FEBS Letters 585 (2011) 2676–2681

journal homepage: www.FEBSLetters.org

A yeast two hybrid screen identifies SPATA4 as a TRAPP interactor

Débora Teixeira Duarte [a,1], Sokunthear Hul [a,1], Michael Sacher [a,b,*]

[a] Concordia University, Department of Biology, Canada
[b] McGill University, Department of Anatomy and Cell Biology, Canada

ARTICLE INFO

Article history:
Received 1 April 2011
Revised 25 July 2011
Accepted 26 July 2011
Available online 4 August 2011

Edited by Gianni Cesareni

Keywords:
TRAPP
Vesicle tethering
SPATA4
Spermatocyte
Yeast two-hybrid

ABSTRACT

The TRAPP vesicle-tethering complex consists of more than 10 distinct polypeptides and is involved in protein transport. Using the C2 subunit as bait we identified SPATA4, a spermatocyte-specific protein of unknown function, as an interacting partner in a yeast two hybrid screen. Further studies indicate SPATA4 interacts with the C2 portion of the TRAPP complex. SPATA4 fractionates with both cytosolic and nuclear fractions suggesting it may have several distinct functions. SPATA4 is one of only three human proteins that contain a DUF1042 domain and we show that C2 does not interact with another one of the DUF1042 domain-containing proteins. Our results suggest a role for SPATA4 in membrane traffic and a specialized function for TRAPP in spermatocytes.

Structured summary of protein interactions:
C2 physically interacts with **SPATA4** by two hybrid (View Interaction 1, 2)
C2 physically interacts with **POSTN** by two hybrid (View interaction)
C2L physically interacts with **REPS2** by two hybrid (View interaction)
C2L physically interacts with **TRAPPC3** by two hybrid (View interaction)
C2 physically interacts with **LAP3** by two hybrid (View interaction)
C2 physically interacts with **SPATA4** by anti bait coimmunoprecipitation (View interaction)
C2L physically interacts with **SPATA22** by two hybrid (View interaction)
SPATA4, **C2** and **C3** colocalize by cosedimentation through density gradient (View interaction)
SPATA4 binds to **C2** by pull down (View interaction)
C2 physically interacts with **TRAPPC3** by two hybrid (View interaction)
C2L physically interacts with **SPATA4** by anti tag coimmunoprecipitation (View interaction)
C2 physically interacts with **REPS2** by two hybrid (View interaction)
SPATA4 and **C2** physically interact by molecular sieving (View interaction)
C2L physically interacts with **LAP3** by two hybrid (View interaction)
C2 physically interacts with **SPATA22** by two hybrid (View interaction)
C2L physically interacts with **POSTN** by two hybrid (View interaction)

1. Introduction

The process of vesicle-mediated delivery of both membranes and proteins to their proper intracellular location requires many factors acting in a coordinated manner. Many unanswered questions remain with respect to the molecular mechanisms regulating these processes.

The complexes that tether these vesicles to the acceptor compartment have been well-studied at the structural level [1]. One such complex called TRAPP functions in the early portion of the secretory pathway leading to transport to the Golgi [2]. TRAPP or subunits within the complex have been proposed to carry out numerous functions including vesicle tethering [3,4], nucleotide exchange for several small GTPases [5–8], regulation of gene expression [9,10] and to contribute to Golgi morphology [8,11]. To fulfill all of these functions, the complex interacts with specific vesicle coat proteins, GTPases, transcription factors and likely other proteins. Curiously, a mutation in one subunit called TRAPPC2 (henceforth called C2) has been tied to a skeletal defect called spondyloepiphyseal dysplasia tarda (SEDT) [12]. Interestingly, while most SEDT patients have mutations that lead to a truncated C2 protein, one patient with a D47Y missense mutation was identified. Since this mutation is in a region of the protein that to date

* Corresponding author. Address: Concordia University, Department of Biology, 7141 Sherbrooke Street West, Montreal, Quebec, Canada H4B 1R6.
E-mail address: msacher@alcor.concordia.ca (M. Sacher).
[1] These authors contributed equally to this work and are listed alphabetically.

0014-5793/$36.00 © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2011.07.040

has not been shown to interact with any other TRAPP protein, it was suggested that it may interfere with an interaction between TRAPP and an as yet unidentified binding partner [13].

To better understand the regulation of TRAPP, we decided to look for proteins that interact with C2. Using a yeast two-hybrid screen, we show that a spermatocyte-specific protein of unknown function called SPATA4 binds specifically to this subunit. SPATA4 binds to the C2 portion of TRAPP and co-fractionates with the high molecular weight pool of C2. Ectopically expressed SPATA4 displays a cytosolic and nuclear localization. Our data suggest a role for SPATA4 in membrane traffic in spermatocytes and imply a specialized function for the TRAPP complex in these cells.

