Title: Dual TORCs driven and B56 orchestrated Signaling Network guides eukaryotic cell migration

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Running Title: Signaling networks of TorC2, TorC1, and PP2A/B56 organize eukaryotic cell migration.

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ABSTRACT

Different types of eukaryotic cells may adopt seemingly distinct modes of directional cell migration. However, several core aspects are regarded common whether the movement is either ameoboidal or mesenchymal. The region of cells facing the attractive signal is often termed leading edge where lamellipodial structures dominates and the other end of the cell called rear end is often mediating cytoskeletal F-actin contraction involving Myosin-II. Dynamic remodeling of cell-to-matrix adhesion involving integrin is also evident in many types of migrating cells. All these three aspects of cell migration are significantly affected by signaling networks of TorC2, TorC1, and PP2A/B56. Here we review the current views of the mechanistic understanding of these regulatory signaling networks and how these networks affect eukaryotic cell migration.

1. Regulation of PP2A System

PP2A is one of the major phosphatases that regulate large number of critical cellular events in virtually

all eukaryotic organisms. Unlike kinases, phosphatases are generally regarded to be pleiotropic, which hampers the progress of unveiling their specific cellular functions. The PP2A holoenzyme system consists of three classes of core subunits: the catalytic C, the scaffolding A, and the regulatory B. The B subunit plays multiple regulatory roles such as defining enzymatic specificity, subcellular location, and/or effector pathways and thus pivotal in achieving specific tasks of PP2A system. The B subunit is classified into four subclasses: B, B' (B56), B", or B"'.

Among these multiple B subunits, B56 is one of the critical regulatory subunits of the PP2A: B56 is modulating diverse cellular events such as cell cycle, differentiation, cancer, cell polarization, migration, and stress signaling in diverse organisms such as yeast, *Dictyostelium*, plant, and animals (Eichhorn et al., 2009, Gutiérrez-Caballero et al., 2012, Kurimchak and Graña, 2015, Lillo et al., 2014, Mumby, 2007, Rahikainen et al., 2016, Seshacharyulu et al., 2013, Stamos and Weis, 2013, Jansen and Goris, 2001).

Dictyostelium PP2A system includes a catalytic C subunit, a scaffolding A subunit, two B subunits (PR55), and a B56 (PsrA) subunit. Attempts to generate *Dictyostelium* knockout cells for the C and A subunits were unsuccessful, suggesting that they may be essential for cell growth (Murphy et al., 1999). We have previously isolated *Dictyostelium B56* gene and generated B56 knockout (*psrA⁻*) cells and uncovered that B56 is modulating development through GSK3 and chemotaxis through F-Actin remodeling (Lee et al. 2008, Pino et al., 2015).

In addition to the regulatory B subunits, PTPA and PTPB are distinct type of regulatory proteins that can alter the substrate specificity of PP2A from phosphor-serine/threonine to phosphor-tyrosine residues (Fig. 1, i). The other type of PP2A associating protein is TAP42 or human α4 protein (Fig. 1, ii). Unlike other types of PP2A regulatory proteins, TAP42 was shown to facilitate the dissociation of B subunits through the association with the C subunit and interferes with the dephosphorylation reaction (Jiang et al., 2013). Furthermore, TAP42 mediated inhibition of PP2A seems to be pleiotropic. TAP42 association with PP2A holoenzyme was enhanced when the target of rapamycin complex 1 (TorC1) was activated. The core of TorC1 signaling is discussed in the latter part of this review. Interestingly, cells harboring mutation in TAP42 displayed near complete resistance to Rapamycin, a TorC1 inhibitor (Di Como and Arndt, 1996).

In addition to the above-mentioned PP2A regulatory proteins that affect PP2A through assembly control, several studies uncovered that PP2A function can also be post-translationally modified. The well-characterized methylation of Leu^{309} of the C subunit was suggested to stimulate the formation of B subunit containing holoenzyme (Ogris et al., 1997, Bryant et al., 1999) and the phosphorylation of Tyr^{307} of the C

subunit was shown to be inhibitory (Hu et al., 2009) (Fig. 1, iii). Given that PP2A function heavily depends on the type of the associated B subunit, it is not surprising to find that B, B56, and B" subunits are targets of phosphorylation. Protein Kinases A enhanced the PP2A catalytic activity by phosphorylating B56 subunits (Fig. 1, iv) (Hong et al., 2008, Ahn, et al., 2007, Usui et al., 1998). In addition, PKC is reported to inhibit PP2A function through phosphorylating B56α (Fig. 1, v) (Kirchhefer et al., 2014). Another example is ERK2, which is a target of PP2A/B56 holoenzyme, but it can also antagonize PP2A function by dissociating B56 from the holoenzyme by phosphorylation as discussed later (Fig. 1, vi).

