Minireview

Regulation of Tiam1–Rac signalling

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Abstract The GTPases of the Rho family are molecular switches that play an important role in a wide range of cellular processes and are increasingly implicated in tumourigenesis. Unlike what was found for the Ras oncogenes in tumours, hardly any activating mutations have been found in the genes encoding Rho proteins. In the past, we have identified Tiam1 (T-lymphoma invasion and metastasis) as a specific activator for the Rho-like GTPase Rac. In vivo, Tiam1 deficiency protects against Ras-induced skin carcinogenesis, underscoring the consequences of deregulated signalling for the onset and progression of tumours. Thus, an important level of regulation of signalling via the Rho-like GTPases comes from the specific control of their activators. In this paper, we review what is known on the specific regulation of Tiam1 signalling towards Rac.

Key words: Tiam1; Rac; GTPase; Signalling; Guanine nucleotide exchange factor; Tumourigenesis

1. Introduction

The Rho-like GTPases (which include Rho, Rac and CDC42) are molecular switches that regulate a wide variety of cellular processes. They are crucial for cytoskeletal rearrangements necessary for cell motility, are involved in cell cycle progression and regulate gene transcription. It is becoming increasingly clear that they play an important role in tumourigenesis [1]. E.g. both Rho and Rac are involved in the transformation of cells by the Ras oncogene [2,3]. However, unlike what was found for Ras, very few (if any) activating mutations are found in the genes encoding Rho-like proteins. But given the importance of the GTPases in a spectrum of biological activities, it comes as no surprise that aberrant regulation of their activity can lead to several abnormal cellular phenotypes.

Like the Ras oncogene, these Rho-like GTPases cycle between an inactive, GDP-bound state and an active, GTP-bound state. Activation is accomplished by the release of Rho guanine nucleotide dissociation inhibitor (Rho-GDI) and exchange of GDP for GTP, catalysed by a class of molecules known as guanine nucleotide exchange factors (GEF). When bound to GTP, the GTPases undergo a conformational change which allows them to bind to their respective downstream effector molecules and thereby to transmit the signal. Proteins that increase the intrinsic rate of GTP hydrolysis of the GTPase, known as GTPase-activating proteins (GAPs), inactivate the GTPase and thus shut down the signalling. Thus, the activity of Rho-like GTPases is determined by the amount of protein in the GTP-bound form and the time during which it is active. This activation state is therefore dependent on the balance of activities of GEFs and GAPs. In turn, these regulatory molecules themselves (GEFs and GAPs) are also under tight control.

In the past, we have identified the Tiam1 protein (for T-lymphoma invasion and metastasis) as a specific GEF for the Rho-like GTPase Rac [4]. Although this function of Tiam1 has been well-established [5], the specific regulation of its activity is far from being fully understood. In this review, we will focus on the ways in which the activity of Tiam1 is regulated. Apart from increased or decreased expression, GEFs are basically regulated at four different levels in the cell: these include (relief of) intramolecular inhibition, changes in intracellular localisation, post-translational modifications and interaction with other proteins [6] and all of these seem to apply to Tiam1. Insight into the biochemical mechanisms governing the activity and localisation of GEFs such as Tiam1 may lead to a better understanding of the specific spatio-temporal activation of Rho-like GTPases and the various ways in which this signalling may be deregulated in diseases, notably in cancer.

2. Tiam1 regulation; (de-)phosphorylation, phosphoinositol binding and stability

2.1. Tiam1 structure, auto-inhibition and protein stability

We originally identified Tiam1 in a retroviral insertional mutagenesis screen for genes that confer an invasive phenotype to otherwise non-invasive murine T-lymphoma cells [4]. The protein possesses the characteristic Dbl homology (DH)–pleckstrin homology (PH) domain combination, always found in members of the Dbl-like family of exchange factors (Fig. 1). The human Tiam1 gene shows a high degree of homology to its murine counterpart and is widely expressed [7]. The Tiam1 protein is 1591 amino acids long and contains several distinct

