Summary
Research was focused on how a single double-strand break – a model of low-dose ionizing radiation-induced DNA damage – could be studied in a simple model system, budding yeast. Breaks were induced in several different ways. We used the site-specific HO endonuclease to create a single DSB in all cells of the population so that its fate could be extensively analyzed genetically and molecularly. We also used two heterologous systems, the plant DS element and the Rag1/Rag2 proteins, to generate different types of DSBs, these containing hairpin ends that needed to be cleaved open before end-joining could take place. All three approaches yielded important new findings. We also extended our analysis of the Mre11 protein that plays key roles in both NHEJ and in homologous recombination. Finally we analyzed the poorly understood recombination events that were independent of the key recombination protein, Rad52. This line of inquiry was strongly motivated by the fact that vertebrate cells do not rely strongly on Rad52 for homologous recombination, so that some clues about alternative mechanisms could be gained by understanding how Rad52-independent recombination occurred. We found that the Mre11 complex was the most important element in Rad52-independent recombination.

A. Identification of new genes involved in NHEJ

(Valencia et al. 2001) A major finding was a new factor involved in nonhomologous end-joining (NHEJ). This paper identified NEJ1 (later shown to have the human homolog Cernunnos/XLF) as a key factor in NHEJ, working in convert with DNA ligase 4 and Xrcc4 (Lif1).

B. A new form of NHEJ: microhomology-mediated end-joining (MMEJ)

(Ma et al. 2004) A second major discovery, initiated in my lab, was that there was a distinct, Ku-indpendent pathway of NHEJ, which involved more "trimming" of the ends of a double-strand break (DSB) and the use of microhomology at the junctions. This was the first report of such a pathway, which is now recognized as a major alternative Ku-independent end-joining pathway in mammals.

C. Processing and end-joining of hairpin ends in budding yeast.

(Yu et al. 2004) It was not known if yeast could open hairpin ends and join them by NHEJ. Exploiting a plant Ds transposable element that could be activated in budding yeast, we showed that excision of the element left hairpin ends that could be opened and ligated to the opposite opened end. A key discovery was that the junctions frequently had P (palindromic) nucleotides that would be expected if the hairpin ends were cleaved not at the tip but on the hairpin stem and then opened by a helicase. This type of event is a hallmark of events in the human immune system; thus we had developed a yeast model in which to study this process in detail. We showed that both Mre11 endonuclease activity and the Sae2 protein (CtIP in humans) are required for the cleavage and that a homolog of human Artemis protein (Pso2) played a key role in excision.

(Clathworth et al. 2003) An even more direct use of yeast to study V(D)J recombination events came from our collaboration with Oettinger's lab to show that we could generate cleavage and NHEJ of authentic V and J region sequences by expressing the Rag1 and Rag2 proteins in budding yeast. This was an important step in developing an in vivo system where extensive genetic analysis could be carried out.

D. Analysis of Mre11 mutations

(Lee et al., 2002) The Mre11 complex (with Rad50 and Xrs2) plays central roles in NHEJ and in homologous recombination. We analyzed a series of mutations of evolutionarily conserved histidines in the catalytic site. We found that several of these mutations disrupted the entire MRX complex and conferred severe phenotypes, however mutation of H125, shown by others to eliminate endo- and exonuclease activity, had very weak phenotypes and did not affect checkpoint responses, 5' to 3' resection or telomere length. In addition we showed that two mutations exhibited intergenic complementation, suggesting that Mre11 functioned as a multimer.

E. Analysis of *RAD52*-independent recombination.

(Coïc et al. 2007). Haber and Hearn (1985) described *RAD52*-independent recombination in a diploid heteroallelic for mutations in the *his4* gene. A notable feature of RAD52-independent events is that nearly all of them were associated with a crossover on the *HIS4*-containing recombinant and the concomitant loss of the recombining partner. In the more recent work we found that UV irradiating *rad52* cells results in an increase in overall recombination frequency, comparable to increases induced in wild-type (WT) cells, and surprisingly results in a pattern of recombination products quite similar to *RAD52* cells: gene conversion without exchange is favored, and the number of $2n - 1$ events is markedly reduced. Both spontaneous and UV-induced *RAD52*-independent recombination depends strongly on Rad50, whereas *rad50*Δ has no effect in cells restored to *RAD52*. The high level of noncrossover gene conversion outcomes in UV-induced *rad52* cells depends on Rad51, but not on Rad59. Those outcomes also rely on the UV-inducible kinase Dun1 and Dun1's target, the repressor Crt1, whereas gene conversion events arising spontaneously depend on Rad59 and Crt1. Thus, there are at least two Rad52-independent recombination pathways in budding yeast.