Several recent structural and bioinformatics analysis discovered that B56 binds to its target through either the central region or the C-terminal edge of the pseudo heat domain. The central region recognizes B56 targets that contain LxxIxE motif (Hertz et al., 2016, Wang et al., 2016), whereas the C-terminal end region binds to a distinct type of targets such as Shugoshin (Xu et al., 2009) as shown in figure 1.

2. Eukaryotic cell migration modulated by PP2A/B56 and TorC2 signaling network.

2. 1. TorC2 signaling network

Directional cell migration is one of the core mechanisms that are essential for a large number of eukaryotic organisms to survive. A number of laboratories have been investigated the mechanisms of external signal sensing and the internal networks that translate the sensing event to polarized F-Actin remodeling during the last several decades and uncovered that the molecular mechanisms of these events were highly conserved from *Dictyostelium discoideum* and mammalian leukocytes. In both *Dictyostelium* and leukocytes, extracellular chemoattractants activate G-protein coupled receptors (GPCR), resulting in the activation of heterotrimeric G-proteins and the small GTPases Ras and Rap, which in turn lead to the activation of PKBs through PI3K, PDK, and TorC2 (Target of Rapamycin Complex 2) (Funamoto et al., 2002, Sasaki et al., 2004, Cai et al., 2010, Kamimura et al., 2010 Khanna et al., 2016).

TorC2 signaling network is widely conserved in diverse type of eukaryotic organisms except plants. Recent studies discovered that TorC2 signaling network significantly affect F-Actin remodeling and directional cell migration. In contrast to TorC1, TorC2 is insensitive to Rapamycin due to the absence of Raptor. As discussed later, more upstream regulatory mechanism for TorC1 activation has been discovered compared to that of the TorC2, but recent studies using *Dictyostelium* uncovered that chemoattractant induced activation of TorC2 is mediated through small GTPases Ras and Rap, which is reminiscent of the finding that TorC1 is regulated through the small GTPase Rheb and Rag. It is, however, yet to be determined if mammalian TorC2 is also

controlled by Ras and Rap similarly to that of *Dictyostelium*.

Extensive investigation of the signaling network modulating Akt and TorC2 in *Dictyostelium* cells uncovered significant details of the molecular mechanisms of Akt and TorC2 mediated Actin cytoskeletal remodeling and directional cell migration (Artemenko et al., 2014, Devreotes and Horwitz, 2015). Several other recent studies also reported that TorC2 heavily affects Actin Network assembly and neutrophil chemotaxis (Liu et al., 2010a, Diz-Muñoz et al., 2016) or mast cell chemotaxis (Kuehn et al., 2011). Liu and others (2010a) also showed that TorC2 suppressed Myosin II activity in neutrophil.

In response to chemoattractant stimulation, *Dictyostelium* cells locally activate several Ras proteins at the leading front that include RasG, RasC and Rap1. RasC, in parallel with RasG, mediates activation of TorC2 and subsequent activation of Akt kinases PKBA and PKBR1 at the leading edge (Fig. 2A, iv) (Insall et al., 1996, Kae et al., 2007, Kamimura et al., 2008, and Lee et al., 2005). A recent study uncovered that Rap1 Gef protein GflB is activated by GTP-Gα2 (Liu et al., 2016).

One of the Tor associating subunits Rictor, which distinguishes TorC2 from TorC1, has been shown to affect Actin cytoskeleton and thus essential for efficient chemotaxis of *Dictyostelium*, neutrophil, and cancer cell metastasis (Chen et al., 1997, He et al., 2013, Agarwal et al., 2013, Zhang et al., 2010). In addition, several studies demonstrated that Rictor can be either positively or negatively modulated by distinct kinases. One study using mammalian system showed that inhibitor of nuclear factor κ-B kinase (IKK) phosphorylates and activates Rictor and thus functions positively to TorC2 activation (Fig. 2A, i) (Xu et al., 2013). Other studies showed that GSK3 (Fig. 2A, ii) or S6K1 (Fig. 2A, iii) phosphorylates and inhibits Rictor and interfere TorC2 function (Julien et al., 2010, Chen et al., 2011). As described below, S6K1 may also inhibit TorC2 through Sin1 in addition to Rictor.

Another TorC2 subunit Sin1 mediates chemoattractant induced GTP-Rap1 association with TorC2 (Fig. 2A, iv) (Khanna et al., 2016). Several other mammalian studies also uncovered that TorC2 component Sin1 is also a target of Akt, S6K, and Tor kinase. However, upon Sin1 phosphorylation by these kinases, various outcomes including contrasting effects on TorC2 activity were unveiled, indicating the complexity of Sin1 mediated regulation of TorC2. Akt mediated phosphorylation of Sin1 at Thr86 stimulated TorC2 activity, which in turn induced the full activation of Akt at both AL and HM sites (Humphrey et al., 2013). However, Lin and others (2013) showed that in response to insulin stimulation, Sin1 became dually phosphorylated at T86 and T398 by S6K1 and dissociated from the TorC2, which led to inactivate the TorC2 (Fig. 2A, iii). Lst8 is a TorC2

subunit shared by *Dictyostelium* and mammalian system, of which ablation severely affected F-Actin remodeling and chemotaxis (Lee et al., 2005). In contrast to mammalian TorC2, no Deptor homolog was identified in *Dictyostelium* yet.

