



# Chaperonin point mutation enhances cadmium endurance in *Saccharomyces cerevisiae*

Ankita Dube · M. Anaul Kabir

Received: 30 March 2021 / Accepted: 25 May 2021 / Published online: 28 May 2021  
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

## Abstract

**Objective** To study the effect of the mutation in conserved G412E in Cct7p subunit of CCT complex on its cellular fate.

**Results** TriC/CCT is a dynamic multimeric protein that assists in protein folding in an energy-dependent manner. A point mutation in the ATP binding pocket in the equatorial domain of the Cct7p subunit delays the doubling time. The cell size was twice the wild type, and the formation of protein aggregates suggests disturbed folding of the proteins. Upon growing in stressful conditions of arsenous acid and cadmium chloride, the mutant was lethal in  $As^{3+}$  but grew well in  $Cd^{2+}$  with 10.5  $\mu g$  cadmium uptake  $mg^{-1}$  compared to the wild type. The increased expression of vacuole transporters *YCF1* and *BPT1* by ten-fold and two-fold in mutant indicates the metal transportation to the vacuole.

**Conclusion** CCT complex was vulnerable to the mutation in G412E in the Cct7p subunit of protein folding molecular machinery. Interestingly, already

stressed cells provided robustness against oxidative stress and cadmium sequestration in the vacuole.

**Keywords** TriC/CCT · Protein aggregation · Cadmium · Vacuole sequestration · YCF1 · BPT1

## Introduction

Chaperones are required for proper folding of the nascent polypeptide synthesis ensue in the lumen of the cell. These chaperones bind non-covalently with the exposed hydrophobic surfaces of newly formed polypeptides to prevent them from misfolding. Molecular chaperones are essentially not an element of the final form of a protein. The chaperonin TriC/CCT complex folds approximately 10% of cellular proteins, including cytoskeleton protein actin and tubulin (Thulasiraman et al. 1999; Yam et al. 2008). The CCT complex is composed of 16 subunits encoded by eight paralogous essential genes *CCT1-8*. The arrangement of subunits is in a ring-shaped manner containing eight related but different subunits. Two such rings are stacked upon each other to assemble into a cylindrical structure with a central cavity. This cavity provides a secluded environment for native protein to fold into its three-dimensional functional form. Each subunit comprises three domains- apical, intermediate, and equatorial. The apical domain

---

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10529-021-03151-9>.

---

A. Dube · M. A. Kabir (✉)  
Molecular Genetics Laboratory, School of Biotechnology,  
National Institute of Technology Calicut, Calicut,  
Kerala 673601, India  
e-mail: anaulk@nitc.ac.in

interacts with the substrate to be folded by the complex and contains a helical extension that forms the lid. It has open and close forms that enable the folding of the substrate by binding and hydrolysis of ATP in the equatorial domain. Intermediate domain coordinates between these two domains (Spiess et al. 2004; Gestaut et al. 2019). Each subunit has a distinct affinity to ATP, and hydrolysis occurs sequentially, aiding its diverse substrate folding (Shimon et al. 2008; Gruber et al. 2017).

Loss of function mutation in any of the subunits often disturbs protein folding. Mis-folded proteins have exposed hydrophobic moiety that may form aggregates with other polypeptides present in overly crowded cytosol. In neurodegenerative disorders like Alzheimer's (AD) and Huntington's (HD) diseases, cellular protein aggregation occurs in neurons. A mutation altering aspartate to glutamate in the P-loop of ATP binding pocket in Cct3p leads to loss of interaction with Q/N rich protein aggregates (Carmichael et al. 2000; Nadler-Holly et al. 2012). Oxidative stress and protein aggregates are indicators of neurodegenerative disorders. Oxidative stress causes oxidative peroxidation of unsaturated fatty acids and produces 4-hydroxy-2, 3-neonenal (HNE). In Alzheimer's disease, levels of HNE in plasma are high (Selley et al. 2002). A decline in glutathione levels was seen in Parkinson's disease and determined as a potential biomarker in the disorder's progression (Mischley et al. 2016). *Saccharomyces cerevisiae* is a well-established eukaryotic model system with a defined genetic system and relatively facile genetic manipulation methods. It has been extensively used to understand many human diseases due to the similarity of core biological processes. For example, the expression of alpha-synuclein A53T mutant induces abnormal folding of HD proteins in yeast models, leads to the discovery of CCTs ability to alleviate the phenotype (Sot et al. 2017).

The GGG (412–414 in Cct7p) motif of ATP binding pocket in the equatorial domain is conserved from bacterial counterpart GroEL to human CCT. Moreover, the motif is conserved amongst the eight subunits of yeast except in Cct6p and Cct8p, in which GAG sequence is present instead of GGG. As the GGG motif occurs in the equatorial domain, it is predicted to have a role in ADP binding (unpublished docking data; Chagoyen et al. 2014). The subunits Cct6p and Cct8p remain ADP-bound and do not

participate in the ATPase cycle of the complex (Jin et al. 2019). Mutation in the GGG motif of Cct1p leads to temperature-sensitive phenotype, whereas the same mutation in Cct6p does not affect its growth (Lin et al. 1997; Ursic et al. 1994). Herein, we have created a point mutation by modifying glycine to aspartic acid at 412 position (G412E) of the amino acid sequence in Cct7p to study the role of the conserved motif GGG (412–414) of ATP binding pocket. The mutation caused growth defects, altered cell morphology, and protein accumulation. We have also observed a possible cross-talk between aggregation and oxidative stress pathways.

## Materials and methods

### Media and growth condition

*Escherichia coli* strain XL1-Blue was routinely grown at 37 °C in YT medium (0.5% yeast extract, 1% tryptone, 0.5% sodium chloride). 100 µg Ampicillin ml<sup>-1</sup> was added for isolation of the plasmid. YPD (1% yeast extract, 2% peptone, 2% dextrose) and SD (synthetic dextrose) media were prepared as described. 5-Fluoro orotic acid (5-FOA), an analog of uracil, was used to evict the plasmid containing the *URA3* gene. For FOA plate preparation, 1 mg 5'-FOA ml<sup>-1</sup> was added after autoclaving the media. YPD was used for growth assay; 1 mM arsenous acid, 1 mM sodium arsenate, 2 mM copper sulphate, 2 mM zinc sulphate, 1 mM cadmium chloride, and 1% lactic acid were added wherever required. The spot assay was done on the solid medium by applying dilutions of 10<sup>4</sup>, 10<sup>3</sup>, 10<sup>2</sup>, and 10<sup>1</sup> colony forming unit (CFU) per spot.

