

Chapter 19

TRiC/CCT Chaperonin: Structure and Function



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Abstract The eukaryotic group II chaperonin TRiC/CCT assists the folding of 10% of cytosolic proteins including many key structural and regulatory proteins. TRiC plays an essential role in maintaining protein homeostasis, and dysfunction of TRiC is closely related to human diseases including cancer and neurodegenerative diseases. TRiC consists of eight paralogous subunits, each of which plays a specific role in the assembly, allosteric cooperativity, and substrate recognition and folding of this complex macromolecular machine. TRiC-mediated substrate folding is regulated through its ATP-driven conformational changes. In recent years, progresses have been made on the structure, subunit arrangement, conformational cycle, and substrate folding of TRiC. Additionally, accumulating evidences also demonstrate the linkage between TRiC oligomer or monomer and diseases. In this review, we focus on the TRiC structure itself, TRiC assisted substrate folding, TRiC and disease, and the potential therapeutic application of TRiC in various diseases.

Keywords Chaperonin · TRiC/CCT · Structure · Function · Substrate folding · ATP-driven conformational changes · Cryo-EM

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Introduction

Many proteins need assistance to fold into their native state and achieve functional activity after release from ribosome into the crowded environment of the cell (Fig. 19.1) (Kim et al. 2013). Correspondingly, cells have evolved a system of molecular chaperones and quality control machineries, often called the “protein homeostasis” network. Proteins from this network function to bind unfolded or misfolded polypeptides and induce their folding, sequester them, or facilitate their degradation (Kim et al. 2013; Willison 2018). The chaperonins, a critical group of molecular chaperones, are large double-ring complexes of 800–1000 kDa built of 7–9 subunits per ring, and each ring provides a central cavity for ATP-driven chaperonin-mediated protein folding cycle (Hartl and Hayer-Hartl 2002; Horwich et al. 2007; Willison 2018).

Chaperonins have been classified into two groups (Horwich and Willison 1993), including group I chaperonins presented in prokaryotes (GroEL/GroES), mitochondria (Hsp60/Hsp10), and chloroplasts (Rubisco subunit binding protein), and group II chaperonins presented in archaea (thermosome, Mm-cpn) and eukaryotes (TCP-1 ring complex, TRiC, or chaperonin-containing TCP-1, CCT) (Fig. 19.2a–c). Group I chaperonin consists of a detachable co-chaperonin, while group II chaperonin has a build-in lid in the apical domain that can shut close the chamber (Fig. 19.2a–c). The archaea group II chaperonin has eight or nine subunits per ring, while the eukaryotic group II chaperonin has eight paralogous subunits. The eukaryotic group II chaperonin TRiC/CCT (~960 kDa) comprises of two hetero-oligomeric stacked rings with eight distinct subunits each in a certain arrangement. The paralogous subunits share about 23–35% and 27–39% sequence identity for yeast and bovine TRiC, respectively (Frydman et al. 1992; Ursic and Culbertson 1991). An individual TRiC subunit has three domains: an equatorial domain (E domain) that contains the ATP-binding site and forms the intra- and inter-ring contacts, an apical domain (A domain) that comprises the interaction sites with target proteins, and an intermediate domain (I domain) that connects the other two domains (Fig. 19.2d) (Ditzel et al. 1998; Waldmann et al. 1995; Klumpp et al. 1997).

Coordinated with its ATP-driven conformational cycle, TRiC can assist the folding of approximately 10% of cytosolic proteins, including the cytoskeleton proteins actin and tubulin, which are also redundant substrates of TRiC (Khabirova et al. 2014; Waldmann et al. 1995; Llorca et al. 1999, 2000), cell cycle regulator CDC20 (Camasses et al. 2003), and G-protein signaling related element G β (Plimpton et al. 2015). TRiC is also essential for the folding of many proteins involved in oncogenesis, such as p53, VHL tumor suppressor, and STAT3 (Trinidad et al. 2013; Kasembeli et al. 2014; McClellan et al. 2005), and for the folding of retinal developmental factors, including transducin α and PEX7 (Tables 19.1 and 19.2) (Hunziker et al. 2016; van den Brink et al. 2003). Hence, dysfunction of TRiC is closely related to cancer and neurodegenerative diseases