Together with TorC2, another serine/threonine kinases PDK1/2 also contribute to the full activation of PKBA and PKBR1 in *Dictyostelium* similarly to mammalian system. Both PKBA and PKBR1 need to be phosphorylated at the activation loop (AL) and the hydrophobic motif (HM) site by PDKs and TorC2, respectively during the chemotaxis (Fig. 2A, v) (Meili *et al.,* 1999, Kamimura *et al.,* 2008, Kamimura and Devreotes, 2010, Liao *et al.,* 2010, Cai *et al.,* 2010). Consistently, mammalian PDK1 was also shown to be essential for the neutrophil chemotaxis (Yagi et al., 2009).

2.2 Interaction of B56 with TorC2 signaling network

Downstream targets of activated TorC2 include several kinases such as Akt (Fig. 2A, v) and PKA (Fig. 2A, vi). Akt mediates a large number of modulations of signal transduction events that include, but not limited to, lamellipodia and adhesion complex remodeling as discussed later. Through modulating Akt activity, PP2A/B56 may optimally regulate TorC2 driven spatiotemporal remodeling of leading edge formation and adhesion complex formation. TorC2/PKA mediated activation of PP2A/B56 may constitute a positive feedback by activating Ras proteins that function upstream of TorC2 (Fig. 2A, vi).

A number of kinases are known to be involved in the modulation of TorC2, but the elucidation of the mechanism of the dephosphorylation of TorC2 components, its upstream and downstream signaling components is still in progress. Previous studies demonstrated that a B56 subunit inhibits Akt in *C. elegans* and in differentiated 3T3-L1 adipocyte cells (Padmanabhan *et al.,* 2009). Another line of studies also showed that PP2A/B56 could dephosphorylate phospho-Erk2 and phospho-Akt through scaffolding protein IEX-1. IEX-1 functions as a scaffolding platform for Erk2 and PP2A/B56, and thus facilitates Erk2-mediated phosphorylation of B56 that stimulates B56 dissociation from PP2A core dimer, which then no longer inhibits Erk2 and Akt (Letourneux et al., 2006, Rocher et al., 2007). Furthermore, studies using mammalian cells demonstrated that insulin mediated activation of PDK and TorC2 not only activated Akt, but also stimulated the formation of PP2A/B56 holoenzyme complex, which in turn dephosphorylated phospho-Akt   (Rodgers et al., 2011). These studies indicate that B56 mediated Akt inhibition is widely conserved among eukaryotes.

Regulation of TorC2 signaling network through PP2A system is not limited to TorC2 itself or its downstream signaling network. In *Dictyostelium*, the PP2A core dimer was shown to be essential for activating

Ras and its downstream Akt kinases (Charest et al., 2012) and the regulatory B56 subunit was essential for the proper activation of RasD and RasC, but necessary for properly maintaining low basal activity of TorC2/PKB activities (Pino et al., 2015). Furthermore, B56 preferentially associated with GDP-RasC, GDP-RasD, but not with their GTP forms. When these GTPases are active, the level of Ras-B56 association significantly decreased (Pino et al., 2015).

2.3 TorC2 and Actin cytoskeletal remodeling

Dictyostelium cells provided significant insight on the mechanism of eukaryotic chemotaxis. One of the major regulatory modules is the Ras-TorC2-Akt signaling axis as discussed earlier (Swaney et al., 2010). TorC2 mediated activation of Akt was shown to be essential for regulating the dynamic remodeling of F-Actin at the leading edge of chemotaxing *Dictyostelium* cells. Here, Akt phosphorylates a group of target proteins, which mediate spatio-temporal remodeling of the leading edge lamellipodia (Cai et al., 2010): multiple small GTPases regulators (RasGEF and RacGAPs), Talin, PI5K, and p21 activated kinase (Pak) (Fig. 2B, i).

The nascent adhesions are connected to the actin cytoskeleton in the anterior region of migrating cells through multiprotein complexes that include F-Actin binding proteins such as Talin and Vinculin and signaling proteins such as FAK and Paxillin (Figure 2B). FAK and Paxillin containing adhesion complexes are positively regulated by both TorC2 and TorC1 as described later (Liu et al., 2008, Jacinto et al., 2004). In addition, TorC2, PKB and PIP5K were shown to be necessary for Talin association with integrin receptor at the adhesion complex (Fig. 2B, ii) (Le et al., 2015). Talin/Vinculin/integrin complex was shown to be necessary for TorC2 activation indicating the presence of a positive feedback regulation between TorC2 and adhesion complex (Fig. 2B, iii) (Sen et al., 2014).

