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Joana M Santos, Fatima-Zohra Braikia, Chloé Oudinet, Audrey Dauba, Ahmed Amine Khamlichi. Two modes of cis-activation of switch transcription by the IgH superenhancer. Proceedings of the National Academy of Sciences of the United States of America, 2019, 116 (29), pp.14708-14713. 10.1073/pnas.1902250116 . hal-02361175

**HAL Id: hal-02361175**

**<https://hal.science/hal-02361175>**

Submitted on 13 Nov 2019

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# Two modes of *cis*-activation of switch transcription by the *IgH* superenhancer

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Edited by Matthew D. Scharff, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, and approved June 14, 2019 (received for review February 7, 2019)

B cell isotype switching plays an important role in modulating adaptive immune responses. It occurs in response to specific signals that often induce different isotype (I) promoters driving transcription of switch regions, located upstream of the Ig heavy chain (*IgH*) constant genes. The transcribed switch regions can recombine, leading to a change of the constant gene and, consequently, of antibody isotype. Switch transcription is controlled by the superenhancer 3' regulatory region (3'RR) that establishes long-range chromatin *cis*-interactions with I promoters. Most stimuli induce more than one I promoter, and switch transcription can occur on both chromosomes. Therefore, it is presently unknown whether induced I promoters compete for the 3'RR on the same chromosome. Here we performed single-chromosome RT-qPCR assays to examine switch transcription monoallelically in the endogenous context. We show that there are two modes of 3'RR-mediated activation of I promoters: coactivation and competition. The nature of the inducing signal plays a pivotal role in determining the mode of activation. Furthermore, we provide evidence that, in its endogenous setting, the 3'RR has a bidirectional activity. We propose that the coactivation and competition modes mediated by the 3'RR may have evolved to cope with the different kinetics of primary immune responses.

B lymphocyte | immunoglobulin locus | enhancer | promoter | switch transcription

The *IgH* locus is one of the most complex loci in mammals, and its expression is regulated in a cell-type and developmental-stage manner (1, 2). Two types of recombination take place at the *IgH* locus. In developing B cells, variable, (diversity), and joining segments [V(D)J] assembly generates the variable region genes encoding antigen-binding sites. Upon antigen challenge, mature B cells undergo class switch recombination (CSR) in which a selected constant (C) gene is brought next to a rearranged VDJ gene, enabling isotype switch from the initially expressed IgM to one of the downstream isotypes (IgG, IgE, or IgA), diversifying antibody effector functions (3, 4).

CSR takes place between highly repetitive sequences [called switch (S) sequences] that must be transcribed for CSR to occur. Switch transcription initiates from upstream I promoters (Fig. 1A) and is induced by various stimuli, including antigens, mitogens, and cytokines. These stimuli direct which isotype (I) promoter is activated, and therefore the S sequence that will undergo recombination. Switch transcription produces long noncoding RNAs that render S sequences accessible to the Activation-Induced cytidine Deaminase enzyme, which initiates DNA breaks at the  $\Sigma$  donor region and the transcribed downstream acceptor S regions ( $\Sigma$ ,  $\Sigma$ ,  $\Sigma$ ) (3, 4). CSR often occurs on both alleles, and  $\Sigma$  donor regions generally recombine with the same acceptor switch regions on the two chromosomes (e.g., refs. 5 and 6).

Complex loci in higher eukaryotes often contain multiple gene promoters that are activated by distant enhancers. Despite extensive efforts, it remains unclear whether a shared enhancer coactivates two promoters or if target promoters compete for enhancer activity (e.g., refs. 7 and 8). The major element controlling switch transcription is a superenhancer called 3' regulatory region (3'RR) located downstream of the *IgH* locus. The

3'RR is composed of four enhancers that synergistically activate the I promoters (9), and deletion of 3'RR severely impairs switch transcription and CSR (10).

In cultured B cells, most stimuli activate more than one I promoter and previous mutational studies on B cell populations concluded that I promoters compete for 3'RR activity (e.g., refs. 11–15). Notably, insertional studies suggested that, for example, while lipopolysaccharide (LPS) stimulation activates  $\Sigma$ 3 and  $\Sigma$ 2b promoters, IL-4 stimulation activates  $\Sigma$ 1 and  $\Sigma$ , which out-compete  $\Sigma$ 3 or  $\Sigma$ 2b promoters for 3'RR and extinguish  $\Sigma$ 3 and  $\Sigma$ 2b transcription (13). Whereas a recent single-cell study using tagged I promoters proposed that allelic I promoters are stochastically activated (16), activation of I promoters within each allele has never been addressed. It therefore remains uncertain whether I promoters that are located on the same chromosome and that respond to the same stimulus compete for 3'RR activity. Here, we have designed a single-chromosome RT-qPCR assay to examine, on a monoallelic basis, I promoter activation by the 3'RR in the endogenous context. We show that there are two modes of 3'RR long-range activity, depending on the inducing signal.

