HIGHLIGHTS

LYMPHOCYTE DEVELOPMENT

Damage limitation

Sun-block, anti-oxidant supplements, organic food — we go to great lengths and expense to protect ourselves from cancer by essentially trying to keep our genomes intact. But everyday, the integrity of our genomes is deliberately breached millions of times. These breaks occur in developing lymphocytes as they rearrange their V (variable), D (diversity) and J (joining) gene segments to form antigen receptors, and they might be one reason why lymphoid malignancies are relatively common. What is amazing is that most of us survive all this genomic cutting and pasting. Clearly, there must be mechanisms that keep V(D)J recombination on track. Now, two studies in *The EMBO Journal* describe protective mechanisms that are intrinsic to the V(D)J recombinase-activating genes 1 and 2 (RAG1/2).

RAG1/2 cleaves DNA at specific sites that flank V, D and J genes. But the RAG proteins can also mediate transposition, a reaction that can potentially lead to chromosomal translocations. Evidence that this reaction occurs *in vivo* has been difficult to find but, recently, translocations with all the hallmarks of RAG-mediated transposition were reported in human T-cell lymphomas, which proves that the threat is real.

Several damage-limitation mechanisms have been invoked to explain the rarity of RAG-meditated transposition *in vivo*. One proposal is that transposition might be limited by the activity of 'dispensable' domains outside the core catalytic unit of the RAG proteins (the amino-terminal region of RAG1 and the carboxy-terminal region of RAG2). This possibility was tested by Tsai and Schatz, and Elkin *et al*. Both groups found that, *in vitro*, full length RAG2 was less effective at mediating transposition than a truncated 'core' RAG2, although its capacity to cleave was the same or even better. So, the carboxy-terminal region of RAG2 can protect against transposition. This protective mechanism seems to operate after DNA cleavage, by inhibiting the capture of the target DNA.

In addition, noting that many other transposases are regulated by nucleotides, Tsai and Schatz examined the effects of various nucleotides on RAG1/2 activity. Physiological levels of GTP selectively inhibited transposition *in vitro*. And although they could not show GTP binding to RAG1 or RAG2, they did find a motif in RAG1 that resembled a nucleotide-binding domain. By mutating this motif, they generated a RAG1 protein that was resistant to GTP-mediated inhibition of transposition. This mechanism also seems to block the capture of the target DNA.

But do these test-tube mechanisms operate *in vivo*? It is certainly plausible and would be consistent with the idea that RAG proteins have a secondary function, acting as a scaffold for the resolution phase of the V(D)J recombination reaction. But a definitive answer must await an *in vivo* assay for RAG-mediated transposition.

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References and links

ORIGINAL RESEARCH PAPERS Elkin, S. K. *et al*. The C-terminal of RAG2 protects against transposition *in vitro*. *EMBO J*. **22**, 1931–1938 (2003) | Tsai, C. L. & Schatz, D. G. Regulation of RAG1/RAG2-mediated transposition by GTP and the C-terminal region of RAG2. *EMBO J*. **22**, 1922–1930 (2003)

WEB SITES

David Schatz's lab: http://www.hhmi.org/research/investigators/schatz.html

Marjorie Oettinger's lab: http://www.hms.harvard.edu/dms/bbs/bulletin/issues/2002/01/oettinger.html