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Research Article

The interactome of CCT complex – A computational analysis

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ABSTRACT

The eukaryotic chaperonin, CCT (Chaperonin Containing TCP1 or TriC-TCP-1 Ring Complex) has been subjected to physical and genetic analyses in *S. cerevisiae* which can be extrapolated to human CCT (hCCT), owing to its structural and functional similarities with yeast CCT (yCCT). Studies on hCCT and its interactome acquire an additional dimension, as it has been implicated in several disease conditions like neurodegeneration and cancer. We attempt to study its stress response role in general, which will be reflected in the aspects of human diseases and yeast physiology, through computational analysis of the interactome. Towards consolidating and analysing the interactome data, we prepared and compared the unique CCT-interacting protein lists for *S. cerevisiae* and *H. sapiens*, performed GO term classification and enrichment studies which provide information on the diversity in CCT interactome, in terms of protein classes in the data set. Enrichment with disease-associated proteins and pathways highlight the medical importance of CCT. Different analyses converge, suggesting the significance of WD-repeat proteins, protein kinases and cytoskeletal proteins in the interactome. The prevalence of proteasomal subunits and ribosomal proteins suggest a possible cross-talk between protein-synthesis, folding and degradation machinery. A network of chaperones and chaperonins that function in combination can also be envisaged from the CCT interactome-Hsp70 interactome analysis.

1. Introduction

The proper three-dimensional structure of a protein is indispensable for its function. Protein folding is thus an important cellular event, in which several enzymes, regulators and specialised machinery take part. Chaperones and chaperonins constitute the protein-folding machinery. CCT (Chaperonin Containing TCP1 or TriC-TCP-1 Ring Complex) are eukaryotic chaperonin complexes, hetero-oligomeric in structure, made up of two rings stacked back-to -back, enclosing a cavity where proteins fold as ATP binds to the complex. They are grouped together with the archaeal thermosomes while the bacterial and organellar chaperonins constitute a separate group (reviewed by Hartl, 1996; Horwich et al., 2007).

Defects in protein folding have been implicated in many medical conditions. Protein misfolding and subsequent aggregation are clinical features of several neurodegenerative diseases like Alzheimer's disease (AD), Parkinson's disease and Huntington's disease (Ross and Poirier, 2004). The major substrates of CCT are proteins with high beta sheet propensity, which is a characteristic of misfolded proteins in neurodegenerative disorders (reviewed by Broadley and Hartl, 2009). CCT is found to be directly linked to polyQ protein disorders. Huntingtin (Htt) was identified as a substrate of CCT, with which it interacts in a subunit-specific manner. Deficiency of CCT6 increased the aggregate formation and over-expression of CCT1 inhibited aggregation (Tam et al., 2006). Impairment of CCT function was found to enhance toxicity due to Htt aggregation, while its over-expression suppressed the aggregation, when analysed in a yeast model (Behrends et al., 2006) suggesting that deficiency of CCT may be a potential cause of neurodegenerative diseases. The TriC subunits are underexpressed in the foetal brains of Down's syndrome patients, an AD-related disease (Yoo et al., 2001).

Defects in CCT have been implicated in other disease conditions as well. The disruption of CCT activity affects rod outer segment morphogenesis which in turn leads to retinal degeneration (Posokhova et al., 2011). Mutation in CCT5 (A492G) resulting in the substitution of a highly conserved histidine with arginine leads to autosomal recessive mutilating sensory neuropathy (Bouhouche et al., 2006). Mutation in CCT4 (G1349A) causes hereditary sensory neuropathy (Lee et al., 2003). The role of CCT in cancer has also been investigated (Boudiaf-Benmammar et al., 2013; Yokota et al., 2001).

Yeast CCT and human CCT, made up of eight subunits (Cct1p-Cct8p), are structurally and functionally similar. Yeast CCT has been

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subjected to structural and functional studies to explain the basic mechanism underlying eukaryotic chaperonins. CCT mutations in yeast have been found to result in aberrant phenotypes that include cytoskeleton defects, growth defects, sensitivity to anti-mitotic drugs, cell duplication defects, abnormal nuclear distribution and so on (Ursic and Culbertson, 1991). They have been employed in many genetic studies to characterise the eukaryotic chaperonin as well as to identify proteins that interact with it (Dekker et al., 2008; Kabir and Sherman, 2008). The substrate binding region of recombinant yeast CCT1 (apical domain) given exogenously was found to inhibit the aggregation and reduce the cellular toxicity of huntingtin in rat-derived cells, thus proving the therapeutic potential of CCT complex as well as the functional association of yeast and mammalian protein folding systems (Sontag et al., 2013). Yeast CCT is more characterised and can act as a system to improve our understanding of human CCT.