## Results

### Single-Chromosome RT-qPCR Analysis of Allele-Specific Transcription.

We used stimulation conditions that robustly induce two I promoters (Fig. 1A and B). To detect allele-specific switch transcription, we purified IgM-expressing splenic B cells from different mouse lines and then immediately single-sorted them or placed them in culture in stimulation media (Fig. 1B). We were able to efficiently perform RT-qPCR with three distinct primer pairs per single cell (SI Appendix, Fig. S1 and Datasets S1–S3).  $\Sigma$

## Significance

Immunoglobulin isotype switching plays an important role in adaptive immune responses. It requires transcription of switch regions from inducible promoters controlled by a long-range superenhancer (3'RR). Most stimuli induce more than one promoter on both chromosomes. Thus, it is unknown whether isotype (I) promoters compete for the 3'RR on the same chromosome. By performing allele-specific RT-qPCR assays, we show that the 3'RR activates promoters by either co-activation or competition and that the nature of the inducing signal plays a pivotal role in determining the activation mode. The two activation paths may have evolved to cope with different kinetics of primary immune responses.

Author contributions: A.A.K. designed research; J.M.S. performed research; F.-Z.B., C.O., and A.D. contributed new reagents/analytic tools; J.M.S. analyzed data; and J.M.S. and A.A.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

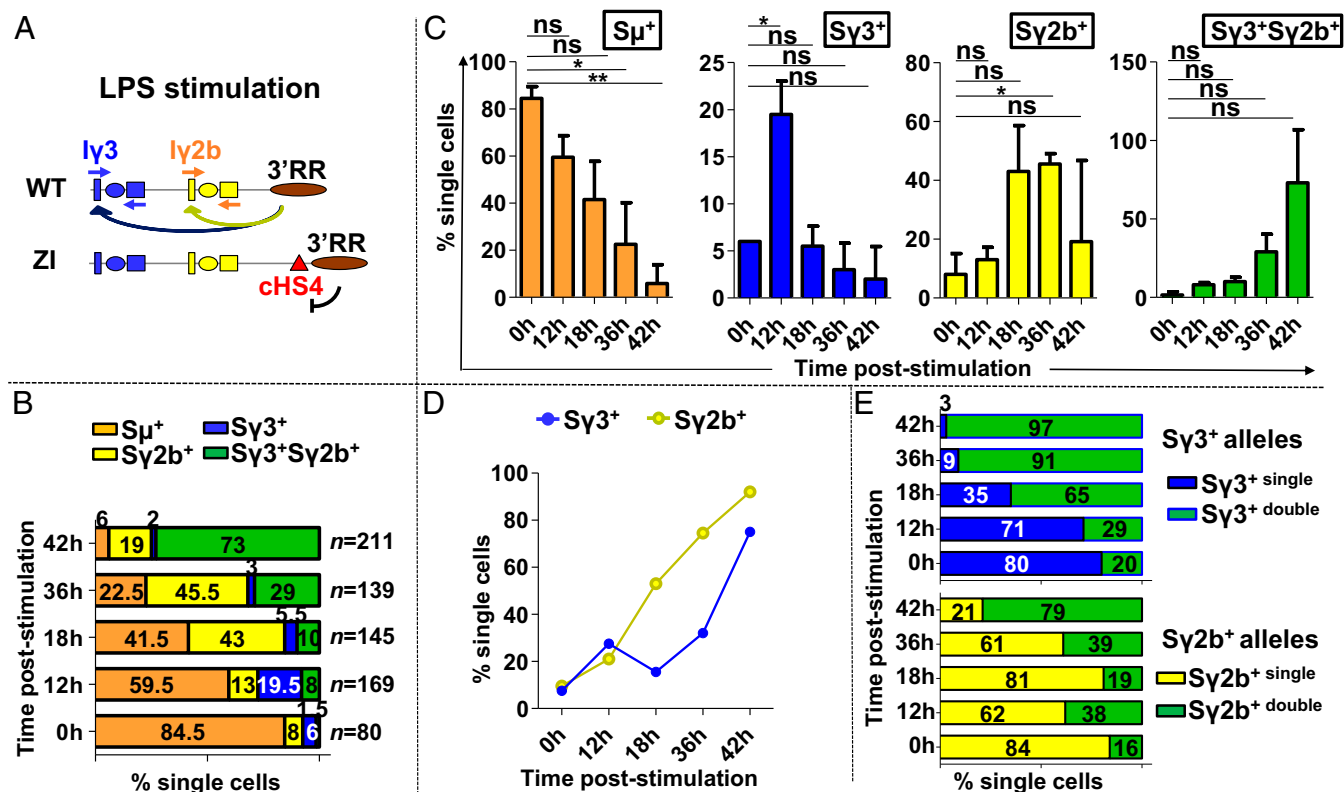
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This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1902250116/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1902250116/-DCSupplemental).

