ty may lead to the synthesis of more of the small RNAs that guide the mRNA-degrading ribonuclease to its targets.

Such RNA amplification could help explain how RNAi spreads through plants and other organisms. As Hannon notes, "You either have to have amplification, or the enzyme [degrading the mRNA] has to be ferocious." More work will be needed to establish exactly what the RNA polymerase does in RNA, but other investigators now have evidence pointing to some involvement. In today's issue of *Cell*, both Baulcombe's team and Vaucheret's describe an RNA polymerase from *Arabidopsis* plants that is needed for RNAi.

How RNAi helps the organism

Although much of the evidence for RNAi comes from experiments in which researchers have artificially perturbed cells by putting in foreign nucleic acids, they are finding that it provides essential services for the organism. Several groups, including Baulcombe's and that of Vicki Vance of the University of South Carolina in Columbia, have evidence that plants use RNAi as a defense against infection with viruses.

It turns out that when viruses invade plant cells, the cells silence the viral genes needed for reproducing and spreading. Such silencing may be triggered by the double-stranded RNAs that plant viruses produce as part of their life cycle. Indeed, Baulcombe, Vance, and others have shown that, in the continuing evolutionary war to survive and reproduce, plant viruses have evolved genes that enable them to suppress silencing.

RNAi may also help keep the transposable elements that litter genomes from jumping around and causing harmful mutations. Both Plasterk's team and Mello, Fire, and their colleagues found that mutations that knocked out RNAi in *C. elegans* led to abnormal transposon movements. "Transposons were jumping out all over the place," Plasterk says. "These experiments tell us that RNAi's function is to protect your genome from transposons."

There are also hints that RNAi may be important in embryonic development. For example, Eleanor Maine of Syracuse University in New York and her colleagues found that the Ego1 protein, which is needed for germ line development in *C. elegans*, is structurally related to the RNA polymerase made by the *Neurospora qde1* gene. Her evidence also suggests that Ego1 participates in RNAi in worms. When she knocks out the gene, she finds the resulting animals are defective in RNAi directed at some genes expressed in the worm germ line.

Moreover, Florence Wianny and Magdalena Zernicka-Goetz of the University of Cambridge, U.K., have shown that they can elicit RNAi against certain genes in early mouse embryos by injecting them with the corresponding double-stranded RNAs. This indicates that RNAi could be used to inactivate specific genes in mammals, just as in the worm and fly, and could thus be a valuable tool for studying gene function in mammalian development.

Although the explosion of recent results has provided a good start toward understanding RNAi, researchers know that many questions still remain to be answered. They have to pin down the functions of the genes they have identified so far, and they say there are additional genes waiting in the wings to be identified.

Then there is the big question of whether RNAi, which is posttranscriptional and occurs in the cytoplasm, ties together in any way with the transcriptional silencing known to happen in the nucleus. Again, there are hints that it might. Plant researchers in particular have found that genetic manipulations that trigger RNAi often correlate with addition of methyl groups to the corresponding genes. Such methylation can lead to transcriptional shutdown of genes, but it's unclear which comes first in this situation.

Baulcombe and his colleagues have suggested that methylation of transgenes that have become inserted in the genome might lead to formation of abnormal transcripts, rather than complete transcriptional inhibition. These aberrant RNAs might then be selected for copying by an RNA polymerase to make double-stranded RNAs, thus triggering posttranscriptional silencing. But other researchers, such as Michael Wassenegger of the Max Planck Institute for Biochemistry in Martinsreid, Germany, and Marjorie Matzke of the Austrian Academy of Sciences in Salzburg, have found that certain RNA constructs can lead to methylation of the corresponding gene—an indication that the RNA is somehow talking back to the DNA. If confirmed, Mello says, "such retrograde flow of information would be really remarkable."

But much of what researchers have already learned about RNAi has been remarkable. As Vance puts it, "It's been so incredibly cool."

