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APPL1 is a Multi-Functional Endosomal Signaling Adaptor Protein

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Abstract

Endosomal adaptor proteins are important regulators of signaling pathways underlying many biological processes. These adaptors can integrate signals from multiple pathways via localization to specific endosomal compartments, as well as through multiple protein-protein interactions. One such adaptor protein that has been implicated in regulating signaling pathways is the adaptor protein containing a pleckstrin-homology (PH) domain, phosphotyrosine binding (PTB) domain, and leucine zipper motif 1 (APPL1). APPL1 localizes to a subset of Rab5-positive endosomes through its Bin-Amphiphysin-Rvs (BAR) and PH domains, and it coordinates signaling pathways through its interaction with many signaling receptors and proteins through its PTB domain. This review discusses our current understanding of the role of APPL1 in signaling and trafficking, as well as highlights recent work into the function of APPL1 in cell migration and adhesion.

Keywords

Trafficking; cell migration; adhesion; Akt; endosomes; and Rab5

Introduction

Signaling endosomes are believed to function as platforms for integrating distinct communication pathways within cells[1]. Endosome-associated adaptor proteins are critical in mediating this crosstalk between signaling pathways because of their ability to interact with multiple proteins[1]. APPL1, also known as DIP13α, is a 709-amino acid adaptor protein that is receiving increasing attention because it can facilitate signaling pathway crosstalk on endosomal surfaces. For example, APPL1 has been proposed to mediate crosstalk between Wnt and insulin signaling pathways by bringing together proteins involved in these pathways on endosomes[1, 2]. In this review, we will highlight the role of APPL1 as an adaptor protein and how APPL1 coordinates signaling and trafficking events to regulate cellular processes, including cell migration and adhesion.

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APPL1 integrates signaling crosstalk via multiple domains that mediate protein and lipid interactions[3, 4]. The N-terminal BAR domain is implicated in sensing or inducing membrane curvature[5, 6], while both the central PH and C-terminal PTB domains have been shown to bind to phospholipids[7]. Indeed, multiple studies have demonstrated the ability of APPL1 to bind phosphoinositides, including PtdIns(3)P, PtdIns(4)P, PtdIns(5)P, PtdIns(3,4)P_2 and PtdIns(3,5)P_2, and PtdIns(3,4,5)P_3[7, 8]. Through its BAR domain, APPL1 can oligomerize into homodimers (APPL1-APPL1) or heterodimers with APPL2 (APPL1-APPL2)[8]. APPL1 is unique from other BAR domain-containing proteins, in that the BAR and PH domains of APPL1 together form a functional domain that binds the small GTPase Rab5 (Figure 1). Mutagenesis studies have revealed that Rab5 binds to both the BAR and PH domains of APPL1, as Rab5 could not interact with APPL1 lacking one of them[9]. The BAR-PH domain of APPL1 also binds Rab21[9], which is similar in structure to Rab5[10]. The PTB domain enables interaction of APPL1 with a number of receptor proteins, including epidermal growth factor receptor (EGFR)[4], tropomyosin receptor kinase A (TrkA)[11], deleted in colorectal cancer (DCC)[12], adiponectin receptor (AdipoR1)[13], insulin receptor (IR)[14], follicle stimulating hormone receptor (FSHR)[15], androgen receptor (AR)[16], and N-methyl-D-aspartate (NMDA) receptors[17], to regulate signaling[7]. At its C-terminus, APPL1 binds the PSD-95/Discs-large/ZO-1 (PDZ) domain of the adaptor protein GAIP-interacting protein C terminus, member 1 (GIPC1)[18], a protein involved in loading cargoes onto vesicles through its interaction with the actin motor protein myosin VI[19] (Figure 1).

Moreover, APPL1 associates with at least thirty-three unique proteins (Table 1), and possibly many more, as indicated by numerous studies (data deposited in BioGrid dataset: https://thebiogrid.org/117522/summary/homo-sapiens/appl1.html)[20]. It is highly implausible that APPL1 interacts with all of these proteins simultaneously. More likely, APPL1 binds a subset of these proteins in a cell type dependent manner to regulate specific signaling pathways. Furthermore, interactions between APPL1 and some of the listed proteins may not be direct since they were found by immunoprecipitation assays. Thus, more reconstitution studies should be performed to define proteins that directly interact with APPL1. Nevertheless, the number of putative interactors of APPL1 suggests its great potential to regulate a variety of processes.

