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Review

Palmitoylated transmembrane adaptor proteins in leukocyte signaling

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ABSTRACT

Transmembrane adaptor proteins (TRAPs) are structurally related proteins that have no enzymatic function, but enable inducible recruitment of effector molecules to the plasma membrane, usually in a phosphorylation dependent manner. Numerous surface receptors employ TRAPs for either propagation or negative regulation of the signal transduction. Several TRAPs (LAT, NTAL, PAG, LIME, PRR7, SCIMP, LST1/A, and putatively GAPT) are known to be palmitoylated that could facilitate their localization in lipid rafts or tetraspanin enriched microdomains. This review summarizes expression patterns, binding partners, signaling pathways, and biological functions of particular palmitoylated TRAPs with an emphasis on the three most recently discovered members, PRR7, SCIMP, and LST1/A. Moreover, we discuss in silico methodology used for discovery of new family members, nature of their binding partners, and microdomain localization.

Contents

1. Introduction

The family of transmembrane adaptor proteins (TRAPs) represents a heterogeneous group of proteins that differ in the number and character of interacting partners and expression pattern as well as in their biological role. However, the unifying function of TRAPs is their involvement in the signaling pathways by facilitating protein–protein interactions and recruitment of proteins or protein complexes into specific membrane-proximal compartments and microdomains. The localization of most TRAPs to the plasma membrane predisposes them to act in the proximal events of various signaling pathways, either as positive or negative regulators of signaling. Leukocyte TRAPs can be divided into three subfamilies: immunoreceptor-associated TRAPs (TCRζ, FcRγ, DAP10, DAP12), palmitoylated TRAPs (LAT, PAG/Cbp, NTAL/LAB, LIME, PRR7, SCIMP, LST1/A), and TRAPs that are neither directly associated with any immunoreceptor nor palmitoylated (e.g. LAX, SIT, TRIM) [1–5]. A special case is GAPT which contains a potential pamitoylation motif but whose putative palmitoylation has not been experimentally addressed [6].

This review focuses on the palmitoylated TRAPs (pTRAPs) in leukocytes. Palmitoylation is a reversible lipid modification of juxtamembrane cysteine residues in many transmembrane or soluble cytoplasmic membrane-associated proteins [7]. Numerous proteins involved in

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proximal leukocyte signaling are palmitoylated (Fig. 1) [8]. The addition of palmitate to proteins is catalyzed by members of DHHC palmitoyltransferases (23 family members in humans) that reside in plasma membrane or membranes of Golgi or endoplasmic reticulum [9].

Palmitoylation can regulate multiple aspects of protein biology, including protein conformation, membrane microdomain association, protein–protein interactions, and other posttranslation modifications [10]. In case of LAT, a prototypic TRAP, palmitoylation mediates both plasma membrane localization and microdomain targeting [11,12]. Known pTRAPs share the following structural properties: a short N-terminal extracellular part (up to 20 amino acids) without receptor or receptor-like properties, single transmembrane domain, lack of any enzymatic domain, and presence of at least one protein–protein interacting motif in the intracellular part.

Four pTRAP family members were identified by the year 2003 [4]. Recently, the family has expanded by the characterization of three new members in our laboratory [1–3]. Here, we review functional and structural properties of known pTRAPs with the emphasis on the recently discovered family members (Fig. 2) and general features of pTRAPs such as microdomain localization, binding partners, and in silico approaches that were employed to identify the majority of the known family members.

2. "Old" pTRAPs

This chapter briefly summarizes the main findings about the well-established pTRAPs and one putative pTRAP identified before the year 2004. For a more detailed overview, we refer to recent review articles focused on the individual adaptors [13–17].

The first member of the pTRAP family, Linker of activation for T cells (LAT) was identified as a protein expressed in T cells that became strongly phosphorylated upon T-cell receptor (TCR) stimulation [18]. Phosphorylation of LAT, mediated by ZAP70, enables association of LAT with various effector molecules, including Grb2, GADS, PLCγ, and SLP76 [19,20]. LAT is a key component of the TCR signaling pathway that orchestrates formation of a signaling complex where individual molecules interact with each other, leading to the signal propagation and eventual activation of the cell [21]. LAT-deficient Jurkat T cells are unable to respond to the TCR stimulation [22,23]. Mice deficient in LAT have a severe defect in early thymocyte development and are devoid of peripheral T cells [24]. Further analysis demonstrated that LAT is also expressed in NK cells, megakaryocytes, mast cells [25,26], platelets [27,28], and pre-B cells [29]. LAT-deficiency in mast cells leads to markedly decreased responses to FcεRI activation and LAT-deficient mice are therefore resistant to IgE-mediated passive systemic anaphylaxis [30].