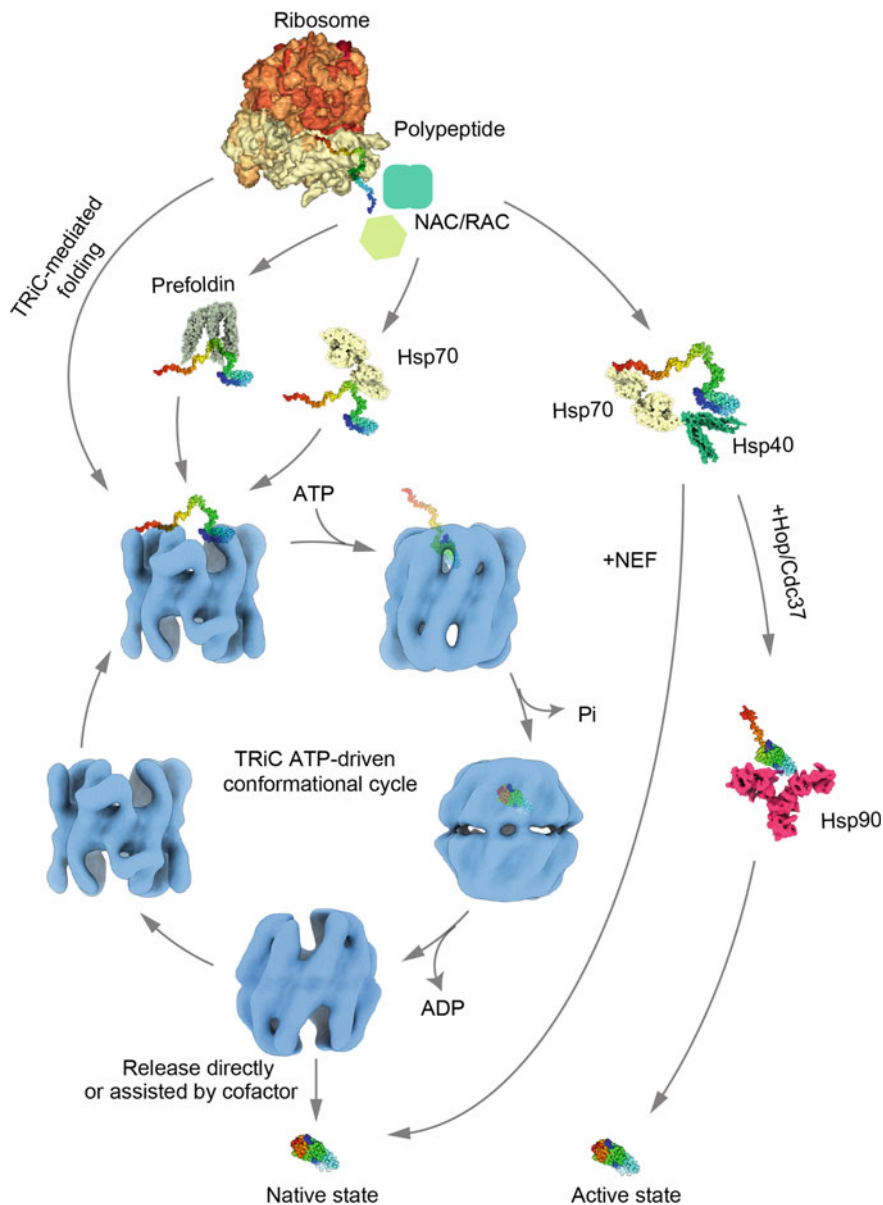


Fig. 19.1 Proposed TRiC-mediated substrate folding pathway. The scheme shows the downstream protein folding pathway from the ribosome. Hsp70 serves as the hub of the chaperone network, which cooperates with Hsp40s and nucleotide exchange factors (NEFs) to fold $\sim 20\%$ of the proteome. TRiC binds and helps fold $\sim 10\%$ of cytosolic proteins directly or with the assistance of cofactors (prefoldin, Hsp70) for efficient substrate delivery. TRiC-mediated substrate folding is regulated through its ATP-driven conformational changes. Then substrate is released from the central cavity of TRiC to achieve its native state, which sometimes needs the help of cofactor, such as Phlp1. In addition, eukaryotes also engage the Hsp90 chaperone system to catalyze the activation of metastable proteins *via* the Hsp70 system and the co-chaperone Hop

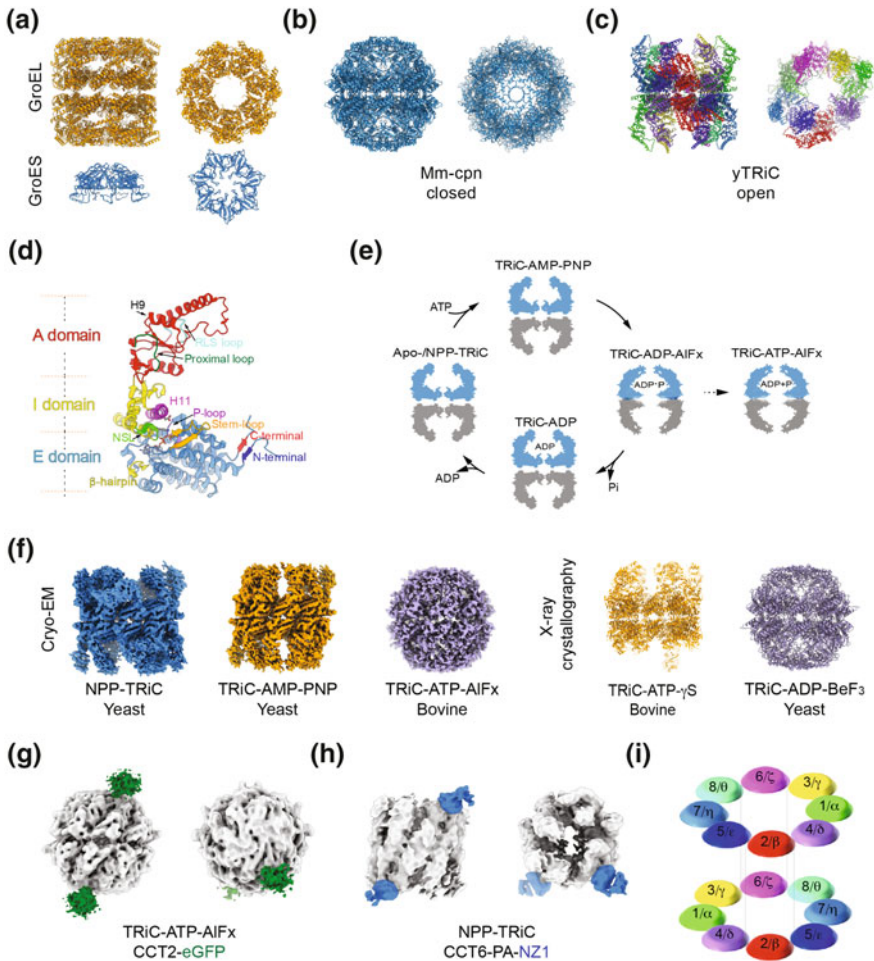


Fig. 19.2 TrIC structural studies and subunit arrangement. **a** The crystal structures of GroEL (PDB ID: 1SS8, orange) and co-chaperone GroES (PDB ID: 1PCQ, cyan). **b** The crystal structure of archaea group II chaperonin Mm-cpn (PDB ID: 3RUQ). **c** The cryo-EM-structure-derived pseudo atomic model of yeast TrIC at open NPP state with different subunits in distinct colors (PDB ID: 5GW4). **d** The three domains of a TrIC subunit, with the key structural elements labeled. **e** A cartoon diagram illustrating TrIC ATPase cycle. **f** Available relative high-resolution structures of TrIC at different nucleotide states, including the cryo-EM structures of NPP-TrIC (EMDB: 9540), TrIC-AMP-PNP (EMDB: 9541), and TrIC-ATP-AIFx (EMDB: 5415), X-ray structures of TrIC-ATP- γ S (PDB: 4B2T) and TrIC-ADP-BeF₃ (PDB: 4V8R). **g** Illustration of using YISEL method to identify the location of CCT2 subunit in TrIC complex. The map of the closed yeast TrIC is in grey, with eGFP (dark green) tagged to CCT2 subunits (one in each ring). **h** Illustration of using YISPANL method to identify the location of CCT6 subunit in TrIC complex. The map of open yeast TrIC is in grey, with NZ-1 fab (sky blue) tagged to CCT6 subunits. **i** Determined TrIC subunit arrangement

