

## Sharon Dent: The unfolding SAGA of chromatin-modifying proteins

Dent studies how chromatin-modifying proteins regulate development and cancer.

Histone modifications come in many flavors—phosphorylation, methylation, acetylation, and ubiquitination—that interact with each other to regulate the folding state of chromatin, with profound effects on gene expression. For example, histone acetylation reduces the affinity of histone N termini for DNA, promoting chromatin relaxation and allowing transcription factors easy access to DNA. Such modifications are essential for regulating the changes to genome organization and gene expression that take place during organismal development and cellular differentiation.

Studying chromatin modifications is the research calling of Sharon Dent, Chair of the Department of Epigenetics and Molecular Carcinogenesis at the University of Texas MD Anderson Cancer Center. Her efforts have helped untangle the developmental (1, 2) and regulatory (3–5) functions of proteins involved in histone modification. As it turns out, these sometimes don't involve histones at all (4, 5). We recently called her to learn more.

### CONTROVERSIAL IDEAS

*You worked on *Tetrahymena* as a postdoc with David Allis...*

*Tetrahymena* is interesting because it has one very small nucleus that is never transcribed, and another larger one—with something like 40 times the number of chromosomes of the smaller nucleus—that provides all the transcriptional activity. It's a cool system for comparing how chromatin state affects gene expression. Of course, back then it was very controversial that chromatin affected transcription at all, so it was an interesting time to come into the field.

I had a really great time in Dave's lab. He is still a fantastic friend and mentor to this day. In his lab I focused on how phosphorylation changes in the linker histone H1 affect inter-nucleosome interactions and chromosome folding.

But, although *Tetrahymena* is great for biochemistry, it's very difficult to do genetics with it. I knew that I wanted to move into a more genetically tractable organism, so I went to Robert Simpson's lab at NIH to learn how to use budding yeast as a model organism. There, I showed that the repressor proteins that regulate mating type have a profound effect on the organization of nucleosomes. It was the first time that anyone showed that, although it was a bit controversial because it was hard to prove beyond a shadow of a doubt that the location of the nucleosome was what was causing repression of a gene. But it sure was a striking correlation. I used that work to launch my own lab at MD Anderson.

*By the time you started your own lab, had opinions on how histone modifications affect transcription changed?*

It had progressed somewhat, mostly through the work of Michael Grunstein and Mitch Smith, who showed that mutations at the sites of posttranslational modifications in histone tails could generate a change in transcriptional outcome. But the real breakthrough came in 1996, when my former mentor, David Allis, identified the first histone acetyltransferase protein.

That was a big breakthrough for two reasons. One, people had been looking for those enzymes since the '60s. Everyone was expecting it to be a single protein that had a lot of activity, and in fact it turned out that the protein David found, Gcn5, had already been identified as a transcriptional regulator through genetic experiments in yeast. Second, it provided a Rosetta Stone. Once we knew what one histone acetyltransferase looked like, we could scan the genome for similar proteins. It also spurred interest in the other histone modifications, so the field really took off.

**"Back then it was very controversial that chromatin affected transcription."**



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Sharon Dent

### JUST THE BEGINNING

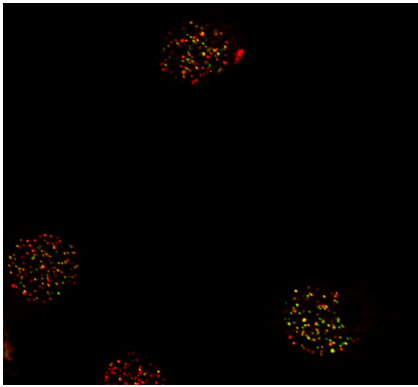
*How did that discovery affect your own research?*

When Dave sequenced Gcn5 and realized that it was the *Tetrahymena* version of a yeast protein, he called me and asked if I would clone the yeast gene and express it in vitro, to see if it had the same activity. So of course we said, "Yeah, we'll get right on that!"

That was actually one of the last experiments I did with my own two hands. [Laughs] I cloned the gene, and my postdoc Diane Edmondson expressed it in bacteria. Then we sent the protein to Dave, and his group did the histone acetyltransferase assays. It worked beautifully.

