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Review

Coupling receptor tyrosine kinases to Rho GTPases—GEFs what's the link

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Abstract

Rho GTPases are molecular switches involved in the regulation of many cellular processes. This review summarizes work examining how stimulation of receptor tyrosine kinases (RTKs) leads to the activation of Rho guanine nucleotide exchange factors (GEFs) and their Rho GTPase substrates. The collective findings strongly suggest that RTK signaling to Rho proteins is a general signal transduction mechanism, like RTK mediated activation of phosphatidyl inositol 3-kinase, phospholipase Cγ, and the mitogen activated protein kinase (MAPK) pathway. More than half of the 58 known human RTKs activate at least one Rho family member. Likewise, 16 Rho GEFs directly interact with and/or are phosphorylated by a RTK. The specificity of receptor tyrosine kinase/Rho GEF signaling seems to be somewhat promiscuous. There several cases where multiple RTKs activate the same Rho GEF and where a single RTK can activate multiple Rho GEFs. Expression analysis indicates that the average human tissue contains transcripts for 33 RTKs, 34 Rho GEFs, and 14 Rho GTPases with each tissue containing a unique complement of these proteins. Given the promiscuity of RTKs for Rho GEFs, Rho GEFs for Rho GTPases, and the large number of these proteins expressed in cells, a complex combinatorial network of proteins in these families may contribute to coding specific signals and cell responses from RTKs.
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Keywords: Rho; Rho GEF; Rho GTPase; Receptor tyrosine kinase; Signal transduction; Pleckstrin homology domain

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1. Introduction

Many of the pathways that transduce signals from cell surface receptors to the nucleus involve at least one G-protein.

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G-proteins act as molecular switches being inactive when bound to GDP and bind a variety of effector proteins when bound to GTP in their active conformation. G-proteins are controlled by guanine nucleotide exchange factors (GEFs) which destabilize the inactive GDP/G-protein complex inducing release of GDP, and binding of cytosolic GTP, activating the G-protein. GTPase activating proteins (GAPs) induce the hydrolysis of GTP bound to G-proteins deactivating the G-protein switch and guanine nucleotide dissociation inhibitors can sequester G-proteins bound to either GTP or GDP.

Many receptors are known to activate G-proteins. The majority of receptors in mammalian proteomes are serpentine G-protein coupled receptors (GPCRs) and the principal signaling mechanism for receptors in this family involves direct activation of heterotrimeric G-proteins and their downstream signaling pathways. Another superfamily of G-proteins (Ras) encompasses the Ras, Rab, Arf, Ran, and Rho families [1]. In general, Rabs and Arfs are involved in protein trafficking, Ras is involved in signal transduction, Ran is involved in nuclear export and Rho proteins are involved in modulation of the actin cytoskeleton. Rho proteins in their active GTP-bound form, bind to a rapidly expanding group of effector proteins that effect many cell processes [2]. Through binding these effectors, Rho family members play important roles as controllers of actin cytoskeleton, and related cell motility and chemotactic responses. In addition, Rho GTPases and the Rho GEFs that activate these Rho proteins play additional roles in cell adhesion, cytokinesis, cell-cycle progression, macropinocytosis, endocytosis, membrane trafficking, and signal transduction [3].

The Rho family GTPases are activated by Rho GEFs. Because Rho proteins affect so many different cellular processes, they are attractive candidates for processing incoming receptor signals similar to the activation of heterotrimeric G proteins by GPCRs. The general role of Rho GTPases in signal transduction was last reviewed in 2000 [4]. At the time of this review, several GPCRs were known to activate a Rho GTPase and p115, PDZ, or Lbc Rho GEFs were activated by G_{α} subunits of heterotrimeric G-proteins. The activation of Rho GTPases by GPCRs, was recently reviewed [5]. These reviews do not cover the now emerging connection between the receptor tyrosine kinase and Rho GTPase families. Over the past 5 years the coupling between receptor tyrosine kinases and activation of Rho GTPases has become evident. Activation of Rho GTPases, is probably a general property of receptor tyrosine kinase signaling and is the subject of this review.