TorC2 was also shown to be essential for rear end retraction. Chemoattractant mediated activation of TorC2 stimulates intracellular cAMP production, which in turn activates PKA and thus inhibits the small GTPase RhoA and stimulates Myosin-II mediated rear-end contraction (Fig. 2B, iv) (Liu et al., 2010a). Interestingly, Ravi and others (2015) showed that PP2A activates RhoGAP and thus suppress RhoA activity (not clear if B56 is involved). In contrast, Ahn and others (2007) reported that PKA activates PP2A by phosphorylating B56δ at Ser566. Thus TorC2 may inhibit Rho associated kinase (ROCK) and activates PP2A/B56 to drive Myosin II dephosphorylation to suppress the microfilament assembly and contraction at inappropriate locations.

Several cytoskeletal elements enriched at the adhesion complexes were also shown to be affected by

TorC2 (Figure 2). Multiple studies showed that TorC2 regulates the dynamics of and localization of adhesion complexes during the migration (Lamonille et al., 2011, Jacinto et al., 2004, Sato et al., 2016, Sen et al., 2014). Jacinto and others (2004) showed that TorC2 can activate Paxillin (Fig. 2B, v) and Sen and others (2014) demonstrated that TorC2 and Akt localize to the nascent adhesion, where vinculin is essential for TorC2 activation (Fig. 2B, iii). Myosin-II activity was essential for recruitment of Rictor and Akt to the focal adhesion complex (Sen 2014). Interestingly, Akt activates PIP5K, which in turn regulates Talin activity (Le et al., 2015) in mammalian system, which is reminiscent of the finding that Talin couples the actomyosin cortex to the plasma membrane during rear end retraction in chemotaxing *Dictyostelium* cells (Tsujioka et al., 2012).

3. Eukaryotic cell migration modulated by PP2A/B56 and TorC1 pathway.

3.1. TorC1 signaling network and PP2A/B56

Akt activation by chemoattractant, as discussed earlier, is central in phosphorylating a group of proteins essential for remodeling Actin cytoskeleton at the leading edge of *Dictyostelium* cells. Another critical target of Akt is RhebGAP protein TSC1/2. TSC1/2 is major regulator of another Tor kinase containing multi-protein complex TorC1. TorC1 is distinct from TorC2 in that its subunit composition is different and its sensitivity to Rapamycin. The role of TorC1 in the regulation of cellular energy metabolism is central and thus drew huge attention. Recent studies, however, uncovered that TorC1 also plays a significant role in eukaryotic cell migration. The small GTPases Rheb was shown to be positive upstream regulators of TorC1 (Fig. 2C, i), of which regulation is mediated through several distinct kinases and GTPase Activating Proteins (GAPs). Kinases such as Akt, ERK2, RSK, CDK1, IKKβ inhibit TSC1/2 (Fig. 2C, ii) and thus activate Rheb proteins and other groups of kinases that include AMPK and GSK3 inhibit TorC1 by activating TSC1/2 (Fig. 2C, iii). TorC1 is also activated by the small GTPase Rag, of which activation depends on the activation of the kinase MAP4K3 (Fig. 2C, iv) (Yan, et al., 2010).

Interestingly, many of the TorC1 regulators are the targets of B56 (Figure 2C). The fully active Akt are phosphorylated at T308 and S473. B56β and B56δ were shown to decrease the phosphorylation levels of these two critical residues. B56β and B56γ were shown to decrease the phosphorylation levels of ERK1/2 at T^{202}. B56γ decreased phosphorylation of T^{172} of AMPK and T^{398} phosphorylation of S6K1. Furthermore, B56ε decreased phosphorylation of S^{170} of MAP4K3 (Yan et al., 2010).

3.2 TorC1 signaling network and Actin cytoskeletal remodeling

The TorC1 and TorC2 signaling networks interact with each other through several feedback loops. One of the major TorC1 activator Akt requires TorC2 mediated phosphorylation on the so-called hydrophobic motif (S^{473}) to become fully active, but TorC2 activity declines as TorC1 increases Rictor phosphorylation (T^{1135}) through S6K1 and thus forms a negative feedback loop (Fig. 2D, i).

It was mentioned earlier that the FAK and Paxillin containing adhesion complexes are a target of TorC2 during the cell migration (Fig. 2B). In addition, TorC1 also modulates the FAk and Paxillin containing adhesion complexes. Liu and other (2008) reported that TorC1 target S6K1 affects tyrosine phosphorylation of FAK and Paxillin and thus regulates focal adhesion formation in chemotaxing cells (Fig. 2D, ii). Activation of FAK by S6K1 will lead to rear end retraction through activation of RhoA (Fig. 2D, iii) (Tomar, et al., 2009). TorC1 may also activate Rho signaling axis through Tap42 mediated inhibition of PP2A/B56 (Fig. 2D, iv), which consequently will activate Rho small GTPAses. Liu and others (2010b) showed that S6K1 is required to properly express and activate small GTPases RhoA, Rac1, and Cdc42 and thus renders Lamellipodia formation and cell migration in a Rapamycin sensitive manner (Fig. 2D, v).

