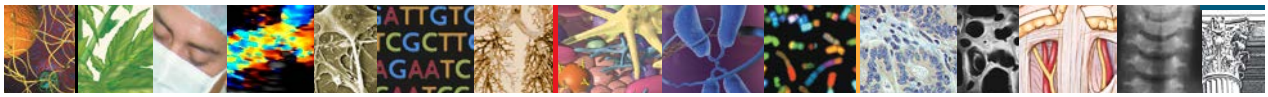

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Perspective

Accolades for the DNA Damage Response

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The importance to human health of the DNA damage response is obvious to anyone in the medical field. Syndromes arising from mutations in DNA damage–response genes — such as ataxia telangiectasia, Bloom syndrome, Fanconi's anemia, and xeroderma pigmentosum — are well established. The predisposition to cancer conferred by mutations in DNA-repair genes — such as breast and ovarian cancer from mutations in homologous recombination genes and colon cancer from mutations in mismatch-repair genes — is also well known (see table). Moreover, although DNA-damaging agents cause human disease, they are also widely used as cancer therapeutics. It's therefore not surprising that the 2015 Albert Lasker Award for Basic Medical Research is being given to two scientists who have elucidated the DNA damage response: Evelyn M. Witkin for her work in bacteria and Stephen J. Elledge for his work in eukaryotes.

Witkin's scientific career began in the 1940s, just as the field of bacterial genetics was developing. Research had already established that x-rays and ultraviolet (UV) radiation induce mutations in drosophila — findings with clear medical ramifications. But *Escherichia coli* provided a more tractable system, facilitating understanding of this phenomenon. For her doctoral thesis, Witkin isolated the first *E. coli* mutants that were resistant to UV radiation; she found that of approximately 1000 bacteria, only 4 survived a high dose of UV radiation. Resistance to UV radiation was heritable, and resistant bacteria showed similar amounts of DNA damage and similar rates of excision of the damaged DNA as the UV-sensitive parental cells. Witkin went on to show that these bacteria were also resistant to x-rays.

These simple, elegant experiments led to a finding with profound consequences. Witkin observed that when irradiated, the *E. coli* formed snakelike filaments up to hundreds of times the length of the cell, owing to a failure of cell division, and eventually most of the cells died. The rare resistant mutants, however, continued dividing rather than forming filaments. Over the years, similarities between filamentation and another radiation-induced phenomenon (excision of a virus integrated in a bacterial chromosome, or prophage induction) led Witkin to hypothesize that bacteria normally express a repressor that keeps filamentation in check but that DNA damage leads to its

DNA Damage Response Genes Mutated in Human Diseases.

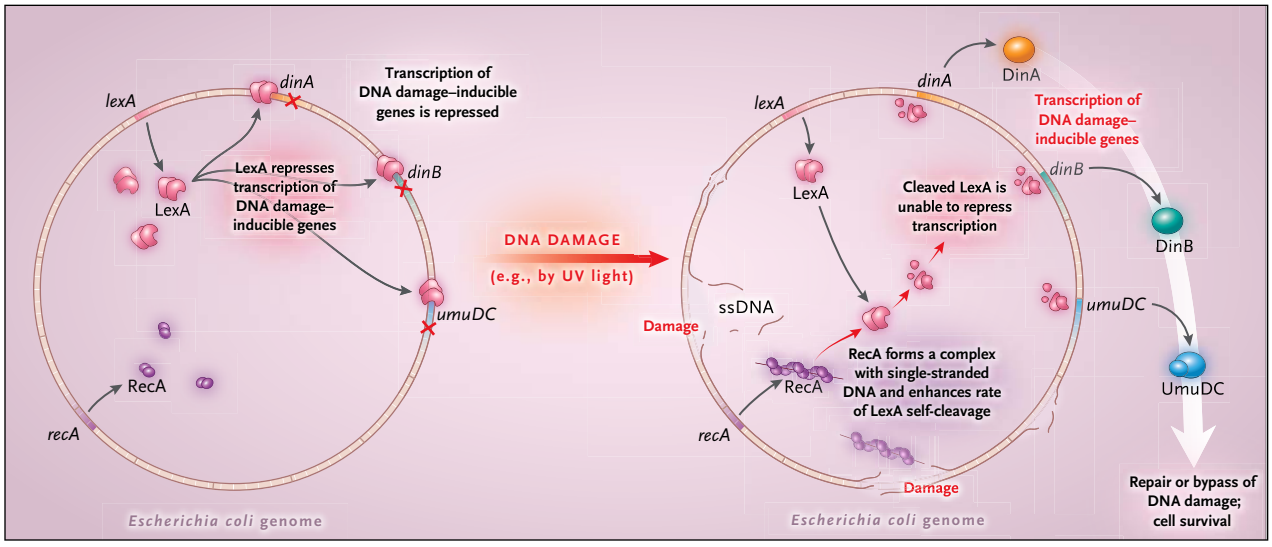
Type of DNA Repair or Signaling	Genes Mutated	Phenotypes
Defective DNA repair pathway		
Homologous recombination	*BRCA1*, *BRCA2*, *PALB2*, *RAD51C*, *RAD51D*	Breast, ovarian, and other cancers; developmental abnormalities
Interstrand crosslink repair	*FANCs*	Fanconi's anemia: developmental abnormalities, bone marrow failure, cancer
Mismatch repair	*MLH1*, *MSH2*, *MSH6*, *PMS2*	Colon and other cancers
Nonhomologous end-joining	*LIG4*, *NHEJ1*, *DCLRE1C*	Immunodeficiency, growth defects, microcephaly (*LIG4*, *NHEJ1*)
Nucleotide excision repair	*XPA*, *XPB*, *XPC*, *XPD*, *XPE*, *XPF*	Xeroderma pigmentosum: photosensitivity, skin cancer
Single-strand break repair	*APTX*, *TDP1*	Ataxia, neurodegeneration, hypercholesterolemia
Telomere maintenance	*DKC1*, *RTEL1*, *TERC*, *TERT*	Bone marrow failure, abnormal skin pigmentation, nail dystrophy
Transcription-coupled repair	*CSA*, *CSB*, *UVSSA*	Cockayne's syndrome: developmental and neurologic abnormalities, photosensitivity
Translesion synthesis	*POLH*	Photosensitivity, skin cancer
Helicases	*BLM*, *WRN*, *RECQL4*	Growth defects and cancer, aging (*WRN*, *RECQL4*)
Defective DNA damage signaling or repair		
Damage signaling	*TP53*, *CHEK2*	Breast cancer, sarcoma, other cancers
Double-strand breaks	*ATM*, *MRE11*, *NBS1*, *RAD50*	Ataxia (*ATM*, *MRE11*), immunodeficiency (*ATM*, *MRE11*, *NBS1*), cancer (*ATM*, *NBS1*), growth defects and microcephaly (*NBS1*, *RAD50*)
Replication stress	*ATR*	Seckel's syndrome: microcephaly