Published online July 2, 2019.





**Fig. 2.** Monoallelic coactivation of *Iy3* and *Iy2b* promoters upon LPS stimulation. (A) Scheme representing the two alleles of ZI/WT mice. On the ZI allele, a constitutive insulator (cHS4) has been inserted upstream of 3'RR, thus blocking activation of *Iy3* and *Iy2b* promoters. Switch transcription can occur only on the WT allele. (B) Percentages of single- and double-expressers upon single-chromosome RT-qPCR. Purified B cells from  $n = 2-3$  ZI/WT mice were grown in the absence (0 h) or presence of LPS for 12–42 h. The percentages of Sμ<sup>+</sup> (orange), Sy3<sup>+</sup> (blue), Sy2b<sup>+</sup> (yellow), and Sy2b<sup>+</sup>Sy3<sup>+</sup> (green) change with time ( $n$  indicates the number of single cells analyzed per time point). (C) Evolution of the total number of single- versus double-expressers with time after stimulation (same data as for B). (D) Evolution of the total number of Sy3<sup>+</sup> or Sy2b<sup>+</sup> alleles (single- plus double-expressers) with time after stimulation (same data as for B). (E) Graphic representation of the percentage of single Sy3 (blue) or single Sy2b (yellow) expressers versus double-expressers (green) contributing to the total number of Sy3<sup>+</sup> or Sy2b<sup>+</sup> alleles with time post stimulation (same data as for B).

(15). We reasoned that that might reflect an initial higher number of Sy3-expressing alleles.

To analyze monoallelic transcription, we created hemizygous mice in which one chromosome carried the 5'hs1RI deletion whereas the other chromosome was derived from ZI mice (Fig. 3A). Purified resting or LPS-activated splenic B cells were single-cell-sorted and assayed for monoallelic transcription of Sy3 and Sy2b, as described above. Interestingly, and in agreement with the high levels of Sy3 transcripts detected in resting B cell populations (15), already at 0 h, over 40% of alleles expressed Sy3, while only 6.5% transcribed Sy2b and 4% coexpressed Sy3 and Sy2b (Fig. 3B–D). Upon LPS stimulation, while the number of Sy2b expressers tended to increase, the number of Sy3 expressers kept decreasing to a minimum of 5% at 42 h ( $P = 0.005$  between 0 and 42 h) (Fig. 3B–D). Importantly, there was a dramatic increase in the number of Sy3<sup>+</sup>Sy2b<sup>+</sup> alleles with time ( $P = 0.0006$  between 0 and 42 h) (Fig. 3B–D), and at 42 h 67% of alleles were double-expressers (Fig. 3B). Accordingly, the majority of Sy3<sup>+</sup> and Sy2b<sup>+</sup> at this time corresponded to Sy3<sup>+</sup>Sy2b<sup>+</sup> alleles (Fig. 3D). The percentage of single- and double-expressers remained virtually unchanged between 36 and 42 h ( $P = 0.8956$ ) (Fig. 3B and C and SI Appendix, Figs. S3 and S6), indicating that the population had reached equilibrium at 36 h. Moreover, since the number of Sy3<sup>+</sup> alleles kept decreasing (Fig. 3E), while the percentage of both Sy2b<sup>+</sup> and Sy3<sup>+</sup>Sy2b<sup>+</sup> increased (Fig. 3B and C), the *Iy2b* promoter was likely activated on alleles that had already activated the *Iy3* promoter.