### Vectors, plasmid constructions and primers

The yeast vectors pRS315, pRS316, and pRS11326 were used for maintaining constructs and assessing the phenotypes. The plasmid pUC19 was used for deletion construct. The *CCT7* gene (2153 bp) was amplified by PCR using primers KC124 and KC125 cloned into pJET1.2/blunt cloning vector. Subsequently, it was subcloned into pRS316 at BamHI-SalI site generating the plasmid pKA376. Upstream (591 bp) and downstream (506 bp) sequences of *CCT7* were amplified using the primers KC126/KC127 and KC128/KC129. The PCR amplified products were cloned into vector

pTZ57R/T. The primers KC120 and KC121 were used to amplify 1205 bp *TRP1* gene and cloned into pJET1.2/blunt cloning vector. Plasmid pUC19 was used as a primary vector for plasmid constructs. 1.2 kb *TRP1* gene was cloned in BamHI-SalI site and 591 bp BamHI-SacI upstream fragment was cloned in the BamHI-SacI site of pUC19. SphI-SalI 506 bp downstream fragment was cloned in the above construct in SphI-SalI site. The cassette was released by digesting with SacI and transformed into yeast strain B-8728 containing a copy of *CCT7* in pRS316 plasmid. Gene deletion was done using the homologous gene disruption method. The primers KC316/KC317 were used for internal amplification and KC542/KC129 were used for junction checking to verify the gene deletion. A point mutation was created from GGG to EGG of *CCT7* using primers KC474/KC475 with *CCT7* cloned in BamHI-SalI site of pRS315 using site-directed mutagenesis kit. The primers KC589/KC590 were used to amplify the *HSP104* gene (3181 bp, promoter and 3' end forming sequences) and cloned in BamHI-XhoI site of pRS11326 for the aggregation visualization. Subsequently, a DNA fragment (750 bp) containing GFP was cloned at BamHI site to generate the plasmid pKA844. The GFP-tagged cassette was transformed in wild-type and mutant yeast (Sambrook and Russell 2001). The lithium acetate/polyethylene glycol method was used to transform yeast cells (Hinnen et al. 1978). All the strains, plasmid constructs and primers used in the study are listed in Supplementary Tables 1, 2, and 3.

#### Atomic adsorption spectroscopy

Fresh yeast cells were inoculated in 20 ml of YPD, grown till the early log phase. 1 mM CdCl<sub>2</sub> was added to it and grown for 16 h at 30 °C, initial pH 6. Conditions were altered accordingly to study the effect of contact time and pH. The culture was centrifuged at 5000 rpm for 5 min, and the supernatant was analyzed for residual cadmium by Perkin Elmer AAS, PinAAcle 900F. The *Q*, uptake capacity (μg mg<sup>-1</sup>) and *E*, removal efficiency (%) was calculated by

$$Q = \frac{(C_i - C_e)}{M} \times V \quad (1)$$

$$E = \frac{(C_i - C_e)}{C_i} \times 100 \quad (2)$$

where *C<sub>i</sub>* (μg ml<sup>-1</sup>) is the initial concentration, *C<sub>e</sub>* (μg ml<sup>-1</sup>) is the final concentration of cadmium, *M* (mg) is the dry weight of yeast, and *V* (ml) is the volume (Pérez-Marín et al. 2007).

#### Microscopic methods

Strains were grown till the early-log phase then 1 mM CdCl<sub>2</sub> was added and grown for 4 h with and without cadmium. Cells were collected and washed with distilled water. Slides were prepared in 1% agar and seen under the Nikon ECLIPSE Ti-E fluorescent microscope. For electron microscopy, cells were spread over 1% agar, layered the coverslip, and desiccated. These were then sputtered with gold particles for 15 s and imaged using scanning electron microscope Hitachi SU6600 (Japan). 5 μl of 2 mg neutral red ml<sup>-1</sup> was added to 25 μl of cell culture and incubated for 5 min to visualize vacuoles, and slides were seen under the optical microscope (Corbacho et al. 2010).

#### RNA preparation and real-time PCR

Total RNA isolation was done using the hot acid-phenol method as described. Eppendorf NanoDrop spectrophotometer was used for estimation of RNA concentration. DNaseI treatment was given to remove any DNA contamination in RNA samples and verified with standard PCR. cDNA from RNA samples were prepared using Thermo Fisher Scientific RevertAid first strand cDNA synthesis kit. Real-time quantitative PCR was performed using Bio-Rad CFX96 Real-time system in 20 μl reactions taking 25 ng cDNA μl<sup>-1</sup> in each sample using Thermo Fisher Scientific Maxima SYBR Green/ROX qPCR Master Mix. The in-built Bio-Rad system protocol (CFX\_2 step ampl protocol) was used for RT-qPCR with additional melt curves after the amplification. Briefly, denaturation was done at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 55 °C for 30 s. After amplification, melt curves were run at 65–95 °C for 5 s at increment of 0.5 °C. The gene expression difference was calculated as fold change (2<sup>-ΔΔC<sub>T</sub></sup>) using the ΔΔC<sub>T</sub> method (Schmittgen and Livak 2008). ΔC<sub>T</sub> is difference between C<sub>T</sub> of target gene and C<sub>T</sub> of reference gene and ΔΔC<sub>T</sub> is difference between ΔC<sub>T</sub> of mutant and

