Profile of C. David Allis

Without histone proteins, DNA strands would be little more than chaotic tangles. In the last decade, biologists and biochemists have found that histones, essential for packaging DNA in all eukaryotic cells, are not merely storage structures, but they also provide an active platform for DNA modification and remodeling, resulting in dynamic regulation of the genetic template. Biochemist C. David Allis has helped elucidate much of this knowledge and has played a major role in ushering in the modern era of chromatin biology. Consisting of DNA, histones, and other proteins, chromatin is the building block of chromosomes, and modifications to histones and chromatin may help extend the informational content of DNA.

Elected to the National Academy of Sciences (NAS) in 2005, Allis is the Joy and Jack Fishman Professor and head of the Laboratory of Chromatin Biology at The Rockefeller University (New York). In his Inaugural Article in this issue of PNAS, Allis and postdoctoral associate Sandra B. Hake (1) propose that modifications of DNA histones produce a genomic "barcode" that contains important epigenetic instructions influencing gene expression and cellular development.

Getting into a "Real Lab"

Born and raised in Cincinnati, OH, Allis entered the University of Cincinnati in 1969 as a biology major, fully intending to attend medical school. As his senior year approached, Steven Keller, one of his cell biology professors, suggested that he get into a "real lab" before committing to medical school. Keller set up an interview for Allis with Michael Bharier at the University of Cincinnati medical school. "I went over and interviewed. Remarkably, Dr. Bharier took me into his lab group and turned me loose on a project. I went into a cold room, fractionated an extract, and to my surprise, it had a personality. I became hooked, and the rest is history," says Allis about his first foray into research. He stayed in Bharier's laboratory for a senior thesis project (2). "Keller and Bharier saw a scientific spark in me, and they kindled it. I am glad that there are dedicated teachers like them who strive to make a difference in their students," he says.

With medical school forgotten, despite the wishes of his friends and parents, Allis enrolled at Indiana University (Bloomington, IN) for graduate studies.

C. David Allis

"I'd gotten hooked on developmental biology; it was one of the classes I really enjoyed," he says. He studied development in *Drosophila*, concentrating on pole cells and polar granules with NAS member Anthony P. Mahowald as his supervisor. "It was a terrific lab environment. The people were great, and for where I was at that stage of my career, the science was exactly what I wanted to do," he says.

Chromatin Fascination Begins

After finishing his Ph.D. in biology in 1978, Allis went to the University of Rochester (Rochester, NY) for a postdoctoral fellowship with Martin Gorovsky. There, Allis surprised people, including Mahowald, when he switched from studying *Drosophila* to studying chromatin in the ciliated protozoan *Tetrahymena*. "I was getting interested in chromatin, which is what I still study. This was a relatively new topic back then. It wasn't by any means the raging topic that it is today, and to think about doing chromatin biology in a super-low, offbeat critter was not fashionable," he says. Talking about Gorovsky, Allis says, "I think I gravitated to [his] genuinely caring personality and his ability to do remarkable biochemistry on chromatin using the unique biology of this organism." Working in Gorovsky's laboratory, as well as in Mahowald's previously, has influenced Allis's own laboratory philosophy. "Mahowald and Gorovsky were terrific mentors. Both of those lab environments were fun and enjoyable for me because of the people who formed the lab. It's a model atmosphere that I've tried to mirror in my own lab. If I could keep the science on a high keel, but also keep the atmosphere fun, I felt that would be a worthwhile long-term goal," he says.

After his postdoctoral research, Allis accepted an assistant professor position in the Department of Biochemistry at the Baylor College of Medicine (Houston, TX) in 1981. "When I was hired, it was pretty unpopular to work on chromatin, and combining that with a ciliated protozoan, forget it! I had two big strikes against me, but I never sensed that from my chair, Salih Wakil. I felt a huge amount of genuine interest and support from Salih for what I did as a junior faculty member. I never felt like a second-class scientist. When you're junior and young and trying to get over all the hurdles and make tenure, it is wonderful to have that support," Allis says. Over the next decade at Baylor, he moved up the ranks to associate professor and then full professor in the Departments of Biochemistry and Cell Biology.

A Head for HAT

In the early 1990s, many laboratories raced to find proteins that modified histones. Allis won that race in 1996 when he identified the first transcription-associated histone, acetyltransferase (3). He and his graduate student Jim Brownell isolated the protein from *Tetrahymena* cultures and found that the protein, known as Gcn5 in yeast, was a histone acetyltransferase (HAT) that adds an acetyl group to an exposed lysine in histones. "The HAT that we discovered in the protozoan model was already known to function in some unclear way as a positive transcription regulator in yeast. Not necessarily binding to DNA directly, it was probably working through an activator, maybe bridging somehow a property between target activators and the basal transcription machinery. Only in a few discussion[s] did anyone suggest that Gcn5 might be affecting chromatin," says Allis. Identifying HAT came about through a stroke of luck. "Ironically, we probably only succeeded in doing this in the protozoan because the enzyme was super-active in our activity gel assay. It was a little bit of good luck. If we had been doing

