CHROMATIN STRUCTURE

Molecular Machines May Aid Gene Expression

Each human cell contains a full 2 meters of DNA. To solve what could be the ultimate storage problem, the DNA is wound like thread around a spool of proteins known as histones, forming the complexes known as nucleosomes. These, in turn, make up the chromosomes that fit easily in the cell nucleus. Some geneticists speculate, however, that such compulsively neat storage raises problems of its own: Like clothes in an overpacked closet, the DNA is hard to get at. Now those researchers are glimpsing how the cell exposes the genes to the enzymes and other building materials needed to transcribe them into RNA.

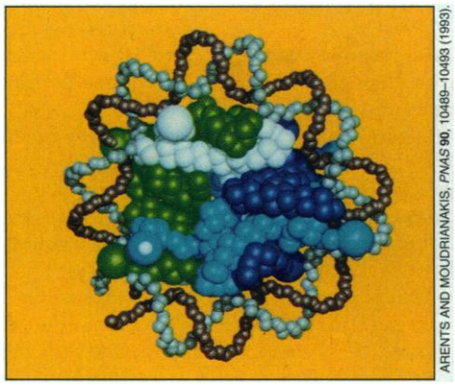
In the last 18 months, chemists, molecular biologists, and geneticists have unveiled a battery of molecular machines, energy-consuming proteins in the cell's nucleus, that disrupt the histones. Apparently working in conjunction with transcription factors—DNA binding proteins that trip switches called regulatory elements to turn genes on and off—these protein complexes remodel the nucleosomes, allowing the transcription machinery access to the DNA.

The discovery is pumping new life into the old idea that histones regulate genes by selectively exposing them to transcription. That theory took brief hold in the 1970s after researchers studying the structure of chromatin, the DNA-protein mixture that makes up the chromosomes, discovered the thread-and-spool structure of the nucleosomes. It fell into disrepute, however, as evidence grew that genes are regulated mainly by transcription factors. Now it seems that histones may have a part to play in gene regulation after all.

Histone disruption "may supply an additional layer to [gene] regulation that overlays the control exerted by transcription factors," says molecular biologist Peter Becker of the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. A second line of research, meanwhile, is showing how some histones can have a targeted effect on the activity of genes, suppressing the activity of some and not others in a phenomenon called gene silencing (see box). Still, researchers warn that many of the histone results are preliminary, and some are controversial. "The field is in flux. There are new results every month, and the models change every month," says Becker.

There had, in fact, been hints all along that chromatin structure might be important for gene activity. Arrays of nucleosomes roughly similar to those in the living nucleus can be reconstructed in the test tube. Genes in such nucleosome templates are transcribed into RNA far more slowly than genes in naked DNA. Many other experiments have shown, conversely, that when genes are activated in yeast, fruit fly, and mammalian nuclei, the structure of the nucleosomes containing their regulatory sequences changes. But it remained unclear whether such disruption is needed for gene activation to occur, or is simply its consequence.

An inkling that nucleosome disruption may be a separate step that gets genes ready for transcription came in a surprise finding last year. While studying activation of heat shock genes, a team led by molecular biologist Carl Wu of the National Cancer Institute (NCI) in Bethesda, Maryland, found



ARENTS AND MOUDRIANAKIS, *PNAS* 90, 10489–10493 (1993).

Heart of the matter. DNA in the nucleus is wound around histone cores.

that adding GAGA, the transcription factor that helps activate the genes, to nucleosome preparations containing GAGA's regulatory sequence changed the structure of the nucleosomes. Previous work had led the researchers to expect that, Wu says. What was surprising was that the transcription factor alone couldn't modify nucleosome structure. "It required ATP absolutely," he says. There's no evidence that GAGA itself consumes energy, so the result, which appeared in the 10 February 1994 issue of *Nature*, implied that, besides transcription factors, "you needed a specialized energy-consuming machine to destabilize the nucleosome," says Wu.

A few months later, a team led by genetic biochemist Craig Peterson of the University of Massachusetts Medical Center in Worcester and molecular biologist Jerry Workman of Pennsylvania State University in University Park identified a candidate for the molecular machine: A humungous protein complex called SWI/SNF (pronounced "switch/sniff"). Yeast geneticists had suspected for several years that SWI/SNF—which contains at least 11 different SNF proteins and SWI proteins, one of which can break down ATP—plays a role in histone-gene interactions. They'd found, for example, that disabling any one of six of the protein subunits blocks the activity of large numbers of yeast genes, and that the effect is reversed by disabling the genes encoding histones. One interpretation of the "double mutant" experiments was that SWI/SNF releases certain genes from the histone's repressive influences.