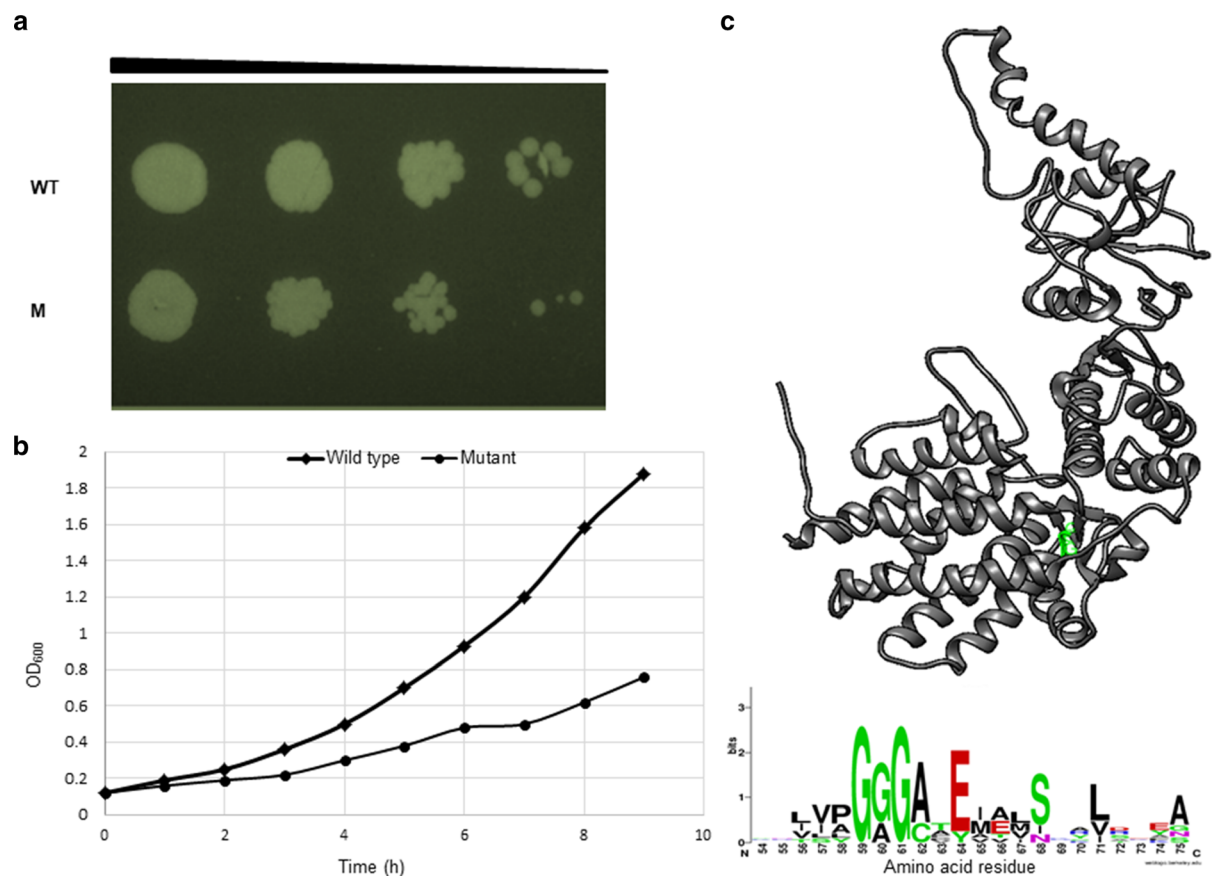
wild type strain. Triplicates were performed in all the PCR reactions.

### Bioinformatics methods and tools

Peptide sequences of eight yeast CCTs were retrieved from the *Saccharomyces* Genome Database. Prime module (version 11.1) of Schrödinger, (Schrödinger, LLC, New York, NY, 2017) was used to predict three-dimensional structures. Template structures 6KRD\_8 was selected based on the percentage identity. ATP docking was carried out using swissdock online tool (Grosdidier et al. 2011). Clustal omega was used for multiple sequence alignment of protein sequences. Web logo model for conserved motif was prepared using consurf (Ashkenazy et al. 2016; Sievers et al. 2011).

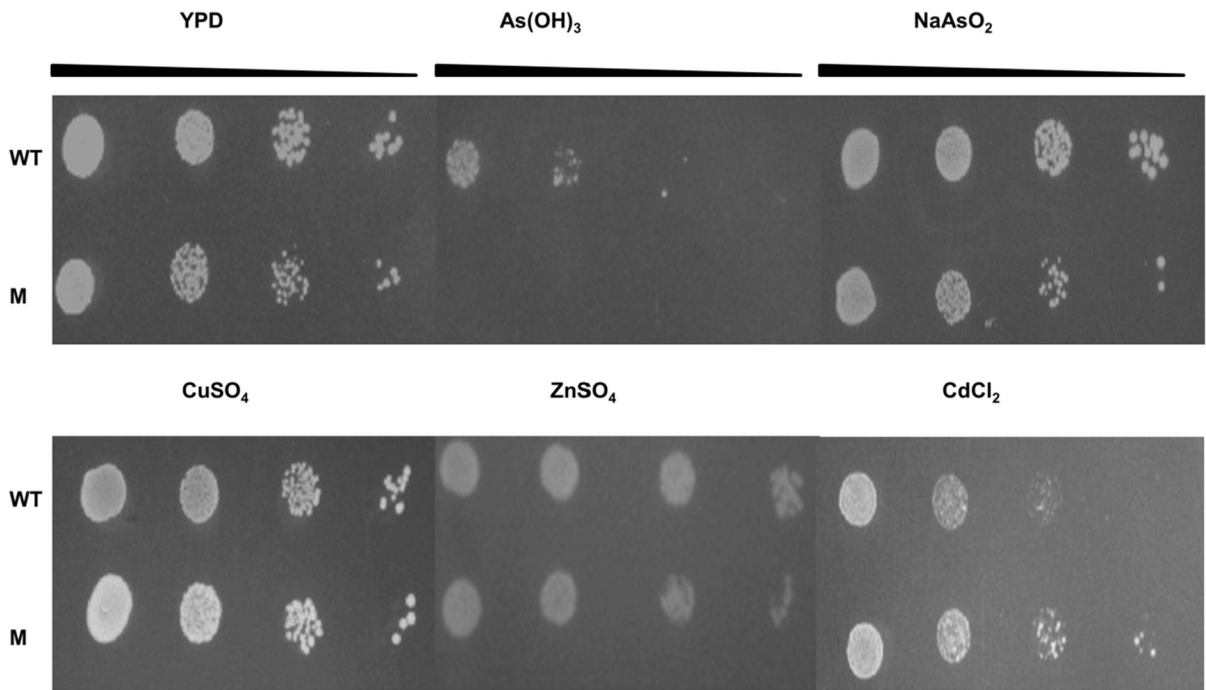
### Results and discussion

CCT subunits have varying affinity to ATP of Cct4p, Cct5p, Cct2p, Cct1p, Cct7p, Cct3p, Cct6p and Cct8p in descending order. These subunits are characterized on the basis of affinity hierarchy into two asymmetrical planes of high and low-affinity subunits. Cct4p, Cct5p, Cct2p, Cct1p have higher affinity than subunits Cct3p, Cct6p, and Cct8p, while the status of Cct7p remains ambiguous (Amit et al. 2010; Reissmann et al. 2012; Zang et al. 2016). The four conserved loop motifs LGPKG, ITKDG, GDGTT, and GGG are identified in equatorial domains of chaperonins (Kabir et al. 2011). These motifs are differentially conserved among subunits of yeast CCTs and attribute to asymmetry to ATPase activity (Chagoyen et al. 2014). The P-loop GDGTT is extensively studied in