2. Materials and methods

A detailed materials and methods can be found in the Supplementary data.

2.1. Yeast two hybrid screen

The yeast two hybrid screen was performed as described in the Matchmaker™ Pretransformed Libraries User Manual (Clontech). The identity of positive clones was performed by polymerase chain reaction using the oligonucleotides pGAD-F-ID and pGAD-R-ID (Table S1) followed by sequence analysis. Plasmids used in this study are listed in Table S2.

2.2. Co-immunoprecipitation and gel filtration

Samples for co-immunoprecipitation (CoIP) contained 1 mg of lysate in phosphate buffered saline and 2 μg of IgG. For Western analysis, primary antibodies used were mouse anti-myc (Upstate) and mouse anti-FLAG (Sigma). Secondary antibody used was peroxidase-labelled goat anti-mouse IgG (Kirkegaard & Perry Laboratories).

2.3. Recombinant protein preparation

Cells were grown at 37 °C and protein production was induced by the addition of IPTG. Cells were collected by centrifugation and resuspended in the appropriate column buffer for maltose binding protein (MBP) fusion proteins, glutathione-S-transferase (GST) fusion proteins or polyhistidine (His)-tagged proteins.

Purification of MBP fusion protein using amylose resin (New England Biolabs), GST fusion protein using glutathione sepharose beads (GE Healthcare) and His-tagged protein using Ni^{2+}-NTA Agarose (Qiagen) were as per the manufacturer's instructions.

2.4. In vitro binding assay

In vitro binding assays contained 0.1–0.5 μM of either MBP or MBP-SPATA4 with increasing amounts (0–0.5 μM) of either subcomplexes composed of C2/His-C3/C5 or His-C3/C5, His-C2 or a peptide comprising the two C-terminal helices of C2 (H2/H3 C2) fused to GST. Samples were treated with amylose resin, washed and detected by western analysis using affinity purified polyclonal antibody recognizing C2 or anti-GST (Sigma).

2.5. Fluorescence microscopy

Cells were fixed 48 h after transfection in cold methanol or 4% paraformaldehyde. The cells were visualized on a Zeiss Axioplan Fluorescence microscope using a 63× oil EC Plan-Neofluar objective. Images were overlayed using Adobe Photoshop.

2.6. Live cell imaging

Cells were visualized either 24 or 48 h post-transfection. Images were captured on a Leica DMI6000 B inverted microscope coupled with a Hamamatsu C10600 ORCA-R2 digital camera.

2.7. Cellular fractionation

Cytosolic and nuclear fractionation were performed as previously described [14] except that sucrose was added to buffer N after homogenization of the cells. For western blot analysis, equal volumes of the normalized fractions were analyzed.

3. Results

3.1. Identification of SPATA4 as a C2 binding partner

To begin to understand the regulation of the function of TRAPP, we undertook a yeast two-hybrid (Y2H) screen using C2 as the bait. A normalized, human cDNA library in prey plasmids, produced from multiple tissues, was used in the screen. After the initial screen, >180 potential interactors were identified. Potential interactors were narrowed down by all of the following methods: (i) duplicates were identified by colony PCR and *Alu*I restriction analysis; (ii) plasmids were rescued and tested for autoactivation; (iii) plasmids were re-tested to remove false-positives. This process left 6 potential interactors (Table 1) including the C3 subunit of TRAPP which was previously shown to directly interact with C2 [13].

The six interactors were tested for specificity by checking their ability to interact with the TRAPP subunit C2L which is closely related to C2 [11]. Only SPATA4 discriminated in its ability to bind to C2 and C2L while the remaining interactors bound to both proteins (Fig. 1). For this reason, we chose to focus the remainder of this study on SPATA4.

SPATA4 is a highly conserved protein that has been found in numerous species [15–19]. Human SPATA4 is 305 residues in length and contains a DUF1042 domain of unknown function. Sequence analysis of the cDNA clone isolated in our screen revealed a frameshift that resulted in a 38 amino acid truncation of the protein from the carboxy-terminus suggesting that this region is not important for its interaction with C2 (see below).

3.2. C2 and SPATA4 interact in vivo

To confirm the Y2H results, we examined the ability of C2 and SPATA4 to interact in vivo. As shown in Fig. 2A, FLAG-SPATA4 co-precipitated with myc-C2 (lane 4). We noted that the levels of FLAG-SPATA4 were considerably higher when the protein was co-expressed with myc-C2 compared to when it was expressed in its absence (compare lanes 1 and 2). This result was seen numerous times, however the basis for it remains unclear. Although an interaction between SPATA4 and C2L was not detected by Y2H (see above), we found that myc-C2L could also precipitate

Table 1
C2 interactors identified in yeast two-hybrid screen.