The crucial role of LAT in the T cell function prompted research on whether a LAT paralog is present in mature B cells that are practically devoid of LAT. One such candidate termed Non-T-cell activation linker (NTAL alias LAB, LAT2) was independently described by our group and Zhang's [31,32]. NTAL is expressed in B cells, NK cells, monocytes, mast cells, and activated T cells [31–33]. Triggering of B-cell receptor (BCR), Fc receptors, or TCR induces phosphorylation of NTAL mediated by Syk or ZAP70 and enables recruitment of Grb2, Gads and SLP76, but not PLCγ [31,32,34–36]. Initial experiments demonstrated that ectopic expression of NTAL in LAT-deficient Jurkat cells can partially rescue TCR signaling defects [31,37]. Transgenic expression of NTAL enabled murine $LAT^{-/-}$ T cells to pass through thymic selection and these mice subsequently developed severe T cell lymphoproliferative disorders and organomegaly [31,32,34,37]. Interestingly, similar phenotype was described in knock-in mice expressing LAT with mutated PLCγ binding site [38,39]. All these data indicated that ectopically expressed NTAL can substitute for LAT to some extent as a positive regulator of TCR signaling. Therefore, it was surprising that mature B cells derived from NTAL-deficient mice had no overt phenotype [40]. Moreover,

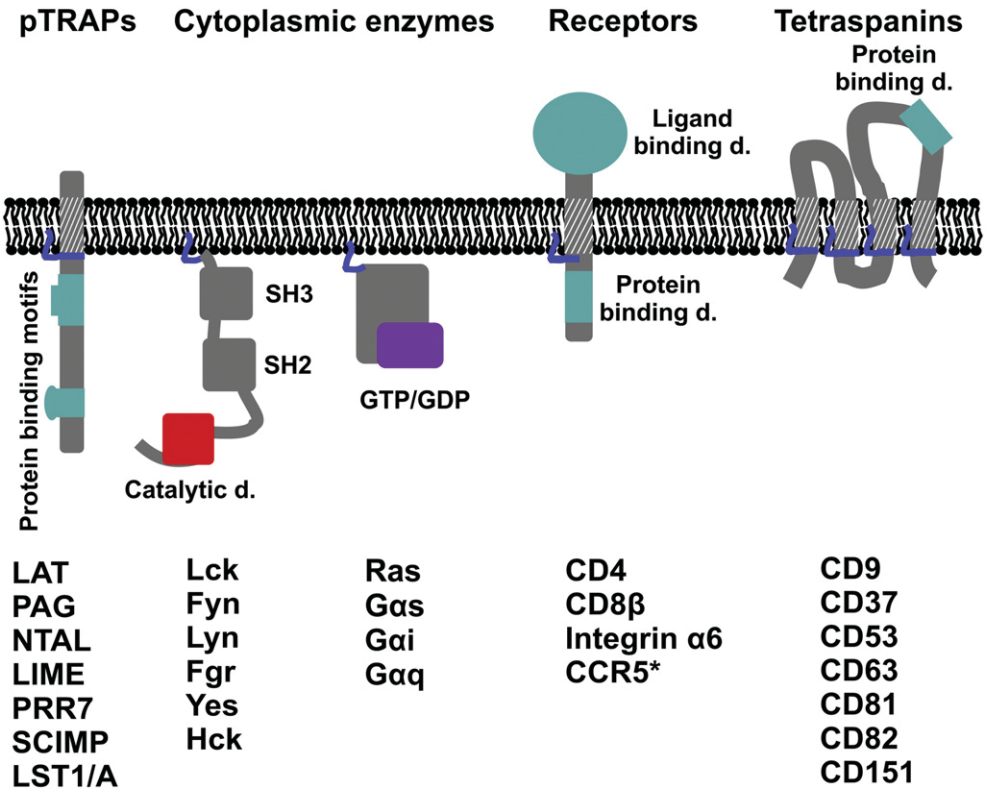

Fig. 1. Important palmitoylated proteins in leukocyte signaling. pTRAPs, soluble cytoplasmic enzymes, receptors, and tetraspanins represent the most important groups of palmitoylated proteins involved in leukocyte signaling. CCR5 has 7 transmembrane domains that are not shown in the scheme. d.: domain.

