"Modifying" My Career toward Chromatin Biology

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Stepping in the Batter’s Box

Like most, if not all, graduate students, I faced some hurdles along the way that seemed higher than the rest. In my doctoral program at Indiana University in Bloomington, a required, but to me dreaded, seminar course in genetics stood in the way. It was a small class that was directed by a professor well known for demanding painful excellence from all those who enrolled. My self-confidence was shaky, but I knew that I did not want to strike out.

The First Pitch

To clear this intimidating bar, I chose my topic carefully and overcompensated in its preparation. I began the seminar covering the high points of a provocative theory, the concept of “the nucleosome” as an organizing principle for eukaryotic genomes, a compelling synthesis put forward by Roger Kornberg in 1974 (1). I went on to review experimental data in support of his theoretical model, which were micrococcal nuclease digestions that revealed chromatin’s repeating nature, electron microscopic evidence of its “beads-on-a-string” particulate character, and the elegant determinations of the pairwise associations of specific histone dimer pairs that might explain the core particle’s stoichiometry and assembly properties. The climax of my talk was a paper that had caught my attention in a reasonably new journal, Cell (true, but not wanting to date myself). In this 1975 study, Chambon and colleagues (2) elegantly showed how four core histones packaged and condensed predictable lengths of adenovirus-2 DNA. I was fascinated by this “seeing-is-believing” study; my enthusiasm was hard to contain, slipping into my seminar. Even as I was giving the talk, it occurred to me that this might have been one of my better seminars in graduate school: every word seemed to come out right. I jumped over the hurdle and finished the talk by being hooked on chromatin.

Strike One

Despite my unbridled enthusiasm for the general topic of chromatin, many notables in gene regulation were skeptical. At that time, chromatin was viewed by most as simply a passive packaging scheme for DNA, essentially acting little more than “salt” to help DNA fold through charge effects. To be fair, chromatin studies in this era paled in comparison with the more exciting studies on transacting transcription factors that were all the rage, well deserving of their top billing. After all, these factors engaged defined DNA control elements, such as enhancers and promoters, and they played key roles in the recruitment of RNA polymerase and company. Moreover, well defined paradigms of gene regulation had been elegantly worked out in prokaryotic models that lay the groundwork for these exciting studies. Histone proteins were viewed as only being in the way of where all of this exciting action took place. My career choice to study histone biology was a steep uphill climb, especially given the popular notion that histones did not really matter in gene regulation.
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**Strike Two**

My passion to enter the chromatin field as a “newbie” dictated my choice of postdoc laboratory. Many of what I perceived to be the “star gene expression labs” were filled, and I was told to apply elsewhere or to wait in line for an opening in the group, which might never come. Making matters worse, I had wanted to pursue chromatin problems in a developmental biology context, likely sticking with *Drosophila*, the organism on which my doctoral studies had been centered. However, none of the fly labs I visited seemed interested in studying chromatin. I returned to Bloomington, disappointed and confused at my first tastes of rejection. I had no “plan B.”

**Ball One**

A plan B came from the surprising suggestion of one of my co-workers in the lab of Anthony Mahowald, Kathy Karrer. Kathy suggested to me that I consider the ciliated protozoan *Tetrahymena* as a model organism for chromatin studies. Being from Bloomington, I had heard of another protozoan, *Paramecium*, from the long-standing work in the Sonnenborn-Preer labs located one floor above my Ph.D. laboratory. However, at first glance, the idea to work on such an offbeat critter seemed to be out of the question. I was not even sure how to spell *Tetrahymena*. More specifically, Kathy suggested the name of Martin Gorovsky at the University of Rochester in New York, whose lab had not only worked out conditions to grow large quantities of this organism, which permitted biochemical studies, but had also developed methods to isolate purified populations of macronuclei (the active somatic nucleus) from the neighboring micronuclei (the inactive germ line nucleus). With no disrespect to anyone from upstate New York, I worried about the amount of snow that I might see in Rochester, but I knew that we were on borrowed time. Again, but now as a matter of renewal. I knew that we were on borrowed time. Again, I was confident that we could deal with whatever the winters would bring to Rochester. I was more worried about pursuing chromatin biology in ciliates, referred to as “pond scum” by some, and that it might be a big mistake.

