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ALTHOUGH the various cells in your body are all endowed with the same genetic blueprint, they fulfill specialized roles as bone cells, retinal cells, muscle cells, and the like. They do so by keeping various sets of genes switched on or off. Some cells maintain their roles for a long time. The neurons in your brain, for example, never divide, but they keep looking and behaving like nerve cells throughout most of your life. Other cells are different. The top layer of skin cells, the epidermis, is replaced about every five weeks, from constantly dividing stem cells in the deeper layers of that tissue. Those stem cells always produce new skin cells, and not, for example, muscle cells. Therefore, when called for, the system that switches different genes on or off must also be a mechanism that can be passed on from parent cell to daughter cell every time there is a cell division.

In my article in last month's issue, I referred to chemical modifications surrounding and attaching to DNA, modifications that switch different sets of genes on or off without altering the underlying genetic code. But what do such "epigenetic" modifications actually look like, and how do they work? Biologists are beginning to find out.

The first epigenetic modification to be identified is known as DNA methylation. Methylation means the addition of a methyl group to another chemical, in this case DNA. A methyl group is very small. It's just one carbon atom linked to three hydrogen atoms. The DNA code is embodied in much larger components, known as nucleic acid bases. There are four types--adenine, cytosine, guanine, and thymine--often referred to as the DNA alphabet: ACGT. They are arranged in pairs, commonly depicted as forming the rungs of the twisted, ladderlike DNA molecule (the famed double helix). Cytosine (C) is the only one of the four DNA bases that gets methylated [see illustration on page 32].

The methylation reaction is carried out in our cells, and those of most other organisms, by enzymes called DNA methyltransferases, or DNMTs (there are several kinds). The enzymes that add the methyl group to DNA can be described as "writers" of the epigenetic code. These enzymes have a particular preference when it comes to adding a methyl group to a C. Usually there has to be a G immediately downstream from the C on its strand, or side, of the DNA ladder, a sequence known as CpG. (The two DNA strands are coded in opposite directions, so "downstream" depends on which side you are looking at--the "ladder" metaphor isn't quite adequate.) That's probably enough about what methylation is; what does it do?

When a gene is switched on, the sequences of its bases are faithfully transcribed to make messenger RNA (mRNA). The mRNA then carries these protein-making instructions to the structure in the cell that assembles the corresponding protein. In the early 1980s it was shown that if you injected DNA into mammalian cells, the amount of methylation on the injected DNA affected how many copies of the mRNA were produced. The greater the degree of methylation on the injected DNA, the less transcription occurred. In other words, high levels of DNA methylation were associated with switching genes off.

The key work in establishing the importance of methylation in mammalian cells came out of the laboratory of Adrian Bird, at the University of Edinburgh. In 1985 he published a key paper showing that most CpG motifs were not randomly distributed throughout the genome. Instead, the majority of CpG pairs were concentrated just upstream of certain genes, in the "promoter" region. Promoters are the stretches of the genome to which the DNA transcription machinery binds and starts copying DNA to form mRNA.

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Regions with high concentrations of CpG motifs are called CpG islands. The promoters of about 60 percent of protein-coding genes contain CpG islands. When these genes are active, the levels of methylation in the CpG islands are low. The CpG islands tend to be highly methylated only when the genes are switched off.

For quite some time there was considerable debate about what this association meant. It was the old cause-or-effect debate. One interpretation was that genes were repressed by some unknown mechanism and then the DNA became methylated. In this model, DNA methylation was just a consequence of gene repression. The other interpretation was that the CpG islands became methylated, and it was this methylation that switched the gene off. In this model, the epigenetic modification actually causes the change in gene expression.

Although there is still the occasional argument about this among competing labs, the vast majority of scientists in this field now believe that the data generated in the quarter century since Bird's paper are consistent with the second, causal model. Under most circumstances, methylation of the CpG islands at the start of a gene turns that gene off.

