent dementia from occurring."

Endocrine disruptors. Chemicals like phthalates (which are used to soften plastic), DDT, and PCBs can disrupt normal activity in the body by mimicking or blocking estrogen or other hormones. Epigenetics may help explain how even trace amounts of those compounds can cause havoc years later.

Take bisphenol A (BPA), which is used to make some hard plastic food containers and the linings of most food and beverage cans.

"When we expose mice in the womb to levels of BPA comparable to what people are exposed to, we see sets of genes that become over-methylated and sets of genes that become under-methylated," explains Dana Dolinoy, assistant professor in environmental health sciences at the University of Michigan School of Public Health.¹⁶

"We were able to reverse this effect of BPA by feeding the pregnant mothers a high-methyl donor diet with lots of folic acid or a diet with lots of soy and its phytoestrogen genistein," adds Dolinoy.¹⁷

So should women who could become pregnant load up on soy or get more than the recommended intake of folic acid, which is a B vitamin?

No, cautions Dolinoy.

"You don't know whether the epigenetic changes from lots of soy or folic acid will be good or bad because it depends on where they occur," she says.

"Hypermethylation of an oncogene can be great, but at a tumor-suppressing gene, it's not. And you have no control over where the methyl groups from soy or folic acid are hitting."

¹ *Mol. Cell Biol.* 23: 5293, 2003.

² *Environ. Health Perspect.* 114: 567, 2006.

³ *Epigenomics* 3: 503, 2011.

⁴ *FASEB J.* 20: 506, 2006.

⁵ *J. Natl. Cancer Inst.* 105: 694, 2013.

⁶ *J. Natl. Cancer Inst.* 105: 678, 2013.

⁷ *Reprod. Toxicol.* 20: 345, 2005.

⁸ *Proc. Natl. Acad. Sci. U S A* 105: 17046, 2008.

⁹ *Pediatrics* 118: e1644, 2006.

¹⁰ *J. Clin. Endocrinol. Metab.* 94: 4275, 2009.

¹¹ *PNAS* 2013. doi:10.1073/pnas.1216959110.

¹² *BMC Medicine* 11: 29, 2013.

¹³ *Diabetes* 2013. doi:10.2337/db12-1306.

¹⁴ *PLoS One* 4: e6617, 2009.

¹⁵ *Neurobiol. Aging* 31: 2025, 2010.

¹⁶ *Birth Defects Res. A Clin. Mol. Teratol.* 88: 938, 2010.

¹⁷ *Proc. Natl. Acad. Sci. U S A* 104: 13056, 2007.