**Ball Two**

My postdoc years in the Gorovsky lab were good ones, maybe enriched by confinement in the lab during the long and gray winter months in Rochester. *Tetrahymena* had been good to me, leading to the discovery of macronuclear histone hyperacetylation, histone variants, and so on. I was psyched to ride this momentum to my first faculty position at Baylor College of Medicine in Houston, Texas. While in the biochemistry department there, I made the intellectual (and emotional) commitment to focus my young group on purifying what I believed would be a worthwhile goal: the histone acetyltransferase(s) (HATs) responsible for bringing about the steady-state hyperacetylation I had observed in macronuclear histone preparations during my postdoctoral stint. Moreover, I believed that purified macronuclei were going to give me an excellent “enriched” starting source of this long sought-after enzyme activity that had eluded other groups. This was my lab’s single main goal; long hours lay ahead in the cold room.

**Check Swing**

Meanwhile, elegant studies carried out by Michael Grunstein at UCLA, Mitch Smith at the University of Virginia, and colleagues using the “awesome power of yeast genetics” to dissect histone function had begun to take the chromatin field by storm. Searching for invisible bands tracking HAT activity on hundreds of silver-stained gels seemed much less rewarding. Moreover, cold room work was cold and uninviting, even to me. Again, but now as a group leader, I wondered to myself if we could pull this HAT purification off, even with the biological strengths of *Tetrahymena*. The ultimate unwelcome news came out of left field in 1988. In a high-profile paper, Grunstein and colleagues (3) showed that the highly conserved N-terminal domain of histone H4 was dispensable in yeast. Everyone at Baylor (and elsewhere) read *Cell*, and many of my colleagues, including my chair, questioned what this study meant for my research program and my future. After all, if removing the H4 N-terminal tail took away all of the well known sites of acetylation (lysines at positions 5, 8, 12, and 16) and the yeast was still viable, what did this say about the importance of histone acetylation? The simplest interpretation of their findings was that histone acetylation, at least in H4, was unimportant! Was this my “strike three?” Worse yet, one of my key R01 grants from the National Institutes of Health, all centered on the enzymology and function of histone acetylation, was up for its first competing renewal. I knew that we were on borrowed time. Again, I had no plan B.
Full Count

Enter Jim Brownell, a talented graduate student who remarkably seemed to like working with enzymes, even in a cold room. Perhaps my enthusiasm for the importance of histone acetylation and HATs, no less using *Tetrahymena*, had rubbed off on Jim? In a fairly short order, Jim bravely joined my group, got hooked on this mysterious ciliate HAT activity, and made magic happen. An ingenious in-gel HAT assay, when applied to his most purified fraction (itself isolated from 200 liters of *Tetrahymena* macronuclei), led Jim to a few SDS gel lanes, microsequencing, and cloning of “the band.” A 55-kDa polypeptide (p55) with strong HAT activity in the in-gel HAT assay had reared its head. The gene encoding p55 was cloned and sequenced (the old-fashioned way). Jim was richly rewarded for his cold room Herculean efforts. *Tetrahymena* p55 matched a previously described yeast transcriptional co-activator, Gcn5p, studied by Leonard Guarente and colleagues at the Massachusetts Institute of Technology. Their work suggested that Gcn5p played an important yet unknown positive role in transcriptional activation in yeast; however, its biochemical function remained unclear. Jim’s work demonstrated that both *Tetrahymena* p55 and yeast Gcn5p were endowed with a novel, never-before-seen catalytic function: the job of enzymatically modifying histones. *Tetrahymena* p55 was the first transcription-associated (nuclear) acetyltransferase to be discovered.

Home Run

Jim’s 1996 paper (4) turned out to be a landmark paper in the field (selected as a *Cell* Classic and annotated by Jerry Workman at the Stowers Institute for Medical Research in 2014) (Fig. 1). Jim’s findings were all the more remarkable when viewed in the context of a paper published the following month by Stuart Schreiber and colleagues (5) at Harvard University, who independently identified the first histone deacetylase and linked a mammalian histone deacetylase to an already known transcription repressor in yeast. Taken together, these two papers registered a one-two punch in 1996 that set the stage for what has become the modern era of chromatin biology. As a footnote, the Brownell *et al.* paper was published on my forty-fifth birthday (March 22) that year.

