

## Dam! A New Method for Pinpointing Gene Transcription

In February, the human genome was published in science journals, the result of the labor of a multitude of scientists around the world. Now researchers face the daunting task of explaining how human genes turn on to produce the proteins they encode. What activates T cells in the immune system, for example, to respond to an infection? How do cells in the womb know they must grow and divide to form an eye or an arm or a set of nerve cells?

The answer lies with transcription factors, which account for as much as 10 percent of the genome in humans and other organisms. Transcription factors, which are themselves proteins, travel to a cell's nucleus to turn genes on, telling them to start producing their particular proteins. Or, they may turn genes off. Either way, they send their message by binding to the end of a gene and recruiting the cell's machinery to spur the gene into action.

Despite their large numbers and critical role, transcription factors remain poorly understood. The newly published genome, in other words, is like an instruction manual describing thousands of parts that no one is quite sure how to spur into action. In large part, that's because researchers lack an effective method for matching up transcription factors with their "gene targets." The most widely used method, called chromatin immunoprecipitation, is toxic to cells and often ineffective.

Bas van Steensel, a former postdoctoral fellow with HHMI investigator Steve Henikoff and now the head of a lab at the University of Amsterdam, came up with a better approach. He and Henikoff collaborated to develop a technique that may enable research on transcription factors to make significant progress.

At the time, they weren't even thinking about transcription factors. Henikoff, a

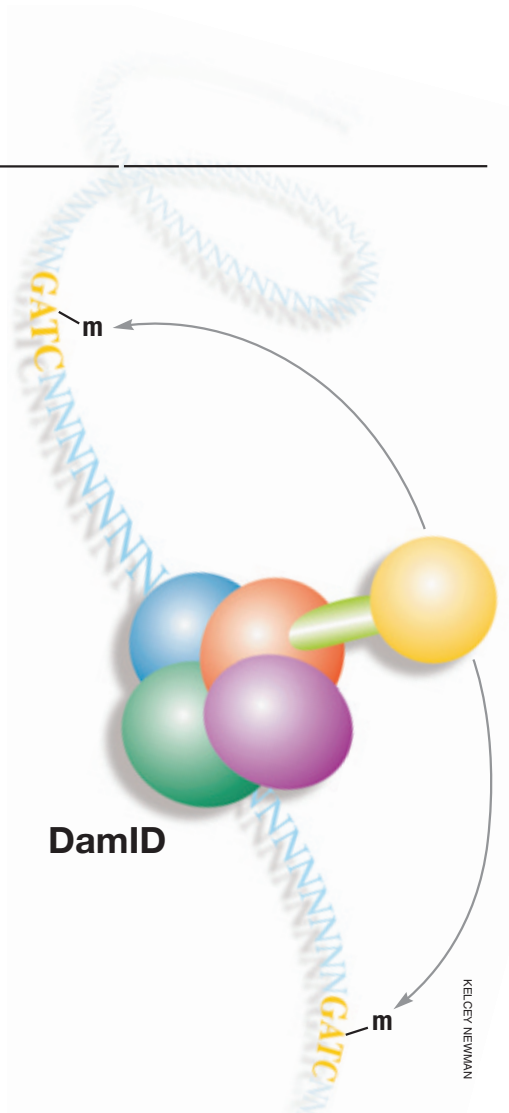
member of the Fred Hutchinson Cancer Research Center in Seattle, has a long-standing interest in gene function and the structure of chromatin, the mass of proteins surrounding chromosomes that plays a role not only in transcription but also in such functions as cell division and DNA synthesis. He and van Steensel set out to track the destinations of these mysterious proteins, which meant using living cells and ruled out the use of chromatin immunoprecipitation because of its toxic effects.

The two scientists pondered possible alternatives. They needed a signal of some kind to show them where each protein was binding to its target on the DNA. So, they fused the proteins to a chemical signal: an enzyme called adenine DNA methyltransferase, known as Dam.

In the method they developed, each protein, accompanied by Dam, is sent into a genome to find its matching DNA sequence. In the case of transcription factors, the protein latches on to its target gene, thereby sending the signal to begin the transcription process. The protein's traveling partner, Dam, also grabs hold of the DNA and attaches a group of chemicals called methyls to the DNA flanking the gene. The scientists then cut up and remove the DNA near the methyl groups, and these fragments reveal the sequence of the DNA that matches the protein.

Dubbed the DamID method, this new tool, which should accelerate the study of transcription factors, at first could be used only to examine a single target at a time. That was a problem because, as Henikoff points out, a single transcription factor may actually "bind to dozens or hundreds of targets throughout the genome."

