Sorbitol and PKC1 overexpression alleviate temperature sensitivity in chaperonin mutants of *Saccharomyces cerevisiae*

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Abstract

CCT (Chaperonin containing TCP-1) is a constitutively expressed eukaryotic chaperonin complex involved in the proper folding of proteins like actin and tubulin. Temperature sensitive mutants of CCT complex have been employed in various genetic screens, acting as models to study human CCT, the defects of which are implicated in disease conditions like neurodegeneration. Mutants of CCT complex are sensitive to cell wall stress agents. In this study, we have tested the effects of sorbitol and protein kinase C overexpression on the temperature sensitivity of *cct* mutants. We report that both the factors alleviated temperature sensitivity of *cct* mutants, indicating the possible role of CCT in maintaining cell wall integrity in *S. cerevisiae*.

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Figure 1. Suppressors of temperature sensitivity in CCT complex chaperonin mutants: A) Effect of osmotic stabilizer on the temperature sensitivity of *cct* mutants. Strains B-8728 (WT), DUY326 (*cct1-2*) and DDY229 (*cct4-1*) spotted on YPD and incubated at (a) 30°C (b) 35°C and (c) 37°C. Strains B-8728 (WT), DUY326 (*cct1-2*) and DDY229 (*cct4-1*) spotted on YPD+1M Sorbitol and incubated at (d) 30°C (e) 35°C and (f) 37°C. B) Effect of PKC1 and HSP82 overexpression on temperature sensitivity of *cct1-2*. The mutant *cct1-2* transformed with pRSII326 (empty vector), PKC1, HSP82, and CCT1 spotted on uracil-omission media after serial dilution and incubated at (a) 30°C and (b) 37°C.
Description

TRiC (TCP-1 Ring Complex) or CCT (Chaperonin Containing TCP-1) is a multi-subunit, double-ringed, ATP-driven cytosolic eukaryotic chaperonin complex that is constitutively expressed and facilitates the folding of several proteins like actin and tubulin (Frydman et al. 1992; Llorca et al. 1999; Berger et al. 2018). Its role in many cellular processes like differentiation, viral replication, and heart contractility were elucidated recently (Melkani et al. 2017; Knowlton et al. 2018; Ohhara et al. 2019). Its functional defects are also associated with neurodegenerative diseases and cancer (Tam et al. 2006; Bouidaf-Benmammar et al. 2013). Temperature sensitive mutants of *Saccharomyces cerevisiae* CCT have been used in different studies, especially those involving genetic screens to identify CCT-interacting proteins (Kabir and Sherman 2008). The *CCT1–CCT8* genes encode eight different subunits of the complex; the mutant allele *cct1-2* has G423D replacement in the conserved ATP binding/hydrolysis domain in the subunit Cct1p. *cct4-1* (G345D), the mutation in subunit Cct4p, affects ATP binding, intra and inter-ring co-operativity (Shimon et al. 2008). *cct1-2* and *cct4-1* grew well at 30°C (permissive temperature), showed retarded growth at 35°C (semi-permissive temperature) and failed to grow at 37°C (restrictive temperature).

Their sensitivity to cell wall antagonists like SDS and Congo red are suggestive of cell wall defects in *cct* mutants (Narayanan et al. 2016). Are the temperature sensitivity and cell wall defects linked in *cct* mutants? To answer this, we studied the effects of two different suppressors of cell wall defects on the temperature sensitivity of *cct* mutants.

Osmotic stabilisers like sorbitol are known to rescue cell-wall integrity mutants by preventing cell lysis (Ribas et al. 1991). We observed that the growth of *cct1-2* and *cct4-1* mutants was restored in presence of 1M sorbitol both at the semi-permissive and restrictive temperatures (Fig. 1A). Mutants in MAPK-activation pathway undergo cell lysis at elevated temperatures, due to the unrepaired cell wall defects (Kamada et al. 1995). The osmoremedial nature of temperature sensitivity in the *cct* mutants prompted us to study the role of cell wall biogenesis in temperature sensitivity. Towards this end, we overexpressed the *PKC1* gene in *cct1-2*. The *PKC1* gene in *S. cerevisiae* encodes protein kinase C, homolog of human protein kinase C, an important enzyme in the maintenance of cell wall integrity (Levin 2005). Overexpression of *PKC1* resulted in the rescue of the temperature-sensitive phenotype of *cct* mutants at 35°C and 37°C (Fig. 1B).

Wild type copy of the *CCT1* gene was also overexpressed that served as the positive control; the overexpression enabled growth at 35°C and 37°C, confirming that the defects observed in the mutant were indeed due to compromised CCT function. *HSP82*, a yeast *HSP90* homolog, was overexpressed in *cct1-2* to check if more copies of another chaperone can reverse the defects in the mutant. But, unlike *PKC1*, *HSP82* on overexpression failed to make an impact on the temperature sensitivity of *cct* mutants. Similar observations were made for *HSP40* and *HSP90–* overexpression of *PKC1* was found to ameliorate the temperature sensitivity in these heat shock protein mutants (Wright et al. 2007). Our results suggest the role of CCT, a constitutively expressed cytosolic chaperonin, in maintaining cell wall integrity in *S. cerevisiae*.