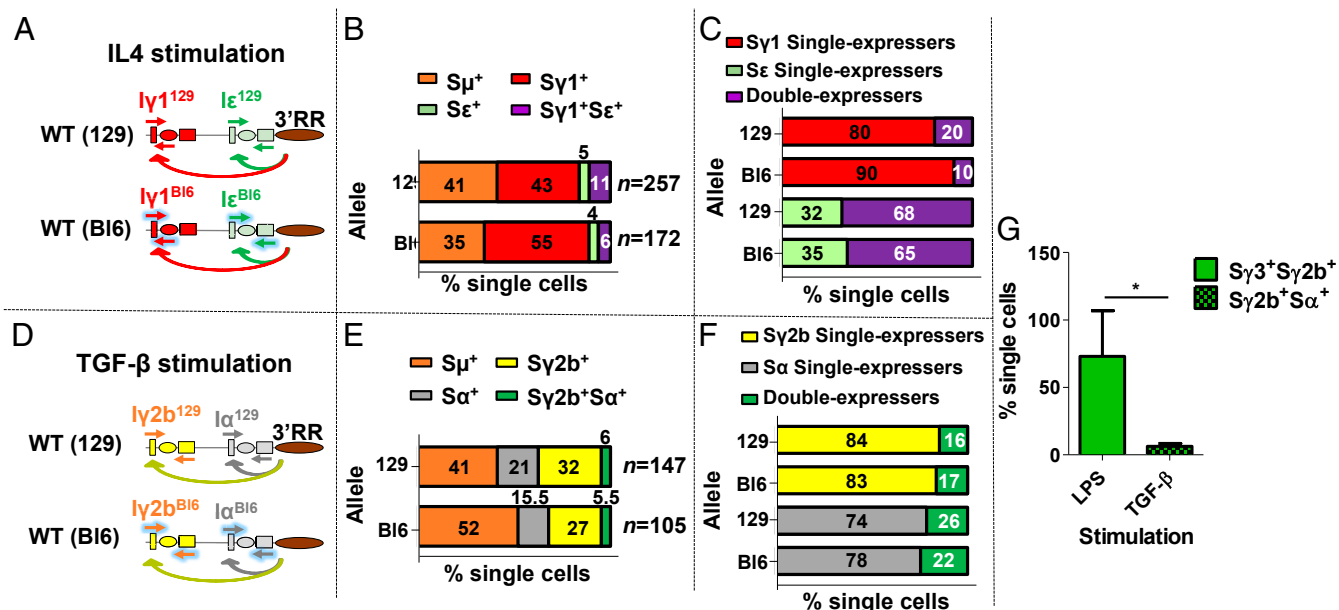
Thus, initial activation of the *Iy3* promoter did not preclude monoallelic activation of the *Iy2b* promoter.

#### I Promoters Induced by Different Stimuli Compete for 3'RR Activity.

The data above suggest that, after LPS stimulation, Sy3<sup>+</sup>Sy2b<sup>+</sup> coexpressing alleles are in the majority. However, stimuli other than LPS trigger activation of more than one promoter pair (Fig. 1A). We wondered whether 3'RR-mediated coactivation of I promoters is a general feature or if instead I promoters activated by other stimulations compete for the 3'RR. In the absence of mutations that fully repress transcription from promoter pairs, we took advantage of the existence of polymorphisms between the γ1, γ2b, ε, and α constant genes of 129Sv1 and C57BL/6J mouse strains and designed polymorphic primers that distinguish transcripts originating from one or the other allele (SI Appendix, Table S1). We obtained “heterozygous” mice (here called 129/Bl6) resulting from the crossing between 129Sv1 (129) and C57BL/6J (Bl6) WT mice (Fig. 4A) and validated the strain specificity of the polymorphic primers at the population and single-cell levels (SI Appendix, Fig. S7).

