traits of the offspring. Darwin's influence
s myopically focused on identifying that
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oscopic analyses revealed that the hered-
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hread-like structures that become visible
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second strand of DNA, a loosely wrapped configuration known as the "double helix." The genius of this system is that the sequences of DNA bases on both strands are mirror images of each other. When the two strands of DNA unwind, each single strand contains the information to make an exact, complementary copy of itself. So through a process of separating the strands of a double helix, DNA molecules become self-replicating. This observation led to the assumption that DNA "controlled" its own replication . . . it was its own "boss."

The "suggestion" that DNA controlled its own replication *and* served as the blueprint for the body's proteins led Francis Crick to create biology's Central Dogma, the belief that DNA rules. The dogma is so fundamental to modern biology it is essentially written in stone, the equivalent of science's Ten Commandments. The dogma, also referred to as "the Primacy of DNA," is a fixture of every scientific text.

In the dogma's scheme of how life unfolds, DNA perches loftily on top, followed by RNA. RNA is the short-lived Xerox copy of the DNA. As such, it is the physical template encoding the amino acid sequence that makes up a protein's backbone. The Primacy of DNA diagram provides the logic for the Age of Genetic Determinism. Because the character of a living organism is defined by the nature of its proteins and its proteins are encoded in the DNA, then by logic, DNA would represent the "first cause," or primary determinant of an organism's traits.

The Human Genome Project

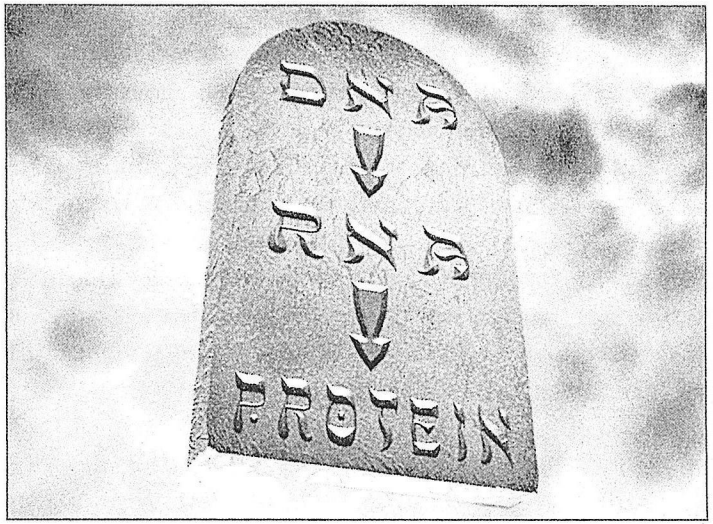
After DNA achieved superstar status, the remaining challenge was to create a catalog of all the genetic stars in the human firmament. Enter the Human Genome Project, a global, scientific effort begun in the late 1980s to create a catalog of all the genes present in humans.

From the outset, the Human Genome Project was a massively ambitious one. Conventional thought held that the body needed

one gene to provide the blueprint for each of the 100,000 plus different proteins that make up our bodies. Add to that at least 20,000 regulatory genes, which orchestrate the activity of the protein-encoding genes. Scientists concluded that the human genome would contain a minimum of 120,000 genes located within the twenty-three pairs of human chromosomes.

But that wasn't the whole story. A cosmic joke was unfolding, one of those jokes that periodically unsettle scientists convinced they have discovered the secrets of the universe. Consider the impact of Nicolaus Copernicus' discovery published in 1543 that the Earth was not the center of the universe, as was thought by the scientist-theologians of the day. The fact that the Earth actually revolved around the sun and that the sun itself was not the center of the universe undermined the teachings of the Church. Copernicus' paradigm-busting discoveries launched the modern, scientific revolution by challenging the presumed "infallibility" of the Church. Science eventually displaced the Church as Western civilization's source of wisdom for understanding the mysteries of the universe.

