STIMULUS REPORT

blood advances

YY1 controls Eμ-3′RR DNA loop formation and immunoglobulin heavy chain class switch recombination

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Key Points

- Transcription factor YY1 regulates the *IgH* Eμ-3′RR long-distance DNA loop without the YY1 transcriptional activation domain.
- YY1 constructs that rescue the Eμ-3′RR DNA loop also restore CSR strongly arguing for the necessity of this long-distance DNA loop for CSR.

Introduction

Immunoglobulin heavy chain (*IgH*) class switch recombination (CSR) is a crucial immune function that produces immunoglobulin isotypes with distinct effector functions.[1] CSR is initiated by the introduction of double-stranded DNA breaks by activation-induced DNA deaminase (AID) at switch sequences nearby Cμ (Sμ) and targeted C_H exons (Sγ1, Sγ2a, Sγ2b, etc). Repair of these breaks results in linkage of the VDJ exon with the switched C_H region. The 200-kb region from the VDJ exon to the terminal Cα exon forms a loop involving interactions between sequences nearby the Eμ intronic enhancer and the 3′ regulatory region (3′RR) located at the end of the *IgH* locus.[2-4] Although enhancers associated with the 3′RR are essential for CSR,[5,6] the role of Eμ in this process is more complex.[7-9] Eμ deletion impairs, but does not abolish CSR, suggesting either the V_H promoter 3 kb upstream from Eμ contacts the 3′RR or the V_H promoter may substitute for Eμ activity on Eμ-deficient alleles. Consistent with prior convention, we refer to these interactions as the Eμ-3′RR loop.

Although the Eμ-3′RR DNA loop has been described, its necessity for CSR is uncertain, and the proteins that control DNA interactions are poorly understood. Previously, we found that conditional ablation of transcription factor YY1 causes a significant drop in CSR.[10] YY1 physically interacts with AID and regulates its nuclear accumulation.[10] We proposed that YY1 controls CSR, at least in part, by regulating the amount of nuclear AID. However, YY1 is also known to impact long-distance DNA contacts in a number of systems.[11-16] Given the importance of YY1 in CSR and long-distance DNA loops, we tested the importance of YY1 for Eμ-3′RR DNA loops.

Methods

Splenic B cells and YY1 deletion

Isolation of splenic follicular B cells and treatment with recombinant TAT-CRE enzyme to knock-out YY1 have been described.[10]

Retroviral constructs and transduction

Virus production of various YY1 constructs and transduction of splenic B cells are described in the supplemental Methods.

Fluorescent in situ hybridization

Position-specific 10-kb probes were generated by polymerase chain reaction using BAC templates with primers as described.[14,15] fluorescent in situ hybridization (FISH) was performed as described,[15] with additional details in the supplemental Methods.

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The full-text version of this article contains a data supplement.

