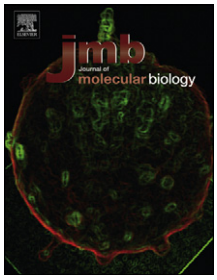

# Oligomerization of the UapA Purine Transporter Is Critical for ER-Exit, Plasma Membrane Localization and Turnover

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## Abstract

Central to the process of transmembrane cargo trafficking is the successful folding and exit from the ER (*e*ndoplasmic *r*eticulum) through packaging in COPII vesicles. Here, we use the UapA purine transporter of *Aspergillus nidulans* to investigate the role of cargo oligomerization in membrane trafficking. We show that UapA oligomerizes (at least dimerizes) and that oligomerization persists upon UapA endocytosis and vacuolar sorting. Using a validated bimolecular fluorescence complementation assay, we provide evidence that a UapA oligomerization is associated with ER-exit and turnover, as ER-retained mutants due to either modification of a Tyr-based N-terminal motif or partial misfolding physically associate but do not associate properly. Co-expression of ER-retained mutants with wild-type UapA leads to *in trans* plasma membrane localization of the former, confirming that oligomerization initiates in the ER. Genetic suppression of an N-terminal mutation in the Tyr motif and mutational analysis suggest that transmembrane α-helix 7 affects the oligomerization interface. Our results reveal that transporter oligomerization is essential for membrane trafficking and turnover and is a common theme in fungi and mammalian cells.


## Introduction

In eukaryotes, polytopic transmembrane proteins, such as transporters, channels and receptors, are co-translationally integrated in the ER (*e*ndoplasmic *r*eticulum) membrane and subsequently follow a vesicular secretory pathway for targeting to their final destination, this being the plasma or organellar membranes [1,2]. Central to the process of transmembrane protein exit from the ER is the concentrative packaging of protein cargoes in cytoplasmically budding COPII vesicles [3–8]. Assembly of the COPII coat on the ER membrane occurs in a stepwise fashion, beginning with recruitment of the GTPase Sar1, which recruits the heterodimeric Sec23/24. The Sec23/24 makes additional interactions directly with the membrane. Sec24 serves as the principle cargo binding adaptor. Following pre-budding complex formation, heterodimers of Sec13/31 are recruited via interaction between Sec23 and Sec31, and this interaction drives membrane curvature. In addition to the need for proper cargo folding [7,9,10], the process of ER-exit also requires the presence of specific ER-exit motifs on the cytoplasm-facing side of cargo proteins, usually in their N- or C-terminal region. Such short motifs include di-basic, tri-basic, di-acidic, di-leucine or tyrosine-based signals, several of which interact with the Sec23p–Sec24p complex in a Sar1p-dependent way [3,11–14]. Disruption of these motifs, similar to cargo misfolding, leads to ER retention. After vesicle formation, downstream events lead to uncoating of transport vesicles and recycling of the COPII coat components [4,8]. COPII vesicle membrane cargoes are sorted in the *cis*-Golgi and eventually in the *trans*-Golgi network, an important sorting station where cargoes are packaged into distinct transport vesicles and eventually targeted to various membrane destinations [15]. Although our understanding of COPII-mediated vesicle formation has developed substantially over the past two decades, many details of this process remain unresolved.

 

The short cargo motifs required for ER-exit are believed to interact mainly with one of three binding sites on the COPII coat component, Sec24 [16]. The majority of ER-exported membrane proteins, however, carry no known export signal in their sequence. Thus, either new signals remain to be identified or something else drives their recruitment into COPII vesicles. As many membrane proteins form oligomers prior to export from the ER, combinatorial signals have been postulated to link oligomerization to efficient export [17]. For a yeast COPII binding cargo receptor protein and its mammalian homologue (Emp47p, a type I membrane protein), oligomerization is required for its export from the ER but is not required for efficient binding of COPII subunits in the pre-budding complex [18]. This shows that oligomerization acts downstream from the cargo–Sec24 interaction. Very recently, Springer *et al*. showed that regulated oligomerization induces the packaging of a membrane protein into COPII vesicles independently of any putative ER-exit motif [19]. Oligomerization or assembly of cargo proteins seems important for ER-exit of some other cargo proteins, including SNARE molecules or G-protein-coupled receptors [20–22]. Oligomerization of neurotransmitter (e.g., dopamine and serotonin) transporters has also been shown to occur in the ER and is maintained both at the cell surface and during trafficking between the plasma membrane and endosomes [23–30]. The human blood–brain barrier glucose transport protein GLUT1 also forms homodimers and homotetramers in detergent micelles and in cell membranes, which in turn seems to determine its function [31].