Geneticists experienced a comparable shock when, contrary to their expectations of over 120,000 genes, they found that the entire human genome consists of approximately 25,000 genes. (Pennisi 2003a and 2003b; Pearson 2003; Goodman 2003) More than eighty percent of the presumed and *required* DNA does not exist! The missing genes are proving to be more troublesome than the missing eighteen minutes of the Nixon tapes. The one-gene, one-protein concept was a fundamental tenet of genetic determinism. Now that the Human Genome Project has toppled the one-gene for one-protein concept, our current theories of how life works have to be scrapped. No longer is it possible to believe that genetic engineers can, with relative ease, fix all our biological dilemmas. There are simply not enough genes to account for the complexity of human life or of human disease.



The Central Dogma. The dogma, also referred to as the Primacy of DNA, defines the flow of information in biological organisms. As indicated by the arrows, the flow is only in one direction, from DNA to RNA and then to protein. The DNA represents the cell's long-term memory, passed from generation to generation. RNA, an unstable copy of the DNA molecule, is the active memory that is used by the cell as a physical template in synthesizing proteins. Proteins are the molecular building blocks that provide for the cell's structure and behavior. DNA is implicated as the "source" that controls the character of the cell's proteins, hence the concept of DNA's primacy that literally means "first cause."

I may sound like Chicken Little shouting that the genetics sky is falling. However, you don't have to take my word for it. Chicken Big is saying the same thing. In a commentary on the surprising results of the Human Genome Project, David Baltimore, one of the world's preeminent geneticists and a Nobel Prize winner, addressed the issue of human complexity (Baltimore 2001):

"But unless the human genome contains a lot of genes that are opaque to our computers, it is clear that we do not gain our undoubted complexity over worms and plants by using more genes.

"Understanding what does give us our complexity—our enormous behavioral repertoire, ability to produce conscious action, remarkable physical coordination, precisely tuned alterations in response to external variations of the environments, learning, memory, need I go on?—remains a challenge for the future."

As Baltimore states, the results of the Human Genome Project force us to consider other ideas about how life is controlled. "Understanding what does give us our complexity . . . remains a challenge for the future." The sky *is* falling.

In addition, the results of the Human Genome Project are forcing us to reconsider our genetic relationship with other organisms in the biosphere. We can no longer use genes to explain why humans are at the top of the evolutionary ladder. It turns out there is not much difference in the total number of genes found in humans and those found in primitive organisms. Let's take a look at three of the most studied animal models in genetic research, a microscopic nematode roundworm known as *Caenorhabditis elegans,* the fruit fly, and the laboratory mouse.

The primitive *Caenorhabditis* worm serves as a perfect model for studying the role of genes in development and behavior. This rapidly growing and reproducing organism has a precisely patterned body comprised of exactly 969 cells and a simple brain of about 302 cells. Nonetheless it has a unique repertoire of behaviors and most importantly, it is amenable to genetic experimentation. The *aenorhabditis* genome consists of approximately 24,000 genes. (Blaxter 2003) The human body, comprised of over fifty trillion cells, contains only 1,500 more genes than the lowly, spineless, thousand-celled microscopic worm.

The fruit fly, another favored research subject, has 15,000 genes. (Blaxter 2003; Celniker, et al, 2002) So the profoundly more complicated fruit fly has 9,000 fewer genes than the more primitive *Caenorhabditis* worm. And when it comes to the question of mice and men, we might have to think more highly of them or less of ourselves; the results of parallel genome projects reveal that humans and rodents have roughly the same number of genes!

Cell Biology 101

In retrospect, scientists should have known that genes couldn't provide the *control* of our lives. By definition, the brain is the organ responsible for controlling and coordinating the physiology and

behavior of an organism. But is the nucleus truly the cell's brain? If our assumption that the nucleus and its DNA-containing material is the "brain" of the cell, then removing the cell's nucleus, a procedure called enucleation, should result in the immediate death of the cell.

And now, for the big experiment . . . (Maestro, a drumroll if you please).