2. Receptor tyrosine kinases that activate Rho proteins

About 35 years ago, through studies applying insulin, epidermal growth factor, and nerve growth factor to cells, the first cell-surface receptors were identified [6–10]. In the early 1980s, the Insulin receptor was shown to possess tyrosine autokinase activity and this family has become known as the receptor tyrosine kinase (RTK) family [11,12]. The human genome encodes 58 RTKs as recognized to date [13] (Fig. 1). Several years later the connection between growth factor stimulation and Ras activation paved the way for unraveling of the mitogen activated protein kinase pathway [14]. In addition

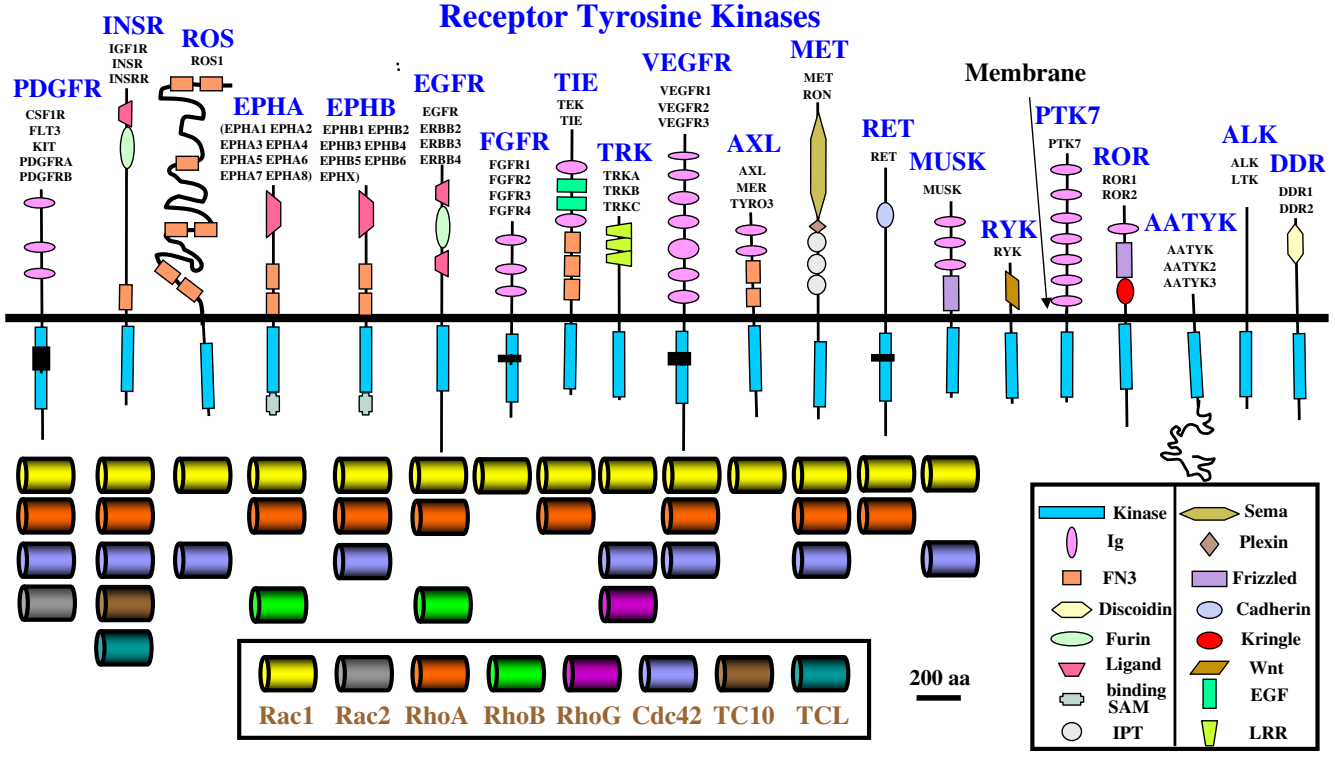

Fig. 1. Rho GTPases activated by different receptor tyrosine kinase subgroups. Schematic diagram shows Rho GTPases that are activated by RTK subgroups. Subgroups are as previously defined [13]. Specific Rho GTPases that are activated by RTKs are provided in Table 1. Key shows the domains of RTKs, and Rho GTPases are color coded. A scale for the RTKs is provided.

to the MAPK pathway, RTKs commonly activate signal transduction pathways leading from phosphatidyl inositol 3-kinase (PI3K) to Akt and involving phospholipase Cγ1 signaling through IP3 and diacylglycerol [15,16].