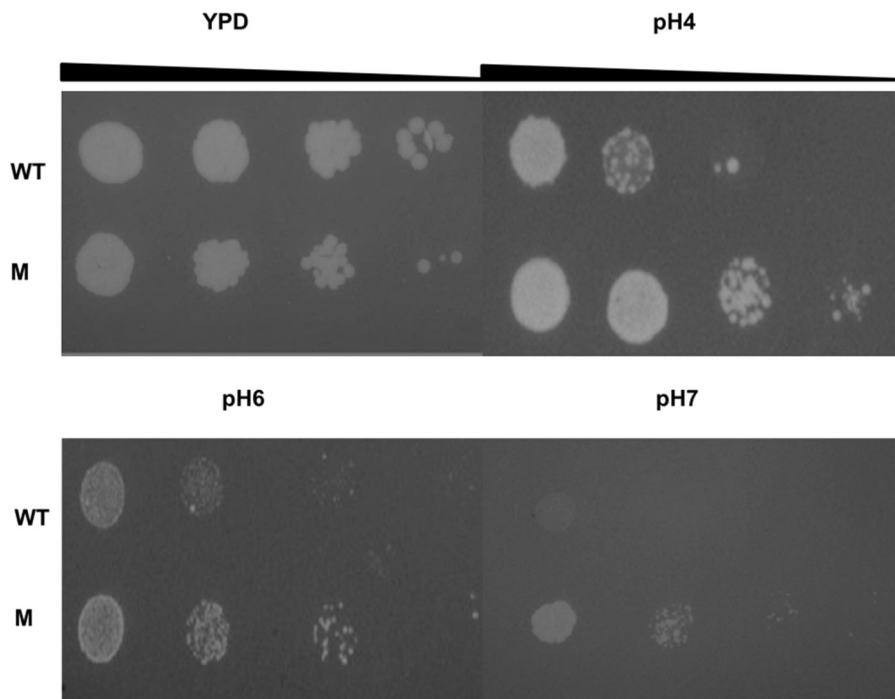


**Fig. 1** Growth assay: **a** Spot assay of wild type and mutant on YPD plate at dilutions  $10^4$ ,  $10^3$ ,  $10^2$ , and  $10^1$  CFU per spot as indicated. **b** Liquid growth assay of wild type and mutant on YPD **c**. Modelling of Cct7p depicting location of conserved

glycine residue at 412 position of equatorial domain and ConSurf model represents the conserved glycine residue in all eight subunits



**Fig. 2** Spot assay of wild type and mutant on YPD plate containing 1 mM of arsenous acid, 1 mM of sodium arsenate, 2 mM of copper sulphate, 2 mM of zinc sulphate and 1 mM of cadmium chloride at 30 °C at dilutions  $10^4$ ,  $10^3$ ,  $10^2$ , and  $10^1$  CFU per spot as shown



**Fig. 3** Spot assay wild type and mutant on YPD plates containing 1 mM Cadmium chloride at 30 °C at pH4, 6 and 7 at dilutions  $10^4$ ,  $10^3$ ,  $10^2$ , and  $10^1$  CFU per spot as indicated

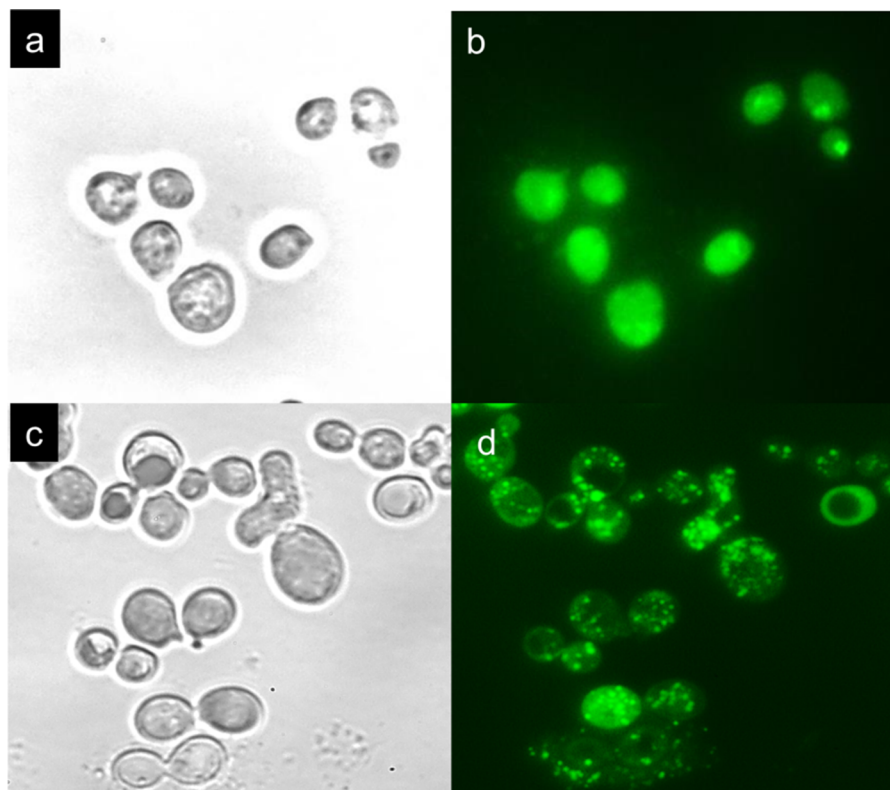


all eight subunits of yeast CCTs by creating residue modification. High-affinity CCT1 and low-affinity CCT6 on mutating in GGG motif are temperature sensitive and phenotypically unaffected respectively (Lin et al. 1997; Ursic et al. 1994). However, CCT7 remains poorly understood in the context of its binding affinity to ATP. Hence we have made an effort to incorporate the mutation G412E in the conserved GGG region of CCT7 and analyzed it. In haploid *Saccharomyces cerevisiae* strain B-8728, the *CCT7* gene in the genome was deleted by homologous recombination, providing an extra copy of the gene in pRS316 plasmid (*CEN6 URA3*). The size of the deletion cassette used was 2.2 kb. The accurate deletion was confirmed using two sets of PCR primers to amplify the 5' and 3' junctions (Supplementary Fig. 1 a.). One of the two primers used for junction bind in the marker sequence, and the other corresponds to the sequence outside the flanking sequences used for deletion cassette. The primer KC542/KC317 and KC129/KC316 were used to check upstream and