APPL1 in trafficking

APPL1, which localizes to early endosomes via interaction with Rab5 as well as lipid binding[9], may comprise a transient and very early compartment in the endocytic pathway[21]. In support of this, highly motile tubulovesicular transport carriers that traffic receptors and fuse to early endosomes were shown to contain APPL1[22]. APPL1 positive vesicles may serve as a precursor for more mature, early endosome antigen (EEA1)-positive, endosomes[21, 23]. Indeed, little to no colocalization occurs between APPL1 and EEA1, although both are Rab5 effectors[4]. Furthermore, EEA1 competes with APPL1 for Rab5 binding on endosomes, and upon a phosphoinositide switch, APPL1 is lost and EEA1 is gained, giving more evidence to a model where APPL1 endosomes mature into EEA1 endosomes[21]. Interestingly, another Rab5 effector, WD Repeat and FYVE Domain Containing 2 (WDFY2), partially colocalizes with both APPL1 and EEA1[21]. After APPL1

was lost from WDFY2 compartments, these compartments then fused to form larger endosomes, which then acquired EEA1 and lost WDFY2[21].

However, recent evidence suggests that a subset of APPL1 endosomes make up a distinct early endosome compartment that can be very stable[24]. Mathematical modeling argues against the hypothesis of APPL1 endosome maturation into EEA1-positive ones as an obligatory mechanism along the endocytic route[24]. Moreover, APPL1 compartments can act as sites of cargo sorting, which enable recycling of cargo back to the plasma membrane[24]. In support of this, the endosomal sorting complex required for transport (ESCRT)-0 was recently shown to mark an APPL1-independent route for trafficking to EEA1-positive endosomes, indicating that there are alternative ways of endosome maturation[25]. A subset of APPL1 endosomes most likely mature into and/or bidirectionally exchange cargo with EEA1 endosomes, whereas another subset directly sort cargo for recycling[24].

APPL1 has also been implicated in the regulation of trafficking, which is crucial for modulating signals from receptors. For instance, APPL1 is linked to EGFR trafficking by modulating Rab5 activation[26]. EGFR is quickly internalized after activation by EGF, in a Rab5-dependent manner. Then it is trafficked to early endosomes for sorting into recycling endosomes or lysosomes for degradation. Overexpression of APPL1 decreases Rab5 activation and subsequently inhibits internalization of EGFR, which reduces degradation of the receptor. Conversely, APPL1 depletion increases Rab5 activation, resulting in increased internalization and trafficking of EGFR to lysosomes to regulate EGFR protein levels and signaling[26]. Thus, APPL1 is an important regulator of endocytic trafficking, and further studies will be needed to reveal the mechanisms by which APPL1 regulates the trafficking of a variety of receptors.

APPL1 in signaling

In addition to its roles in endocytic trafficking, APPL1 also regulates signaling events by interacting with receptors and other signaling proteins. APPL1 binds various signaling proteins, including the serine/threonine kinase Akt[3], p110α and p85 subunits of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)[16], insulin receptor substrate proteins 1 and 2 (IRS1/2)[14], and the Rab5 effector oculocerebrorenal syndrome of Lowe (OCRL)[27] (Figure 1). As a signaling adaptor protein, APPL1 is important for mediating signaling specificity[1]. For example, APPL1 regulates phosphorylation of glycogen synthase-3 beta (GSK3-β) by Akt, playing a role in cell survival. However, APPL1 is not required for Akt-mediated activation of tuberous sclerosis complex 2 (TSC2), which plays a role in growth control[1, 2].

APPL1-mediated signaling is largely coordinated through its PTB domain, located near the C-terminus. This allows APPL1 to couple trafficking and signaling, as lipid binding and Rab5 interaction (accomplished through the BAR and PH domains located near the N-terminus of the protein) would not interfere with APPL1 interaction with signaling proteins[1]. The PTB domain of APPL1 is similar to that of another adaptor protein Shc, which recognizes the NPXpY consensus sequence in its interacting proteins[28]. However,

the binding of most PTB domains is independent of tyrosine phosphorylation[29], and this seems to be the case for APPL1[11, 14, 28]. Indeed, APPL1 has been shown to interact exclusively with the inactive (unphosphorylated) form of Akt2[3]. Similarly, binding between the PTB domain of APPL1 and TrkA or AdipoR1 does not depend on the presence of phosphotyrosine[11, 13]. Mutation of all tyrosine residues in AdipoR1 had no effect on APPL1 binding[13]. Thus, it is likely that APPL1 mediates its signaling interactions through a novel mechanism, independent of tyrosine phosphorylation, and this will require further studies to elucidate.

