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Deciphering the DNA Damage Response

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This year's Albert Lasker Basic Medical Research Award honors Evelyn Witkin and Stephen J. Elledge, two pioneers in elucidating the DNA damage response, whose contributions span more than 40 years.

Bacterial or human cells devote significant resources to maintain the integrity of their genomes. Among the most severe challenges are sources of DNA damage such as ultraviolet (UV) light and other chemical agents that alter DNA bases. Crosslinking of DNA bases creates blocks to normal DNA replication that must be removed or bypassed. Genome integrity is also assaulted by ionizing radiation and other clastogens that cause double-strand breaks that must be rejoined, either by nonhomologous end-joining or by homologous recombination. For repair to be successful, it must be completed before the cell divides; incompletely replicated chromosomes become trapped, while acentric broken chromosome segments get lost or mis-segregated. Failures of the DNA damage response are a common cause of cancer in humans. To assure that repair is accomplished before cell division, cells have evolved complex surveillance mechanisms to identify DNA damage, to impose checkpoints that arrest cell division until repair is completed, and to assure that an appropriate DNA repair response is launched. This year's Lasker prize honors two visionary scientists whose experiments and revolutionary insights set the stage for our present understanding of these critical processes.

Evelyn Witkin's contributions to the field of DNA repair began with her first publication, in 1946, identifying a mutant *E. coli* strain that is resistant to both UV light and X-rays. She noted that this strain does not show the usual delay in cell division or the filamentous elongation before cell division that is normally seen in wild-type strains. Subsequently, she noted many similarities between UV-induced filament formation and the UV-induced activation of the dormant phage λ. After moving from Cold Spring Harbor Laboratory to the State University of New York Downstate Medical Center, Witkin pursued these ideas while also providing key insights into the process of UV-induced mutagenesis, identifying a "dark repair" process in addition to the photo-reversal of pyrimidine dimers and suggesting the existence of error-prone DNA polymerases.

In her seminal paper (Witkin, 1967), Evelyn Witkin invoked the ideas recently proposed by Jacob and Monod of a repressor that inhibits both UV-induced phenomena and that itself would be inactivated by UV irradiation. By this time, it was already known that phage λ itself has a repressor that is inactivated by UV light. Witkin proposed that the presence of UV photodimers triggers the inactivation of a common repressor that would then allow the expression of genes, which in turn would promote both the arrest of cell division and the induction of the phage. Subsequently, Miro Radman, a postdoctoral fellow in Paris, circulated a letter in 1971 to leading scientists in the field, proposing the concept of a general "SOS response." Radman's ideas were not formally published and widely available until 1975, but Witkin quickly embraced this idea. She summarized the rapidly growing body of knowledge in a comprehensive review on "Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*" (Witkin, 1976).

Witkin and Elledge at Graham Walker's induction into the National Academy of Sciences in 2013. Photo credit: Gordon Walker.

Soon after Witkin advanced her hypothesis, several labs identified key elements of this regulation. Activation of phage λ and inhibition of cell division depend on the recombination protein RecA. Witkin herself, now at Rutgers University, showed that UV-induced mutagenesis depends on an apparently UV-induced error-prone form of DNA replication controlled by LexA (Witkin and George, 1973), which proved to be the repressor of many SOS genes. The induction of phage λ depends on the proteolytic cleavage of the λ repressor, a process involving RecA. Initially RecA was thought to be a protease as well as the central recombination protein, but subsequent work has shown that RecA, which forms a filament on single-stranded DNA that is created at stalled replication forks (i.e., after UV irradiation) or on the resected single-stranded ends of X-ray broken DNA ends, acts as an allosteric effector to promote the autocleavage of both LexA and the λ repressor.

In the more than 40 years since Witkin's hypothesis began to take molecular shape, the SOS response has emerged as a much more complex network of responses to DNA damage. Nearly 70 genes have been identified that either regulate LexA response or are regulated directly by LexA and are induced by

DNA damage. These genes have been identified by reporter gene fusions to various promoters and subsequently by gene expression profiling or by bioinformatics approaches using the consensus SOS repressor sequence. Among the processes controlled by SOS are cell division, nucleotide excision repair, DNA repair by recombination, and translesion DNA polymerases. Witkin's decisive role in understanding the bacterial DNA damage response has been recognized by many awards, most notably the National Medal of Science in 2004.

