

### **Analysis of T Cell Receptor:CD3 Complex Internalization in T Cell Development and Function**

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The TCR is a multimeric complex consisting of an  $\alpha\beta$  heterodimer and six CD3 chains ( $\zeta\eta\epsilon\delta\gamma\epsilon$ ). We have previously shown that wild-type but not tailless TCR:CD3 complexes are rapidly internalized when ectopically expressed on 293T cells, confirming suggestions that TCR internalization is mediated by motifs in the CD3 cytoplasmic domains. A number of studies have documented the interaction of the adaptor protein complex 2 (AP-2), involved in the transport of proteins from the plasma membrane, with the TCR:CD3 complex. However, it is still unclear what motifs mediate TCR internalization and what role this process plays in T cell development and function. We have identified two motifs that are essential for TCR internalization: a dileucine motif in CD3 $\zeta$  and several YXXL motifs in CD3 $\eta$ , with CD3 $\zeta$  being the most important. Furthermore, we have shown that AP-2 $\gamma$  specifically interacts with tyrosine residues in CD3 $\zeta$ , and to a lesser extent CD3 $\eta$ , but not CD3 $\epsilon$  and CD3 $\delta$  using a yeast two-hybrid assay. Importantly, this interaction was lost following mutation of the ITAM residues. Studies are underway to further define the residues that interact with the AP complex and mediate TCR internalization. Mice that lack the ability to internalize TCR are being generated. TCR expression in mice lacking the CD3 chains can be restored using a novel Picornavirus 2A peptide-linked multi-cistronic vector and retroviral-mediated stem cell gene transfer. Analysis of mice restored with wild-type and the mutant CD3 chains identified *in vitro* will allow us to assess the role of TCR internalization in T cell development and function.

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