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An autoregulatory mechanism imposes epigenetic control on the V(D)J recombinase

Inventory of Supplemental Information

1. Supplemental figures and legends

Fig. S1 amplifies the data presented in Fig. 1A, B and C. Fig. S1A is a diagram of mutants assayed in Fig. 1A, B and C. Fig. S1B, C, H and I provide data that validate or extend the interpretations of Fig. 1A and B. Fig. S1D, E, F, G, J and K provide data that confirm the results represented by Fig. 1A and C.

Fig. S2 supports and extends results presented in Fig. 1F and G. Fig. S2A and B support our description of the scheme used to purify RAG protein. Fig. S2C and D replicate the assay of Fig. 1F and G under conditions in which the 23-RSS substrate is radiolabeled, rather than the 12-RSS substrate.

Fig. S3 provides the burst kinetic data used to normalize activities of wild-type and mutant RAG proteins for comparison in assays for catalysis and substrate binding.

Fig. S4 provides data that support experiments to assess the effects of the RAG-2 388/405A₁₈ mutation on RAG activity. Fig. S4A and B demonstrate that the 388/405A₁₈ mutant retains the ability to bind H3K4me₃; these panels are related to Figs. 2, 3 and 4. Fig. S4C and D confirm and extend the results of Fig. 2. Fig. S4D and E support Fig. 4 by providing reference measurements obtained with wild-type RAG-2.

2. Supplemental table

Table S1 displays for ease of comparison the values for substrate affinity and catalytic rate constant obtained for RAG variants in Figs. 3 and 4 and Fig. S4.

3. Supplemental experimental procedures

Expression constructs with list of primers used to construct mutants, detailed protocols for protein purification, extrachromosomal recombination assays, assays for endogenous recombination, burst kinetic analysis, coupled DNA cleavage, DNA nicking and surface plasmon resonance.

4. Supplemental references (9)