One particular LexA-regulated "bypass" DNA polymerase, encoded by the UmuDC operon, provides the link between Evelyn Witkin and Stephen Elledge. Witkin had generously supported the research of young researchers such as Graham Walker (in his case, Witkin carried a sensitive UV dose meter from Paris to Cambridge, MA, so that Walker could carry out his experiments). Elledge and Walker (Elledge and Walker, 1983) cloned the UmuC and UmuD open reading frames. UmuD is cleaved in a RecA-dependent fashion to a smaller, active form called UmuD′. Witkin herself showed that there is a third key RecA-mediated activity, independent of the operon's induction by LexA or the cleavage of UmuD.

In Walker's lab, Elledge first displayed his remarkable facility in creating novel genetic screens and molecular tools for the analysis of complex regulation, inventing phasmid vectors for the complementation of *E. coli* mutants. Indeed, throughout his career, Elledge has invented remarkable research tools and genetic screens, most recently a powerful new method for profiling human populations with a drop of blood, using a synthetic human virome to detect anti-viral antibodies.

After earning his Ph.D., Elledge moved to Ron Davis' lab at Stanford for his postdoctoral work, making the transition from prokaryotes to the emerging eukaryotic model system, budding yeast. Elledge's initial goal was to use phage-expression techniques to screen for yeast's RecA on the assumption that an anti-RecA antibody would find the gene. Instead, he accidentally pulled out the gene encoding the small subunit of ribonucleotide reductase, Rnr2 (Elledge and Davis, 1987). (It would take another 5 years before yeast's Rad51 protein would be shown to be similar to RecA.) Elledge demonstrated that *RNR2* mRNA is strongly induced by DNA damage and realized that this might be a tool through which he could interrogate the regulatory pathway responsible for RNR2's induction.

Elledge's characterization of *RNR2* regulation quickly established that it does not fit the paradigm of the *E. coli* response: its expression is still induced by UV in the absence of protein synthesis. A 42-bp regulatory region would confer damage inducibility to a reporter gene but does not share sequence similarity with the LexA binding site. Importantly, a protein kinase, Dun1, was found to be a key regulator of RNR gene expression after blocking DNA replication (Zhou and Elledge, 1993). Dun1 itself is activated for autophosphorylation in response to DNA damage. This finding established that DNA damage is indeed transduced by signal transduction, through a protein kinase, and was the first demonstration of what is now called the DNA damage response (DDR) pathway.

The concept of a DNA-damage-dependent cell-cycle delay in eukaryotes was intuited by Tobey (Tobey, 1975) studying drug and UV-sensitive mutants in fission yeast, and the concept of a DNA damage checkpoint was first articulated by Weinert and Hartwell (Weinert and Hartwell, 1988), who demonstrated that mutants in the *RAD9* gene, while proficient for repair of X-ray induced lesions, are X-ray sensitive because they fail to arrest prior to mitosis and thus give cells sufficient time to repair lesions before chromosome segregation. Several additional mutations, including mutants in *MEC1* (mitotic entry checkpoint) and *RAD53* (originally identified as an X-ray sensitive mutation), had been identified by Weinert et al. (Weinert et al., 1994), but the biochemical activities of these gene products were unknown. Elledge's lab contemporaneously identified S-phase arrest-defective (*sad*) mutants, including an allele of *RAD53* and an allele of *MEC1*, which later proved to be yeast's homolog of the ATR kinase (Allen et al., 1994). Allen et al. showed first that Rad53 is itself a protein kinase. Second, Rad53's kinase activity is required for the activation of Dun1, and Dun1 remains unphosphorylated in the *rad53* mutant. Third, Rad53 is involved in the control of three distinct checkpoints: a pause in G1 after DNA damage, the failure to induce RNR genes in response to a replication block, and a failure to delay mitosis in the face of unrepaired DNA damage. At this point, it became clear that the response to DNA damage in eukaryotes was not going to be similar to the regulation of the LexA repressor. The studies of eukaryotic regulation "implicate protein phosphorylation in the cellular response to DNA damage and replication blocks" (Allen et al., 1994).