Table 19.1 Involvement of TRiC in diseases

Disease		TRiC or subunits	Biological behavior	Substrate	References
Neuro-related diseases	Huntington's disease	CCT1, CCT5	Inhibit aggregation	Huntingtin	Shahmoradian et al. (2013), Darrow et al. (2015), Sontag et al. (2013)
	Parkinson's disease	CCT2, CCT3, CCT6	Inhibit amyloid fiber assembly of α -synuclein A53T	α -synuclein A53T	Sot et al. (2017)
	Alzheimer's disease	TRiC	Inhibit A β toxicity	A β peptide	Khabirova et al. (2014)
	Cockayne syndrome	CCT4, CCT5	Interaction	CSA	Pines et al. (2018)
	Hereditary sensory neuropathy	CCT4	C450Y (Mutation)		Lee et al. (2003)
	Mutilating sensory neuropathy	CCT5	H147R (Mutation)		Bouhouche et al. (2006)
	Neuronal Apoptosis	CCT8	up-regulated		Wu et al. (2015b)
Eye-related diseases	Leber Congenital amaurosis	CCT2	T400P, R516H (Mutation)		Minegishi et al. (2016)
	Achromatopsia	TRiC	Folding	Transducin α	Kohl et al. (2002), Koulikovska et al. (2005), Farr et al. (1997)
	Refsum disease	TRiC	Folding	Peroxisomal targeting signal 2 receptor protein	Siegers et al. (2003), van den Brink et al. (2003), Erdo et al. (2004)
	Usher syndrome	TRiC	Folding	Myosin	Srikakulam and Winkelmann (1999), Adato et al. (1997), Erdo et al. (2004)

(continued)

Table 19.1 (continued)

Disease		TRiC or subunits	Biological behavior	Substrate	References
Cancer	Breast cancer	CCT1, CCT2, CCT5	Up-regulated	p53	Guest et al. (2015), Ooe et al. (2007)
	Colorectal cancer	CCT2, CCT6	Up-regulated		Qian-Lin et al. (2010), Coghlin et al. (2006)
	Glioma	CCT8	Up-regulated		Qiu et al. (2015)
	Hepatocellular cancer	CCT3, CCT8	Up-regulated		Zhang et al. (2016), Wei et al. (2016), Cui et al. (2015), Huang et al. (2014), Yokota et al. (2001)
	Lymphoma	CCT1, CCT8	Up-regulated		Yin et al. (2016), Xudong Jiang et al. (2015)
	Small cell lung cancer	CCT2	Up-regulated		Carr et al. (2017)
	SC/ASC, AC	CCT2	Up-regulated		Zou et al. (2013)
	Uterine Cancer	CCT2	Interaction	β -tubulin	Lin et al. (2009)

such as Huntington's, Alzheimer's, and Parkinson's diseases (Chen et al. 2014; Bassiouni et al. 2016).

Our knowledge of the TRiC complex has been summarized in a large number of superb reviews (Willison 2018a, b; Balchin et al. 2016; Lopez et al. 2015; Broadley and Hartl 2009; Dunn et al. 2001; Skjaerven et al. 2015; Fernandez-Fernandez et al. 2016). Our current review will mainly focus on the recent progresses on the structure and function of TRiC complex and TRiC related diseases.

TRiC Molecular Structure

Structural Studies of TRiC and Its ATP-Driven Conformational Cycle

Purification of such a large eukaryotic complex as TRiC is usually challenging. There are several established purification protocols for direct purification of TRiC from mammalian tissues or cells (Lewis et al. 1992; Frydman et al. 1992; Knee et al. 2013; Gao et al. 1992), as well as from yeast (Pappenberger et al. 2006; Zang et al. 2016). In addition, a method was also reported to reconstitute human TRiC by co-expressing its eight distinct subunits in BHK-21 cells (Machida et al. 2012).

Table 19.2 Involvement of TRiC in biological system/process

Biological system/ Process	Organism	Related subunits	Substrate	References
MCC assembly	Human	TRiC	CDC20	Kaisari et al. (2017)
Autophagy	Human	CCT2, CCT5, CCT7		Pavel et al. (2016)
Sarcomere Z-disk	<i>Dario rerio</i>	CCT5 (Mutation G422V)		Berger et al. (2018)
Invasion	<i>C. elegans</i>	CCT5		Matus et al. (2010)
Cell polarity	Yeast	TRiC		Liu et al. (2010)
Morphogenesis	Yeast	CCT8		Rademacher et al. (1998)
Spermatogenesis	<i>Flatworm</i>	CCT1, CCT3, CCT4, CCT8		Counts et al. (2017)
Fibroblast motility	Human	CCT7		Satish et al. (2010)
Proteostatic control of telomerase function	Human	TRiC	TCAB1	Freund et al. (2014)
Cancer signaling	Human	CCT3	STAT3	Kasembeli et al. (2014)
Apoptosis/ autophagy/cell motility	Human	TRiC		Fontanella et al. (2010)
Myeloid leukemia	Human	CCT6, CCT8	AML1-ETO	Roh et al. (2016a, b)
Atherosclerosis development	Human	TRiC	LOX-1	Bakthavatsalam et al. (2014)
Mediating cellular responses to oxygen	Human	TRiC	PHD3	Masson et al. (2004)
Chromatin remodeling	Human	TRiC	HDACs	Guenther et al. (2002)
Cilia biogenesis	<i>Tetrahymena</i>	CCT1, CCT3, CCT4, CCT7		Soues et al. (2003), Seixas et al. (2010), Cyrne et al. (1996)
Proper formation of microvilli in intestinal cells	<i>C. elegans</i>	TRiC		Saegusa et al. (2014)
Lifespan extension	<i>C. elegans</i>	CCT8		Noormohammadi et al. (2016)
Fragile X mental retardation	<i>Drosophila</i>	CCT3, CCT4, CCT7		Monzo et al. (2010)
Stem cell function	<i>Arabidopsis</i>	CCT8		Xu et al. (2011)
TFIID assembly	Human	TRiC	TAF5	Antonova et al. (2018)

Chaperonins are molecular machines that assist protein folding by undergoing ATP-dependent conformational changes that are coordinated in time and space owing to complex allosteric regulation (Fig. 19.2e). Since the early days, both negative staining-electron microscopy (NS-EM) and cryo-electron microscopy (cryo-EM) have been used to investigate the complex structure itself and the ATP-induced conformational changes of TRiC (Lewis et al. 1992; Frydman et al. 1992; Gao et al. 1992; Llorca et al. 1998). Moreover, small angle X-ray scattering (SAXS) analysis also suggested that ATP hydrolysis is needed for the lid closure of TRiC (Meyer et al. 2003).

Furthermore, reference-free 2D analysis of cryo-EM data showed the asymmetric nature among the eight distinct subunits of bovine TRiC (Rivenzon-Segal et al. 2005). A cryo-EM structural study revealed that the inter-domain motions lead to lid closure in bovine TRiC, which is radically different from those of group I chaperonins despite their overall structural similarity (Booth et al. 2008). Our previous asymmetric cryo-EM 3D reconstructions on TRiC in different nucleotide states demonstrated that TRiC changes its intra- and inter-ring subunit interaction pattern during the ATPase cycle, with all the subunits in each ring highly asymmetric in the apo state, whereas all the nucleotide-containing states tend to be more symmetrical. Moreover, the eight subunits in each ring form four subunits pairs, including CCT2-CCT4, CCT1-CCT3, CCT6-CCT8, and CCT7-CCT5; and there exists a two fold axis between its two rings, resulting in two homotypic subunit interactions across the rings (Cong et al. 2010, 2012).