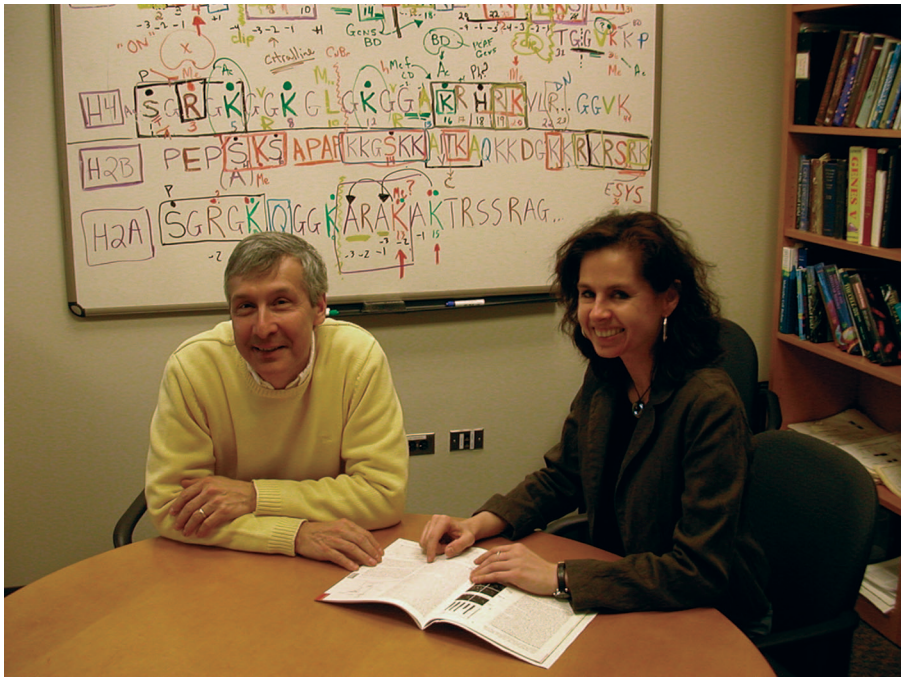
---

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 6428.

these experiments in any other model, I don't know if we would have seen the activity so clearly," he says.

A month after finding HAT, Allis characterized an enzyme that removes the acetyl group from histones. Identified previously by Stuart Schreiber and colleagues, this histone deacetylase enzyme was related to a transcriptional corepressor in yeast (4). "It couldn't have been a more wonderful one–two punch. I am not sure that the chromatin field has been the same since," says Allis.

With these findings of two opposing enzymes that add and subtract acetyl groups to and from histones to enhance or repress transcription, a number of ideas fell into place. "It wasn't rocket science to figure out this enzymatic pair of reactions might function as an on/off switch," says Allis. "Whether you bought into the full importance of chromatin, or whether you just enjoyed it as a topic, you almost couldn't turn your back on what these findings were saying. Most people thought chromatin was just a passive platform that wraps the DNA. But those two papers made people think about a more active process in which chromatin truly participates," he says.



Allis and postdoctoral associate Sandra B. Hake in front of diagrams of N-tail sequences and covalent modifications of histones H2A, H2B, and H4.

Making Marks

Since 1996, the field of chromatin biology has exploded. In addition to acetyl groups, methyl groups can also be added to histones, and the N- and C-terminal tails can be phosphorylated and ubiquitylated. Alone or in combination, these modifications can silence or enhance transcription and contribute to chromosomal dynamics such as mitosis and meiosis (5). "Whether they are acetyl groups, or a methyl group, or yet another chemical modification, we're really getting at a very exquisite biological sensor of basic metabolism. It's maybe not a coincidence that the acetyl group for HAT enzymes comes from acetyl-CoA, a fundamental metabolite," Allis says.

The current challenge for chromatin biologists is to find out how histone modifications, or DNA "marks," are read and propagated. For Allis, it boils down to "marks, writers, and readers. Once you've written the marks, how do you read them? We're becoming aware of a class of proteins that are now under intense scrutiny in our lab and others that we call chromatin effectors, but a more simplified term would be reader." Some readers have been identified already. "Proteins with bromodomains read acetyl marks, those with chromodomains read methyl marks," he says. In both cases, "it's very exciting that there are 'Velcro' patches on these effector proteins for grabbing chromatin, some of which have been crystallized with cognate histone peptides leading to their examination at atomic resolution. Often they are found in transcription factors or chromatin-associated factors that have to do with chromosome replication and repair," Allis explains.

Barcodes and Epigenes

Allis and others propose that histone modifications constitute the foundation of an "epigenome," which could potentially be heritable, like DNA. He stresses that these ideas are put forward as a testable hypothesis that remains to be proven. "Some of us have called it a code, others a pattern, and even others have called it an epigenetic indexing system," he says. In his PNAS Inaugural Article, Hake and Allis (1) hypothesize that modifications to H3 histones on DNA produce a readable barcode signal that contains information in the chromatin structure. The signal could allow cellular machinery to identify euchromatin and heterochromatin and to interpret signals associated with epigenetic changes that control gene expression and development and that influence heredity.

"People don't just talk about the genome any more, they talk about the epigenome, something beyond the DNA."