To determine the extent of coactivation of *Iy1* and *Iε* promoters on the same allele, purified splenic B cells from 129/Bl6 mice were stimulated with IL4 for 42 h, single-sorted, and assayed for allele-specific transcription of Sy1 and Se. Each single-cell plate was tested for one allele; that is, in “129 plates,” we detected 129-specific Sy1 (Sy1<sup>129</sup>) and Se (Se<sup>129</sup>) transcripts, while in “Bl6 plates,” we checked transcription of Bl6-specific Sy1 (Sy1<sup>Bl6</sup>) and Se (Se<sup>Bl6</sup>) (Fig. 4A). We found that, while half





**Fig. 4.** Promoters that respond to stimuli other than LPS compete for the 3'RR activity. (A) Scheme representing the two WT alleles from 129Sv1 and C57BL/6J strains.  $I\gamma 1$  and  $I\epsilon$  promoters are induced by IL4. The  $S\gamma 1$  and  $S\epsilon$  transcripts originating from the two alleles can be distinguished by using polymorphic primers. (B) Percentages of single- and double-expressers upon single-chromosome RT-qPCR. Purified B cells from  $n = 2$  129Sv1/C57BL/6J mice were grown in the absence (0 h) or presence of IL4 for 42 h. The percentages of  $S\mu^+$  (orange),  $S\gamma 1$  (red),  $S\epsilon$  (green), or both  $S\gamma 1$  and  $S\epsilon$  (purple) are indicated ( $n$  indicates the number of single cells analyzed). (C) Graphic representation of the percentage of  $S\gamma 1$  single-expressers (red) or  $S\epsilon$  single-expressers (green) versus double-expressers (purple) contributing to the total number of  $S\gamma 1^+$  and  $S\epsilon^+$  cells in each allele (same data as in B). (D)  $I\gamma 2b$  and  $I\alpha$  promoters are induced by TGF-β. (E) Percentages of single- and double-expressers upon single-chromosome RT-qPCR. Purified B cells from  $n = 3$  129Sv1/C57BL/6J mice were grown in the absence (0 h) or presence of TGF-β for 42 h. The percentages of  $S\mu^+$  (orange),  $S\gamma 2b^+$  (yellow),  $S\alpha^+$  (gray), or  $S\gamma 2b^+S\alpha^+$  (green) are indicated ( $n$  indicates the number of single cells analyzed). (F) Graphic representation of the percentage of  $S\gamma 2b$  single-expressers (yellow) or  $S\alpha$  single-expressers (gray) versus double-expressers (green) contributing to the total number of  $S\gamma 2b^+$  and  $S\alpha^+$  cells in each allele (same data as in E). (G) Comparison of the percentage of  $S\gamma 2b$  double-expressers after LPS ( $S\gamma 3^+S\gamma 2b^+$ ) or TGF-β ( $S\gamma 2b^+S\alpha^+$ ) stimulation (same data as for B and Fig. 2B).

of I promoters by the 3'RR superenhancer in response to different inducers.

Upon LPS stimulation, the 3'RR coactivated mainly  $I\gamma 3$  and  $I\gamma 2b$  promoters, and the  $I\gamma 2b$  promoter was often activated on alleles that had previously activated  $I\gamma 3$ . The lack of significant competition between the two promoters suggests that 3'RR activity, RNA polymerase II, and transcription factors are not limiting, that both promoters are in close proximity to the 3'RR, and that initial activation of one promoter does not exclude activation of the other. The latter point was made clear when we examined  $5'hsIRI^A$  alleles, which are initially almost exclusively  $S\gamma 3^+$  and progressively become  $S\gamma 3^+S\gamma 2b^+$  double-expressers. The large number of  $5'hsIRI^A$  alleles transcribing  $S\gamma 3$  before LPS stimulation suggests that the  $I\gamma 3$  promoter already lies in close proximity to the 3'RR at this time. Indeed, chromosome conformation capture studies detected a relatively high frequency of cross-linking between the 3'RR and  $I\gamma 3$  in resting B cells (21–23). These findings are consistent with a model in which the  $I\gamma 3$  promoter is first activated due to its proximity to the 3'RR (15), while  $I\gamma 2b$  is recruited to the 3'RR- $I\gamma 3$  hub after LPS stimulation only. This indicates that 3'RR,  $I\gamma 3$ , and  $I\gamma 2b$  lie in an LPS-induced productive spatial proximity where factors such as RNA polymerase II and transcriptional/architectural factors may contribute to bridge (8) the 3'RR with  $I\gamma 3$  and  $I\gamma 2b$ .

Transcription occurs in bursts (8, 24) and, suggesting that coactivation and bursting are not mutually exclusive processes, it has been reported that promoters sharing a distant enhancer are activated in coordinated transcriptional bursts (25). Our assay cannot determine if the  $I\gamma 3$  and  $I\gamma 2b$  promoters are activated simultaneously in double-expressing alleles. However, non-synchronized activation would require fast shift of the 3'RR from one promoter to the other, and given the high percentage of

cotranscribing alleles and the comparable half-lives of transcripts, this is unlikely.

