The Chaperonin Containing TCP-1: Interactions with the Mammalian Cytoskeleton

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Abstract

The eukaryotic Chaperonin Containing TCP-1 (CCT) is a heterooligomeric chaperonin essential for enabling the cytoskeletal proteins actin and tubulin to fold to their native state. The eight CCT subunits are encoded by individual genes and are present in cells as the ~960kDa oligomer, as components of micro-complexes, and as monomeric subunits. In addition to the well-characterised substrate folding mechanism of the CCT oligomer, roles for CCT subunits as monomeric proteins are emerging. The work in this thesis illustrates the dependence upon functional CCT and its roles in cytoskeletal organisation.
Levels of functional CCT have far-reaching implications for cellular functions dependent upon an intact cytoskeleton and siRNA targeting of CCT subunits results in growth arrest and reduced levels of native actin and tubulin. Targeting each CCT subunit individually by siRNA revealed different effects upon cytoskeletal organisation, suggestive of distinct roles for specific CCT monomers. Cell shape and microfilament polymerisation are influenced by CCT monomers with CCTε levels appearing particularly important for these processes. Furthermore, CCTε and to a lesser extent ζ and θ were found to co-localise to microfilaments and CCT subunits associated with non-soluble protein assemblies following detergent extraction are predominantly monomeric. Gelsolin, an actin filament severing and capping protein was identified as a CCT-binding protein, providing another link between CCT activity and cytoskeletal organisation. Although shown to bind the CCT oligomer with some degree of specificity, it is most likely that gelsolin does not represent a CCT folding substrate. siRNA of CCT subunits influences gelsolin levels differently depending upon the target subunit, suggestive of some regulation between CCT monomers, F-actin, and gelsolin levels.
The work presented in this thesis indicates that CCT influences the mammalian cytoskeleton far beyond its involvement in folding newly synthesised actin and tubulin polypeptides and implicates CCT subunits in their monomeric assembly state as likely perpetrators of such activity.

Keywords: CCT, TRiC, Chaperonin, Molecular chaperone, Cytoskeleton, Actin, Tubulin, Gelsolin

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