

## Treasures in the Trash

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### What genetic researchers used to call junk DNA may conceal the most important medical secrets of all.

When researchers began mapping the genome, aiming to decode the entire human gene sequence, they expected to eventually locate 100,000 or more active genes. After completing the genome map in 2001, they were startled to find that humans have only 25,000 active genes. The lowly roundworm has almost as many (19,000).

That means the active genes contain a mere 1.5% of the 3 billion units of DNA that make up our genetic structure. The rest is "dark" or "junk" DNA, long presumed to be present for no particular reason.

But researchers are now finding this junk DNA, overlooked for decades by geneticists, may actually not be junk at all. They are finding hints of an enormous and previously unimagined command-and-control apparatus that regulates what our 25,000 genes do and how the body is assembled. Junk DNA, when it goes wrong, may be a culprit in major killers ranging from cancer to diabetes to infectious disease.

That insight could unearth hundreds of new targets for experimental drugs that had been aimed only at working genes. "It's sort of like *Antiques Roadshow*," says Harvard genome scientist John Quackenbush, who has long argued that the junk DNA could be vital. "You look in the closet full of junk and find out you have a Picasso."

"This will revolutionize human genetics over the next few decades," says David Haussler, a Howard Hughes investigator at UC, Santa Cruz who was on the government team that decoded the human genome. He predicts that most disease-causing genetic flaws will be found lurking in our junk DNA.

The dark DNA "may be even more important" than active genes in causing disease, says Isaac Bentwich, chairman of Israel's Rosetta Genomics. Founded in 2000, Rosetta has applied for patents on 200 dark genes. He hopes for new treatments and diagnostic tools for lung cancer, prostate cancer and other diseases.

Bentwich's team is focusing on something called microRNA, a promising target discovered in the sea of junk DNA only five years ago. MicroRNAs essentially quell a gene's workings, and they already have been linked to diabetes, hepatitis C, leukemia, lymphoma and breast cancer.

"It's a revolution in how we understand the genome and how the cell functions," says MIT Nobel laureate Phillip Sharp. "There's a whole new frontier there." Sharp and a few microRNA researchers have founded Alnylam Pharmaceuticals to invent RNA-based drugs to treat Parkinson's, cystic fibrosis and spinal injury. The firm has development deals with Merck and Novartis, and such rivals as San Francisco's Sirna Therapeutics are in pursuit.

The new view of junk DNA overturns 50 years of dogma in molecular biology. James Watson and Francis Crick discovered DNA's structure in 1953. The double-helix, twisted ladder of chemical base pairs is carried on the 23 pairs of chromosomes that inhabit every cell in the body. The human genome contains 3 billion base pairs (or "letters") of DNA, and one active gene can span thousands of base pairs.

For decades scientists have zoomed in on the active genes that carry instructions for making hundreds of thousands of proteins; this protein production is carried out by DNA's doppelgänger, RNA. The dark genes were dismissed as debris left over from millions of years of evolution.

Now that junk-DNA theory looks to be ... junk. Huge expanses of dark DNA are nearly identical in numerous species, from flies to rats to humans. That nature has conserved the dark stuff for millions of years is a clear indication that it must do something crucial, biologists say.

Some say that the dark DNA that plays a crucial role in the body could occupy at least 4% of the genome--almost three times the portion taken up by active genes. A better guess is that a huge 40% to 70% of the whole DNA sequence is dark

DNA with secret powers, posits Peter Andolfatto, a fruit-fly geneticist at UC, San Diego.

An early hint that junk DNA might hold treasures came in 1993, when Victor Ambros of Dartmouth Medical School studied a mysterious genetic mutation in worms that prevented them from maturing from the larva stage to adult. To his astonishment, he found that the mutation existed not in active genes but in dark DNA that controlled a tiny strand of RNA, the first microRNA whose main function was to turn off another gene.

The result remained an isolated curiosity until 2000, when Harvard Medical School geneticist Gary Ruvkun found a second microRNA in worms and showed it also was present in people. That suggested there might be a whole realm of mysterious and undetected RNA master controllers inside cells. Soon several supercomputers tried tracking them down--and quickly found them, in abundance. Now researchers figure 300 to 1,000 human microRNAs form a hidden layer of control, helping determine which genes are turned off when. They may help regulate 30% or more of all proteins.

