AUTHOR COMMENTARIES - 2009

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Barry Wanner & Kirill Datsenko
Featured Paper from *Essential Science Indicators*SM

According to Essential Science Indicators *from Thomson Reuters, the paper ranked at #2 in the field of Microbiology is "One-step inactivation of chromosomal genes in* Escherichia coli *K-12 using PCR products," (Datsenko KA, Wanner BL,* Proc. Nat. Acad. Sci. *97[12]: 6640-5, 6 June 2000), with 2,023 citations up to December 31, 2008.*

The paper's authors are Dr. Barry Wanner and Dr. Kirill Datsenko, both of whom hail from the Department of Biological Sciences at Purdue University in West Lafayette, Indiana. Dr. Wanner is a full professor in the department, and Dr. Datsenko is a research associate there. Dr. Wanner has published 100 manuscripts and book chapters, including 31 papers recorded in Essential Science Indicators *as cited a total of 2,932 times, and Dr. Datsenko's record includes 9 papers cited a total of 2,118 times.*

In the interview below, ScienceWatch.com *correspondent Gary Taubes talks with both authors about this paper and its impact on the research community.*

SW: In 2000, you published a methods paper in *PNAS* that now has over 2,000 citations: "One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products." Why is this method of gene inactivation so special? What exactly does it do?

Wanner: The method itself is actually a very rapid and efficient method for making mutations. As geneticists who study physiology, one of the key ways in which we do our work is basically to break a pathway—break something in a cell—and see what's broken and how that affects the physiology or growth of the cell. This method was a way to target particular genes for interruption. Thirty years ago, when I started in this field, we would look for random mutations and then identify the gene that was mutated, that was responsible, and try to understand the process. Now we have 4,000 or 5,000 genes, or DNA sequences that we believe are genes, and we have a way of targeting each of them with high precision.

That's what this method is all about. It's a way to do reverse genetics very efficiently. It makes making mutations easy so you can focus on trying to determine the function of the genes, on the biology rather than the labor. It's a technology, one that's quite simple to use and can be used in different ways, not just knocking out genes but modifying them.

SW: How much easier does it make it?

Wanner: Prior to this work it would take maybe two to three weeks to make a single mutation from start to end. Using this method, as we are

now, with robots involved, we can make hundreds of mutations in a single day.

+enlarge

Coauthor
Kirill A. Datsenko

SW: What were you working on in the 1990s that led up to this work?

Wanner: I've been studying signal transduction using *E. coli* as a model cell for a long time. This cell was the focus of both my Ph.D. work and postdoctoral research—in particular signal transduction and the question of how a signal gets across a membrane. I've continued to study that at a more and more detailed level ever since.

Datsenko: I was trained as a classical microbiologist. I started my career studying the phosphotransferase system in the phytopathogenic bacteria Erwinia, and when I moved to Purdue, I shifted my research to *E. coli* because that was the focus of the laboratory. That was in early 1999.

SW: What gave you the idea for your gene-inactivation method in *E. coli*?

Wanner: What prompted this particular study was actually a paper published in 1993, reporting the development of a similar method for a different organism, a eukaryote—*Saccharomyces cerevisiae*. What it did was make gene disruption extremely simple in yeast, but nothing like it existed for other organisms at the time. That method was actually used later to make the first knockout organisms, which were also done in *Saccharomyces cerevisiae*. So I started work on this as a part-time project, along with other members of my group who had left the lab by the time Kirill arrived. I had actually done some of the last experiments on this technique with my own hands. Once Kirill arrived, a new person in the group, it became his project as an introduction to the lab.

SW: Why Kirill? Was there something unique about his expertise?

Wanner: It was more a matter of timing. He had just gotten his Ph.D. in Moscow before joining my group, and he was interested in learning bacterial genetics. The last person other than myself to work on this was a post-doc who had left the laboratory more than a year earlier. The other researchers in my lab were grad students with other projects to work on. So Kirill got it.

SW: Were you aware at the time how significant this could be?

Datsenko: I have to admit that before this paper was published, I didn't think it was such a big deal. For me it started like a side project: I was supposed to develop this technique as a tool to isolate a certain mutant; at that time, it was not the main focus of my research. It wasn't until November 1999, when Dr. Wanner came into the lab and congratulated me because the paper had been accepted in *PNAS* that I realized it was something special.

SW: What was the major obstacle or challenge that had to be overcome to make this method work?

Wanner: We always knew the method would work—based on earlier results we had obtained. The problem was that it was extremely inefficient. Until Kirill persevered with it, getting it to the point that it was 100% reliable, it was never efficient enough to use.

SW: Was there one particular breakthrough that made it more efficient or just a series of minor fixes and improvements?

Wanner: The method was working all along but the problem was that we would isolate maybe 1,000 cells on a petri dish and only a few of those would be correct. We had a way of identifying those one or two that were correct, and what Kirill started doing was looking at the other 998 or 999 and trying to figure out what was wrong with them.

So his breakthrough was to find out what was wrong with the majority—this high background—and then how to eliminate the background so we'd end up with just the one or two that were correct. Well, maybe there were 10,000 cells on a plate and 10 and or 20 that were correct. Either way, what we did was just eliminate the background—the incorrect cells. So Kirill was tinkering to find out what was wrong with them and that was the real technological breakthrough. Other people had always focused on the correct cells; Kirill figured out what was wrong with the incorrect ones and how to eliminate them.

