# Specific Aims

Failure of cells to respond to DNA damage is a key mechanism of toxicity to environmental agents and a primary step in the onset of cancer. To obtain a global perspective on these events, we are using systematic experimental and computational approaches to map the molecular networks induced by DNA damage. Since DNA damage response pathways are conserved across the eukaryotic kingdom, our studies are focused on the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*—impacting our basic knowledge of genotoxicity while keeping within species for which systems data are easily obtained.

In our past five years of funding (NIH grant R01-ES14811), significant progress was made in mapping the architecture of transcriptional networks responding to DNA damage and in perturbing these networks to reveal their key state transitions. In the present proposal, we turn our attention to the global networks of damage-induced kinases and other signaling proteins which lead to the transcriptional responses we have observed.

**Specific Aim 1. Develop dynamic genetic interaction maps of signaling in response to DNA damage.**

Synthetic Genetic Arrays (SGA) and Epistatic MiniArray Profiles (E-MAP) allow measurement of synthetic lethal and epistatic interactions in a semi-quantitative high-throughput format. All previous E-MAPs have been static; therefore, we will create a series of *dynamic* E-MAPs induced specifically by different DNA damaging agents. All possible double deletions will be constructed among a set of ~500 core signaling genes including all kinases, phosphatases, transcription factors, genes required for resistance to DNA damage, and yeast orthologs of human oncogenes. Growth rates will be measured across a panel of ten DNA damaging agents in both budding and fission yeasts. Our preliminary data show that dynamic genetic interactions are far preferable to static interactions for identifying genes involved in DNA repair or DNA damage responses.

**Specific Aim 2. Connect transcription factors to upstream kinases through sets of co-regulated genes.**

In our prior funding period, we performed a series of genome-wide expression and chromatin IP experiments to link yeast transcription factors (TFs) to genes they directly regulate during the DNA damage response. Here, we perform a series of parallel experiments for kinases, in which kinase mutants are profiled to identify genes whose expression is both damage- and kinase-dependent. Kinase/TF pairs that regulate similar damage-responsive genes will be investigated for functional association, by seeking specific phosphorylation sites on the TF and exploring the functional consequences of TF phosphorylation on DNA repair.

**Specific Aim 3. Assemble conserved signaling modules categorized by phenotype or repair function.**

Using the Cytoscape platform, we will develop novel statistics and algorithms to analyze the genetic and kinase-TF interaction networks generated in the previous two aims. These methods will be used to assemble signaling modules that are conserved across species and dynamically activated by general DNA damage stress or by specific agents. Dynamic interactions and modules will be categorized with respect to their specific DNA repair defects, using assays for abnormal cell cycle progression (i.e., checkpoints), elevated formation of Rad52 and other foci (markers for repair and DNA damage responses), and accumulation of mutations or genome rearrangements. Network modules in various phases of development and categorization will be made publicly available through the CellCircuits database.

**Deliverables.** Successful execution of these aims will lead to a number of concrete deliverables:

1. A web-accessible database of DNA damage genetic interactions, integrated across ten conditions and two model species.
2. An accompanying set of module maps, built on the genetic interaction data and navigable within Cytoscape, for understanding the higher-order structure of the interactions and facilitating their comparison across species and damaging conditions.
3. Linkages between yeast genetic interactors and their human orthologs, with special attention to interactions likely to occur in humans.

A comprehensive set of DNA-damage-induced genetic networks and signaling modules will provide an important tool for use in ***understanding genetic polymorphisms that predispose an individual to environmental DNA damage.*** In addition, such networks are a predictive tool for synthetic lethality and epistasis in human cancer.