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Meeting abstract

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Real time monitoring of B cell antigen receptor-proximal events by fluorescence lifetime imaging

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Many molecular events downstream of B cell antigen receptor engagement have been elucidated by different genetic and biochemical approaches. These studies revealed that a hallmark of BCR signalling is the assembly of a multiproteincomlplex comprising at least the SH2 domain-containing adapter protein of 65 kDa (SLP-65), Bruton's tyrosine kinase (Btk) and Phospholipase C-γ2 (PLC-γ2). Only in context of this so called Ca^{2+} initiation complex PLC-γ2 is activated and produces the second messengers Diacylglycerol and Inositol-1,4,5-trisphosphate. Hence, SLP-65 provides a molecular platform that links BCR engagement to the regulation of important transcription factors and the reorganization of the cytoskeleton. However, little is known about the kinetics and subcellular dynamics of Ca^{2+} initiation complex assembly. Here we report a real time imaging approach to monitor BCR-induced changes in the three dimensional structure of SLP-65. For this aim different dichroic fluorescent SLP-65 variants were expressed in *slp65*-/- DT40 B cells. In resting cells the conformation of SLP-65 allows for fluorescence resonance energy transfer (FRET) between both fluorophors that was monitored by measuring the fluorescence lifetime of respective donor fluorophors. BCR engagement significantly attenuated the efficiency of FRET indicating an induced conformational change in SLP-65. The kinetics of these changes correlated with that of BCR-induced Ca^{2+} mobilization.

Hence, this novel approach makes it possible to analyze the spatial and temporal dynamics of BCR-induced Ca^{2+} initiation complex assembly.