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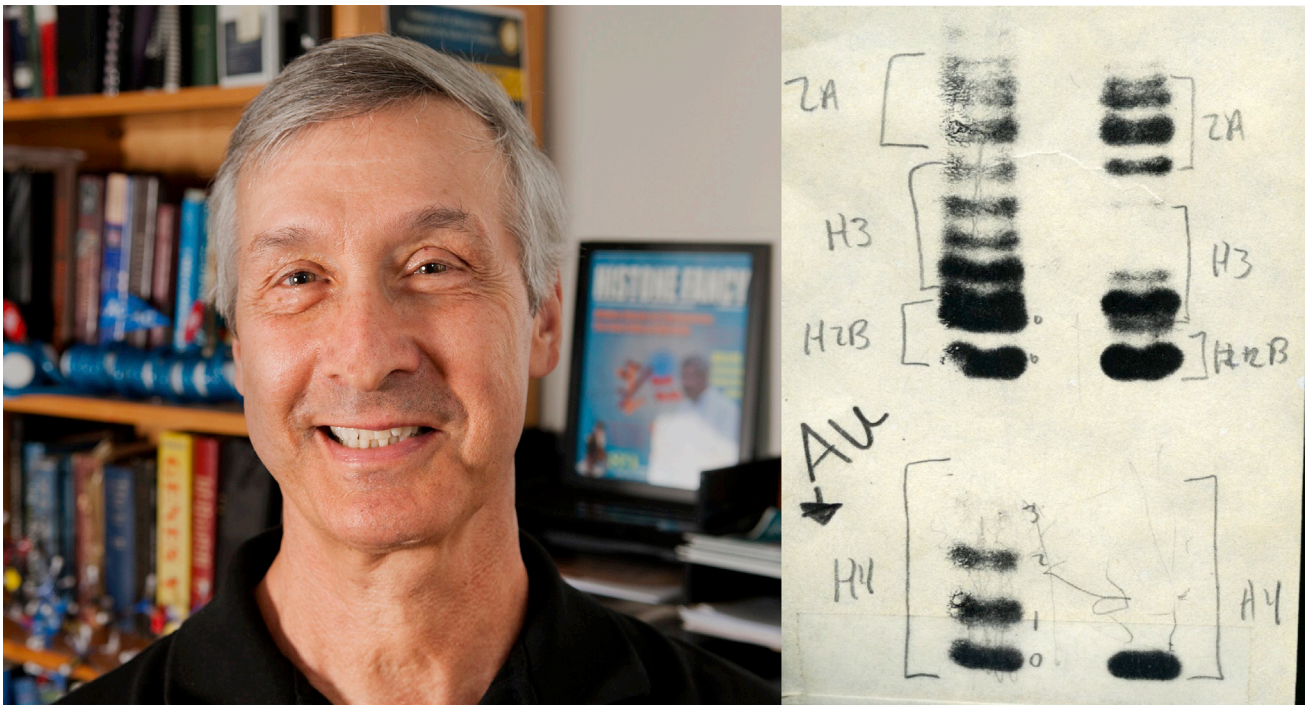

Pursuing the Secrets of Histone Proteins: An Amazing Journey with a Remarkable Supporting Cast

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Receiving any award for achievements in basic biomedical research, let alone one as prestigious as a Lasker Award, prompts me to ask a simple question: *how did I get here?* My answer has two parts: not by design and certainly not alone. When I entered the field of histone biology as a postdoc, a rich older literature suggested that histone proteins might be worth a career investment. This was a gamble, but in short, I found histone proteins interesting. While relatively poor in molecular weight, histones were potentially rich in biologically meaningful information, as different gel systems revealed a large variety of patterns in electrophoretic properties. But was any of this heterogeneity physiologically relevant? That histones might be important was supported by their highly conserved nature and their undisputed role as fundamental protein organizers of the chromatin. Well before my time, numerous labs had documented a wealth of post-translational modifications (PTMs) on histones and hypothesized that these PTMs might alter chromatin structure and function by influencing histone-DNA and histone-histone contacts. Eukaryotic genomes demanded packaging within a chromatin environment, but was this packaging anything more than just nature's clever way to make sure large genomes fit into tight nuclear spaces? I was hoping that there was more gold left to be mined from these "simple" proteins and their well-documented covalent decorations. I had no plan B, and nothing could have been further from my mind than a Lasker Award.