Following IL4 or TGF-β stimulation, the majority of alleles displayed promoter competition. Nonetheless, the patterns were different. While, similarly to previous findings (16),  $S\gamma 1^+$  single-expressers prevailed over  $S\epsilon^+$  single-expressers, there were similar percentages of single  $S\gamma 2b^+$  and  $S\alpha^+$  alleles. This might be due to the intensity of the signal, the promoters' strength and their affinity for transcription factors, the kinetics of transcription factor assembly, preferential interactions between individual 3'RR enhancers and I promoters (22, 26), or, as is likely the case for  $I\gamma 1$ , association with a proximal (27) and a distal enhancer (28, 29).

CSR to IgE can occur directly ( $S\mu/S\epsilon$ ) or sequentially ( $S\mu/S\gamma 1$  and then  $S\epsilon$ ) (e.g., refs. 30–33). While direct  $S\mu/S\epsilon$  switching could occur on  $S\epsilon^+$  alleles, sequential switching could occur on  $S\gamma 1^+S\epsilon^+$  double-expressers. This does not exclude the possibility that  $I\epsilon$  promoter can be activated in an IgG1-expressing B cell intermediate (having deleted  $I\gamma$  subsequent to  $S\mu/S\gamma 1$  switching), which may be relevant to IgG1-driven generation of IgE-producing cells in secondary responses (34).

Previous analyses of the well-studied  $\beta$ -globin locus led to conflicting results regarding promoter competition and the effect of proximity between promoter and  $\beta$ -globin superenhancer for promoter activation (e.g., refs. 35–37). Nevertheless, the most recent evidence shows that, in their endogenous context, two distant but developmentally synchronized promoters can be coactivated by the  $\beta$ -globin superenhancer and that the previously activated proximal promoters do not interfere (37). The  $\beta$ -globin and  $IgH$  superenhancers differ not only in terms of tissue specificity, but also in the fact that, while the former is developmentally regulated, the latter is induced, at least in activated mature B cells. Significantly, prevalence of  $I\gamma 1$  over  $I\epsilon$  and the

relative balance between activation of  $I\gamma 2b$  and  $I\alpha$  occur despite the 3'RR-proximal location of the  $I\epsilon$  and  $I\alpha$  promoters. Moreover, coactivation of  $I\gamma 3$  and  $I\gamma 2b$  takes place despite initial activation of the more distant  $I\gamma 3$  promoter. Additionally,  $I\gamma 1$ , located between  $I\gamma 3$  and  $I\gamma 2b$  promoters, does not interfere with  $I\gamma 3$  and  $I\gamma 2b$  coactivation not because it is activated at a distinct developmental stage, but due to the nature of the inducer. Clearly, the physical arrangement of I promoters along the locus does not play a significant role in the mode of 3'RR-mediated activation. The type of activation may instead be the most important factor regarding how the promoter is activated. The  $I\gamma 2b$  promoter was almost always coactivated with  $I\gamma 3$ , after LPS stimulation, and almost never coactivated with  $I\alpha$ , upon TGF- $\beta$  stimulation.

We have previously proposed that the 3'RR,  $I\gamma 3$ , and  $I\gamma 2b$  proximal positioning evolved, at least in part, to enable rapid generation of IgG3 and IgG2b by marginal-zone B cells, which mainly switch to IgG3 and, to a lesser extent, IgG2b, in response to T cell-independent antigens (15). Fast coactivation of  $I\gamma 3$  and  $I\gamma 2b$  promoters may have also evolved as a safety mechanism to cope with the presence of a nonexpressed allele in the same cell. In contrast, it takes days for germinal centers to form and CSR and selection mechanisms to proceed in T-dependent responses. Therefore, the fact that  $I\gamma 3$  and  $I\gamma 2b$  are mainly coactivated while other promoters mostly compete for 3'RR activity may relate to the kinetics of primary immune responses against T-independent and T-dependent antigens.

Our results showing that the  $IgH$  superenhancer can activate transcription from two promoters in the same allele, regardless

of the position of the promoter, may also be relevant for the onset of cancer. The 3'RR can long-range-activate the translocated *c-myc* oncogene (38). The translocated *c-myc* can accompany CSR to downstream S regions during tumor progression (39), and the coactivating capacity of the 3'RR may facilitate this process by activating both the translocated *c-myc* and the I promoter of the target S sequence.

