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Structural insights into assembly of TRAPPII and its activation of Rab11/Ypt32

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Abstract

Transport protein particle (TRAPP) complexes belong to the multisubunit tethering complex. They are guanine nucleotide exchange factors (GEFs) that play essential roles in secretory and endocytic recycling pathway and autophagy. There are two major forms of TRAPP complexes, TRAPPII and TRAPPIII, which share a core set of small subunits. TRAPPIII activates Rab1, while TRAPPII primarily activates Rab11. A steric gating mechanism has been proposed to control the substrate selection *in vivo*. However, the detailed mechanisms underlying the transition from TRAPPIII's GEF activity for Rab1 to TRAPPII's GEF activity for Rab11 and the roles of the complex-specific subunits in this transition are insufficiently understood. In this review, we discuss recent advances in understanding the mechanism of specific activation of Rab11/Ypt32 by TRAPPII, with a particular focus on new findings from structural studies.

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Introduction

The nobel prize in physiology or medicine 2013 was awarded jointly to three scientists for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells. Cells produce molecules such as hormones, neurotransmitters, cytokines, and enzymes that have to be delivered to other places inside the cell, or exported out of the cell, at exactly the right moment and position to keep the proper function of the cells. These molecules were known to be packaged into vesicles, and their transportation in the cell is driven primarily by vesicle-mediated membrane trafficking in the secretory and endocytic pathways [1]. Vesicle-mediated membrane trafficking is a highly sequential process including vesicle formation, vesicle translocation to, vesicle tethering on and vesicle fusion with the target compartment [2]. Among them, tethering is the initial interaction between a vesicle and its target membrane and thus plays a critical role to guide vesicles to specific membranes [2]. It is highly regulated by tethering factors together with Rab/Ypt guanosine triphosphatases (GTPases) and their diverse downstream effectors [3,4]. The roles of tethering factors involve acting as linkers between different target membrane and transport vesicles through specifically binding the coat proteins [5,6], being effectors or guanine nucleotide exchange factors (GEFs) for Rab/Ypt GTPases [4], and promoting the organization of the SNARE proteins involved in the vesicle fusion [7]. Almost all tethering factors fall into two categories: the long coiled-coil proteins and the multisubunit tethering complexes (MTCs) [8]. The former includes p115, Uso1, GM130, etc. [9] and MTCs comprise HOPS, CORVET, exocyst, COG, GARP, Dsl1 and TRAPP complexes [2]. They exist on a variety of compartments and contribute to vesicle fusion specificity.

The TRAPP (transport protein particle) complexes, originally identified from yeast in 1998, are highly conserved across all known eukaryotes [10–13]. In most species studied to date, including *Saccharomyces cerevisiae*, *Aspergillus nidulans*, *Drosophila*, and human being, there are two forms, TRAPPII and TRAPPIII, with TRAPPI now thought to appear only *in vitro* and result from dissociation of other two TRAPPs either *in vivo* or during extract preparation [14–17]. TRAPP complexes act as GEFs for Rab GTPases to convert them from the inactive GDP-bound form to the active GTP-bound form. Specifically, TRAPPIII activates Rab1, while TRAPPII primarily activates Rab11 and also has some activity on Rab1, based on genetic, biochemical and cellular studies [18–24]. Thus, they function in the endoplasmic reticulum to cis-Golgi transport, the maturation of Golgi cisternae, the endosome-to-Golgi transport, and the biogenesis of autophagosomes [5,6,10,13,21,25,26]. Consistent with the TRAPP complexes acting in key membrane trafficking pathways, variations in the genes

encoding their subunits result in a spectrum of human diseases including neurodevelopmental disorders, muscular dystrophies and skeletal dysplasias [27–30].