In this work, we use the *Aspergillus nidulans* purine transporter UapA as a model transmembrane cargo to investigate the role of cargo oligomerization in ER-exit, plasma membrane localization and turnover. UapA is an $H^+$/uric acid-xanthine symporter consisting of 14 transmembrane segments (TMS) and cytoplasmic N- and C-termini. It is the founding member of the ubiquitously conserved Nucleobase-Ascorbate Transporter family [32–34]. The choice of UapA follows from the uniquely detailed current knowledge of its structure, function and regulation of expression, together with preliminary genetic evidence suggesting that UapA might oligomerize [35]. Inactive UapA mutants, unlike active wild-type UapA, cannot be endocytosed in response to substrate transport but can do so when co-expressed with active UapA. The simplest explanation for this phenomenon, called *in trans* endocytosis, is that UapA molecules oligomerize (at least dimerize) in the plasma membrane so that it is sufficient to have only a fraction of active molecules to recruit or activate the endocytic machinery and thus internalize both active and non-active UapA molecules [35,36]. Here, we provide multiple lines of evidence that UapA dimerizes (oligomerizes) in the ER membrane and provide evidence for a link among oligomerization, ER-exit and subsequent membrane trafficking. Our results are discussed in relation to similar findings concerning the role of oligomerization of mammalian transporters.

## Results

### Biophysical evidence for UapA dimerization

We have recently isolated a specific mutant with exceptional stability for performing biophysical studies [37]. This mutant has a missense mutation replacing a Gly with a Val residue in TMS10, in addition to a deletion removing the first 11 N-terminal amino acids. A GFP-tagged version of UapA-G411VΔ1-11 is normally secreted and localized in the plasma membrane of *A. nidulans* or *Saccharomyces cerevisiae* (data not shown). The mutant exhibits highly reduced transport activity but retains substrate binding, strongly indicating that the gross folding of the transporter is not significantly affected [38]. UapA-G411VΔ1-11 was purified after heterologous expression in *S. cerevisiae* and used in static light-scattering measurements. As shown in Fig. 1, the measured molecular mass for UapA-G411VΔ1-11 is 140 ± 4.2 kDa. Given that the predicted molecular mass of the monomeric form of UapA-G411VΔ1-11 is 60,138 kDa, our data support that UapA can form dimers.

### *In vivo* indirect evidence for UapA oligomerization in the plasma membrane

We have shown before that endocytosis of non-active UapA molecules occurs when these are co-expressed with active UapA molecules. This phenomenon of *in trans* endocytosis occurs even when the active UapA molecule cannot, by itself, be endocytosed due to the presence of mutation Lys572Arg, which prevents HulA/ArtA-dependent ubiquitination [35,36]. To further investigate whether the non-ubiquitylated mutant version UapA-K572R can be itself endocytosed *in trans* when expressed with active UapA molecules, we constructed a GFP-tagged UapA-K572R (UapA-K572R-GFP) and expressed it in a genetic background that hyperexpresses untagged wild-type UapA molecules due to a promoter mutation [39]. Results in Fig. 2 show that UapA-K572R-GFP is efficiently internalized upon imposing endocytic conditions (ammonium or uric acid addition), solely when co-expressed with wild-type UapA molecules. As UapA-K572R-GFP is a non-ubiquitylated version of UapA and ubiquitination is absolutely necessary for endocytosis, the most rational explanation for our results is that the mutant molecules are internalized due to their tight dimerization/oligomerization with wild-type UapA molecules.