SW: How do you eliminate the incorrect ones?

Wanner: The method is based on synthesizing DNA using PCR. In order to do that, you use a template,

and the problem was that the template plasmid was undergoing other types of recombination events—the template itself was actually the source of the background. We worked out a couple of ways to prevent the template plasmid from entering the cells. Simple chemical purification of the PCR product was insufficient. We always had some template plasmid present.

One approach was to use a template plasmid that would be unable to replicate; another way was to use a certain type of restriction enzyme that restricted or digested only the template plasmid after amplification. Using the two together we completely eliminated this background problem.

SW: Was it immediately clear from the response of the community that you had achieved something particularly significant in the field?

"...even a simple organism like E. coli *has 4,000 nonessential genes."*

Datsenko: Actually, the opposite. I remember when Dr. Wanner first reported about our procedure at a meeting, before the paper was published in *PNAS*, no one got excited. Nobody asked any questions. They treated it as just another boring report. That was it.

Wanner: Every two or three months, a group of us from universities in the Midwest would get together and discuss our research. I had given a presentation describing this work at one of these meetings. I was quite excited about it because we were in the business of making mutations and that's what this method allowed us to do. But it didn't seem to be well appreciated at that meeting. Maybe a couple of people came up to talk to me about it afterward.

Malcolm Casadaban, a geneticist from the University of Chicago, said it was the best thing since baked bread or something like that. He was a very bright guy and he was very enthusiastic. But most people just didn't get it. Maybe my presentation wasn't as clear as it could have been and Dr. Casadaban understood anyway, because he was a geneticist.

Shortly after that, I presented it at another meeting of geneticists and they were all quite excited. Ever since then, we've been getting requests for this system from a wide range of people, even those who have no expertise in genetics. In fact, half a dozen have visited the lab to learn the technique, although we never thought that was necessary. Still, we continue to get requests, usually one or two a day, for the actual system and the necessary materials.

SW: Why did you decide to publish it in *PNAS* rather than more of a specialty journal?

Wanner: Previously I had published three or four papers in *PNAS*, and I wanted a journal that is read widely. We might have published in other excellent journals like *Molecular Microbiology,* although this was a methods paper and it doesn't take methods papers, or the *Journal of Bacteriology*, which does take methods, but I thought this deserved a broader audience.

SW: Was there any element of serendipity in making this PCR method work?

Wanner: I'm a great believer in serendipity in science, but that wasn't the case here. This one worked through perseverance and because we tried a number of approaches until we hit on the right one.

SW: Are there competing techniques for accomplishing targeted gene disruption in *E. Coli*?

Wanner: Francis Stewart in Germany had published a method that was analogous to ours, but much less efficient and not very practical. He had published his paper before ours in *Nature Biotechnology*, but I didn't actually become aware of it until I was writing up our manuscript.

During the summer of 1999 I did speak to Don Court at NIH, who had developed a method very similar to ours. We had exchanged strains and things and I discussed with him specific problems we were having with our background. In fact, I cited him in the acknowledgements for these discussions, because he told me of a method he was using that reduced the background in his case.

We ended up using both his approach as well as the digestion approach using these restriction enzymes. He also published about the same time we did—also in *PNAS*. And he has gone on to develop this technology much farther. We were never interested in developing our technology so much as using it for research.

SW: How are you using the method in your research now?

Wanner: We're working in collaboration with Hirotada Mori's group at the Nara Institute of Science and Technology in Japan. The work is largely done there, although both Kirill and I are co-authors. We've employed this technology to make a complete knockout set of all nonessential genes of *E coli*. We've recently gone farther in a manuscript I'm now writing, making a second knockout set. The reason for

making two is we're embarking on making double knockouts to look at how one gene interacts with another gene, doing these genetic interactions genome wide. We published the first two manuscripts on this last summer in *Nature Genetics*, along with Carol Gross's group at UCSF, and Jack Greenblatt and Andrew Emili's group at University of Toronto. We developed the technology in collaboration with Mori and have now published six or seven papers with him since around 2003.

What we're gearing up to do is use these double knockouts with genome-wide scans. We're using double plates that have 1,536 wells—96 x 16—and that's because even a simple organism like *E. coli* has 4,000 nonessential genes. It has about 4,400 genes total. We want to make doubles of all 4,000. That's 16 million strains and it has to be done in quadruplicate for statistical reasons. This is very, very high throughput. And the wealth of information we're getting out of this is just amazing. We've already been discussing this work at meetings. Some of it is now reported, most is not. Already we're getting requests for the system from other groups.

SW: Did the 1999 *PNAS* paper change the way you saw your career progressing?

Datsenko: It allowed me to establish many research relationships with scientists around the world, which resulted in multiple successful collaborations.

Wanner: I have actually become better known myself for this technology than any of the actual science I've done, either before or since.

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Barry Wanner & Kirill Datsenko's current most-cited paper in *Essential Science Indicators*, with 2,023 cites:

Datsenko KA, Wanner BL, "One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products," *Proc. Nat. Acad. Sci. USA* 97(12): 6640-5, 6 June 2000. Source: *Essential Science Indicators* from Thomson Reuters.

KEYWORDS: CHROMOSOMAL GENES, ESCHERICHIA COLI, E. COLI, PCR, REVERSE GENETICS, MUTATIONS, TEMPLATE PLASMA, DOUBLE KNOCKOUTS, GENOME-WIDE SCANS.

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