Regulation of the ER Ca^{2+} sensor STIM1 at the single-molecule level

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Precise regulation of store-operated calcium entry (SOCE) is essential for human health, as demonstrated by multiple serious pathologies induced by gain- or loss-of-function mutations in associated STIM and Orai genes. The primary regulator of SOCE is the ER Ca^{2+} sensor STIM1, which in resting cells is inactive and diffuse throughout the ER membrane but after ER Ca^{2+} release accumulates at ER-plasma membrane junctions where it binds, traps, and activates mobile Orai1 (CRAC) channels to activate SOCE. The coiled-coil 1 (CC1) domain of STIM1 sequesters the CRAC activation domain (CAD/SOAR) of STIM1 to prevent SOCE in resting cells. Following ER Ca^{2+} depletion CC1 is released, unveiling the CAD and extending it towards the plasma membrane where it can bind and activate Orai1. To better understand the changes in STIM1 structure as it transitions between inactive and active states, we applied a single-molecule FRET (smFRET) approach. Time-resolved smFRET measurements between >50 locations in the purified cytosolic domain of STIM1 (ctSTIM1; aa 234-685) were used to determine the arrangement and dynamics of interacting CC1 and CAD domains. Our results suggest that in the inactive state, CC1 binds to the CAD in a way that will sequester it against the ER membrane with the CAD apex facing the ER. Activation of ctSTIM1 by the Stormorken's syndrome mutation R304W released CC1 from CAD and triggered a conformational change in the CAD apex, a region thought to bind Orai1. The results imply that during activation STIM1 must undergo a remarkably large conformational change in which the CAD domain rotates 180o and translocates >10 nm toward the plasma membrane to bind Orai1. Potential intermediate states in this activation sequence will be discussed to help understand how this massive protein rearrangement occurs.