BIRD WENT ON TO INVESTIGATE how DNA methylation switches genes off. He showed that 'there's a protein that binds to methylated CpG sites. It is called Methyl CpG binding protein 2, or for short, MeCP2. But MeCP2 doesn't act as an additional modification of the DNA. Its role is to enable the cell to interpret the modifications on a DNA region. MeCP2 is an example of a "reader" of the epigenetic code.

Once MeCP2 binds to the methylated C in a gene promoter, it not only hinders DNA transcription on its own but also attracts other proteins that help to switch the gene off. Where genes and their promoters are very heavily methylated, binding of MeCP2 seems to be part of a process whereby that region of a chromosome gets shut down almost permanently. The DNA becomes incredibly tightly coiled, and the gene transcription machinery can't get access to the alphabet of bases to make mRNA copies.

This is one of the reasons why DNA methylation is so important. Remember those neurons that faithfully do your brainwork from youth into old age? DNA methylation has kept certain regions of their genome incredibly tightly compacted and certain genes completely repressed. That is why your brain cells never produce hemoglobin, for example, or digestive enzymes.

But what about the other situation, the example of skin stem cells dividing very frequently but always just creating new skin cells, rather than some other cell type such as bone? In this situation, the pattern of DNA methylation is passed from the dividing "mother" cell to the two "daughter" cells.

First, when the two strands of the DNA double helix separate, each reconstitutes its missing half, because each can act as a template for a new strand. Then along comes one of the DNA methyltransferases, DNMT1. It can recognize whether a CpG motif is methylated on the original strand that acted as the template. If it is, DNMT1 will put a methyl group in the same position on the new strand, and so the pattern of DNA methylation is faithfully reproduced on the double helix. [See illustration at right]

The daughter cells therefore end up with the same DNA methylation patterns as the parent cell. As a consequence, they will repress the same genes as the parent cell, and the skin cells will stay skin cells.

NEURONS ARE VERY DIFFERENT from skin cells. If both cells types use DNA methylation to switch off certain genes, they must be applying it in different patterns. Neurons and skin cells would both need to repress sets of genes that make a cell a liver cell, for example, but a neuron also silences the skin-cell instructions in the genome, and a skin cell returns the favor.

The key to how different cell types can use the same mechanism to create such different outcomes from the same genome lies in how DNA methylation gets targeted to different regions of the genome in different cell types. This takes us into the second great area of molecular epigenetics: proteins.

DNA is often described as if it's a naked molecule, DNA and nothing else. When we visualize it in our minds, a DNA double

helix looks something like a very long twisted ladder. But inside a cell, it's actually nothing like that, and many of the great breakthroughs in epigenetics came about when scientists began to appreciate this fully.

DNA is intimately associated with proteins, and in particular with proteins called histones. At the moment the most attention in epigenetics and gene regulation is focused on four particular histone proteins, called H2A, H2B, H3, and H4. These histones have a structure known as "globular," as they are folded into compact ball-like shapes. However, each also has a loose floppy chain of amino acids that sticks out of the ball, which is called the histone tail. Two copies of each of these four histone proteins come together to form a tight structure called the histone octamer, so called because it's formed of eight individual histones.

It might be easiest to think of this octamer as eight Ping-Pong balls stacked on top of each other in two layers. DNA coils tightly around this protein stack, like a long licorice whip around marshmallows, to form a structure called the nucleosome. One hundred forty-seven base pairs of DNA coil around each octamer [see illustration on page 34].

If we had read anything about histones even just fifteen years ago, they would probably have been described as simply "packaging proteins," and left at that. It's certainly true that DNA has to be packaged. The nucleus of a cell is usually only about 10 microns in diameter--that's 1/100th of a millimeter--and if the DNA in a cell were just left all floppy and loose, it could stretch for 2 meters. The DNA is curled tightly around the histone octamers, and the resultant nucleosomes are strung together in a chain that is tightly coiled yet again, stacking and packing all those histone "spools" of DNA closely on top of one another into a short, thick fiber called chromatin, the material of our chromosomes.