Indeed, the notion of a cascade of phosphorylation signals, and the counteracting dephosphorylations that must help terminate the checkpoint response, is the primary theme of eukaryotic cell-cycle regulation in response to DNA damage. On top of this scheme is another feature especially of higher eukaryotes, the self-destruction of cells with DNA damage by apoptosis, mediated principally through the p53 gene.

When Elledge set up his own lab at Baylor College of Medicine in 1989, he soon began to work on mammalian cells as well as yeast, embracing questions of the role of cyclin-dependent kinases in cell-cycle control. The yeast work quickly informed recent discoveries in the DNA damage response in mammalian cells. Soon thereafter, Elledge's lab showed that Mec1 and another kinase Tel1 phosphorylate and regulate Rad53 (Sanchez et al., 1996) and later that Tel1 phosphorylates yet another protein kinase Chk1. In collaboration with Errol Friedberg's lab, they established that Mec1 is a homolog of the mammalian ATM gene, whose mutant cells show many defects in response to DNA damage. In fact, Mec1 later proved to be the homolog of another PI3K-like kinase, ATR, while Tel1 is the closer homolog to the ATM-related ATR gene. As the work progressed, it became evident that the PI3K-like kinases Mec1 and Tel1 sat atop a protein kinase cascade whose immediate downstream targets include Rad53 and Chk1 (Matsuoka et al., 1998). In a flurry of other papers, the Elledge lab demonstrated the parallels between the Mec1/Tel1 regulation of Rad53 and Chk1 with the mammalian ATM/ATR control of Chk1 and Rad53's mammalian homolog, Chk2, respectively (Liu et al., 2000; Matsuoka et al., 1998;

Sanchez et al., 1999). The link between ATR and cell-cycle control became stronger when Elledge's lab, in collaboration with Helen Piwnica-Worms, demonstrated that Chk1 phosphorylates the key Cdk2 regulator, Cdc25 phosphatase (Sanchez et al., 1997), and later, with Elledge's long-time collaborator Wade Harper, showed that this phosphorylation triggers the degradation of Cdc25 and imposes cell-cycle arrest (Jin et al., 2003). Of course, while celebrating Stephen Elledge, it is important to remember that there were many important contributions by other labs as these ideas blossomed. To cite only a few, Yosef Shiloh's group first cloned and sequenced ATM; Michael Kastan demonstrated a key control by ATM of p53; Antony Carr and Karlene Cimprich characterized ATR; and Paul Russell and Paul Nurse outlined the phosphoregulation of the Cdk2 kinase.

The outlines of the full DDR are still being inked in. After moving to Harvard Medical School, Elledge's lab continued to enlarge the domain of DDR responses. A phosphoproteomic screen identified more than 700 in vivo substrates of ATM and ATR, implicating ATM/ATR control of processes as diverse as kinetochore function, regulation of the cytoskeleton, control of ubiquitylation, and protein degradation by both the proteasome and autophagy. Most recently, his lab has carried out an analysis of proteins recruited to DNA-damaged chromatin and a quantitative "atlas" of ubiquitylation and acetylation associated with the DDR. In addition, Elledge's masterful reviews have guided the field. Stephen Elledge's insights into the eukaryotic DNA damage response have led to many awards, most recently the Rosenstiel Award and the Canada Gairdner Award in 2013.

Both bacteria and eukaryotes exhibit a complex DDR, but this is one of those fascinating instances in which there has been little evolutionary conservation of the mechanisms to achieve a common goal. Our present understanding of these processes owes much to Evelyn Witkin and Stephen Elledge, the two winners of this year's Albert Lasker Award in Basic Medical Research.

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