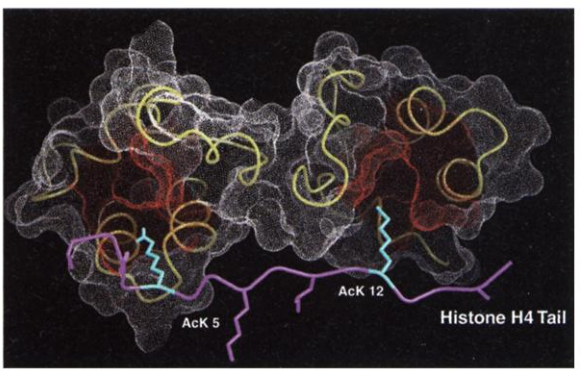
—JEAN MARX

NEWS

Matching the Transcription Machinery to the Right DNA

The structure of a tandem set of folds called bromodomains reveals how they help set the stage for transcription

Gene transcription in the nucleus is a bit like an elaborate wedding at St. Patrick's Cathedral in New York City. Imagine that the "aisle" is the DNA of a gene that's going to be transcribed—that is, copied into a messenger RNA, as the first step in protein synthesis. The ushers and bridesmaids are the proteins that line up along the DNA to prepare it for the enzymes that will do the copying. In either case, before the walk down the aisle can occur, all sorts of players must get in their proper places—despite what often seems like total chaos.



Close connection. The configuration of paired bromodomains lets them bind to the acetylated histone tail. AcK 5 and 12 are acetylated histone lysines.

CREDIT: JACOBSON *ET AL.*

Cell biologists have long wondered how this molecular event is choreographed. Now they have a new clue about how one key member of the transcription wedding party, a protein called $TAF_{II}250$, knows where to stand. Researchers knew that this protein is an essential part of the transcribing machinery—a complex of many proteins, some in common for all genes and some unique to particular gene targets—but its role was unclear. Work described on page 1422 by structural biologist Robert Tjian and his colleagues at the University of California, Berkeley, may now provide an answer: The protein helps direct the transcription machinery to the right DNA targets and gets the DNA into the correct configuration for transcription to occur.

Combining both structural analysis of a portion of $TAF_{II}250$ and biochemical studies of its behavior, the Berkeley workers find that the protein apparently homes in on one of the histone proteins that wraps the DNA and forms

beadlike structures called nucleosomes. In particular, the work suggests that two protein motifs in $TAF_{II}250$ called bromodomains recognize and bind to two acetyl groups added to the histone. Such histone acetylation is known to be a signal for activating gene transcription and was at one time thought to work by disentangling the DNA from the nucleosome. Only then did the transcription machinery have access to DNA, or so cell biologists thought.

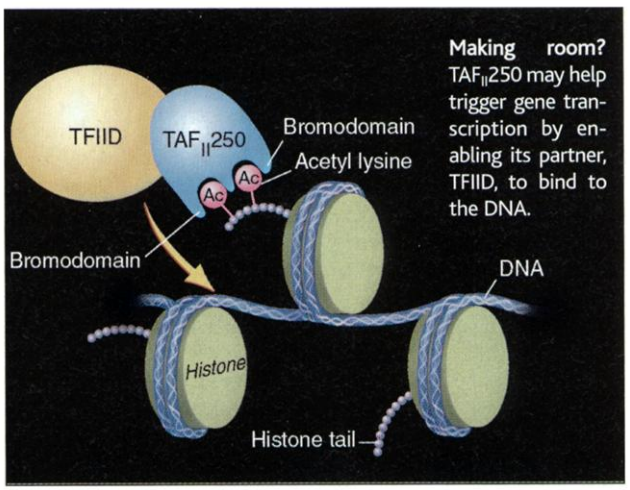
But the Tjian team's findings suggest that nucleosomes play a more dynamic role in controlling gene expression: It looks as though the proteins involved in transcription might actually start to work while the DNA is still wrapped up in the nucleosome. "It seems pretty clear that there are different states of wrapping," with transcription proteins helping to control how much unraveling of the DNA occurs, says Steve Buratowski, a molecular biologist at Harvard Medical School in Boston. Until now, most test tube studies of transcription have involved naked DNA, but this work indicates that researchers need to start looking at the DNA in nucleosomes as well.

The new findings also add to a growing body of evidence that bromodomain recognition of acetyl groups plays an important role in bringing about transcription. The strength of that recognition suggests, too, that bromodomain-acetyl connections assist in other cellular transactions. "I am very excited about this paper," says Ming-Ming Zhou, a structural biologist at Mount Sinai Medical School in New York City, whose work first hinted at that possibility. What's more, he adds, given how common bromodomains are, "I won't be surprised if nature doesn't use bromodomains to mediate many protein-protein interactions."

