# HIGHLIGHTS



*V(D)J* RECOMBINATION

## Split ends and hairpins

The process of *V(D)J* recombination — through which the variable domains of immunoglobulins and T-cell receptors are assembled — occurs by an ordered series of steps. One of these is the formation of DNA hairpins at the 'coding' ends of the variable (*V*), diversity (*D*) or joining (*J*) elements. These hairpins are subsequently opened, and the free DNA ends are pasted together by the non-homologous end joining (NHEJ) pathway.

Although the so-called 'RAG complex' is known to generate the hairpins, the factor that then opens them has proved elusive. Hence the excitement caused by a report in *Cell* from Klaus Schwarz, Michael Lieber and colleagues, who now identify this factor as a protein called Artemis. Not only that, but they show that Artemis is regulated by a component of the NHEJ pathway — the catalytic subunit of the DNA-dependent protein kinase (DNA-$PK_{cs}$).

Mice that lack DNA-$PK_{cs}$ show a strikingly similar phenotype to human cells that lack Artemis, so the authors wondered whether the two proteins might be involved in similar steps of the process — possibly as a complex. They used immunobead pulldown assays to show that DNA-$PK_{cs}$ and Artemis indeed form a complex *in vitro*, and confirmed this result *in vivo* by co-immunoprecipitation experiments.

As DNA-$PK_{cs}$ is a kinase, the authors next showed that it can phosphorylate Artemis. To test what effect this might have, they studied the activity of Artemis in the presence and absence of DNA-$PK_{cs}$. They found that Artemis alone is a 5′ to 3′ exonuclease; that is, it nibbles single-stranded DNA from the 5′ end. Add DNA-$PK_{cs}$, however, and this activity changes dramatically — Artemis switches to being an endonuclease, with a preference for cleaving within DNA at single-stranded/double-stranded DNA junctions.

Mice that lack DNA-$PK_{cs}$ or Artemis cannot complete the hairpin-opening step of *V(D)J* recombination, so Schwarz, Lieber and colleagues next asked whether DNA-$PK_{cs}$–Artemis might act on hairpins. Although Artemis alone had no effect, in the presence of DNA-$PK_{cs}$ it was able to cleave and open hairpins, and both the kinase activity of DNA-$PK_{cs}$ and its physical presence in a complex with Artemis were required for this effect.

The acid test was whether DNA-$PK_{cs}$–Artemis would open hairpins generated by the RAG complex. Schwarz, Lieber and colleagues used three different experimental configurations, and in each case they detected hairpin opening. In other words, "this is the first efficient opening of RAG-generated hairpins by any vertebrate nuclease in $Mg^{2+}$-containing solutions".

As well as unravelling two of the mysteries of *V(D)J* recombination — the identity of the hairpin-opening activity and the physiological substrate of DNA-$PK_{cs}$ — this study sheds light on the mechanism of

NHEJ. Although there is no requirement for a hairpin-opening activity in this process, the observed role of DNA-$PK_{cs}$–Artemis in processing DNA overhangs means that it should facilitate the ligation of DNA ends. The DNA-$PK_{cs}$–Artemis complex is therefore involved not only in immunoglobulin and T-cell-receptor development, but also in the response to double-stranded DNA breaks.

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### References and links

**ORIGINAL RESEARCH PAPER** Ma, Y. *et al.* Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and *V(D)J* recombination. *Cell* **108**, 781–794 (2002)