In more recent work, reported in the 1 July 1994 issue of *Science*, Peterson and Workman provided direct evidence for that idea. They found that when they added purified histones to test tubes containing the transcription factor GAL4 and its target regulatory element, GAL4 binding to the element dropped to one hundredth of its normal level. Adding SWI/SNF and ATP to the histone-containing preparations, however, brought GAL4 binding up again, by 30-fold. "It looked like SWI/SNF might be loading the transcription factor onto the nucleosomes," says Peterson.

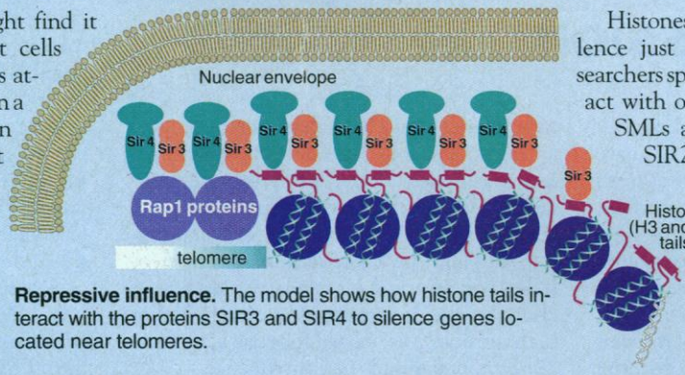
But some researchers suspect that SWI/SNF's apparent nucleosome remodeling activity is a test-tube artifact. "What raises the red flag," says biochemist James Kadonaga of the University of California, San Diego, "is that they have to add so much" SWI/SNF to restore binding. What's more, he says, it's not clear that the mononucleosome assay used "is a good model for chromatin."

Those concerns don't worry Peterson, however. "You'll never prove [SWI/SNF's role] without a shadow of a doubt. But, hey, some people say HIV doesn't cause AIDS," he says. What's more, Peterson now has evidence from living cells that confirms the importance of SWI/SNF. In yet-to-be-published work, Peterson has found that blocking the production of the SWI/SNF subunit SWI1 in modified yeast cells prevents GAL4 from binding to its regulatory elements. "The role in vivo is very similar to the role in vitro," says Peterson, although the cells in the experiment were modified to ensure that GAL4 regulatory sites were embedded in nucleosomes, which is not usually the case.

The SWI/SNF complex may be only the first of several molecular machines that disrupt chromatin. NCI's Wu has isolated from the fruit fly another protein that upsets the structure of nucleosomes in an energy-dependent manner. Wu has since purified the protein, which he calls NURF, for nucleosome remodeling factor, but says he can't discuss its characteristics because they will be reported in an upcoming issue of the journal *Cell*. EMBL's Becker has still another candidate for an energy-consuming nucleosome disrupter—a protein he named CHRAC for

Histones Hush Yeast Mating Genes

Although a mere human might find it difficult to fathom why, yeast cells sometimes find other yeast cells attractive. Yeasts usually indulge in a rather tame form of reproduction called vegetative budding, but every so often some yeast cells convert to the "a" type, and others to the "α" type. These a and α cells find each other alluring and fuse, in the yeast version of sex.



Repressive influence. The model shows how histone tails interact with the proteins SIR3 and SIR4 to silence genes located near telomeres.

Now studies of how yeasts prepare for sex are helping resolve an issue that has plagued histone researchers. For decades, these researchers have sought evidence that histones, which are in intimate contact with DNA throughout the genome, can regulate specific genes (see main text). And the study of yeast sex has shown that in one situation they can. Molecular geneticist Michael Grunstein and his colleagues at the University of California, Los Angeles, have direct evidence that histones help regulate the genes that trigger the two mating types, which are located in the silent mating loci (SML).

For good measure, a team led by Daniel Gottschling of the University of Chicago and Grunstein's team have also shown that histones have a specific effect on the telomeres, the structures at the chromosome tips that suppress activity of genes in the near vicinity. (Also see article by Zakian on p. 1601.) Taken together, "it's the most remarkable data," says molecular biologist Gary Felsenfeld of the National Institutes of Health in Bethesda, Maryland.

