People & Ideas

Michael Buszczak: Tracking the big game in stem cell identity

Buszczak is exploring the regulation of proteins that control stem cell identity in the fly.

Gene expression in stem cell populations is controlled by mechanisms such as chromatin organization and translational repression. Michael Buszczak is hot on the trail of the proteins involved in these events.

Buszczak's quest to explore the processes and proteins controlling fly development started during his graduate work with Bill Segraves and Lynn Cooley at Yale, where he studied control of a critical checkpoint in oogenesis (1). While still there, he began the hunt for new proteins involved in the process by randomly inserting GFP throughout the fly genome (an approach known as protein trapping) and setting up a high-throughput approach to identify targets with interesting expression patterns.

Buszczak extended this strategy during his postdoctoral studies in Allan Spradling's laboratory at the Carnegie Institution, using the traps to probe for factors that control gene expression (2, 3). Now with his own laboratory at UT Southwestern, Buszczak is using his protein trap database (4) as well as new targeted knockout approaches to identify and observe proteins involved in stem cell maintenance (5). We tracked him down to talk about his safari through the fly genome, and where he's setting his sights next.

ON SAFARI

Where did you grow up?

I grew up in Connecticut, in a town called Windsor, which is just north of Hartford. After fourth grade, we lived in South Africa for four years, and then moved back to Windsor.

Did you ever go on Safari while you were in Africa?

One of my fondest memories of South Africa was a trip that I took with my class. We spent an entire week camping and backpacking in one of the game parks with a ranger and our teacher. We saw all sorts of wildlife: lions, elephants, and rhinoceroses. There was even one night where we had to sleep in a tree because the ranger saw evidence of hyenas in the area, and he was worried that they'd come poking around at night. I guess hyenas have a tendency to nip little bits off you while you're sleeping, and then run away.

Ouch! That'd hurt! What was it like sleeping in a tree?

[Laughs] It was scary and exciting. We were on this platform, pretty high up, with no railings. I don't think the ranger was exaggerating, either, because that night we definitely heard some rustling noises down below.

Do you camp out much these days, in trees or not?

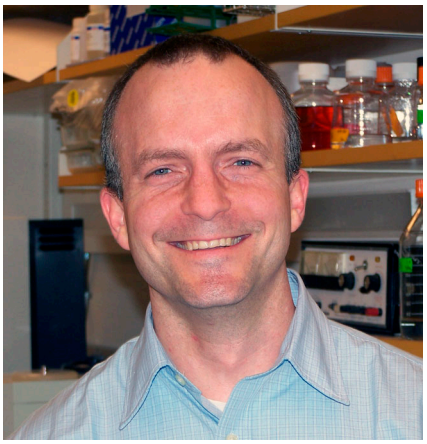
I like to do outdoor activities and visit new places. That's actually one of the reasons I like being at UT Southwestern now. Of course the scientific environment here is really supportive, but I also like its location in the middle of the country—in striking distance for lots of neat places. I'm married and have two kids, and I want to expose them to new places and things, kind of like what I got to experience as a child. This year we went to the Grand Canyon. It was the first time I'd ever been there.

> "One of the really great things about the *Drosophila* community is that people tend to be extremely generous."

TRACKING NEW GAME

Were your safaris what got you interested in science?

My dad had a real interest in science. My parents always made books available and really helped to generate an interest in me, so science appealed to me from a very early age. But because of a fantastic high school English teacher, I actually started college at Tufts as an English major. I realized within the first two years, though, that English and creative writing were not going to be my strongest suits. I finished my English major, but then fell in love again with science, particularly genetics, and decided to do a biology major in my last two years.



Michael Buszczak

How did you come to study* Drosophila *oogenesis as a graduate student?

My first laboratory rotation at Yale was with Bill Segraves, who studied the steroid response hierarchy in *Drosophila*. Flies have this steroid hormone ecdysone, which regulates molting, but Bill had also identified some ecdysone pathway mutations that looked like they had some female-sterile phenotypes. I eventually decided to join Lynn Cooley's laboratory, but I wanted to keep working on steroid hormones, so Bill served as a coadvisor for me.

We figured out that ecdysone works reiteratively during oogenesis. Not only does it control molting patterns and metamorphosis, but it also controls female reproduction. There's a point in mid-oogenesis called vitellogenesis where the oocyte gets loaded up with lots of yolk proteins, which will provide energy and various nutrients in early embryogenesis. It represents a huge energy investment, so there's a control point there: if there's anything wrong with the egg chamber at that point, it dies. We found that ecdysone was a major player in this checkpoint.