Interactor	cDNA accession number
LAP3 – leucine aminopeptidase 3	NM_015907.2
REPS2 – RALBP1 associated Eps domain containing 2	NM_001980975.1
POSTN – periostin, osteoblast specific factor	NM_006475.1
SPATA22 – spermatogenesis associated 22	NM_032598.3
SPATA4 – spermatogenesis associated 4	NM_144644.2
TRAPPC3 – trafficking protein particle complex 3	NM_014408.3

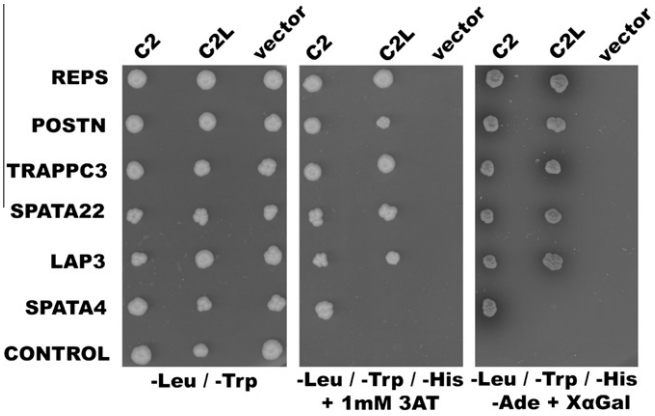

Fig. 1. SPATA4 interacts with C2 by yeast two-hybrid. Yeast cells harboring C2 or C2L in the bait plasmid pGBKT7, or an empty bait plasmid (vector) and the prey plasmid pGADT7 with the inserts indicated (see Table 1) were grown on medium lacking tryptophan and leucine, lacking tryptophan, leucine and histidine with 1 mM 3-amino-1,2,4-triazol (3-AT), or lacking tryptophan, leucine, histidine and adenine with X-α-Gal. SPATA4 interacted specifically with C2 and not C2L under the two experimental conditions tested.

FLAG-SPATA4 (Fig. 2B, lane 3). This is likely due to the fact that C2L is precipitating TRAPP which contains C2 [11]. FLAG-SPATA4 did not co-precipitate with myc-ECT2, a protein unrelated to C2 used as a negative control. Identical results were obtained when anti-FLAG was used as the precipitating antibody. These results confirm the Y2H interaction and suggest that SPATA4 interacts with TRAPP.

3.3. SPATA4 binds to the TRAPP complex

To further confirm the interaction between SPATA4 and C2 we performed an in vitro binding assay using MBP-SPATA4 and His_6-C2. As shown in Fig. 3A, we were unable to detect binding between these proteins above background levels in this system. When the binding assay was performed using a heterotrimeric form of C2 bound to its neighboring subunits (C2/His-C3/C5 heterotrimer) efficient binding was readily seen (Fig. 3B). It is noteworthy that the binding to the heterotrimeric complex was sufficiently strong that the levels of MBP-SPATA4 were reduced fivefold and the levels of the C2/His-C3/C5 were reduced twofold in the assay compared to monomeric C2. An interaction between glutathione-S-transferase (GST)-tagged C2L and MBP-SPATA4 could not be detected (not shown). Interestingly, when just a heterodimer of His-C3/C5 was used, binding of MBP-SPATA4 could be readily detected (Fig. 3C). These results suggest that SPATA4 preferentially interacts with the C2-localized portion of TRAPP and also recognizes a region on the C3/C5 dimer.

Consistent with the above notion, lysates fractionated by size exclusion chromatography showed that FLAG-SPATA4 was found in a high molecular weight fraction that also contained TRAPP-associated C2 (Fig. 3D). Significantly, there was no pool of SPATA4 even in fractions that contained non-TRAPP-associated C2. This fractionation was not affected by co-expression of myc-C2 (not shown) except that the levels of SPATA4 were greater when the two proteins were co-expressed as stated above. This high molecular weight pool of FLAG-SPATA4 coimmunoprecipitated with several TRAPP proteins (Fig. 3E). Collectively, our results suggest that SPATA4 binds to the TRAPP complex through the C2-containing end.

