and adult mice can export T cells to the periphery in the absence of signals from CCR7. The authors suggest that CCR7-dependent migration to the medulla is more important for the export of T cells from the thymus in newborns than in adult mice, and they highlight the fact that REL-B-deficient mice, which lack thymic medullas, can export functional T cells.

These two studies show that signalling through CCR7 is important for intrathymic migration of T cells, but there is clearly more to be learned.

Elaine Bell

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B CELLS

AID provided by RPA

Antibody diversification through somatic hypermutation (SHM) — the process by which non-templated point mutations are introduced into the variable region of immunoglobulin heavy and light chains, thereby enabling the selection of B cells that generate higher-affinity antibodies — requires the single-stranded DNA (ssDNA) deaminase known as activation-induced cytidine deaminase (AID). However, the way in which AID is targeted to sites of mutation was unclear until a paper published in *Nature* identified replication protein A (RPA) as crucial for this process.

More than half of all SHMs occur around a DNA motif known as the RGYW motif (where R denotes A or G, Y denotes C or T, and W denotes A or T), leading to the suggestion that unknown factors might target AID to these sites. To identify these putative cofactors Chaudhuri *et al.* used an *in vitro* assay for AID activity. This assay relied on the observation that although AID purified from B cells can by itself deaminate ssDNA templates, it cannot by itself deaminate an actively transcribed double-stranded DNA SHM substrate (that is, a substrate containing multiple RGYW motifs that cannot form R loops — structures generated during transcription that displace the non-template strand as ssDNA). The ability of protein fractions isolated from B cells to facilitate AID deamination of the SHM substrate led to the identification of a heat-labile complementing factor (CF) of 120–170 kDa.

In further experiments, a 30–35 kDa protein that was highly enriched in fractions containing CF activity was shown to associate with AID. Subsequent analysis identified this as the 32 kDa subunit of RPA (RPA32). RPA is heterotrimeric — consisting of a 70 kDa (RPA70) and a 14 kDa (RPA14) subunit, in addition to RPA32 — and

has previously been implicated in DNA replication, recombination and repair. Confirmation of RPA as the CF was provided by the observation that recombinant human RPA containing all three subunits could replace CF in facilitating AID deamination of the actively transcribed SHM substrate. As well as complexes that contain AID, RPA and RGYW-containing DNA substrates, smaller complexes that contain only RPA and the RGYW-containing substrate were observed. Because these smaller complexes were not detected when the deamination reaction was inhibited, the authors suggest that AID-mediated deamination leads to the release of AID from the AID–RPA–DNA complex and that the DNA-bound RPA might enable the recruitment of DNA-repair proteins.

Interestingly, AID that was purified from the 293 non-lymphoid cell line ectopically expressing AID was unable to mediate deamination of the actively transcribed SHM substrate in the presence of RPA, and RPA and AID did not associate in this cell line. Because AID was found to be more highly phosphorylated in B cells than in the 293 cell line, these results indicate that the AID–RPA interaction is B-cell specific and that this specificity probably depends, at least in part, on the post-translational modification of AID.

This study identified RPA as the factor that targets AID to RGYW DNA motifs — that is, the sites of SHM — and it defined a mechanism by which SHM is a B-cell specific event. Further studies will focus on whether RPA has a similar function in class-switch recombination (which also requires AID), an outcome that the authors' initial experiments support.

Karen Honey

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