Jane Skok: Choreography of allelic exclusion

After a decade-long hiatus from science, Jane Skok returned to the lab to study the mechanism that allows each B cell to secrete antibodies that recognize only one target.

Antibody diversity is generated during B cell development by the rearrangement of variable (V), diversity (D), and joining (J) segments of immunoglobulin (Ig) genes. Both alleles of each Ig locus can initiate rearrangement, but only one allele successfully rearranges. As soon as this occurs, the other allele is prevented from following suit. This mechanism, known as allelic exclusion, guarantees that each B cell produces antibodies specific for just one antigen. In her laboratory at New York University (NYU), Jane Skok studies how exclusion is established.

"I didn't want to be doing nothing for the rest of my life; I wanted it to have some meaning."

Skok's initial foray into research involved dissecting the genetics of the complement cascade with Ellen Solomon at Cancer Research UK (1). But immediately after earning her Ph.D., Skok left science to care for her ill child. When she returned nearly a decade later, she had to start from scratch. After getting up to speed with a Master's in immunology, she joined Amanda Fisher's lab, where she gravitated toward B cells and the question of allelic exclusion.

Skok has since helped define some of the steps that make exclusion possible. She discovered that the excluded Ig allele gets sent to repressive DNA domains near the centromere, whereas the productive allele remains within accessible domains (2–4). She later focused on one of the more curious aspects of allelic exclusion. During rearrangement, the Ig locus contracts, allowing V, D, and J segments to get close to each other and join up. Skok found that the successful rearrangement of one allele triggers locus relaxation, thereby stopping rearrangement of the other allele (5). She is now hunting for the proteins and pathways that direct the contortion and movement of Ig loci.

A PROMISING START

What inspired you to become a scientist?
There are a mixture of artists and scientists in my family, and I enjoyed both disciplines. But solving problems was much more satisfying to me, so I chose science.

Tell me about your graduate degree in genetics.
I worked on the genetics of the first component of the complement system, C1q, in Ellen Solomon's lab. We showed that C1q is encoded by distinct sets of genes in fibroblasts and serum. This was my first paper, which I published in a relatively short time in *Nature*. This gave me an unrealistic outlook of what I would face in the future. I remember thinking, "Oh, wow, this is so easy."

What happened then?
My second child got sick. It's very hard to work when you have young children. But when you've got a sick child and you're in and out of hospitals all the time, it's almost impossible. I managed to finish my Ph.D., but I stopped after that for almost ten years.

A PROLONGED STOP

What did you do during that time?
I took care of my child until she got better and then had two more children. I enjoyed being with my children but was bored without the challenge of a career. I've always enjoyed art, so I went to art school. Although that was a good experience, it wasn't very challenging. I missed having the pressure of a focused job. So I decided to go back to science.

What did the kids think about that decision?
I don't think the older ones minded because they were growing up and didn't need me as much. I went back soon after my youngest daughter was born. She was too young to know any better and hadn't begun to talk yet—so she couldn't tell me anything.

Jane Skok

What was it like to return to science after ten years?
I hadn't kept up with the literature and had forgotten a lot of what I had learned. I decided to start by doing a Master's degree, thinking that it would be a good way of learning and getting oriented in whatever field I was going to go into. I decided to study immunology because I'd had some exposure to it when I was working on C1q in Ellen's lab.

But it was a tough beginning because immunology is not the easiest subject to go into. I was reading like crazy to make sense of it all. But between the children and the studying, it was pretty hard going. It was also quite a challenge making my brain work after ten years. I had zero confidence at that point and felt that I wasn't going to make it.

What made you keep going?
The thought that if I didn't get back into it, I'd be standing in the same place in another ten years' time. That was a scary thought because I didn't want to be doing nothing for the rest of my life; I wanted it to have some meaning.

During my Master's, I did a short research project with David Gray, who was a great mentor. He's one of those people who can see the point of something very clearly without getting bogged

