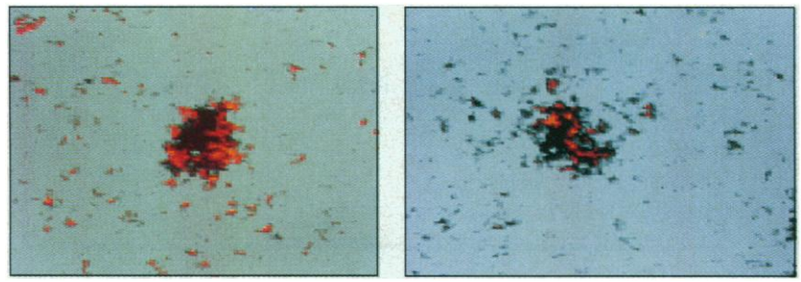
Opening the Way to Gene Activity

A flurry of activity during the past 9 months has brought the chemical modification of histone proteins to the fore in the regulation of gene expression

For decades, molecular biologists bent on understanding how the cell controls the activity of its genes rarely paid much attention to the genes' milieu. In the living cell, the DNA of the genetic material is tightly bound up with histones and other proteins, which together form the chromatin. Even though biologists suspected that the histone's grip had to be loosened before other proteins could turn genes on and off, chromatin is "so complex and so messy that there was very little attempt to really look at it," recalls developmental geneticist John Lucchesi of Emory University in Atlanta. But results from several labs are now making it impossible for researchers to ignore chromatin structure and its modifications any longer. Indeed, says molecular biologist Kevin Struhl of Harvard Medical School, "The whole field has literally exploded in the past 9 months."



BAZETT-JONES *ET AL., NUCLEIC ACIDS RESEARCH* **24**, 321 (1996)

Loosening up. Unacetylated nucleosomes *(left)* are compact, with DNA (red) tightly wound around a core of histone proteins. Acetylation causes the nucleosomes to spread out and the DNA to elongate *(right)*.

In that explosion, researchers have begun to identify parts of the machinery that alters chromatin structure so that gene-regulating and transcribing proteins can do their job. The findings all support the notion that a chemical reaction called acetylation, in which simple chemical groups known as acetyls are added to the histones, is important. The modified proteins then hold less tightly to the nearby DNA, opening the way to activating gene expression. The idea isn't new, but there was little direct evidence for it because researchers could not get their hands on the enzymes that add and remove the acetyl groups. All of that has changed now with the identification of four distinct nuclear histone acetylating enzymes and five more enzymes that undo the reaction by removing acetyls from histones.

Besides pinning down these enzymes, the new work links them to gene expression and to changes in cell growth. All four acetylating enzymes have turned out to be proteins already known to associate with transcription factors, the proteins that regulate gene expression. And their activity has been intimately linked to the control of the cell cycle, the carefully choreographed changes in gene expression and other cellular activities that culminate in cell division. There are even indications that abnormal acetylation can lead to cancer development. "It looks like [acetylation] is a fundamental cell regulatory process," says organic chemist Stuart Schreiber of the Howard Hughes Medical Institute at Harvard University, whose team discovered the first histone deacetylase.

Anticipating acetylation

Cell biologists first began to suspect that acetylation might help regulate gene expression in 1964, when Vincent Allfrey of the Rockefeller Institute (now Rockefeller University) in New York City discovered that histones are sometimes heavily acetylated. In the nucleus, the DNA is arranged in nucleosomes, beadlike structures consisting of a DNA strand wrapped around a histone core that are connected to one another by other histone molecules. And Allfrey noted that the chemical modification appeared to reduce how tightly the histones associate with the DNA in the nucleosomes.

Allfrey's evidence also suggested that these changes influence gene activity. He found that unacetylated histones appeared to inhibit the transcription of DNA into RNA, the first step in gene expression, while histone acetylation reduced this inhibition. This led him to suggest that the acetyl groups, which become attached to the positively charged amino acid lysine, lessen histones' attractiveness to nearby DNA by neutralizing the proteins' positive charge. This change would then make it possible for the proteins needed for gene activity to get close enough to interact with the DNA. Conversely, Allfrey predicted, removal of the chemical side groups would close the door on gene transcription by restoring histone's tight connections with DNA.

