Cell

Accidents and Damage Control

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My work uncovering the eukaryotic DNA damage response pathway is a tale of serendipity and four amino acids. After graduate school at MIT, I joined Ron Davis's lab as a post-doc initially to work on plants but became interested in developing gene-targeting methods for mammals. I had reasoned, naively, that the protein responsible for homologous recombination in mammals would be related to the master regulator of recombination in bacteria, recA. RecA protein had the ability to initiate recombination in vitro on its own using ssDNA and ATP. Therefore, I decided to use the mammalian recA protein to coat ssDNA and inject the complex into cells where it would find its homologous sequence and initiate recombination. Voila, mission accomplished, right? The problem was that there was no mammalian recA gene or protein known. In fact, no yeast recA gene had even been identified yet, and it was quite possible that the recombinase in mammals was unrelated to recA.

"'I have good news and bad news…I identified your gene.' And the bad news? 'It is not recA.'"

This was more than a minor setback. I had to figure out a way around it and decided to see if yeast had a related protein to bacterial recA using antibodies to recA. Sure enough, budding yeast had a single band of molecular weight 44 kDa, just a little bigger than the bacterial protein. My strategy to isolate the gene was to use lambda gt11 cloning, a method to identify genes encoding proteins using antibody detection of proteins expressed in plaques. Using the antibodies to bacterial recA, I isolated the gene encoding the 44 kDa protein and showed that it encoded the protein I had observed. There was just the small matter of sequencing it to establish the degree of identity. This had to be done by hand in 1985. I finally got the sequence, and a friend of mine, Andy Buchman, offered to analyze the sequence for me, as he had expertise in computer analysis of sequences and access to what must have been a minuscule database of previously sequenced genes.

I went away for the weekend and came back and asked Andy if he had found anything. He said, with a bemused look on his face, "I have good news and bad news." I said, "Give me the good news first." He replied, "I identified your gene." And the bad news? "It is not recA." The gene I had cloned was the small subunit of ribonucleotide reductase, which I named *RNR2*, a gene involved in nucleotide metabolism.

I compared the sequences, and the only thing they had in common was that the last four amino acids were identical. I showed by deletion analysis that they indeed comprised the cross-reacting epitope. At this point, I was devastated. I definitely was going nowhere on this recombination idea, and nucleotide metabolism was last thing I wanted to work on. I thought, "This could only have been worse if I cloned a histone!" So I set the project aside and worked on some of my other projects.

However, things took an unexpected turn when David Stillman came through Stanford on the job circuit. I had a chance to talk with him and told him my story, and he said that ribonucleotide reductase was interesting because it was a tightly cell-cycle-regulated activity. That caught my attention. I started thinking about ribonucleotide reductase again and remembered an experiment I performed when I still thought the gene might be *recA*. I had done a Northern blot in the presence of a DNA damaging agent, 4-nitroquinoline-1-oxide, and the *RNR2* mRNA went up almost 20-fold like recA. This was an unusually strong effect. I began to examine what agents induced this gene and found that those that blocked DNA replication, like hydroxyurea and MMS, had by far the largest effects.

This got me thinking about how much cells must care about the capacity to synthesize DNA. I thought there must be a way to sense what is happening at stalled or damaged replication forks, perhaps through some signal that might be transduced to the promoter of *RNR2*, which could be a stepping stone into this pathway. And if this signal transduction pathway did indeed exist, it was

Steve Elledge in his lab at the Baylor College of Medicine, circa 1996.

likely to control much more than just *RNR2* gene expression. I published this work in 1987, noting that "it would be of great interest to investigate the signal responsible for the DNA damage inducibility of *RNR2*" and anticipated "that a detailed analysis of the *RNR2* promoter would allow us to identify the sequences involved in its complex regulation and through these, the genes responsible for that regulation."

As it turned out, *RNR2* was in fact my entry point to the signal transduction pathway I had envisioned. Upon dissecting the *RNR2* promoter, I found that it was under active repression and identified enhancer elements that could confer inducibility on a heterologous promoter. In late 1987, I also isolated the genes that encoded the large subunit of ribonucleotide reductase using the human gene as a probe and found two genes, *RNR1*, which showed strong cell cycle regulated transcription, and *RNR3*, which was induced over 100-fold with replication stress!

"There was indeed a signal at the replication fork, just as I had imagined several years earlier."

