The three dimensional spatial organization of the Igh locus directs class switch recombination

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Immunoglobulin (*Igh*) class switch recombination (CSR) is induced in mature B lymphocytes by activation-induced cytidine deaminase (AID). The *Igh* locus contains eight constant (C_H) region genes with their attendant switch (S) regions and germline transcript (GLT) promoters (with the exception of Cδ) that are bracketed by the intronic Eμ and the 3'Eα regulatory region located at the 5'- and 3'-ends, respectively (fig. 1A). S regions are related but non-identical 1-10 kb stretches of repetitive DNA. AID initiates formation of DNA double strand breaks (DSB) in S regions that flank Cμ and downstream C_H regions. DSB intermediates are resolved via intrachromosomal deletion using a distinct nonhomologous end joining (NHEJ) pathway. Ig class switch recombination is governed by long-range interactions between enhancers and promoters to activate transcription and modulate chromatin accessibility to AID. In mature resting B cells, the Eμ and 3'Eα enhancers are in close spatial proximity and form a chromatin loop (fig. 1B). B cell activation leads to enhanced interaction between Eμ and 3'Eα as well as cytokine dependent recruitment of the germline transcript (GLT) promoters to the Eμ:3'Eα complex that enables transcription of S regions targeted for CSR. This looped structure facilitates S-S synapsis since Sμ is proximal to Eμ and a downstream S region is co-recruited with the targeted GLT promoter to Eμ:3'Eα complex (fig. 1B). We used the chromosome conformation capture (3C) and 3C carbon copy (5C) techniques to assess the contribution of cis-elements within the Igh locus and trans-acting factors to Igh three dimensional organization. 5C studies reveal that the Igh locus is compartmentalized into three subsections; 1) 3'Eα and flanking regions, 2) an area spanning all the S-C_H regions and 3) the μ–δ loci. Unexpectedly, the γ1 GLT promoter appears to be a central organizing element regulating access of other GLT promoters to the 3'Eα enhancer. Our studies also reveal differential regulation of Eμ:3'Eα interactions as compared to association of the γ1 GLT promoter with 3'Eα. Eμ:3'Eα interactions are dependent on CTCF, cohesin and 53BP1. Association of the γ1 GLT promoter region with 3'Eα requires the integrity of the γ1 promoter and presence of trans-acting STAT6 but is independent of CTCF, cohesin or 53BP1. Our studies reveal remarkable integration of three dimensional chromatin organization properties of the Igh locus which concomitantly regulate GLT expression and AID accessibility together with spatial proximity between the S regions targeted for CSR. This work was supported by the NIH, USA (RO1AI052400 to A. L. K.).

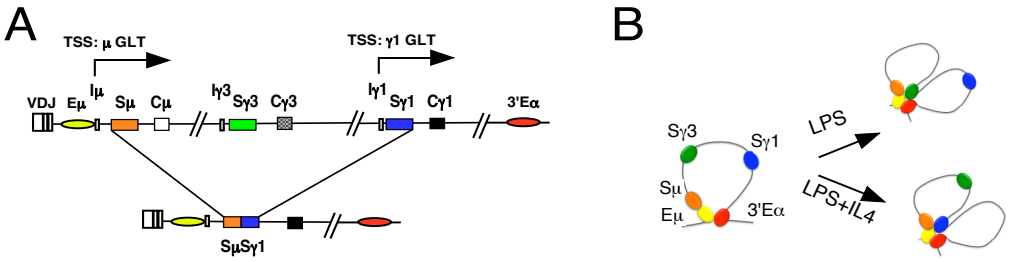

Figure 1. LPS and LPS+IL4 induce the expression of the γ3 or γ1 GLT and CSR between Sμ/Sγ1 or Sμ/Sγ3, respectively. **A)** The Igh locus prior to and following CSR is diagrammatically depicted. Transcription start sites (TSS) for the μ and γ1 GLTs are indicated. **B)** The looped *Igh* locus in which the Eμ:3'Eα enhancers interact in resting B cells is shown. Following activation with LPS or LPS+IL4 the appropriate GLT promoters interact with the 3'Eα in a cytokine dependent fashion.