The composition of the TRAPP complex varies among species (Table 1). In *S. cerevisiae*, two TRAPP complexes share a hetero-heptameric core consisting of Bet3 (two copies), Bet5, Trs20, Trs23, Trs31, and Trs33 [31]. TRAPPII contains four additional proteins (Trs120, Trs130, Trs65, and Tca17) [14], and TRAPPIII has one additional Trs85 [32]. The composition of TRAPPII in *A. nidulans*, another fungus, is the same as that in *S. cerevisiae*, but its TRAPPIII contains Tca17 and Trs85 together with additional three subunits TRAPPC11, TRAPPC12 and TRAPPC13, which are absent in *S. cerevisiae* [17]. Compared with fungal TRAPPII, TRAPPII in metazoans (*Drosophila* and human) lacks Trs65 [33]. The TRAPPIII composition in metazoans is the same as that in *A. nidulans* [17,33]. Despite the different compositions, the essentiality for viability of subunits is also differ between species [17,33–35]. Mutations in most core subunits in all species result in lethality, although Trs33 is essential in *Drosophila* but nonessential in fungi [17,33,35]. Strikingly, the two TRAPPII specific subunits Trs120 and Trs130 are essential in fungi but nonessential in metazoans [17,33,35]. Conversely, the TRAPPIII specific subunit Trs85 is nonessential in fungi but essential in metazoans [17,33,35,36]. The limited conservation of the essentiality for viability of TRAPP subunits from yeast to human suggests that the regulation of TRAPP complex activities may be different among species. Indeed, the molecular mechanisms that mediate GEF specificity of TRAPP complexes, such as the specific activation of Rab11 by TRAPPII but not TRAPPIII, are critical for understanding their roles in membrane trafficking and are not fully characterized.

In the past few years, many studies have reported on TRAPP complexes. In this review, we will focus on recent structural studies of TRAPPII and their complex with the substrate Ypt32 (the Rab11 paralog in yeast),

Table 1

TRAPP complexes subunits.

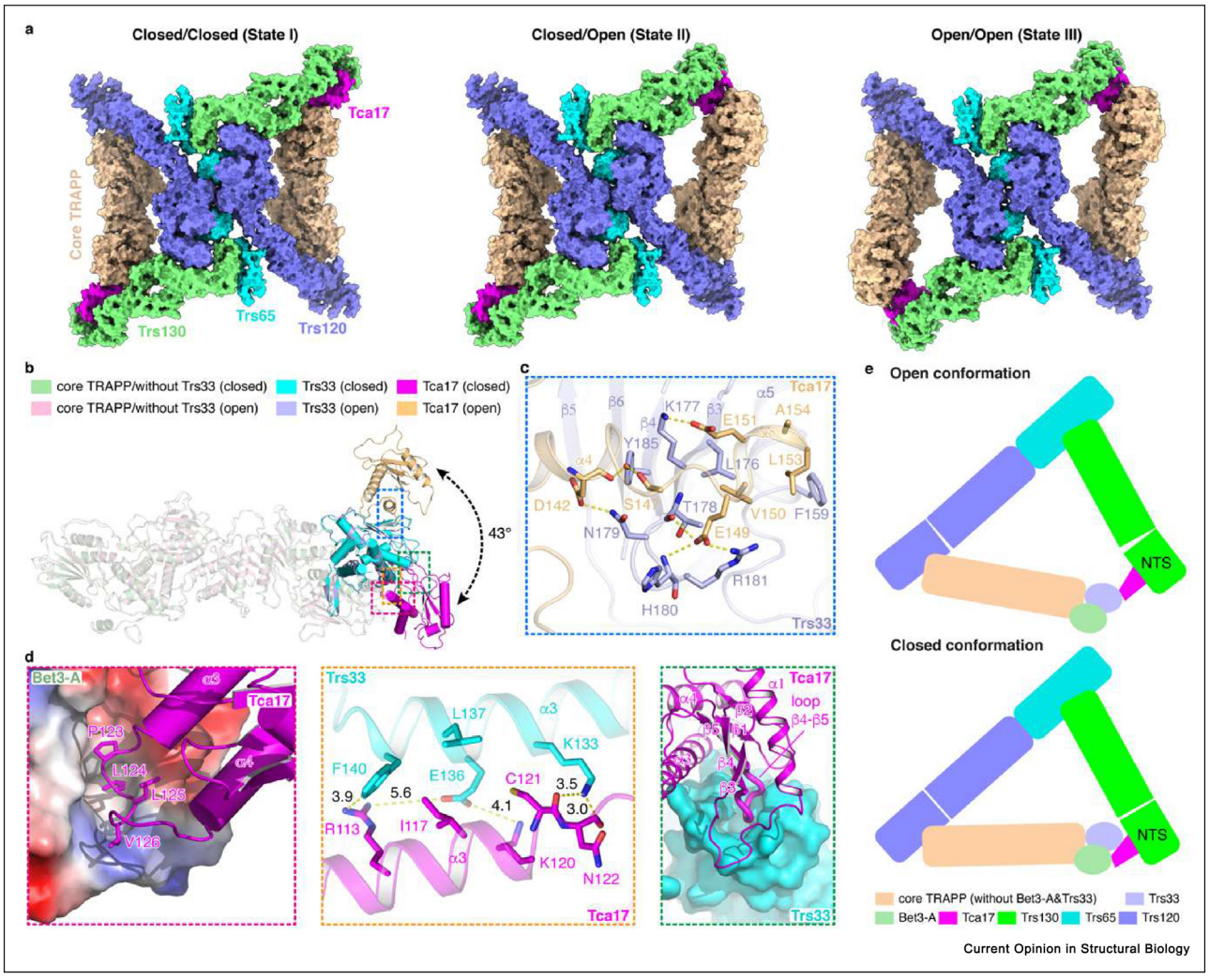
Yeast		*A. nidulans*		*Drosophila*		Human[a]	
Subunit	**Phenotype**	**Subunit**	**Phenotype**	**Subunit**	**Phenotype**	**Subunit**	**Phenotype**
Bet5	lethal	Bet5	lethal	TRAPPC1	/	TRAPPC1	lethal
Trs20	lethal	Trs20	lethal	TRAPPC2	lethal	TRAPPC2	viable
Tca17	viable	Tca17	lethal	TRAPPC2L	/	TRAPPC2L	viable
Bet3	lethal	Bet3	lethal	TRAPPC3	lethal	TRAPPC3, 3L	lethal
Trs23	lethal	Trs23	lethal	TRAPPC4	lethal	TRAPPC4	lethal
Trs31	lethal	Trs31	lethal	TRAPPC5	/	TRAPPC5	lethal
Trs33	viable	Trs33	viable	TRAPPC6	lethal	TRAPPC6A, B	viable
Trs85	viable	Trs85	viable	TRAPPC8	lethal	TRAPPC8	lethal
Trs120	lethal	Trs120	lethal	TRAPPC9	viable	TRAPPC9	viable
Trs130	lethal	Trs130	lethal	TRAPPC10	viable	TRAPPC10	viable
		TRAPPC11	/	TRAPPC11	lethal	TRAPPC11	lethal
		TRAPPC12	/	TRAPPC12	/	TRAPPC12	viable
		TRAPPC13	/	TRAPPC13	/	TRAPPC13	viable
Trs65	viable	Trs65	viable				

