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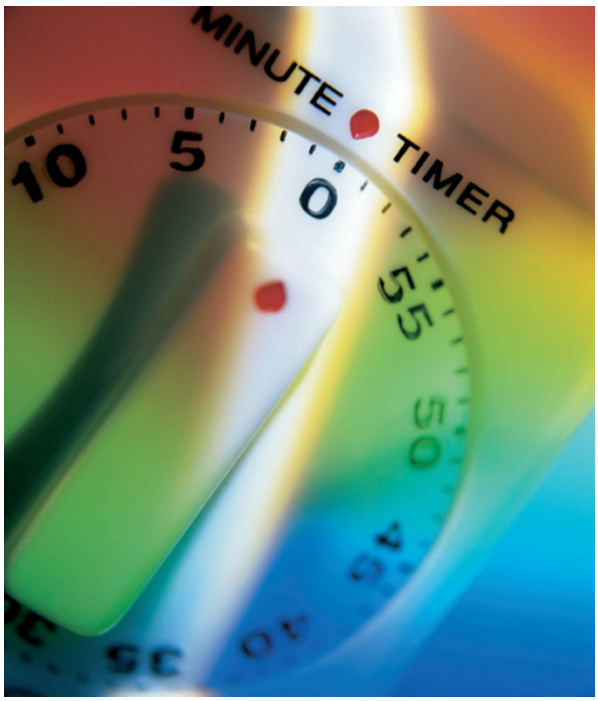
GENE REGULATION

Timing is everything

The enormous diversity of the T-cell receptor (TCR) repertoire is generated by recombination of variable (V), diversity (D) and joining (J) gene segments. These segments must be joined in the correct linear orientation to generate a functional TCR, but they must also be rearranged sequentially in the correct order. This study describes a mechanism to explain this temporal regulation of gene rearrangement on the basis of preferential targeting of the recombination machinery.



Recombination between appropriate gene segments is mediated by binding of the recombination-activating proteins (RAG1 and RAG2) to the recombination signal sequences (RSSs) that flank V, D and J gene segments according to the '12–23 rule', which allows recombination only between RSSs that have spacers of different length (12 base pairs or 23 base pairs, known as 12-RSS and 23-RSS, respectively). This ensures that V, D and J gene segments are joined in the correct sequence and orientation. However, until now it has been unclear how D_β–J_β joining normally precedes V_β–DJ_β rearrangement of the mouse TCRβ locus (*Tcrb*).

The authors identified a binding site for the transcription factor complex AP1 in the 3′ 23-RSS of mouse D_β, which is the first gene segment to be recombined (with the 5′ 12-RSS of J_β). This AP1-binding site was highly conserved in the D_β 23-RSSs of other species but was not found in the D_β 5′ 12-RSS or in other RSSs flanking *Tcrb* gene segments. The expression of FOS, which is a component of the AP1 complex, was upregulated during VDJ_β recombination in double-negative (DN) thymocytes and then downregulated after recombination was complete, and FOS was shown to bind to the putative AP1-binding site in the D_β 23-RSS. FOS was also found to interact with the RAG proteins in precipitation assays and to increase the deposition of RAG proteins onto the D_β 23-RSS. This resulted in a significant increase in the formation of D_β 23-RSS recombination products when FOS was overexpressed in an *in vitro* recombination assay.

In vivo, FOS-deficient mice had marked inhibition of thymocyte development with a block at the DN2 and DN3 stages, which is consistent with the decrease in D_β–J_β recombination that was observed. Furthermore, several V_β–D_β rearrangements were detected in thymic DNA from FOS-deficient mice, but not wild-type mice, which indicates that *Tcrb* recombination ordering is affected in the absence of FOS.

Therefore, these results support a 'RAG deposition' hypothesis to explain the ordering of *Tcrb* recombination, whereby RAG proteins are preferentially targeted to the D_β 23-RSS by FOS binding to promote recombination of this segment first. Interestingly, deletion mutants were used to show that the transcriptional activity of FOS is not required for this regulatory mechanism, which is the first example of a tissue-specific role for FOS that does not involve transcriptional activity.

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ORIGINAL RESEARCH PAPER Wang, X. *et al.* Regulation of *Tcrb* recombination ordering by c-Fos-dependent RAG deposition. *Nature Immunol.* 25 May 2008 (doi:10.1038/ni.1614)