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Certain regions of our chromosomes have an extreme form of that sort of structure almost all the time. These tend to be regions that don't act as genes or code for any proteins. Instead, they are structural regions, such as the very ends of chromosomes, or areas that are important for separating chromosomes after DNA has been duplicated for cell division. The regions of DNA that are really heavily methylated also have this hyper-condensed structure, and the methylation is very important in establishing this configuration. It's one of the mechanisms used to keep certain genes switched off for decades in long-lived cell types such as neurons.

[ILLUSTRATION OMITTED]

But what about those regions that aren't screwed down tight, where there are genes that are switched on or have the potential to be switched on? This is where the histones really come into play. A substantial number of the breakthroughs in this field have come from the lab of David Allis at The Rockefeller University in New York. In a remarkable flurry of papers in 1996, he and his colleagues showed that histone proteins were chemically modified in cells, and that this modification at specific nucleosomes increased expression of nearby genes

The histone modification that David Allis identified is called acetylation. This is the addition of a chemical group called an acetyl, in this case to a specific amino acid named lysine on the floppy tail of one of the histones. Like DNA methylation, lysine acetylation is an epigenetic mechanism for altering gene expression that doesn't change the underlying gene sequence.

So back in 1996 there was a nice simple story. DNA methylation turned genes off, and histone acetylation turned genes on. But gene expression is much more subtle than genes being either on or off. Gene expression is rarely an on-off toggle switch; it's much more like the volume dial on a traditional radio. So perhaps it was unsurprising that there turned out to be more than one histone modification.

In fact, more than fifty different epigenetic modifications to histone proteins have been identified since Allis's initial work, both by him and by a large number of other laboratories. These modifications all alter gene expression, but not always in the same way. Some histone modifications push gene expression up, others drive it down. The pattern of modifications is referred to as a histone code. The problem epigeneticists face is that this is a code that is extraordinarily difficult to read.

IMAGINE A CHROMO-some as the trunk of a very big Christmas tree. The branches sticking out all over the tree are the histone tails, and these can be decorated with epigenetic modifications. We pick up the gold balls and we put one, two or three gold balls on some of the branches. We also have green icicle decorations, and we can put either one or two of these on some branches, some of which already have gold balls on them. Then we pick up the red stars, but are told we can't put these on a branch if the adjacent branch has any gold balls. The silver snowflakes and green icicles can't be present on the same branch. And so it goes on, with increasingly complex rules and patterns.

Eventually, we've used all our decorations and we wind the lights around the tree. The bulbs represent individual genes. By a magical piece of software programming, the brightness of each bulb is determined by the precise conformation of the decorations surrounding it. The likelihood is that we would really struggle to predict the brightness of most of the bulbs, because the pattern of Chrisunas decorations is so complicated.

That's where scientists currently are in terms of predicting how all the various histone modification combinations work together to influence gene expression. It's reasonably clear ill many cases what individual modifications can do, but it's not yet possible to make accurate predictions from complex combinations. There are major efforts being made to learn how to understand this code, with multiple labs throughout the world collaborating or competing ill the use of the fastest and most complex technologies to address this problem.

Some of the key evidence comes from developmental biology, the field from which many great epigenetic investigators have emerged. When an egg and a sperm fuse, they create a single cell, which is called the zygote. The zygote then divides to form two cells, these divide to form four, and so on. Very quickly, daughter cells start to take on discrete functions. Waves of gene expression and epigenetic modifications follow on from one another.

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A useful analogy for this is the game of Mousetrap, first produced in the early 1960s and still on sale today. Players have to build a Rube Goldberg--like mousetrap during the course of the game. The trap is activated at one end by the simple act of releasing a ball. This ball passes down and through all sorts of contraptions, including a slide, a kicking boot, a flight of steps, and a man jumping off a diving board. As long as the pieces have been put together properly, the whole ridiculous cascade operates perfectly, and the toy mice get caught under a net. If one of the pieces is just slightly misaligned, the crazy sequence judders to a halt and the trap doesn't work.

In the developing embryo, the genes and epigenetic proteins work together in a seamless, orderly procession, just like the events in Mousetrap once the ball has been released. Sometimes a cell will express a little more or a little less of a key factor, one whose expression depends on a finely balanced threshold. This has the potential to alter the developmental path that the cell takes.

