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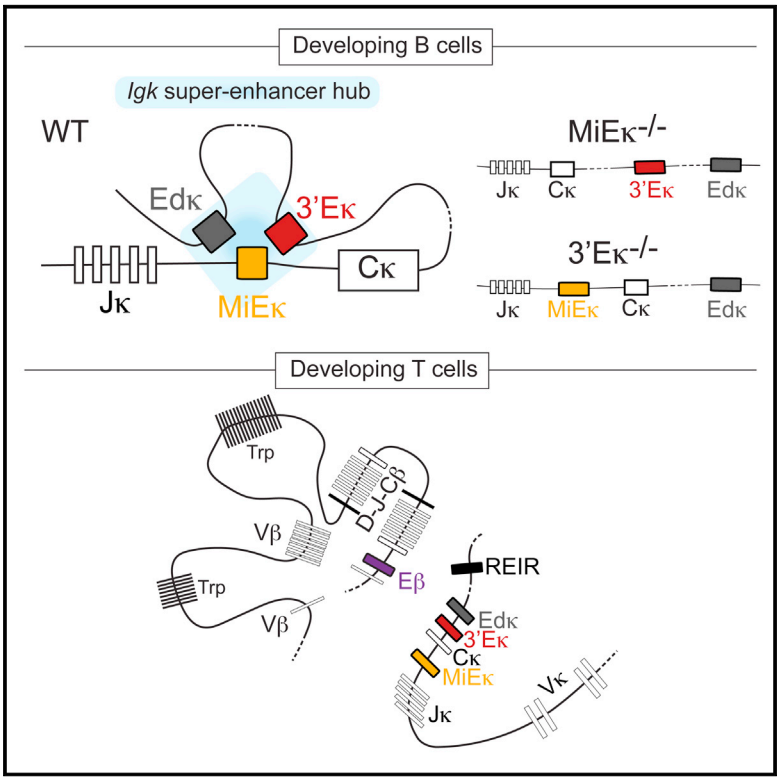
Cell Reports

Active and Inactive Enhancers Cooperate to Exert Localized and Long-Range Control of Gene Regulation

Graphical Abstract



Highlights

- Synergistic contacts between the elements of a super-enhancer contribute to activity
- *Igk* enhancers play diverse roles in lymphocyte development
- The *Igk* enhancer, MiEκ, contributes to regulation of *Tcrb* recombination
- Enhancer sharing can involve active as well as inactive regulatory elements

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In Brief

Proudhon et al. show that, in B cells, synergistic chromatin contacts between the individual elements of the *Igk* super-enhancer contribute to the transcriptional output of all partner enhancers. In T cells, one component of the *Igk* enhancer cluster cooperates with the *Tcrb* enhancer to exert control over *Tcrb* recombination.

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Active and Inactive Enhancers Cooperate to Exert Localized and Long-Range Control of Gene Regulation

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SUMMARY

V(D)J recombination relies on the presence of proximal enhancers that activate the antigen receptor (AgR) loci in a lineage- and stage-specific manner. Unexpectedly, we find that both active and inactive AgR enhancers cooperate to disseminate their effects in a localized and long-range manner. Here, we demonstrate the importance of short-range contacts between active enhancers that constitute an *Igk* super-enhancer in B cells. Deletion of one element reduces the interaction frequency between other enhancers in the hub, which compromises the transcriptional output of each component. Furthermore, we establish that, in T cells, long-range contact and cooperation between the inactive *Igk* enhancer MiEκ and the active *Tcrb* enhancer Eβ alters enrichment of CBFβ binding in a manner that impacts *Tcrb* recombination. These findings underline the complexities of enhancer regulation and point to a role for localized and long-range enhancer-sharing between active and inactive elements in lineage- and stage-specific control.

INTRODUCTION

B and T lymphocyte development is driven by V(D)J recombination, a process through which V (variable), D (diversity) and J (joining) coding gene segments within each of the seven antigen receptor (AgR) loci are rearranged to create a vast repertoire of receptors (Alt et al., 2013). This process is important because lymphocytes require a set of receptors that can recognize and respond to a wide variety of foreign antigens as part of the adaptive immune response. V(D)J rearrangement is mediated by the recombination-activating gene (RAG) complex, which targets the recombination signal sequences (RSSs) that flank each V, D, and J gene segment (Schatz and Swanson, 2011). RAG creates a synapse between two segments, introducing breaks that are then ligated together via non-homologous end joining.

Recombination occurs in a lineage-specific manner so that *T cell receptor* (*Tcr*) and *immunoglobulin* (*Ig*) loci are only fully rearranged in T and B cells, respectively. In addition rearrangement is ordered by stage within a given lineage. In B cells, the *Ig heavy chain* (*Igh*) is rearranged at the pro-B cell stage of development prior to *Ig light chain* (κ or λ) rearrangement in pre-B cells, while in T cells the T cell receptor beta locus (*Tcrb*) is recombined in $CD4^{-}CD8^{-}$ double negative DN2/3 cells prior to T cell receptor alpha (*Tcra*) recombination in double positive (DP) cells. As the RAG proteins and their RSS targets are the same for each AgR locus, lymphocytes restrict rearrangement by controlling the accessibility of the individual loci (Yancopoulos and Alt, 1985). Opening up of the loci occurs at multiple levels including DNA demethylation, acquisition of active histone marks, initiation of sense and antisense germline transcription, and nucleosome repositioning (Johnson et al., 2010).

The AgR loci have served as a rich model system for analyzing the impact of nuclear organization and chromatin architecture on gene regulation (Proudhon et al., 2015). The first evidence that shuttling of loci between repressive and active nuclear compartments (the nuclear lamina or pericentromeric heterochromatin [PCH] and accessible euchromatic regions) has an impact on gene regulation came from tracing the movements of AgR loci during development (Goldmit et al., 2005; Kosak et al., 2002; Roldán et al., 2005). Moreover, studies focusing on chromatin architecture demonstrated that reversible changes in "locus contraction" alter the conformation of each locus, bringing mid and distal V gene segments into contact with proximal DJC domains thereby enabling recombination to occur between widely separated gene segments (Fuxa et al., 2004; Roldán et al., 2005; Sayegh et al., 2005; Skok et al., 2007). These changes are