The scientist drags our unwilling cell into the microscopic operating arena and straps it down. Using a micromanipulator, the scientist guides a needle-like micropipette into position above the cell. With a deft thrust of the manipulator, our investigator plunges the pipette deep into the cell's cytoplasmic interior. By applying a little suction, the nucleus is drawn up into the pipette, and the pipette is withdrawn from the cell. Below the nucleus-engorged pipette lies our sacrificial cell—its "brain" torn out.

But *wait!* It's still moving! My God . . . the cell is still *alive!*

The wound has closed and like a recovering surgical patient, the cell begins to slowly stagger about. Soon the cell is back on its feet (okay, its pseudopods), fleeing the microscope's field with the hope that it will never see a doctor again.

Following enucleation, many cells can survive for up to two or more months without genes. Viable enucleated cells do not lie about like brain-dead lumps of cytoplasm on life-support systems. These cells actively ingest and metabolize food, maintain coordinated operation of their physiologic systems (respiration, digestion, excretion, motility, etc.), retain an ability to communicate with other cells, and are able to engage in appropriate responses to growth and protection requiring environmental stimuli.

Unsurprisingly, enucleation is not without side effects. Without their genes, cells are not able to divide, nor are they able to reproduce any protein parts they lose through the normal wear and tear of the cytoplasm. The inability to replace defective cytoplasmic proteins contributes to mechanical dysfunctions that ultimately result in the death of the cell.

Our experiment was designed to test the idea that the nucleus is the "brain" of the cell. If the cell had died immediately following enucleation, the observations would have at least supported that belief. However, the results are unambiguous: enucleated cells still exhibit complex, coordinated, life-sustaining behaviors, which imply that the cell's "brain" is still intact and functioning.

The fact that enucleated cells retain their biological functions in the absence of genes is by no means a new discovery. Over a hundred years ago, classical embryologists routinely removed the nuclei from dividing egg cells and showed that a single, enucleated egg cell was able to develop as far as the blastula, an embryonic stage consisting of forty or more cells. Today, enucleated cells are used for industrial purposes as living "feeder" layers in cell cultures designed for virus vaccine production.

If the nucleus and its genes are not the cell's brain, then what exactly is DNA's contribution to cellular life? Enucleated cells die, not because they have lost their brain but because they have lost their reproductive capabilities. Without the ability to reproduce their parts, enucleated cells cannot replace failed protein building blocks, nor replicate themselves. So the nucleus is not the brain of the cell—the nucleus is the cell's gonad! Confusing the gonad with the brain is an understandable error because science has always been and still is a patriarchal endeavor. Males have often been accused of thinking with their gonads, so it's not entirely surprising that science has inadvertently confused the nucleus with the cell's brain!

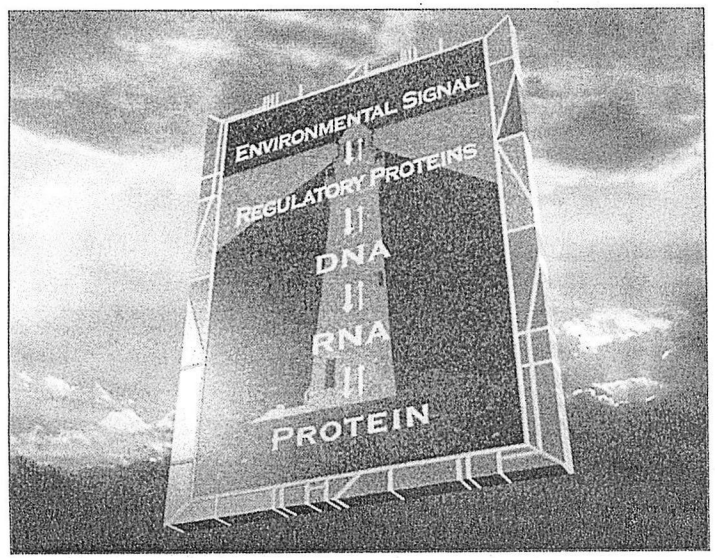
Epigenetics: The New Science of Self-Empowerment