Since then, the link between the amount of acetylation of the histone cores and the rate of transcription has strengthened. But because no one could pin down any enzymes capable of adding or removing acetyl groups, researchers were unable to take the work any further. "A lot of people tried, we tried, but nobody had fingered a polypeptide that was responsible for the acetyltransferase activity," says cell biologist C. David Allis of the University of Rochester in New York.

The closest anyone came was in 1995, when Rolf Sternglanz from the State University of New York, Stony Brook, and shortly thereafter, Dan Gottschling, now at the Fred Hutchinson Cancer Research Center in Seattle, and their colleagues found an enzyme that adds acetyl groups to newly formed histones. But that reaction, which occurs even before the histones move into the nucleus, could not have anything to do with gene control.

The nuclear acetylating enzymes that are more likely to do the job apparently are too scarce to show up in the standard protein-sorting methods. That same year, however, in what Allis describes as perhaps "a last-ditch effort," he and James Brownell in his lab developed a new way to track down the elusive nuclear acetylating enzymes.

Brownell first mixed histones into a gel used to sort proteins by size. After separating proteins in nuclear extracts on this modified gel, he then added radioactively labeled acetyl coenzyme A as a source of acetyl groups. He and Allis reasoned that the previously undetectable band of acetylating enzyme would transfer the labeled acetyl groups to the histones in the gel. The presence of these easily detectable, radiolabeled histones would then indicate their quarry's location in the gel.

Because the ciliated protozoan *Tetrahymena* is known to have a lot of acetyl groups attached to its histones, Brownell and Allis used extracts of this organism's nuclei in their search. It turned out to be a good choice. Their radiolabeling technique produced a band of acetate-labeled histone, and by what Allis describes as "brute-force" methods they collected and purified

enough of the protein located at that band site to determine a partial amino acid sequence. Jianxin Zhou in Allis's lab then synthesized the DNAs that could encode this amino acid sequence and used them as probes to find and clone the gene encoding the protein, which they named HAT A (for histone acetyltransferase type A).

A search of existing gene databases for genes whose sequences resemble those of the *HAT A* gene yielded an unexpected but exciting result. As Allis's team reported in March 1996, the *Tetrahymena* HAT enzyme turned out to be very similar to a protein called Gcn5p (for general controlled nonrepressed protein) that had been discovered first in 1992 by a yeast geneticist, George Thireos, at the Institute of Molecular Biology and Biotechnology in Heraklion on the island of Crete, and later again by Leonard Guarante of the Massachusetts Institute of Technology (MIT) during a search for yeast genes that affect transcription. But beyond playing a role in transcription, "it was completely unclear what these genes [and their proteins did]," says Harvard's Struhl.

The Allis team's discovery that their HAT A is very similar to Gcn5p raised the possibility, however, that Gcn5p is itself a histone acetyltransferase. And indeed, working with Sharon Roth at the M. D. Anderson Cancer Center in Houston, the researchers found that Gcn5p works in yeast much as HAT A does in *Tetrahymena*. "It was a somewhat startling result," recalls Harvard yeast geneticist Fred Winston. "It brought two things together that hadn't been brought together before: something that was a transcription factor was [also] a histone modifier." Adds Struhl: "It turned everything [regarding acetylation and transcription] from this vague correlation to specific molecules."

ROUNDUP OF NUCLEAR HISTONE ACETYLTRANSFERASES AND DEACETYLASES TO DATE

Nuclear Histone Acetylases	Organism	Complexes With	Histones Modified
HAT A (Gcn5p)	*Tetrahymena* yeast, human	ADA2, ADA3, other ADAs?	H3, H4
PCAF	human	p300/CBP	H3, H4
p300/CBP	human	CREB, c-Jun c-Fos, MyoD	H2A, H2B, H3, H4
$TAF_{II}230/250$	human, *Drosophila*, yeast	a subunit of TFIID	H3, H4
Histone Deacetylase	**Organism**	**Complexes With**	**Histones Modified**
DHAC1 (RPD3)	human	?	H4 ?
	Drosophila, **Xenopus*, yeast, *mouse, *nematode	?	H3, H4†
HDA1	yeast	HDA2, HDA3	H3, H4†
HOS1	yeast	?	H3, H4†
HOS2	yeast	?	H3, H4†
HOS3	yeast	?	H3, H4†

*Tentative. †H2A, H2B not tested.

