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By their impact on nuclear organisation, enhancers are master regulators of cell fate.[1,2] The immunoglobulin heavy chain (IgH) locus undergoes numerous changes (such as transcription, accessibility, DNA breaks and mutations) throughout B-cell differentiation. Several of these events are controlled by the IgH 3' regulatory region (3'RR). The 3'RR is the master control element of mature B-cell IgH transcription,[3] somatic hypermutation (SHM),[4,5] conventional class switch recombination (CSR),[6-11] and locus suicide recombination (LSR).[12] In contrast the 3'RR was expected to be dispensable for V(D)J recombination.[13,14] During B-cell development, the heavy and light chain loci are poised for their VDJ and VJ rearrangements, respectively. The IgH locus rearranges first with D-J joining at the pro-B-cell stages followed by V-DJ joining at the pre-B-cell stage. The Ig*k* locus is poised for VJ rearrangements at the pre-B cell stage. A transient association (*trans*-mediated by Igκ enhancer elements) between IgH and Ig*k* loci has been demonstrated at the pre-B cell stage.[15,16] Recent, unexpected and novel findings reported that the 3'RR acts as a *cis* transcriptional silencer of sense and antisense germinal V, D and J transcription at the pro-B cell stage.[17,18] In light of these intriguing 3'RR features, we undertook the current study to determine if such a *trans* silencing effect could also be found on Ig*k* transcription.

Our research has been approved by our local ethics committee review board (Comité Régional d'Ethique sur l'Expérimentation Animale du Limousin, Limoges, France) and carried out according the European guidelines for animal experimentation. Pro-B cell experiments were performed with RAG-deficient ($RAG^{-/-}$) and double $3'RR^{6}$-RAG-deficient ($\Delta 3'RR–RAG^{-/-}$) mice developed in our animal facility. Femoral pro-B cells were recovered with the EasySep^{TM} mouse B-cell isolation Kit (STEMCELL Technologies, France). RNA was extracted using Trisol (ThermoFisher Scientific) according to manufacturer's instructions. Two pooled RNA samples (from four to six mice) were obtained for each genotype. RNA libraries were obtained using TruSeq Stranded Total RNA with Ribo-Zero Gold (Illumina),

according to manufacturer's instructions. RNAseq experiments were done by the genomics platform of Nice Sophia Antipolis as previously reported.[7,8,18] Data were deposited in Gene Expression Omnibus under the accession number GSE117449. Mature B-cells ($CD43^-$ splenocytes) were obtained from four 129 *wt* mice (Charles Rivers Laboratories, France) and four Δ3'RR mice (in a 129 background) before and after 48h of *in vitro* stimulation ($1x10^6$ cells per ml in RPMI 1640 with 10% fetal calf serum) with 5μg/ml LPS. Two pooled RNA samples (from two mice each) were obtained for each genotype. RNAseq experiments were done as above and RNAseq data were deposited with the accession number GSE90760.

Femoral pro-B cells were isolated from $RAG^{-/-}$ and Δ3'RR-$RAG^{-/-}$ mice to explore potential transcriptional cross-talk between IgH and Igκ loci in immature B-cells. A schematic representation of these two loci is reported in Figure 1A. RNAseq experiments led to an unexpected novel finding. Deletion of the IgH 3'RR enhancer markedly enhanced sense and antisense transcription of the Igk locus in *trans* in pro-B cells (Figure 1B). This effect was not found in mice deficient for the IgH Eμ enhancer (ΔEμ-$RAG^{-/-}$ mice); the Eμ enhancer (located in bleu in Figure 1A) being the major control element for IgH VDJ recombination (data not shown).[2] As a positive control we found no such *trans* effect on the Igλ locus. We next examined if this effect could be detected in mature B-cells. Deletion of the 3'RR had no *trans* silencer (nor activator) effect on Igk (and Igλ) transcription in resting and LPS-stimulated splenocytes (Figure 1C and 1D). These results are expected since a close association between the Ig*k* and IgH loci has not been reported in mature B cells.

The concept of a pro-B 3'RR *cis*-mediated transcriptional silencing activity was first reported (using RT-QPCR) by Braikia and coll,[17] and recently confirmed (using RNAseq analysis) by us.[18] The current study is the first report of a *trans* silencing effect of the 3'RR. This effect is found at the Ig*k* locus known to have a feedback inhibition effect on the establishment of allelic exclusion of the IgH locus in pre-B cells. The present study reinforces the concept of a

mutual crosstalk through enhancer/silencer effects between IgH and Ig*k* loci during immature B-cell stages. It is possible that the *trans* silencer effect of the 3'RR on Igκ transcription would use the same mechanism as that of its *cis* silencing effect on transcription of V, D and J segments of the IgH locus. The 3'RR *trans* silencing on the Igκ locus would be of interest to prevent its usage until the end of IgH D-J recombination. Clearly the resolution of how the 3'RR mediates *trans* transcriptional silencing on the Igκ locus is an exciting challenge to meet.

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Legend to Figure

Figure 1: Influence of the 3'RR enhancer on Igκ transcription in pro-B cells.

A: Schematic representation of the IgH, Igκ and Igλ loci (not to scale). B: Igκ and Igλ sense (top) and antisense (bottom) transcription in pro-B cells of $RAG^{-/-}$ and $RAG^{-/-}$Δ3'RR mice. C: Igκ and Igλ sense and antisense transcription in resting splenocytes of 129 *wt* mice and Δ3'RR mice. D: Igκ and Igλ sense and antisense transcription in LPS-stimulated splenocytes of 129 *wt* mice and Δ3'RR mice. Same mice as in C. E: Quantitative representation for C_κ and C_λ transcription (in reads per million). Error bars show extreme values of 2 independent experiments. Same samples as in C and D

A

Igκ on chromosome 6

Igλ on chromosome 16

IgH on chromosome 12

B

C

D

E

Igκ locus

Igλ locus

Pro-B cells

B splenocytes

resting

LPS-stimulated

Mean expression (rpm)

RAG-/-

RAG-/- Δ 3'RR

wt

Δ 3'RR