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Syk dependent pre-TCR signaling precedes ZAP-70 protein upregulation

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Development of thymocytes occurs through very discrete and well-characterized stages that include specific checkpoints. Passage through the two major checkpoints critically depends on the signals generated by a T-cell receptor (TCR) based mechanism. Both stages, the early pre-TCR  $\beta$ -selection signal and the  $\alpha$  $\beta$ TCR positive selection signal („DP% stage), depend on Src-family and Syk-family kinases sequential activation for efficient signal generation. It is well established that Lck is the major Src-family kinase for both checkpoints, and also for antigen receptor signaling in peripheral T cells. ZAP-70 is known to be critical for positive selection and peripheral T cell signaling, but it is unknown if ZAP-70 or Syk is preferentially employed during the earliest checkpoint, pre-TCR signaling. Genetic ablation of either gene does not overtly impair progression at this stage, but the double  $\beta$ knock-out, completely blocks thymocyte development at this early stage, proving the requirement of at least one of the kinases. We have closely examined the expression patterns of these kinases during all stages of T cell development and find that ZAP-70 protein is not upregulated until immediately after pre-TCR signaling. This is soon followed by Syk downregulation as previously found in this laboratory. We also find that deletion of one gene does not result in compensatory upregulation of the other. We postulated that Syk-deficient thymocyte fitness should be decreased when compared to wild-type or even ZAP70 deficient thymocytes. Competitive repopulation assays confirmed this hypothesis; Syk deficient thymocytes rearrange TCR $\beta$  chains properly but do not enter blast phase efficiently and consequently decrease in frequency between DN3 and DN4 relative to wild-type or ZAP-70 deficient cells. We find that ZAP-70 deficient thymocytes progress through  $\beta$ -selection properly, demonstrating the specificity requirement of Syk for optimal pre-TCR signaling. Thus, we find that Syk is preferentially expressed and employed during pre-TCR signaling and that ZAP-70 protein is not upregulated until after this checkpoint.