I wanted to work more with Gcn5, but there were lots of other people working on the biochemistry of the protein, so I decided to do something different. Actually, Diane strongly influenced my thinking on this. She pointed out that we now knew the identity of this gene, but no one else had this information, so we should immediately begin making mutations in mice. I'm a yeast geneticist, but luckily Diane had trained with Eric Olson and was very good at mouse genetics. She teamed up with a student in the lab, Allison Xu, and created the Gcn5 knockout mouse, which turned

IMAGE COURTESY OF DR. BOYKO ATANASSOV



**Loss of Gcn5 affects turnover of the telomere cap protein Trf1 (green; telomeres in red).**

out to have an interesting developmental phenotype. Over the years since, we've been mutating different domains in Gcn5 to see how different parts of the protein affect mouse development.

*The Gcn5 knockout mouse doesn't get very far developmentally...*

The complete knockout dies right after gastrulation, but it doesn't die at the two-cell embryo stage, which tells you that Gcn5 is doing something more specific than just being a general transcription factor. We wanted to know how much of that phenotype was due to the loss of the histone acetyltransferase activity versus deletion of other domains in the protein, so we made mutations in the catalytic site of Gcn5 and created a homozygous mouse. That mouse also died as an embryo, but it developed until about mid-gestation, and it had some very strong and unexpected neural tube closure defects.

Then we played detective because we were really interested in why the catalytic site mutant mice were different from the knockout mice. About that time, a couple of groups reported that the complex that contains Gcn5 had a second enzymatic activity—a deubiquitinase called Ubp8 in yeast and Usp22 in mammals. We were able to show that a large part of the difference in the phenotypes we were seeing in our mutant mice actually had to do with Gcn5's influence upon Usp22 activity. The phenotype didn't have much to do with histones at all. Instead, we found that Usp22 affects the stability of telomeres by deubiquitinating telomere cap proteins, and this is dysregulated in the absence of Gcn5.

**WHAT'S IN A NAME?**

*You've found unexpected effects for other so-called "histone-modifying proteins"...*

I was talking to a new student in the lab, Ke Zhang, about crosstalk between different histone modifications. For example, histone lysine methylation can affect phosphorylation or methylation at another site, so one could imagine there could be regulatory cascades based on patterns of histone modifications. I thought we could take advantage of the awesome power of yeast genetics by mutating the enzymes that regulate these modifications and looking for either suppression or enhancement of phenotypes.

I actually wrote down a list of at least 11 different histone modifying enzymes, and I asked Ke to start making single and double mutations in all of them, to see if any combination of mutations enhanced or suppressed certain phenotypes. This was a pretty ambitious project but Ke is very industrious, and it didn't take her very long to come back with something really interesting. In this case she didn't have to make the mutation; there was already a well-characterized mutation in an Aurora kinase gene, called Ipl1 in yeast, that's very important in regulating mitosis. Ke found that if she took cells that had that mutation in Ipl1 and deleted the methylase Set1, those cells got much healthier. They were able to survive at temperatures that normally completely eliminated Ipl1 function.

We immediately thought, "Great! Ipl1 phosphorylates a serine on histone H3 and Set1 methylates a lysine on histone H3, so we've got a new example of crosstalk between these histone modifications." We tested that by making mutations in the histone and seeing if we got the same phenotype that we got when we mutated the enzyme. It didn't work. [Laughs]

At that point we could have hung it up. But, although we didn't know any of Set1's substrates, we did know Ipl1's substrates, and one particularly important Ipl1 substrate is a kinetochore protein called Dam1. Ke found that mutating a particular lysine

in Dam1 had the same effect as Set1 loss. We ultimately showed that Dam1 is methylated by Set1. We currently have some really interesting data on Set1 and mitotic checkpoints that we hope to publish eventually.

*Does Gcn5 remain a major focus in your lab?*

What I'm most interested now—not surprisingly, since I work at a cancer center—is how Gcn5 contributes to cancer. Our paper this year in *Genes and Development* indicated that the complex that contains Gcn5, the SAGA complex, is an important coactivator for Myc, both in embryonic

stem cells and during reprogramming of somatic cells back to a stem cell state. This begs the question of whether Gcn5 is important for turning on genes in cancers that overexpress Myc. We're also interested in defining the normal functions of Usp22, because its overexpression is associated with highly aggressive cancers, so

it could give us some insight into cancer formation and progression.

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**"What I'm most interested now... is how Gcn5 contributes to cancer."**



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**When she's not in the lab, Dent enjoys dancing with her husband and pampering her dog, Poco.**