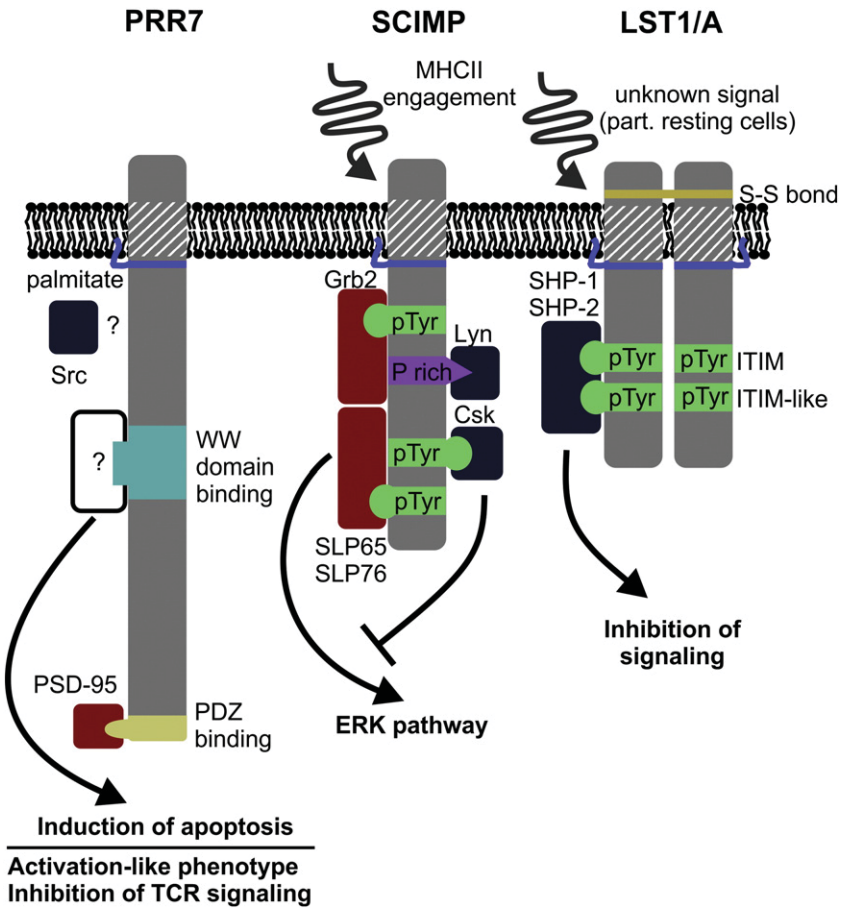

Fig. 2. Schematic representation of new pTRAP family members. The structural motifs of SCIMP, LST1/A, and PRR7 adaptor proteins and their known binding partners are depicted. The arrows show their role in the signaling pathways. See the text for details and references.

NTAL acts as a negative regulator of FcεRI signaling in normal mast cells, but positively regulates FcεRI signaling in LAT-deficient mast cells [41–43]. The crosstalk between NTAL and LAT was further demonstrated in activated T cells that express both LAT and NTAL. NTAL-deficient activated T-cells are hyperresponsive to the TCR re-stimulation due to hyperphosphorylation of LAT and these mice develop spontaneous autoimmune syndrome [33]. Altogether, it seems that NTAL acts as negative regulator of LAT when both proteins are expressed simultaneously. The precise mechanism of the interplay between NTAL and LAT is poorly understood. LAT-independent roles of NTAL were documented in cells that do not express LAT. NTAL promotes the internalization of B-cell receptor via the recruitment of Vav and Grb2-Dynamin complex in B lymphocytes [44,45]. NTAL inhibits signaling of TREM-1 receptor in a myeloid cell line U937 expressing only low levels of LAT [46]. NTAL activates Erk pathway but inhibits proximal signaling in TREM-2 signaling in macrophages [47]. NTAL is involved in Dectin-2 signaling pathway by inhibiting β-catenin pathway in dendritic cells and thus promotes clearance of fungal infections [36]. Low expression of NTAL in childhood T-cell acute lymphoblastic leukemia (T-ALL) predicts poor response to prednisone therapy [48]. Accordingly, over-expression of NTAL sensitized T-ALL-derived Jurkat cell line to anti-CD3-induced or methylprendisolone-induced cell death, indicating that NTAL might act as a tumor suppressor in T-cells and/or T-cell precursors [49].

The third member of the pTRAP family, Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG alias Cbp) is a ubiquitously expressed protein that was discovered by our group as a strongly phosphorylated molecule present in lipid rafts (LR) [50] while Kawabuchi et al. identified PAG as a Csk-interacting protein [51]. Csk is a cytoplasmic kinase that negatively regulates Src family kinases (SFKs). Because SFKs associate with membranes, recruitment of Csk to the membrane proximity might facilitate the Csk-mediated inhibition of SFKs [52]. The hypothesis that PAG could be a global regulator of Csk recruitment to the plasma membrane was substantiated by finding that overexpression of PAG, but not its mutant unable to bind Csk, impairs TCR signaling [53]. Moreover, PAG was shown to be transiently dephosphorylated upon TCR stimulation leading to dissociation of Csk [50,54]. In contrast to T cells, FcεRI crosslinking on surface of mast cells led to enhanced phosphorylation of PAG that promoted Csk recruitment and inhibition of SFKs [55]. Therefore, the observation that PAG-deficient mice have no overt phenotype in leukocyte development, signaling, or immune responses was rather unexpected and indicated that other protein(s) might complement PAG deficiency [56,57]. Apart from its role in regulation of SFK, PAG was also suggested to anchor LRs to cytoskeleton via interaction with EBP50/Ezrin, thus potentially regulating the lateral movement of LRs [58,59].