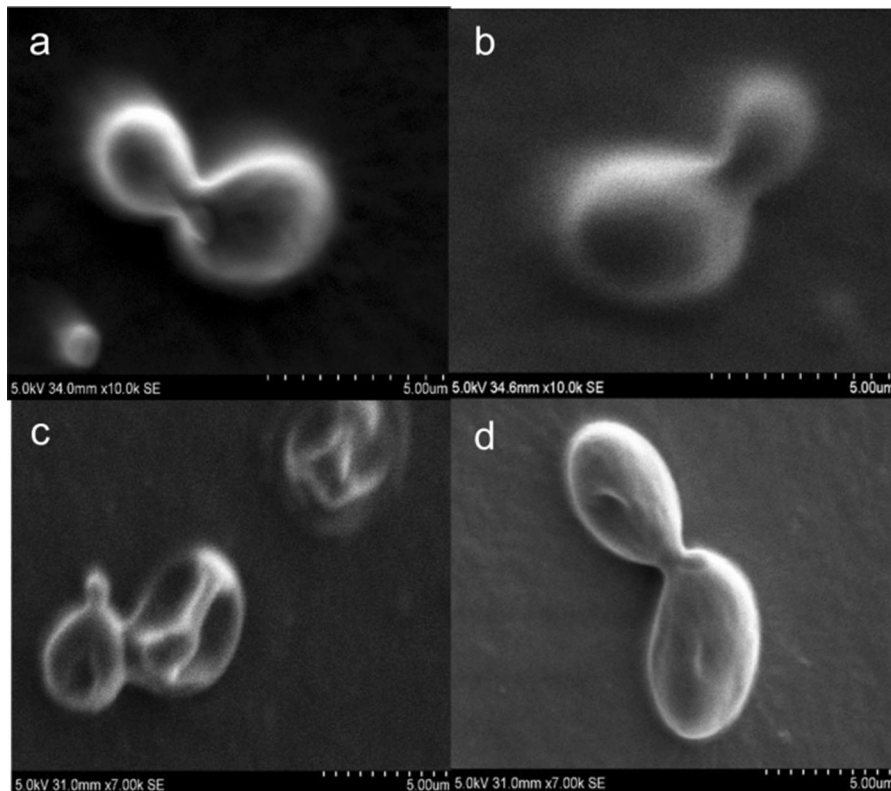
downstream junctions, respectively. The expected size for upstream junction PCR product was 921 bp and 850 bp for the downstream junction (Supplementary Fig. 1a and 1b). The *cct7* deletion mutants were selected on the plate containing 5-fluoroorotic acid (FOA). As *CCT7* is an essential gene, deletant mutant will not grow on FOA plate. The wild type copy of *CCT7* in pRS316 (*CEN6 URA3*) was replaced by mutant *CCT7* (G412E) present in pRS315 (*CEN6 LEU2*) by plasmid shuffling.

#### Effect of mutation on growth

CCT mutants have growth defects, as reported in earlier works (Lin et al. 1997; Narayanan et al. 2016). G412E mutant in Cct7p subunit has visible growth retardation in complete media at optimum temperature (Fig. 1). Doubling time was  $3.47 \pm 0.2$  h and  $2.05 \pm 0.2$  h of mutant and wild type, respectively. Actin is obligate TriC/CCT substrate that plays a central role in cytokinesis. Cdh1p and Cdc20p are



**Fig. 4** Florescent microscopy: Cells were transformed with plasmid containing HSP104 tagged with GFP to detect protein aggregates. **a** and **b** are wild type, **c** and **d** are mutants under bright field and FITC

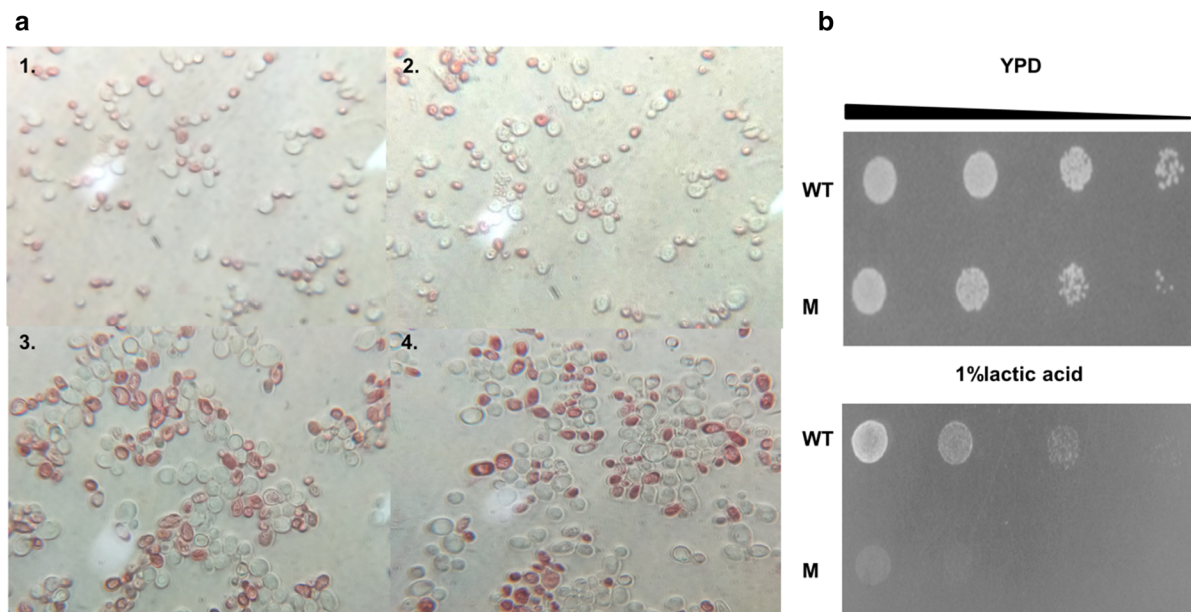


**Fig. 5** Scanning electron microscopy images of **a** Wild type, **b** Mutant **c** Wild type treated with cadmium chloride and **d** Mutant treated with cadmium chloride