APPL1 allows the signaling events to occur after internalization of receptors into compartments termed signaling endosomes[1]. During endocytosis, signaling endosomes can be trafficked to specific locations within the cell to mediate certain signaling cascades. One example can be regulation of lysophosphatidic acid (LPA)-induced signaling by APPL1 and its interacting partner GIPC1[18]. Depletion of GIPC1 promoted lysophosphatidic acid receptor 1 (LPA_1)-mediated Akt signaling on APPL1 endosomes. At the same time trafficking of the receptor into EEA1-positive early endosomes that attenuates signaling is restricted. When LPA_1 is in the APPL1 compartment, Akt signaling continues, while trafficking to EEA1 attenuates LPA_1-mediated signaling[18]. Therefore, APPL1 may represent an important mode of regulation of signaling events through endosomes.

APPL1-regulated signaling is not limited to signaling endosomes. APPL1 couples the trafficking of receptors into early endosomes with the transmission of signals to the nucleus, through the interaction of APPL1 with Rab5. Upon GTP hydrolysis of Rab5, APPL1 is lost from endosomes and translocates to the nucleus, where it stimulates changes in chromatin remodeling and transcription[4]. In support of this, APPL1 interacts with and modulates the functions of histone deacetylases (HDAC)1-3[30, 31] to influence the expression of cyclin-dependent kinase inhibitor 1 ($p21^{CIP1}$)[30]. APPL1 also forms a complex with the tumor repressor Reptin in complex with HDAC1 to relieve translational repression and promote transcription of Wnt-signaling target genes[32]. Moreover, APPL1 binds Dishevelled 2 (Dvl2) and enhances its ability to promote non-canonical Wnt signaling through the transcription factor activating protein 1 (AP-1). This is dependent on the endosomal localization of APPL1[33]. As such, APPL1 seems to regulate post-internalization signaling by coupling signaling and transcription to endocytic trafficking.

In addition to regulating signaling specificity, APPL1 facilitates crosstalk between multiple signaling pathways; one example is the synergism between the adiponectin and insulin signaling pathways. APPL1 associates with AdipoR1 upon stimulation with adiponectin, leading to downstream phosphorylation of 5’ adenosine monophosphate-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK)[28]. Co-treatment with adiponectin and insulin usually results in Akt phosphorylation, but not in cells depleted of APPL1[28]. Furthermore, APPL1 mediates sensitization of insulin signaling through adiponectin. Treatment of C2C12 cells with adiponectin leads to phosphorylation of APPL1 at Ser401. Co-treatment with insulin mediates the association of phosphorylated APPL1, IR, and IRS1/2 to allow for insulin signaling[14]. Together, this evidence highlights an important role for APPL1 as a signaling adaptor which mediates signaling pathways coupled to endosomal trafficking.

APPL1 as a regulator of cell migration

While the functions of APPL1 in trafficking and signaling have been studied, the role of APPL1 in regulating cell migration is still poorly understood. This was initially explored in a study using murine embryonic fibroblasts (MEFs) from the APPL1 knockout mouse. Although APPL1 is dispensable for development, fibroblasts require APPL1 for proper Akt signaling during hepatocyte growth factor (HGF)-mediated survival and cell migration[34].

The importance of APPL1 in the regulation of cell migration has also been documented in cancer cell line models. A study by Broussard et al. showed that cell lines exhibiting lower migratory rates express higher levels of APPL1 in comparison to highly motile cancer cell lines[35]. It is therefore hypothesized that alterations in APPL1 expression levels affect cell migration. In support of this hypothesis, expression of APPL1-GFP in HT1080 fibrosarcoma cells leads to a decrease in cell migration speeds, which is dependent on endosomal localization of APPL1 and its ability to coordinate signaling. Mechanistically, APPL1 inhibits Akt activity within adhesions and downstream of the tyrosine kinase Src (Figure 2a). Indeed, APPL1 decreases Src-mediated tyrosine phosphorylation of Akt, and this Akt activation is necessary for migration. As a result, APPL1-GFP-expressing cells exhibit slower migration due to impaired turnover of leading edge adhesions[35].