We recently reconstructed two cryo-EM structures of yeast TRiC in a newly captured nucleotide partially preloaded (NPP) state (termed NPP-TRiC) and in the ATP-bound state at ~ 4.6 Å resolution (Zang et al. 2016). Our NPP-TRiC map showed that CCT2 subunit-pair forms an unexpected Z-shaped feature (red subunit pair, Fig. 19.2c), which conformation is different from that of bovine TRiC in the apo state, whose CCT2 subunit is intrinsically dynamic with its A domain mostly missing (Cong et al. 2010, 2012). ATP binding could induce a dramatic conformational change of CCT2 subunit, which could be either unbent or stabilized for yeast or bovine TRiC, respectively, constructing a typical inward tilting conformation as the other subunits. Strikingly, for yeast TRiC, CCT3, CCT6, and CCT8 subunits have preloaded nucleotide from the environment of the yeast cell (Zang et al. 2016). Overall, TRiC has evolved into a complex that is structurally divided into two sides and reveals a staggered ATP binding mechanism. Interestingly, there are extra densities blocking the two chambers of yeast TRiC in both NPP-TRiC and TRiC-AMP-PNP maps as well as in the bovine apo-TRiC and TRiC-AMP-PNP maps, which may be correlated to the N- and C- termini and might be involved in the complex assembly and allosteric regulation (Cong et al. 2012; Zang et al. 2016).

There are biochemical investigations and computational analyses on the subunit specificity of TRiC in ATP usage. It has also been suggested the ATP binding affinity varies among the eight distinct subunits of TRiC. A recent report has shown that within one ring of bovine TRiC, only four subunits (CCT1, CCT2, CCT4, and CCT5) bind ATP at its physiological concentration (Reissmann et al. 2012). Additionally, it has also been reported that the most dramatic phenotypic effects are

associated with mutations in the nucleotide-binding pockets of CCT4, CCT2, CCT5, and CCT7 of yeast TRiC; however, similar mutations do not markedly affect CCT3, CCT6, and CCT8 (Amit et al. 2010), which is consistent with our observation that there are preloaded nucleotides in these three subunits (CCT3/6/8) (Zang et al. 2016). Moreover, equivalent mutations in the eight subunits of TRiC at the positions involved in ATP hydrolysis led to dramatically different phenotypes, indicating each subunit may have different function and play distinct role in TRiC ATPase hydrolysis (Amit et al. 2010). By measuring emissions from chaperonin-bound fluorescent nucleotides, it was suggested that TRiC either hydrolyzes no ATP or hydrolyzes about four ATPs in each of the two rings at the same time (Jiang et al. 2011). By measuring the amount of phosphate generated by ATP hydrolysis as a function of time, it has been indicated that TRiC is in an equilibrium with a large number of conformational states, and that “conformational selection” by ATP takes place before hydrolysis (Korobko et al. 2016). Diffracted X-ray tracking experiment by characterizing *Chaetomium thermophilum* TRiC variants containing ATPase-deficient subunits showed that ATP-induced motion in a ring is in an asymmetric manner (Yamamoto et al. 2017). All the above studies show that TRiC subunits consume ATP in an asymmetric manner, coordinated in time and space owing to complex allosteric regulations, and each subunit has its own functional specificity. However, the underlying structural basis of this specificity and the evolutionary benefit need further investigation.

TRiC Subunit Arrangement

To better understand the subunit specificity in ATP usage and allosteric regulation, as well as in substrate recognition and folding, it is essential to address the long-standing question regarding the subunit arrangement of the TRiC complex. In the early studies, TRiC micro-complexes (comprised of subsets of the constitutively expressed TRiC subunits) were examined and implied the unique topology of TRiC subunits (Liou and Willison 1997). In the past decade, extensive efforts have been made to determine the subunit arrangement of this complex molecular machine (Fig. 19.2f) (Dekker et al. 2011; Munoz et al. 2011; Cong et al. 2010; Liou and Willison 1997; Kalisman et al. 2012). Recently, the arrangement has been better determined by chemical cross-linking and mass spectrometry (XL-MS) (Kalisman et al. 2012; Leitner et al. 2012). Moreover, computational approaches have been used to allocate the subunits in the X-ray structures of yeast TRiC in the presence of ATP-BeFx and bovine TRiC in the presence of ATP- γ S, which confirmed the XL-MS result (Kalisman et al. 2013). However, due to the structural similarity among TRiC subunits and resolution limitation, the conclusions have been inconsistent across studies (Dekker et al. 2011; Munoz et al. 2011; Cong et al. 2010; Liou and Willison 1997; Kalisman et al. 2012). Recently, combining the cryo-EM reconstruction of yeast TRiC at the open NPP state with our recently developed yeast internal-subunit eGFP labeling (YISEL) strategy and yeast inner-subunit

PA-NZ-1 labeling (YISPANL) strategy, we unambiguously identified the subunit locations in the open-state TRiC (Fig. 19.2g–i) (Zang et al. 2018; Wang et al. 2018), and the subunit ordering determined in this way is in agreement with the previous XL-MS result (Kalisman et al. 2012; Leitner et al. 2012).

Structural Study on TRiC with Substrate

TRiC plays an important role in the maintenance of protein homeostasis. Therefore, capturing high-resolution structural information of TRiC in complex with substrate is of great significance for our understanding of the mechanism of how TRiC recognizes and folds substrate proteins through its ATP-driven conformational cycle. However, due to the relative low binding efficiency between TRiC and the pretreated unfolded substrates (by applying urea or GuHCl *in vitro*), and the potential conformational heterogeneity of bound substrate, so far the available structural information of TRiC-substrate remains limited.

Along this line, a number of efforts have been made to investigate the TRiC-substrate complex mainly using cryo-EM. Early cryo-EM studies of TRiC in complex with denatured actin (Llorca et al. 1999) or denatured tubulin (Llorca et al. 2000) in the open state indicated that actin binds below the helical protrusions with multiple TRiC subunits, while tubulin interacts directly with the helical protrusion of two TRiC subunits. Subsequently, structural and functional analyses of Huntingtin-TRiC complex uncovered the A domains of CCT1 and CCT4 involve in the inhibition of the aggregation process of Huntingtin, the causative agent of Huntington's disease (Tam et al. 2006; Darrow et al. 2015; Sontag et al. 2013; Shahmoradian et al. 2013). Recently, the cryo-EM structure of cancer-related protein AML1-ETO in complex with human TRiC revealed the subunit-specific interaction, primarily through its DNA binding domain with two TRiC subunits (Fig. 19.3a) (Roh et al. 2016a, b).