components of the mitotic checkpoint complex (MCC). The disassembly of the MCC complex by CCT facilitates cells to move in anaphase (Kaisari et al. 2017; Llorca et al. 2000). Similarly, slow growth was seen in a strain carrying *cct7* mutation in the conserved GDGTT motif of ATP binding pocket (Amit et al. 2010). Substrate folding takes place by open and close conformation cycle, which is powered by ATPase activity. Strain with the G345D mutation in the hinge region of *cct4p* has slower ATP hydrolysis (Shimon et al. 2008). Glycine at 412 position is localized close to P-loop (GDGTT) of the binding pocket; mutation will likely disturb the ATP binding. Docking of individual subunit study shows that wild-type Cct7p binds tightly than mutant *cct7p* (Supplementary Fig. 2). The yeast strain carrying mutant *cct7* was investigated in various stress conditions to assess its sensitivity. Cell growth was at par with optimum conditions at acidic to alkaline pH, oxidizing agent hydrogen peroxide and redox agent dithiothreitol

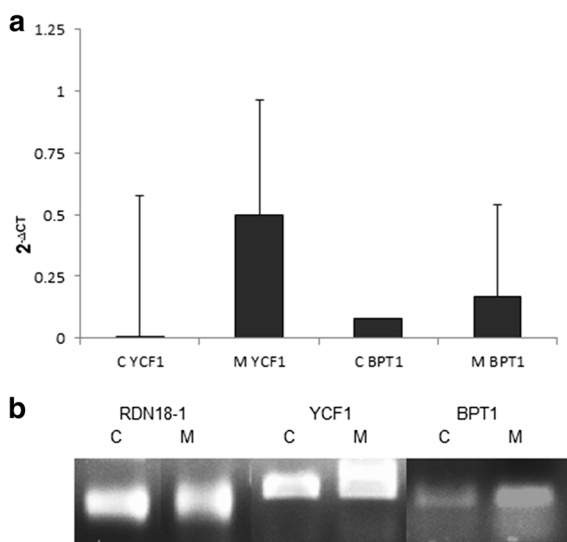
(DTT) however lesser than wild type (Supplementary Fig. 3).

Metal ions bind to the thiol group of cysteine residue and affect cellular proteins. Metal ions have a propensity to produce reactive oxygen species that causes protein damage. Mutant viability was tested against different metal salts, namely zinc chloride, copper sulphate, cadmium chloride, arsenous acid, and sodium arsenate. Except for arsenous acid (III), no further growth differences were observed than wild-type strain. On the contrary to  $As^{3+}$ , cells were growing better than wild type in 1 mM cadmium chloride (Fig. 2, Supplementary Fig. 4).  $As^{3+}$  inhibits CCT complex and tubulin, actin polymerization by binding to its monomer (Pan et al. 2010; Thorsen et al. 2009). Cadmium produces protein aggregates in yeast; Heat shock proteins (HSPs) are up-regulated in the presence of both  $As^{3+}$  and  $Cd^{2+}$  (Jacobson et al. 2017).



**Fig. 6** **a** Microscopy images of wild type (1, 2) and mutant (3, 4) grown in YPD and YPD with cadmium chloride respectively stained with vacuole staining dye neutral red. **b** Spot assay of

parent strain B-8728, wild type and mutant on YPD plates containing 1% lactic acid at 30C as indicated



**Fig. 7** Real time PCR for vacuole pumps genes *YCF1* and *BPT1* in wild type and mutant **a** Bar graph of  $2^{-\Delta CT}$  values of RT qPCR as depicted. **b** Relative amplification for C-DNA of wild type and mutant was loaded in lane 1, 3, 5 and 2, 4, 6 respectively. Lane 1, 2; 3, 4 and 5, 6 contains reference gene *RDN18-1*, *YCF1* and *BPT1* respectively on 2% agarose gel

#### Adsorption of cadmium in the mutant

Mutant is growing in Cd (II) better than the wild type; tolerance to the metal ion is attributed to the outflux of metal ion or hyperaccumulation. Cadmium uptake efficiency is  $10.5 \mu\text{g cadmium mg}^{-1}$  and  $9 \mu\text{g cadmium mg}^{-1}$  for mutant and wild type, respectively. Cells were grown for 16, 24, and 48 h to study the effect of contact time on cadmium adsorption of mutant. The removal capacity was 42.75%, 46.70%, and 65.17% for 16, 24, and 48 h respectively. Mutant and wild type were grown in pH 4, 6, and 7 with 1 mM  $\text{CdCl}_2$ . Mutant can grow better at acidic pH4 with a removal capacity of 48.97% compared to pH6 with 42.75% (Fig. 3). Cadmium removal capacity was favoured by acidic pH4 and 48 h contact time.

#### Aggregation of the protein

The heat shock protein 104 (Hsp104) is a cytosolic protein that, on the incidence of protein aggregates, gets recruited on-site and stimulates Hsp70 to bind this complex and assists in clearance. To visualize protein aggregates, Hsp104 was tagged with GFP and expressed in both wild type and mutant. In wild type, Hsp104 was dispersed all over the cytosol while



aggregates were present in the mutant. These aggregates may have a protective function against oxidative stress. The amyloid plaque has metal chelation and antioxidant properties at macromolecular metal levels (Baruch-Suchodolsky and Fischer 2008, 2009; Carija et al. 2017). The aggregate formation was seen in both wild type and mutant when grown in the presence of cadmium (Jacobson et al. 2017) (Fig. 4).