Several studies suggest that APPL1 is involved in integrin trafficking, which is critical for cell adhesion and migration. In endothelial cells, GIPC1 interaction with the glycoprotein neuropilin-1 (Nrp1) was shown to promote internalization of active α5β1 integrin into Rab5-positive vesicles, which is then recycled to the cell surface near adhesion sites[36] (Figure 2b). This is crucial for cell adhesion to the extracellular matrix protein fibronectin as well as fibronectin fibrillogenesis. Because APPL1 interacts with both GIPC1 and Rab5, Valdembri et al. suggest that APPL1 is involved in this pathway (Figure 2b)[36]. Future studies are needed to determine the importance of APPL1-dependent regulation of integrin internalization for cell migration.

More recently, APPL1 was implicated in the regulation of integrin trafficking in Arf6-mediated adhesion and migration. Arf6 has been paradoxically shown to both increase and decrease cell adhesion by accelerating integrin recycling and by increasing integrin internalization, respectively. Chen et al. demonstrated that these opposite effects on integrin trafficking and focal adhesion size are accomplished by spatially separating two Arf6 GTPase activating proteins (GAPs), namely ArfGAP with Coiled-Coil, Ankyrin Repeat and PH Domains 1 (ACAP1) and ArfGAP with RhoGAP Domain, Ankyrin Repeat and PH Domain 2 (ARAP2)[37]. ACAP1 was shown to promote recycling of integrins, while ARAP2 instead decreases integrin recycling (Figure 2c). ARAP2 colocalizes with Arf6 and APPL1 in distinct structures that are separate from ACAP1/Arf6 recycling endosomes. Knockdown of ARAP2 enhances trafficking of β1 integrin, and reduces β1 integrin transit from APPL1 to EEA1 vesicles (Figure 2c), whereas overexpression of ARAP2 leads to an increase in adhesion size. The former phenotype has been also reported for cells overexpressing APPL1. It is plausible that impaired trafficking of integrins by ARAP2 would favor adhesions that turn over more slowly. Since APPL1 has been shown to reduce adhesion turnover[35], it is tempting to speculate that APPL1 and ARAP2 are both involved

in this process. On the other hand, ACAP1, which promotes integrin recycling, localizes to an APPL1-negative Arf6 compartment that has a different effect on Arf6-mediated cell adhesion. Interestingly, an inhibition of Akt prevents ACAP1-mediated integrin recycling[38]. As APPL1 downregulates Akt activation during cell migration, this could mean that APPL1 promotes ARAP2 activity by inhibiting ACAP1.

APPL1 and APPL2: similarities and contrasts

APPL2 is a 669-amino acid protein that shares 54% identity with APPL1[4]. APPL1 and APPL2 share similar BAR, PH, and PTB domains, as well as a C-terminal SEA motif for binding PDZ domains[12]. APPL1 and APPL2 share some similar roles. For instance, APPL1 and APPL2 both contribute to cell survival, but this occurs through distinct signaling pathways[2, 40]. APPL1 regulates Akt signaling to mediate cell survival, requiring its endosomal localization[2]. On the other hand, APPL2 decreases gene expression of apoptosis-related genes, and this effect on cell survival is independent of its endosomal localization[40]. Furthermore, the two proteins may have some redundant roles, as evidenced by the APPL1 knockout mouse, which is viable, with no obvious phenotypes. MEFs from the APPL1 knockout mouse exhibit impaired Akt signaling in response to HGF stimulation, and this effect is aggravated by APPL2 depletion[34]. However, APPL1 and APPL2 are not fully redundant, as the two proteins display some differences in binding partners. For instance, both APPL1 and APPL2 interact with Rab5[9], but APPL2 cannot bind Rab21; conversely, Rab22a, Rab24, and Rab31 can bind APPL2, but not APPL1[39]. APPL1 and APPL2 also exhibit some opposing functions; for example, APPL1 is a positive regulator of AdipoR1 signaling, whereas APPL2 is a negative regulator[41]. APPL2 has not been as well studied as APPL1, and further studies on APPL2 will be important to reveal redundant and/or unique novel functions of APPL2.

Future directions concluding remarks

APPL1 is an important adaptor protein for coordinating both signaling and trafficking events within cells in order to regulate processes such as cell migration and adhesion. Although the role of APPL1 in signaling and trafficking has been well characterized, future studies are needed to understand the complexity of APPL1 endosome formation and exchange and/or maturation with other Rab5-positive endosomes, and how this affects signaling on endosomes. A number of intriguing questions still remain. What is the importance of the ability of APPL1 to bind to unphosphorylated tyrosine residues? Is it to prevent signaling until the bound protein is released from the APPL1 compartment? Are there other receptors or signaling proteins that interact with APPL1? How does APPL1 mediate signaling specificity to coordinate multiple signaling pathways?