There are few clear definitions from the common sequence or structural features of TRiC substrates except for WD40-domain containing proteins, which have been described to be an important family of TRiC substrates (Lopez et al. 2015; Willison 2018b). The WD40-domain containing proteins usually consist of a 7-bladed- β -propeller and could coordinate multi-protein complex assemblies, where the repeating units serve as a rigid scaffold for protein interactions. A number of TRiC substrates have been found to have the WD40 β -propeller features such as TCAB1, CDC20, and G β (Freund et al. 2014; Kaisari et al. 2017; Plimpton et al. 2015; Miyata et al. 2014). To overcome the low binding efficiency problem, researchers purified the TRiC substrate, human G β , from insect cells. In this way, the potentially partially folded human G β may retrieve the intact insect TRiC from the cells. Then the cryo-EM structures of G β -TRiC and PhLP1-G β -TRiC complexes were reconstructed, revealing a mechanism for G β folding and G $\beta\gamma$ dimer assembly assisted by TRiC (Plimpton et al. 2015). Recently, it has been reported that TRiC

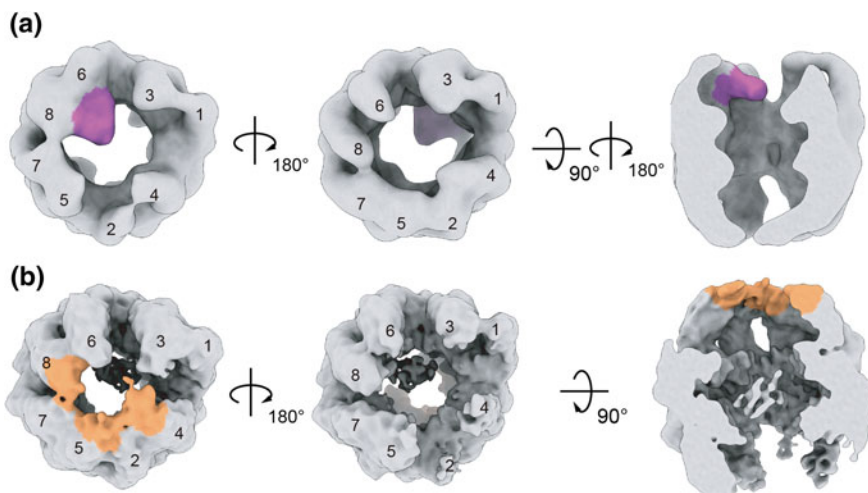


Fig. 19.3 Cryo-EM structures of TRiC in complex with substrate. **a** Cryo-EM structure of TRiC-AML1-175 (EMDB: 6227, the map is rendered according to the recommended threshold). TRiC density is colored in grey and AML1-175 in purple. Subunits are also labeled. **b** Cryo-EM structure of TRiC-actin (EMDB: 0017, the map is rendered according to the recommended threshold). TRiC density is colored in grey and actin density in orange

could capture TAF5, assist its WD40 domain folding and subsequent handover to TAF6-TAF9 and ultimately holo-TFIID formation (Antonova et al. 2018).

There are also X-ray structures of actin-TRiC in the presence of ADP-BeFx in the closed state at 3.8 Å resolution, revealing residual density in one of the TRiC cavities as unfolded actin interacting with multiple TRiC subunits (Dekker et al. 2011), and of tubulin-TRiC with ATP-γS in the open state at 5.5 Å resolution, which also allocated part of the substrate density contacting with three TRiC subunits (Munoz et al. 2011). A recent study combining cryo-EM, fluorescence correlation spectroscopy (FCS), and hydrogen/deuterium exchange coupled to mass spectrometry (H/DX-MS) determined the conformational progression of actin as it is folded by bovine TRiC. This analysis revealed that actin binds to five TRiC subunits (CCT2/4/8/7/5), and captured the dynamic interaction locations through the ATP-driven TRiC-mediated actin folding cycle (Fig. 19.3b) (Balchin et al. 2018).

Key Structural Elements of Group II Chaperonin

To-date, many key structural elements of group II chaperonins involved in nucleotide consumption, subunit coordination, and substrate recognition have been delineated (Fig. 19.2d). The GDGTT motif, called the p-loop and located in the

E domain of individual subunits of group II chaperonins, involves ATP binding and is strictly conserved in all chaperonins (Amit et al. 2010). The nucleotide-sensing loop (NSL) located in the I domain monitors the presence of the γ -phosphate of ATP (Pereira et al. 2012; Zang et al. 2016). The β -hairpin in the E domain of almost every archaea and eukaryotic group II chaperonin subunit has been suggested to play a role covering the entrance of the nucleotide pocket (Pereira et al. 2012; Zang et al. 2016). Between two adjacent subunits of group II chaperonins within a ring, there are contacts linking their E domains *via* a mixed 4-stranded β -sheet involving the N- and C- termini of one subunit and the stem-loop of the neighboring subunit that communicates with its nucleotide pocket (Zhang et al. 2010; Pereira et al. 2010; Ditzel et al. 1998). In addition, the proximal loop (PL) in the A domain recognizes substrate together with its neighboring α -helix 9 (H9), while the release loop for substrate (RLS) is responsible for evicting bound substrate into the TRiC chamber during TRiC ring closure (Lopez et al. 2015; Joachimiak et al. 2014). Still, it needs further examination on how these structural elements coordinate together to perform allosteric conformational transitions in ring closure and substrate folding.

Structure of TRiC with Co-chaperone

Although TRiC does not need a co-chaperone to cover the central cavity forming a closed ring, it has co-chaperone or cofactors to deliver unfolded/partially folded substrates, or to accelerate the substrate folding process (Fig. 19.1). There are several cofactors for TRiC: (1) prefoldin, a hexameric chaperone, binds specifically to chaperonin and transfers target protein (mainly actin) to it (Vainberg et al. 1998); (2) phosducin-like protein, including three known families of phosducin-like proteins participating in G β -protein signaling, which have also been implicated in other process, such as the folding of actin and tubulin (Plimpton et al. 2015; Stirling et al. 2006; Humrich et al. 2005); (3) Hsp70, another heat-shock protein that was found to be involved in substrate delivery to TRiC (Cuellar et al. 2008; Melville et al. 2003). Therefore, understanding the interaction mechanism of TRiC with cofactors may provide a thorough picture of TRiC-assisted substrate folding. The cryo-EM map of the TRiC-PFD complex indicates that PFD (human) binds to both rings of TRiC (bovine) in a unique configuration, suggesting a substrate handoff mechanism from PFD to TRiC (Martin-Benito et al. 2002). The cryo-EM structure of bovine TRiC-Hsc70 also supports the notion that Hsc70 may deliver the unfolded proteins to the substrate receiving region of TRiC, and it has been postulated that in eukarya, TRiC and Hsc70 evolved as a concerted action that makes the folding task more efficient (Cuellar et al. 2008). Recently, a cryo-EM structure of insect TRiC in complex with the co-chaperone phosducin-like protein 1 (PhLP1, human) and substrate G β (human) was reported. This study indicated that PhLP1 binding could stabilize the G β fold, disrupting interactions with TRiC and releasing a PhLP1-G β dimer for assembly with G γ (Plimpton et al. 2015). Still, there are remaining obscure questions regarding the role that co-chaperone plays in cooperating with

TRiC that need to be addressed, such as in what situation co-chaperone binds substrate, and when and how co-chaperone delivers substrate to TRiC or helps release substrate from TRiC.