#### Vacuole sequestration of cadmium and altered cell morphology

The mutant's cell size was enlarged and is roughly double the size of the wild type (Fig. 5). Larger cell size can be due to prominent vacuoles in the cell (Chan and Marshall 2014). For visualization, wild type and mutant were then stained with vacuole dye neutral red (Corbacho et al. 2010), mutants were able to retain more stain compared to wild type (Fig. 6a). It is evident that mutant has more pronounced vacuoles and possibly assisting in withstanding cadmium. Mutant was sensitive to lactic acid. Lactic acid causes vacuole fragmentation (Suzuki et al. 2012) (Fig. 6b). Aggregated proteins are subjected to degradation via proteasome and vacuolar pathways. Cct1-1, Cct1-2, Cct1-3, Cct2-1 and Cct6-50 are also sensitive to lactic acid. (Petroi et al. 2012; Narayanan et al. 2016) Metal chelation and vacuole sequestration are often adopted strategies in metal tolerant strains. Ycf1p and Bpt1p are vacuole ABC transporters (Jacobson et al. 2017; Sharma et al. 2002). These transporters influx metal ions bound complexes of glutathione to the vacuoles. Relative expression of the *YCF1* and *BPT1* genes was enhanced by ten-fold and two-fold in the mutant, respectively. *RDN18-1* was taken as the reference gene (Fig. 7). The mutation leads to enhanced expression of the two genes supported by sensitivity to lactic acid and thus prepared mutants for detoxification of  $\text{Cd}^{2+}$ .

#### Conclusion

The chaperonin CCT complex is a central molecule that, along with translation machinery, HSPs and ubiquitin, tightly maintains cellular protein homeostasis. Loss of proper folding or degradation leads to disturbance in cell physiology, such as in neurodegenerative disorders. The Cct7p subunit of the CCT

complex has exhibited sensitivity to G412E mutation. Cells were enlarged, has slow doubling time, and presence of protein aggregates. Surprisingly, the mutant was generally resistant to oxidative stress and accumulated cadmium better than the wild type. An increase in the expression level of metal ion vacuolar transporters genes, *YCF1* and *BPT1*, in mutant suggests metal influx to vacuoles. This study provides a cross-talk between protein folding and stress pathways. Further research is needed to understand how a mutation in CCT prepares the cell for oxidative stress and overexpression of vacuolar transporters.

**Acknowledgements** We are thankful to Dr. B. Das, Jadavpur University, Kolkata for providing basic *Saccharomyces* plasmid, Dr. P. J. Bhatt, IIT Bombay and Dr. F. Sherman for XL1 and B8728. We thank Dr. George K. Varghese, NIT, Calicut for permitting and Ms. Ashwini S. K. for helping out with AAS facility and Dr. Rathinaswamy K, NIT, Calicut and Mr. Sushobhan Mahanty to help with florescent microscopy, Mr. Dileep Pullepu for his inputs in manuscript writing.

**Author contributions** MAK and AD conceived the study. AD performed the research; all authors were involved in analysis of the data. AD wrote and MAK revised and confirmed the manuscript.

**Funding** This study was supported by council of scientific and Industrial Research (CSIR) grant (No: 37 (1571)/12/EMRII).

#### Declarations

**Conflict of interest** All authors declare that they have no conflict of interest.

#### References

- Amit M, Weisberg SJ, Nadler-Holly M, McCormack EA, Feldmesser E, Kaganovich D, Willison KR, Horovitz A (2010) Equivalent mutations in the eight subunits of the chaperonin CCT produce dramatically different cellular and gene expression phenotypes. *J Mol Bio* 401(3):532–543
- Ashkenazy H, Abadi S, Martz E, Chay O, Mayrose I, Pupko T, Ben-Tal N (2016) ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. *Nucleic Acids Res* 44(W1):W344–W350
- Baruch-Suchodolsky R, Fischer B (2008) Soluble amyloid  $\beta$ 1–28–Copper(I)/Copper(II)/Iron(II) complexes are potent antioxidants in cell-free systems. *Biochemistry* 47(30):7796–7806
- Baruch-Suchodolsky R, Fischer B (2009) A $\beta$ 40, either soluble or aggregated, is a remarkably potent antioxidant in cell-free oxidative systems. *Biochemistry* 48(20):4354–4370