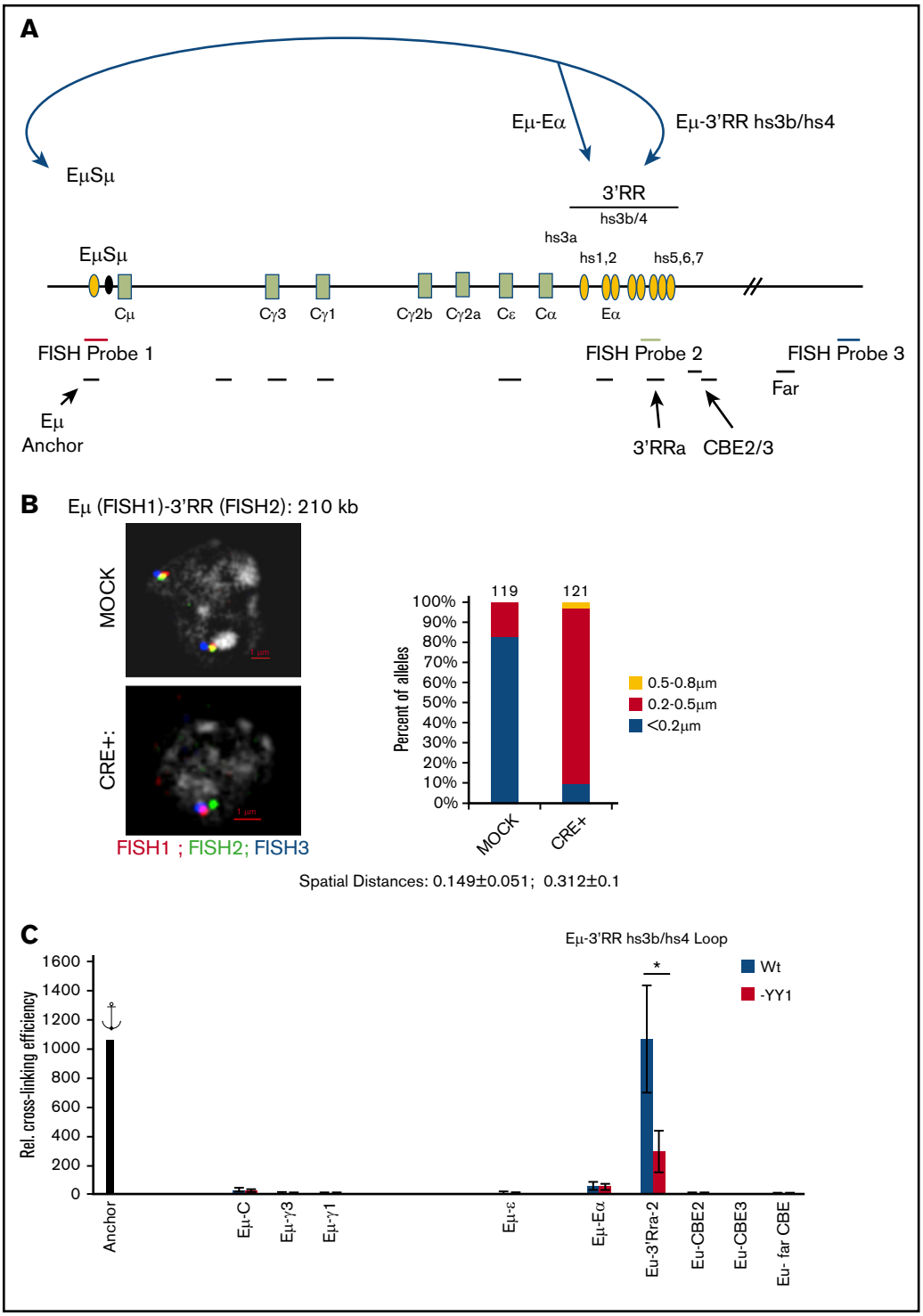

Figure 1. YY1 is required for long-distance DNA loops detected by 3D FISH and 3C. (A) Map of the 240-kb 3′ region of the mouse *IgH* locus. Yellow ovals represent Eμ and 3′RR enhancers or boundary elements; the black oval represents the Sμ sequence, and green boxes represent various constant region exons. FISH probes 1, 2, and 3 are indicated by red, green, and blue lines, respectively. The red probe detects the Eμ region; the green probe detects the 3′RR region, and the blue probe lies downstream of the IgH locus and is used for orientation. Positions of primers used in 3C assays are shown as well as the regions of DNA contacts (blue curved line with double-headed arrows). (B) 3D FISH results in activated $yy1^{f/f}$ splenic B cells that were either Mock treated or TAT-CRE treated to delete the *yy1* gene. The right panel shows percentage of alleles with probe signals separated by various distances. Total number of nuclei analyzed is indicated above the bars. Average spatial distances between FISH1 and FISH2 probes were determined as in Guo et al[15] and are 0.149 ± 0.051 μm and 0.312 ± 0.1 μm for Mock- and TAT-CRE–treated cells, respectively. (C) YY1 is required for long-distance DNA contacts detected by 3C assays. YY1 deletion greatly reduced the Eμ-3′RR DNA loop. YY1 was deleted in $yy1^{f/f}$ splenic B cells by TAT-CRE, and 3C assays were performed with Mock- or TAT-CRE–treated splenic B cells. Blue bars represent Mock samples, and red bars represent TAT-CRE samples. Asterisks indicate $P < .05$. All experiments were repeated 3 to 5 times.