Lck-interacting transmembrane adapter (LIME) was simultaneously characterized by two groups [60,61], being currently the last pTRAP (co) discovered by a conventional approach [61]. It is expressed predominantly in B cells and T cells and its phosphorylation is triggered upon antibody-mediated crosslinking of CD4 or CD8 [61]. Known LIME interacting partners are Csk and SFK members, Lck and Fyn, indicating that LIME could be a regulator of SFKs [61]. LIME interacts with several other proteins involved in signal transduction pathways, including SHP-2, Grb2, PI3K, and Gads [60,62]. LIME was shown to contribute to TCR-induced adhesion and formation of an immunological synapse via stimulation of Vav-dependent actin polymerization [62]. However, no overt phenotype was observed in LIME-deficient mice and the biological role of LIME is still to be explained [63].

GAPT is a Grb2 binding TRAP expressed mainly in B cells and to lesser extent in mast cells and dendritic cells, but not in T cells. GAPT

contains a putative palmitoylation site but its palmitoylation has not been addressed experimentally. The biological role of GAPT is poorly understood, as GAPT-deficient mice showed generally normal phenotype. The only detected phenotypic changes in GAPT-deficient mice were enhanced anti-BCR induced proliferation of B cells and increased number of marginal zone B cells in aged mice [6].

3. PRR7

One of the recently described pTRAPs emerging from our in silico screens is Proline-rich protein 7 (PRR7), a poorly characterized protein originally identified in forebrain tissue [64]. Although PRR7 is expressed predominantly in the neuronal tissue, its mRNA and protein product were detected in other tissues including lymphoid organs [3]. Comparison of PRR7 orthologs among jawed vertebrates revealed three highly conserved regions: 1. extracellular, transmembrane, and juxtamembrane parts involving the palmitoylation motif, 2. intracellular WW domain binding site, and 3. C-terminal PDZ binding domain that was previously shown to interact with a neuronal protein, PSD-95 [3,64].

PRR7 over-expression in Jurkat T-cell line induced apoptotic cell death, activation-like phenotype (CD69 expression and enhanced IL-2 production after PMA/ionomycin stimulation), and TCR unresponsiveness, probably as a consequence of Lck down-regulation [3]. How PRR7 mechanistically mediates these effects is not completely understood. Experiments with C-terminally truncated PRR7 mutants showed that the intact WW-domain binding site is required for the induction of apoptosis. The WW domain binding motif contains tandem group 1 WW domain binding consensus motifs (PPXY) and exhibits striking homology to the respective part of WBP-1 and WBP-2 (WW domain binding proteins) [3,65,66]. Most likely, PRR7 triggers apoptosis via binding of a WW domain containing protein. Whether the two other hallmarks of PRR7 over-expression (activation-like phenotype and TCR hyporesponsiveness) depend on such an interaction remains to be elucidated. Moreover, PRR7 interacts with Src kinase. The nature and role of this interaction is unknown [3]. Although the expression of PRR7 in the Jurkat model exceeded its physiological levels, PRR7 is up-regulated upon T-cell activation, suggesting that PRR7 might regulate survival and TCR sensitivity of activated T cells.

PRR7 is palmitoylated but its possible localization into particular membrane microdomains remains unclear because PRR7 is rapidly internalized from the plasma membrane. Truncated PRR7 lacking the transmembrane domain induced apoptosis efficiently, indicating that membrane localization is dispensable for this effect.

4. SCIMP

Our search for additional pTRAPs recruiting Csk to the plasma membrane revealed a protein that we termed SLP adapter and Csk-interacting membrane protein (SCIMP) [2]. This molecule contains a submembrane palmitoylation motif, several tyrosine residues that upon phosphorylation enable binding of Csk, Grb2, SLP65 or SLP76, and is constitutively associated with a SFK member, Lyn. Strong SCIMP expression was detected only in immune tissues (spleen and bone marrow) and detailed analysis revealed that SCIMP is confined to professional antigen presenting cells — dendritic cells, B cells, and macrophages.

