Fluorescence Detection of Recombination in Mice

Although homologous recombination can provide an efficient means for repairing and tolerating DNA damage, mitotic recombination events between misaligned sequences can lead to loss of genetic information (e.g., deletions and translocations). In addition, recombination between homologous chromosomes can result in cancer-promoting loss of heterozygosity (LOH) events. Since most people inherit two functional copies of each tumor suppressor gene, one allele from each parent, a mutation in one allele is generally inconsequential to a cell. However, in a cell that carries one wild-type and one mutant allele of a tumor suppressor gene, loss of the remaining wild-type copy (LOH) can increase the likelihood of a cell becoming a tumor. Mitotic recombination has been estimated to be the underlying cause of LOH 25-50% of the time.

Given that mitotic recombination may be essential to the process of tumorigenesis, it is critical that we learn more about the genetic factors that modulate cellular susceptibility to DNA damage-induced homologous recombination.

Our goal is to develop tools that will allow us to better understand the underlying molecular basis for DNA damage-induced recombination and to determine how repair of DNA lesions modulates susceptibility of mammalian cells and mice to homologous recombination. In order to study DNA damage-induced homologous recombination, we have developed a system that exploits fluorescence to rapidly quantify mitotic recombination events in mammalian cells. Using recombinant DNA technology, we have constructed a substrate that contains two non-functional, partially-truncated copies of the enhanced yellow fluorescent protein (EYFP) expression cassette. Mitotic recombination between these truncated EYFP sequences can restore expression of EYFP. Thus, the frequency of homologous recombination can be quantified by determining the proportion of cells expressing EYFP using fluorescence activated cell sorting (FACS).

Employing both transgenic (microinjection) and knock-out technologies, we have engineered mice that carry this fluorescent recombination substrate in their genomes. Primary ear fibroblasts derived from these adult mice undergo spontaneous recombination at a median frequency of 6/10^6. Ventral skin cells isolated from these mice (and not expanded in culture) show a median recombination frequency of 1.3/10^6, which is still readily detectable by FACS. When challenged with the potent recombinogen and cancer chemotherapeutic Mitomycin C, primary ear cells exhibit a clear dose response, indicating that these mice can be used to detect DNA damage-induced recombination events.

A scanning two-photon microscope system is currently being optimized for the purpose of rapidly quantifying recombination events (fluorescent cells) in mouse tissues and to determine the particular cell type(s) that have undergone recombination. We anticipate that this combination of genetic and mechanico-optical engineering will make it possible for the first time to study mitotic homologous recombination in situ in multiple tissues of a mouse. Ultimately, these mice can be crossed with mice carrying a variety of engineered genetic defects to determine how specific genetic traits affect cellular susceptibility to damage-induced mitotic recombination in vivo.