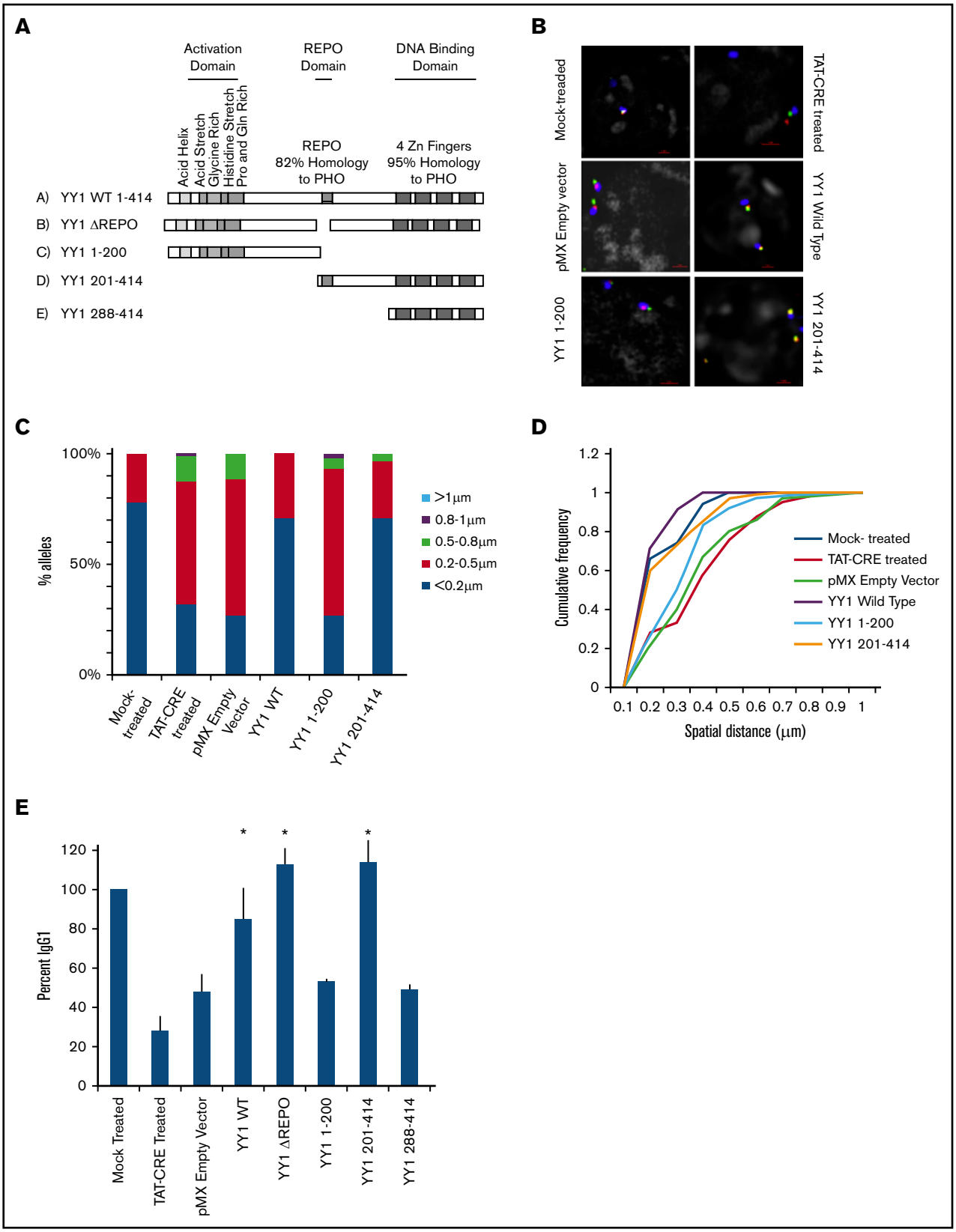

Figure 2. The YY1 C-terminal half is sufficient for rescue of DNA loops between the Eμ and 3′RR enhancers, and for CSR in endogenous YY1-deleted primary splenic B cells. (A) Maps of YY1 constructs used to rescue YY1-conditionally deleted splenic B cells. The transcriptional activation domain resides near the amino terminus spanning amino acids 16-99.[25] The REPO domain involved in YY1 recruitment of PcG proteins to DNA lies between residues 201 and 226.[17] The 4 zinc fingers that constitute the

Chromosome conformation capture

Chromosome conformation capture (3C) analyses are described in supplemental Methods.

RNA transcript analyses

Microarray methods and analyses are described in the supplemental Methods.

Results and discussion

Eμ-3′RR DNA loop formation is YY1 dependent

The 3′ region of the *IgH* locus involved in CSR is shown in Figure 1A. The DNA loop formed between the Eμ and 3′RR enhancer regions in activated splenic B cells can be detected by 3D FISH using probes that hybridize nearby the Eμ and the 3′RR enhancers (FISH probes 1 and 2, respectively; Figure 1A).[14] To determine the importance of YY1 for Eμ-3′RR loop formation, YY1 was ablated ex vivo with recombinant TAT-CRE (supplemental Figure 1A-B). YY1 deletion resulted in a three- to eightfold drop in immunoglobulin G1 CSR similar to our previous work[10] (supplemental Figure 1C). Strikingly, ablation of YY1 also dramatically reduced Eμ-3′RR DNA loop formation (Figure 1B; supplemental Table 1).

Chromosome conformation capture (3C) assays can also identify the Eμ-3′RR long-distance loop.