The current findings grew out of work Grunstein performed a few years ago. He noticed that yeast cells with certain mutations in the amino-acid tails of two histone proteins, designated H3 and H4, have trouble mating, although the cells were otherwise normal. Grunstein's team soon pinpointed the source of the yeast impotence: Normally, only one SML is activated at a time, but in the mutant cells both were active. As a result, the yeast cells fail to produce pheromones and so lose their attraction for one another. Gottschling, Grunstein, and their colleagues found that those same histone mutations abolish the repressive effect of yeast telomeres on nearby genes, without affecting other genes.

Histones don't have the specificity to silence just these particular genes. So researchers speculated that histone tails interact with one or more proteins specific to SMLs and telomeres—perhaps RAP1, SIR2, SIR3, and SIR4, proteins already known to be required for silencing SMLs and genes near telomeres. Results reported in the 24 February issue of *Cell* by the Grunstein team have confirmed that suspicion.

To tag normal and mutant H3 and H4, researchers genetically engineered hybrid proteins in which the enzyme glutathione S-transferase (GST) was fused to the histone tails. After mixing each hybrid one at a time with each of the RAP and SIR proteins, the researchers then used glutathione-coated beads that bind GST to precipitate the hybrid histones, which carried with them the proteins they bound.

The result was clear-cut, says Grunstein. Hybrids constructed with normal H3 or H4 tails bound SIR3 and SIR4. But hybrids made with mutant tails did not. The experiment "brings the chemistry and genetics together. The regions [of H3 and H4] that are required for silencing in vivo are the regions that bind SIR3 and SIR4 in vitro," he says.

And while the initial experiments were done in the test tube, in the past few months, Grunstein and his postdoc Andreas Hecht have found evidence that histones and SIR proteins behave in the same way in live yeast. Hecht found that he could precipitate from cell extracts a clump of proteins including SIR3, SIR4, H3, and H4, indicating that in living cells the four proteins are bound in a large complex. The evidence suggests that "histones are involved in silencing [the SML and the telomeres], and they do that by interacting with the SIR proteins," says Grunstein.

And Grunstein suspects that the histones' sphere of influence doesn't end with yeast SML and telomeres. "It's a leap," he says, "but perhaps histones interact with other proteins in other parts of the genome" to regulate other genes. That remains to be seen. But for now, histone enthusiasts have at least one firm example of how their favorite proteins can have specific gene control functions.

–R.N.

chromatin accessibility activity.

The Becker team stumbled across CHRAC this summer while investigating whether any DNA binding protein other than transcription factors could gain access to DNA in the nucleosomes. The answer proved to be yes. The researchers found that even DNA-cutting restriction enzymes, which come from bacteria, organisms that have no nucleosomes, remodel nucleosome templates. And, once again, ATP was required. Like transcription factors, "restriction enzymes don't normally require energy," says Becker. So using "brute-force biochemistry," he and his colleagues went in search of a putative energy-consuming cofactor for the enzymes, isolating CHRAC, which appears capable of remodeling nucleosomes, suggesting that it too helps expose genes for transcription.

And no one thinks that will be the end of the list. Indeed, at the Chromatin and Transcription meeting held from 24 to 29 June in Snowmass, Colorado, Workman's postdoc Thomas Owens-Hughes announced that he had isolated a protein from human cells that consumes energy and disrupts nucleosomes when GAL4 fastens to its binding site. They call this protein NDF for nucleosome disruption factor. "The present conclusion is that there is not one chromatin remodeling activity, but various activities," Becker says.

But despite the wealth of new information, there are still some big unknowns, says molecular biologist Richard Young of the Whitehead Institute in Boston. "How," he asks, "is [SWI/SNF] being brought to the regulatory sequences where they supposedly do their remodeling?" What's more, the most controversial idea, that the molecular machines fine-tune the activity of individual genes by acting on histones and nucleosome structure, is unproven. "Even for genes where SWI/SNF is clearly required for absolute expression, there's no evidence that it is also part of normal regulatory control," says geneticist Fred Winston of Harvard Medical School.

That notwithstanding, biochemist Roger Kornberg of Stanford University in Palo Alto, California, takes an optimistic view. "The remodeling of chromatin for transcription is a far bigger story than we know at the moment," he says. "It's going to be the life's work for the next generation."

–Rachel Nowak