One, called microRNA 375, can block insulin secretion and may be involved in diabetes. Another, microRNA 122, is found in the liver and is used by the hepatitis C virus to help it replicate; it could be a target for future drugs for the disease. "MicroRNAs are almost ideal drug targets. The field is exploding," says Rockefeller University's Markus Stoffel. He and colleagues at Alnylam recently created new RNA drugs that bind to and break down specific microRNAs. When they tested one such RNA drug in mice, cholesterol levels fell 44%.

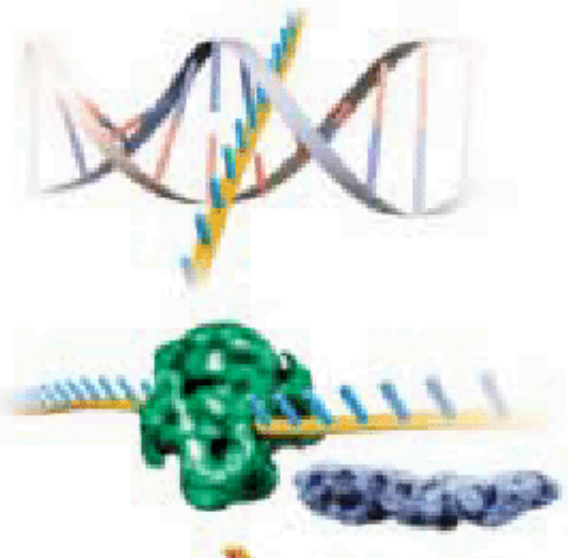
MIT and Harvard researchers showed in June that 217 microRNAs were altered in a wide array of tumors. Ohio State University researchers, writing in the *New England Journal of Medicine* in October, found that levels of just 13 microRNAs predicted how quickly a common type of leukemia would progress. Moreover, of 75 patients studied, 11 patients with this leukemia had mutations in their microRNA that may have caused the disease. This was the first time such mutations had been found. "When I was giving talks about microRNAs three years ago, people thought I was from Mars," says cancer geneticist Carlo Croce, who led the research.

But even microRNA, one of myriad types of RNA, accounts for only a tiny fraction of junk DNA. What the rest does is still mostly a mystery, but almost certainly it is doing something. Many areas of the genome previously thought to be barren actually are churning out huge quantities of RNA, chipmaker Affymetrix has found. There may be hundreds of thousands of RNA molecules of various kinds that boss around our genes, says Affymetrix research director Thomas Gingeras.

Even dark DNA that doesn't ever transform into microRNA or a protein-coding active gene may have crucial functions. A recent study examined a three-year-old Japanese girl with a DNA mutation that caused her to have two thumbs on each hand and seven toes on each foot. The mutation turned out to be in the junk DNA. Astoundingly, the junk DNA mutation somehow had disrupted the function of a distant development gene that lies a million DNA letters away.

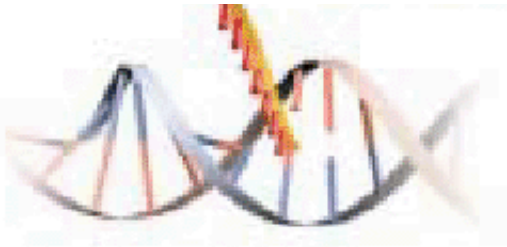
## All Jammed Up

**Old dogma held that DNA was merely a recipe book for proteins. MicroRNAs show that it can work in previously unimagined ways. Here is what they do.**

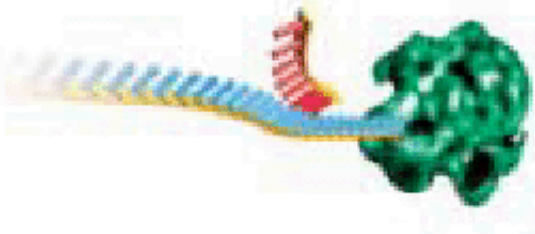


**1** A gene encoded in DNA is copied into an RNA message that is sent into the cell.

**2** The RNA message is read by a molecular factory called a ribosome, which uses it as a blueprint to produce a protein.



**3** But it turns out that DNA also makes various tiny microRNAs; these also drift out into cells.



**4** When a microRNA binds to a specific RNA message, it gums up protein production.