Studies focusing on APPL1 in the context of cell migration are only beginning to emerge, and thus, much remains to be learned. However, multiple proteins that interact with APPL1 have been implicated in cell migration, and could represent feasible mechanisms for APPL1-mediated cell migration. For instance, Rab5, in addition to its roles in regulating early endosome dynamics, is known to promote cell migration in a number of ways[42]. Rab5 localizes to the leading edge of migrating cells and promotes lamellipodia formation[42, 43].

Mechanistically, Rab5, downstream of Caveolin1, promotes activation of the GTPase Rac by recruiting the guanine nucleotide exchange factor (GEF) Tiam1[43–45]. APPL1 signaling endosomes could therefore be important for coordinating the signals leading to Rac activation. Moreover, Rab5 localizes to focal adhesions[46], associates in a complex with focal adhesion proteins vinculin and paxillin, and also promotes focal adhesion disassembly[47]. There is already evidence for the role of APPL1 in focal adhesion turnover[35]; therefore, the interaction between APPL1 and Rab5 might be important for focal adhesion dynamics. APPL1 also interacts with Rab21, a poorly studied Rab protein involved in the endocytic pathway. Rab21 associates with and controls trafficking of integrins to regulate cell migration[48]. Since APPL1 has already been shown to bind to Rab5 and Rab21[9, 37, 42], APPL1 may also be involved in Rab21-mediated integrin trafficking.

The integration of signaling and trafficking through adaptor proteins, such as APPL1, is an intriguing area of research that is still not well understood. Signaling specificity and crosstalk between multiple signaling pathways are complex, and studying APPL1 may provide a greater understanding into how signaling is controlled. Further studies into APPL1 will lend insight in the molecular mechanisms underlying trafficking and signaling, as well as cell migration and adhesion.

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Abbreviations

APPL1	adaptor protein containing a pleckstrin-homology domain, phosphotyrosine binding domain, and leucine zipper motif 1
BAR	Bin-Amphiphysin-Rvs
PH	Pleckstrin Homology
PTB	Phosphotyrosine binding
EGFR	epidermal growth factor receptor
TrkA	tropomyosin receptor kinase A
AdipoR1	adiponectin receptor
IR	insulin receptor
GIPC1	GAIP-interacting protein C terminus, member 1
EEA1	early endosome antigen
IRS1/2	insulin receptor substrate proteins 1 and 2

GSK3-β	glycogen synthase-3 beta
GAPs	GTPase activating proteins
WDFY2	WD repeat and FYVE domain containing 2
LPA_1	lysophosphatidic acid receptor 1
HGF	hepatocyte growth factor
ACAP1	ArfGAP with Coiled-Coil, Ankyrin Repeat and PH Domains 1
ARAP2	ArfGAP with RhoGAP Domain, Ankyrin Repeat and PH Domain 2

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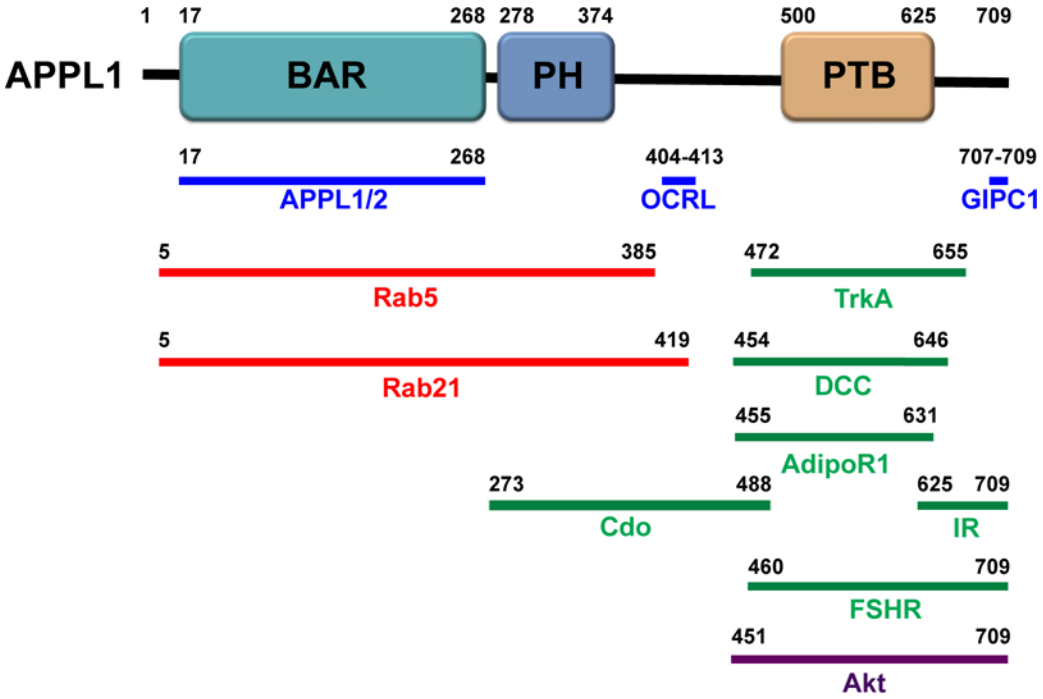