The ability to recruit SLP65 or SLP76 indicated that SCIMP could act as a signal propagating molecule (similarly to LAT), while Csk binding suggested rather a role as a negative regulator of membrane proximal signaling (similarly to PAG). It was therefore intriguing that antibody-mediated crosslinking of a chimeric protein, consisting of extracellular part of CD25 fused to SCIMP, induced calcium flux and phosphorylation of Akt and Erk, demonstrating that SCIMP is able to propagate signals. All these events were dependent on the presence of the SLP65 binding site [2]. By contrast, mutation of the Csk binding site enhanced all signaling events, suggesting that Csk binding negatively regulates SCIMP signaling itself.

Albeit SCIMP is palmitoylated, it is not targeted to LRs. Instead, SCIMP is present in tetraspanin-enriched microdomains (TEM), together with MHCII molecules. Accordingly, SCIMP co-localizes with several tetraspanin family members in uropods of migrating cells and in immunological synapse [67,68].

Given the shared expression pattern and subcellular localization of SCIMP with MHCII, we hypothesized that SCIMP might be involved in MHCII signaling [2,69,70]. Indeed, antibody-mediated crosslinking of MHCII molecules on the surface of splenic B cells induces phosphorylation of SCIMP and association with SLP76 and Grb2 [2]. A knock-down of SCIMP leads to premature termination of MHCII-induced Erk signaling in K46 B-cell line [2]. This is rescued by re-expression of wild type SCIMP, but not a mutant SCIMP unable to bind SLP65. Future research should address what the physiological role of SCIMP is in MHCII signaling and whether other receptor(s) employ SCIMP for signal propagation.

5. LST1

The Leukocyte-specific transcript 1 (*LST1*) gene is located in immunologically important MHC class III locus [71,72]. Numerous alternatively spliced *LST1* transcripts were reported in human cells. These mRNAs would eventually translate into a large number of particular transmembrane and cytosolic proteins [71–75]. However, three research groups detected monomeric LST1 as a single band by Western blotting suggesting a limited variability of LST1 on the protein level [1,76,77]. Interestingly, under non-reducing conditions LST1 is detected as disulfide-linked dimers, indicating that only transmembrane form(s) of LST1 might be present [1,76]. We have recently used a monoclonal antibody raised against amino acids 66–80 of human LST1/A (clone LST1/02) to detect an LST1 isoform that shared an apparent molecular weight and some predicted features (tyrosine phosphorylation, palmitoylation, homodimerization, membrane targeting) with LST1/A, the only isoform conserved among mammalian species [1]. This isoform was identified only in leukocyte-rich tissues and in myeloid leukocyte subsets. An RT-qPCR using primers recognizing only two canonical *LST1* isoforms, including *LST1/A*, revealed the same expression pattern as our antibody staining. Altogether, we concluded that the characterized myeloid isoform is most probably LST1/A (Table 1) [1].

Schiller et al. generated a monoclonal anti-human LST1 antibody using a peptide corresponding to amino acids 85–97 of human LST1/A for immunization (clone 7E2) that stained two bands in Western blotting in reduced samples (interpreted as monomers and dimers) [77]. The lower band had similar apparent molecular weight (~16 kDa) as the isoform detected by the LST1/02 antibody under reducing conditions. However, the protein recognized by this antibody was ubiquitously expressed in all analyzed cell lines of hematopoietic and non-hematopoietic origin [77]. As LST1/02 and 7E2 antibodies recognized different epitopes in the LST1 sequence, a possible explanation of their differential staining was that they are specific for distinct isoforms. However, all canonical LST1 isoforms contain either both epitopes or neither of them (Table 1). Further experiments, including direct side by side comparison of 7E2 and LST1/02 antibodies, are required to reconcile these contradictory observations.

We characterized LST1/A as a transmembrane adaptor protein. It contains ITIM and ITIM-like motifs with high homology to similar sequences in CD33-related Siglecs [1]. These receptors, expressed mainly on different leukocyte populations, bind sialic-acid residues on various glycoproteins and act as negative regulators of immunoreceptor signaling. CD33-related Siglecs recruit inhibitory effector molecules, including phosphatases SHP-1 and SHP-2, to the plasma membrane in a phosphorylation-dependent manner [78]. Accordingly, LST1/A interacts with SHP-1 and SHP-2 via the ITIM and ITIM-like motifs [1]. Unlike Siglecs, LST1 cannot act as a receptor per se due to its very short extracellular part.

Table 1
Overview of human LST1 isoforms.
The table shows canonical human LST1 isoforms and their amino acid numbers and indicates presence of a transmembrane domain (TM), ITIM and ITIM-like motifs. It is shown if the protein includes epitopes recognized by LST1/02 (SSEGPDLRGRDKRGT) [1] and 7E2 (RADYACIAENKPT) [77] antibodies and if the encoding mRNA is recognized by primers used for RT-qPCR detection of *LST1/A* transcript [1]. LST1/A isoform is underlined.