3.4. Defining the regions of interaction between SPATA4 and C2

Since SPATA4 was identified through its interaction with C2, we sought to determine which regions of SPATA4 and C2 mediate the interaction between the proteins. Although largely similar, the three-dimensional crystal structure of C2 as part of the heterotrimeric complex differs slightly from uncomplexed C2 [13,20]. Specifically, helix 1 is extended in the heterotrimeric complex by incorporating additional residues on both the amino- and carboxy-terminal sides. We used several C2 mutants to determine whether SPATA4 interacts with this helix. First, the amino-terminal 91 residues of C2 containing helix 1 (spanning residues 31–54 in C2 in the heterotrimeric complex) was cloned into the bait vector and tested for an interaction with SPATA4. As demonstrated in

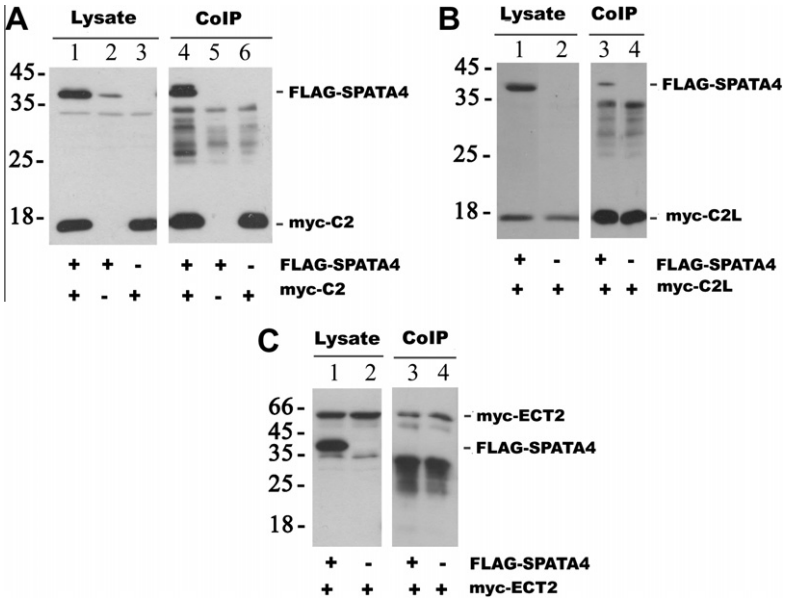

Fig. 2. SPATA4 and C2 interact in vivo. Lysates were prepared from HEK293T cells co-transfected with plasmids expressing: (A) lanes 1 and 4: FLAG-SPATA4 with myc-C2; lanes 2 and 5: FLAG-SPATA4 with pRK5MYC; lanes 3 and 6: pFLAGCMV6a with myc-C2; (B) lanes 1 and 3: FLAG-SPATA4 with myc-C2L; lanes 2 and 4: pFLAGCMV6a with myc-C2L; (C) lanes 1 and 3: FLAG-SPATA4 with myc-ECT2 (amino acids 421–883); lanes 2 and 4: pFLAGCMV6a with myc-ECT2 (amino acids 421–883). Samples were immunoprecipitated (co-IP) with rabbit anti-C2 (A) or rabbit anti-myc (B, C). Precipitates were analyzed by western blotting using mouse anti-myc and mouse anti-FLAG IgG.

