ABSTRACT

THE ROLE OF SWITCH REGION DNA AND PROTEIN FACTORS IN IMMUNOGLOBULIN CLASS SWITCH RECOMBINATION

By

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Immunoglobulin class switch recombination (CSR) is a recombination event that changes the heavy chain constant region of an antibody while maintaining the same variable region. The process of CSR alters the effector function of the antibody without altering antigen specificity, which ensures efficient pathogen clearance. Defects in CSR can result in hyper-IgM syndrome, autoimmune diseases and chromosomal translocations that result in lymphoid malignancies. Thus, understanding the mechanism of CSR will give us better insight into these diseases. The work presented in this dissertation aims to improve our understanding of the molecular mechanism of CSR.

CSR occurs via a cut and paste mechanism that involves the generation and repair of DNA double stranded breaks (DSBs) within intronic regions known as switch regions. The first part of this thesis aims to identify sequence features within switch regions that are important for CSR. We show that WGCW (W=A/T) motifs, which occur at a high frequency in switch regions, are important determinants of CSR efficiency.

The next study focuses on elucidating the mechanism of DSB formation during CSR. It is known that uracils within switch regions are recognized and excised by Uracil DNA Glycosylase to result in abasic sites. AP endonucleases then create a nick at abasic sites and two closely spaced nicks on both DNA strands can act as DSBs. However the identity of the AP endonuclease that creates DSBs during CSR was unclear. Our work shows that APE1, the major AP endonuclease in mammalian cells, is essential for CSR.

Finally, we focused on understanding the mechanism of DSB repair by studying the role of DNA ligases in CSR. Mammals have three DNA Ligases, each of which are conventionally thought to have distinct, non-overlapping functions. By creating cells that lack one or two DNA ligases we were able to better understand their role in CSR as well as in other aspects of DNA metabolism. Our results show a remarkable level of functional redundancy between the three DNA ligases. We show that DNA Ligase I, previously thought to be essential for joining Okazaki fragments during DNA replication, is dispensable for cell viability, a number or DNA repair pathways as well as for CSR. In addition we also constructed and characterized a cell line with only one DNA ligase, Ligase III. These cells are viable and show no increased hypersensitivity to a number of DNA damaging agents. Our results show a previously unanticipated level of redundancy between DNA ligases in CSR as well as in DNA replication and repair. These studies have improved our understanding of the molecular mechanism of CSR. In addition the creation of cell lines lacking APE1 and DNA ligases will provide important tools for the study of DNA repair and replication.