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TANGO1 Facilitates Cargo Loading at Endoplasmic Reticulum Exit Sites

Kota Saito,[1] Mei Chen,[3] Fred Bard,[4] Shenghong Chen,[5] Huilin Zhou,[5] David Woodley,[3] Roman Polischuk,[6] Randy Schekman,[7] and Vivek Malhotra[1,2,*]

[1]Department of Cell and Developmental Biology, CRG-Centre de Regulacio Genomica
[2]Institutcio Catalana de Recerca I Estudis Avancats
Dr. Aiguader, 88 08003 Barcelona, Spain
[3]Department of Dermatology, USC/Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA 90033, USA
[4]Institute of Molecular and Cell Biology, 61 Biopolis Drive, 138673 Proteos, Singapore
[5]Department of Cellular and Molecular Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
[6]Department of Cell Biology and Oncology, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro (Chieti), Italy
[7]Department of Molecular and Cellular Biology, HHMI, University of California, Berkeley, Berkeley, CA 94720, USA
*Correspondence: vivek.malhotra@crg.es
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SUMMARY

A genome-wide screen revealed previously unidentified components required for transport and Golgi organization (TANGO). We now provide evidence that one of these proteins, TANGO1, is an integral membrane protein localized to endoplasmic reticulum (ER) exit sites, with a luminal SH3 domain and a cytoplasmic proline-rich domain (PRD). Knockdown of TANGO1 inhibits export of bulky collagen VII from the ER. The SH3 domain of TANGO1 binds to collagen VII; the PRD binds to the COPII coat subunits, Sec23/24. In this scenario, PRD binding to Sec23/24 subunits could stall COPII carrier biogenesis to permit the luminal domain of TANGO1 to guide SH3-bound cargo into a growing carrier. All cells except those of hematopoietic origin express TANGO1. We propose that TANGO1 exports other cargoes in cells that do not secrete collagen VII. However, TANGO1 does not enter the budding carrier, which represents a unique mechanism to load cargo into COPII carriers.

INTRODUCTION

Newly synthesized secretory proteins exit the endoplasmic reticulum (ER) in so-called COPII-coated vesicles (Bonifacino and Glick, 2004; Lee et al., 2004). A large number of proteins are required for the biogenesis of these vesicles, including chaperones and enzymes that fold the newly synthesized protein in the lumen of the ER, receptors that bind secretory cargo and help their recruitment into the newly forming carriers, structural coat proteins that help mold the flat ER membrane into a bud and promote its growth into a vesicular element, and components that catalyze the budding of cargo-loaded carriers from the ER (Herrmann et al., 1999; Lee et al., 2004).

COPII coat-mediated budding of vesicles from the ER is relatively well characterized. Two layers of coat proteins comprised of a Sec23/24 inner layer and a Sec13/31 outer layer are recruited to the membrane surface by Sar1-GTP at specific ER exit sites that are marked by the protein Sec16 (Bonifacino and Glick, 2004; Lee et al., 2004). Less is known about how cargo proteins are collected into nascent vesicles. Whether it is a passive process (bulk flow) or actively mediated by cargo receptors has been debated extensively (Wieland et al., 1987; Lee et al., 2004). Support in favor of the bulk flow proposal, however, is waning by recent growing evidence that cargo loading into COPII carriers utilizes receptors and chaperones (Lee et al., 2004). The receptors identified thus far appear to exit with the outgoing cargo, whereas the chaperones mostly stay behind in the ER (Lee et al., 2004).

Prominent examples of cargo receptors are ERGIC 53 for the transport of coagulation factors V and VIII, cathepsin C and Z, and α1-antitrypsin; Erv29p/Surf4 for the export of soluble cargo proteins such as precursor of α factor mating pheromone; and Emp24p for GPI-anchored membrane protein Gas1p (Belden and Barlowe, 2001; Muniz et al., 2000; Nichols et al., 1998; Nyfeler et al., 2008; Vollenweider et al., 1998). Additional relatives of these three proteins have also been identified (Baines and Zhang, 2007). Another well-characterized cargo receptor is the KDEL receptor, which traffics KDEL-containing proteins from the Golgi to the ER (Munro and Pelham, 1987; Pfeffer, 2007). Chaperones primarily assist in the folding of newly synthesized proteins and, therefore, are required for export from the ER. In addition to the conventional chaperones, BAP31 and the yeast-specific Shr3p also appear to function as cargo receptors (Baines and Zhang, 2007; Kota et al., 2007). It has been estimated that as many as 35% of the entire human genome enters the secretory pathway at the ER. Considering the quantity and the diversity of cargo exported by this compartment, the number of receptors identified thus far seems surprisingly low.

We performed a genome-wide screen in *Drosophila* tissue culture S2 cells to identify transport components (Bard et al., 2006). This screen revealed a number of previously unidentified genes required for transport and Golgi organization (TANGO

genes). TANGO1 from this collection emerges as a guide for loading the cargo molecule collagen VII into COPII carriers. Our findings provide further proof that collagen VII export from the ER is not by bulk flow.