A relationship between radiation-sensitive *RAD* mutants and defects in cell-cycle delays in response to DNA damage had been noted previously in 1976 in *S. pombe* by Nasim and colleagues and in mammalian cells by Painter and Young in 1980—who found that cells from patients with ataxia telangiectasia fail to slow S phase in the presence of DNA damage. In 1988, Weinert and Hartwell described another such mutant, this time in a budding yeast gene, *RAD9*, that also failed to arrest the cell cycle in response to DNA damage, and this was referred to as a cell-cycle checkpoint. With all this in mind, I began to wonder about connections between my pathway and *RAD* genes. However, I found that *RNR* transcription was still induced in *rad9* mutants when replication was stalled, so there was much more to be learned on these connections.

I started my own lab in the Biochemistry Department at the Baylor College of Medicine in 1989. There, I found a vibrant environment and began what would become a life-long collaboration with my fellow faculty member Wade Harper, studying the mammalian cell cycle and discovering Cdk inhibitors and the SCF ubiquitin ligases. My big early break in the RNR regulation project came when my first graduate student, Zheng Zhou, joined my lab. Zheng was a force of nature, and she set up a genetic selection for constitutive and uninducible *RNR* transcription (*CRT*) mutants. We found *RNR3* to be constitutively expressed in mutants in DNA polymerase alpha, providing a direct link to the replication fork, mutants in other components of nucleotide metabolism, and mutants in a transcriptional repressor complex whose DNA binding component encoded by *CRT1/RFX1* was later shown by a post-doctoral fellow Mingxia Huang to undergo inhibition in response to DNA damage, thereby establishing the mechanism of transcriptional induction. However, the DNA damage uninducible mutants (*DUN*) were the most revealing. One was found to be DNA polymerase II by my student Anthony Navas, providing yet another link to the fork. Another was found to be a protein kinase encoded by the DNA damage uninducible 1 gene, *DUN1*. *DUN1* was the most exciting because protein kinases are agents of signal transduction. However, it might have merely been constitutively required for some aspect of the pathway and might not actually transduce the signal. Fortunately, Zheng did the hard experiment and looked at the activity of Dun1 in vivo using metabolic labeling with ^{32}P-labeled organic phosphate and showed that Dun1 became highly auto-phosphorylated in response to DNA damage This established that the presence of DNA damage was indeed transduced by signal transduction and was the first demonstration of what is now known as the DNA damage response pathway, the DDR.

Those early years in my lab witnessed a flurry of activity, with another student, Jim Allen, identifying the S phase arrest defective (*SAD*) genes that became known as *RAD53* and *MEC1*, alleles of which were also identified by Weinert and colleagues. Jim and Zheng showed that Rad53 was a protein kinase acting upstream of Dun1. This was the first step in identifying what we subsequently discovered to be a protein kinase cascade that activated Rad53 and Dun1 in response to agents that blocked replication. The work that followed in my lab identified further components of the pathway, with another student, Brian Desany, showing that the essential function of the *MEC1/RAD53* pathway was to regulate DNA synthesis, as even transient inhibition of DNA synthesis in hypomorphic mutants killed cells by preventing their ability to complete DNA replication. Meme Alcasabas in my lab then discovered *MRC1*, with Alex Osborn then showing that it traveled with the fork and was phosphorylated by Mec1 and that this phosphorylation was required for