Besides modulating adhesion complexes, S6K1 signaling network also affect Actin cytoskeletal remodeling at the leading front. Berven and others (2004) demonstrated that activation of TorC1 and S6K were required to form a caveolin-enriched F-Actin structure at the leading edge of fibroblast (Fig. 2D, vi). These studies are thus consistent with the earlier studies that uncovered that TorC1/S6K1 signaling significantly affects cell migration of multiple types of mammalian cells (Poon et al., 1996, Sakakibara, et al., 2005, Attuob et al., 2000, Berven et al., 2004, Wong et al., 2004, Wan et al., 2005, Liu et al., 2006, Liu et al., 2008, Liu et al., 2010b, Zhou and Wong, 2006).

3.3 TorC1 and Actin cytoskeletal remodeling in Plants

Interestingly, although the presence of TorC2 in plant cells is not clear, plants do have TorC1 components, PDK, and PP2A/B56 (Maegawa et al., 2015, Otterhag et al., 2006, Dobrenel et al., 2011, Ahn et al., 2011, Sommer et al., 2015). PP2A has been shown to be essential for light induced and F-Actin dependent chloroplasts movement in plant (Wen et al., 2012). The B56γ and B56ζ subunits of plant PP2A system were shown to be necessary for optimal growth of Arabidopsis under normal condition (Konert et al., 2015). Several members of the plant B56 family were shown to bind to WtsE, a LxxIxE motif containing bacterial Type-III

effector, which facilitates bacterial infection to plant cells (Jin et al., 2016). In addition, the plant PP2A-B56γ reduces ROS production by inhibiting ROS generating Oxidases and is also involved in the regulation of salicylic acid-dependent pathogenesis responses (Durian et al., 2016). Furthermore, it is also well known that plant cell morphogenesis is dependent on the small GTPases ROP (Rho related protein from plant) mediated F-Actin remodeling (Vernoud et al., 2003, Hussey et al., 2006). Although plant cells have no direct Ras ortholog, multiple ROPs exist and some may mediate the F-Actin remodeling events (Hussey et al., 2006).

Conclusion

The interactions and the consequences of the evolutionarily conserved core regulatory circuits, PP2A/B56, TorC2, and TorC1 are reviewed here in the context of eukaryotic cell migration. The three regulatory signaling modules of cells migration, anterior F-Actin remodeling, rear end contraction, and the dynamics of cell-to-matrix adhesion, were revisited as the effectors of TorC2, TorC1, and PP2A/B56. Anterior F-Actin remodeling is mediated through TorC2/Akt (Fig. 3, i) and TorC1/S6K1 signaling axes (Fig. 3, ii). Talin/Vinculin containing adhesion complexes are regulated by TorC2/Akt/PIP5K (Fig. 3, iii) and Paxillin/FAK adhesion complexes are activated by both TorC2 (Fig. 3, iv) and TorC1 (Fig. 3, v). The Rho dependent back retraction is antagonistically regulated by the two Tor complexes: TorC2 inhibits Rho thorough PKA and/or PP2A/B56 (Fig. 3, vi) and TorC1 could activate Rho either through FAK/Paxillin complex (Fig. 3, vii) or through TAP42/PP2A/B56 signaling axis (Fig. 3, viii).

Survey of the presence of LxxIxE motifs in the above-mentioned components of TorC1 and TorC2 signaling networks uncovered potential B56 targets that may associate directly with B56, which include Tor kinase, Rictor, S6K1, ERK1/2, plant MAPK Mpk6. Interestingly proteins involved in the regulation of Ras proteins in *Dictyostelium* such as RasGefA, RasGefH, RasGefF, RasGefI, and scaffolding proteins Sca1/2 contain the LxxIxE motif. The presence of LxxIxE motif in these proteins invites an experiment to determine their association with B56, which will facilitate deeper mechanistic understanding of PP2A/B56 mediated regulation of TorC2 and TorC1 signaling network.

FIGURE LEGENDS

Figure Legends

Figure 1. Protein phosphatase 2A is comprised of three components: the catalytic C, the scaffolding A, and the

regulatory B subunits. Structural and bioinformatics analysis unveiled that B56, one of the B subunits, binds to its target either through the central region or the C-terminal edge of the pseudo heat domain. The central region recognize B56 targets that contain LxxIxE motif (Hertz et al., 2016, Wang et al., 2016), whereas the C-terminal end region binds to distinct type of target such as Shugoshin (Xu et al., 2009). Number of B56 genes in yeast, fungi, protist, plants, and human is shown. A single *B56* gene exists in *Dictyostelium discoideum*. PTPA and PTPB is competing with the regulatory B subunits to associate with the AC core dimer (i). TorC1/TAP42 inhibits the PP2A catalytic activity (ii). Src tyrosine kinase phosphorylates and inhibits the catalytic PP2A subunit (iii). TorC2/PKA signaling axis activates B56/PP2A (iv) whereas Protein Kinase C and ERK1/2 phosphorylate and inhibit B56 (v and vi).