Three years ago when Zhou first decided to study bromodomains, researchers knew that these protein motifs are very common—they've been found in approximately 50 proteins—but their function was largely a mystery. In cells, bromodomain proteins associate with the so-called HAT enzymes (for histone acetyltransferases), which add acetyl groups to the amino acid lysine in histones and are thus involved in regulating gene transcription. One possibility, which had been suggested by cell biologist C. David Allis of the University of Virginia in Charlottesville, is that bromodomains direct HATs to acetylated histones, where they can enhance the acetylation and activate gene transcription. His work had indicated that $TAF_{II}250$ was itself a HAT enzyme (*Science*, 10 January 1997, p. 155).

To explore whether bromodomains do in fact recognize acetylated histones, Zhou turned to a bromodomain-containing protein called P/CAF. He and his colleagues first worked out the general structure of the P/CAF bromodomain. Then they made a synthetic version and tested its ability to bind to bits of proteins that had acetyl molecules attached to some of their amino acids. As they reported last year in the 3 June issue of *Nature*, the bromodomain tends to stick to acetylated lysines. "We were pleased," recalls Allis, because that result supported his ideas. Still, he says, "the binding constants were really pretty wimpy," which left him wondering if the binding was physiologically relevant.

Meanwhile, Tjian's group was doggedly pursuing the structures and functions of the transcription complex and decided to take a closer look at the $TAF_{II}250$ protein, which has two bromodomains side by side. After first making crystals of the piece of $TAF_{II}250$ containing the bromodomains, Tjian and his colleagues used x-ray crystallography to determine its structure to a resolution of 2 angstroms. This revealed that each of the two bromodomains has a pocket just the right size to fit an acetylated lysine.



Making room? $TAF_{II}250$ may help trigger gene transcription by enabling its partner, TFIID, to bind to the DNA.

ILLUSTRATION: C. CAIN

Tjian's team then tested the fit between the $TAF_{II}250$ segment containing the bromodomains and small synthetic proteins carrying pairs of acetylated lysines at varying distances. The researchers found that the $TAF_{II}250$ bromodomain region bound more tightly to some of the test peptides than others. The best fit seemed to be with a peptide having the same acetylation pattern as the H4 type of histone, with the acetyl groups separated by seven amino acids. Its binding to the double bromodomain was 70 times stronger than the binding Zhou found with the single bromodomain protein—a finding that Allis calls "exciting."

It suggests that in cells, proteins such as P/CAF, with just one bromodomain, likely pair up with a molecular partner with another bromodomain to find a particular pattern of acetylated lysines. If their bromodomains align correctly, their weak ability to connect to a histone tail could improve dramatically, he suggests. There they may help unwind DNA and promote transcription.

The strength of the double bromodomain connection needs to be tested in live cells, but already cell biologists are seeing how the result might fit into their view of how transcription occurs. They are coming to think that DNA becomes acetylated only in specific spots, likely near a promoter, a DNA sequence just upstream of a gene that acts as its on-off switch. Nobody really knows what puts the first acetyl groups on the histones, but once there, they may flag down proteins such as $TAF_{II}250$ with double bromodomains. Then $TAF_{II}250$ could add more acetyl groups to the histone, and the resulting hyperacetylation could help promote the binding of other proteins needed to get transcription going.

In this way, "you essentially could kill two or three birds with one stone," Buratowski says. "You have a positive feedback loop, and in addition you have a way to recruit [other proteins] to the site." Moreover, in the April issue of *Genes and Development*, Buratowski described a double bromodomain protein related to $TAF_{II}250$ that associates with the transcription machinery in yeast—suggesting that these double bromodomains are key for transcription in a wide range of organisms.

Equally intriguing is the possibility that the configuration of the bromodomains relative to one another might help determine just which genes out of the many thousands in a cell are to be turned on at any one time. "There could be a whole language of bromodomains," says Buratowski. Whereas $TAF_{II}250$'s bromodomains recognize and bind to acetylated lysines about seven amino acids apart, another protein's bromodomains could be configured to recognize those closer together or farther apart. As a result, Allis suggests, $TAF_{II}250$ and other transcription factors could be targeted to promoters for specific genes.

He and others plan to explore these ideas by looking at other proteins with bromodomains. Tjian, meanwhile, has kept his sights on the transcription machinery that $TAF_{II}250$ is part of. These results, along with new, albeit fuzzy, structures of transcription complexes (*Science*, 10 December 1999, p. 2153), have invigorated his quest to understand the actions not just of $TAF_{II}250$, but of all the players in the transcription "wedding." "The transcription machinery is unbelievably complex," he admits, "but we will absolutely be able to figure it out."

–ELIZABETH PENNISI