Hake and Allis (1) propose that the mammalian genome is indexed by histone H3 variants in a nonrandom fashion that reflects assembly mechanisms, "personalized" chaperone proteins, and exchange factors. The H3 variants control whether genes are constitutively expressed or silent. Passing on the variants between cell generations could be the process by which signals for genomic imprinting are copied and inherited. Their model suggests that mammals have evolved an additional way of regulating their genetic information over many cell generations. "H3.1 and H3.2, for example, only differ in one amino acid, yet they are marked by different covalent modification signatures, behave differently in some assays, and function differently," says Allis.

"This pattern is somewhat clear with respect to histone methylation and transcription activity. For H3, at a particular lysine, K4, if it's methylated it's a very robust 'on' mark. On the same H3 at the lysine on the tail, K9, if it's methylated, that looks very 'off,'" says Allis (6). The pattern repeats itself over long stretches of DNA. "Elegant studies have systematically marched down the genome where a particular mark occurs

for thousand of kilobases of DNA," he says, "but then you hit a boundary, and you get a DNA element where suddenly, in a couple kilobases it changes its personality. It might be appropriate to think of it as an indexing system" (7). In women, who have two X chromosomes, one is inactivated, and "it looks like the lion's share of that regulation is epigenetic" (8).

Today, the search is on for readers that can interpret these epigenetic signals at the histone, nucleosome, or multiple-nucleosome level. "In our current understanding, 'Velcro' readers are best known as reading single histone marks. Recently, it has become clear that neighboring marks can act as switches that serve to eject these chromatin-bound effectors," says Allis (9, 10).

Beyond DNA

Another major research problem that faces Allis is how histone-marker signals are templated or inherited between cell divisions and organismal development. "People don't just talk about the genome any more, they talk about the epigenome, something beyond the DNA," he says. In DNA, inheritance is well understood with Watson–Crick pairing, "but we don't have that same understanding for chromatin or chromatin marks. We don't have that understanding how you template histone marks and propagate them faithfully," he says. As an example, Allis points to the cell-lineage problem, where a differentiated cell must somehow tell all of its daughter cells to keep its cellular identity. This control may be exerted through histone markers, but much more needs to be learned, says Allis.

Epigenetic markers on histones may explain some of the questions surrounding cloning and the differences between adult and embryonic stem cells, as well as the transitions from totipotency to pluripotency, and to more restricted and fully differentiated cells. "Animal cloning has been very inefficient and difficult. A lot of people think that maybe the problem step or one of the significant barriers are these epigenetic histone marks. Maybe these marks have to be stripped or reprogrammed during early development. It's like erasing the blackboard and starting over. I think we are going to see many more developmental biology labs paying much more attention to chromatin modeling and modifications," Allis says.

Epigenetics may also be able to provide insight for diseases that cannot be explained by simple genetic inheritance. "Some complex disorders like depression, bipolar disorder, and autism, these things don't always have a clearly genetic explanation. The idea that you could have two monozygotic twins where one is normal and one is autistic, how do you explain that?" asks Allis.

After publishing his first paper on HAT, Allis told his wife, Barbara, that "histone modifications were going to stay hot for about 6 months, tops," he says. That was 10 years ago. Today, with many new questions to answer, Allis has no plans to change his field of study from histone and chromatin biology. Married with three children, Allis splits his time between Manhattan and Princeton, NJ, where he also has a home. Like many researchers, Allis says he is grateful to his family, "who have been remarkable in putting up with me and my science." And he also pays tribute to those he has worked with for his career achievements. "One real pleasure of the NAS election was an opportunity to reflect on all of the talented and dedicated students and postdocs who made the science happen over the years. They are a big reason for any of my successes; they are the unsung heroes," he says.

Philip Downey,
Freelance Science Writer

1. Hake, S. B. & Allis, C. D. (2006) *Proc. Natl. Acad. Sci. USA* **103,** 6428–6435.
2. Bharier, M. & Allis, C. D. (1974) *J. Bacteriol.* **120,** 1434–1442.
3. Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y. & Allis, C. D. (1996) *Cell* **84,** 843–851.
4. Taunton, J., Hassig, C. A. & Schreiber, S. L. (1996) *Science* **272,** 408–411.
5. Strahl, B. D. & Allis, C. D. (2000) *Nature* **403,** 41–45.
6. Boggs, B. A., Cheung, P., Heard, E., Spector, D. L., Chinault, A. C. & Allis, C. D. (2001) *Nat. Genet.* **30,** 73–76.
7. Noma, K., Allis, C. D. & Grewal, S. I. S. (2001) *Science* **293,** 1150–1155.
8. Heard, E., Rougeulle, C., Arnaud, D., Avner, P., Allis, C. D. & Spector, D. L. (2001) *Cell* **107,** 727–738.
9. Fischle, W., Wang, Y. & Allis, C. D. (2003) *Curr. Opin. Cell Biol.* **15,** 172–183.
10. Fischle, W., Tseng, B. S., Dormann, H. L., Ueberheide, B. M., Garcia, B. A., Shabanowitz, J., Hunt, D. F., Funabiki, H. & Allis, C. D. (2005) *Nature* **438,** 1116–1122.