[a]Essentiality for human cell viability [35].

Yellow background: TRAPPII subunits

Red: TRAPPIII subunits

Figure 1



Structure of the intact yeast TRAPPII complex. (a) At least three states were observed for the intact yeast TRAPPII complex. State I contains both monomers in the closed conformation, state II contains both monomers in the open conformation, and state III consists of one monomer in open conformation and the other in closed conformation. **(b)** Tca17 adopts a completely different set of interactions with the core in the open and closed states. **(c)** Close-up view of the interaction between Tca17 and Trs33 in the open conformation. **(d)** Close-up view of Tca17 binding to Bet3-A and Trs33 in the closed conformation. Left panel: a loop of Tca17 is docked into a hydrophobic groove of Bet3-A. Middle panel: the helix α3 of Tca17 makes extensive interactions with the helix α3 of Trs33. Right panel: the loop β4-β5 of Tca17 is nestled in one groove of Bet3-A. **(e)** Schematic diagram showing the different interaction between the core and Tca17 and the conformational change of Trs130-NTS in the open and closed states.

which can help us understand the mechanism of the specific activation of Rab11/Ypt32 by TRAPPII.

Structure of the intact yeast TRAPPII complex

Previous study using negatively stained single-particle EM showed that yeast TRAPPII dimerizes into a three-layered, diamond-shaped structure. However, this structure lacked Tca17, which is one subunit of TRAPPII, and was at a low resolution (~30 Å) [37]. With the improvement of sample preparation and the development of the cryo-EM instrumentation and computational methodology, two groups resolved the cryo-EM structures of the intact yeast TRAPPII and its complex with Rab11/Ypt32 at the near atomic resolution independently [38,39].