In conclusion we show that the  $IgH$  superenhancer *cis*-activates I promoters through two modes, coactivation and promoter competition. The nature of the inducer is critical in determining the prevalence of one mode over the other.

## Materials and Methods

**Mice and Ethical Guidelines.** Two to three mice of each genotype were used per experiment, and each experiment was repeated at least two times. We used mice of both genders at 5–7 wk. All experiments on mice were carried out according to the CNRS ethical guidelines and were approved by the Regional Ethical Committee (accreditation no. E31555005).

Additional materials and methods are provided in *SI Appendix*.

**ACKNOWLEDGMENTS.** We thank the Institut de Pharmacologie et de Biologie Structurale (IPBS) animal facility, the Imaging Core Facility TRI-IPBS, in particular Emmanuel Nasser, and the Purpan CPTP cytometry platform for their excellent work. TRI-IPBS has the financial support of ITMO Cancer Aviesan (Alliance Nationale Pour les Sciences de la Vie et de la Santé, National Alliance for Life Science and Health) within the framework of Cancer Plan. This work was supported by the Institut National du Cancer (INCA\_9363, PLBIO15-134), the Agence Nationale de la Recherche (ANR-16-CE12-0017), and the Fondation ARC pour la Recherche sur le Cancer (PJA 20141201647).