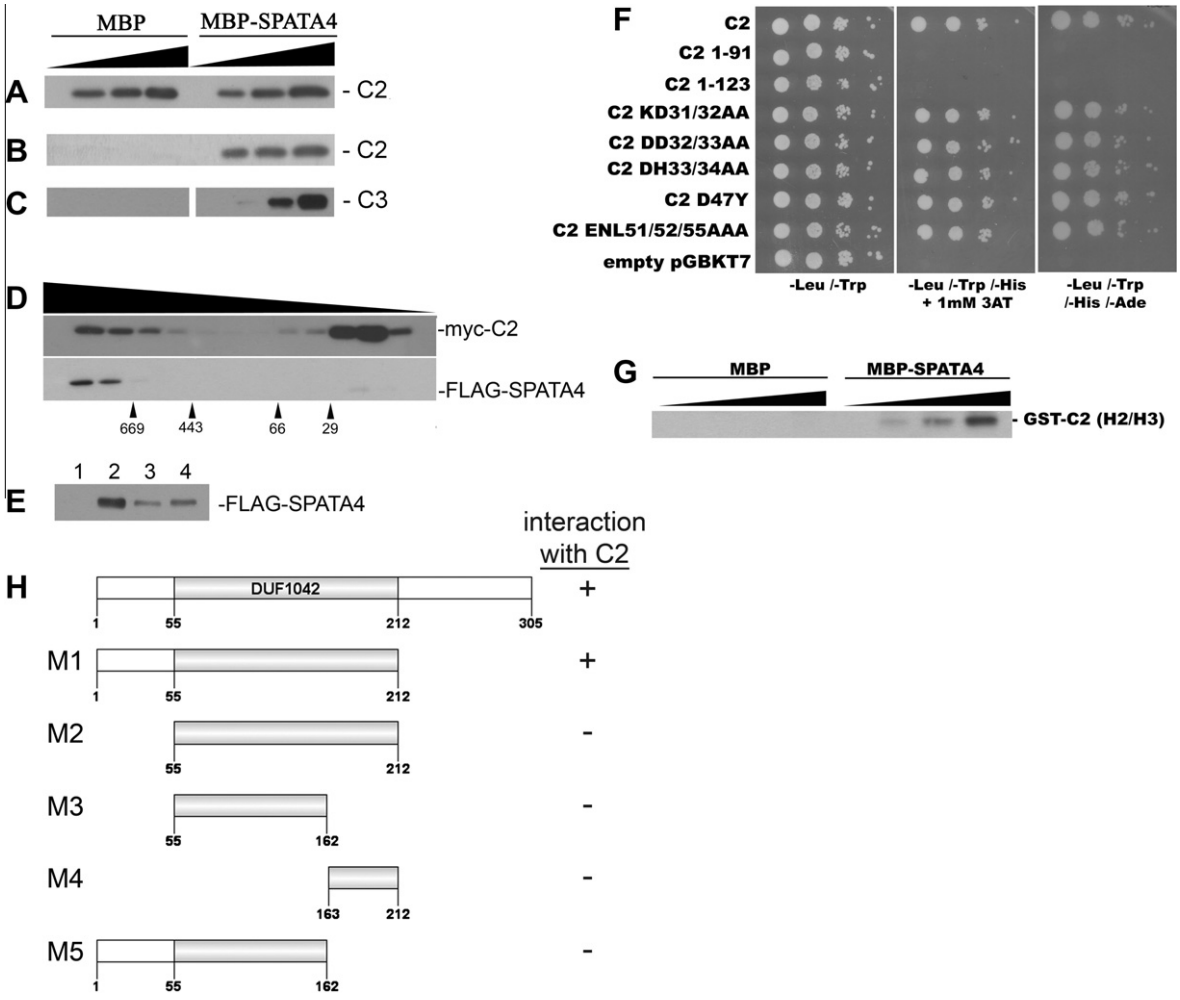

Fig. 3. SPATA4 binds to TRAPP. MBP or MBP-SPATA4 were subjected to an in vitro binding assay as described in Section 2 using amylose resin to pull down MBP and MBP-SPATA4 with increasing amounts of either His-C2 (A), C2/His-C3/C5 (B) or His-C3/C5 (C). Samples were probed by western blotting using anti-C2 (A, B) or anti-C3 (C) IgG. (D) Lysates were prepared from HEK293T cells transfected with a plasmid expressing FLAG-SPATA4 or myc-C2. Lysates were fractionated on a Superdex™ 200 column and fractions were analyzed by western blotting using anti-myc and anti-FLAG antibodies. (E) FLAG-SPATA4 fractions from (D) were untreated (lane 1) or immunoprecipitated with anti-C2 (lane 2), anti-C3 (lane 3) or anti-C11 (lane 4) IgG and probed with anti-FLAG IgG. (F) Full length C2 or the indicated fragments and mutants were tested for an interaction with SPATA4 by yeast two hybrid. Serial dilutions were spotted on medium lacking either leucine and tryptophan, leucine, tryptophan and histidine with 3-AT, or leucine, tryptophan, histidine and adenine. (G) MBP or MBP-SPATA4 were subjected to an in vitro binding assay as in (A) with increasing amounts of GST fused to residues 91–140 of C2 (helices 2 and 3; GST-C2(H2/H3)). Samples were probed by western blotting using anti-GST IgG. (H) SPATA4 constructs were tested for interaction with C2 by yeast two hybrid (see Supplementary Fig. 1). The SPATA4 fragments generated are (human numbering): M1 (1–212), M2 (55–212), M3 (55–162), M4 (163–212) and M5 (1–162).

Fig. 3F, an interaction was not detected. We then mutated the highly conserved additional residues (31–34 and 51–55) that are incorporated into helix 1 in the heterotrimeric form of C2 into alanines. All of these mutant forms of C2 retained their ability to interact with SPATA4 (Fig. 3F). Finally, we found that the pathogenic D47Y SEDT mutation in helix 1 of C2 had no effect on binding to SPATA4 (Fig. 3F). These results argue against a role for the involvement of helix 1 of C2 with SPATA4. We then focused our attention to the carboxy-terminal portion of C2 which contains two antiparallel helices called helix 2 and helix 3 (H2/H3) which were deleted in the amino-terminal construct described above. As shown in Fig. 3G, GST-tagged recombinant H2/H3 (residues 91–140) was indeed able to bind MBP-SPATA4. Consistent with these results, a construct lacking the final 17 residues of C2 that represent H3 failed to interact with SPATA4 (Fig. 3F). These results suggest that the carboxy-terminal helix of C2 is necessary for interaction with SPATA4.

