Sea region are broken or idle; that means "it is now much more difficult to estimate water resources than it was 20 years ago," says Rodda.

As for the United States, the problem is in river gages, which have dropped by about 6% over the last decade. Most of these stations are paid for with collaborative agreements between the U.S. Geological Survey (USGS) and more than 800 state and local agencies, but local priorities often shift, leading to loss of gages with long-term data, says Robert Hirsch, chief hydrologist at the USGS.

What's more, U.S. stations that record flow on small, free-flowing rivers have dropped by 22% since 1971. These stations are funded solely by the USGS, which has seen its gage budget shrink 9% in the past decade. The loss is "disturbing," says Hirsch, especially because these naturally flowing rivers show how streams respond to changes in land use and climate, and so are vital to climate models.

International officials have begun to address the problem, with several initiatives already under way. The World Bank has agreed to finance 25 new stations in Uzbekistan for a total of $2.5 million. And the World Hydrological Cycle Observing System, a program launched by WMO in 1993, is establishing 50 new monitoring stations in 10 countries in southern Africa. In the United States, Hirsch says he's beginning to see signs that Congress takes the problem seriously, and he hopes that next year's budget will include a $2.5 million boost for gages.

But even with new stations, coverage in the developing world will be sparse. And in the meantime, valuable climatic records are not being kept, says Schulze. "There is going to be a 10- to 20-year gap; it's data lost forever." For towns like Falmouth—where the upriver gage is still out of action—the losses might be even greater. **–ERIK STOKSTAD**

CELL BIOLOGY

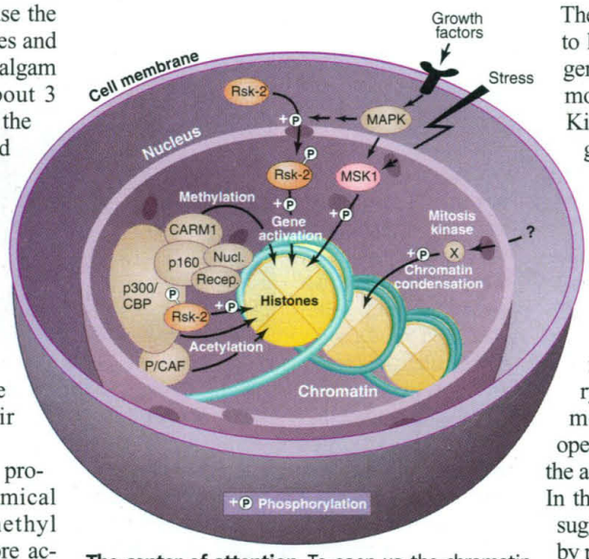
How Chromatin Changes Its Shape

A variety of modifications affect the protein-DNA complex known as chromatin, causing it to loosen—or tighten—as needed for cell function

Buried in the depth of the cellular nucleus, the long threads of DNA are the ultimate control center of the cell. But how the many molecular players that convey to the genetic material the signals it needs to determine what to do has been a mystery, because the DNA is literally balled up with histones and other proteins in a tightly wound amalgam known as chromatin. Beginning about 3 years ago, cell biologists discovered the first crack in DNA's armor. They found that when a small chemical entity called an acetyl group is glued to specific places on the histone proteins, the chromatin fiber opens up, allowing the cell's gene-reading apparatus to gain access to the genetic material (*Science*, 10 January 1997, p. 155). New work is now revealing other ways in which histones can be coaxed to loosen—or tighten—their grip on the DNA.

In recent months, researchers have produced evidence that two other chemical appendages—phosphate and methyl groups—can also make the DNA more accessible when they are attached to histones, boosting the activity of specific genes. One of those modifications, histone phosphorylation, also appears to be involved in other types of chromatin remodeling, such as the chromosome condensation that takes place prior to cell division. How the phosphorylation can have such different effects—opening up the chromatin for gene expression, but condensing it for cell division—is still an open question. But the finding suggests that histone modifications may have an impact far beyond gene activation.