RESULTS

TANGO1 Localizes to ER Exit Sites

Drosophila TANGO1 contains an SH3-like domain at its N terminus, followed by a potential transmembrane (TM) domain, a coiled-coiled domain, and a C-terminal, proline-rich domain (PRD). The mammalian ortholog of TANGO1 was identified by sequence comparison, cloned, and HA-epitope tagged at its C terminus. The domain organization of human TANGO1 is basically the same as *Drosophila* TANGO1 except for two closely situated potential transmembrane domains that were found in the databases (amino acids 1143–1165 and 1183–1205) (Figure 1A). A polyclonal antiserum was generated against the PRD (C-terminal amino acids 1884–1898) of TANGO1 and affinity purified. HA-tagged TANGO1 was expressed in COS7 cells and immunoprecipitated with anti-HA antibody and blotted with either anti-HA or anti-TANGO1 antibodies. Both antibodies recognized the exogenously expressed, tagged protein (Figure 1B, lanes 1 and 2). TANGO1 migrates slower than the expected molecular mass on SDS-PAGE. To detect the endogenous protein, HeLa cell extracts were either immunoprecipitated with anti-TANGO1 antibody followed by western blotting with anti-TANGO1 antibody (Figure 1B, lane 3), or cell extracts from HeLa (Figure 1B, lane 4) and A431 (Figure 1B, lane 5) were directly western blotted with anti-TANGO1 antibody. These experiments revealed a major polypeptide of > 250 kDa, which has the same mobility as the exogenously expressed, HA-tagged TANGO1. This indicates that we have cloned the mammalian TANGO1 and the antibody is suitable for further characterization of this polypeptide. It has been reported that the N-terminal 125 amino acid, which includes the SH3-like domain of TANGO1, is secreted (Bosserhoff et al., 2004). This portion has been given the name TANGO (the name is coincidently the same as our transport and Golgi organization) and MIA3 (melanoma inhibitory activity 3) (Bosserhoff et al., 2004). The reported sequence contains a termination codon at the 376 base pair region of TANGO1 and is listed as US patent WO 00/12762 (Bosserhoff et al., 2004); however, there are no such sequences in the NCBI databases. Our RT-PCR also did not reveal a termination codon at this site. Moreover, the database search for other species, including *Drosophila*, zebrafish, and mouse, did not reveal termination codons at the reported site. The origin of TANGO, therefore, remains speculative, but it is possible that, under certain conditions, a spliced variant of TANGO1 is secreted. However, our results here describe the full-length TANGO1 (1907 amino acids), which is localized to the ER exit sites and is not secreted (see below).

The affinity-purified antiserum was used to visualize the localization of endogenous TANGO1 protein in HeLa cells. The antibody staining localized to punctate structures, 80% of which colocalized with transfected Sec16L, a bona fide component of ER exit sites (Figure 1C, panel 1). The antibody also displayed a 60% colocalization with the endogenous ER exit site protein Sec31A (Figure 1C, panel 2). ERGIC-53 of the intermediate compartment (or ERGIC) appears as a collection of perinuclear elements and punctate elements that are distributed throughout the cytoplasm. There was only partial colocalization (30%) between TANGO1 and the dispersed (peripheral) ERGIC-53 (Figure 1C, panel 3). No colocalization was observed between TANGO1 and the perinuclear ERGIC-53 and between TANGO1 and β-COP (Figure 1C, panels 3 and 4). Thus, TANGO1 is localized to the majority of ER exit sites.

HeLa cells were transfected with control or TANGO1-specific siRNA. Immunofluorescence microscopy and western blotting with anti-TANGO1 antibody was used to determine the depletion of endogenous TANGO1. Immunofluorescence microscopy revealed the loss of TANGO1 staining from the ER exit sites (Figure S1A available online). Western blotting revealed > 90% reduction in TANGO1 protein levels (Figure S1B). Thus, TANGO1 localizes to the ER exit sites, the anti-TANGO1 antibody is suitable for analysis of the endogenous protein, and the siRNA efficiently depletes TANGO1 in mammalian cells.

The Topology of TANGO1 at ER Exit Sites

To determine the topology of TANGO1, we made use of the fact that digitonin can be used to selectively permeabilize the plasma membrane, and, after paraformaldehyde (PFA) fixation, treatment of the same cells with Triton X-100 permeabilizes both plasma membrane and intracellular membranes. The cytoplasmically oriented region of the protein should be visible by fluorescence microscopy in cells treated with digitonin, whereas detection of the luminal domain of a membrane protein should require treatment with Triton X-100. As shown in Figure 2A, an antibody that recognizes the luminal domain of ERGIC-53 required Triton permeabilization for binding to its cognate epitope. In contrast, GM130, which is peripherally attached to the cytoplasmic surface of *cis*-Golgi cisternae, was visible by immunofluorescence microscopy in cells treated with digitonin only (Figure 2A).

The anti-TANGO1 C-terminal-specific antibody was, therefore, used to reveal the orientation of this domain across the ER. As shown in Figure 2A, digitonin treatment followed by fixation with PFA was sufficient to visualize endogenous TANGO1 protein with the C-terminal specific, anti-TANGO1 antibody. This suggests that the C-terminal residues face the cytoplasmic side. When TANGO1 is expressed exogenously in cells and fixed with PFA, it yields a more diffused ER staining, but this should not affect the topology of the protein. To determine the orientation of the N-terminal SH3 domain, we generated a tagged version of TANGO1 in which a FLAG tag was placed between the signal sequence and the SH3 domain and an HA tag was introduced at the C terminus of TANGO1 (FLAG-TANGO1-HA). The transfected HeLa cells were treated with digitonin followed by fixation and incubated with either anti-FLAG or HA antibodies (stage I). The cells were then washed, treated with Triton X-100, and incubated with anti-TANGO1 antibody (stage II). The transfected cells were identified by higher TANGO1 staining compared with the nontransfected cells. These cells were also visualized for the staining with anti-FLAG or HA antibodies. Although HA antibody gave a strong signal in these cells (Figure 2B, lane 2), the N-terminally localized FLAG epitope was not detected in transfected