- Carija A, Navarro S, de Groot NS, Ventura S (2017) Protein aggregation into insoluble deposits protects from oxidative stress. *Redox Biol* 12:699–711
- Carmichael J, Chatellier J, Woolfson A, Milstein C, Fersht AR, Rubinsztein DC (2000) Bacterial and yeast chaperones reduce both aggregate formation and cell death in mammalian cell models of Huntington's disease. *Proc Natl Acad Sci USA* 97(17):9701–9705
- Chagoyen M, Carrascosa JL, Pazos F, Valpuesta JM (2014) Molecular determinants of the ATP hydrolysis asymmetry of the CCT chaperonin complex. *Proteins* 82(5):703–707
- Chan YH, Marshall WF (2014) Organelle size scaling of the budding yeast vacuole is tuned by membrane trafficking rates. *Biophys J* 106(9):1986–1996
- Corbacho I, Teixidó F, Velázquez R, Hernández LM, Olivero I (2010) Yeast vacuole staining using quinacrine and neutral red. *Microorg Ind Environ World Sci*. [https://doi.org/10.1142/9789814322119\\_0142](https://doi.org/10.1142/9789814322119_0142)
- Gestaut D, Limatola A, Joachimiak L, Frydman J (2019) The ATP-powered gymnastics of TRiC/CCT: an asymmetric protein folding machine with a symmetric origin story. *Curr Opin Struct Biol* 55:50–58
- Grosdidier A, Zoete V, Michielin O (2011) SwissDock, a protein-small molecule docking web service based on EADock DSS. *Nucleic Acids Res* 39:W270–W277
- Gruber R, Levitt M, Horovitz A (2017) Sequential allosteric mechanism of ATP hydrolysis by the CCT/TRiC chaperone is revealed through Arrhenius analysis. *Proc Natl Acad Sci USA* 114(20):5189–5194
- Hinnen A, Hicks JB, Fink GR (1978) Transformation of yeast. *Proc Natl Acad Sci USA* 75(4):1929–1933
- Jacobson T, Priya S, Sharma SK, Andersson S, Jakobsson S, Tanghe R, Ashouri A, Rauch S, Goloubinoff P, Christen P, Tamás MJ (2017) Cadmium causes misfolding and aggregation of cytosolic proteins in yeast. *Mol Cell Biol* 37(17):e00416–e00490
- Jin M, Han W, Liu C, Zang Y, Li J, Wang F, Wang Y, Cong Y (2019) An ensemble of cryo-EM structures of TRiC reveal its conformational landscape and subunit specificity. *Proc Natl Acad Sci USA* 116(39):19513–19522
- Kabir MA, Uddin W, Narayanan A, Reddy PK, Jairajpuri MA, Sherman F, Ahmad Z (2011) Functional subunits of eukaryotic chaperonin CCT/TRiC in protein folding. *J Amino Acids* 2011:843206
- Kaisari S, Sitry-Shevah D, Miniowitz-Shemtov S, Teichner A, Hershko A (2017) Role of CCT chaperonin in the disassembly of mitotic checkpoint complexes. *Proc Natl Acad Sci USA* 114(5):956–961
- Lin P, Cardillo TS, Richard LM, Segel GB, Sherman F (1997) Analysis of mutationally altered forms of the Cct6 subunit of the chaperonin from *Saccharomyces cerevisiae*. *Genetics* 147(4):1609–1633
- Llorca O, Martín-Benito J, Ritco-Vonsovic M, Grantham J, Hynes GM, Willison KR, Carrascosa JL, Valpuesta JM (2000) Eukaryotic chaperonin CCT stabilizes actin and tubulin folding intermediates in open quasi-native conformations. *EMBO J* 19(22):5971–5979
- Mischley LK, Standish LJ, Weiss NS, Padowski JM, Kavanagh TJ, White CC, Rosenfeld ME (2016) Glutathione as a biomarker in Parkinson's disease: associations with aging and disease severity. *Oxid Med Cell Longev* 2016:9409363
- Nadler-Holly M, Breker M, Gruber R, Azia A, Gymrek M, Eisenstein M, Willison KR, Schuldiner M, Horovitz A (2012) Interactions of subunit CCT3 in the yeast chaperonin CCT/TRiC with Q/N-rich proteins revealed by high-throughput microscopy analysis. *Proc Natl Acad Sci USA* 109(46):18833
- Narayanan A, Pullepu D, Reddy PK, Uddin W, Kabir MA (2016) Defects in protein folding machinery affect cell wall integrity and reduce ethanol tolerance in *S. cerevisiae*. *Curr Microbiol* 73(1):38–45
- Pan X, Reissman S, Douglas NR, Huang Z, Yuan DS, Wang X, McCaffery JM, Frydman J, Boeke JD (2010) Trivalent arsenic inhibits the functions of chaperonin complex. *Genetics* 186(2):725–734
- Pérez-Marín AB, Zapata VM, Ortuño JF, Aguilar M, Sáez J, Lloréns M (2007) Removal of cadmium from aqueous solutions by adsorption onto orange waste. *J Hazard Mater* 139(1):122–131
- Petroi D, Popova B, Taheri-Talesh N, Irmiger S, Shahpasandzadeh H, Zweckstetter M, Outeiro TF, Braus GH (2012) Aggregate clearance of  $\alpha$ -synuclein in *Saccharomyces cerevisiae* depends more on autophagosome and vacuole function than on the proteasome. *J Biol Chem* 287(33):27567–27579
- Reissmann S, Joachimiak LA, Chen B, Meyer AS, Nguyen A, Frydman J (2012) A gradient of ATP affinities generates an asymmetric power stroke driving the chaperonin TRiC/CCT folding cycle. *Cell Rep* 2(4):866–877
- Sambrook JF, Russell DW (2001) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, New York
- Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 3(6):1101–1108
- Selley ML, Close DR, Stern SE (2002) The effect of increased concentrations of homocysteine on the concentration of (E)-4-hydroxy-2-nonenal in the plasma and cerebrospinal fluid of patients with Alzheimer's disease. *Neurobiol Aging* 23(3):383–388
- Sharma KG, Mason DL, Liu G, Rea PA, Bachhawat AK, Michaelis S (2002) Localization, regulation, and substrate transport properties of Bpt1p, a *Saccharomyces cerevisiae* MRP-Type ABC Transporter. *Eukaryot Cell* 1(3):391–400
- Shimon L, Hynes GM, McCormack EA, Willison KR, Horovitz A (2008) ATP-induced allostery in the eukaryotic chaperonin CCT is abolished by the mutation G345D in CCT4 that renders yeast temperature-sensitive for growth. *J Mol Biol* 377(2):469–477
- Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7(1):539
- Sot B, Rubio-Muñoz A, Leal-Quintero A, Martínez-Sabando J, Marcilla M, Roodveldt C, Valpuesta JM (2017) The chaperonin CCT inhibits assembly of  $\alpha$ -synuclein amyloid fibrils by a specific, conformation-dependent interaction. *Sci Rep* 7:40859–40859
- Spieß C, Meyer AS, Reissmann S, Frydman J (2004) Mechanism of the eukaryotic chaperonin: protein folding in the

- chamber of secrets. *Trend Cell Biol* 14(11):598–604. <https://doi.org/10.1016/j.tcb.2004.09.015>
- Suzuki T, Sugiyama M, Wakazono K, Kaneko Y, Harashima S (2012) Lactic-acid stress causes vacuolar fragmentation and impairs intracellular amino-acid homeostasis in *Saccharomyces cerevisiae*. *J Biosci Bioeng* 113(4):421–430
- Thorsen M, Perrone GG, Kristiansson E, Traini M, Ye T, Dawes IW, Nerman O, Tamás MJ (2009) Genetic basis of arsenite and cadmium tolerance in *Saccharomyces cerevisiae*. *BMC Genomics* 10(1):105
- Thulasiraman V, Yang CF, Frydman J (1999) In vivo newly translated polypeptides are sequestered in a protected folding environment. *EMBO J* 18(1):85–95
- Ursic D, Sedbrook JC, Himmel KL, Culbertson MR (1994) The essential yeast *Tcp1* protein affects actin and microtubules. *Mol Biol Cell* 5(10):1065–1080
- Yam AY, Xia Y, Lin HTJ, Burlingame A, Gerstein M, Frydman J (2008) Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. *Nat Struct Mol Biol* 15(12):1255–1262
- Zang Y, Jin M, Wang H, Cui Z, Kong L, Liu C, Cong Y (2016) Staggered ATP binding mechanism of eukaryotic chaperonin TRiC (CCT) revealed through high-resolution cryo-EM. *Nat Struct Mol Biol* 23(12):1083–1091

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.