Figure 2. (A) Signaling network that regulate TorC2. TorC2 activity is regulated either at the level of the subunit assembly or the TorC kinase activity. The kinase IKK activates TorC2 (i) but several other kinases such as GSK3 and S6K1 inhibit TorC2 (ii and iii). In *Dictyostelium*, Ras and Rap proteins function positively at the upstream of TorC2 (iv) and PP2A/B56 seems necessary for Ras activation (viii). Interestingly, TorC2 may activate PP2A/B56 through PKA and thus potentially form positive feedback loop (iv, vi, and viii). In addition to PKA, TorC2 activates Akt (v). Akt, unlike PKA, is a target of PP2A/B56 mediated inhibition (vii).

(B). Regulatory network that orchestrate TorC2 mediated Cytoskeletal remodeling. In *Dictyostelium*, TorC2 activates Akt kinases, which in turn phosphorylate multiple proteins that mediate F-Actin remodeling at the leading edge of a migrating cell (i). In addition, Akt may also modulate Talin/Vinculin containing adhesion complexes (ii). The Talin/Vinculin complex may activate TorC2 and thus may form a positive feedback loop (iii). TorC2 may affect rear end retraction through either activating PKA (iv) or modulating Paxillin/FAK adhesion complex (v). Considering that these two singling axes are antagonistic, the signaling output from TorC2 to the Rho mediated rear end retraction is likely dependent on the strength of each signaling in a cell type specific manner.

(C). PP2A/B56 mediated regulation of TorC1 signaling network. A number of kinases that are known to modulate the TorC1 activity are also known to be targets of PP2A/B56. Kinases that regulate the Rheb GAP protein TSC1/2, the critical upstream regulator of TorC1 activity (i), include Akt, ERK1/2, and AMPK. These kinases are the known targets of PP2A (ii and iii). Another kinase MAP4K3 is known to regulate the small GTPase Rag and thus regulate TorC1 and is the target of PP2A/B56 (iv). Lastly, PP2A/B56 inhibits TorC1 target S6K1 (v). The types of B56 isoforms and their targets are denoted in the diagram.

(D). TorC1 signaling network and the cytoskeletal remodeling in the context of cell migration. In addition to the

previously described role of TorC2 in the cytoskeletal remodeling (Fig. 2B), TorC2 may also affect the process by activating TorC1 through Akt (i), which will eventually inhibit TorC2 through S6K1 as a negative feedback loop. S6K1, a TorC1 downstream signaling components, may activate Paxillin/FAK including adhesion complex (ii) and thus activate Rho small GTPase (iii). Another well-characterized target of TorC1 is TAP42, through which TorC1 may inhibit PP2A/B56 (iv). Inhibition of PP2A/B56 would derepress Rho activity at the rear cell end. In addition, S6K1 was shown to affect lamellipodia formation through affecting expression of small GTPases RhoA, Rac, and Cdc42 (v). Finally, TorC1/S6K1 signaling axis was shown to affect caveolin-enriched F-Actin structure at the leading edge (vi).

Figure 3. Summary of TorC2 and TorC1 mediated orchestration of cytoskeletal remodeling in migrating cell. The leading edge of a migrating cell display extensive F-Actin remodeling mediated by either TorC2/Akt (i) and TorC1/S6K1 (ii). Dynamic turnover of adhesion complexes are essential part of cell migration and are targets of TorC2/Akt/PIP5K signaling (iii and iv) and TorC1/S6K1 (v). Rho activity may negatively be affected through TorC2 and PKA signaling axis (vi) or positively thoroughly Paxillin/FAK adhesion complex (vii) and TorC1/TAP42 mediated inhibition of PP2A/B56 (viii).