"There's no reason to believe that a lot of these [chromatin-remodeling] activities won't function in other processes," such as DNA replication, recombination, or repair, says biochemist Jerry Workman of Pennsylvania State University in University Park. Indeed, the findings point to a picture of chromatin as a kind of giant receptor complex that can pick up and integrate a variety of external and internal signals and can change its appearance accordingly—opening up to permit access or winding into tighter coils. "It's like a Morse code where the different modifications could work in various combinations to bring about a biologically meaningful response," suggests biochemist C. David Allis of the University of Virginia, Charlottesville, whose team is at the forefront of the work.



The center of attention. To open up the chromatin so that gene expression can occur, some transcription factors and coactivators attach different chemical tags such as acetyl or methyl groups to certain histone proteins. Similarly, growth factors, stress, and other external signals lead to histone phosphorylation to activate the appropriate response genes. But histone phosphorylation is also a driving force behind the condensation of chromatin into the densely packed chromosomes needed for proper cell division.

ILLUSTRATION: K. SUTLIFF

The two faces of histone phosphorylation

The first clue that adding phosphate groups to histone might alter chromatin and allow genes to be expressed came in 1991 from molecular biologist Louis Mahadevan of King's College in London. When certain growth factors stimulate cells, their signals are transmitted to the nucleus through the so-called MAP (mitogen-activated protein) kinase cascade—a series of kinase enzymes, each of which activates the next kinase in line by adding phosphate groups to it. Mahadevan observed that one of the five major histone proteins, H3, is phosphorylated as a result of growth factor treatment, and speculated that this change opens up the chromatin structure and allows the activation of the growth-promoting genes. In the years since, more and more evidence suggesting that MAP kinases activate genes by phosphorylating histones trickled in from Mahadevan, as well as from James Davie's lab at the University of Manitoba in Winnipeg. It took researchers several years to piece together what exactly was happening at the very end of the cascade, however.

One clue came from Allis and his colleagues. In the course of other work on histone phosphorylation, they had generated an antibody that recognizes H3 only when it has a phosphate on a specific amino acid, serine 10. When the researchers tested the antibody on growth factor–stimulated cells, they saw that it clustered in some 100 sharply

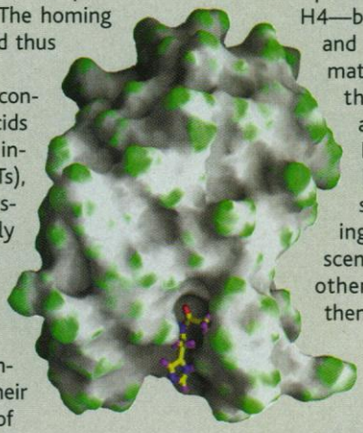
Clinging to Histones

Structural biologist Ming-Ming Zhou of the Mount Sinai School of Medicine in New York City finds nothing more enticing than the intimate relations of proteins—often a key to understanding how they perform their functions in the cell. In his latest achievement, Zhou has taken a close look at a protein region, or "domain," that may guide proteins to specific sites on chromatin, the complex of DNA and proteins making up the chromosomes. The homing may be a step in the remodeling of chromatin and thus in gene regulation (see main text).

The region in question is the bromodomain, a conserved sequence containing roughly 100 amino acids found in some 30 chromatin-associated proteins, including all nuclear histone acetyltransferases (HATs), enzymes that add acetyl groups to certain of the histone proteins found in chromatin. This apparently loosens up the chromatin, thus allowing gene expression to occur. Even though scientists discovered the bromodomain in 1992, exactly how it might contribute to these activities has been unclear. "Bromodomains kept showing up in all these interesting proteins, but nobody had a clue about their function," says molecular biologist Robert Kingston of Massachusetts General Hospital in Boston.