Figure 1. Schematic of the domain structure of APPL1 with binding sites of interacting proteins

Numbers correspond with amino acid residues. Numbering of APPL1 domains is based on Li et al.[7]. Interacting proteins are labeled as follows: red, trafficking proteins; purple, signaling proteins; blue, proteins involved in both signaling and trafficking; green, receptors.

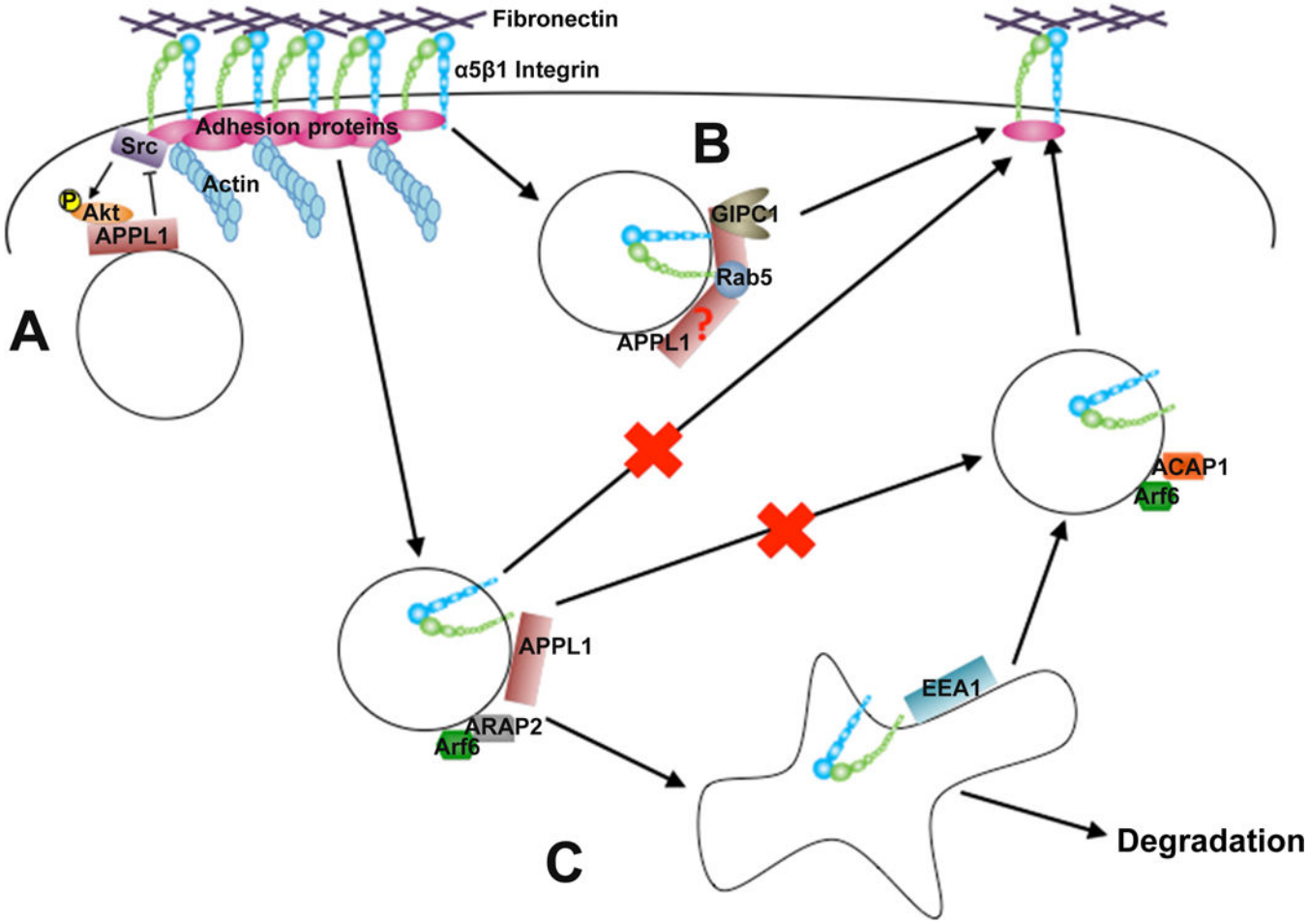

Figure 2. Function of APPL1 in cell migration and adhesion

(A) APPL1 decreases migration speeds by inhibiting Akt tyrosine phosphorylation by Src within adhesions. This process required endosomal localization of APPL1. Circle represents APPL1-positive early endosomes. (B) Rab5 and GIPC1 promote internalization of active α5β1 integrin from adhesions, which is subsequently recycled to newly forming adhesions. APPL1 may be involved in this process. Circle represents Rab5-positive early endosomes that may contain APPL1. (C) APPL1 colocalizes with the Arf6 compartment containing the GAP ARAP2, which promotes transition of integrins from APPL1-endosomes to EEA1-endosomes, and may block transition of integrins to recycling endosomes. Arf6 compartments containing ACAP1, however, promote recycling of integrins. Circles represent Arf6 compartments marked by either ARAP2/APPL1 or ACAP1. Irregular shaped compartment represents EEA1-positive early endosomes.

Table 1

List of APPL1 interactions identified through BioGRID database and literature searches. At least 33 proteins interact with APPL1. Protein interactions identified by BioGRID through high throughput methods have been excluded. For full list, see https://thebiogrid.org/117522/summary/homo-sapiens/appl1/html[20].