Cryo-ET of TRiC with Substrate

It is exciting to directly visualize locations of TRiC in the cell and to understand how TRiC assists substrate folding in situ. In recent years, cryo-electron tomography (cryo-ET) has emerged as a powerful tool for direct visualization of the molecular organization of cellular landscapes, with the potential to reach near-atomic resolution (Beck and Baumeister 2016; Mosalaganti et al. 2018; Guo et al. 2018). Cryo-ET has been used to visualize the interacting location of TRiC on the mutant huntingtin exon fibril, containing an expanded polyglutamine tract with 51 residues (mhttQ51) and resolved a 3D structure of TRiC interacting with mhttQ51, which provided a structural description for TRiC's inhibition of mhttQ51 aggregation in vitro (Shahmoradian et al. 2013). Recently, using cryo-ET and sub-tomogram averaging, the structure and cellular interactions of poly-GA aggregates (poly-Gly-Ala aggregates resulting from aberrant translation of an expanded GGGGCC repeat in C9orf72) within intact neurons was visualized, in which TRiC molecules were also found to locate between poly-GA ribbons (Guo et al. 2018). These studies pave the way for further understanding of the function of TRiC in substrate folding or its role in hindering substrate protein aggregation.

Structure of Mutated TRiC and Homo-Oligomer of TRiC Single Subunit

TRiC consists of eight distinct subunits which bring extra complexity to its architecture and functional specificity. Each subunit of TRiC may play a distinct role in the assembly, allosteric coordination, and substrate processing of TRiC complex (Spiess et al. 2004). Some TRiC subunits are able to form a biologically active homo-oligomer when respectively expressed in *Escherichia coli*. It has been shown that human CCT4 and CCT5 could form biologically active homo-oligomer independently, and a cryo-EM reconstruction of CCT5 homo-oligomeric ring suggests an overall TRiC-like structure (Sergeeva et al. 2013). Intriguingly, similar to WT TRiC, the synthetic CCT5 homo-oligomer complex was found to be able to cap mHTT fibrils at their tips and inhibit mHTT aggregation, revealed by cryo-ET analysis (Darrow et al. 2015), and it is also able to promote the disassembly of the mitotic checkpoint complex (MCC) in the same fashion as TRiC (Kaisari et al. 2017). Moreover, two mutations, C450Y of CCT4 and H147R of CCT5, were identified in hereditary sensory neuropathies (HSNs) (Bouhouche et al. 2006;

Lee et al. 2003). NS-EM study revealed that CCT4-C450Y forms few ring-shaped species, whereas WT CCT4, CCT5-H147R, and WT CCT5 can form similar ring structures (Sergeeva et al. 2014). Recently, the X-ray structures of human CCT5 and CCT5-H147R recombinant complexes were resolved, respectively, which demonstrate the mutation may impact the allosteric cooperativity of TRiC (Pereira et al. 2017).

Functions of TRiC

Substrate Folding Assisted by TRiC

Although the mechanism of TRiC-substrate recognition and binding need further investigation, it has been demonstrated that substrate motifs are recognized by a cleft formed between the H9 and PL in the A domains of TRiC individual subunits (Fig. 19.2d), and this cleft contains subunit-specific patterns of polar and hydrophobic residues (Joachimiak et al. 2014). Thus, the diversification of TRiC subunits, especially in the A domain regions, enables TRiC to recognize and bind diverse substrates (Yamamoto et al. 2017). Considering that there are usually multiple TRiC subunits involving in substrate binding (Lopez et al. 2015), this explains why TRiC can assist up to 10% of cytosolic proteins to fold in the cell. Moreover, TRiC plays an important role in the folding of newly synthesized proteins (Frydman et al. 1994; Yam et al. 2008), but it can also prevent the aggregation of proteins with polyglutamine regions (Kitamura et al. 2006; Tam et al. 2006), thus potentially contributing to the suppression of mis-folding in diseases such as Huntington's, Parkinson's, and Alzheimer's diseases. Still, for the folding state changes of substrates along with the ATP-driven ring closure process, TRiC remains to be further explored.

TRiC and Diseases

TRiC and Neurodegenerative Diseases

Many neurodegenerative disorders, such as Alzheimer's, Parkinson's, and polyglutamine diseases, as well as amyotrophic lateral sclerosis (ALS), are characterized by conformational changes in proteins that result in misfolding, aggregation and intra- or extra-neuronal accumulation of amyloid fibrils, molecular chaperones including TRiC provide the first line of defense (Table 19.1) (Muchowski and Wacker 2005; Roh et al. 2016b). Recent studies have investigated the role of TRiC in neurodegenerative diseases (Voisine et al. 2010; Liebman and Meredith 2010). It has been biochemically shown that TRiC can inhibit several aggregation variants

both in vitro and in vivo (Kitamura et al. 2006; Tam et al. 2006; Behrends et al. 2006) and in cooperation with the Hsp70 system (Behrends et al. 2006). Moreover, a cryo-ET analysis provided a structural description for TRiC's inhibition of mhttQ51 aggregation in vitro (Shahmoradian et al. 2013).

Moreover, genetic defects in CSA, a 7-bladed WD40 protein, mostly give rise to Cockayne syndrome, which is characterized by premature aging, progressive mental and sensorial retardation, microcephaly, severe growth failure, and cutaneous photosensitivity. TRiC has been shown to interact with CSA through its WD40 domain, thereby regulating CSA stability (Pines et al. 2018). In addition, several individual subunits of TRiC have been reported to be related to hereditary sensory neuropathy, neuronal apoptosis or neurological defects (Lee et al. 2003; Bouhouche et al. 2006; Wu et al. 2015b; Matsuda and Mishina 2004) (described elsewhere in this chapter).

TRiC and Eye Related Diseases

It had been reported that several retinal developmental factors are the client of TRiC, such as myosin, the cause of Usher syndrome (Bonné-Tamir et al. 1994; Adato et al. 1997), transducin α , the cause of Achromatopsia (Kohl et al. 2002), and peroxisomal targeting signal 2 receptor protein, the cause of Refsum disease (Srikakulam and Winkelman 1999; Farr et al. 1997).