With chromatin being so fundamental, I reasoned that I could pursue the inner-most secrets of histones in any eukaryotic organism. *Tetrahymena*, a single-celled, ciliated protozoan, seemed

Acid-urea gel revealing the distinct histone ladders in *Tetrahymena* macro- and micronuclei, performed by C. David Allis (left)

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particularly attractive to me. Each vegetative cell contains two functionally distinct genomes: a somatic, transcriptionally active macronucleus sitting side by side with its cozy neighbor, a germline, transcriptionally silent micronucleus. Martin Gorovsky and his colleagues had championed this system for histone and chromatin biology studies, and their foundation allowed this newbie to learn the ropes of this off-beat model, making many of my lab's future studies possible. The decision to exploit *Tetrahymena*'s strengths in my own young lab was a good one. I charged forward with enthusiasm and one overarching goal: to use macronuclei as an enriched source for the biochemical purification of the enzyme(s) responsible for ''writing'' acetyl marks onto histones in the first place (known as a histone acetyltransferase or HAT, for short). I reasoned that macronuclei, loaded with hyperacetlylated histones I had observed as a postdoc, might have more of this enzyme or a more active form of it. I knew from attending chromatin meetings that this was a long sought after, but elusive, activity. Numerous top-flight labs had tried to purify this enzyme with little or no success. Was *Tetrahymena* going to be my secret weapon? I wasn't sure, but my colleagues and I were determined to give our best shot.

What followed were long hours in the cold room looking for what seemed to be more challenging than a needle in a haystack. Gel after gel of our most highly purified HAT fractions revealed no convincing band(s) or, worse, suggested that we had purified the HAT to a point that it was now invisible, even with silver staining. In contrast, Michael Grunstein had turned to budding yeast to exploit the ''awesome power of yeast genetics'' to address fundamental questions in histone and chromatin biology. This was their strength but not ours. Well before any HAT had been identified, a paper from the Grunstein lab dealt my lab what I thought was a career-ending death blow. Their genetic experiments demonstrated that the extreme amino terminus of histone H4 was dispensable in yeast. This removal of the H4 N-tail did away with all known acetylation sites in H4, and for the most part, yeast were fine without it (with the notable exception that the silent mating loci were now derepressed; Kayne et al., 1988, Cell *55*, 27–39). This paper took the chromatin field by storm and caused me (and others) to seriously question the centerpiece goal of my lab—the enzymology and function of histone acetylation. How ironic that 30 years later, I am a co-recipient of a Lasker Award in Basic Medical Research with Michael Grunstein. Histone acetylation *is* important; Allfrey had had it right.

Indeed, the general view that histone acetylation was a critically important ''switch'' for gene activation from chromatin templates was championed by Vincent Allfrey (Allfrey et al., 1964, Proc. Natl Acad. Sci. USA *51*, 786–794), who did all of his work at The Rockefeller University long before I ever dreamed that I might be there. Would knowledge of the enzyme system responsible for bringing about the steady-state balance of histone acetylation provide a critical test of his hypothesis? Fortunately for me, Jim Brownell, a talented PhD student, joined my quest to identify the elusive ciliate HAT. Jim devised a clever in-gel HAT assay to detect a promising HAT band from macronuclear extracts. In a gusty Herculian effort, Jim then purified the 55 KDa (p55) band from 200 L of starting *Tetrahymena* cultures, permitting its cloning and identification. The ciliate enzyme proved to be an already known homolog of a known transcriptional co-activator in yeast, Gcn5, leading to a paradigm shift in how genes are regulated from chromatin templates (Brownell et al., 1996, Cell *84*, 843–851). Minimally, this work suggested that histones and their acetylation were doing more than just the passive packaging of eukaryotic genomes. In rapid succession, other members of the transcription apparatus were found to possess HAT activity. The implications were immediate and potentially far-reaching. Histones and their modifications were active participants in gene regulation. In truth, I had never seen this coming.

1996 also ushered in a remarkable independent discovery by Stuart Schreiber and his colleagues of the first-ever enzyme activity that acted in opposition to the forward reaction catalyzed by HATs (Taunton et al., 1996, Science *272*, 408–411). A histone deacetlyases (HDAC or ''eraser'') had reared its head through their clever use of trapoxin as an affinity hook to purify the responsible target. This discovery not only completed the loop as to how the steady-state balance of histone acetylation is brought about but also provided the critical link between a HDAC, and therefore histone deacetylation, and a known transcriptional co-repressor in yeast, Rpd3. Importantly, HATs and HDACs provided the scientific community with new targets to modulate the previously ''undruggable'' process of gene transcription. Several of these drugs are now FDA approved with more on the horizon, and they are helping cancer patients lead healthier lives. 1996 proved to be a breakout year in ushering in what some refer to as the modern era of chromatin biology. It is a year I will never forget.

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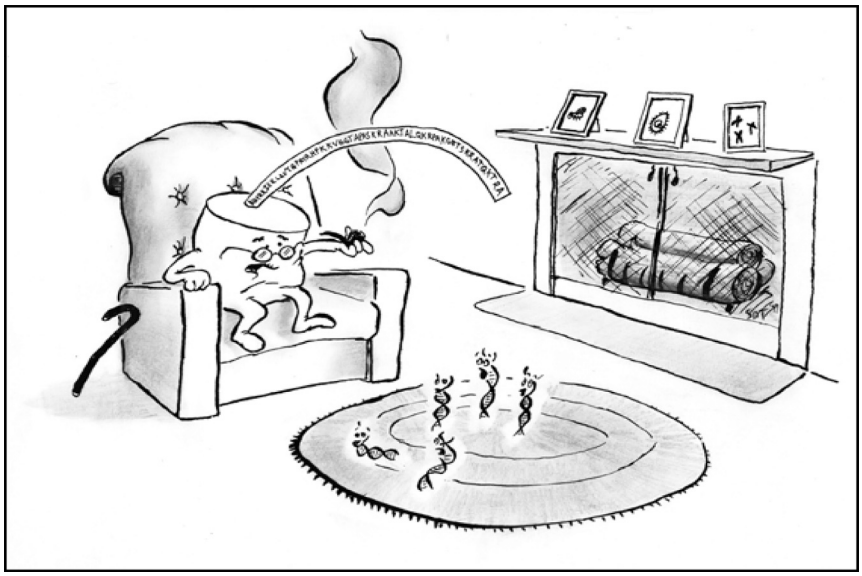