Isoform	GenBank accession #	Amino acids	TM	ITIM/ITIM-like	LST1/02 epitope	7E2 epitope	qRT-PCR primers
Isoform 1 alias LST1/P	NP_009092.3	104	+	+	+	+	−
Isoform 2	NP_995309.2	66	+	−	−	−	−
Isoform 3	NP_995310.2	66	−	+	+	+	+
Isoform 4 alias LST1/A	NP_995311.2	97	+	+	+	+	+
Isoform 5	NP_995312.2	59	−	−	−	−	−
Isoform 6	NP_001160010.1	73	−	−	+	+	−
LST1/C	AF000424.1	91	−	+	+	+	−
LST1/K	Y18487.1	59	+	−	−	−	−
LST1/J	Y18486.1	43	−	−	−	−	−
LST1/E	AF000426.1	53	−	+	+	+	−
LST1/L	Y18488.1	51	−	ITIM-like only	+	+	−
LST1/M	Y18489.1	34	−	−	−	−	−
LST1/N	Y18490.1	13	−	−	−	−	−

Co-crosslinking of a chimeric protein, consisting of LST1/A fused to extracellular portion of CD25, with Fc receptors by anti-CD25 antibodies in THP-1 and U937 myeloid cell lines increased phosphorylation of LST1/A, enhanced the association with SHP1 and SHP2, and inhibited the Fc receptor-mediated signaling [1]. This proved that LST1/A has the ability to inhibit signaling but the identity of receptors associated with LST1/A and signaling pathways regulated by LST1/A remains unknown. One hint might be localization of LST1/A in TEMs [1]. TEMs contain numerous receptors and the LST1/A could be physically or functionally linked to some of them.

LST1 has also been described as an inducer of nanotube formation [76,79]. LST1 was shown to co-immunoprecipitate with RalA, M-Sec, and exocyst components (mainly Sec5) indicating a role of LST1 in the assembly of multicomponent nanotubes promoting protein complex at the plasma membrane [79]. However, the particular part of LST1 involved in any of these interactions has not been mapped. Thus, it remains to be elucidated if LST1 interacts with the proteins involved in nanotube formation directly.

6. Bioinformatic tools for identification of novel pTRAPs

In silico approach has been widely used for identification of homologs of particular proteins or partial protein sequences. Bioinformatic search can be used even for proteome-wide identification of whole protein families. A good example could be protein tyrosine phosphatases that contain a conserved amino acid sequence signature in their catalytic domain, enabling their easy identification [80]. Although pTRAPs share some structural elements, the lack of a family-specific feature shared by all known members makes the identification of all pTRAPs in the proteome complicated. Although the palmitoylation site could be found in all pTRAPs by definition, there is no clear consensus sequence for palmitoylation. The only general rule in the potentially palmitoylated cysteine residue is in membrane proximity [81]. Nevertheless, in silico screening has been used for the discovery of majority of known pTRAPs using common palmitoylation motifs in pTRAPs (CxxC or CxC), phosphotyrosine interaction motifs, and transmembrane domains as query sequences. Most useful open tools include a predictor of transmembrane domains TMHMM and a motif finder ScanProsite [82,83], but a lot of subsequent work has been done manually.

NTAL was the first pTRAP (co)identified via bioinformatic tools [32]. The authors searched for LAT-like proteins containing: 1. four to five YxN Grb-2 phospho-tyrosine binding motifs; 2. a predicted transmembrane domain; and 3. a potential palmitoylation site. The second in silico identified pTRAP was LIME [61]. The criteria for the search were based on the structure of known adaptor proteins LAT, NTAL, and PAG: 1. CxxC palmitoylation motif; 2. a short extracellular part (5–50 amino acids) followed by a transmembrane α-helix; and 3. at least one phospho-tyrosine motif Yxx[V/L/I] in the intracellular part. Criteria used for identification of GAPT (palmitoylation motif, YxN Grb2-binding motif, and predicted transmembrane domain) were only slightly changed [6]. However, the subtle differences in the definition of the palmitoylation and phosphotyrosine motifs prevented the simultaneous identification of both adaptors in either of these two screenings.

More recently, PRR7 and LST1/A were identified in a human proteome-wide search focused on proteins fulfilling similar criteria that were used for the identification of LIME [3]. One of the changed criteria was less stringent definition of the palmitoylation motif (CxC or CxxC) that enabled the identification of LST1/A [1,3]. The last published screening identified SCIMP using only two criteria: Csk-binding phosphotyrosine motif (Y[A/S/T]X[V/P][N/Q/C][K/R]) and a prediction of a transmembrane domain [2].

