A. Specific Aims

Antigen receptor genes are assembled from their component gene segments by a series of site-specific DNA recombination reactions known as V(D)J recombination. Each rearranging gene segment is flanked by a conserved recombination signal sequence (RSS) which is recognized by a common recombinase which includes the RAG1 and RAG2 proteins. Seven complex genetic loci in B and T cells undergo V(D)J recombination in a highly regulated fashion. The goal of this research project is to understand the mechanisms whereby a common V(D)J recombinase which recognizes a conserved RSS can result in an exquisitely regulated pattern of gene-segment rearrangement.

A.1 To determine the role of nucleosomes in the regulation of Igκ locus rearrangement. Our preliminary experiments showed that the V(D)J recombinase could not recognize RSS targets if they were packaged into a nucleosome structure. We propose experiments to extend these observations by 1) determining what fraction of the Jκ gene segments are in a nucleosomal structure in cells undergoing V-to-Jκ gene rearrangement as compared with non-lymphoid cells; 2) determining if nucleosomes are phased across the Jκ cluster in κ rearranging cells; 3) determining whether nucleosome remodeling complexes can alter the accessibility of the Jκ cluster in native or reconstituted chromatin.

A.2 To determine the mechanism by which the TCRβ enhancer (Eβ) regulates D-to-Jβ recombination during T cell development. During an analysis of V(D)J recombination in $E\beta^{-/-}$ mice we made the surprising observation that this enhancer plays an important role in both recombinational accessibility and coding joint formation. We plan to test the possibility that this may be due to Eβ's ability to recruit Ku and DNA-PK to the rearrangement complex or that it is involved in holding broken coding ends together through interaction with the nuclear matrix. In addition, we plan to use a ~130kb BAC clone of the TCRβ locus to construct a transgenic reporter construct for use in experiments aimed at delineating with precision the DNA sequences involved in accessibility and joining.

A.3 To determine whether the V(D)J recombinase catalyzes the transposition of DNA signal sequences during normal or pathological lymphocyte development. Recent biochemical studies have shown that the RAG proteins can catalyze transposition of RSS sequences. In vitro, this reaction is characterized by the efficient insertion of an excised fragment with RSS ends into a target DNA molecule resulting in a three to five nucleotide target site duplication. We have developed an inverse PCR assay capable of detecting signal sequence transposition in genomic DNA. In this aim we propose experiments to measure whether, and how frequently, the V(D)J recombinase mediates signal sequence transposition in vivo and whether this frequency is affected by several genetic disorders which are associated with lymphoid malignancy.

A.4 To determine the function of the nonessential domain of RAG2. Extensive mutagenesis studies performed by other labs has defined core domains of the RAG proteins. Core RAG1 and core RAG2 are capable of catalyzing efficient RSS cleavage in vitro and complete gene rearrangement in vivo (in transfected cells). The nonessential domain of RAG2, however, is highly conserved (>60% amino acid similarity) amongst all species which use V(D)J recombination to generate antigen receptor diversity. In order to uncover potential regulatory functions of the nonessential domain, we plan to target the endogenous RAG2 locus in ES cells to generate a mutant allele capable of expressing only this core domain protein. We will then analyze lymphocyte development in mice expressing only core RAG2 and in heterozygotes. These analyses might uncover previously unsuspected regulatory roles for the nonessential domain of this highly conserved protein.

A.5 To biochemically purify and molecularly clone accessory factors required for the recognition and cleavage of RSSs in complex DNA templates. Preliminary experiments revealed that factors in addition to RAG1, RAG2 and HMG1 are required for efficient RSS cleavage in complex templates in vitro. Such factors might bind DNA in a sequence-specific fashion (i.e. such as a transcription factor) and then recruit the recombinase by protein-protein interaction, or alternatively might form a complex with the RAGs and alter their ratio of specific to non-specific DNA binding activity. This aim involves an attempt to purify factors which improve the efficiency of the recognition and cleavage steps of V(D)J recombination on genomic DNA templates and to determine whether coding joint formation on a cosmid template requires transcriptional enhancer or other non-RSS sequences.