Molecular elucidation of the CBM complex in NF-kappaB activation by using single molecule imaging and structural studies

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Abstract

NF-κB family proteins are evolutionarily conserved master regulators of immune and inflammatory responses. NF-κB signaling has a crucial role in regulating the activation, proliferation and effectors functions of lymphocytes in adaptive immune responses. The CARMA1/Bcl10/MALT1 (CBM) complex is the central mediator of TCR and BCR induced NF-κB activation in lymphocytes. Mutations, chromosomal translocations and over-expressions of CBM component proteins have been shown to directly lead to non-Hodgkin's lymphomas. Elucidating the molecular basis of assembly and activation of the CBM complex could provide a platform for mechanistic understandings of the associated lymphomas and potential therapeutic target identification. Our research goals are to investigate the CBM mechanism in the NF-κB activation pathway by single molecule imaging and structural studies on the CARMA1/Bcl10, BCl10/MALT1 and CBM complex by combining electron microscopy and X-ray crystallography. We have succeeded in purifying the CARMA1/Bcl10/MALT1 complex and shown by negative staining EM that the CBM complexes form filamentous structures as well as BCl10 only. *In vitro*, polymerization assay indicated that CARMA1 nucleates Bcl10 filament assembly, which could promote downstream MALT1 activation. Based on the 3D map from negative stained electron microscopy and high-resolution individual structures of CARMA1/Bcl10 CARD domains, we reconstituted a structure model of CARMA1/Bcl10 filament. Single molecule imaging enables us to track after the filament formation of labeled BCl10 filaments and the nucleation of BCl10 by labeled CARMA1 and determine the filaments formation rates *in vitro* supported by kinetic studies. In addition, we could determine that polymerization BCl10 has polarity and the filament growth is only at one end. In addition, Real time Super Resolution Microscopy (STORM) measurements showed a surprising funding that CARMA1(8-302) is segmented along the filaments and incorporated within BCl10 filaments. At the moment, we are pursuing the determination of cryo-EM structures of BCl10 and BCl10/ MALT1 complex.