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NORMAL DEVELOPMENT has yielded important evidence of the significance of epigenetic modifications in controlling cell fate. Cases where development goes awry have also shown us how important epigenetics can be. For example, a 2010 publication in Nature Genetics identified the mutations that cause a rare disease called Kabuki syndrome. It is a complex developmental disorder with a range of symptoms that include mental retardation, short stature, facial abnormalities, and cleft palate. The paper showed that Kabuki syndrome is caused by mutations in a gene called MLL2. The protein the gene specifies is an epigenetic writer: its talent is to add a methyl group to an amino acid, lysine, located at a specific position on the tail of histone H3. Patients with this mutation are unable to write their epigenetic code properly, and this leads to their symptoms.

Human diseases can also be caused by mutations in enzymes that remove epigenetic modifications--"erasers" of the epigenetic code. A gene called PHF8 specifies a protein that can remove a methyl group from a lysine at a different position on histone H3. A mutation in that gene causes a syndrome of mental retardation and cleft palate. In these cases, the patient's cells put epigenetic modifications on without problems, but don't remove them properly when that is called for.

It's interesting that although the proteins involved in these two syndromes have different roles, the clinical symptoms caused by mutations in these genes have overlaps in their presentation. Both lead to cleft palate and mental retardation. Both of those symptoms are classically regarded as reflecting problems during development. Epigenetic pathways are important throughout life, but seem to be particularly significant during development.

In addition to these epigenetic code writers and erasers, there are more than 100 proteins that act as readers of this histone code by binding to epigenetic marks. These readers attract other proteins and build up complexes that switch on or turn off gene expression. This is similar to the way that MeCP2 helps turn off expression of genes that are carrying DNA methylation.

In a very important way, however, histone modifications are different from DNA methylation. Once a DNA region has become methylated, it will tend to stay methylated under most conditions. That's why this epigenetic modification is so important for keeping neurons as neurons, and why there are no teeth in our eyeballs. DNA methylation can be removed in cells, but usually only under very specific circumstances.

Most histone modifications are much more plastic. A specific modification can be put on a histone at a particular gene, removed, and then later put back on again. This happens in response to all sorts of stimuli from outside the cell nucleus. The stimuli can vary enormously. In some cell types, the histone code may change in response to hormones. These include insulin signaling to our muscle cells, and estrogen affecting the cells of the breast during the menstrual cycle. In the brain the histone code can change in response to addictive drugs such as cocaine, whereas in the cells lining the gut, the pattern of epigenetic modifications will alter depending on the amounts of fatty acids produced by the bacteria in our intestines. These changes in the histone code are one of the key ways in which nurture (the environment) interacts with nature (our genes) to create the complexity of every higher organism on earth.

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Histone modifications also allow cells to "try out" particular patterns of gene expression, especially during development. Genes become temporarily inactivated when particular histone modifications that drive gene expression down are established on the histones near those genes. If there is an advantage to the cell in those genes being switched off, the histone modifications may last long enough to lead to DNA methylation. If enough DNA methylation takes place, expression of the gene will shut down semipermanently. In extreme circumstances, the whole chromosome region may become hyper-compacted and inactivated for multiple cell divisions, or for decades in a non-dividing cell like a neuron.

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Next month, in a final installment of "Epigenetics in Action," I will explore a tantalizing and highly controversial question, which challenges everything we thought we knew about inheritance: can epigenetic changes be carried over from one generation to the next?

Nessa Carey was employed at the Metropolitan Police Forensic Science Lab in London before earning a degree in immunology and a doctorate in virology. She then followed the academic route of post-doc and university lecturer, becoming a senior lecturer in Molecular Biology at Imperial College, London, where she led a research team investigating a genetic disorder that gets worse as it passes down through the generations in an affected family. Subsequently, for the past ten years, she has worked in the biotech and pharmaceutical industry. Carey lives in Bedfordshire, England (www.nessacarey.co.uk).

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Top of page