Besides the pTRAPs, a non-palmitoylated TRAP, LAX, was identified using a bioinformatic search for LAT-related proteins with similarities to amino acid sequence 160–180 of human LAT [84].

In silico searches have identified 5 pTRAPs and 1 TRAP with predicted palmitoylation and display substantial advantage over conventional "wet" methods (yeast two hybrid system, biochemical isolation followed by sequencing or mass spectrometry) [31,50,51,60]. Despite these benefits, the employed bioinformatic tools for the identification of novel pTRAPs had two major drawbacks. First, a lot of false positives (e.g. enzymes) were generated and had to be manually sorted out. Second, special care had to be paid to the definition of the searched motifs. The use of stringent consensus sequences in the phosphotyrosine or palmitoylation motifs eliminates not only some false positives but also relevant hits. Thus, some unknown pTRAPs might be still hiding in the human proteome and probably even a larger number of pTRAPs remain to be characterized in less studied organisms.

7. Binding partners

The ability to bind specific partners determines the biological role of adaptor proteins, including pTRAPs, in signal transduction cascades. Although each pTRAP exhibits a unique set of interacting proteins, there are certain common features. One of the shared properties of pTRAPs is a link to tyrosine phosphorylation. This phenomenon is caused by their position in proximal signaling pathways, where tyrosine phosphorylation is a typical way of transducing signals. All of the known pTRAPs are phosphorylated at specific tyrosine residues, usually in an inducible manner. These phosphotyrosines act as docking sites for SH2 domains of the interacting proteins. The second relation between pTRAPs and tyrosine phosphorylation is the fact that tyrosine kinases

and phosphatases are typical interactors with pTRAPs. All pTRAPs, with the exception of LAT, NTAL and GAPT, are known to bind at least one tyrosine kinase or phosphatase (Table 2). Three pTRAPs (PAG, LIME, and SCIMP) are able to bind both a SFK and Csk, a negative regulator of SFKs. In case of PAG, the simultaneous binding of a SFK and Csk was viewed as negative regulation of cellular SFKs [50,51]. However, ultimate evidence for the role of PAG as a global regulator of the SFKs' activity is still missing. Based on the observations of SCIMP, the recruited Csk might locally regulate signals emanating from the pTRAP-bound SFK. In this case, the Csk binding acts as a negative feedback for the pTRAP signaling, not as a feature to regulate SFKs globally.

Cytosolic adaptor proteins (e.g. Grb2, GADS, SLP65) are a well-represented group of pTRAP binding proteins (Table 2). They recruit additional proteins to the signalosome nucleated by the pTRAP and help to transduce the signal from the plasma membrane downstream to the cytosol.

8. Membrane microdomains

LRs and TEM represent two different types of membrane microdomains with specific composition of accommodated proteins [85]. These microdomains represent platforms favoring interactions among their resident proteins. Whereas LR formation is mostly based on aggregation of certain lipids (e.g. cholesterol, sphingolipids), TEMs are organized by protein–protein interactions among members of tetraspanin family [86–88]. LRs and TEMs are biochemically isolated as detergent resistant membranes (DRMs) and residence of a protein in LRs/TEMs can be determined by its presence in soluble/insoluble fractions after the cell lysis in particular detergents. Whereas TEMs are resistant only to mild detergents (e.g. Brij 98, CHAPS) and soluble in more stringent detergents (NP40, Triton X-100), LRs are insoluble in both [2,87–89]. The putative residence in TEMs can be additionally confirmed by co-immunoprecipitation with tetraspanin proteins [2,88,90,91].

Interestingly, LRs as well as TEMs are enriched for palmitoylated proteins and accordingly, palmitoylation of several proteins, including LAT, was shown to be indispensable for their targeting to LRs or TEMs [11,92–95]. All four initially described pTRAPs (LAT, NTAL, PAG, LIME) localized to LRs [1,2,31,50,61,96,97]. Thus, palmitoylation was considered as a LR-targeting motif for transmembrane adaptors [4,98]. Surprisingly, recently described SCIMP and LST1/A are not associated with LR, but localize to TEMs instead [1,2]. The determinants of LR vs. TEM targeting still remain to be elucidated. However, the two groups of pTRAPs exhibit strikingly different sequences of the palmitoylation site. Palmitoylation motif of SCIMP and LST1/A consists of two cysteines separated by a single hydrophobic amino acid (C[V/L]C). In contrast, the palmitoylation motifs of LR-resident pTRAPs include two cysteines separated by two amino acids, of which at least one is polar or basic (CxxC) (Table 2). The importance of palmitoylation for the microdomain targeting of pTRAPs is underscored by the fact that non-palmitoylated TRAPs occupy distinct membrane microdomains [4,84]. For instance, LAX and TRIM are present in recently described "heavy" DRMs and SIT localizes to detergent soluble membranes [99].