Several studies suggested an essential role of TRiC in ciliogenesis. The most abundant clients of TRiC are represented by cytoskeletal proteins from the actin and tubulin families (Gao et al. 1992; Grantham et al. 2006; Hanafy et al. 2004; Vinh and Drubin 1994; Stemp et al. 2005; Chen et al. 1994). It has been proven that TRiC plays an essential role in the biogenesis of vertebrate photoreceptor sensory cilia (Sinha et al. 2014). Additionally, suppressing TRiC activity in mouse photoreceptors results in the malformation of the retinal outer segment, a cellular compartment responsible for light detection, and triggers rapid retinal degeneration (Posokhova et al. 2011). The activity of TRiC is also essential for the assembly of the BBSome complex (Zhang et al. 2012), a complex thought to control trafficking of molecules into the cilium (Nachury et al. 2007; Loktev et al. 2008). As a result, knockdown of TRiC in zebrafish results in a Bardet-Biedl-like syndrome, a human genetic disorder resulting in obesity, retinal degeneration, polydactyly, and nephropathy (Seo et al. 2010).

G β , as part of G $\beta\gamma$ (G protein $\beta\gamma$) and RGS9-G β_5 heterodimers, plays an essential role in photo-transduction. Both are obligate dimers that rely on TRiC and its co-chaperone PhLP1 to form a complex from their nascent polypeptides (Tracy et al. 2015; Willardson and Howlett 2007). Moreover, two mutations T400P and R516H in CCT2 subunit has been reported to be related to Leber congenital amaurosis (LCA), a hereditary early-onset retinal dystrophy that is accompanied by severe macular degeneration (Minegishi et al. 2016).

TRiC and Cancer

TRiC has been found to be related to oncogenesis, probably through the interaction of TRiC with oncogenic clients which modulate the cancer cells (Roh et al. 2015). Studies have indicated that both TRiC and Hsp70 are required for correct folding of newly translated von Hippel-Lindau (VHL), which is coupled to assembly of a ternary complex with its partner proteins elongin BC (McClellan et al. 2005; Feldman et al. 2003; Melville et al. 2003; Feldman et al. 1999). Loss of VHL function is associated with a number of inherited and spontaneous tumors (Kaelin 2002). Moreover, TRiC also contributes to the folding and function of STAT3, a transcription factor for the transmission of peptide hormone signals from receptors on the extracellular membrane to nucleus, which is up-regulated in many pathological conditions, including cancer and inflammatory diseases (Kasembeli et al. 2014).

p53 is a transcription factor that mediates tumor suppressor responses. Correct folding of the p53 protein is essential for these activities, and point mutations that induce conformational instability of p53 are frequently found in cancers. It has been shown that folding of WT p53 is promoted by the interaction with TRiC. Depletion of TRiC in cells results in the accumulation of misfolded p53, leading to a reduction in p53-dependent gene expression. Although the most straightforward model for the role of TRiC is to assist in the correct folding of newly synthesized p53 (Malcikova et al. 2010), it is also possible that TRiC binding to p53 prevents its aggregation (Trinidad et al. 2013).

TRiC expression is closely correlated with cell growth and is markedly enhanced at early S phase of the cell cycle in mouse and human cultured cells (Yokota et al. 1999). Furthermore, specific TRiC subunits have been found to be up-regulated in the development of diverse cancers (Table 19.1). For instance, CCT1 and CCT2 have been reported to be essential for survival and proliferation of breast cancer (Guest et al. 2015). The expression levels of CCT3 mRNA and protein are up-regulated in hepatocellular carcinoma cells (HCC) cell lines, and over expression of CCT3 in the nuclei of cancerous cells is associated with HCC progression. CCT3 may be a target that affects the activation of STAT3 in HCC (Cui et al. 2015).

CCT8 has been reported to be dysregulated in several tumor tissues. In glioma, high expression of CCT8 is significantly associated with shorter overall survival. CCT8 can regulate the proliferation and invasion of glioblastomas (Qiu et al. 2015). Similarly, CCT8 expression is increased in hepatocellular carcinoma specimens, and knockdown of CCT8 can inhibit the proliferation of HCC (Huang et al. 2014). Also, CCT8 is highly expressed in proliferating germinal center cells and in progressive lymphomas. High expression of CCT8 is significantly associated with shorter overall survival in patients with diffuse large B-cell lymphoma (Yin et al. 2016). Further study is needed to address the following questions: whether the TRiC subunits in cancer act as a monomer, a homo-oligomer, or a TRiC complex; if it functions as a monomer, does it work as a chaperone with ATPase activity or just

transiently interacts with a certain substrate? Hence, elucidating the roles of TRiC in oncogenesis may provide new strategies for related cancer therapy.

Potential Therapeutic Applications of TRiC

TRiC Subunit Can Serve as a Biomarker for Related Disease

Recently, accumulating evidences have suggested the relationship between specific TRiC subunits and disease, and several TRiC subunits have been found to be over-expressed in certain diseases (Table 19.1). Therefore, over-expressed specific TRiC subunit could be used as a clinical marker for related disease.

The expression of CCT5 along with RGS3 and YKT6 genes are up-regulated in p53-mutated breast tumors and are associated with a resistance to docetaxel, and this might be clinically useful in identifying the subset of breast cancer patients who may or may not benefit from docetaxel treatment. Treatment of human breast cancer cell line MCF-7 with siRNA specific for CCT5 resulted in a significant enhancement of docetaxel-induced apoptosis and this may provide new insights into the molecular mechanism of resistance to docetaxel (Ooe et al. 2007). It has been reported that CCT5 also shows higher expression in non-small cell lung cancer (NSCLC) tissues and could be used as a biomarker in the diagnosis of NSCLC in an early stage (Gao et al. 2017). Moreover, the expression of CCT2 has been shown to be elevated in urine of patients with type 2 diabetic mellitus in the hyperfiltration stage. Hyperfiltration in early diabetic nephropathy (DN) may be detectable by measuring urine CCT2, which can be a novel and valuable biomarker for clinical evaluation (Wu et al. 2015a). Increased CCT7 has been observed in fibrous diseases such as Dupuytren's contracture (Satish et al. 2013) and skin contractive scars (Satish et al. 2010), and CCT7 has been indicated to be a unique potential marker for active fibroblasts (Bai et al. 2015).

Potential Application of TRiC in Preventing MHTt (Mutant Huntingtin) Aggregation

TRiC has been suggested to be able to prevent mHtt aggregation in cells (Behrends et al. 2006; Kitamura et al. 2006; Tam et al. 2006, 2009), and the underlying inhibition mechanism of TRiC against mhttQ51 aggregation *in vitro* has been investigated (Shahmoradian et al. 2013). Besides, the CCT5 homo-oligomer can cap mHtt fibrils in a similar fashion to the TRiC complex. These results suggest the option of exploring the use of the TRiC complex or CCT5 as a reagent to prevent mHtt aggregation, which may contribute to the development of HD therapy (Darrow et al. 2015). Moreover, it has been reported that the CCT1 subunit is sufficient to inhibit aggregation and reduce mHtt-mediated toxicity in mouse N2a neuronal cells (Tam et al. 2006), and the exogenous delivery of the 20 kDa

recombinant apical domain of CCT1 (ApiCCT1) can inhibit aggregation of recombinant mHtt *in vitro* (Sontag et al. 2013). More remarkably, applying ApiCCT1 to the cortical compartment of Htt disease mouse model can rescue impaired anterograde transport of brain-derived neurotrophic factor (BDNF) (Zhao et al. 2016). Therefore, there is a potential application for ApiCCT1 to be explored in the treatment of HD.