"Histone Tales" by Sean Taverna, Johns Hopkins Medical School, given to C. David Allis. Histone H3 is depicted as a "teacher" to DNA "students," and pictures on mantel depict *Tetrahymena*

Caught up in the notion that *histones matter*, my lab and many others have gone on to dig deeper into histones' best-kept secrets. In the 20-plus years that followed the discovery of HATs and HDACs, numerous labs have contributed to the fields of histone and chromatin biology in ways that have had clear and enormous implications for science and medicine. Groundbreaking discoveries have documented a close interplay between cancer genomes and the epigenome, representing a paradigm shift in our understanding of cancer epigenetics. Mutations have been identified in cancer patient genomes that map to a remarkably wide range of ''epigenetic regulators,'' including much of the machinery dedicated to the dynamic regulation of chromatin. DNA methylation is equally important. Driven by cancer-genome sequencing initiatives, a remarkably wide range of histone-modifying and chromatin-remodeling activities have proven to be effective drug targets in several human cancers (Pfister and Ashworth, 2017, Nat. Rev. Drug Discovery *4*, 241–263). Histone and chromatin biology matter.

The elegant studies of Grunstein and others taught us that ''histone genetics,'' carried out in organisms like budding yeast, can give incredible insights into histone and chromatin biology. Histone genetics is tractable in these simple modes due in part to their small copy number. In humans, as in other mammals, the genes encoding histone proteins exist in large gene families making histone genetics unrealistic. Recently, however, monoalleic, high-frequency, missense mutations have been identified in a collection of pediatric gliomas and bone and cartilage tumors. Even the most ardent fans of histones, like myself, did not see this coming. Remarkably, many of these amino acid substitutions occur at or near sites of well-studied histone PTMs, suggesting that mistakes made in setting these marks can lead to human disease. While relatively few of the histone mutations have been studied in great detail, early results suggest that some act as gain-of-function, ''driver'' mutations in various cancers. Deciphering how mutations of histone genes in multipotent stem cells alter normal pathways of chromatin function remains a critical issue. Whether and how these mutations (and others being identified) lead to significant changes in the transcriptome and epigenome, which in turn promote tumorigenesis, is not clear. It is our general view that understanding fundamental mechanisms that govern these changes will lead to new drugs and therapies against cancer and a variety of other human pathologies.

I am humbled and honored to be a co-recipient of the 2018 Lasker Basic Medical Research Award with Michael Grunstein. In many ways, his studies inspired my own. As well, my own lab's contributions rest on many shoulders, far too many to mention. This includes not only my lab colleagues, past and present, but also the many collaborators with whom I have had the pleasure to work along my journey. They continue to inspire me with their passion and commitment to do what we do; I am a better scientist because of them. Doing science is interesting, fundamentally

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important, and richly rewarding. I will never forget Jim Brownell holding up a piece of X-ray film showing that recombinant Gcn5 from yeast displayed a clear band of HAT activity in his in-gel assay. I had never seen Jim dance around the lab grinning from ear to ear; it still provides me with a wonderful memory. The *Tetrahymena* p55 band was even more intense and considerably more active than Gcn5 homologs expressed from yeast, fly, and mammalian sources. I have often wondered what would have been our fate if we had attempted our HAT quest from any other model organism. To all those who have educated, supported, and inspired me and put up with my unbridled enthusiasm for just about everything, and definitely all-things histone, I am extremely grateful. I have profited from excellent role models at every stage of my career; their mentorship has shaped me in profound ways. Along the way, I have also learned from wonderful lab members who have put in the long hours, done the actual experiments, made the intellectual and emotional commitments to difficult lab problems, and made many sacrifices to make it all happen. Any of my successes are their successes. I also have an amazing family who have simply put up with me and always given me a supporting nod whenever I told them just how cool histone proteins are. This happened far too often.

In closing, my scientific journey has been wonderful, and I am delighted to share this Lasker Award with Michael Grunstein and such a terrific supporting cast. How did I get here? Not alone. I look forward to more secrets learned from histone proteins and the machines that evolved to deal with the ''chromatin problem.'' My hope is that these insights will ultimately help people live healthier lives. Many key contributions came before me in the field of histone biology, and more will surely follow. Watching the next generation of young scientists take over the reins has been, and will continue to be, one of the more rewarding aspects of my career.