Report

IL-7R signaling activates widespread V_H and D_H gene usage to drive antibody diversity in bone marrow B cells

Graphical abstract

Authors

Amanda Baizan-Edge, Bryony A. Stubbs, Michael J.T. Stubbington, Daniel J. Bolland, Kristina Tabbada, Simon Andrews, Anne E. Corcoran

## Correspondence

anne.corcoran@babraham.ac.uk

In brief

Baizan-Edge et al. show that the interleukin-7 receptor drives antibody repertoire diversity. Deletion of the IL-7R impairs usage of most V_H genes and D_H genes in V(D)J recombination of the Igh, causing a severely restricted repertoire. Defects include reduced Igh antisense

Highlights

- The IL-7R drives recombination of V_H and D_H genes in the immunoglobulin heavy chain

- Deletion of the IL-7R impairs usage of all except 3′ V_H and flanking D_H genes

- IL-7R loss diminishes large-scale V_H and D_H antisense transcription in the Igh

- IL-7R loss causes reduced expression of B lineage transcription factors and targets

[1]Nuclear Dynamics Programme, Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK
[2]Lymphocyte Signaling and Development Programme, Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK
[3]Bioinformatics Group, Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK
[4]Present address: Centre for Gene Regulation and Expression, School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK
[5]Present address: 10x Genomics, 6230 Stoneridge Mall Road, Pleasanton, CA 94588, USA
[6]Present address: Palaeogenomics, PalaeoBARN, School of Archaeology, University of Oxford, Oxford OX1 3TG, UK
[7]Lead contact
*Correspondence: anne.corcoran@babraham.ac.uk
 https://doi.org/10.1016/j.celrep.2021.109349

SUMMARY

Generation of the primary antibody repertoire requires V(D)J recombination of hundreds of gene segments in the immunoglobulin heavy chain (*Igh*) locus. The role of interleukin-7 receptor (IL-7R) signaling in *Igh*

A diverse antibody repertoire requires inclusion of all available V_H and D_H genes. Large-scale processes, including non-coding transcription and Ig locus contraction, are thought to facilitate accessibility of distal 5′ V_H genes to the recombination center over the DJ region, where the recombination activating gene (RAG) 1 and 2 bind (Ji et al., 2010, Corcoran et al., 1998; Bolland et al., 2004; Yancopoulos and Alt, 1985, Chowdhury and Sen, 2003; Fuxa et al., 2004; Jhunjhunwala et al., 2008; Sayegh et al., 2005; Stubbington and Corcoran, 2013). Nevertheless, V_H genes recombine at widely different frequencies; frequently recombining V_H genes also have one of two local active chromatin states (Bolland et al., 2016).

IL-7R$\alpha^{-/-}$
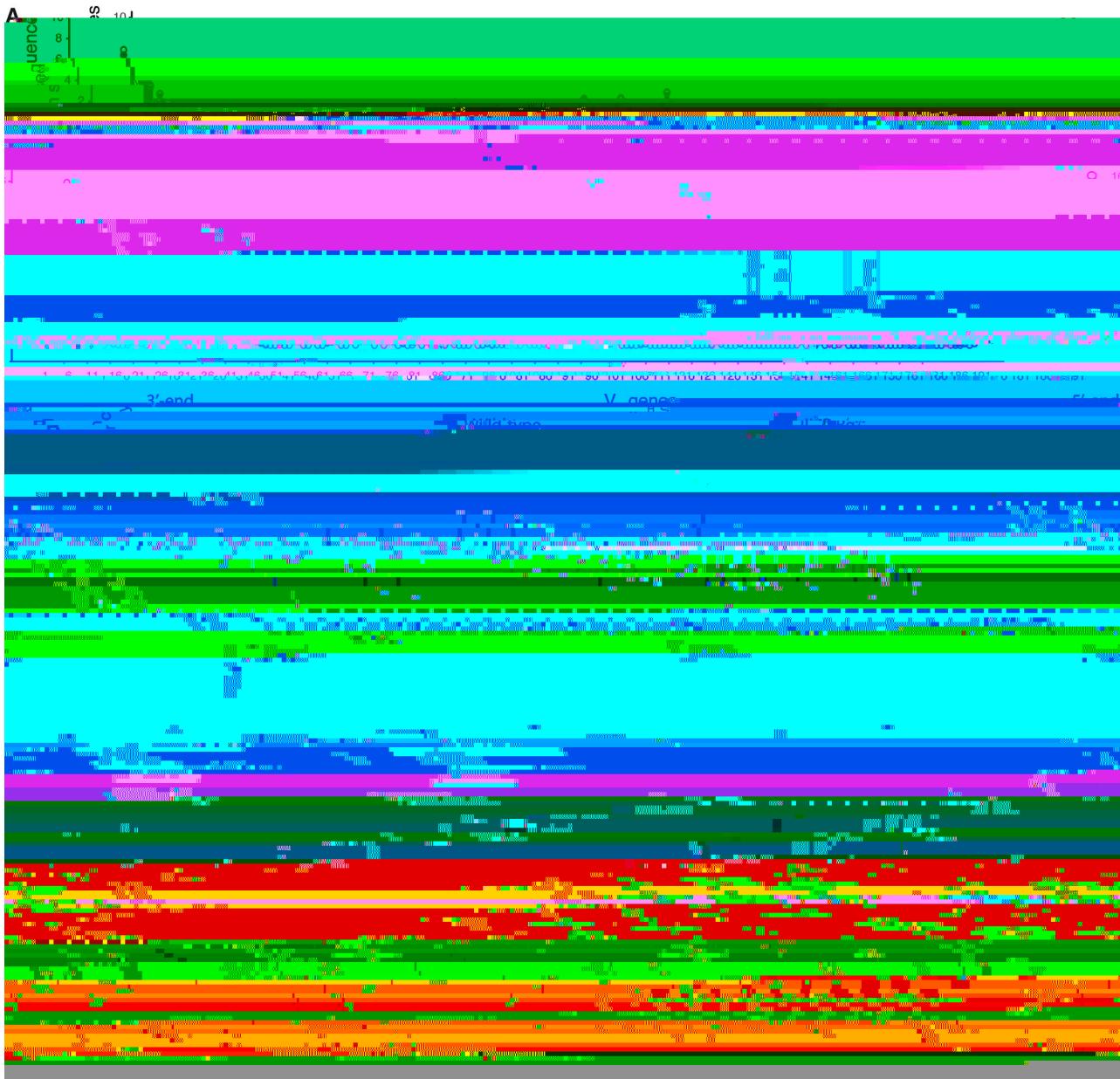
Figure 1. IL-7Rα$^{-/-}$ pro-B cells and V$_H$ and D$_H$