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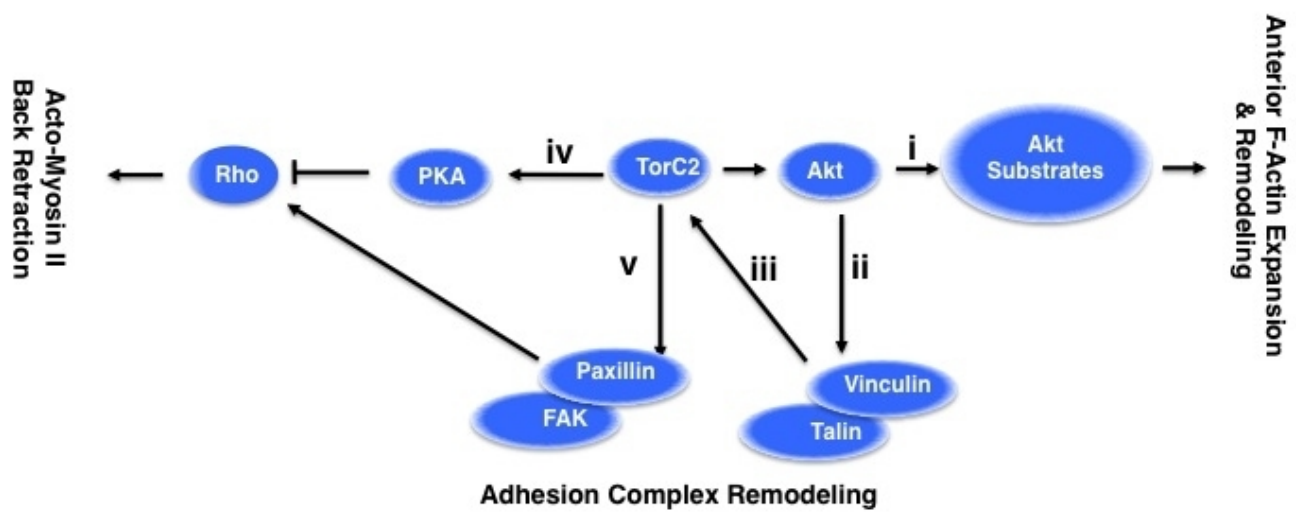
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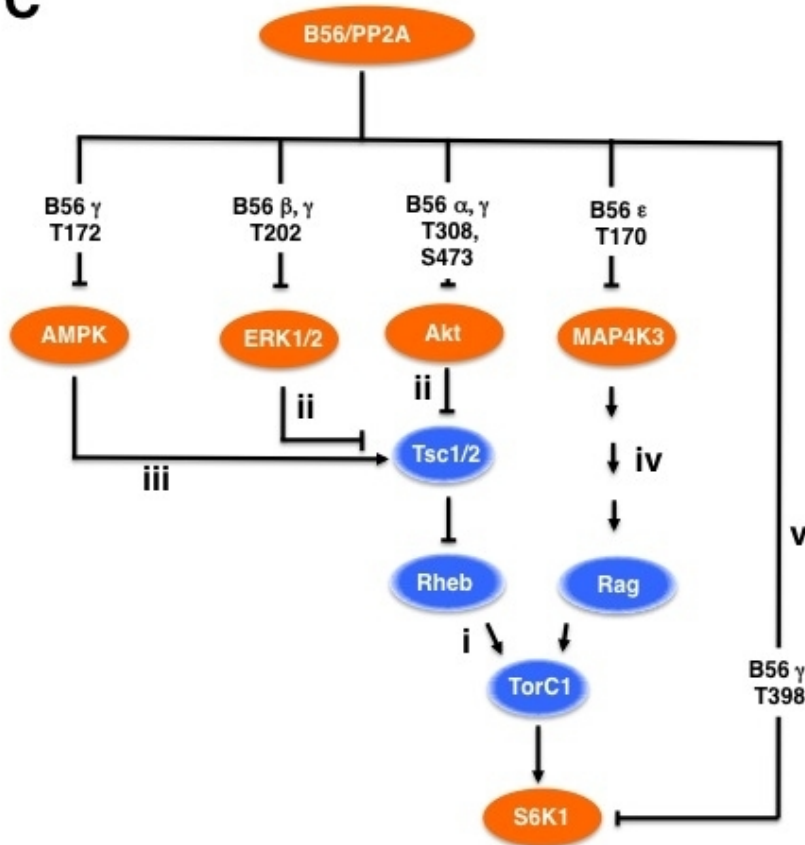
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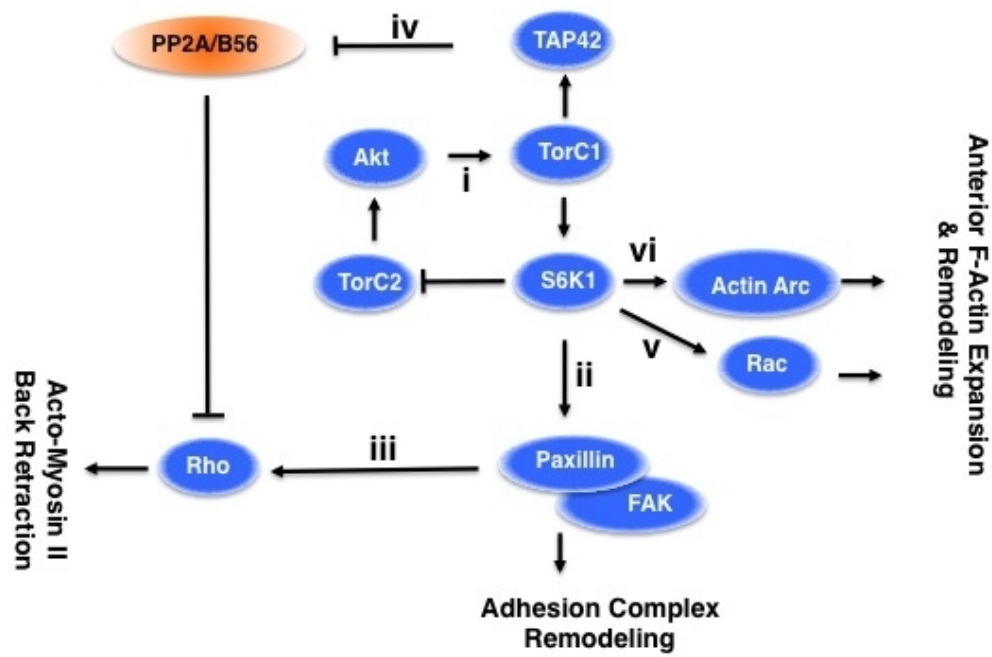Fig. 2