inactivation (analogous to the inactivation of the viral repressor that results in prophage induction).[1] She also proposed the existence of error-prone repair mechanisms that cause mutations but allow survival of *E. coli* treated with UV radiation, ultimately verifying Miroslav Radman's proposal that this error-prone repair, like filamentation, was induced by DNA-damaging agents[2] — a phenomenon that Radman dubbed "SOS" mutagenesis. The existence of a number of inducible functions, coordinately expressed in response to agents that damage DNA and impair DNA replication, was thus postulated, and clear evidence of a set of DNA damage–inducible (din) genes was provided by Kenyon and Walker a few years later.

The coordinated response to DNA damage in bacteria — the SOS response — is elegant (see figure). The repressor is the LexA protein; in the presence of the RecA protein bound to a single strand of DNA, LexA self-cleaves, inducing genes that ultimately promote the survival of the cell (or its viruses). Curiously, the *E. coli* strain Witkin used that filaments in response to DNA damage and then dies is defective in a protein that allows cells to resume cell division after DNA repair has been completed — one of those serendipitous conditions that facilitate scientific discovery.

Stephen Elledge, the other Lasker honoree, began his graduate work when research into the SOS response was running full tilt. As a student, he cloned a locus critical to error-prone repair of DNA (i.e., for UV-induced mutagenesis, *umuDC*). Subsequent studies by others showed that this locus encodes a DNA polymerase that can replicate across DNA lesions, providing a mechanism for DNA damage–induced mutagenesis.

Given that the agents that induce the SOS response in bacteria were known to be carcinogenic in mammals, it seemed likely that eukaryotic cells would also mount a coordinated response to DNA damage. When, as a postdoctoral fellow, Elledge was searching for the eukaryotic homologue of RecA, he stumbled upon DNA damage–induced regulation of ribonucleotide reductase, the enzyme required for the production of nucleotides for DNA synthesis. He later capitalized on this fortuitous discovery to elucidate the DNA damage response in yeast, which halts cell division until DNA damage is repaired. He uncovered what he called DNA damage–uninducible (dun) genes, which when mutated were unable to induce the enzyme.[3] One



The SOS Response in *Escherichia coli*.

LexA represses expression of a number of genes by binding to their promoters. When DNA damage occurs, RecA-single-strand DNA complexes form and act as a coprotease for the self-cleavage activity of LexA. As a result, several DNA damage–inducible (din) genes are expressed, including DNA polymerases, as shown. More than 40 genes are induced in the SOS response that promote repair of DNA damage and survival.

of these encoded a nuclear protein kinase which itself is phosphorylated when DNA damage occurs. Further work identified an upstream kinase, which controls cell-cycle progression and DNA damage–induced transcription.

Elledge's work in yeast thus established that the DNA damage response in eukaryotes is a signal transduction pathway involving a kinase cascade. He was able to demonstrate the conservation of the response by identifying mammalian homologues of yeast kinases that prevent cell-cycle progression in response to DNA damage: CHK1 and CHK2, the latter of which increases risk for breast cancer when mutated. There are now many genes known to encode DNA damage–response proteins and, when mutated, to cause disease — breast, ovarian, pancreatic, prostate, and colorectal cancers, in particular.

CHK1 and CHK2 are phosphorylated by ATR and ATM, which are the earliest kinases to respond to DNA damage. ATM primarily responds to breaks in both strands of the DNA helix (double-strand breaks), whereas ATR responds to single-stranded DNA that, for example, has been generated during replication stress. The response to single-stranded DNA as a damage signal is thus conserved from bacteria to humans.[4]

Analysis of ATM and ATR kinase substrates has revealed that the scale of the DNA damage response is enormous: more than 700 human proteins are phosphorylated at sites recognized by these kinases.[5] Some of these proteins sense DNA damage or regulate cell division and DNA repair, and others are involved in transcription, splicing, and cellular metabolism as part of interconnected networks. Many of the phosphorylated substrates are tumor suppressors or are mutated in genetic syndromes. Phosphorylation is often observed on multiple components of a single pathway, allowing amplification of the DNA-damage signal. Given the complexity of the DNA damage response, it is not surprising that disturbances to it have such a profound effect on human health.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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