Genes-as-destiny theorists have obviously ignored hundred-year old science about enucleated cells, but they cannot ignore new research that undermines their belief in genetic determinism. While the Human Genome Project was making headlines, a group of scientists were inaugurating a new, revolutionary field in biology

called *epigenetics*. The science of epigenetics, which literally means "control above genetics," profoundly changes our understanding of how life is controlled. (Pray 2004; Silverman 2004) In the last decade, epigenetic research has established that DNA blueprints passed down through genes are not set in concrete at birth. Genes are not destiny! Environmental influences, including nutrition, stress, and emotions, can modify those genes without changing their basic blueprint. And those modifications, epigeneticists have discovered, can be passed on to future generations as surely as DNA blueprints are passed on via the double helix. (Reik and Walter 2001; Surani 2001)

There is no doubt that epigenetic discoveries have lagged behind genetic discoveries. Since the late 1940s, biologists have been isolating DNA from the cell's nucleus in order to study genetic mechanisms. In the process they extract the nucleus from the cell, break open its enveloping membrane, and remove the chromosomal contents, half of which is made up of DNA and half of which is made up of regulatory proteins. In their zeal to study DNA, most scientists threw away the proteins, which we now know is the equivalent of throwing the baby out with the bathwater. Epigeneticists are bringing back the baby, by studying the chromosome's proteins, and those proteins are turning out to play as crucial a role in heredity as DNA.

In the chromosome, the DNA forms the core, and the proteins cover the DNA like a sleeve. When the genes are covered, their information cannot be "read." Imagine your bare arm as a piece of DNA representing the gene that codes for blue eyes. In the nucleus, this stretch of DNA is covered by bound regulatory proteins, which cover your blue-eye gene like a shirtsleeve, making it impossible to be read.



Primacy of Environment. The new science reveals that the information that controls biology starts with environmental signals that, in turn, control the binding of regulatory proteins to the DNA. Regulatory proteins direct the activity of genes. The DNA, RNA, and protein functions are the same as described in the Primacy of DNA chart. Note: the flow of information is no longer unidirectional. In the 1960s, Howard Temin challenged the Central Dogma with experiments that revealed RNA could go against the predicted flow of information and rewrite the DNA. Originally ridiculed for his "heresy," Temin later won a Nobel Prize for describing reverse transcriptase, the molecular mechanism by which RNA can rewrite the genetic code. Reverse transcriptase is now notorious, for it is used by the AIDS virus' RNA to commandeer the infected cell's DNA. It is also now known that changes in the DNA molecule, such as adding or removing methyl chemical groups, influence the binding of regulatory proteins. Proteins must also be able to buck the predicted flow of information, since protein antibodies in immune cells are involved with changing the DNA in the cells that synthesize them. The size of the arrows indicating information flow are not the same. There are tight restrictions on the reverse flow of information, a design that would prevent radical changes to the cell's genome.

How do you get that sleeve off? You need an environmental signal to spur the "sleeve" protein to change shape, i.e., detach from the DNA's double helix, allowing the gene to be read. Once the DNA is uncovered, the cell makes a copy of the exposed gene. As a result, the activity of the gene is "controlled" by the presence