[2,3] Using the Eμ region as anchor, we probed the *IgH* 3′ region by 3C assay in the presence and absence of YY1. Initial 3′RR contacts were observed at the Eα region as previously observed[2] (Figure 1C Eμ-Eα peak; supplemental Figure 2 with expanded scale). Deletion of YY1 caused a 50% drop in DNA contacts (supplemental Figure 2). Scanning further, we observed the dramatic Eμ-3′RR loop that makes Eμ contacts within the hs3b/hs4 region of the 3′RR enhancer (Figure 1C Eμ-3′Rra-2 peak). Deletion of YY1 caused a striking fivefold drop in this loop (Figure 1C). This loss was specific as there was little impact on DNA loops at the Igκ locus using the Igκ E3′ enhancer as anchor (supplemental Figure 3). Thus, our 3C experiments also showed the Eμ-3′RR DNA loop is YY1 dependent.

The C-terminal half of YY1 is sufficient for controlling DNA loops and CSR

YY1 contains a number of domains with distinct functions (Figure 2A). We tested the ability of YY1 mutants that ablate 1 or more functions to rescue the Eμ-3′RR DNA loop after YY1 ablation. Mock-treated samples showed 80% of cells with Eμ-3′RR loops, whereas DNA loops were lost in TAT-CRE–treated cells due to YY1 deletion (Figure 2B-D). Transduction with empty vector failed to rescue the Eμ-3′RR DNA loop (Figure 2B-D). On the contrary, wild-type YY1 rescued the Eμ-3′RR DNA loop, whereas the 1-200 mutant failed in this rescue (Figure 2B-D). Of particular importance, the 201-414 mutant lacking the activation domain but containing the REPO and DNA binding domains completely restored the Eμ-3′RR DNA loop (Figure 2B-D; supplemental Table 1).

In parallel, we explored the ability of YY1 mutants to rescue CSR. Treatment with TAT-CRE resulted in a fourfold drop in CSR (Figure 2E). Empty vector failed to rescue CSR, whereas vector expressing wild-type YY1 and the YY1ΔREPO mutant fully restored CSR (Figure 2E). The YY1 1-200 and YY1 288-414 mutants failed to restore CSR, but the YY1 201-414 mutant, which rescued the Eμ-3′RR DNA loop, completely restored CSR (Figure 2E; supplemental Figure 4B-H). We conclude that the C-terminal half of YY1 is sufficient for rescuing both the Eμ-3′RR DNA loop and the CSR.