Recent studies from multiple laboratories indicate that, in addition to Ras activation, stimulation of RTKs can induce Rho GTPase activation. Of the 58 RTKs, more than half are now known to activate at least one of the 20 Rho family members (Fig. 1, Table 1). Fig. 1 and Table 1 show that many RTKs activate more than one Rho protein, potentially linking receptor tyrosine kinases to a multitude of signaling cascades and effects on different cellular processes. RTKs can be divided into 20 subgroups and at least one member of each RTK subgroup can activate a Rho GTPase (Fig. 1) with the exceptions of the PTK7, RYK, ROR, AAATYK, ALK, and DDR subgroups [13]. While some RTKs are not known to activate Rho proteins, in general these receptors have not been well characterized and future studies will likely reveal that all RTKs can activate multiple Rho GTPases. Consistent with coupling of RTK to Rho GTPases, stimulation of several RTKs leads to rearrangement of the actin cytoskeleton, which is likely mediated through their activation of RhoGTPases [17–19].

There are 20 known Rho GTPases and 2 more distantly related mitochondrial Rho GTPases encoded in the human genome [20]. 8 Rho proteins (Rac1, Rac2, RhoA, RhoB, RhoG, TC10, TCL, and Cdc42) are known to be activated by RTKs (Fig. 1, Table 1). Like RTKs, many Rho GTPase family members have not been well studied, and may be unidentified downstream targets of RTKs. Most studies have thus far focused on Rac1, RhoA and Cdc42, likely because these were among the first identified Rho proteins, with established assays for measuring activation of these GTPases both in vitro and in living cells.

Similar to the activation of Ras and the MAPK pathway by RTKs, Rho GTPases are typically activated within minutes of RTK stimulation. For example, Angiopoetin-1 stimulation of TIE2 activates RhoA within 2 min; a similar results is observed for NGF induced activation of Rac1 through TRKA [21–23]. EGF induces maximal activation of Cdc42 within 5 min and IGF-1 induces maximal RhoA induction within 2.5 min [24,25]. There are, however, cases where longer time periods are required for Rho GTPase activation. Stimulation of EPHA4 induced phosphorylation of the Vsm Rho GEF and activation of RhoA shows maximal activation after 20 min [26].

Table 1
Summary of Rho GTPases and Rho GEFs activated by receptor tyrosine kinases

[a]Receptor	[b]G-protein	GEFs	Reference
CSF1R	Rac1, Rac2 Cdc42	Vav1	[94]
KIT	Rac1	?	[95]
[c]PDGFR-A	Rac1	Vav2(Rac1,RhoA,Cdc42)	[37]
[c]PDGFR-B	Rac1,RhoA,Cdc42	αPix(Rac1)	[64]
IGF1R	RhoA	Larg(RhoA), Vav3	[25,42]
INSR	Rac1,Cdc42,TC10,TCL	Vav3	[42,96]
ROS	Rac1,Cdc42	Vav3	[42]
EPHA1	Rac1	Ephexin	[58]
EPHA2	Rac1	Tiam	[97]
EPHA3	RhoA	?	[98]
EPHA4	Rac1,RhoA,RhoB	Vsm(RhoA),Vav2(Rac1)	[26,99]
EPHA7	?	Vav2	[38]
EPHB1	Rac1	?	[38]
EPHB2	Rac1,Cdc42	Kalirin,Vav2,αPix(Rac1), Intersectin(Cdc42)	[38;71]
EPHB3	RhoA	Vav2	[38]
EPHB4	?	Vav2	[38]
EGFR	Rac1,RhoA,RhoB	Vav2(Rac1,RhoB),Vav3,βPix,Sos1(Rac1),P-Rex (Rac1)	[37,55,100]
ERBB2	Rac1,Cdc42	βPix	[101]
MUSK	Rac1,Cdc42	?	[102]
RET	Rac1,RhoA	?	[103,104]
MET	Rac1,RhoA,Cdc42	?	[105,106]
FGFR1	Rac1	βPix	[45]
TEK	Rac1,RhoA	Sos	[21]
TRKA	Rac1,RhoG	Kalirin(Rac1,RhoG), βPix,RasGrf1,P-Rex (Rac1),	[23,45,54,69]
TRKB	Rac1	Ras-Grf1,P-REX	[54,69]
TRKC	Rac1,Cdc42	Dbs(Cdc42),RasGrf1,Tiam(Rac1,Cdc42)	[54,56,70]
VEGFR1	Cdc42,Rac1	?	[107]
VEGFR2	Rac1,RhoA,Cdc42	Vav2(Cdc42)	[39,40]
MER	Rac1	Vav1	[52]