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Abbreviations: CaMK-II, Ca^{2+}/calmodulin kinase II; DH, Dbl homology; EDG2, endothelial cell differentiation gene 2; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; HA, hyaluronic acid; HER, heregulin; JIP/IB2, JIP/islet-brain; LPA, lysophosphatidic acid; PH, pleckstrin homology; PI3-kinase, phosphatidyl inositol 3-kinase; PKB, protein kinase B; PKC, protein kinase C; RBD, Ras-binding domain; Tiam1, T-lymphoma invasion and metastasis

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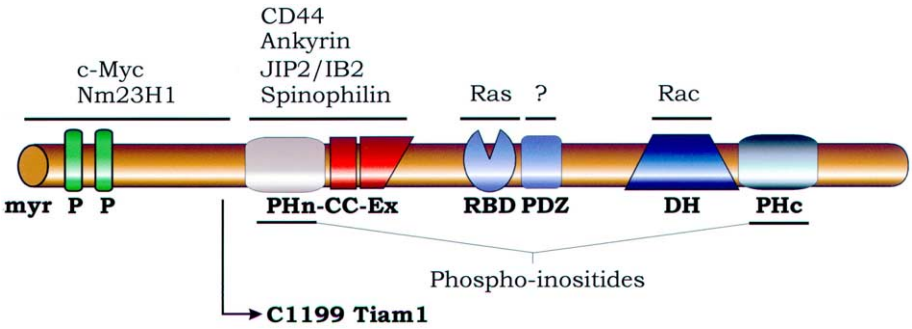

Fig. 1. Schematic representation of the domain structure of Tiam1. The specific domains of Tiam1 are indicated (from the N-terminus onwards): myr: myristoylation site; P: PEST sequence; PHn: N-terminal PH domain; CC: coiled-coil region; Ex: extended structure (without known fold); RBD: Ras-binding domain; PDZ: PSD-95/DlgA/ZO-1 domain; DH: Dbl homology domain and PHc: C-terminal PH domain. Proteins reported to interact with certain regions of Tiam1 are indicated above the structure; domains shown to interact with phosphoinositides are highlighted below the structure. The part of the protein present in the active (C1199) form of Tiam1 is also shown.

domains (Fig. 1). It is myristoylated at its N-terminus [8], contains two N-terminal PEST domains, an N-terminal PH domain (PHn), a coiled-coil region with adjacent sequence (named CC-ex) [9], a Ras-binding domain (RBD; [10]), a PSD-95/DlgA/ZO-1 domain and the characteristic catalytic DH–PH (named PHc) combination. As was shown for other Dbl-like Rho-GEFs (i.e. Dbl, Vav, Asef, Ect2 and Net1), N-terminal truncation of Tiam1 (as in C1199-Tiam1) enhances its in vitro GEF activity and promotes its in vivo association with the plasma membrane [8]. In the case of Vav [11] and proto-Dbl [12], intramolecular interactions between an N-terminal α-helix or coiled-coil domain with the DH or its flanking PH domain, respectively, inhibit the interaction with and therefore GTP exchange on the Rho-GTPase. However, the exact mechanism underlying the N-terminal auto-inhibitory effects of Tiam1 is not yet clear. Like other GEFs, Tiam1 has two consensus PEST domains, located N-terminally of the PHn domain. PEST domains are believed to function by targeting proteins to the degradation machinery, hence increasing their turnover (for review, see [13]). Therefore, deletion of these PEST domains probably enhances the stability and thereby the activity of Tiam1 and other GEFs. Indeed, truncation of the C-terminal PEST domain of proto-Dbl increases its in vivo GEF activity, membrane localisation and