Zhou and his colleagues set out to test one speculation: that bromodomains might be "some sort of homing addresses," guiding proteins that contain the domains to specific sites on the chromatin. In the first stage of their work, the Zhou team used nuclear magnetic resonance to determine the structure of the bromodomain of a HAT protein called P/CAF. As reported in the 3 June issue of *Nature*, this revealed that the P/CAF bromodomain consists of a bundle of four helices that are tightly packed into a more or less cylindrical shape. On one end of the structure, the researchers detected a region that looked like it might be a docking site for other proteins: a little cleft consisting of hydrophobic, or water-shunning, amino acids. "Since hydrophobic amino acids are usually buried inside a protein, we thought this pocket may be a protein-protein interaction site," says Zhou.

To try to find out what the site might recognize, the researchers began mixing the P/CAF bromodomain with various peptides from other proteins, including fragments of histones H3 and H4—both unmodified and modified by acetylation and other changes that have been linked to chromatin remodeling. Zhou and his colleagues found that the P/CAF bromodomain binds only the acetylated H3 and H4 peptides with the acetylated lysine amino acid fitting exactly into the hydrophobic pouch. The results suggest, Zhou says, that "bromodomains may be general binding modules for acetylated histones." In such a scenario, an initial acetylation event could lure in other, bromodomain-containing HATs that would then lead to more widespread histone acetylation and, ultimately, gene activation.



Getting a grip. The binding pocket of this bromodomain is shown with an acetylated lysine homolog (colored molecule at bottom).

Some chromatin aficionados predict that the discovery of the acetyl-lysine–binding capacity of the P/CAF bromodomain is just the first glimpse of a complex homing system that might guide the hundreds of chromatin-binding factors needed to orchestrate the cell's activities. "Acetylation is just the tip of the iceberg. Underneath there's something that's even bigger—the different histone modifications together might act like molecular ZIP codes for the different [chromatin-binding] proteins," says Alan Wolffe, a cellular biologist at the National Institute of Child Health and Human Development in Bethesda, Maryland. **–M.H.**

CREDIT: MING-MING ZHOU

defined spots, or speckles, distributed all over the chromosomes. The number of spots roughly corresponded to the number of genes activated in the early stages of growth factor stimulation. To find the enzyme responsible, Allis's team fractionated cell extracts and came up with a protein with a molecular weight of 90 kilodaltons.

At this point, Allis learned of results from molecular biologist Paolo Sassone-Corsi and human geneticist André Hanauer of the Institute of Genetics and Molecular and Cellular Biology in Strasbourg, France. In 1996, the two had shown that patients suffering from Coffin-Lowry syndrome, a hereditary form of mental retardation that often is accompanied by facial and other deformities, have mutations in the Rsk-2 protein, one of the kinases in the MAP kinase pathway. Sassone-Corsi's group also linked the Coffin-Lowry mutation to a defective response to growth factors, implying that the mutations somehow impede gene activation.

Allis was intrigued by those findings, because Rsk-2 has about the same molecular weight as his mystery enzyme. He sent a sample of his antibody against phosphorylated histone H3 to Sassone-Corsi and his colleagues so that they could look for the punctuated pattern of antibody staining on cells taken from Coffin-Lowry patients. If this pattern did not appear during growth factor–stimulated gene expression, it would be a sign that the Rsk-2 mutation had interfered with histone phosphorylation—which in turn might be the cause for the defective response to growth factors in these cells.

As the researchers reported in the 6 August issue of *Science* (p. 886), the Coffin-Lowry cells showed none of the bright speckles at all, evidence that histone H3 isn't phosphorylated in response to growth factor stimulation. In fact, Sassone-Corsi recalls, the results "were so black and white that the postdoc who had done the experiment thought something went wrong."

