The Mechanistic Basis for Recombinase Upregulation in CD4+CD8+ Thymocytes

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Lymphoid-specific recombinases RAG1 and RAG2 act synergistically to generate a diverse T-cell receptor (TCR) repertoire by catalyzing V(D)J Recombination, a site-specific recombination process that occurs during two stages of thymocyte development and is necessary for the survival and maturation of early T-cells. During the CD4+CD8+ double positive (DP) stage, RAG expression was shown to depend on the enhancer function of an Anti-silencer Element (ASE) distal to the Rag promoters, which tethers to the promoters to dominate the suppressive effects of an intergenic silencer found between the Rag genes. However, the transcription factors responsible for the Rag promoter-ASE interaction are unknown. Through assaying for the luciferase activity of Rag1 or Rag2 promoter-driven reporter plasmid constructs transfected into the thymocyte cell line VL3-3M2, this project determined the functional significance of conserved binding sites for transcription factors GATA3, RUNX1, IKZF1 and E2A on the Rag promoters and ASE and proposes a more complete mechanistic basis for RAG upregulation during the DP stage of thymocyte development: data implicate that GATA3, RUNX1, and E2A proteins bound to the 140bp region of the ASE are essential for Rag promoter-ASE interactions, and that E2A proteins located at the ASE and Rag promoters have critical regulatory roles that help to mediate these interactions, an insight which has important implications for T-cell development and the existence of a functional immune system.

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