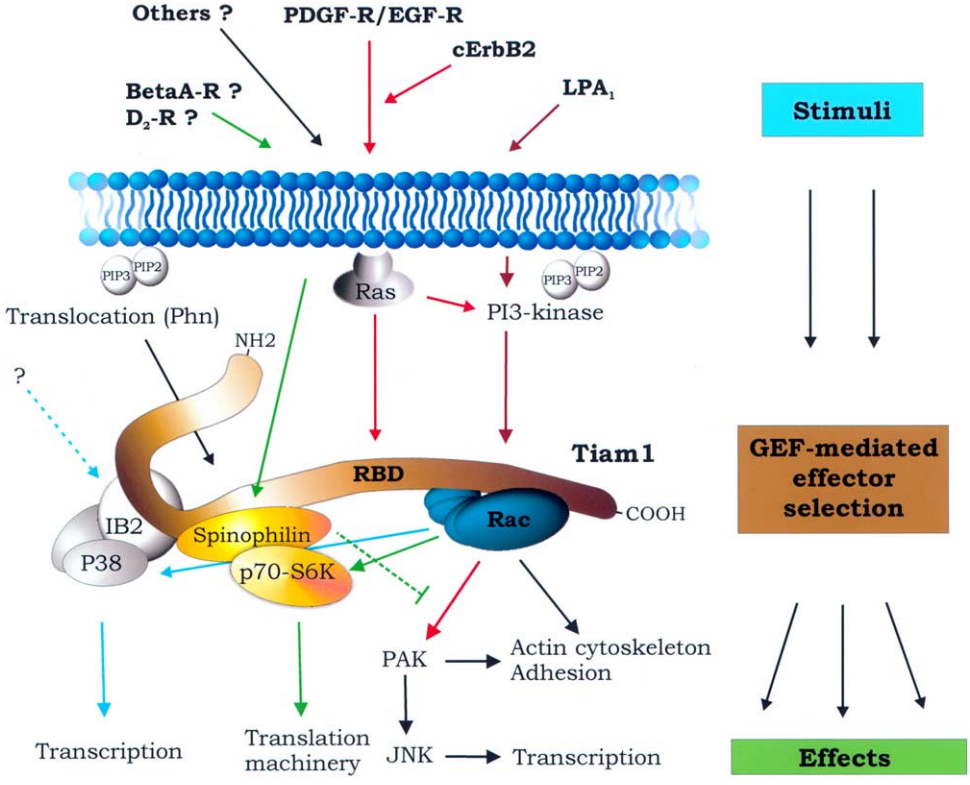

Fig. 2. Tiam1-mediated sorting of upstream signals to downstream effects. Schematic drawing showing pathways known to result in Tiam1 activity or known to depend on Tiam1 function; the specific interactions of Tiam1 with other proteins determine the outcome of the signalling (see also text). Known canonical pathways are indicated by different colours. BetaA-R: β-adrenergic receptor; D_2-R: dopamine-2 receptor; EGF-R: epidermal growth factor receptor; JNK: Jun N-terminal kinase; LPA_1: lysophosphatidic acid receptor-1; PDGF-R: platelet-derived growth factor receptor; PAK: p21-activated kinase; PHn: N-terminal PH domain; PI3-kinase: phosphatidyl inositol 3-kinase; PIP2: phosphatidyl inositol diphosphate; PIP3: phosphatidyl inositol triphosphate; RBD: Ras-binding domain and p70S6K: protein of 70 kDa, S6-kinase.

oncogenic potential [12]. In contrast, the destruction box in the PEST domain of Ras-GRF1/2 is only exposed once it binds to Ras, probably causing Ras-GRF degradation upon activation of its target GTPase [14]. In case of Tiam1, PEST-induced N-terminal degradation may account for enhanced turnover upon activation of Rac. In addition, truncation might be a potential mechanism to activate the protein in vivo, since truncated products with increased half-life are often observed once the full-length Tiam1 protein is expressed in cells (Mertens et al., unpublished results). Following apoptotic stimuli, caspase-mediated cleavage of Tiam1 has been shown to be a mechanism of down-regulating Rac activity and hence inhibiting Rac-induced anti-apoptotic signalling [15].

2.2. Membrane translocation and activation of Tiam1

Membrane translocation of Tiam1 is crucial for its capacity to induce Rac-mediated membrane ruffles and activation of c-Jun N-terminal kinase [8]. The PHn, rather than the PHc domain was shown to be critical for membrane localisation of C1199-Tiam1 [8]. More precisely, the whole PHn-CC-Ex region was shown to be required, since deletion of the PHn, CC or Ex domain abolished translocation to the membrane [9]. Interestingly, we found the isolated PHn-CC-Ex region to reside primarily at the plasma membrane where it could block the function of C1199-Tiam1, indicating that Rac binding nor activation is required for membrane translocation of Tiam1. The N-terminus of Tiam1 also contains a potential myristoylation site. However, this lipid anchor probably only stabilises the protein at the plasma membrane and does not cause its translocation, since myristoylation could not compensate the loss of membrane localisation in a Tiam1 mutant that lacks the PHn domain [8]. Intriguingly, the PHn domain of Tiam1 is a region that has been described to be mutated in cancer [16]. A single point mutation was reported to cause hyperactivity of the protein, as assessed by a colony formation assay. Whether this mutation affects localisation of Tiam1 by interfering with phosphoinositide binding (see below), or interferes with phosphorylation, is not clear.