"It looks like [acetylation] is a fundamental cell regulatory process."
—Stuart Schreiber

Mammalian HATs

The emerging story would soon take an even more intriguing twist, when work already under way in molecular biologist Yoshiro Nakatani's lab at the National Institute of Child Health and Human Development (NICHD) led not only to the discovery of a mammalian counterpart to the yeast acetylating enzyme, but connected such enzymes to the cell's growth-control pathways. Nakatani was studying E1A, an oncogenic protein made by adenovirus that overrides the natural brakes on proliferation in mammalian cells by altering gene transcription. He knew that in order to exert its growth-stimulating effects, E1A has to bind to a large cellular protein, called p300/CBP. Typically, p300/CBP forms a complex with certain transcription factors, helping them promote specific patterns of gene expression. But exactly how E1A binding to p300/CBP releases cell growth controls is unclear. To get a better picture, Nakatani needed to track down p300's normal protein partners. He found one of them, which he called PCAF (for p300/CBP-associated factor), by looking for the mammalian counterpart of Gcn5p.

At that point, the yeast protein was not yet known to be an acetyltransferase. But when Nakatani heard that Allis had shown that Gcn5p has acetylating activity, Xiang-Jiao Yang in his group tested PCAF for similar activity. It, too, was one of those elusive enzymes, he and his colleagues reported in July 1996. And it's not the only one in mammals. As Nakatani continued his work, he and NICHD's Bruce Howard and Vaily Ogryzko began to suspect that p300/CBP itself might be capable of acetylating histones because they could detect acetylation by the p300/CBP-E1A complex even in PCAF's absence. Their hunch proved correct, as the group described in November. Then, less than a month later, Andrew Bannister and Tony Kouzarides of the University of Cambridge in England reported the same result.

The discovery that p300/CBP and PCAF acetylate histones indicates that the reaction is important for turning on a wide range of genes. Although Gcn5p apparently has a rather limited range of action, p300/CBP has "been shown essential for a lot of genes that are acutely regulated," says Alan Wolffe, a biochemist at NICHD. And further evidence that acetylation is a common prerequisite for gene expression came just as 1996 drew to a close when Nakatani and Allis found that a protein called $TAF_{II}230/250$, which is a part of a large transcription factor complex called TFIID, is also a histone-acetylating enzyme. TFIID is necessary for the initiation of transcription of all protein-coding genes, so the result further expands the role of acetylation in gene expression, Allis says.

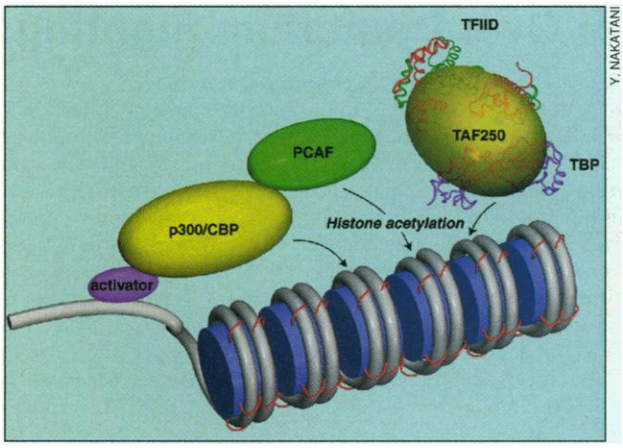
The HATs found so far have somewhat different specificities. For example, while p300/CBP can add acetyl groups to all four histones in the nucleosome core, Gcn5p acetylates only two of them—histones H3 and H4. And the different acetylating enzymes are also specific about which histone amino acids they will acetylate. These differences suggest that the added acetyl groups may be more than just "on" or "off" signals for transcription. They may also serve, Allis says, as "specific flags," for attracting other proteins that can fine-tune gene activity. "Depending on which particular site [on the histone] gets acetylated, it might make a difference in downstream effects with other [molecular] machinery that needs to bind to chromatin," he proposes.