More recently, the Allis team obtained more direct evidence that H3 phosphorylation is linked to the expression of genes involved in growth stimulation. In experiments performed in collaboration with Davie's team and in press at the *Journal of Biological Chemistry*, the researchers used the phospho-H3–specific antibody to fish out histone H3 phosphorylated in the course of a gene response to growth factors, along with the snippets of DNA bound to the modified histone. And lo and behold, they found that two of those snippets came from the *c-fos* and *c-myc* genes, both of which are turned on early in growth factor responses. Taken together, these findings, says Davie, "establish histone phosphorylation as a crucial mechanism for gene regulation."

Rsk-2 may not be the only player in this arena, however. In an upcoming issue of the *EMBO Journal*, Mahadevan's group presents evidence that MSK1, a close relative of the Rsk family, might be the kinase that phosphorylates H3 in response to both growth factors and stress in mouse fibroblast cells. And geneticist Kristen Johansen of Iowa State University in Ames and her colleagues have found that this kinase localizes to areas of high gene activity in fruit fly chromatin, a further hint that it has a role in gene regulation. Because some MAP kinase subtypes don't activate Rsk-2, yet still lead to histone H3 phosphorylation, Mahadevan thinks that MSK1 could be the missing link.

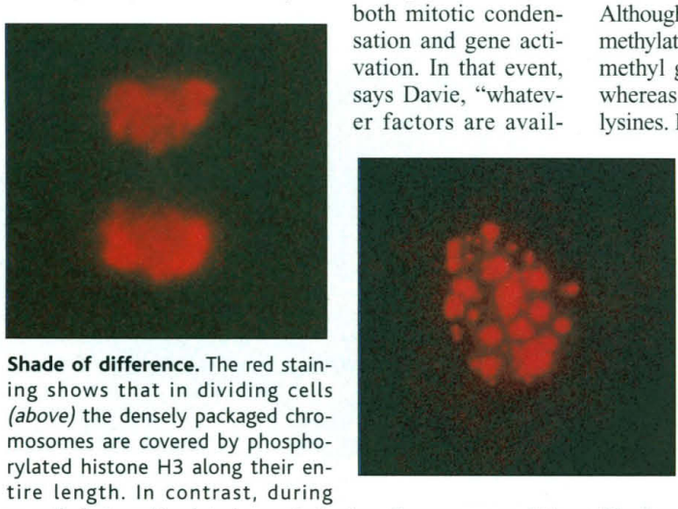
Meanwhile, others were pinning down a

link between histone phosphorylation and a very different process: cell division, or mitosis, when the chromatin condenses and locks up the DNA in tight bundles. The first indication that histone phosphorylation might be important for this process had come in the early 1970s when scientists found that two of the histone proteins, H1 and H3, become highly phosphorylated when the chromosomes start condensing. But researchers couldn't determine whether this was a crucial prerequisite for chromosome condensation or a mere coincidence. "The best we had for a long time was this correlation," Allis says.

Now Allis's team, working with Martin Gorovsky and colleagues at the University of Rochester in New York, has turned to the ciliated protozoan *Tetrahymena thermophila*, which has two types of nuclei with drastically different properties. The *Tetrahymena* macronucleus, which harbors some 90 copies of each of the protozoan's chromosomes, provides all the cellular proteins the organism needs for normal growth. Its chromosomes don't get condensed before the cells divide; instead, the macronucleus simply pinches in half so that each daughter cell ends up with a macronucleus containing about half the multiple genomes.

In contrast, the normally silent micronucleus, which comes into play only when two *Tetrahymena* cells want to have sex—that is, exchange genetic material—contains only two copies of the genome and divides by way of regular mitosis. Thus, Allis and Gorovsky reasoned that if they mutated the histone H3 at its major phosphorylation site, the serine at position 10, any resulting changes might only be manifest in the micronucleus. And that's exactly what they saw.

Their results, reported in the 2 April issue of *Cell*, showed abnormal chromatin condensation and chromosome loss in the micronucleus of H3 mutant strains without any apparent effect on the macronucleus. In addition, antibody studies showed that the phosphorylated H3 covers the chromosomes along their entire length rather than just being found in isolated speckles, as in the growth-stimulated cells. "This establishes the role of H3 phosphorylation in chromatin condensation. The evidence is pretty much cast in stone," says Alan Wolffe, a cellular biologist at the National Institute of Child Health and Human Development in Bethesda, Maryland.



