The Symposium 1 (Preserving Chromosome Continuity When DNA Replication Encounters Damage), generously sponsored by the Fanconi Anemia (FA) Research Fund, focused on the mechanisms by which DNA replication maintains chromosome integrity when various kinds of damage (e.g. oxidative base damage, DNA crosslinks), or atypical DNA sequences (fragile sites) are present. Chromosomal rearrangements are likely a driving force in carcinogenesis. The FA pathway is globally important in preventing persistent double-strand breaks and chromosomal rearrangements.

Tom Glover (University of Michigan) explained how fragile sites are AT-rich regions, often present in very large genes, that are “hot spots” for chromosomal breakage, deletion, duplication, and sister chromatid exchange when DNA replication is stressed with inhibitors such as aphidicolin. Although ~75 fragile sites are identified, 35-50% of gaps and breaks occur at just five sites. Many DNA damage signaling and repair proteins, such as ATR, Chk1, BRCA1, FANCD2, Rad51 and DNA-PK, promote fragile site stability in terms of chromosome integrity as seen at metaphase.

Howard Lieberman (Columbia University) showed that mouse cells lacking RAD9 are sensitive to a wide range of DNA damaging agents as well as DNA replication inhibitors. *Rad9* mutant cells also show elevated spontaneous chromosomal instability measured by aberrations, micronuclei, *hprt* mutations, and sister chromatid exchange. Curiously, Rad9 can bind androgen receptor and prevent it from regulating downstream target genes that are important for prostate function. RAD9 protein is overexpressed in prostate cancer cells and tissue, and siRNA knockdown decreases the tumorigenic potential of prostate cancer cells.

Benjamin Chen (University of Texas SW Medical Center) discussed kinase-mediated signaling in response to UV-C replication stress. Phosphorylation of DNA-PK in the T2609 cluster by ATR is required for repair of replication-associated breaks and cell survival. Both ATR and ATM are activated in S phase by UV-C and contribute to the phosphorylation of H2AX, which presumably marks regions of broken DNA replication forks. This study emphasizes that nonhomologous end joining (NHEJ) is important for coping with replication-associated damage.

Laura Niedernhofer (University of Pittsburgh) addressed the mechanisms by which DNA replication copes with interstrand crosslinks. In humans and mice mutations in the ERCC1-XPF endonuclease produce much more severe phenotypes (growth retardation, accelerated aging) than mutations in other nucleotide excision repair proteins. *Ercc1* or *xpf* mutant cells are unusually sensitive to killing by crosslinking agents. Moreover, FANCD2 monoubiquitination, a key step in the FA pathway, occurs in these mutant cells, but FANCD2-Ub fails to associate with chromatin and FANCD2 focus formation is impaired.

Paul Andreassen (Cincinnati Children's Hospital) discussed how the integrity of the FA pathway depends on the ATR kinase (which has hypomorphic mutations in ATR-Seckel syndrome) as well as RPA, single-strand binding protein. FANCD2 co-localizes with ATR and BRCA1 in response to DNA damage, and knockdown of ATR produces radial chromosomal aberrations that mimic those seen in FA cells. Interestingly, the FANCJ/BRIP1/BACH1 helicase (first identified as a BRCA1 interactor) is required for efficient localization of FANCD2 into nuclear foci and association with chromatin, but not for FANCD2 monoubiquitination.

Larry Thompson (LLNL) discussed the use of CHO-cell knockout mutants for FANCG and RAD51D to gain insights into the role of the FA pathway in mutagenesis. *Fancg* mutant cells have reduced rates of both spontaneous and mutagen-induced (X-rays, ENU, MMS) *hprt* mutations (ascribed to mutant lethality), and the spectrum of spontaneous mutants is shifted towards more deletions. In contrast, *rad51d* HR (homologous recombination) mutant cells have a greatly elevated rate of spontaneous *hprt* mutations, which are almost all deletions. The results argue that the FA pathway promotes NHEJ as well as translesion synthesis and HR.