He and van Steensel went on to make the method more useful by incorporating



*A transcription factor consisting of multiple protein subunits, shown here as colored balls, binds to its DNA target. One of the protein subunits is fused to Dam (the yellow ball connected by a green linker segment). This tethered Dam grabs hold of each nearby "recognition site"—the DNA sequence shown as GATC—and attaches a methyl chemical group (m) to it.*

"gene chips"—microarrays that contain numerous DNA sequences—and tiny colored labels that glow when genes are activated. Now, they're able to isolate not one but many sections of DNA that match up with a transcription factor and to deduce where the sections are located on the helix.

Other researchers have also begun using microarrays to study gene expression patterns, but this new method allows one to "see where transcription

factors actually bind, which may be an important prerequisite for gene expression,” Henikoff says. “In many cases, this approach will give you a more direct readout of gene regulation. People have talked about using microarray analysis to study genes that are coexpressed, but we can do it with the transcription factors themselves and look at where they’re binding genes to activate or silence them. I think that’s the major value of this approach.”

The approach is attracting notice. “The paper by van Steensel and Henikoff describing a method that allows one to find needles in a haystack made me get up from my desk and rush to the laboratory,” says transcription expert Danny Reinberg, an HHMI investigator at the University of Medicine and Dentistry of New Jersey. “It’s allowed us to see the light at the end of the tunnel as we attempt to define one or more genes that are recognized by a protein whose expression causes cells to stop dividing. I predict this technology will be used worldwide.”

In addition to spurring basic research, Henikoff and van Steensel’s technique might be used for diagnostic purposes. For example, some researchers are now using microarray analysis to correlate patterns of gene expression with different types of tumors and with how these types respond to various treatments. “The DamID method can provide the same information,” Henikoff says. “But where microanalysis provides only a single readout regarding the expression pattern of genes, DamID may be able to provide a different readout for each transcription factor that affects a single type of cancer cell. Thus, the method has the potential to greatly increase the power of cancer profiling.”

Indeed, good things may result as scientists identify the binding sites for proteins encoded by the oncogenes and tumor-suppressor genes that loom so large in cancer. “For example,” says Henikoff, “the *myc* oncogene, which is being studied by my Hutchinson colleague Robert Eisenman, encodes a transcription factor that has unknown targets. If our new method proves useful in determining the gene targets for *myc*, then this knowledge may help elucidate how misexpression of *myc* causes cancer.”

Jim Kling

## Fine-Tuning a Blood Pressure Regulator

In a study in mice, researchers have found evidence that high blood pressure may be controllable by drugs that target a particular type of ion channel found in the smooth muscle cells surrounding arteries. The drugs would specifically affect the release of potassium ions, which play important roles in regulating blood pressure.

The research team, led by HHMI investigator Richard W. Aldrich at Stanford University, focused on the so-called BK channels. When a burst of calcium is released from intracellular sites within smooth muscle cells, the BK channels in those cells open, potassium ions flood out, the smooth muscle relaxes and blood pressure goes down. Calcium entering through the surface membrane, however, can also cause blood vessels to constrict, causing high blood pressure.

“Thus, there’s a balance involved in regulating blood pressure,” Aldrich says, “and we are hoping to learn how the BK channel acts to tip that balance toward dilation.”

In their study, reported in *Nature*, the researchers created genetically modified

mice that were normal in every way except that their BK channels lacked a single component, called the  $\beta 1$  subunit. This modification rendered the channels less responsive to calcium release, and the mice developed high blood pressure and other abnormalities caused by chronic hypertension.

The physiological effects were so straightforward that the  $\beta 1$  subunit appears to be a promising target for new antihypertension drugs. “Drugs that change  $\beta 1$  subunit function by altering the channel’s calcium sensitivity could allow control of blood pressure up or down with fewer side effects than current treatments,” Aldrich says.

Having these mice available may also help advance basic research in hypertension. Says Aldrich, “Since we can alter this subunit to affect blood pressure without affecting other systems, we can use it as a model to study hypertension beginning at the molecular level, through cellular physiology, to the pathology and long-term ramifications of the disorder.”

In addition, the study indicates that the gene controlling production of the  $\beta 1$  subunit may be involved in inherited forms of hypertension, some of which have not been pinpointed to specific genes. “These findings suggest that this is a good candidate to examine to see if humans with hypertension have mutations in this gene,” Aldrich says. **H**

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—Richard W. Aldrich



BARBARA RILES