The enhanced activity of truncated (C1199) Tiam1 versus that of the full-length protein is not the mere result of increased protein stability. C1199-Tiam1 is predominantly present at the plasma membrane [8], probably causing it to interact more efficiently with prenylated, Rho-GDI-dissociated Rac [17]. In addition, membrane association brings the catalytic domain of Tiam1 in close proximity with phospholipids, which might facilitate the exchange reaction (see below). It is currently unclear whether Tiam1 can directly trigger the association of Rac with the plasma membrane.

Phosphorylation of Tiam1 is another event that may directly trigger the translocation and/or the activity of the GEF. Tiam1 was reported to be phosphorylated on threonine residues by Ca^{2+}/calmodulin kinase II (CaMK-II) and protein kinase C (PKC) in vitro and upon treatment of Swiss 3T3 fibroblasts with lysophosphatidic acid (LPA) and PDGF in vivo [18,19]. More specifically, activation of the LPA_1 receptor (formerly named EDG2) by its ligand LPA induces a robust Rac activation that is dependent on the GEF activity of Tiam1 [20]. CaMK-II but not PKC was able to stimulate C1199-Tiam1-induced nucleotide exchange on Rac in vitro, although to a maximum of only two-fold. However, the absence of the N-terminal regulatory domain in C1199-Tiam1 makes this result difficult to interpret [18]. Membrane translocation and threonine phosphorylation of membrane-bound Tiam1 upon LPA and PDGF stimulation could only be inhibited by CaMK-II inhibition in NIH 3T3 cells [21]. In line with these observations, PKC inhibition did not affect C1199-Tiam1-induced T-lymphoma cell invasion, a process totally dependent on LPA signalling [22], indicating once more that PKC-mediated Tiam1 phosphorylation probably does not play a direct role in Tiam1–Rac signalling. On the other hand, we found that Ca^{2+} signalling can induce PKC-mediated phosphorylation of Rho-GDI, resulting in release of Rac and its subsequent activation (Price et al., submitted). However, this mechanism of Rac activation does not involve Tiam1. Based on these studies, one can conclude that membrane translocation of Tiam1 is closely associated with its phosphorylation, but that it is unclear whether phosphorylation is causal for translocation or full activation of the protein once it is on the plasma membrane.

Poly-phosphorylated inositol lipids like phosphatidyl inositol diphosphate (PIP_2) and phosphatidyl inositol triphosphate (PIP_3) play an important role in GEF-mediated activation of Rho-GTPases in processes that require actin cytoskeleton rearrangements, such as cell motility and the establishment of cell polarity [23,24]. However, the exact role of inositol phospholipids in regulation of Tiam1–Rac signalling and GEF activation in general has been a matter of debate. Together with Ras-GRF1/2, Tiam1 is the only Dbl family GEF that contains a second PH domain, in addition to the PH domain in the catalytic DH–PH unit. In general, the characteristic role of PH domains (e.g. of those found in pleckstrin, PKB/Akt or P85 phosphatidyl inositol 3-kinase (PI3-kinase)) lies in protein localisation through their ability to bind phosphoinositides at the plasma membrane. In contrast to these PH domains, however, those in the DH–PH tandem of Dbl-like proteins bind phospholipids with low affinity and probably require the synergy with other adaptor modules for efficient membrane localisation. Indeed, the PHn domain of Tiam1 has higher affinity for poly-phosphorylated inositides than the PHc domain, with a preference of PtdIns(3,4,5)P_3 over PtdIns(3,4)P_2 and PtdIns(4,5)P_2 [19,25]. This is in contrast to the PHc domain which has the highest (but relatively low) affinity for PI(3)P [26]. However, lipid binding to the PHc domain has been reported to have a direct effect on the catalytic activity of the associated DH domain.