[a] The FLT3, INSRR, EPHA5, EPHA6, EPHA8, EPHX, EPHB6, ERBB3, ERBB4, RON, FGFR2, FGFR3, FGFR4, TIE, VEGFR3, AXL, Tyro3, RYK, PTK7, ROR1, ROR2, AATYK1, AATYK2, AATYK3, ALK, LTK, DDR1, and DDR2 receptor tyrosine kinases are not know to activate any Rho GTPase or Rho GEF.

[b] There are 20 potential human Rho GTPases (Rac1, Rac2, Rac3, RhoA, RhoB, RhoC, RhoD, RhoG, Cdc42, TC10, TCL, TTF, Rnd1, Rnd2, Rnd3, Chp, Wrch, Rif, RhoBTB1, and RhoBTB2) activated by RTKs and Rho GEFs.

[c] PDGF stimulation induced Rac1, RhoA, and Cdc42 activation and PDGF induces activation of SOS1 to activate Rac1, but it is not known whether these effects are mediated through PDGFRA or PDGFRB [41,55].

Since most Rho proteins are activated within minutes of RTK stimulation, these G-proteins may play an important role in RTK signaling. This hypothesis is supported by several observations. Rac1, RhoG, Cdc42, Chp, RhoA, RhoB, RhoC and TC10 can activate the JNK pathway and may play a role in cases where RTKs are known to activate the JNK/SAPK signaling pathway [27–31]. Rac1 is activated by many RTKs and induces Pak mediated phosphorylation of Raf, which is important for activation of the MAPK pathway [32,33]. RhoC also activates the MAPK pathway, but RTKs that activate RhoC are yet to be identified [34]. After stimulation with ligands, RTKs undergo endocytosis and trafficking, which is important for signaling from signaling endosomes [35,36]. RhoB is involved in EGF stimulated trafficking of EGFR, thus may play a role in modulating RTK signaling from signaling endosomes [35].

3. Rho GEFs that link RTKs to Rho GTPase activation

Since so many RTK are known to activate Rho GTPases, there is a strong link between these protein families. Therefore, one would expect Rho GEFs, as activators of GTPases also to be connected with RTKs. At least 16 of the 69 known Rho GEFs (from humans) have been shown to mediate the connection between RTKs and Rho GTPase activation, as summarized in Table 1.

Several Rho GEFs seem to be somewhat promiscuous, mediating signals from several different RTKs. Vav2 activates Rac1 through stimulation of EPHA4, EPHB2, EGFR, VEGFR2 and at least one of the PDGF receptors [37–41]. EPHA7, EPHB3, and EPHB4 also induce phosphorylation of Vav2 at a tyrosine residue important for its activation [38]. Likewise, α-Pix, β-Pix, Vav1, Vav3, Kalirin, Sos, P-Rex1, and Tiam are known to be involved in Rho signaling from more than one RTK.

On the other hand, a few Rho GEFs seem to be more specific for RTKs. For example, stimulation of the IGF-1 receptor induces Larg-mediated activation of RhoA. Comparison of expression profiles for this pairing with the Unigene gene expression profiler shows 28 of 31 tissues to co-express IGF-1 receptor and Larg transcripts (co-expression plots of Rho GEF/RTK pairings are available in supplemental data). However, IGF-1 also signals through at least one other Rho GEF, Vav3, and we can not rule out the possibility that further characterization will reveal that Larg is also involved in signaling from multiple RTKs.

Many RTKs activate multiple Rho GTPases (Fig. 1), suggesting that RTKs activate GEFs that are promiscuous for Rho substrates or that each RTK may interact with multiple Rho GEFs. The ROS and INSR receptors activate both Rac1 and Cdc42 through Vav3 and no other known Rho GTPases [42]. However, more commonly, multiple Rho GEFs are known to mediate a signal from a specific RTK. This is clearly exemplified by the EPHB2 receptor that signals through Intersectin, Kalirin, Vav2, and β-Pix, as is the case for several other RTKs shown in Table 1.