Shade of difference. The red staining shows that in dividing cells *(above)* the densely packaged chromosomes are covered by phosphorylated histone H3 along their entire length. In contrast, during growth factor–stimulated gene induction, the very same H3 modification is specifically localized to certain response genes *(right)*, as indicated by the speckled pattern.

CREDITS: CLAUDIA CROSIO AND PAOLO SASSONE-CORSI/CNRS

How one and the same event, the phosphorylation of serine 10 in histone H3, can lead to chromatin condensation in one setting and to chromatin unfolding and gene activation in another is still a mystery, however. As yet unpublished work from the labs of both Allis and Mahadevan provide an interesting hint: Phosphorylation in conjunction with some other modification, such as acetylation, tilts the balance in favor of gene activation. "Maybe the effect of H3 phosphorylation depends on what else is going on on that particular chromatin fragment," speculates Penn State's Workman.

An even simpler, albeit not mutually exclusive, scenario holds that H3 phosphorylation initially loosens up chromatin in both mitotic condensation and gene activation. In that event, says Davie, "whatever factors are available at the time this happens will determine the outcome." During mitosis this would be the chromosome-condensing machinery, and during gene activation, transcription factors and coactivators, which might further open up the chromatin by acetylating it.

Methylation joins the club

Further complicating the picture, it now appears that the addition of methyl groups might modify chromatin structure as well. Molecular endocrinologist Michael Stallcup of the University of Southern California in Los Angeles came across this possibility while studying nuclear receptors, proteins that when bound to steroid hormones such as estrogen act as transcription factors that turn on specific target genes. First, though, the nuclear receptors recruit a number of "coactivators," including a protein designated p160. And p160 itself recruits a set of helpers, as the team discovered when they found that it associates with an enzyme called CARM1. CARM1's structure, they found to their surprise, indicates that it serves to add methyl groups to other proteins. (The team described their results in the 25 June issue of *Science*, p. 2174.)

When Stallcup's team tested CARM1 in gene activation experiments, they found that it behaves like a bona fide coactivator, boosting the effect of nuclear receptors and p160 on gene expression. What's more, when the researchers mutated CARM1, disabling its methyl-adding activity, it lost its ability to boost transcription as well. "This isn't a proof that the methyltransferase activity defines how CARM1 works, but it seems weird if it were a pure coincidence," Stallcup says.

Now the hunt is on for CARM1's targets. Although Stallcup found that the enzyme can methylate H3 in the test tube, it attaches the methyl groups to the amino acid arginine, whereas histones are mostly methylated at lysines. For that reason, Davie for one thinks that CARM1's role as a histone methyltransferase still remains to be established; he and others contend that the enzyme might methylate other proteins such as transcription factors or fellow coactivators, regulating their activities, rather than the histone's. Even so, Allis predicts that the study will bolster interest in methylation as a means of reshaping chromatin. "Before Stallcup's paper, methylation wasn't even on people's radar screen," he says.

And the rest?

Many researchers in the field do not expect the current momentum to slow down anytime soon. Histones are among the most heavily modified proteins in the cell, carrying many other attachments besides acetyl, phosphate, and methyl groups. Among their other molecular decorations are the small protein ubiquitin, ADP-ribose, and various sugars. Researchers suspect that some of these, too, will turn out to affect chromatin behavior.

As Allis sees it, "All these [histone] modifications that have been buried in the chromatin literature for years will come back into fashion before long." He cautions, however, that a great deal more work will be required to pin down the role of the modifications and to see in particular how they tie in with the numerous hormones, growth factors, and other external signals that influence cell activities. Stallcup agrees, saying, "We're a long way from having discovered all the players that can open up chromatin." But he adds, "we have at least cracked open the door."

–MICHAEL HAGMANN