Powerful new method allows scientists to probe gene activation

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NYU Langone Medical Center researchers have developed a powerful new method to investigate the discrete steps necessary to turn on individual genes and examine how the process goes wrong in cancer and other diseases. The finding, based on seven years of research and described in the April 9 issue of *Molecular Cell*, allows scientists to investigate the unfolding of DNA, a process required for gene activation.

"The new methodology allows us to examine the steps that turn on individual genes in order to figure out what part of the process breaks down in diseases like cancer, " says Danny Reinberg, PhD, professor of biochemistry at NYU Langone Medical Center and a Howard Hughes Medical Institute Investigator, who led the study. "Right now we have the book with a lot of chapters. The idea now is to read each of those chapters and analyze how things happen. After that, we can start devising assays to test for steps or molecules we want to target," says Dr. Reinberg, who has been studying the molecular processes governing how genetic information is transferred for more than 20 years. He is a leader in the field of epigenetics, which probes the modifications that control when genes are expressed, many of which are linked to a wide variety of diseases.

DNA, in its simplest form, is a long double-stranded helix. Inside the cell's nucleus, however, the helix is further twisted and wrapped around protein complexes to form much more compact fibers called chromatin. For example, chromosome 22, one of the smallest human chromosomes, would be about 1.5 centimeters long as a simple DNA helix, but twisted

around the protein complexes, it is just two micrometers, a 10,000-fold compression.

However, the degree of compaction is dynamic and is part of the way cells control gene transcription. When a gene is inactive, its chromatin is packed together more tightly than when the gene is actively transcribed. Although scientists have known about these changes for years, they didn't know how the cell regulated the shift from the most highly compact fiber, which is 30 nanometers across (about the size of the tiniest particle of smoke from cooking oil), to the less compact one, which is just 11 nanometers. They wanted to understand the process, but faced a major hurdle—no one knew how to recreate a 30 nanometer fiber in a test tube.

With their new study, Dr. Reinberg and his colleagues have cleared that hurdle, allowing the team to begin teasing apart the steps to unwind the fiber and start transcription in a test gene. "It was a lot of background work, but in the end we got a nice story that, I believe, is the only story out there that goes from highly compact chromatin to transcription," says Dr. Reinberg.

Once the fiber was in the assay tubes, the team found that a DNA regulatory protein called the retinoic acid receptor (RAR) could access its binding site on the DNA, even when the chromatin was in its most tightly wound state. When researchers added the hormone that stimulates RAR, called retinoic acid, a derivative of vitamin A, to the system, things started to change. . The hormone-bound RAR began to unwind the chromatin and move aside large protein complexes, called nucleosomes, to make room for other DNA unwinding proteins and transcription factors. As they added in more and more factors, the DNA continued to loosen up, until finally, the team could see transcription start from their test gene PEPCK, which is controlled by RAR.

Significantly, electron microscopy showed that the 30 nanometer fiber formed in the test tube resembles the one in cells. Moreover, as the team added individual factors to the system, they used biochemical assays, such as DNA cutting experiments, to show that the nucleosome movement mimicked what happens when the PEPCK gene is turned on by retinoic acid in living cells.

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