4. RTKs signal through Rho GEFs by multiple mechanisms

Unlike the connection of RTKs to Ras activation, there seems to be no unifying mechanism by which RTKs can activate Rho GTPases. In one type of mechanism, RTKs can activate Rho GTPases indirectly as downstream signaling events. Stimulation of PDGFRB and EPHB2 induces receptor autophosphorylation and docking of the SH2 domain of the p85 subunit of PI3K. PI3K recruits α-Pix which mediates the activation of Cdc42 and Rac1 [43]. Binding of a Nck/Pak complex to the RTK may also be involved in recruiting αPix through a Pak–αPix interaction. Also, stimulation of FGFR1 or TRKA receptors activates the MAPK pathway and Pak, inducing the phosphorylation of β-Pix and activation of Rac1 [44,45].

Although some Rho GEFs are downstream targets of RTKs, it is more common that RTKs directly activate Rho GEFs by several different mechanisms. Like the single-pass transmembrane RTKs, Rho GTPases are also localized to membranes. Rho GTPases have CAAX (Cys–aliphatic–aliphatic–any residue) boxes on their C-termini that mediate lipidation and targeting of Rho GTPases to membrane bilayers. The restriction of RTKs and Rho GTPases to membranes may not be coincidental. Similar to the way in which RTKs activate Ras by recruiting a cytosolic Grb2/Sos complex, Rho GEFs may be recruited to the membrane to mediate RTK activation of Rho proteins. Accordingly, there are now numerous examples of Rho GEFs that are either directly interact with, or are activated directly by stimulation of a RTK (Table 1). The binding of Rho GEFs to RTKs may position the Rho GEF in proximity to its membrane associated substrates.

There are several distinct mechanisms by which Rho GEFs associate with RTKs (Fig. 2). EPHA2 binds directly to Tiam's N-terminal region and Ephrin mediated activation of Rac1 depends on Tiam [46]. Also, the PDZ domain of the Rho GEF Larg interacts with the C-terminal domain of the IGF1R and mediates IGF signaling to RhoA [25]. While these are unique examples of domains in Rho GEF that associate with RTKs, many Rho GEFs seem to be linked to RTKs through phosphorylation mediated recruiting and/or activation of Rho GEFs, or through interactions with the PH domain of the Rho GEF.

5. RTKs phosphorylate Rho GEFs on tyrosine residues, inducing their GEF activity

The mechanisms by which Rho GEFs are regulated have been previously reviewed [47,48]. Perhaps the best understood mechanism of Rho GEF activation is that of Vavs where tyrosine phosphorylation of the Tyr^{172} residue N-terminal to the Dbl-homology domain relieves autoinhibition by allowing access to Rho substrates [49–51].

Several pieces of evidence suggest that RTK mediated tyrosine phosphorylation of Rho GEFs is a widely used mechanism for activation of many Rho GEFs. As detailed below, several different RTKs can phosphorylate Vavs on Tyr^{172}, effecting their GEF activities. Stimulation of the MER RTK induces tyrosine phosphorylation of Vav1, dissociation from

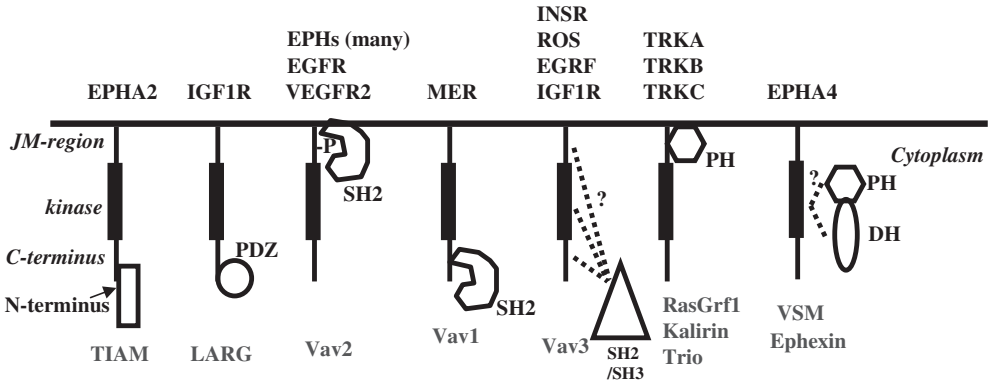

Fig. 2. Domains of Rho GEF that interact with Receptor tyrosine kinases. Several modes by which RTKs interact with Rho GEFs are shown. It is not known which fragments of the EPHA4, INSR, ROS, EGFR, IGF1R interact with Rho GEF. RTKs are labeled black and Rho GEFs are labeled gray. Domains of Rho GEFs that interact with RTK are shown.