On Deck

Post-1996, chromatin biology as a field could proudly edge its nose out of the gene regulation closet. Although not everyone was a “true believer” of chromatin’s importance, many of its harshest critics at least had to pause for some reflection. The common notion that histones were just a passive structure used to wrap DNA into defined units seemed less tenable. As suggested early on by chromatin’s earliest proponents, notably Vincent Allfrey, Morton Bradbury, Colyn Crane-Robinson, and Gordon Dixon, covalent post-translational modification of histones stood as a novel dedicated and reversible “on-off switch,” brought about by defined enzyme activities contained in complexes of the much better accepted transcriptional apparatus. Moreover, modification of histone proteins was not the only mechanism by which variation could be introduced into the chromatin fiber: DNA methylation, chromatin remodeling, exchange of histone variants, and so on were all being advanced by many in the field as playing critical roles in the overall equation of gene regulation. Links to human biology and disease were only beginning to emerge, but we and others sensed that the implications of what was now being collectively described as “epigenetics,” something beyond the DNA itself, were far-reaching.

The fields of chromatin biology and epigenetics have witnessed many more remarkable discoveries made by many groups worldwide since 1996. Although it would be a mistake for me to attempt to list them, I look back on some of my favorites with satisfying pleasure and anticipation of more to come in years ahead. I have been known to describe the complex biochemistry of chromatin regulation to lay audiences by trying to describe the collective covalent marking of chromatin as a “language.” This analogy includes “writers,” “erasers,” and “readers” of the ever-expanding list of covalent post-translational modifications that decorate histone and non-histone proteins. Knowing now that oncology-based drugs have been developed (some of which are approved by the United States Food and Drug Administration) against members of classes of histone modification targets is most gratifying. Helping people live healthier lives is a goal we all can rally behind (Fig. 2).
Bottom of the Ninth

I like to tell seminar audiences that “every amino acid in histones matters.” This is a concept that is hard to test without the awesome power of histone genetics in genetically tractable organisms such as yeast. The year 2012 produced a “game-changing” discovery by two groups working independently and lead by Nada Jabado at McGill University and Suzanne Baker at St. Jude’s Children’s Hospital. In rapid succession, both teams uncovered high-frequency mutations in single copies of human histone H3 genes (6, 7) in a universally fatal type of pediatric brain tumor (Fig. 3). Moreover, these mutations mapped exactly at, or very close to, well known sites of histone modification, such as lysine residues that are modified by acetylation or methylation (e.g. H3 Lys-27). We and others are currently seeking to identify the underlying mechanisms affected by these dominant-acting mutations with the hope that understanding how epigenetic landscapes are altered to contribute to tumorigenesis will lead to new therapies for these affected children. Many of these children die within two years following diagnosis. The stakes are high. This battle must be won (8).

FIGURE 2. David Allis in his office at The Rockefeller University (circa 2014).

FIGURE 3. Mutation in the N terminus of histone H3 inhibits histone lysine methylation in pediatric brain tumors. Shown is a schematic view of a condensed metaphase chromosome unfolding, with the chromatin fiber and a single mononucleosome indicated. The red asterisk over the histone H3 N-terminal tail (blue) denotes the existence of a “oncohistone” mutation at Lys-27. The in situ images in the background denote the staining of H3 Lys-27 methylation (H3K27me3) in a “wild-type” human brain tumor (left) versus a human brain tumor from a patient genotyped with the H3K27M (Lys-27 to Met) mutation (right). Note the striking loss of H3 Lys-27 methylation in the H3K27M tumor sample, except for the contaminating vasculature in the section (see text and Ref. 9 for details).
Extra Innings

I have been fortunate to witness a remarkable twenty-year period of scientific advances since the Brownell (4) and Taunton (5) discoveries of 1996. I have enjoyed a nearly forty-year career in chromatin biology since I made the decision to enter this field as a postdoc. More than once, I questioned my career path, never having a plan B to fall back on. My advice for young scientists would be to follow their hearts, knowing that they will be tested at points and even make some mistakes along the way. Do not be afraid to take risks. For me, “doing science” is both a privilege and a passion. It is the best job in the world, and I would not trade it for anything. Science is a people-driven business, and I truly enjoy all of the many people I have met and interacted with over my career, both inside and outside of my own laboratory. Even if I am not at bat anymore or taking swings in the cold room, I manage a great team. They make it all happen; I just help them swing at the first pitch.

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References