ID	Experimental Method	References
ADIPOR1	Affinity Capture-Western, Protein Fragmentation Complementation Assay, Two-Hybrid, Reconstituted Complex	[13, 49]
ADIPOR2	Affinity Capture-Western, Two-Hybrid	[13]
AKT1	Affinity Capture-Western	[16]
AKT2	Affinity Capture-Western, Reconstituted Complex, Two-Hybrid	[3, 50]
APPL1	Co-crystal Structure, Reconstituted Complex	[9]
APPL2	Affinity Capture-Western	[50]
AR	Affinity Capture-Western	[16]
CDON	Affinity Capture-Western, Two-Hybrid	[51]
DCC	Affinity Capture-Western, Two-Hybrid	[12]
DVL2	Affinity Capture-Western	[33]
EGFR	Co-Localization	[4, 26]
FSHR	Affinity Capture-Western, Two-Hybrid	[15, 52]
GIPC1	Affinity Capture-Western	[11, 18, 53]
HDAC1	Affinity Capture-Western, Reconstituted Complex	[30]
HDAC2	Affinity Capture-Western, Reconstituted Complex	[30]
HDAC3	Affinity Capture-Western	[31]
INPP5B	Affinity Capture-Western	[27]
INSR	Affinity Capture-Western	[14]
IRS1/2	Affinity Capture-Western	[14]
MTA 2	Affinity Capture-Western, Reconstituted Complex	[4, 30]
NTRK1	Affinity Capture-Western	[11]
OCRL	Affinity Capture-Western	[27]
PIKCA	Affinity Capture-Western	[3]
PIK3R1	Affinity Capture-Western	[16]
PRKCZ	Affinity Capture-Western, Reconstituted Complex	[54]
RAB5A	Affinity Capture-Western, Reconstituted Complex	[4, 9, 13]
RAB21	Reconstituted Complex	[9]
RBBP4	Affinity Capture-Western	[30]
RBBP7	Affinity Capture-Western Reconstituted Complex	[30] [4]
RUVBL2	Affinity Capture-Western, Reconstituted Complex	[32]
TGFBR1	Affinity Capture-Western, Co-localization, Reconstituted Complex	[54]
TRAF6	Affinity Capture-Western	[55]
TUBB3	Affinity Capture-Western	[54]