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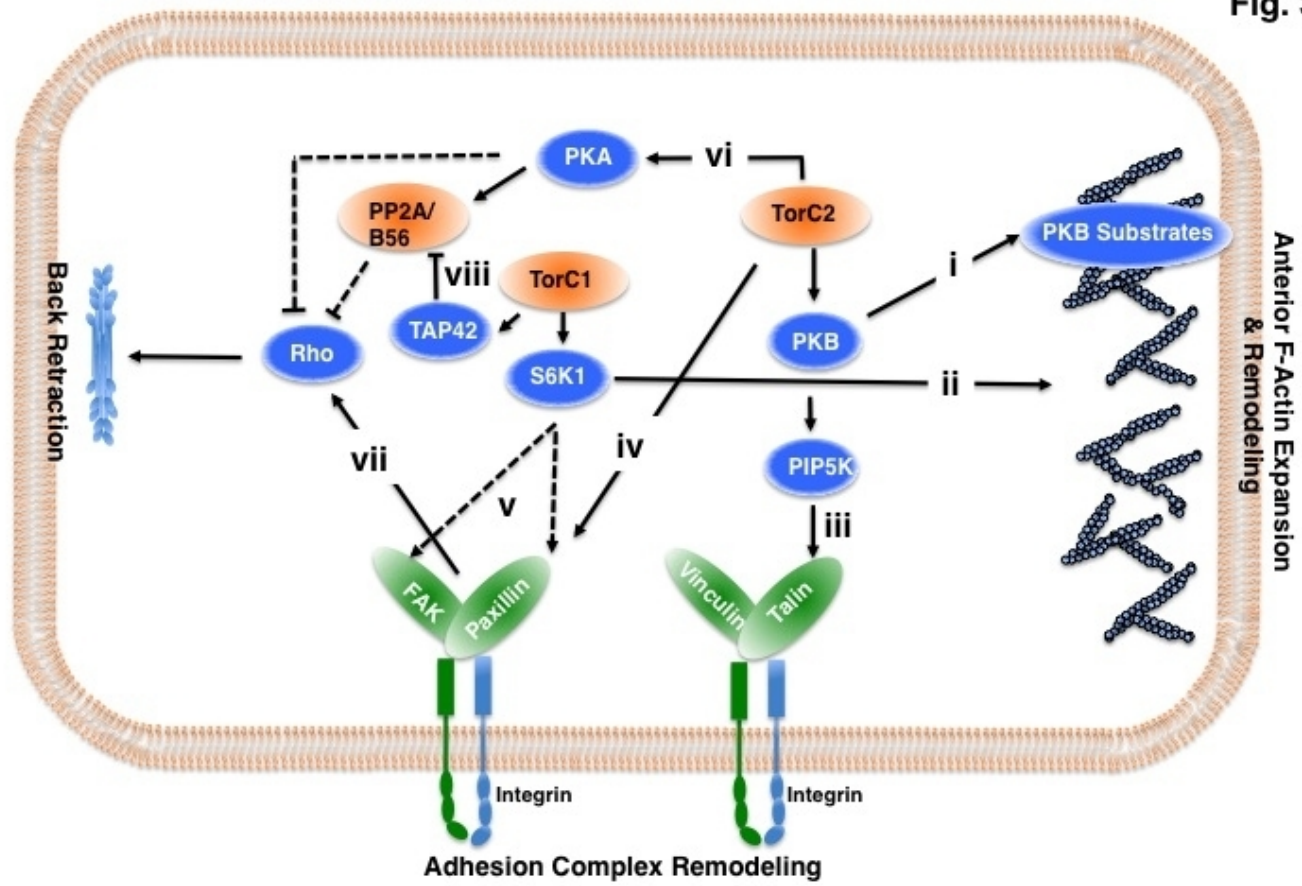**B**

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