To define the portion of SPATA4 that interacts with C2 we cloned portions of the protein into the prey vector and tested their ability to bind to C2 by Y2H. As shown in Fig. 3H and Figure S1, deletion of the entire carboxy-terminal portion of the protein until the DUF1042 domain did not affect its interaction with C2. Further truncations into the DUF1042 domain ablated the interaction suggesting that this domain is required for the interaction with C2. However, the DUF1042 domain (amino acids 55–212) alone was unable to bind to C2. When SPEF1, one of two other DUF1042-containing proteins, was tested for binding to C2, no interaction was detected (not shown). These results suggest that the DUF1042 domain of SPATA4 is necessary but not sufficient for its interaction with C2.

3.5. SPATA4 is found in both the cytosol and in the nucleus

The binding of SPATA4 to TRAPP suggests that SPATA4 should be found in the cytosol. We tested this notion by cell fractionation and fluorescence microscopy using green fluorescent protein (GFP)-tagged SPATA4. Upon fractionation, a portion of GFP-SPATA4 was indeed found in the cytosol (Fig. 4A). A significant portion was also found in the nuclear-enriched fraction consistent with an earlier study [18]. The latter result was not due to cross-contamination between the nuclear and cytosolic fractions since the cytosolic marker (β-COP) and two TRAPP subunits, C2 and C3, were

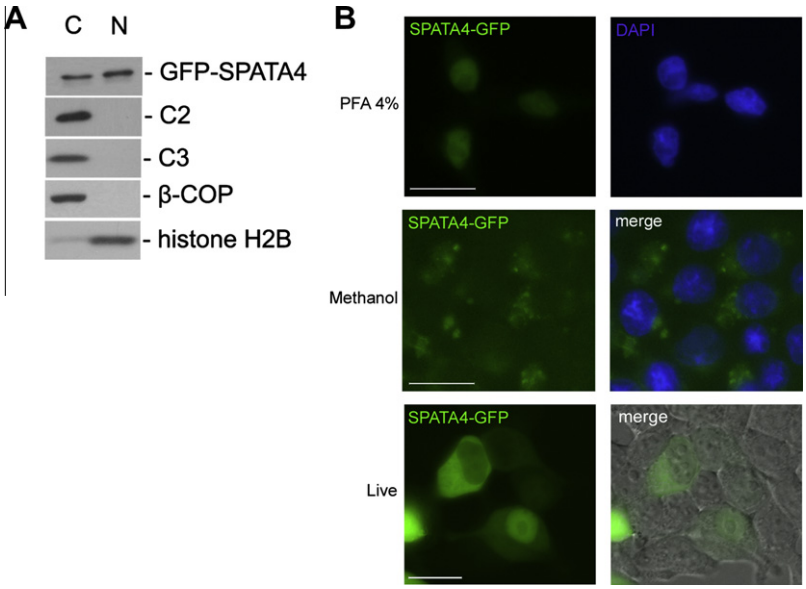

Fig. 4. Localization of SPATA4 in HEK293T cells. (A) HEK293T cells were transfected with pGFP-SPATA4 and harvested 48 h later. The cells were lysed and separated into cytosolic (C) and nuclear fractions (N). Aliquots were analyzed by western blotting using anti-GFP, anti-C2, anti-C3, anti-histone B (nuclear marker) and anti-β-COP (cytosolic marker). (B) HEK293T cells were transfected with pGFP-SPATA4 and visualized after fixation with either paraformaldehyde (top row) or methanol (middle row). The bottom panel shows a representative image of unfixed, live cells. Cytosolic localization is not due to background fluorescence since there are a number of untransfected, non-fluorescent cells in the fields as demonstrated by the DAPI merge for the methanol fixed samples and the DIC merge of the live cells. The scale bars represent 20 μm.

only detected in the cytosolic fraction (Fig. 4A). This result further supports the interaction between C2 and SPATA4 taking place in the cytosol.

We then sought to confirm the biochemical fractionation by fluorescence microscopic localization of GFP-SPATA4. When cells were visualized after fixing with 4% paraformaldehyde, GFP-SPATA4 displayed a nuclear localization in 89.4% of the cells ($n = 47$) (Fig. 4B). Curiously, when cells were fixed with methanol, the localization of GFP-SPATA4 was cytosolic in 89.4% of the cells ($n = 104$) with some cells displaying punctae (Fig. 4B). As neither of these results were consistent with the biochemical fractionation we visualized GFP-SPATA4 in live cells where no fixation method is used. In this case, all of the transfected cells showed the GFP-SPATA4 signal in both the nucleus and the cytosol (Fig. 4B). Although some cells showed a stronger signal in one of these compartments, the GFP-SPATA4 signal in those cells could also be seen in the other compartment. Although it remains to be shown whether GFP-SPATA4 is functional, the localization seen in live cells suggests that GFP-SPATA4 localization is dynamic.