Our attempt is to consolidate, analyse and thus, compare the CCT-interactome data of *S. cerevisiae* and *H. sapiens.* The disease-association of CCT and proteins interacting with it is also studied, to underline the medical significance of the chaperonin complex. This also helps us to evaluate the usefulness of *S. cerevisiae* as a model system to study CCT and in turn, many disease conditions linked to CCT. Interactome analysis can provide information on other functional aspects of CCT. Hsp70p or 70-kDa heat shock protein is another molecular chaperone that has many house-keeping functions in the cell. It can fold newly synthesised proteins, refold misfolded proteins, solubilise aggregated proteins and aid in the transloaction of organellar as well as secretory proteins across biological membranes (Mayer and Bukau, 2005). Hsp70 and CCT were found to exhibit co-operative chaperone activity, interfering with the toxic polyQ protein aggregation pathway. The cooperation of *HSP70* and CCT can be studied in detail by analysing their interactomes.

2. Materials and methods

Owing to the structural and functional similarities, the CCT complex of *S. cerevisiae* and *H. sapiens* were chosen for analysis. The proteins interacting with each of the eight subunits were obtained from the public database BioGRID (The Biological General Repository for Interaction Datasets; Version 3.4; the biogrid.org) (Stark et al., 2006). BioGRID is a public repository that collects data on physical, genetic and chemical interactions. The files were downloaded in Tab 2.0 format and exported to MS Excel for further study. The interactome data of Hsp70 (in *H. sapiens*) and the prefoldin complex (in *H. sapiens* and *S. cerevisiae*) were also obtained the same way.

The redundant interactions were removed from the lists of CCT complex (Filter 1), especially the subunit–subunit interactions, as they appear in the interactome of both the interacting subunits. The list after Filter 1 was used to obtain data on the nature of interactions and related statistics. These steps were carried out for yeast CCT and human CCT separately. The combined CCT interactome (for all the eight subunits) was searched for the prominent interacting proteins and the ones which interact with 7 or more subunits were selected. The duplications of these proteins were removed (Filter 2) to obtain the lists of unique interacting proteins which consists of 369 proteins in *S. cerevisiae* and 365 proteins in *H. sapiens*.

The yeast proteins were subjected to gene list analysis in Saccharomyces Genome Database (SGD) to obtain the homologues. The intersection of the set of homologues and human CCT-interacting proteins was obtained using gene list analysis option in SGD to find the common interacting proteins in both yeasts and humans. These proteins were tested for enrichment of protein domains using STRING 10 (string-db.org) (von Mering et al., 2003). P -value (Bonferroni) of 10^{-1} was applied as the initial cut-off to filter out smaller categories. The most significant terms in the resulting list were chosen for the analyses. The P-values of the terms chosen in STRING were in the range of 10^{-20}–10^{-30}. The intersection of *HSP70* interactome and human CCT interactome was also acquired. The interactome data for CCT complex and prefoldins (*PFDN1-PFDN6* in *H. sapiens* and *PFD1-PFD6* in *S. cerevisiae*) was also subjected to the same analysis. GO classification was performed using PANTHER (**P**rotein **AN**alysis **TH**rough **E**volutionary **R**elationships) (Mi et al., 2013). The genes were classified based on their molecular function, biological process, protein class and pathway. Enrichment tests were done

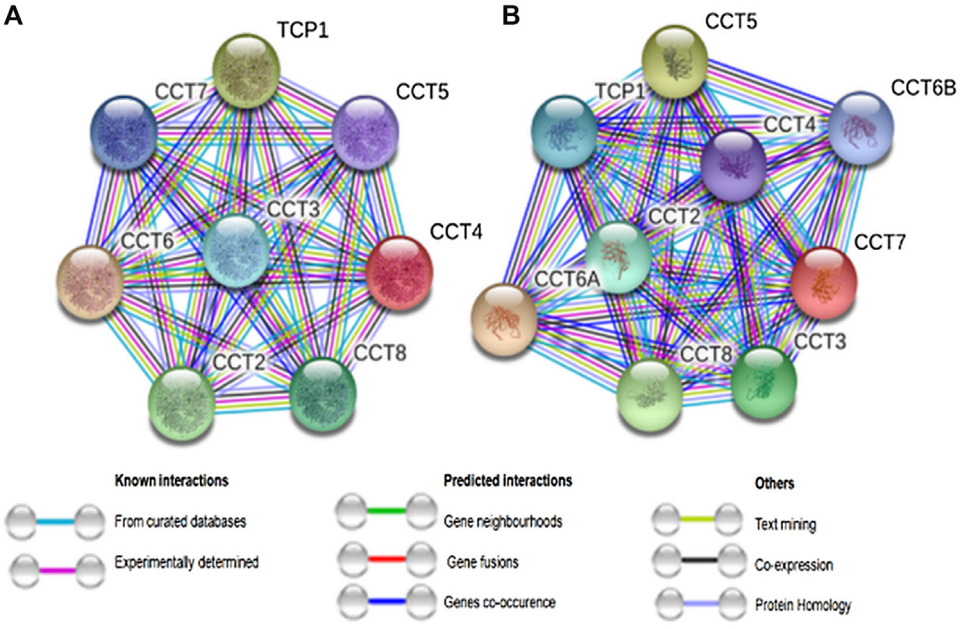

Fig. 1. Interactions within the CCT complex. Different parameters are taken into consideration to build the interaction map of CCT subunits in A. *S. cerevisiae* and B. *H. sapiens* (Using String 10.0).