- D. Jung, C. Giallourakis, R. Mostoslavsky, F. W. Alt, Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Annu. Rev. Immunol.* **24**, 541–570 (2006).
- D. G. Schatz, Y. Ji, Recombination centres and the orchestration of V(D)J recombination. *Nat. Rev. Immunol.* **11**, 251–263 (2011).
- J. Stavnezer, J. E. Guikema, C. E. Schrader, Mechanism and regulation of class switch recombination. *Annu. Rev. Immunol.* **26**, 261–292 (2008).
- C. Boboila, F. W. Alt, B. Schwer, Classical and alternative end-joining pathways for repair of lymphocyte-specific and general DNA double-strand breaks. *Adv. Immunol.* **116**, 1–49 (2012).
- M. Hummel, J. K. Berry, W. Dunnick, Switch region content of hybridomas: The two spleen cell Igh loci tend to rearrange to the same isotype. *J. Immunol.* **138**, 3539–3548 (1987).
- E. Winter, U. Krawinkel, A. Radbruch, Directed Ig class switch recombination in activated murine B cells. *EMBO J.* **6**, 1663–1671 (1987).
- M. Bulger, M. Groudine, Functional and mechanistic diversity of distal transcription enhancers. *Cell* **144**, 327–339 (2011).
- E. E. M. Furlong, M. Levine, Developmental enhancers and chromosome topology. *Science* **361**, 1341–1345 (2018).
- A. A. Khamlichi, E. Pinaud, C. Decourt, C. Chauveau, M. Cogné, The 3' IgH regulatory region: A complex structure in a search for a function. *Adv. Immunol.* **75**, 317–345 (2000).
- C. Vincent-Fabert et al., Genomic deletion of the whole IgH 3' regulatory region (hs3a, hs1.2, hs3b, and hs4) dramatically affects class switch recombination and Ig secretion to all isotypes. *Blood* **116**, 1895–1898 (2010).
- M. Cogné et al., A class switch control region at the 3' end of the immunoglobulin heavy chain locus. *Cell* **77**, 737–747 (1994).
- J. P. Manis et al., Class switching in B cells lacking 3' immunoglobulin heavy chain enhancers. *J. Exp. Med.* **188**, 1421–1431 (1998).
- K. J. Seidl et al., Position-dependent inhibition of class-switch recombination by PGK-neo cassettes inserted into the immunoglobulin heavy chain constant region locus. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3000–3005 (1999).
- Z. Oruc, A. Boumediène, M. Le Bert, A. A. Khamlichi, Replacement of Iggamma3 germline promoter by Iggamma1 inhibits class-switch recombination to IgG3. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 20484–20489 (2007).
- F. Z. Braikia et al., Inducible CTCF insulator delays the  $IgH$  3' regulatory region-mediated activation of germline promoters and alters class switching. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 6092–6097 (2017).
- L. V. Wu, M. J. Stubbington, M. Daly, S. A. Teichmann, C. Rada, Intrinsic transcriptional heterogeneity in B cells controls early class switching to IgE. *J. Exp. Med.* **214**, 183–196 (2017).
- S. C. Li et al., Expression of I mu-C gamma hybrid germline transcripts subsequent to immunoglobulin heavy chain class switching. *Int. Immunol.* **6**, 491–497 (1994).
- J. H. Chung, A. C. Bell, G. Felsenfeld, Characterization of the chicken beta-globin insulator. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 575–580 (1997).
- J. M. Santos et al., Duplication of a germline promoter downstream of the IgH 3' regulatory region impairs class switch recombination. *Sci. Rep.* **8**, 9164 (2018).
- L. Delpy, M. Le Bert, M. Cogné, A. A. Khamlichi, Germ-line transcription occurs on both the functional and the non-functional alleles of immunoglobulin constant heavy chain genes. *Eur. J. Immunol.* **33**, 2108–2113 (2003).
- R. Wuerffel et al., S-S synopsis during class switch recombination is promoted by distantly located transcriptional elements and activation-induced deaminase. *Immunity* **27**, 711–722 (2007).
- M. Sellars, K. Reina-San-Martin, P. Kastner, S. Chan, Ikaros controls isotype selection during immunoglobulin class switch recombination. *J. Exp. Med.* **206**, 1073–1087 (2009).
- A. S. Thomas-Claudepierre et al., Mediator facilitates transcriptional activation and dynamic long-range contacts at the IgH locus during class switch recombination. *J. Exp. Med.* **213**, 303–312 (2016).
- D. Hnisz, K. Shrinivas, R. A. Young, A. K. Chakraborty, P. A. Sharp, A phase separation model for transcriptional control. *Cell* **169**, 13–23 (2017).
- T. Fukaya, B. Lim, M. Levine, Enhancer control of transcriptional bursting. *Cell* **166**, 358–368 (2016).
- J. Laurencikienė, V. Deveikaite, E. Severinson, HS1.2 enhancer regulation of germline epsilon and gamma2b promoters in murine B lymphocytes: Evidence for specific promoter-enhancer interactions. *J. Immunol.* **167**, 3257–3265 (2001).
- M. Z. Xu, J. Stavnezer, Regulation of transcription of immunoglobulin germ-line gamma 1 RNA: Analysis of the promoter/enhancer. *EMBO J.* **11**, 145–155 (1992).
- J. Medvedovic et al., Flexible long-range loops in the VH gene region of the Igh locus facilitate the generation of a diverse antibody repertoire. *Immunity* **39**, 229–244 (2013).
- A. V. Predeus et al., Targeted chromatin profiling reveals novel enhancers in Ig H and Ig L chain loci. *J. Immunol.* **192**, 1064–1070 (2014).
- K. Yoshida et al., Immunoglobulin switch circular DNA in the mouse infected with *Nippostrongylus brasiliensis*: Evidence for successive class switching from mu to epsilon via gamma 1. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7829–7833 (1990).
- R. Mandler, F. D. Finkelman, A. D. Levine, C. M. Snapper, IL-4 induction of IgE class switching by lipopolysaccharide-activated murine B cells occurs predominantly through sequential switching. *J. Immunol.* **150**, 407–418 (1993).
- T. Zhang et al., Downstream class switching leads to IgE antibody production by B lymphocytes lacking IgM switch regions. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3040–3045 (2010).
- D. R. Wesemann et al., Immature B cells preferentially switch to IgE with increased direct S $\mu$  to S $\epsilon$  recombination. *J. Exp. Med.* **208**, 2733–2746 (2011).
- J. S. He et al., IgG1 memory B cells keep the memory of IgE responses. *Nat. Commun.* **8**, 641 (2017).
- X. Hu et al., Promoters of the murine embryonic beta-like globin genes Ey and betah1 do not compete for interaction with the beta-globin locus control region. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1111–1115 (2003).
- W. Deng et al., Reactivation of developmentally silenced globin genes by forced chromatin looping. *Cell* **158**, 849–860 (2014).
- A. Allahyar et al., Enhancer hubs and loop collisions identified from single-allele topologies. *Nat. Genet.* **50**, 1151–1160 (2018).
- M. Gostissa et al., Long-range oncogenic activation of Igh-c-myc translocations by the Igh 3' regulatory region. *Nature* **462**, 803–807 (2009).
- S. Janz, Myc translocations in B cell and plasma cell neoplasms. *DNA Repair (Amst.)* **5**, 1213–1224 (2006).