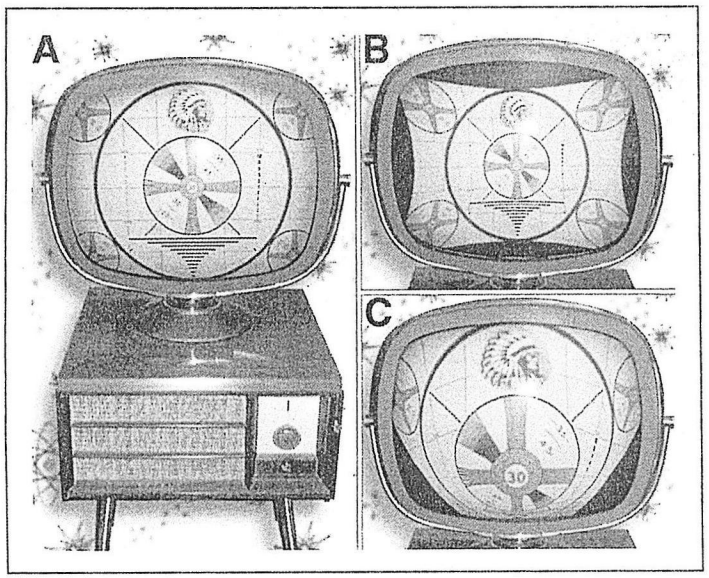
or absence of the ensleeving proteins, which are in turn controlled by environmental signals.

The story of epigenetic control is the story of how environmental signals control the activity of genes. It is now clear that the Primacy of DNA chart described earlier is outmoded. The revised scheme of information flow should now be called the "Primacy of the Environment." The new, more sophisticated flow of information in biology starts with an environmental signal, then goes to a regulatory protein and only then goes to DNA, RNA, and the end result, a protein.

The science of epigenetics has also made it clear that there are two mechanisms by which organisms pass on hereditary information. Those two mechanisms provide a way for scientists to study both the contribution of nature (genes) and the contribution of nurture (epigenetic mechanisms) in human behavior. If you only focus on the blueprints, as scientists have been doing for decades, the influence of the environment is impossible to fathom. (Dennis 2003; Chakravarti and Little 2003)

Let's present an analogy, which hopefully will make the relationship between epigenetic and genetic mechanisms clearer. Are you old enough to remember the days when television programming stopped after midnight? After the normal programming signed off, a "test pattern" would appear on the screen. Most test patterns looked like a dartboard with a bull's eye in the middle, similar to the one pictured on the following page.

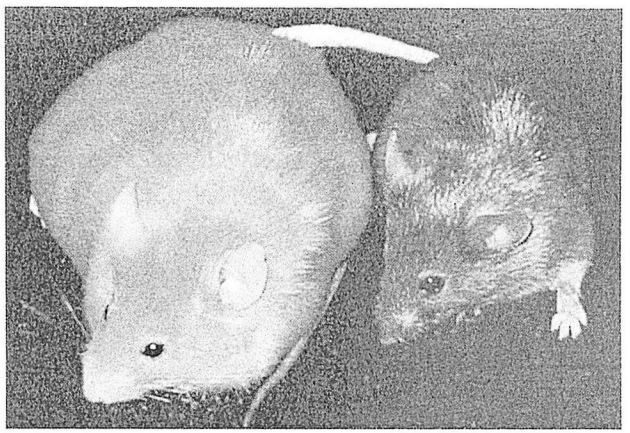
Think of the pattern of the test screen as the pattern encoded by a given gene, say the one for brown eyes. The dials and switches of the TV fine-tune the test screen by allowing you to turn it on and off and modulate a number of characteristics, including color, hue, contrast, brightness, and vertical and horizontal holds. By adjusting the dials, you can alter the appearance of the pattern on the screen, while not actually changing the original broadcast pattern. This is precisely the role of regulatory proteins. Studies of protein synthesis reveal that epigenetic "dials" can create 2,000 or more variations of proteins from the same gene blueprint. (Bray 2003; Schmuker, et al, 2000)



In this epigenetic analogy, the test pattern on the screen represents the protein backbone pattern encoded by a gene. While the TV's controls can change the appearance of the pattern (B and C), they do not change the original pattern of the broadcast (i.e., the gene). Epigenetic control modifies the read-out of a gene without changing the DNA code.