From the face view, the dimeric TRAPPII complex is assembled by two triangle-shaped monomers. Trs120 and Trs130, the two largest subunits in the TRAPP family, together with core TRAPP, constitute the three sides of the triangle, respectively (Figure 1a). Trs20 located at one end of the core TRAPP binds to Trs120, while Tca17 at the other end of the core TRAPP binds to Trs130. Thus, the central core TRAPP appears to be held by a pair of elongated arms composed of the

TRAPPII specific Trs120 and Trs130 subunits (Figure 1a). Detailed analysis indicates that the binding mode between Trs20 and Trs120 is quite similar to that between Tca17 and Trs130, that is, the first α-helix of Trs20/Tca17 locates at the center of the interface and is wrapped by several α-helices from Trs120/Trs130. Actually, this overall arrangement of the interaction also exists between TRAPPC2 and TRAPPC8, and TRAPPC2L and TRAPPC11 in *Drosophila* TRAPPIII [22], and Trs20 and Trs85 in yeast TRAPPIII [40]. Thus, in the TRAPP complexes, the small subunits located at the end of the core may link the core to the elongated arms in a similar manner. Trs65 is located at the vertex of the triangle connected with Trs120 and Trs130 and forms extensive interactions between the two monomers, confirming its established role in dimerization of the TRAPPII complex [14,37]. From the side view, TRAPPII shows a curved shape similar to a single-arch bridge with a height of about 160 Å. The N-terminal α-solenoid (NTS) of Trs130 extends a substantial distance down below the core and is like a leg lifting the active site of the core TRAPP above the membrane to enforce steric gating for counterselection against Rab1 [39,41]. Notably, within the dimeric TRAPPII complex, individual monomers exhibited two major conformations, denoted as the open and closed states (Figure 1a). The most obvious difference between them is the rotation of the core TRAPP and that the open conformation has more internal space within the triangle compared to the closed conformation. This larger space is accomplished by two major structural changes. First, Tca17 adopts a completely different set of interactions with the core subunits in the open and closed conformations (Figure 1b—d). Second, the Trs130-NTS undergoes a rotation by 20.5° to be more distal to its position in the closed state (Figure 1e). There is a long loop upstream of Trs130-NTS that may bestow the movability of Trs130-NTS. As the same active site is used by TRAPP complexes and is expected to be inside the triangle-shaped TRAPPII, the larger space in the open conformation could facilitate Rab11/Ypt32 entrance to and exit from the active site.

