



GÖTEBORGS UNIVERSITET

THE EXTENDED ROLE OF THE MOLECULAR CHAPERONE CCT

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ABSTRACT

The oligomeric chaperone CCT is a large ATP-dependent chaperonin that consists of two rings placed back-to-back with eight different paralogous subunits with a size of ~ 55 kDa that sit in each of the two rings. The function of CCT is mainly to fold the abundant proteins actin and tubulin, components of the cytoskeleton. However, several studies have shown that CCT has a wide diversity of low-abundant substrates. In addition, CCT and monomeric subunits of CCT have been shown to influence cytoskeletal organization and processes that the cytoskeleton mediates. The aim of this thesis was to study the role of CCT beyond the folding of proteins.

We have overexpressed the subunits of CCT as monomers and demonstrated that monomeric CCT δ has an unknown function at the plasma membrane. The overexpression of monomeric CCT δ mainly induced lamellipodia retraction fibres and the function of monomeric CCT δ at the plasma membrane was shown to be dependent on a wild-type ATP-binding site and a wild-type apical domain of CCT δ . By reducing the levels of individual subunits of CCT, we report in a second study a function of CCT ϵ to regulate the activity of the transcription factor SRF, which controls the transcription of cytoskeletal genes such as actin, via the transcription activator MRTF-A. Cells depleted of CCT ϵ have an increased SRF-mediated transcription in an SRF-luciferase gene reporter system. Monomeric CCT ϵ was shown to interact directly with MRTF-A and the interaction site was identified as the apical domain of CCT ϵ and the c-terminal half of MRTF-A. Consistent with an increased SRF-transcription upon the reduction of CCT ϵ levels, the overexpression of monomeric CCT ϵ delayed the translocation of MRTF-A to the nucleus in serum-stimulated cells. In our final study, we addressed the possibility of CCT to affect the number of actin filaments via the interaction between CCT and the actin filament severing protein gelsolin. We showed that CCT binds to the activated severing conformation of gelsolin and that CCT inhibits activated gelsolin to sever actin filaments.

Taken together, we present several studies that independently identify the CCT oligomer, or its individual subunits, to affect processes related to the cytoskeleton. Thus, there is a close interplay between CCT and the cytoskeleton that extends beyond the dependency of actin and tubulin to be folded by CCT.

Keywords: Actin, CCT oligomer, CCT δ , CCT ϵ , Cell morphology, Gelsolin, MRTF-A.