The molecular chaperone CCT: Functions beyond folding

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ABSTRACT

The chaperonin-containing tailless complex polypeptide 1 (CCT) is a eukaryotic ~1 MDa barrel shaped molecular chaperone, built up by eight distinct subunits and is required for the folding of the abundant cytoskeletal proteins actin and tubulin. CCT exists as an assembled oligomer, micro-complexes and as individual subunits in the cell. In addition to folding, CCT is connected to a variety of cellular processes that involve both assembled CCT oligomer and individual monomeric CCT subunits. Monomeric CCTδ translocates to the plasma membrane when overexpressed, indicating a function for CCTδ when not incorporated into the oligomer. One aim of this thesis is to understand the underlying monomeric function CCTδ. The dynactin complex component p150Glued was identified as a binding partner for monomeric CCTδ and together with the transmembrane protein dynAP, p150Glued;CCTδ creates an inward movement of the plasma membrane along microtubules, resulting in a curved membrane. We used fluorescence imaging and ToF-SIMS in cells overexpressing CCTδ and detected an increase in phosphatidylethanolamines (PE), lipids often found in membranes with high curvature. We show that one point-mutation in CCTδ can affect the assembly state of CCT, increasing the CCT oligomer pool and it abolishes the binding of CCTδ to p150Glued. Oligomeric CCT is known to interact with the transcription factor STAT3. Here, we show that STAT3 does not behave as an obligate folding substrate. Instead, IL-6 induced tyrosine phosphorylation is increased when CCT levels are reduced. Furthermore, CCT depletion does not affect other stages of STAT3 activation. Therefore it is possible that CCT regulates STAT3 phosphorylation levels by acting as a sequestering protein for STAT3. CCT oligomer can also interact with the capping and severing protein gelsolin and by high resolution microscopy we localize CCT and gelsolin the edge of protruding lamellipodia where extensive actin filament rearrangement occurs. By cryo-EM imaging using purified gelsolin:CCT complexes gelsolin is seen located deep in the CCT chaperonin cavity, suggesting a sequestering role for CCT. Consistently, in the presence of CCT, gelsolin is protected from caspase-3 cleavage. Taken together, we have identified the mechanism behind monomeric CCTδ at the plasma membrane and two possible sequestering protein interactions for CCT oligomer. Therefore, the work in this thesis extends the understanding of the non-folding properties for CCT.

Keywords: TRiC, chaperonin, CCT oligomer, CCT monomer, cancer, p150Glued, gelsolin, STAT3.