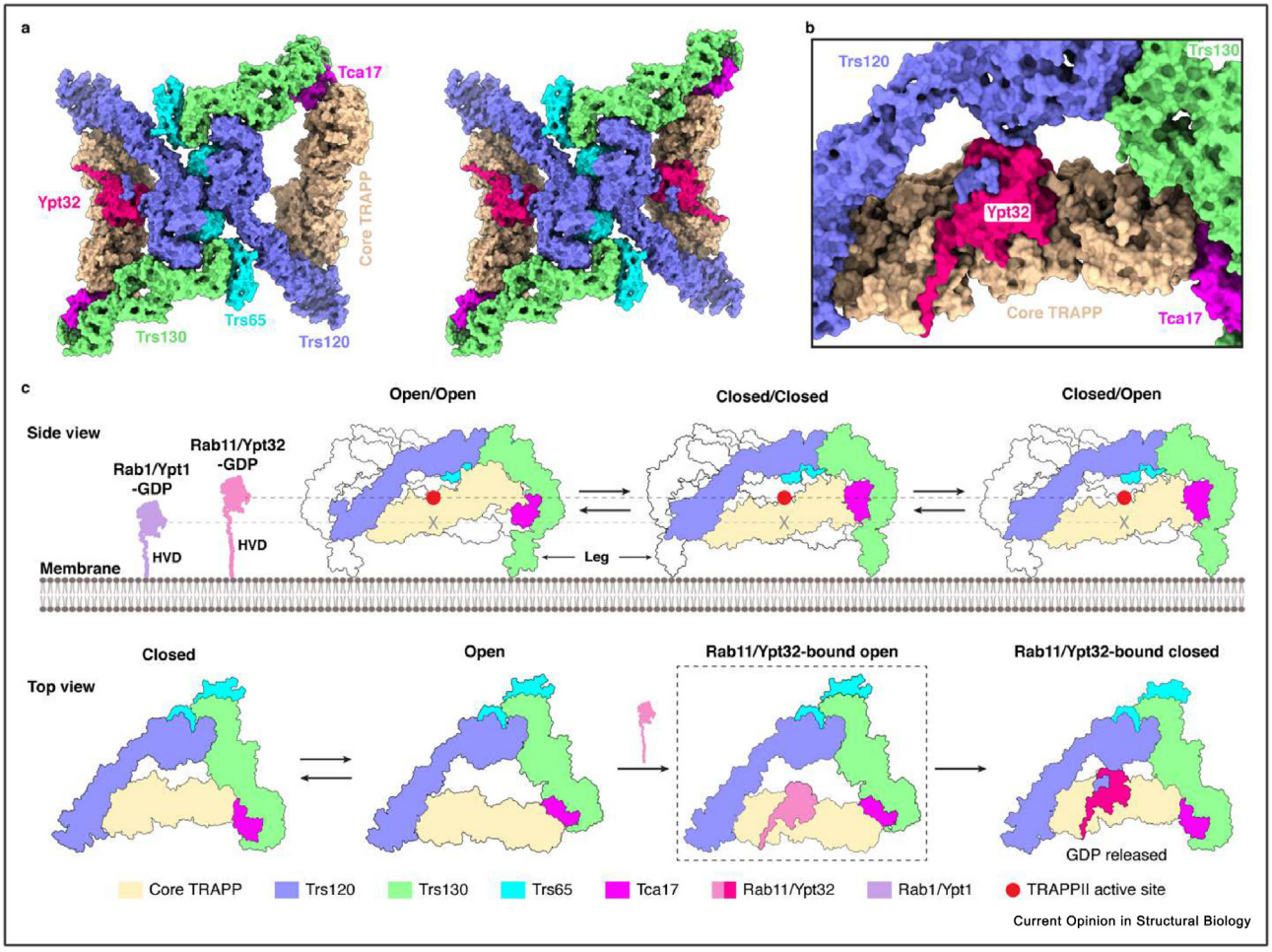
Structure of the yeast Rab11/Ypt32-TRAPPII complex

The Rab11/Ypt32-bound TRAPPII also shows a dimeric architecture, but different from the monomer in the apo state exhibiting both open and closed conformations, the monomer in the Rab11/Ypt32-bound state only captures the closed conformation, suggesting that the closed conformation is the activation intermediate (Figure 2a). Consistent with this, the Rab11/Ypt32 in the Rab11/Ypt32-bound TRAPPII is nucleotide-free and the nucleotide-binding pocket is in a favorable conformation for the nucleotide release [42]. The subunit arrangement of TRAPPII in the TRAPPII-Rab11/Ypt32 complex is almost identical to that of Rab11/Ypt32-free TRAPPII. As expected, Rab11/Ypt32 locates inside the triangle-shaped TRAPPII and both the nucleotide-binding domain (NBD) and the C-terminal region of Rab11/Ypt32 contribute to interactions with TRAPPII. Comparison of the core-Rab1 [43] and core-Rab11/Ypt32 interaction interfaces identified a potentially unfavorable repulsive interaction of the core with Rab11/Ypt32 due to a negatively charged surface of Rab11/Ypt32, which is the reason, at least in part, why Rab11/Ypt32 is a poor substrate of the core [39]. Indeed, except for the interface between NBD of Rab11/Ypt32 and the core, which is very similar with the interaction between the core and Ypt1 (the Rab1 paralog in yeast), the TRAPPII-specific subunits Trs120 makes additional direct contacts with Rab11/Ypt32 on a surface of the GTPase that is distal to its interaction with the core (Figure 2b). The main interaction is contributed by a loop between strands β1 and β2 of Trs120-IgD1 (loop β1-β2). It is nestled in a conserved hydrophobic surface of Rab11/Ypt32. Two other loops of Trs120 make primarily electrostatic interactions with Rab11/Ypt32. Compared to the structure of Ypt31 (the other Rab11 paralog in yeast besides Ypt32) in the GDI-bound state, which is inactive in the cytosol, the interactions between Trs120 loops and Rab11/Ypt32 drive the relocation of the Rab11/Ypt32's Ser-Ala-Leu (SAL) motif, which is involved in the interaction with the guanine base, to adapt to a favorable microenvironment for nucleotide release [38]. Accordingly, although deletion of any single loop resulted in only a minor growth phenotype, deletion of all three loops resulted in a substantial growth defect [39], suggesting the important roles of the additional interactions between Trs120 and Rab11/Ypt32 in TRAPPII function by inducing the conformational changes of Rab11/Ypt32 and/or stabilizing Rab11/Ypt32 at the active site [38,39]. The structure of the Rab11/Ypt32-TRAPPII complex also reveals the interaction between the C-terminal hypervariable domain (HVD) with the Trs31 subunit, which is essential for the binding of Rab11/Ypt32 to TRAPPII [38,39]. Although the Rab1/Ypt1 HVD also binds to the equivalent pocket of Trs31 in TRAPPIII [40], there is little sequence homology shared between the portions of the Rab1 and Rab11 HVDs bound to Trs31, suggesting that both the sequence and length of the HVD can be critical determinants used by GEFs to identify their Rab substrates [39,41].