Methods

Request a detailed protocol

Yeast extract (1%)- Peptone (2%)- Dextrose (2%) (YPD) medium was used to grow the yeast strains. The *cct1-2* and *cct4-1* strains were grown at different temperatures in media with and without sorbitol to study the effect of osmotic stabilizers. DUY326 was transformed with pFR22 (YEpU-PKC1) and pCMS154-HSP82 for the overexpression of *PKC1* and *HSP82*, respectively (Wright et al. 2007) using the lithium acetate/PEG method. The transformants were selected on uracil-omission medium. The plates were incubated at 30 °C for two days and the transformants were subcultured in uracil-omission medium. Approximtely equal number of cells of the transformants were serially diluted, spotted on YPD and incubated at different temperatures to study the phenotype.

Reagents

| Plasmid          | Description                        | Reference/Source |
|------------------|------------------------------------|------------------|
| pRSII326         | 2- micron based multicopy plasmid with *URA3* | Chee et al. 2012 |
| YEpU-PKC1        | *PKC1* cloned in YEp352            | Wright et al. 2007 |
| pCMS154-HSP82    | *HSP82* cloned in pRS426           | Wright et al. 2007 |
| pAB2477          | *CCT1* *URA3* CEN/ARS              |                  |

| Strain | Genotype                  | Figure | Source   |
|--------|---------------------------|--------|----------|
| B-8728 | *MATa* *ura3-52* *trp1-Δ63* *leu2-Δ1* *GAL2* | Figure 1A | Lin et al. 1997 |
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References
Berger J, Berger S, Li M, Jacoby AS, Arner A, Bavi N, Stewart AG, Currie PD. 2018. In Vivo Function of the Chaperonin TRiC in α-Actin Folding during Sarcomere Assembly. Cell Rep 22: 313-322. PMID: 29320728.
Boudiaf-Benmammar C, Cresteil T, Melki R. 2013. The cytosolic chaperonin CCT/TRiC and cancer cell proliferation. PLoS One 8: e60895. PMID: 23613750.

Frydman J, Nimmesgern E, Erdjument-Bromage H, Wall JS, Tempst P, Hartl FU. 1992. Function in protein folding of TRiC, a cytosolic ring complex containing TCP-1 and structurally related subunits. EMBO J 11: 4767-78. PMID: 1361170.

Kabir MA, Sherman F. 2008. Overexpressed ribosomal proteins suppress defective chaperonins in Saccharomyces cerevisiae. FEMS Yeast Res 8: 1236-44. PMID: 18680526.

Kamada Y, Jung US, Piotrowski J, Levin DE. 1995. The protein kinase C-activated MAP kinase pathway of Saccharomyces cerevisiae mediates a novel aspect of the heat shock response. Genes Dev 9: 1559-71. PMID: 7628692.

Knowlton JJ, Fernández de Castro I, Ashbrook AW, Gestaut DR, Zamora PF, Bauer JA, Forrest JC, Frydman J, Risco C, Dermody TS. 2018. The TRiC chaperonin controls reovirus replication through outer-capsid folding. Nat Microbiol 3: 481-493. PMID: 29531365.

Levin DE. 2005. Cell wall integrity signaling in Saccharomyces cerevisiae. Microbiol Mol Biol Rev 69: 262-91. PMID: 15944456.

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: 1609-33. PMID: 9409825.

Llorca O, Smyth MG, Carrascosa JL, Willison KR, Radermacher M, Steinbacher S, Valpuesta JM. 1999. 3D reconstruction of the ATP-bound form of CCT reveals the asymmetric folding conformation of a type II chaperonin. Nat Struct Biol 6: 639-42. PMID: 10404219.

Melkani GC, Bhide S, Han A, Vyas J, Livelo C, Bodmer R, Bernstein SI. 2017. TRiC/CCT chaperonins are essential for maintaining myofibril organization and cardiac physiological rhythm, and lifespan. FEBS Lett 591: 3447-3458. PMID: 28963798.

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: 38-45. PMID: 26992923.

Ohhara Y, Nakamura A, Kato Y, Yamakawa-Kobayashi K. 2019. Chaperonin TRiC/CCT supports mitotic exit and entry into endocycle in Drosophila. PLoS Genet 15: e1008121. PMID: 31034473.

Ribas JC, Diaz M, Duran A, Perez P. 1991. Isolation and characterization of Schizosaccharomyces pombe mutants defective in cell wall (1-3)beta-D-glucan. J Bacteriol 173: 3456-62. PMID: 1828464.

Shimon L, Hynes GM, McCormack EA, Willison KR, Horovitz A. 2008. ATP-induced allosterity in the eukaryotic chaperonin CCT is abolished by the mutation G345D in CCT4 that renders yeast temperature-sensitive for growth. J Mol Biol 377: 469-77. PMID: 18272176.

Tam S, Geller R, Spiess C, Frydman J. 2006. The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. Nat Cell Biol 8: 1155-62. PMID: 16980959.
Wright CM, Fewell SW, Sullivan ML, Pipas JM, Watkins SC, Brodsky JL. 2007. The Hsp40 molecular chaperone Ydj1p, along with the protein kinase C pathway, affects cell-wall integrity in the yeast Saccharomyces cerevisiae. Genetics 175: 1649-64. PMID: 17237519.

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