A newly discovered potential HAT in male fruit flies shows one possible kind of discriminating acetylation reaction: acting only on one chromosome. In *Drosophila*, the male's X chromosome makes up for the fact that it is present in only a single copy by producing as much gene product as do both copies of that same chromosome in the female. Emory's Lucchesi has identified five

proteins that seem to trigger this dosage compensation, as it's called. One of them, he says, looks very much like a HAT. That HAT seems to acetylate a particular lysine on the H4 histones along the X chromosome but doesn't appear to touch histones on other chromosomes. "What we think is that we have a gene that encodes a specific HAT that is targeted to the X chromosome in males and that is responsible for a very well-defined enhancement of transcription," Lucchesi says of *MOF* (for male-absent on first chromosome), the name of the gene.

Cell-cycle regulation

Anything that affects gene expression is likely to influence whether or not cells proliferate, and that goes for HATs as well. Most likely, proteins that normally interact with PCAF and p300/CBP keep the cell cycle in check, presumably by turning on genes that inhibit cell growth while turning off those that might foster cell division. But as Nakatani has shown, E1A binding to p300/CBP displaces PCAF from the complex, and this may alter HAT activity and thus gene acetylation patterns, allowing expression of genes that enable cells to start dividing and perhaps preventing the expression of genes that inhibit cell division.



Acetylase lineup. Many transcription factors and other proteins interact to bring about the reading of DNA (seen here in white, wound around blue histone cores). Several of these, including p300/CBP, PCAF, and TAF_{II} 230/250, are acetylase enzymes, which may add acetyl groups to histones at multiple sites.

Indeed, changes in acetylation may play a role in the uncontrolled cell growth of cancer. In September, two reports—one from yeast geneticist Lorraine Pillus's team at the University of Colorado, Boulder, and the other from Julian Borrow of MIT—hinted at a connection between a certain type of acute myeloid leukemia and p300/CBP. The work links the leukemia to a chromosomal translocation that fuses the gene for the acetylating enzyme with another gene. "This translocation may be misdirecting [p300's HAT] activity," says Pillus. As patterns of acetylation change, genes normally turned off may turn on, facilitating tumor growth.

The regulation of the converse process—the removal of acetyl groups from histones—may be just as crucial to normal cell growth. Harvard's Schreiber discovered the first of the histone deacetylase enzymes responsible for this reaction as part of his efforts to understand how trapoxin, a compound that shows potential for treating cancer, inhibits cell growth and causes tumor cells to revert to their normal, differentiated states.

Others had shown that trapoxin blocks the removal of acetyl groups from histones and in so doing may put the brakes on cell growth by increasing or restoring gene activity that normally helps keep cell division in check. By tracking a protein that trapoxin binds in cancer cells, Jack Taunton in Schreiber's group identified the enzyme that normally removes the acetyl groups from the histones.

And December brought news that deacetylation, like acetylation, may be accomplished by multiple enzymes. Yeast geneticist Michael Grunstein from the University of California, Los Angeles, and his colleagues found that yeast contains five separate versions of Schreiber's deacetylase.

Many researchers expect that even more acetylating and deacetylating enzymes await discovery. "It's put a whole new spin on everybody's work," says Allis. "Now a lot of coactivators are being screened for acetyltransferase activity." At the same time, the researchers who have already found HATs are sorting out the functions of their discoveries. Besides figuring out the specific jobs for each, they want to know how acetylation fits into what else is known about chromatin structure. For example, they want to know whether acetylation leads or follows other pretranscription activities. Does it break open the chromatin initially, or does this type of modification simply help maintain the availability of the genes as DNA is being read?

Allis also provides a note of caution in the newfound excitement over histone acetylation. So far, he says, all the work demonstrating that the new enzymes acetylate or deacetylate histones has been done on free histones. It is less clear how HATs work on histones that are part of a nucleosome or embedded in a series of nucleosomes. And in actual nucleosomes, histone acetylation probably interacts with other types of chemical modifications, such as methylation or phosphorylation, that also aid in chromatin remodeling. How all these fit together, "nobody knows," says Wolffe. Nevertheless, one thing is certain, Allis notes: "The field has come to the understanding that acetylation is an important part of the activation of genes."

Vincent Allfrey should be pleased.

–Elizabeth Pennisi

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