Mechanism of the yeast Rab11/Ypt32 activation by TRAPPII

The structures of yeast TRAPPII in apo and Rab11/Ypt32-bound state, together with biochemical and cellular studies provide a molecular basis for Rab11/Ypt32 activation by the TRAPPII complex (Figure 2c). The regulatory GTPase Arf1 recruits TRAPPII onto the membrane [41,44,45] and may enforce such an orientation of TRAPPII on the membrane surface so that

Figure 2



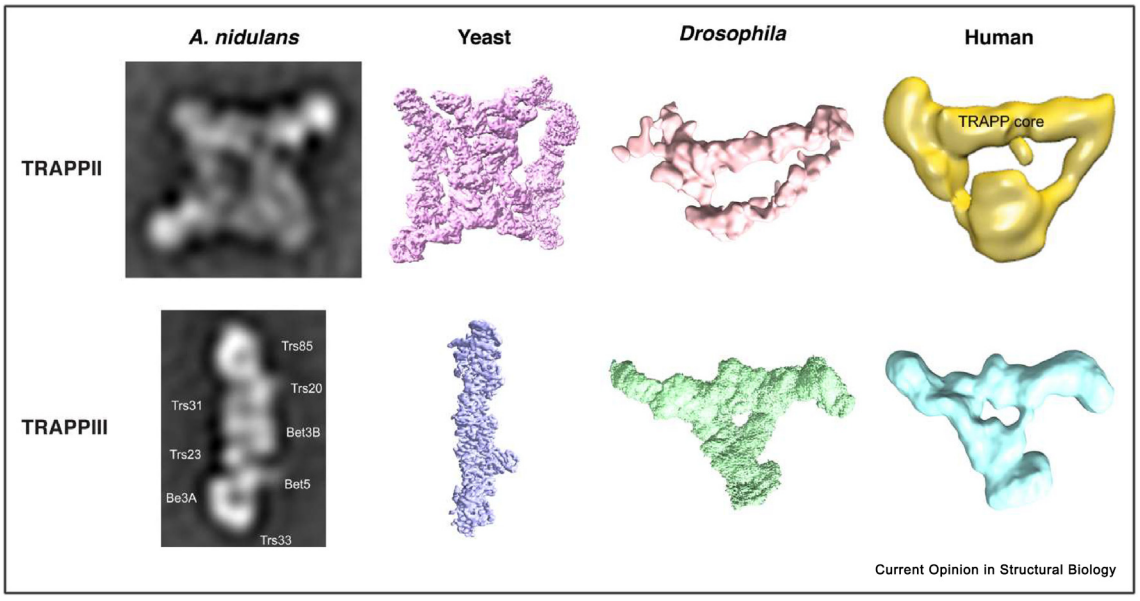
Structure of the yeast Rab11/Ypt32-TRAPPII complex and the model of TRAPPII-mediated Rab11/Ypt32 activation. (a) The monomer in the Ypt32-bound form only captures the closed conformation. **(b)** Close-up view of Ypt32 binding to both core TRAPP and Trs120. **(c)** Upper panel: TRAPPII is recruited onto the membrane and may adopt such an orientation on the membrane surface so that Trs130 molecules provide legs that lift the active site up and away from the membrane to form steric gating, in which the shorter Rab1/Ypt1 HVD is not long enough to enable access to the TRAPPII active site and only Rab11/Ypt32 can bind to TRAPPII specifically. At least three states of TRAPPII were observed and they exist simultaneously and can be transformed into each other. Lower panel: As the open conformation has larger space to accommodate substrates, Rab11/Ypt32 prefers to bind the open monomer. However, the Rab11/Ypt32-bound open state could not be captured probably due to the unstable and transient association (dashed box). Accompanied by the change from the open conformation to the closed conformation, Trs120 provides additional contacts with Rab11/Ypt32, which may stabilize Rab11/Ypt32 at the active site and induce the conformational change of Rab11/Ypt32 to adapt to a favorable microenvironment for the nucleotide release.