4. Discussion

We present evidence that SPATA4, a protein of unknown function, interacts specifically with a protein involved in membrane traffic. Collectively, our data implicate SPATA4 in a role in membrane traffic by virtue of its association with the TRAPP complex. It is noteworthy that a low molecular weight pool of SPATA4, similar to that of C2 with which it interacts, was not detected. Rather, all of the ectopically expressed, cytosolic SPATA4 co-fractionated with TRAPP. The fact that SPATA4 is expressed almost exclusively in spermatocytes, suggests that TRAPP may perform a function specific to these cells. Although subcellular fractionation suggested that a portion of SPATA4 was found in the nucleus, this pool of SPATA4 would not be seen in our gel filtration fractions since the protocol removes nuclei and DNA from the sample prior to size-exclusion chromatography. Given a previous report that C2 is found in the nucleus [21], we cannot rule out a SPATA4–C2 interaction taking place in this compartment. It should be noted, however, that the previous report was based on the fractionation of overexpressed C2 while our fractionation focused on the endogenous protein. In addition, our in vitro binding assay argues against a SPATA4 interaction with non-TRAPP-associated C2. Although SPATA4 was identified in a yeast two hybrid screen using C2 as the bait, SPATA4 bound more efficiently in vitro to His-C3/C5 compared to His-C2. It is possible that the yeast two hybrid interaction may have been mediated by a mixed TRAPP complex composed of human C2 with yeast TRAPP proteins. Such a complex is likely produced in yeast since the human protein complements its yeast ortholog [11,22]. Alternatively, the interaction in vivo is more stable than that in vitro.

A recent study suggests that SPATA4 is localized to the cytosol in the osteoblast cell line MC3T3-E1 where it interacts with and promotes the phosphorylation of the kinase ERK1 [23]. An earlier study reported the localization of GFP-SPATA4 to the nucleus [18]. Our present work shows GFP-SPATA4 localizing to and fractionating with both the cytosol and nucleus in live cells. Clearly, this is a dynamic protein with perhaps several functions. Interestingly, mutations in C2 that lead to the skeletal defect SEDT include carboxy-terminal truncations [24], a region we have defined as important for the interaction with SPATA4. The position of C2 within TRAPP and its interactions with neighbouring subunits leave available this carboxy-terminal helix for interactions with non-stably-associated members of the complex [13]. It remains to be seen whether ablation of the SPATA4–C2 interaction in the cytosol is a contributing factor to SEDT.

A previous study on ectopically expressed SPATA4 in MCF7 cells showed that it increased their growth rate by allowing the cells to progress through S phase more rapidly [17]. While the mechanism for the increased growth rate of these cells is unclear, our present study suggests it may be due to a more active secretory pathway.

The involvement of the DUF1042 domain in binding to C2 is interesting in light of the fact that this domain has been implicated in microtubule interactions [25]. Given that ER-derived carriers migrate along microtubule tracks [26], it is tempting to speculate

that SPATA4 may link TRAPP to the microtubule network to facilitate membrane traffic in spermatocytes. Alternatively, profound changes to the cytoskeletal microtubule network accompany division of spermatocytes and development of spermatids [27]. Such changes may present special needs for membrane traffic and SPATA4 would be well positioned to allow this vesicle tether to adapt to the changing cytoskeletal landscape. In addition, given the link between SPATA4 and ERK1, it is tempting to speculate that this protein of unknown function serves to link TRAPP to ERK1 and thus allow membrane traffic to respond to signaling events as previously suggested [28]. Clearly, further studies on the SPATA4 protein in an appropriate spermatocyte model system are needed to further elucidate the function of this TRAPP-interacting protein.

Acknowledgements

We are grateful to Dr. James Scrivens, Nassim Shahrzad and Adrian Moores for advice, helpful discussions and constructs used in this study, and to Drs. Alisa Piekny and Gerard Dougherty for reagents. D.T.D. is supported in part by a Groupe de Recherche Axé sur la Structure des Protéines graduate recruitment award. This work was funded by grants from the Canadian Institutes of Health Research, the Canada Foundation for Innovation and the Natural Sciences and Engineering Research Council of Canada to M.S.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.07.040.