The DH–PH region of Tiam1 and other GEFs was shown to mediate nucleotide exchange on Rac in vitro, but the addition of phosphoinositides (either dissolved or on vesicles) did not affect the rate of exchange by either the DH–PH region alone or by C1199-Tiam1 [26]. However, other reports claim that PI(4)P [27], PI(3,4)P_2 and PI(3,4,5)P_3 [19] do trigger GEF activity in vitro. Truncation of the PHc domain in C1199-Tiam1 totally abolished its capacity to induce membrane ruffles in cells [27]. In agreement with this observation, mutations in the PHc domain that abolished PI(3)P binding but did not affect the in vitro GEF activity of Tiam1 prevented in vivo Rac activation without affecting the membrane localisation of Tiam1 [28]. Although PI(3)P is mainly found on endosomal compartments, the authors proposed that high local concentrations of PIP_2 or PIP_3 at the plasma membrane might be able to trigger PHc-driven DH activity.

PI3-kinase activity (which produces PI(3,4)P_2, PI(4,5)P_2 and PI(3,4,5)P_3) has often been implicated in the control of

Tiam1-driven activation of Rac, probably through its effect on membrane translocation of Tiam1 [19–21,29]. In MDCK cells, PI3-kinase inhibition blocked C1199-Tiam1's capacity to mediate either cell migration or intercellular adhesion, processes that could be rescued by active (V12) Rac [29]. PI3-kinase activity was also shown to promote C1199-Tiam1-driven Rac activation in Swiss 3T3 cells, a process dependent on the PHn domain [19]. Intriguingly, PIP_3 production did not cause translocation of the isolated PHn and PHc domains to the plasma membrane, nor did PI3-kinase inhibition reduce the ability of C1199-Tiam1 to locate to the plasma membrane and induce membrane ruffles. However, truncation of the PHc domain made C1199-Tiam1-induced membrane ruffling dependent on PI3-kinase activity. In addition, C1199-Tiam1-mediated invasion of T-lymphoma cells is critically dependent on the activity of PI3-kinase (Roovers et al., unpublished results).

Rac has been reported to be a critical component during the first stage of cellular transformation caused by oncogenic Ras [30] and PI3-kinase activity is found to play a pivotal in this activation [2]. In addition to a PI3-kinase-dependent, a PI3-kinase independent Tiam1–Rac signalling pathway has also been described [10]. This study demonstrates that the signalling of activated Ras to Rac via Tiam1 is at least partially independent of PI3-kinase activity. It is tempting to speculate that Ras is able to overcome the dependency of Tiam1 on PI3-kinase for its signalling to Rac, whereas other signalling pathways do require PI3-kinase activity for Tiam1-mediated Rac activation.

Tiam1 might act as a downstream target of cadherin signalling: when the vascular isoform of E-cadherin (VE cadherin) was re-expressed in VE cadherin null cells, this led to an increase in Tiam1 mRNA and protein levels, an increased membrane localisation of Tiam1 and an increase in Rac activity [31]. However, it remains elusive whether Tiam1 can directly interact with the cadherin–catenin complex and whether it is required for Rac activation downstream of cadherin signalling. Tiam1 was also shown to be a target of heregulin 2 (HER2) signalling in MCF7 breast carcinoma cells [32]. The HER2 (or cErbB2) oncogene, a member of the epidermal growth factor receptor family, is frequently over-expressed in mammary tumours and its signalling is associated with enhanced metastatic potential. In fact, upon treatment of MCF7 cells with HER, Tiam1 is phosphorylated and displaced from intercellular adhesions, together with β-catenin and E-cadherin. In addition, Tiam1 was recruited into membrane ruffles and this was associated with increased migratory potential of these cells.

3. Regulation of signalling through protein–protein interactions

Different members of one GEF family are expressed in specific cell types and the activity of a given GEF within one cell is often controlled by a subset of membrane receptors and intracellular proteins. This hints towards a mechanism of spatial regulation of GEF activity and restricts the activation of a given Rho-GTPase to a certain signalling pathway in time and space. Tiam1 has been implicated to directly bind to a plethora of different cytoplasmic and membrane-associated proteins, which couples Tiam1–Rac activity to specific signalling pathways. This is schematically depicted in Fig. 2.