Trs130 provides a leg that lifts the active site up and away from the membrane to form steric gating. Thus, direct recognition of the Rab11/Ypt32 HVD by TRAPPII, together with the steric exclusion of the shorter Rab1/Ypt1 HVD, ensures the specific binding of Rab11/Ypt32 to TRAPPII. As the open conformation has larger space to accommodate substrates, most Rab11/Ypt32 may bind to the open conformation. However, the open monomer bound to Rab11/Ypt32 may be unstable, as such conformation was not observed in the cryo-EM data set. When the open conformation changes to the closed conformation, Trs120 provides additional contacts with Rab11/Ypt32, which may stabilize Rab11/Ypt32 at the active site and induce the conformational change of the SAL motif to adapt to a favorable microenvironment for the nucleotide release.

Structures of TRAPPII from other species

In addition to the high-resolution structures of yeast TRAPPII, structures of TRAPPII from *A. nidulans*, *Drosophila* and human at low-resolutions were also reported (Figure 3). 2D class averages of the negative-staining EM indicated that *A. nidulans* TRAPPII is also a dimer similar to the yeast TRAPPII [17]. Due to the lack of Trs65, structures of TRAPPII from human and *Drosophila* obtained by negative-staining EM [24] and single particle cryo-EM [22], respectively, exhibit a

Figure 3



Structure of TRAPPII and TRAPPIII from different species. Negative staining 2D averages of TRAPPII and TRAPPIII from *Aspergillus nidulans* [17], cryo-EM maps of TRAPPII and TRAPPIII from yeast [38–40] and *Drosophila* [22], and negative staining EM maps of human TRAPPII [24] and TRAPPIII [23] are shown. Note that the *A. nidulans* TRAPPIII shown in the figure lacks four subunits: Tca17, TRAPPC11, TRAPPC12 and TRAPPC13.

monomer with an elongated rod of the dimensions of the core attached to two arms that connect at their opposite ends to form an irregular triangle, similar to the individual monomer in the yeast TRAPPII dimer. Unlike the yeast TRAPPIII that adopts a narrow rod-like structure [40], the TRAPPIII from *Drosophila* and human is also an irregular triangular shape in which the core sits between arms formed from TRAPPC8 and TRAPPC11 with TRAPPC12 and TRAPPC13 attached at the opposite vertex [22,23] (Figure 3). Thus, both TRAPPII and TRAPPIII in metazoan share an architecture that consists of a central core held between two elongated arms.

Perspectives

Extensive functional and structural studies of the yeast TRAPP complex have provided a clear picture of how distinct Rab substrates can be differentiated by different GEFs sharing a common active site. Despite the great progress, many important questions remain to be addressed. In yeast, the substrate specificity of TRAPP complexes is controlled by a steric gating mechanism, in which the different lengths of the HVD acts as a molecular ruler for substrate selection, i.e., the shorter Rab1 HVD tail prevents the access of Rab1 to the TRAPPII active site [39,41]. However, the length of the HVDs in the mammalian homologs of Rab1 and Rab11 differ slightly than in yeast [24], and therefore it remains further investigated whether the metazoan TRAPPII complex also uses steric gating to counterselect against Rab1, and if not, how the metazoan TRAPP complexes achieve Rab specificity. Current TRAPP structures were all states in the solution. Given the enhanced GEF activity on lipid membranes and numerous conformational changes that accompany membrane association, it is very important and of great interest to resolve the structures of TRAPP complexes on membrane to further clarify the mechanism of how TRAPP complexes activate Rab GTPases in native conditions.

Conflict of interest statement

Nothing declared.

Data availability

No data was used for the research described in the article.

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