A. Specific Aims

Normal B cell development depends on the proper assembly of immunoglobulin heavy- and light-chain genes from their component gene segments by the V(D)J recombinase. The process is regulated such that an individual mature B cell expresses a single antigen receptor specificity, a phenomenon known as allelic exclusion. Previous research has uncovered roles for transcriptional regulatory DNA sequences, chromatin structure, and the assembled Ig chains themselves in the regulation of V(D)J recombination. In this competing renewal of a project most-recently reviewed in 1997, we propose a series of experiments aimed at understanding how the V(D)J recombinase is regulated during B cell development.

A.1 To identify the cis-acting DNA sequences and transcription factors involved in the developmentally regulated transcription of the RAG-1 and RAG-2 genes. RAG1 and RAG2 are the only two known essential lymphoid-specific components of the V(D)J recombinase. They are coordinately expressed only in B and T lymphocytes, where transcript levels vary at different stages of development. Inactivation of RAG gene expression is presumably essential for allelic exclusion and continued RAG gene expression is critical for receptor editing. We have identified key DNA sequences and transcription factors necessary for activity of the murine RAG-2 promoter. In addition, we have discovered a RAG locus transcriptional enhancer. We plan to determine the role of this novel enhancer in the developmental regulation of RAG gene expression, and identify additional DNA elements and transcription factors required for regulation of transcription of these important genes.

A.2 To test the hypothesis that infrequent, stochastic activation of the unrearranged κ locus in pre-B cells contributes to the allelic exclusion of Vκ-to-Jκ gene rearrangement. It has been presumed that light-chain allelic exclusion is due to inactivation of the V(D)J recombinase immediately following surface expression of functional IgM. We and others have found, however, that recombinase activity persists in $sIgM^+$ immature B cells, suggesting that additional mechanisms must exist. We plan to test the hypothesis that only a small fraction of Ig κ alleles in a population of pre-B cells is accessible to the recombinase making it unlikely that a given pre-B cell would contain more than one accessible allele. These experiments rely on a novel mouse model system containing a GFP cDNA targeted to the the Jκ1 gene-segment as well as various in vitro assays of chromatin accessibility. If true, this hypothesis would represent a newly appreciated mechanism which contributes to allelic exclusion.

A.3 To determine the molecular mechanism of κ locus activation in BOSC 23 cells transfected with an E47 expression vector. Murre and colleagues recently reported that overexpression of a cDNA encoding the transcription factor E47 results in germline transcription and accessibility of the chromosomal Igμ and Igκ loci to the V(D)J recombinase in the human embryonic kidney cell line, BOSC 23. This finding suggests that E47 can act upon an inactive κ locus and introduce chromatin structural changes which result in accessibility. We plan to establish an inducible gene expression system in BOSC 23 cells and use this system to determine the mechanism of E47-induced κ locus activation as a model for how this locus is first activated during lymphoid development.

A.4 To test three hypotheses regarding the regulation of V_H-to-DJ_H rearrangement during lymphoid development. Regulation of V_H-to-DJ_H rearrangement is responsible in part for the failure of T lymphocytes to express heavy-chain protein and for the allelic exclusion of heavy-chain gene expression in developing B cells. We propose experiments to a) scan the ~90 kb interval between V_H81X and DFL16.1 for DNA sequences which may be involved in regulating V_H-to-DJ_H rearrangement, b) determine whether a Pax-5 (BSAP)-expressing transgene can activate V_H-to-DJ_H rearrangement in developing T cells and c) use a FISH assay to determine whether either of the two heavy-chain alleles in pro-B or pre-B cells is associated with heterochromatic regions within the nucleus.