TRiC Is a Potential Target for Therapeutic Intervention

Cryo-EM analysis revealed that the CCT2 subunit of TRiC interacts with β -tubulin (Llorca et al. 2001). Disrupting the constitutively associated β -tubulin-CCT2 complex can cause severe cell apoptosis in multidrug-resistant MES-SA/Dx5 cancer cells (Harker and Sikic 1985). This finding suggests that the β -tubulin-CCT2 complex may serve as an effective chemotherapeutic target for treating clinical tubulin-binding agent-resistant tumor (Lin et al. 2009).

Breast cancer is the leading cause of death in women between the ages of 35 and 54 (Fiorica 1992). The CCT2 subunit was identified as the intracellular target of CT20p, a peptide that displays cancer (include breast cancer) specific cytotoxicity. The susceptibility of breast cancer cell to CT20p would be increased when over-expressing CCT2. TRiC is thus a potential target for therapeutic intervention for breast cancer treatment (Bassiouni et al. 2016). SMADs are the main signal transducers for receptors of the TGF- β superfamily, which are critically important for regulating cell development and growth. CCT6 has been shown to be the SMAD2 blocker, and silencing CCT6 could result in efficient suppression of metastasis *in vivo* and significantly prolonged the survival of tumor-bearing mice (Ying et al. 2017).

It has been reported that the homozygous cct2-L394H-7del mutation in the zebrafish leads to a small eye phenotype, and injection of RNA encoding wild-type human CCT2 could rescue the small eye phenotype (Minegishi et al. 2018). Also, it has been suggested that two mutations, T400P and R516H, in the CCT2 subunit can evoke leber congenital amaurosis (LCA) (Minegishi et al. 2016). This indicates a potential novel direction for the treatment of the LCA eye disease.

Perspective

In recent years, accumulating evidence has suggested the linkage between TRiC and certain diseases; still, there is a long way to go to fully understand the underlying molecular mechanism. Future atomic-resolution structural details and biochemical analysis of the eukaryotic group II chaperonin TRiC/CCT, may offer the structural basis of its subunit specificity, and provide more mechanistic insights into the allosteric network of TRiC and how this network regulates TRiC conformational transitions. Additionally, how the coordinated conformational transitions

of TRiC correlate with their productive substrate folding is yet to be further elucidated (Fig. 19.4). Moreover, with the development of computational method in cryo-EM, such as manifold embedding algorithm (Frank and Ourmazd 2016), the continuous conformational landscape of TRiC may be delineated, and its free-energy landscape could be mapped out. Furthermore, the *in vivo* study of TRiC in the native environment by cryo-ET and sub-tomogram averaging could enable us to directly visualize the process of TRiC-assisted substrate folding in the cell (Guo et al. 2018; Shahmoradian et al. 2013). Eventually, a combination of all the information on this complex macromolecular machine will facilitate therapeutic strategy development against TRiC-related diseases (Fig. 19.4).

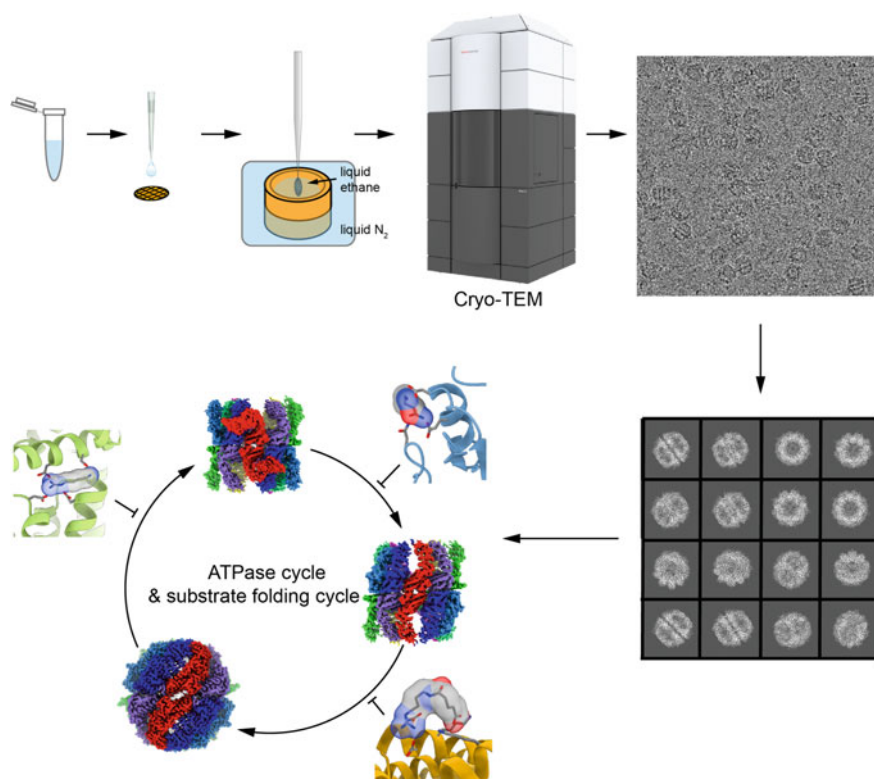


Fig. 19.4 Potential therapeutic development based on high-resolution cryo-EM structural studies of TRiC. Illustration of cryo-EM sample preparation, data collection, 3D reconstruction, model building, and structure-based drug development. The high-resolution reconstruction of an ensemble of TRiC structures in its substrate folding cycle regulated by its ATP-driven conformational cycle, will provide a complete picture of the mechanism of TRiC-assisted substrate folding. This structural information will enable us to design drugs to inhibit the allosteric transition at any desired step, and to block the reorganization of TRiC with specific substrate so as to prevent the development of related diseases such as cancer

Author Declaration

During the page proof process, a cryo-EM study on TRiC in complex with substrate mLST8 was published, revealing the function of TRiC in the folding of mLST8 and in the preparation for the assembly of mLST8 into mTOR complexes (Cuéllar et al. 2019). Moreover, another study resolved the cryo-EM structure of TRiC-PFD complex, established the structural and functional basis for TRiC and PFD cooperation in protein folding, essential for cellular proteostasis (Gestaut et al. 2019).

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