An interesting observation in the field of cancer was that Ras was shown to mediate Tiam1–Rac signalling (see also above) through the direct interaction of Ras with the RBD of Tiam1 [10]. Moreover, the RBD of Tiam1 is required and sufficient for specific binding of Tiam1 to activated Ras. The fact that Tiam1 knock-out mice were proven to be resistant to Ras-induced skin tumours underlines the physiological relevance of the connection between Ras and Tiam1 [33]. Tiam1-mediated Rac activation downstream of oncogenic Ras probably promotes NFκB-mediated cell survival, which in turn promotes tumour initiation. Loss of intercellular adhesion in the absence of Tiam1–Rac signalling was probably causal for the enhanced malignant progression of Tiam1 deficient tumours [33]. In fact, in epithelial MDCK cells, Tiam1–Rac and Ras signalling seemingly oppose each other, since V12Ras-induced epithelial–mesenchymal transition is negatively affected by Tiam1–Rac signalling [29,34]. Increasing Rac activity by introduction of C1199-Tiam1 into MDCK cells promotes E-cadherin-based cell–cell adhesions and thereby prevents cell migration [34,35]. In accordance with these results, down-regulation of endogenous Tiam1 levels in MDCK cells using short-interference RNA technology causes a loss of E-cadherin-based intercellular adhesions (van Es et al., unpublished results). Tiam1-induced restoration of intercellular adhesions in Ras-transformed MDCK cells is independent of its capacity to bind Ras, since deletion of the RBD of Tiam1 does not affect this process (Olivo et al., unpublished results). Thus, Tiam1 either potentiates Ras signalling through direct binding or counteracts its negative effect on intercellular adhesions via an independent signalling pathway.

The first integral membrane protein reported to directly interact with Tiam1 (and more specifically with the PHn-CC-Ex domain) is the hyaluronic acid (HA) receptor isoform $CD44_{v3}$ [36], which is also concurrently over-expressed in mammary gland tumours. In parallel, the same domain of Tiam1 was shown to interact with the cytoskeletal protein ankyrin [37]. In fact, both $CD44_{v3}$ and Tiam1 bind to the ankyrin repeat domains of ankyrin and both $CD44_{v3}$ and ankyrin bind to the same domain of Tiam1. Based on these data one could envisage that Tiam1 binds indirectly to $CD44_{v3}$, using ankyrin as the hub. However, the net result of these interactions for the GEF activity of Tiam1 remains to be determined. In vitro, ankyrin potentiates C1199-Tiam1-mediated nucleotide exchange on Rac, but the sequential steps of Tiam1-induced Rac activation after HA binding to its receptor are still unclear. Data on recruitment of full-length Tiam1 and ankyrin to ligand-bound $CD44_{v3}$ and the resultant effect on Rac activity are lacking.

The principle that proteins interacting with Tiam1 not only control its GEF activity, but also determine the downstream specificity of Tiam1-induced Rac signalling is demonstrated in two recent publications. Yeast two hybrid screens identified the scaffold proteins JIP/islet-brain (JIP/IB2) and spinophilin (or neurabin II) as proteins binding to the PHn-CC-Ex domain of Tiam1 [38,39]. Spinophilin is a ubiquitously expressed protein that binds protein phosphatase 1 (PP1), F-actin filaments, the D2 dopamine receptor and is involved in Rac-mediated actin reorganisation [40,41]. Notably, PP1 bears phosphatase activity towards Tiam1 in vitro [18], but it remains unclear whether spinophilin targets PP1 to Tiam1 and thereby modulates its GEF activity [39]. More importantly, spinophilin binds to and enhances the activity of p70 S6-ki-

nase, one of the direct downstream effectors of Rac. Co-expression of spinophilin and C1199-Tiam1 enhanced S6-kinase activity in a synergistic manner. In contrast, spinophilin binding suppressed Tiam1's ability to activate p21-activated kinase, the downstream target of Rac that mediates actin cytoskeletal reorganisation and thus dictates the downstream effects of Tiam1-mediated Rac activation in a direct manner. Similarly, the pancreatic β-cell/brain-specific protein JIP2/IB2, which is a scaffold for the MLK3-MKK3-p38-mitogen-activated protein (MAP) kinase complex, was demonstrated to couple Tiam1-induced Rac signalling to p38 activity [38]. Ectopic expression of C1199-Tiam1 not only stimulated the build up of a complex of JIP2/IB2 with all components of the p38 signalling cascade, but also enhanced p38 MAP kinase signalling.