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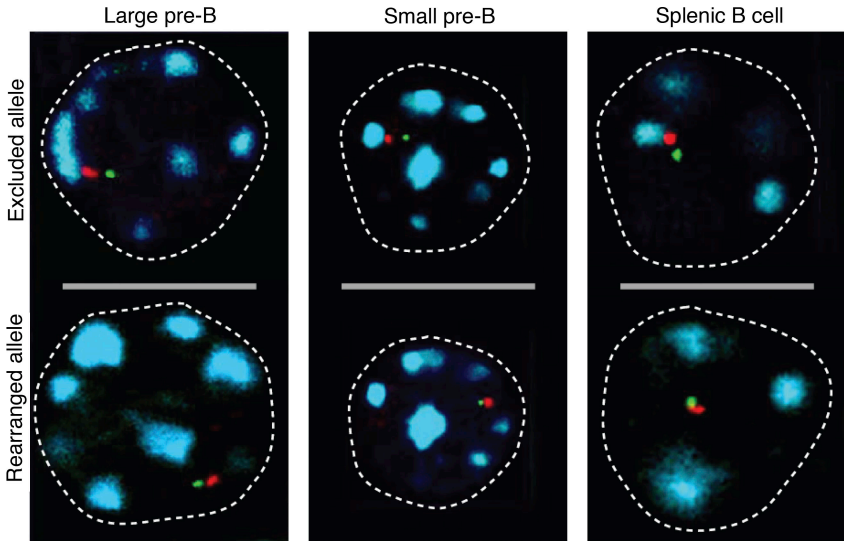

In developing B cells, only the excluded allele (red and green) is positioned at repressive centromeric domains (blue).

down in lots of information—a problem that I was experiencing. He also educated me about funding opportunities and supported me in applying for a Wellcome Trust Career Reentry Award, which is a grant that enables you to get back into science after taking a break. I don't know if there's an equivalent funding program here in the US, but it provides a very good opportunity for people who've had a career break to get back in the game. In the UK, mostly women apply for it. When I later met some of those women, I found out that we had all faced the same problem going back to science: lack of confidence.

BACK ON TRACK

What did you work on when you got back to the lab?

I started to work in David's lab on how cytokines produced by B cells influence the differentiation of T cells. Within a year of getting my funding, he moved to Edinburgh, but luckily, by that time, we had enough data to publish a paper.

Was David's move a setback for you?

No, it actually ended up working in my favor. This is how collaborations and interdisciplinary science comes about. Mandy Fisher, who was in the next building, was looking at the positioning of Ikaros in resting versus activated B cells. I was helping her purify the cells, and when she heard David was leaving, she offered me a position in her lab to look at where Ig genes were positioned in the nucleus. So I was doing new things again and was back in the land of "Don't know anything."

What was your experience in Mandy's lab?

Like David, Mandy is a clear-thinking person who helped me focus. Although I was back to square one again and reading papers and textbooks, I managed to get things working. I showed that one of the Ig alleles is repositioned at repressive pericentromeric domains in activated mature B cells and that this is important for maintaining expression of only one allele. At the end of my time in Mandy's lab I successfully applied for a Wellcome Trust University award, which came with tenure at UCL.

While I was in Mandy's lab, I got to work with Steven Kosak. He had shown that Ig loci are initially located at the cell periphery in non–B cells but then contract and move toward the center in B cells. In collaboration with Steven and also Harinder Singh, I did 3D FISH experiments to visualize the localization of Ig loci in B cells. We found that Ig genes move in from the periphery as they undergo rearrangement and that they contract as they move.

How is this contraction regulated?

This was work I did in collaboration with Meinrad Busslinger in Vienna, who had asked me if I'd be interested in doing some FISH experiments to look at the IgH locus in B cells lacking the transcription factor Pax5. That's when I discovered that Pax5 is necessary for locus contraction of the Ig heavy chain.

Back in my own lab, we soon found that the Ig locus decontracts as soon as the heavy chain has finished rearranging. We then went on to show that contraction also occurs at the Ig kappa (light chain) locus during recombination, and that one allele is repositioned at heterochromatic domains before rearrangement occurs, which is the opposite of what happens with the heavy chain locus. This work was done in collaboration with Yehudit Bergman and later with Marjorie Shapiro and Sean Fitzsimmons.

> "I always want to know the answer to the next question, and I want to know it yesterday."

THE NEXT STEPS

If both alleles contract and can recombine, what prevents simultaneous rearrangement of both alleles?

That's a fantastic question that we're working on at the moment. The fact that only one allele gets functionally rearranged implies that there's a mechanism for preventing simultaneous rearrangement on both alleles. Fred Alt first proposed this idea back in 1992. We are trying to understand how the positioning of the two alleles regulates this and to determine what role the recombinase enzymes play.

Sounds like you've got enough on your plate to stay busy for a while.

I think it's good to have a long-term project because it really gets you focused on something. I didn't really get hooked into science until I got hooked into this project. I am very impatient about getting data because I always want to know the answer to the next question, and I want to know it yesterday.

1. Skok, J., et al. 1981. *Nature.* 292:549–551.
2. Skok, J.A., et al. 2001. *Nat. Immunol.* 2:848–854.
3. Kosak, S.T., et al. 2002. *Science.* 296:158–162.
4. Fuxa, M., et al. 2004. *Genes Dev.* 18:411–422.
5. Roldan, E., et al. 2005. *Nat. Immunol.* 6:31–41.