(A) Recombination frequencies of 195 V$_H$ genes measured by VDJ-seq. WT BM pro-B cells (blue) and IL-7Rα$^{-/-}$ (red) are shown. Two biological replicates are shown as open circles (bar height represents average). Reads for each V$_H$ gene are shown as percentage of total reads quantified. V$_H$ gene number legend is shown in Table S1.

(B) The mean of each V$_H$ gene was divided with the WT mean followed by log2 transformation. Only genes recombining in either genotype are shown. * represents V$_H$ genes with value 0 in IL-7Rα$^{-/-}$ (only in WT). V$_H$ gene raw read counts and recombining/non-recombining classification are shown in Table S1.

(C and D) Log2 values for each gene in graph B (excluding those marked by *) were grouped by (C) evolutionary origin: clan 1 (n = 78), clan 2 (n = 27), and clan 3 (n = 26); ANOVA (degrees freedom [Df] = 2; F-value = 5.39; p = 0.005) and (D) chromatin state: enhancer (n = 68), architectural (n = 30), and background (n = 33); ANOVA (Df = 2; F-value = 4.54; p = 0.012).

(E) Reads for each D$_H$ gene as percentage of total reads quantified for two biological replicates of WT (blue) and IL-7Rα$^{-/-}$ (red) pro-B cells.

genes) and on several middle region families in the architectural state. This distribution also applies to the clans: loss of IL-7R reduces recombination of clan 1 (mostly distal V genes) as well as the middle genes from clans 2 and 3. Importantly, this suggests that the IL-7R does not influence either clans or local chromatin states selectively but rather linear positioning in the Igh V region, i.e., loss of IL-7R impairs recombination of middle and 5′ V genes in the Igh locus.

D_H-antisense) were also analyzed. Although the RNA-seq libraries were not strand specific, these known transcripts are

checkpoint, and MYC were downregulated in the IL-7Rα/Rag2$^{-/-}$ cells (Figures S4B–S4D). Consistent with previous reports, expression of both Ebf1 and Pax5 was substantially reduced in IL-7Rα/Rag2$^{-/-}$ pro-B cells (Table S3

F   4. TF   a a   a   c   a   acc   b

Peaks identified from ATAC-seq by MACS peak calling from two biological replicates.
(A) Sites less accessible in IL-7Rα/Rag2$^{-/-}$ analyzed using DESeq2. TF motif enrichment using HOMER is shown. Relevant significantly en-

IL-7Rα$^{-/-}$ display widespread defects at both stages of Igh recombination. Most importantly, the V_H repertoire was highly biased toward 3′ V_H genes. Reduced use of 5′ V_H genes was much more pronounced than in FL pro-B cells, indicating that IL-7R signaling is specifically needed in the BM to make all V_H genes available for the primary antibody repertoire. The presence of N-additions within IL-7Rα$^{-/-}$ VDJ$_H$ sequences demonstrates that they are derived from BM, not FL, progenitors. Our findings concur with a study in neonatal IL-7Rα$^{-/-}$ BM (Hesslein et al., 2006). Detection of N-additions at D$_H$-J$_H$ junctions indicated that D$_H$ to J$_H$ recombination also took place de novo in the BM. Thus, V(D)J recombination progressed with normal dynamics but severely restricted participation of both V_H and D_H genes. Previous models of a block in B cell development in IL-7Rα$^{-/-}$ BM (Kikuchi et al., 2005; Peschon et al., 1994; Miller et al., 2002; Carvalho et al., 2001) inferred that IL-7Rα$^{-/-}$ BM B cells had originated in the FL, where the IL-7R is not essential (Erlandsson et al., 2004), due to their restricted Vh gene usage. Here, our demonstration that V(D)J recombination occurs de novo in the BM, albeit in the very few remaining B cells, has enabled us to uncover specific roles of the IL-7R in regulating D$_H$ and V_H gene usage in the Igh repertoire in BM pro-B cells.

A previous study showing that IL-7Rα$^{-/-}$ B cell development could be partially rescued by a vav-cre bcl2 transgene, indicating a crucial role in CLP survival (Malin et al., 2010), suggested that IL-7R signaling is not required for BM B cell recombination. However, Bcl2 driven by the Igh Eμ enhancer did not rescue

REFERENCES

Abarrategui, I., and Krangel, M.S. (2006). Regulation of T cell receptor-alpha

Stephan, R.P., Lill-Elghanian, D.A., and Witte, P.L. (1997). Development of B

STAR+METHODS

converted to human gene symbols, and ran with default parameters for genes with BaseMean > = 5. The Molecular Signature Database (MSigDB) hallmark gene sets and the transcription factor targets/regulatory target were used to perform pathway enrichment analysis limiting the output to the top 1000 gene sets.