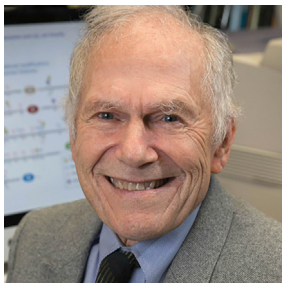
Please cite this article in press as: Conversation with Michael Grunstein, Cell (2018), https://doi.org/10.1016/j.cell.2018.08.008

Cell

Conversation with Michael Grunstein

Together with David Allis, Michael Grunstein just received the Lasker Basic Medical research award. The article that follows is a transcript of a conversation with Jacques Deguine, scientific editor at *Cell*, that was edited for length and clarity.



Jacques Deguine: Dear Michael, thank you again for taking the time to chat, and congratulations on this Lasker Award. As a start, maybe can you tell me how you got into that field? And did you initially want to work on histones, or was there something that spurred you in that direction?

Michael Grunstein: I definitely did not always want to work on histones. It was, to some extent, luck, serendipity, and my own experiences which led me into this field. That occurred about four years after I started at UCLA, and the reason was that I was working on characterizing sea urchin histone gene expression at different stages of development. There was reason to believe that some of the histones were amino acid sequence variants of the major core histones. The main question was how do histone genes and their variants get turned on at one developmental stage and turned off at another?

JD: I have to ask, why was the start in sea urchins? And then, how did you get from that into yeast studies? Sea urchins don't seem like the most obvious model to start in!

MG: Right. I started out working on sea urchins in my post-doctoral work with Larry Kedes at Stanford. And then, I did a second post doc with David Hogness, also at Stanford, who worked on *Drosophila*, the fruit fly. I could have continued studying fly development—that would have been a perfectly good choice.

But in fact, after a few years as a faculty member at UCLA, I wanted to do something different from my mentors. In trying to explain my work to my colleagues, I realized that despite the attractive nature of synchrony in cell division after fertilization of sea urchin eggs, the sea urchin was not suited for my studies. To address the fundamental question of function—for example, why are there histone variants or subtypes in eukaryotes, and how is their expression regulated—required both genetics and biochemistry. Neither was especially convenient using sea urchins. What broke the camel's back were the El Niños of the late seventies that desalinated Pacific coastal waters, triggering the shedding of the urchin gametes.

In contrast, yeast genetics was sophisticated, and yeast was just starting its ascendancy as a model organism for the study of eukaryotic biology. But what you couldn't yet do is reverse genetics: mutating a histone gene *in vitro* followed by gene replacement of the chromosomal allele *in vivo*.

Still, yeast was looking better and better. A paper from Gerald Fink's lab published in 1978 in *PNAS* showed that you could transform yeast with DNA and have it integrate into the genome. And, strikingly, one of the figures in the Hinnen, Hicks, and Fink paper showed what looked like gene replacement. This was earth shattering to me because to my knowledge, this was the first example of DNA transformation of a eukaryotic cell, and gene replacement was possible.

JD: So you had a much more limited set of variants to work with than in sea urchins.

MG: Yes. Lynna Hereford at Brandeis and Mitchell Smith at the University of Virginia had shown that yeast contains two copies of genes for each of the core histones (H2A, H2B, H3, and H4), unlike the 400 or so copies we found in early sea urchin embryos.

Then, John Wallis and Mary Rykowski, my first PhD students, showed that the two copies of the yeast histone H2B were sequence variants of each other and that yeast can grow even with one copy of either H2B gene. When you're asking for a linchpin experiment, for me, that was the one. We could finally construct yeast strains with single histone genes (even those coding for H4 histone) that were to become the targets of reverse histone genetics. This led to the finding that nucleosomes are repressors *in vivo* and that acetylated lysines at the histone H4 N terminus are required for transcription in euchromatin. It also led to the discovery that there is a genetic interaction between the heterochromatin protein Sir3 and deacetylated H4 lysine K16 in heterochromatin. This role of K16

CELL 10360

"I was working on characterizing sea urchin histone gene expression at different stages of development."