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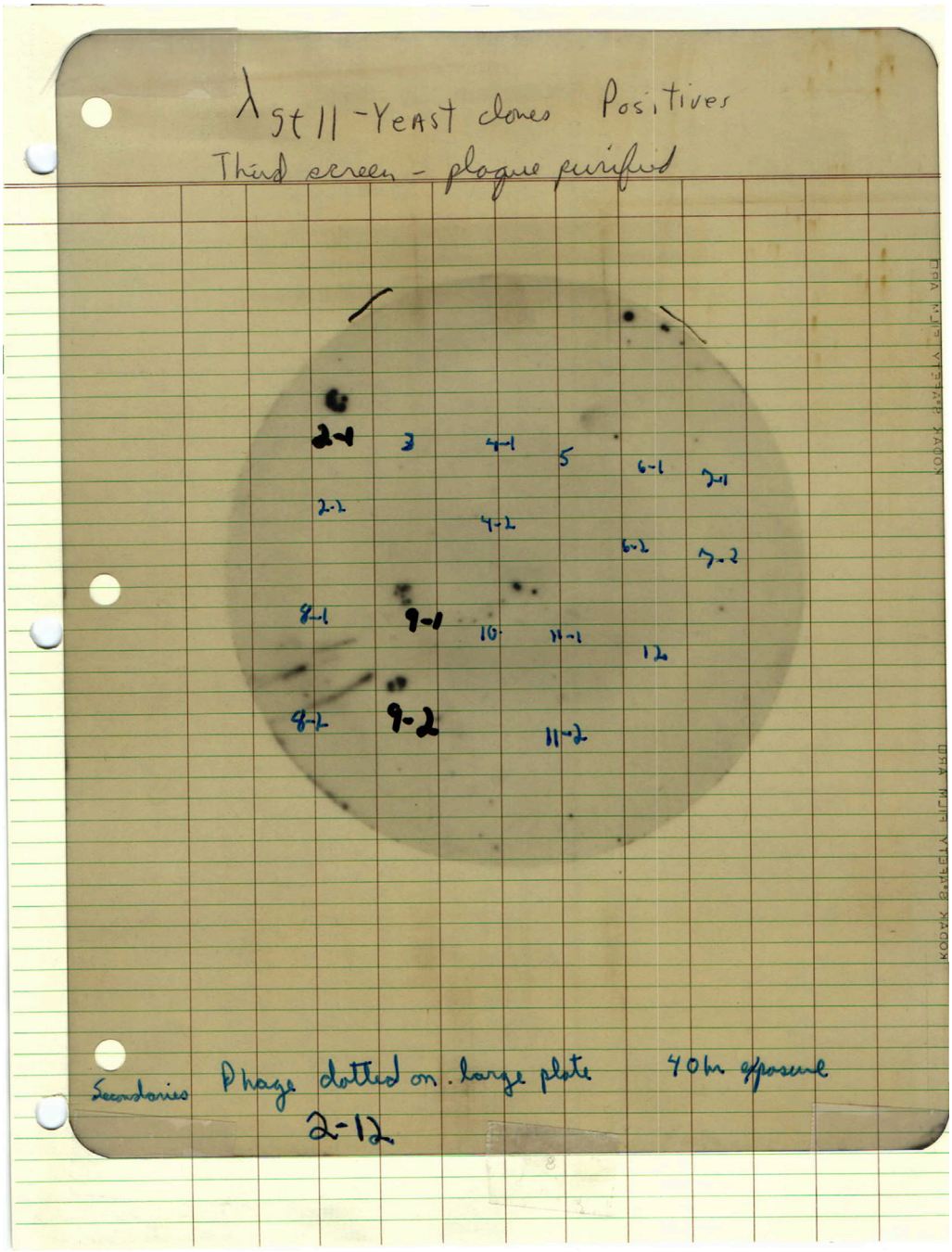

Plaque purification and screening of lambda gt11 clones probed with affinity purified anti-RecA antibodies. Lambda 2-1, 9-1, and 9-2 encode RNR2.

Rad53 activation. There was indeed a signal at the replication fork, just as I had imagined several years earlier.

By 1997, the field had grown tremendously, and many groups had made important contributions throughout, including the fission yeast geneticists Tony Carr, Paul Nurse, Nancy Walworth, and David Beach. They were approaching similar questions from the cell-cycle regulation perspective, which was much easier to study in *S. pombe* than in budding yeast due to its clear mitotic relationship to Cdc2 activation.

This field exploded again when we and others transitioned into mammals and found the overall outline of the pathway to be highly conserved but much more elaborate in humans. Elucidation of the human pathway allowed my post-docs Yolanda Sanchez, Shuhei Matsuoka, Dave Cortez, and Lee Zou to unravel the mechanism of how DNA damage and replication stress are sensed and transduced and revealed the true significance of this complex signal transduction pathway to cellular physiology. Important contributions were also made by the Abraham, Bartek, Carr, Chen, Cimprich, deLange, Dunphy, Gautier, Jackson, Kastan, Livingston, Lucas, Piwnica-Worms, Shiloh, and Yaffe labs and several others. These contributed to important connections being forged throughout the world to human physiology and disease, especially cancer, and drugs inhibiting the DDR kinase cascade are now being actively pursued as cancer therapies.

RecA has always played two roles in bacteria, recombination and control of the SOS DNA damage response. The ironic part of this long, strange trip is that it started out when I initially aimed to exploit the recombinational role of recA and instead discovered, quite accidentally, that eukaryotes have a completely different but analogous pathway to the SOS response, which is the other hat worn by recA.