Parental Life Experiences Shape Their Children's Genetic Character

We now know that the environmentally influenced fine-tuning described above can be passed from generation to generation. A landmark Duke University study published in the August 1, 2003 issue of *Molecular and Cellular Biology* found that an enriched environment can even override genetic mutations in mice. (Waterland and Jirtle 2003) In the study, scientists looked at the effect of dietary supplements on pregnant mice with the abnormal "agouti" gene. Agouti mice have yellow coats and are extremely obese, which predisposes them to cardiovascular disease, diabetes, and cancer.



Agouti Sisters. One year old female genetically identical agouti mice. Maternal methyl donor supplementation shifts coat color of the offspring from yellow to brown and reduces the incidence of obesity, diabetes, and cancer. (Photo courtesy of Jirtle and Waterland©)

In the experiment, one group of yellow, obese, agouti mothers received methyl-group-rich supplements available in health food stores: folic acid, vitamin B12, betaine, and choline. Methyl-rich supplements were chosen because a number of studies have shown that the methyl chemical group is involved with epigenetic modifications. When methyl groups attach to a gene's DNA, it changes the binding characteristics of regulatory chromosomal proteins. If the proteins bind too tightly to the gene, the protein sleeve cannot be removed and the gene cannot be read. Methylating DNA can silence or modify gene activity.

This time the headlines "Diet Trumps Genes" were accurate. The mothers who got the methyl-group-rich supplements produced standard, lean, brown mice, even though their offspring had the same agouti gene as their mothers. The agouti mothers who didn't get the supplements produced yellow pups, which ate much more than the brown pups. The yellow pups wound up weighing almost twice as much as their lean, "pseudo-agouti" counterparts.

The University's photo on the previous page is striking. Though the two mice are genetically identical, they are radically different in appearance: one mouse is lean and brown and the other mouse is obese and yellow. What you can't see in the picture is that the obese mouse is diabetic while its genetically identical counterpart is healthy.

Other studies have found epigenetic mechanisms to be a factor in a variety of diseases, including cancer, cardiovascular disease, and diabetes. In fact, only 5 percent of cancer and cardiovascular patients can attribute their disease to heredity. (Willett 2002) While the media made a big hoopla over the discovery of the BRCA1 and BRCA2 breast cancer genes, they failed to emphasize that ninety-five percent of breast cancers are not due to inherited genes. The malignancies in a significant number of cancer patients are derived from environmentally induced epigenetic alterations and not defective genes. (Kling 2003; Jones 2001; Seppa 2000; Baylin 1997)

The epigenetic evidence has become so compelling that some brave scientists are even invoking the "L" word for Jean Baptiste de Lamarck, the much-scorned evolutionist, who believed that traits acquired as a result of environmental influence could be passed on. Philosopher Eva Jablonka and biologist Marion Lamb wrote in their 1995 book *Epigenetic Inheritance and Evolution—The Lamarckian Dimension:* "In recent years, molecular biology has shown that the genome is far more fluid and responsive to the environment than previously supposed. It has also shown that information can be transmitted to descendants in ways other than through the base sequence of DNA." (Jablonka and Lamb 1995)

We're back to where we started in this chapter, the environment. In my own work in the laboratory, I saw over and over the impact a changed environment had on the cells I was studying. But it was only at the end of my research career, at Stanford, that the message fully sank in. I saw that endothelial cells, which are the blood vessel-lining cells I was studying, changed their structure and function depending on their environment. When, for

example, I added inflammatory chemicals to the tissue culture, the cells rapidly became the equivalent of macrophages, the scavengers of the immune system. What was also exciting to me was that the cells transformed even when I destroyed their DNA with gamma rays. These endothelial cells were "functionally enucleated," yet they completely changed their biological behavior in response to inflammatory agents, just as they had when their nuclei were intact. These cells were clearly showing some "intelligent" control in the absence of their genes. (Lipton 1991)

Twenty years after my mentor Irv Konigsberg's advice to first consider the environment when your cells are ailing, I finally got it. DNA does not control biology, and the nucleus itself is not the brain of the cell. Just like you and me, cells are shaped by where they live. In other words, it's the environment, stupid.