The TSC1-TSC2 complex plays an important role in the mechanistic target of rapamycin (mTOR) signaling pathway that integrates signals from extracellular growth factors, nutrients, energy deficit or inflammation and downstream controls cell metabolism and growth [1]. TSC1 is a ~130 kDa hydrophilic protein that shows no sequence homology to other known vertebrate proteins and likely guides and stabilizes the proper assembly of the TSC1-TSC2 complex [2]. TSC2 is a ~200 kDa protein that contains a conserved 163 amino acid region close to the C-terminus that is homologous to GTPase activating proteins (GAPs). The TSC1-TSC2 complex possesses RHEB GAP activity [3], and conversion of active GTP-bound RHEB into the inactive GDP-bound form by the complex downregulates the activity of mTOR complex 1 (TORC1) [1]. Pathogenic mutations in either TSC1 or TSC2 genes lead to tuberous sclerosis complex (TSC), an autosomal dominant disorder characterized by neurological symptoms, skin and renal abnormalities [4]. Recently, it has been shown that TSC1 and TSC2 assemble into high molecular weight complexes (>1 MDa) [5, 6]. However, the molecular architecture of this complex is unknown. We co-expressed epitope-tagged TSC1 and TSC2 in HEK 293T cells and imaged the affinity purified TSC1-TSC2 complexes by negative stain electron microscopy. Micrographs showed small rings of the complex that had a disk-like appearance. Initial alignments and classification of ~1000 particles using EMAN [7] revealed two kinds of rings: a smaller ring with ~90-Å diameter and 20-Å inner hole that seems to be composed of 5 subunits (Figure 2a) and a larger ring-like structure (~120 Å diameter and ~30 Å inner hole) that may correspond to a hetero-octamer of four TSC1 and four TSC2 subunits, according to the estimated average molecular mass of the complex. Ongoing data analysis aims to identify the different subunits in the two respective complexes and provide the structural basis for their function.


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Fig. 1: Scheme of the mechanistic target of rapamycin (mTOR) pathway. Growth factor signals and energy status regulate the activity of the TSC1-TSC2 complex. The RHEB GAP activity of the TSC1-TSC2 complex inhibits the activity of mTOR complex 1 that controls cell metabolism and growth.

Fig. 2: Epitope-tagged TSC1-TSC2 complexes were affinity purified from HEK 293T cells (inset shows Coomassie-stained SDS-PAGE gel of the purified complex). Electron micrograph of negatively stained complexes shows individual particles that appear as disk-like structures with an overall diameter ~100 Å (arrows).

Fig. 3: Classification of TSC1-TSC2 particles revealed two kinds of ring-like structures. Class average of the larger ring (top left) has ~120-Å diameter and ~30-Å hole, whereas the average of the smaller ring (bottom left) has ~90-Å diameter with ~20-Å hole. Representative particles from each class are shown in the right columns.