T-CELL SIGNALLING

SLAP and CBL cooperate to bring down TCRζ

A recent report in *Nature Immunology* describes evidence to support the hypothesis that the amount of T-cell receptor (TCR) expressed by double-positive (DP) thymocytes is controlled, at least in part, by a molecular pathway involving SLAP (SRC-like adaptor protein)-mediated recruitment of the E3 ubiquitin ligase CBL (Casitas B-lineage lymphoma; also known as c-CBL) to internalized TCR complexes, where CBL ubiquitylates the TCR ζ-chain (TCRζ), thereby targeting TCRζ for degradation.

DP thymocytes express substantially less TCR on their cell surface than do single-positive thymocytes and peripheral T cells. It has been suggested that this enables DP thymocytes to distinguish more sensitively between quantitative differences in signalling initiated by TCR interaction with different peptide–MHC complexes, something that is thought to be important in determining whether a DP thymocyte is positively selected, negatively selected or dies of neglect. Results of previous studies led Myers and colleagues to hypothesize that SLAP and CBL might function in the same molecular pathway to actively regulate TCR expression by DP thymocytes. So, they compared TCR and CD3 expression by DP thymocytes from mice lacking both SLAP and CBL, as well as from mice lacking either SLAP or CBL and from wild-type mice. Expression of TCRβ and CD3ε was substantially higher on the cell surface of $Sla^{-/-}$, $Cbl^{-/-}$ and $Sla^{-/-}Cbl^{-/-}$ DP thymocytes than on wild-type DP thymocytes, and the increase in TCRβ and CD3ε expression was similar for all three mutant cell types. Similarly, degradation of TCRζ was markedly impaired in $Sla^{-/-}$, $Cbl^{-/-}$ and $Sla^{-/-}Cbl^{-/-}$ DP thymocytes compared with wild-type DP thymocytes.

Consistent with this genetic evidence that placed SLAP and CBL in the same molecular pathway, the expression of cell-surface CD3ε and intracellular TCRζ was downregulated in Jurkat T cells that had been engineered to express both SLAP and CBL but not in cells engineered to express only SLAP or CBL alone. This downregulation of CD3ε and TCRζ expression in Jurkat T cells was abrogated if SLAP lacked its myristoylation site, its SRC homology 2 (SH2) domain or its carboxyl terminus, or if CBL lacked its RING (really interesting new gene)-finger domain (the domain of CBL that has E3 ubiquitin-ligase activity). Further analysis showed that expression of SLAP and CBL, but not SLAP or CBL alone, in Jurkat T cells induced ubiquitylation of TCRζ.

The protein tyrosine kinase LCK was also shown to be required for SLAP and CBL to mediate downregulation of CD3ε in Jurkat T cells, so the authors were able to extend their original hypothesis. They therefore propose that LCK phosphorylates TCRζ in fully assembled cell-surface TCR complexes. Also, after internalization, these complexes enter the endosomal compartment where the SH2 domain of SLAP interacts with phosphorylated TCRζ. In turn, SLAP recruits CBL, which ubiquitylates TCRζ, targeting it for degradation so that fully assembled TCR complexes do not recycle and, as a result do not accumulate at the cell surface.

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ORIGINAL RESEARCH PAPER Myers, M. D. *et al.* Src-like adaptor protein regulates TCR expression on thymocytes by linking ubiquitin ligase c-Cbl to the TCR complex. *Nature Immunol.* 4 Dec 2005 (doi:10.1038/ni1291)