"Sometimes you start off in the wrong direction scientifically. But it's no less important than being right because it teaches you what to do next."

deacetylation in silencing helped explain how a ubiquitous protein like H4 can have a specific role in silencing heterochromatin.

JD: What struck me in your description is the importance of non-model organisms as a way to get started and also of model organisms because some of the research is so much easier to carry there. How did you make that transition?

MG: The sequence of histone H4 is extremely conserved in evolution, so it stands to reason that our findings in yeast would apply to mammals. But I had never worked on yeast before, so I took the Cold Spring Harbor Yeast Genetics course, and it was one of the high points of my scientific career. Working hard at the bench in heavy heat, going to the bar afterward, starting the next morning early again. It was wonderful, and everything was done with your own hands. In addition, the course was taught by people like Fink, who lived yeast genetics and who transferred their enthusiasm to the students.

JD: As the fields have gotten bigger and more competitive, do you think some of these experiences or some of that spirit has been a bit lost nowadays?

MG: Right. The chromatin field has become very competitive. We used to joke that there's at least someone working on each amino acid of histone H4, and then it turns out that it is not far from the truth…I think that Jef Boeke and coworkers looked for the function of virtually every amino acid in each of the core histones using mutant libraries. Competition breeds novel approaches.

Of course, the chromatin field has also changed considerably with the influx of hundreds of laboratories, but that is the natural outcome of its success. There is a different spirit. Histone modifications concern not only questions of basic science, but also problems in cancer, neurobiology, and inflammation in human disease.

For these areas, yeast can still be a model organism if the modification in question occurs in yeast. With gene-editing techniques such as CRISPR/Cas9 applied to yeast and human cells, the difference between using yeast and mammalian cells has narrowed. But new functions for histones are still being discovered in yeast. These are likely to be models for other eukaryotes as well.

Naturally, an increase in the number of chromatin scientists and a decrease in federal funding has led to a more difficult environment, to say the least. But we have to look forward, and I am very encouraged by going to seminars where I hear of new approaches to chromatin from extremely talented younger people.

JD: Going back to your work, we talked about how the research unfolded, but was there any unexpected roadblocks along the way?

MG: In 1981, we tried to isolate the histone acetyltransferase from yeast, and we finally published a paper on that in 1984. It was very difficult, and we could get considerable purification but never purity to allow protein sequencing. We didn't pursue the enzyme purification, since the protein-sequencing techniques were not as sensitive as they are today. A decade later, David Allis found that one of the two nuclei of the ciliate Tetrahymena was rich in a histone acetyltransferase whose sequence was homologous to that of the yeast transcription factor GCN5, which helped cement the relationship between

"Then, John Wallis and Mary Rykowski, my first PhD students showed that the two copies of the yeast histone H2B were sequence variants of each other and that yeast can grow even with one copy of either H2B gene. When you're asking for a linchpin experiment, for me, that was the one."

"Papers are always very logical. The reality is less logical."

histone acetylation and transcription. It just goes to show…we started with the wrong model organism for this particular enzyme.

I cannot be so arrogant as to advise younger people on how to do science. I can only comment on the successes and failures in my own career. It was very satisfying to determine novel functions for the mysterious but very important histones. But in some cases, we were sidetracked by tangential projects that were time consuming but had little to do with our focus. Patience and perseverance were important, as some projects took much longer than expected. These were often those of which I was most proud upon their completion and which advanced our goals significantly.

JD: Outside of focus and perseverance, what else do you think was critical in your career?

MG: It was important to choose a direction. Sometimes, you start off in the wrong direction scientifically. But it's no less important than being right, because it teaches you what to do next.

JD: Thank you again for taking the time to discuss you work—it was really interesting for me to know the path you took outside of what's in the paper.

MG: Papers are always very logical. The reality is less logical.

JD: I think that's a great quote to end on! Congratulations again and thank you!

MG: Thank you.