Functional importance of the localization into proper membrane microdomains can be illustrated in the example of LAT. LAT chimeras targeted into "heavy" DRMs or soluble membranes exhibited lower efficiency in promoting TCR signaling [99]. Although the other pTRAPs are studied less intensively than LAT, there are several pieces of evidence indicating that microdomain localization regulates biology of other pTRAPs as well. Translocation of FcγRII into LRs induced either by antibody crosslinking or by lysenin-mediated sphingomyelin aggregation results in phosphorylation of LR resident NTAL [100–102]. Similarly, co-localization of SCIMP and MHCII to TEMs likely enables SCIMP to participate in the MHCII signaling pathway [2].

The issue of microdomain localization of pTRAPs seems to be linked to their physiology and should be a subject of further studies. Open questions include the nature of LR or TEM targeting motifs and the heterogeneity of the two canonical microdomains. The existence of such inner heterogeneity of LRs is suggested by the observation that LAT and NTAL, albeit both resident in LRs, occupy separate membrane segments in mast cells [41]. Furthermore, NTAL is able to communicate with TEMs, as an antibody-mediated crosslinking of tetraspanin CD9 leads to phosphorylation of NTAL and in mast cells. NTAL, but not LAT, partially co-localized with CD9 and this co-localization was further enhanced by anti-CD9 treatment [103]. The heterogeneity of LRs and crosstalk between LRs and TEMs suggest a high level of complexity in the biology of membrane microdomains.

Although it seems that the palmitoylation can regulate localization and function of pTRAPs, we still know little about the identity of respective palmitoyltransferases, turn-over of the palmitoylation of pTRAPs, and the importance of palmitoylation for the function of most pTRAP family members.

9. Summary

The family of pTRAPs currently includes seven true and one putative member. Each pTRAP is unique, because it binds specific proteins, has specific expression pattern, and regulates particular signaling pathway(s). Interestingly, only three pTRAPs (LAT, LIME, and SCIMP) are mostly positive regulators of signaling pathways, while four members inhibit signal propagation (PAG, GAPT, PRR7, and LST1/A) and one has a dual character (NTAL). The functional complementarity between different family members is rare (the only reported case is a partial complementation of LAT deficiency by NTAL). However, the mode of action is shared by all pTRAPs. They recruit positive or negative regulators of signaling into specific membrane microdomains and regulate signaling pathways via facilitating protein–protein interactions. Although there are a lot of open questions concerning the function of particular family members as well as some common issues, such as the role of

Table 2
Overview of known pTRAPs.
The table summarizes main features of known pTRAPs. *Indirect or potentially indirect interaction (not mapped). ‡Predicted, but not verified, palmitoylation sequence. h: human, m: mouse (shown only if the sequences differ). B: B-cells. T: T-cells. DC: dendritic cells. MC: mast cells. NK: nature killer cells. MF: macrophages, P: platelets. Neur: neurons. act.: activated. See the text for details and references.

			Most important binding partners					
	Signaling pathway	Role	Cytosolic adaptors	Kinases	Other enzymes	Expression	Palm. motif	Microdomains
SCIMP	MHCII	+	Grb2, SLP65, SLP76	Csk, Lyn		B, DC, MF	CVC	TEMs
PRR7	TCR	−	PSD-95	Src*		Neurons, act. T	CCFC	Unknown
LST1/A	unknown	−	M-Sec*, Sec5*		SHP-1, SHP-2, RalA*	DC, MF, granulocytes	CLC	TEMs
LAT	TCR, FcεRI	− (+)	Grb2, Gads, SLP76*		PLCγ1, PLCγ2	T, pre-B, NK, MC, P	h: CVHC, m: CVRC	Lipid rafts
NTAL	TCR, FcεRI, BCR, TREM-1, TREM-2, Dectin-2	+/−	Grb2, Gads, SLP76*			Act. T, B, NK, MC, DC, MF	h: CVRC, m: CVHC	Lipid rafts
PAG	TCR, BCR	−	EBP50	Csk, Fyn, Lyn, Src		Ubiquitous	h: CSSC, m: CSTC	Lipid rafts
LIME	TCR	+	Grb2, Gads, SLP76*	Csk, Fyn, Lyn	VAV, SHP-2, PI3K p85	T, B	CTAC	Lipid rafts
GAPT	BCR	−	Grb2*			B, MC, DC	CGIGC‡	Not lipid rafts

palmitoylation and residence in microdomains, pTRAPs represent an important family of regulators of signal transduction pathways.

Conflict of interest

The authors declare that they have no conflict of interest.

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