References

[1] Hughson, F.M. and Reinisch, K.M. (2010) Structure and mechanism in membrane trafficking. Curr. Opin. Cell Biol. 22, 454–460.
[2] Sacher, M. et al. (2008) The TRAPP complex: insights into its architecture and function. Traffic 9, 2032–2042.
[3] Sacher, M. et al. (1998) TRAPP, a highly conserved novel complex on the cis-Golgi that mediates vesicle docking and fusion. EMBO J. 17, 2494–2503.
[4] Sacher, M. et al. (2001) TRAPP I implicated in the specificity of tethering in ER-to-Golgi transport. Mol. Cell 7, 433–442.
[5] Jones, S. et al. (2000) The TRAPP complex is a nucleotide exchanger for Ypt1 and Ypt31/32. Mol. Biol. Cell 11, 4403–4411.
[6] Morozova, N. et al. (2006) TRAPPII subunits are required for the specificity switch of a Ypt-Rab GEF. Nat. Cell Biol. 8, 1263–1269.
[7] Wang, W. et al. (2000) TRAPP stimulates guanine nucleotide exchange on Ypt1p. J. Cell Biol. 151, 289–296.
[8] Yamasaki, A. et al. (2009) MTrs130 is a component of a mammalian TRAPPII complex, a Rab1 GEF that binds to COPI-coated vesicles. Mol. Biol. Cell 20, 4205–4215.
[9] Ghosh, A.K. et al. (2001) A novel 16-kilodalton cellular protein physically interacts with and antagonizes the functional activity of c-myc promoter-binding protein 1. Mol. Cell. Biol. 21, 655–662.
[10] Ghosh, A.K. et al. (2003) Modulation of human luteinizing hormone beta gene transcription by MIP-2A. J. Biol. Chem. 278, 24033–24038.
[11] Scrivens, P.J. et al. (2009) TRAPPC2L is a novel, highly conserved TRAPP-interacting protein. Traffic 10, 724–736.
[12] Gedeon, A.K. et al. (1999) Identification of the gene (SEDL) causing X-linked spondyloepiphyseal dysplasia tarda. Nat. Genet. 22, 400–404.
[13] Kim, Y.G. et al. (2006) The architecture of the multisubunit TRAPP I complex suggests a model for vesicle tethering. Cell 127, 817–830.
[14] Collas, P. et al. (1999) The A-kinase-anchoring protein AKAP95 is a multivalent protein with a key role in chromatin condensation at mitosis. J. Cell Biol. 147, 1167–1180.
[15] Liu, B. et al. (2005) Cloning and expression analysis of gonadogenesis-associated gene SPATA4 from rainbow trout (*Oncorhynchus mykiss*). J. Biochem. Mol. Biol. 38, 206–210.
[16] Liu, S. et al. (2005) Cloning and characterization of zebra fish SPATA4 gene and analysis of its gonad specific expression. Biochemistry (Mosc.) 70, 638–644.
[17] Liu, S.F. et al. (2004) Cloning and characterization of testis-specific spermatogenesis associated gene homologous to human SPATA4 in rat. Biol. Pharm. Bull. 27, 1867–1870.
[18] Liu, S.F. et al. (2004) Cloning of a full-length cDNA of human testis-specific spermatogenic cell apoptosis inhibitor TSARG2 as a candidate oncogene. Biochem. Biophys. Res. Commun. 319, 32–40.
[19] Liu, S.F. et al. (2005) Molecular cloning and bioinformatic analysis of SPATA4 gene. J. Biochem. Mol. Biol. 38, 739–747.
[20] Jang, S.B. et al. (2002) Crystal structure of SEDL and its implications for a genetic disease spondyloepiphyseal dysplasia tarda. J. Biol. Chem. 277, 49863–49869.
[21] Jeyabalan, J. et al. (2010) SEDLIN forms homodimers: characterisation of SEDLIN mutations and their interactions with transcription factors MBP1, PITX1 and SF1. PLoS One 5, e10646.
[22] Gecz, J. et al. (2003) Human wild-type SEDL protein functionally complements yeast Trs20p but some naturally occurring SEDL mutants do not. Gene 320, 137–144.
[23] Wang, X. et al. (2011) Spata4 promotes osteoblast differentiation through Erk-activated Runx2 pathway. J. Bone Miner. Res. doi:10.1002/jbmr.394.
[24] Shaw, M.A. et al. (2003) Identification of three novel SEDL mutations, including mutation in the rare, non-canonical splice site of exon 4. Clin. Genet. 64, 235–242.
[25] Dougherty, G.W. et al. (2005) CLAMP, a novel microtubule-associated protein with EB-type calponin homology. Cell Motil. Cytoskeleton 62, 141–156.
[26] Presley, J.F. et al. (1997) ER-to-Golgi transport visualized in living cells. Nature 389, 81–85.
[27] Lie, P.P. et al. (2010) Cytoskeletal dynamics and spermatogenesis. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365, 1581–1592.
[28] Sacher, M. (2003) Membrane traffic fuses with cartilage development. FEBS Lett. 550, 1–4.