MER and activation of RhoA [52]. Stimulation of EPHA7, EPHB2, EPHB3, or EPHB4 induces tyrosine phosphorylation of Vav2, on Tyr^{172} [38]. Upon PDGF or EGF binding to their receptors, Vav2 becomes phosphorylated on tyrosine and activates Rac1, Cdc42, and RhoA [41]. Stimulation of a VEGFR2/$P2Y_2R$ heteroreceptor complex with UTP induces phosphorylation of Vav2 and activation of RhoA and Rac1 [40]. Activation of ROS, EGFR, IGF1R, and INSR induces tyrosine phosphorylation of Vav3, association with these receptors, and subsequent activation or Rac1 and Cdc42 [42].

Vavs contain SH2 domains which are small domains that bind to phospho-tyrosine, often found in adaptor proteins and other signaling proteins. In addition to Vavs being tyrosine phosphorylated by RTKs, RTK autophosphorylation sites may bind SH2 domains of Vavs recruiting them to activated receptors. Consistent with this hypothesis, the SH2 domain of Vav2 mediates its interaction with autophosphorylation sites in the C-terminal domain of EGFR [53]. Likewise, the C-terminal portion of the MER kinase domain interacts with the SH2 domain of Vav1, although the interaction is phospho-tyrosine independent. Vav2 interacts with phosphorylated tyrosine residues in the juxtamembrane region of EPHA4, which is likely through the SH2 domain of Vav2 [38]. Vavs are the only Rho GEFs that have SH2 domains, thus recruiting of Rho GEFs through their SH2 domain is not a general property of Rho GEFs.

Several other Rho GEFs are tyrosine phosphorylated by RTKs and may be activated by a mechanism similar to that of Vavs. These Rho GEFs include RasGrf1, Sos1, Dbs, and Vsm. RasGrf1 binds to the juxtamembrane region of TRKA, TRKB, and TRKC and is tyrosine phosphorylated by stimulation of these RTKs [54]. Stimulation of EGFR induces tyrosine phosphorylation of Sos1 [55]. Like Vavs and RasGrf1, tyrosine phosphorylation of Sos1 induces GEF activity toward Rac1 [37,54,55]. PDGF also stimulates activation of the Rho GEF activity of Sos1, but it is unknown whether this is due to PDGFRA or PDGFRB [55]. TRKC activation of Cdc42 is mediated trhough Dbs. Dbs associates directly with TRKC and TRKC phosphorylates Dbs on tyrosine residues [56]. EPHA4 induces tyrosine phosphorylation of Vsm and mediates RhoA activation [26].

6. Pleckstrin homology domains that mediate RTK–Rho GEF interactions

Pleckstrin homology domains are best known for their interaction with phosphoinositide lipids and are present in many proteins including nearly all Rho GEFs. In addition to tyrosine phosphorylation, several studies suggest that the PH domains of Rho GEFs may play a major role in coupling several RTKs to Rho GEFs. The PH domain of Vav2 is required for EGF stimulated activation of Vav2 and Rac1. This is thought to be mediated through PI3K production of PIP3 and PIP3 binding to the PH domain of Vav2 and activating its GEF activity [53]. RasGrf1 binds to the juxtamembrane region of TRKA, TRKB, and TRKC through its PH domain. RasGrf1 is tyrosine phosphorylated by TRKA, TRKB and TRKC on the PH domain [54]. Like RasGrf1, the Rho GEF Kalirin binds TrkA through its N-terminal PH domain and mapping experiments show that it binds to the juxtamembrane region of TRKA as well [23] (Martin R. Schiller, unpublished observation). It is interesting that in the case of TRKA, the PH domains are binding to the juxtamembrane regions of the receptors which clearly play an important role in activation of the RTK [57]. Thus, while RTKs clearly activate Rho GEFs, Rho GEFs may also play a role in activation of the RTKs. Consistent with this hypothesis the TRKA receptor in PC12 cells transfected with an antisense Kalirin construct, is inefficiently autophosphorylated in response to NGF stimulation [23]. The PH domains of Vsm and Ephexin Rho GEF may also be involved in EPHA4 activation of RhoGTPase, but this may also be in part due to the Dbl homology domains as well [26,58]. It will be interesting to see if PH domains play a broader role in RTK/Rho GEF pairings and activation of RTKs (Fig. 2).

