Cell

Beginner's Luck

Evelyn Witkin

On June 5, 1944, I discovered a radiation-resistant mutant of *E. coli* in my first experiment at the Cold Spring Harbor laboratories. That's where the new field of bacterial genetics was germinating. I was a Columbia University graduate student, there to learn how to handle *E. coli* so I could do my doctoral research with bacteria.

I had planned to study the mechanism of induced mutation in *Drosophila.* Then, in 1943, Luria and Delbruck established that bacteria have genes like other organisms. I was so excited about the great potential value of bacteria for research in genetics that my advisor, Theodosius Dobzhansky, suggested that I switch from *Drosophila* to *E. coli* and that I spend the following summer at Cold Spring Harbor, tooling up for the change.

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I found an impressive group of scientists gathered at Cold Spring Harbor that summer. There was talk everywhere—at meals, at the beach, in the labs—of the new work on bacteriophage and bacteria and of its potential impact in genetics. Looking back, I think I may have been sensing the early stirrings of the coming revolution in molecular biology.

On my first day there, Dr. Milislav Demerec, director of the laboratories, handed me a culture of *E. coli* and pointed me toward a germicidal ultraviolet (UV) lamp, saying "Go, induce mutations."

My mutant, which could tolerate 100 times more UV or X-rays than its parent strain, created something of a stir among the scientists at Cold Spring Harbor. Salvador Luria, Max Delbruck, and Barbara McClintock, among others I had been thrilled to meet that summer, seemed genuinely interested in it. Soon, with the approval of my advisor, it became the subject of my Ph.D. dissertation.

It turned out that the mutant was no more resistant to UV than most wild-type strains of *E. coli* such as K-12. What was remarkable was the extreme UV sensitivity of the parent strain, the wild-type strain B, which happened to be used at Cold Spring Harbor.

Why was the B strain so sensitive to radiation? The answer became evident when I exposed B bacteria to UV at a dose that killed 99% of them, spread them on agar, and examined them periodically under a microscope. By three hours of incubation, every cell had grown, without dividing, to form a snake-like filament 50–100 times normal length and then stopped growing and died. Filamentous growth was lethal.

At the same UV dose, the mutant cells grew and divided like the unirradiated controls, forming microcolonies of 50–100 cells in 3 hr. UV caused an irreversible arrest of cell division in the B strain, but not in the mutant, which I named strain B/r.

It was Barbara McClintock who had advised me, early and often, to study my bacteria under the microscope, the better to develop a kind of cross-species empathy, "a feeling for the organism," as an aid to intuition. It was good advice.

Evelyn Witkin in her laboratory at the Waksman Institute at Rutgers University in 1981. Looking on are her graduate students Owen MCCall (left) and Howard Lieberman (right).

After receiving my Ph.D. from Columbia in 1947, I began my forty-five year investigation of the mechanism of UV mutagenesis. But I never lost my fascination with filamentous growth, and I managed to steal hours at the microscope, on and off over several years, to watch the UV-sensitive bacteria grow and die virtually before my eyes. I began to form an image in my mind, hazy at first, then quite vivid, of what could be happening inside those cells between UV irradiation and death by filamentation.

I noticed that, at a low UV dose that allowed about 50% survival, all the irradiated bacteria began to grow as filaments. About half of them grew into full-length filaments and never recovered cell division ability. The other half, however, started, between two and three hours, to pinch off cells of normal length at one end of the filament before it quite reached full length. By three hours, a small microcolony with a long tail appeared at that location.

After exposure to a very low sublethal UV dose, most of the bacteria showed no sign of filamentous growth by three hours, having formed microcolonies like those seen in the unirradiated controls. However, when examined earlier, between one and two hours, a few bacteria (perhaps 5%–10%) had a short tail attached to a growing microcolony, as if they had started to grow as incipient filaments, but had recovered cell division activity quite early.

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The duration of cell division arrest in strain B after UV was clearly dose dependent. Gradually, I came to "see," in the YouTube of my imagination, a scenario that could account for all of these observations. It went like this:

1. UV irradiation triggers synthesis of a cell division inhibitor.
2. The number of molecules of inhibitor synthesized is UV dose dependent, increasing with increasing dose.
3. When a cell grows to twice its length, a septation site, where cell division normally occurs, forms on the membrane at the midpoint of the elongated cell.
4. Cell division cannot occur if the septation site is bound by an inhibitor molecule.
5. Lethal filamentous growth appears when inhibitor molecules are abundant enough to bind all successive septation sites that form in three hours.
6. Cell division resumes at one end of the growing filament after all inhibitor molecules have been bound to septation sites and are effectively titrated out. Cell division resumes at the next septation site to be formed.

This was the 1940s, before Watson and Crick and before Monod and Jacob. Fast forward to 1967, when I noticed a number of striking similarities between λ prophage induction and filamentous growth in UV-irradiated *E . coli* B. Luria had just shown that λ repressor is destroyed in the course of prophage induction. I proposed that a bacterial gene encoding a cell division inhibitor, and possibly other bacterial genes, might be controlled by repressors similar enough to λ repressor to respond to the same induction signal.

I believe that linking prophage induction and filamentous growth as UV co-inducible was a step toward my recognition of *E. coli's* SOS response, with Miroslav Radman, about five years later. The SOS response now comprises over forty DNA damage-inducible genes, all sharing a common

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Evelyn Witkin and scientist Arnold Sparrow in Cold Spring Harbor in 1947. Courtesy of Cold Spring Harbor Laboratory Archives.

repressor. Collectively, they promote the repair of genetic damage and the survival and reproduction of the damaged cell and of the population.

One of the SOS genes is *sfiA*(*sulA*), which encodes a cell division inhibitor. My beginner's luck, the mutation in strain B/r, maps in *sfiA* and inactivates the inhibitor, increasing resistance to UV by preventing filamentous growth.

I have little doubt that my readiness to propose the co-inducibility of filamentous growth and λ prophage was subliminally informed by the hours I spent at the microscope twenty-some years earlier, mesmerized by watching the transformation of bacteria into snakes.