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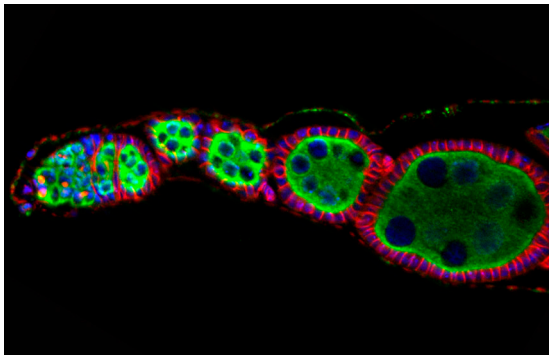

***Drosophila* egg chambers developing in an ovariole.**

SETTING (PROTEIN) TRAPS

How did you develop the protein trapping approach?

Toward the end of my graduate work, I was talking to Lynn and another graduate student and we had this idea of doing what's called a protein trap. That's where you take a histologically scorable marker—in our case, GFP—and mobilize it around the genome to look for new insertions that give you interesting expression and localization patterns. I ran this idea by one of my friends, and he told me "Oh, I just heard this talk; somebody's already done that." [Laughs] It's one of these things where you have something up on the chalkboard and it looks so great, and then it's crushing to hear somebody else is already doing it. I contacted the person, Bill Chia, and my friend was right; Bill's laboratory was working it up. But Bill was really generous and totally willing to give us the reagents to do it ourselves. That's one of the really great things about the *Drosophila* community: people tend to be extremely generous.

So we started up our screens. The events we were looking for are extremely rare, with hit rates of one in every 1,000 or 2,000. Using a microscope you can hope to sort maybe 2,000 embryos an hour, so you could find one or two events per hour. But Lynn had recently got her hands on an automatic embryo sorting machine. With the sorter, you could process up to 72,000 embryos an hour and find hundreds of hits in a day. It's a great hypothesis-generating tool.

> "You can process up to 72,000 embryos an hour... it's a great hypothesis-generating tool."

And then you transitioned to studying gene expression in stem cells as a postdoc?

When I went looking for a postdoc, I wanted to take this technology with me. I ended up deciding to go to Allan Spradling's laboratory at Carnegie—he's a brilliant scientist and everyone that I talked to said that he would be a fantastic person to work for. He's heavily involved in the *Drosophila* disruption project, where they're using transposable elements to tag or mutate every open reading frame in the *Drosophila* genome. His laboratory already had this great pipeline for generating new mutant lines and sequencing them. In talking to Allan, he said, "Oh, it'd be great. You could generate the lines and then we can have them sequenced, using our infrastructure that's already in place." I thought that was potentially really powerful and would accelerate the pace at which the screen could be conducted.

Another reason I chose Allan's laboratory was that I had a strong interest in nuclear architecture. A lot of people in his group study stem cells, so I thought it would be a good place to explore how stem cells organize their chromatin. Our idea was to use the protein trap collection to find genes involved in chromatin programming in stem cells. We looked for proteins that were expressed, say, in stem cells and their early progeny, or for traps that were only expressed as those cells were undergoing differentiation. We used the expression pattern as an entry point to study the function of specific genes in those cells.

And now you're extending this work in your own laboratory at UT Southwestern?

Yes. We're actually moving beyond that on a couple of different fronts. We're following up on some of the chromatin work, but we're also becoming very interested in translational regulation as well, which has been known for some time to play a critical role in germline stem cell maintenance and differentiation. We're currently writing up a manuscript on a protein whose human homologue has been implicated in regulating alternative splicing: we have evidence to suggest that it might also be involved in regulating translation. We're also following up on Scrawny, which is a ubiquitin-specific protease that I studied as a postdoc, which we now think might be involved in gene silencing. And finally, we're embarking on a project to do targeted knockouts of chromatin regulatory enzymes to get a more comprehensive understanding of chromatin programming within stem cells.

1. Buszczak, M., et al. 1999. *Development.* 126:4581–4589.
2. Kelso, R.J., et al. 2004. *Nucleic Acids Res.* 32:D418–D420.
3. Buszczak, M., and A.C. Spradling. 2006. *Genes Dev.* 20:977–989.
4. Buszczak, M., et al. 2007. *Genetics.* 175:1505–1531.
5. Buszczak, M. et al. 2009. *Science*. 323:248-251.

Buszczak and family take in the view at the Grand Canyon.