The interaction of Rho GEFs with RTKs through PH domains is also interesting for several addition reasons. Nearly all Rho GEFs contain PH domains. Since PH domains have limited homology with each other, these domains have the potential to encode specificity for RTK interactions. This said, there are examples where Rho GEFs can interact with multiple RTKs. Since the PH domains of Ras-GRF1 and Kalirin interact with the juxtamembrane region of TRKA, the PH domains may

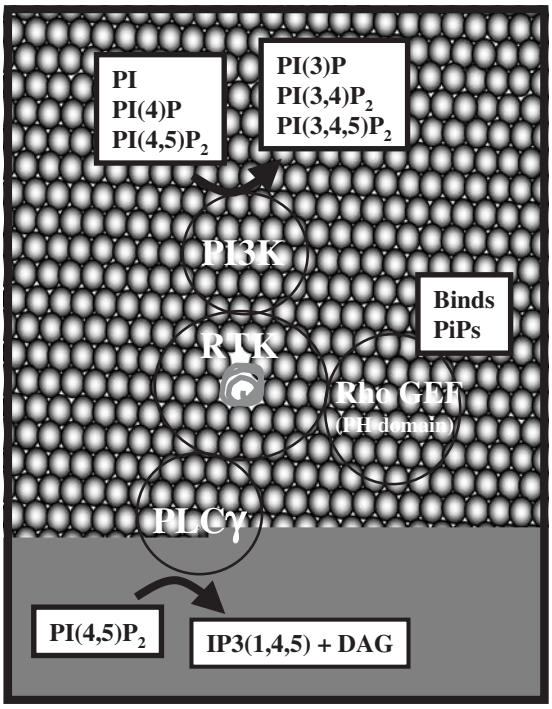

Fig. 3. Role of phosphoinositides in signaling from Receptor tyrosine kinases. View of RTK and associated signaling proteins looking down on the cytoplasmic membrane. Stimulation of RTKs induces activation of phosphatidyl inositol 3-phosphate kinase (PI3K), catalyzing the synthesis of several phosphoinositides. Phospholipase Cγ (PLCγ) is also activated, generating IP3 diacylglycerol (DAG). Several Rho GEFs which bind to RTKs are known to be regulated by phosphoinositides.

also interact simultaneously with phosphoinositide lipids in the membrane and the RTK. This is an interesting possibility considering the role of phosphoinositides in signaling from RTKs through PI3K and PLCγ and in activation of Rho GEFs (Fig. 3) [43,59–65]. It is noteworthy that the PH domains of SH2B, APS and PLCγ also bind to RTKs [66–68]. Furthermore, antisense to PLCγ1 and overexpression of the PLCγ-PH domain inhibits insulin receptor mediated phosphorylation, similar to the effect of the Kalirin PH domain on TRKA phosphorylation [23,68]. These experiments support a possible role for PH domain in the RTK activation mechanism.

7. Other Rho GEF domains that bind RTKs

The previous sections summarize the importance of binding of RTKs to SH2 and PH domains in several Rho GEFs. Several other domains in Rho GEFs are involved in binding RTKs. EPHA2 binds directly to Tiam through its N-terminal region and Ephrin induced activation of Rac1 depends on Tiam (Fig. 2) [46]. Also, the PDZ domain of the Rho GEF Larg interacts with the C-terminal domain of the IGF1R and mediates IGF signaling to RhoA [25]. P-Rex1 Rho GEF is involved in TRKA mediated activation of Rac. Several domains including its DH domain are important for the effects of P-Rex1 on TRKA [69]. We must, however, consider that many of these interactions have been determined by co-immunoprecipitation and may not represent direct interactions. The interaction of RTKs with different domains of RhoGEFs may provide some specificity of RTK/RhoGEF interactions.