YY1 regulation of genes required for DNA looping and CSR

The ability of the YY1 201-414 mutant (lacking the transactivation domain) to rescue the Eμ-3′RR DNA loop as well as CSR argued that DNA looping and CSR do not require YY1 transactivation function. RNA transcript studies showed YY1 deletion decreased expression of genes involved in nucleotide binding, glycolysis, protein folding, lipid biosynthesis, and mitochondrial function (supplemental Figure 5A), and caused increased expression of genes involved in chromatin organization, proteolysis, translation, apoptosis, and non–membrane-bound organelles (supplemental Figure 5A).

Nearly all genes implicated in long-distance DNA interactions (44/48 genes) or CSR (42/47 genes) were not differentially expressed (supplemental Figure 5B blue and green dots, respectively; supplemental Tables 2 and 3). Gene Set Enrichment Analysis demonstrated that DNA looping and CSR genes were not enriched in our dataset. Therefore, YY1 does not likely transcriptionally regulate genes that control DNA looping or CSR.

Our studies define a critical role for YY1 controlling the Eμ-3′RR long-distance DNA loop and strongly argue for its role in CSR. We propose that YY1 plays a largely structural role in regulating long-distance DNA loops. We previously showed that YY1 physically interacts with components of the PcG, condensin, and cohesin complexes,[17-19] each of which can function in long-distance DNA interactions.[20-22] DNA-bound YY1 may provide a platform for recruitment of these complexes (directly or indirectly) to mediate long-distance DNA interactions. Deletion of the Eμ YY1 binding region minimally impacts loops and CSR, suggesting other binding sites may compensate for this loss, or YY1 bound to the 3′RR contacts proteins bound near the VDJ promoter or switch sequences. YY1 levels increase after splenic B-cell activation,[23] and highest expression levels are in germinal center B cells.[24] YY1 may inducibly interact with proteins to nucleate recruitment to DNA. Proteomic and chromatin immunoprecipitation sequencing experiments may identify key YY1-interacting proteins, and whether they are recruited to specific sites within the immunoglobulin loci. Our findings here provide foundational insight into a critical immune function.

Figure 2. (continued) DNA binding domain lie at the C-terminus, spanning amino acids 298-414.[25] (B) 3D FISH results with the probes shown in Figure 1A in primary *yy1*$^{f/f}$ splenic B cells that were mock treated, TAT-CRE treated, or TAT-CRE treated plus rescue with either vector alone or vector expressing full-length YY1, YY1 1-200, or YY1 201-414. Transduced cells were isolated by FACS for expression of green fluorescent protein (linked in the vector), and the Eμ-3′RR DNA loop was measured by 3D FISH. Equivalent expression of each construct was verified (supplemental Figure 4A) (C) Separation between probes was measured after image deconvolution from at least 100 nuclei. Bar graphs show the percentage of alleles in which the distance between probes fell in the ranges shown by different colors. (D) Cumulative frequencies of FISH probe spatial distances. (E) Rescue of CSR with each construct is shown relative to the level of CSR observed with mock-treated *yy1*$^{f/f}$ splenic B cells (no YY1 deletion). Asterisks denote significant change from vector alone ($P < .001$). All experiments were repeated 3 to 4 times. pMX, Moloney murine leukemia virus vector X; WT, wild type.

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Authorship

Contribution: P.M., T.G., V.J., A. Basu, V.S., M.L.A., and R.S. designed the experiments; P.M., T.G., A. Basu, C.T.B., V.S., F.G., M.L.A., and R.S. evaluated data; P.M., T.G., V.J., V.S., F.G., A. Basu, and A. Banerjee performed experiments; M.L.A. and R.S. wrote the manuscript; evaluation and edits were performed by all the authors.

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