Several recent reports further add candidates to the growing list of interacting partners of Tiam1. Interestingly, these proteins claimed to interact with Tiam1 are well known players in the field of cancer. Firstly, the N-terminus of Tiam1 was found to interact with the tumour suppressor Nm23H1 [42]. Although the NDP-kinase activity of Nm23H1 can generate Rac-GTP in vitro, over-expression of Nm23H1 in Rat1 cells inhibited Tiam1-mediated activation of Rac and Tiam1-induced membrane ruffling. However, Nm23H1 also functions as a GAP for the GTPase Rad, which hints towards the possibility that the in vivo effects of Nm23H1 expression on Tiam1–Rac signalling may be due to its function as GAP towards Rac (which has not been excluded), rather than its direct inhibitory interaction with Tiam1. In the process of adherens junction disassembly in MDCK cells, activation of the GTPase Arf6 was claimed to cause an inhibition of Tiam1–Rac signalling by means of Nm23H1 [43]. However, over-expression of Nm23H1 did not alter Rac activity in the presence of high levels of endogenous Tiam1. Furthermore, the kinase-dead (H118C) mutant of Nm23H1, that was claimed to inhibit Tiam1 activity in vivo, did not induce adherens junction disassembly. Therefore, the net effects of Nm23H1 on Tiam1-induced Rac activity remain rather obscure.

A final, rather awkward binding partner of Tiam1 is the oncogene c-Myc [44]. This interaction was reported to be dependent on the Myc-box II domain of c-Myc and to require the N-terminus (upstream of the PHn domain) of Tiam1. The authors describe nuclear localisation of Tiam1, although the protein lacks a putative nuclear localisation signal itself and clearly does not co-translocate together with c-Myc upon over-expression. This questions the biological relevance of the proposed interaction. Moreover, Tiam1 was reported to inhibit c-Myc-induced transcription and apoptosis in a Rac-independent manner, although C1199-Tiam1 expression facilitated c-Myc-induced apoptosis.

In the case of JIP2/IB2, spinophilin, CD44 and ankyrin, the PHn-CC-Ex domain of Tiam1 is a minimal prerequisite for binding to these proteins. Interestingly, the interacting domains of these proteins do not share any sequence or obvious structural homologies, which raises the question of how the recognition of these multiple interactors is accomplished. The coiled-coil region of Tiam1 is a good candidate to be involved in these multiple interactions, since coiled-coil domains are flexible and often found to mediate protein–protein interactions [45]. Of all described binding partners of Tiam1, only spinophilin has a coiled-coil domain that was indeed reported to mediate the interaction. Moreover, the coiled-coil domain of Tiam1 may render flexibility on the surrounding PH and Ex domains, which might represent the actual binding sites for these proteins. The fact that $CD44_v$ isoforms, ankyrins (ANK 1 and 2) and spinophilin are ubiquitously expressed proteins also raises the problem of steric hindrance: although some of these proteins might bind in synergy, many of these putative interactions are probably mutually exclusive. It is therefore probable that Tiam1 resides in different micro-domains of the cell, dependent on its interaction with these proteins. Alternatively, if the amount of Tiam1 is limited within the cell, regulated (over-)expression of one of its binding partners might determine the outcome of Tiam1–Rac signalling. Such a mechanism might account for the seemingly paradoxical roles Tiam1–Rac signalling plays in tumourigenesis.

4. Concluding remarks

For a large part it is unknown which stimuli, known to result in activation of Rac, utilise the GEF activity of Tiam1 to induce this Rac activation. Since the number of GEFs known to date largely outnumbers the number of GTPases, one may suspect a great redundancy of GEF function. This redundancy is partially abrogated by the tissue- and cell type-specific expression of different GEFs. In addition, there is a growing body of evidence that supports the notion that the specific GEF involved in the activation of a given GTPase co-determines the downstream effects. Also, as several isoforms of Rac have been identified over the last few years, it remains to be determined which specific Rac isoform is involved in a specific signalling pathway that results in Tiam1 activity.

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