8. Domains of RTKs that bind Rho GEFs

In addition to knowing which domains of RhoGEFs bind to RTKs, to understand the relationship between Rho GEFs and RTKs it is also important to know which domains of the RTK interact with the Rho GEF (Fig. 2). RTKs have juxtamembrane regions, tyrosine kinase domains, and a C-terminal tail. Like the different domains of Rho GEFs that bind RTKs, all three regions of RTKs are known to interact with RhoGEFs. Both RasGrf1 and Kalirin interact with the juxtamembrane regions of TRKA [54] (Schiller, unpublished observation). The C-terminal extension of MER and IGF1R RTKs mediate their interaction with Vav1 and Larg, respectively [25,52]. The kinase domain of TRKC interacts with Dbs [56]. Although indirect, the kinase domain of the PDGFRB interacts with β-PIX through Nck and the p85 subunit of PI3K [43]. Thus, it is possible that some of these interactions affect RTK kinase activity, while other, such as those in the C-terminal domain are more involved in Rho GEF recruitment.

In addition to these mapped interactions, several Rho GEFs interact with RTKs, but the domains mediating these interactions are not yet characterized. In Schwann cells, NT3 stimulation of TRKC results in activation of Tiam1 and Rac1 [70]. Stimulation of TIE2 (TEK) induces activation of Rac1 and RhoA through Sos [21]. Ligand binding to EPHB2 induces activation of Rac which is dependent on the GEF activity of Kalirin [71].

9. Cells may contain many RTK, Rho GEF and RhoGTPase family members

Since the RTK, Rho GEF, and RhoGTPase families have many family members that are not well characterized, we wanted to better understand the potential relationship between these families. To do so, expression profiles for human transcripts in 31 different tissues were compared using the Gene expression profile viewer in the Unigene database (see supplemental data) [72]. Expression of Rho proteins in the 31 tissues varied, ranging from 8 Rho transcripts present in the small intestine to all 20 in lung, with an average of 14 Rho members/tissue. Rac1, RhoA, and Cdc42 were transcripts were present in nearly all tissues, however, some Rho GEFs such as RhoD, Rnd2, TCL, and Rac3 were expressed in a small subset of tissues and may have very specialized functions. A text file containing raw data for these expression profiles is in supplemental data. Assuming that there are 150,000 transcripts/cell, in general, more than 10 Rho GTPases were present in tissues at greater than 5 transcripts/cell, thus would not be considered rare transcripts in these tissues. Even considering the limitation that this is an analysis of tissues and not individual cells, the large number of transcripts for different GTPases suggest that most cell types express many Rho family members.

Likewise, analysis of 53 of the 58 RTKs that have EST expression data for these 31 tissue samples showed that tissues express an average of 33 RTKs with tissues spanning from 7

exists for the Rho GEF/Rho GTPase system to encode specific RTK responses. Using the Unigene gene expression profiles (see supplemental data) the average human tissue has transcripts for 33 RTKs, 34 Rho GEFs, and 14 Rho GTPases, thus there are over 15,000 possible signaling combinations. This does not even consider the diversity of tyrosine phosphatases, GAPs and downstream effectors, alternatively spliced transcripts, relative affinities of the various proteins, or differing responses to different RTK ligands. Furthermore, Rho GEFs also have many different domains that could recruit other signaling proteins. Although all tissues express a large number of transcripts from these families, each tissue has a specific expression profile for members of these families. This analysis only assesses transcript levels; translation control, protein half-lives, and RNA half-lives will also likely play a role in these cell-specific expression profiles.

Given the average numbers of Rho GEFs and Rho GTPases in cells, plus the ability of many Rho GEFs to activate multiple Rho GTPases, and examples of interaction of individual RTKs with multiple Rho GEFs, extreme care must be taken in interpretation of experiments where the levels of a single protein are altered. Thus, RNAi, constitutively active and dominant negative mutants, and overexpression studies of single proteins have the potential to perturb a balanced combinatorial network of multiple Rho GEFs and Rho GTPases. Therefore, just because altered expression or mutants proteins produce an effect, these effects can not be readily directly attributed to individual proteins. However, such studies are useful in determining whether a particular protein may be associated with some process.

The diversity of Rho GEF/RTK interactions, apparent cell specific expression profiles, and diversity of GTPases activated by different RTKs in different cells lead us to propose that Rho GEFs and Rho GTPase may play an important role in encoding specific responses of RTKs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cellsig.2006.01.022.

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