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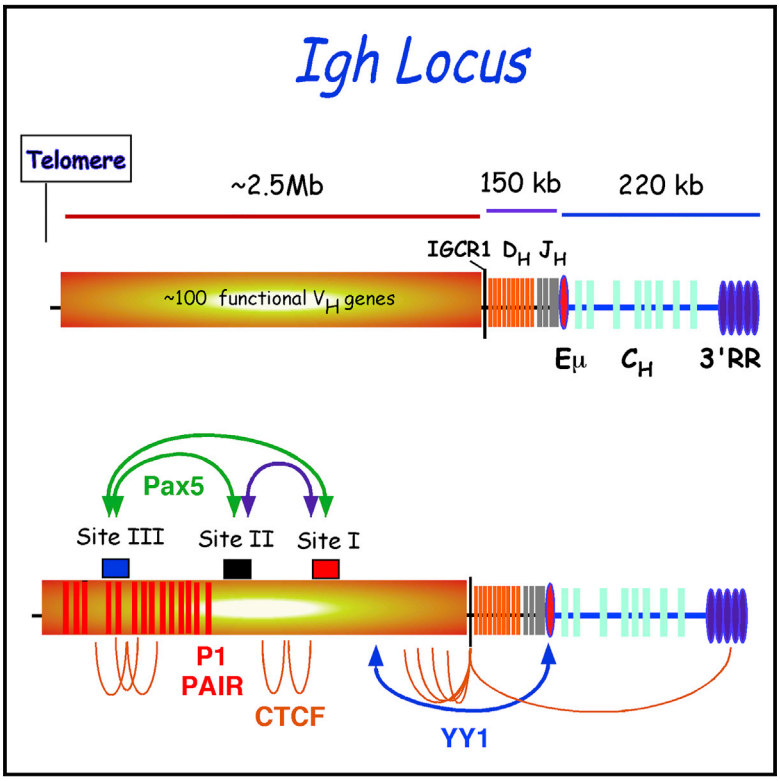
# Extremely Long-Range Chromatin Loops Link Topological Domains to Facilitate a Diverse Antibody Repertoire

## Graphical Abstract

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## In Brief

Using chromosome conformation capture technology, Montefiori et al. define the molecular architecture supporting large-scale Igh locus contraction at the pro-B cell stage of development. Pax5 deficiency leads to loss of a subset of these long chromatin loops suggesting a multilayered mechanism by which $V_H$ gene usage is controlled.

## Highlights

- Igh locus contraction occurs in pro-B cells prior to VDJ joining
- Pro-B cell-specific chromatin looping at the multi-megabase scale defines locus contraction
- A subset of these exceptionally long chromatin loops are Pax5 dependent
- VH gene rearrangement is dependent upon independently regulated chromatin topologies

## Accession Numbers

GSE76086

Montefiori et al., 2016, Cell Reports *14*, 896–906
February 2, 2016 
http://dx.doi.org/10.1016/j.celrep.2015.12.083

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CellPress

Cell Reports
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# Extremely Long-Range Chromatin Loops Link Topological Domains to Facilitate a Diverse Antibody Repertoire

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http://dx.doi.org/10.1016/j.celrep.2015.12.083



## SUMMARY

**Early B cell development is characterized by large-scale *Igh* locus contraction prior to V(D)J recombination to facilitate a highly diverse Ig repertoire. However, an understanding of the molecular architecture that mediates locus contraction remains unclear. We have combined high-resolution chromosome conformation capture (3C) techniques with 3D DNA FISH to identify three conserved topological subdomains. Each of these topological folds encompasses a major $V_H$ gene family that become juxtaposed in pro-B cells via megabase-scale chromatin looping. The transcription factor Pax5 organizes the subdomain that spans the $V_HJ558$ gene family. In its absence, the J558 $V_H$ genes fail to associate with the proximal $V_H$ genes, thereby providing a plausible explanation for reduced $V_HJ558$ gene rearrangements in Pax5-deficient pro-B cells. We propose that *Igh* locus contraction is the cumulative effect of several independently controlled chromatin subdomains that provide the structural infrastructure to coordinate optimal antigen receptor assembly.**

## INTRODUCTION

The mechanisms that govern V gene usage in VDJ rearrangements are central to understanding the formation of the BCR and TCR repertoires. Chromatin conformation and coordinated chromosomal movements govern the clustering of genes in transcription machines and the matrix of interactions specifying regulatory element associations. The *Igh* locus undergoes several different chromosomal movements that ensure developmental-stage and lineage-specific DNA recombination and transcription including relocation from the nuclear periphery to the center and re-organization of the *Igh* locus chromatin topology during B cell ontogeny (Fuxa et al., 2004; Kosak et al., 2002; Sayegh et al., 2005). In the mouse, there are ~100 functional $V_H$ gene segments that are scattered over 2.5 Mb of the *Igh* locus that must recombine with a rearranged $DJ_H$ element assembled from 1 of 8–12 $D_H$ and one of four $J_H$ gene segments. In primary pro-B cells of the bone marrow (BM), RAG recombinase mediates V(D)J or VJ joining for both Ig H and L chain genes. However, the molecular mechanism by which the distal $V_H$ genes gain spatial proximity to the rearranged $D_HJ_H$ gene segments remains obscure.

Chromatin compaction has been studied extensively by cytological methods. Three-dimensional (3D) DNA fluorescent in situ hybridization (FISH) studies in pro-B cells indicate that the Igh locus contracts, and this process is inferred to juxtapose distal $V_H$ genes near to proximal $D_H$ segments to promote V(D)J joining (Fuxa et al., 2004; Jhunjhunwala et al., 2008; Kosak et al., 2002). Locus contraction requires the transcriptional regulators, Pax5, YY1, and Ikaros (Fuxa et al., 2004; Liu et al., 2007; Reynaud et al., 2008). Loss of Igh locus compaction is correlated with the biased usage of the proximal $V_H$ gene segments (Hesslein et al., 2003). The degrees of locus compaction are inferred from relationships of interprobe nuclear distances versus genomic distances. However, FISH-based measurements have limited resolution (100–1,000 nm), and it has been difficult to ascertain the identity of specific DNA sequences that mediate locus contraction. The advent of chromosome conformation capture (3C) and related methods allows examination of pairwise chromatin interactions at the molecular level (~1–100 nm) in cell populations (Gibcus and Dekker, 2013). 3C-based methods can delineate long-range chromatin looping interactions and have