V(D)J RECOMBINATION

RAG recombination centres

The recombination of variable (V), diversity (D) and joining (J) gene segments that form immunoglobulins and T cell receptors is essential for the generation of a highly diverse repertoire of antigen receptors. V(D)J recombination is initiated by binding of recombination-activating gene 1 (RAG1) and RAG2 to recombination signal sequences (RSSs) that flank these gene segments. Schatz and colleagues have now directly assessed RAG protein binding at antigen receptor loci *in vivo* and found that binding occurs at small regions of active chromatin encompassing J (and where present J-proximal D) segments of Igκ, Igh, Tcrα and Tcrβ, which they have termed recombination centres. RAG1 and RAG2 can bind independently at these sites, and the formation of the recombination centres is tightly regulated during lymphocyte development.

The authors generated mutant mice in which the RAG proteins could interact with and bind to DNA normally without initiating V(D)J recombination and assessed RAG protein binding, as well as the chromatin activation status, in purified lymphocyte populations using chromatin immunoprecipitation.

At the Igκ locus of pre-B cells, the RAG proteins associated with the Jκ but not Vκ gene segments. The binding region contained highly active chromatin, as determined by high levels of histone 3 trimethylation at lysine 4 (H3K4me3) and H3 acetylation, as well as RNA polymerase II occupancy. Binding of RAG1 and RAG2 could occur independently of each other, suggesting the possibility that RAG1 and RAG2 may be differentially recruited into the recombination centre. Rearrangement of the Igκ locus is upregulated during pro-B to pre-B cell transition, and binding of the RAG proteins at the Jκ gene segments and the level of active chromatin at these sites was highly increased in pre-B cells compared with pro-B cells.

Similarly, RAG protein binding (both together and separately) at the Tcrα locus of pre-T cells occurred at the Jα cluster in a region of active chromatin. By contrast, the recombination centre had low levels of H3K4me3 in pro-T cells, in which recombination does not occur, and RAG proteins did not bind to any of the Tcrα gene segments analysed in these cells.

In pro-B cells, RAG protein binding at the Igh locus occurred at the Jh and proximal DQ52 gene segments but not at other D or V gene segments. Again, RAG binding strongly correlated with high levels of active chromatin. However, although RAG2 could bind in the absence of RAG1, RAG1 required the presence of RAG2 for binding. Finally, RAG protein binding (both together and separately) occurred at Dβ–Jβ clusters but not at other regions of the Tcrβ locus in pro-T cells. Of note, RAG binding at these sites in the Igh and Tcrβ loci persisted in pre-B and pre-T cells, respectively.

Further analyses showed that the RSSs were required for strong binding of RAG1 and RAG2 to the recombination centres, and mutations in RAG1 at three known RSS-binding residues decreased RAG1 recruitment to the RSSs. RAG2 could also bind to numerous sites with high levels of H3K4me3 that lacked RSSs. Mutation of the plant homeodomain (PHD) of RAG2, which abrogates H3K4me3 binding, decreased its association with numerous H3K4me3-containing genes, suggesting that RAG2 binds broadly to the genome, whereas RAG1 binding is restricted to RSSs.

The lineage- and developmental stage-specific binding of RAG proteins to specific recombination centres supports the suggestion that these sites coordinate V(D)J recombination but could also contribute to the aberrant recombination events associated with lymphomas.

Olive Leavy

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