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Diverse roles of the scaffolding protein RanBPM

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Ran-binding protein microtubule-organizing center (RanBPM) appears to function as a scaffolding protein in several signal transduction pathways. RanBPM is a crucial component of multiprotein complexes that regulate the cellular function by modulating and/or assembling with a wide range of proteins in different intracellular regions and thereby mediate diverse cellular functions. This suggests a role for RanBPM as a scaffolding protein. In this article, we have summarized the diverse functions of RanBPM and its interacting partners that have been investigated to date. Also, we have categorized the role of RanBPM into four divisions: RanBPM as a modulator/protein stabilizer, regulator of transcription activity, cell cycle and neurological functions.

Introduction

The Ran-binding protein microtubule-organizing center (RanBPM) was first identified as a 55 kDa RanBPM [1]. A later work by the same group revealed that the full-length RanBPM is a 90 kDa protein encoding a long stretch of proline and glutamine residues in its N-terminal region [2]. The full-length RanBPM protein showed weak interaction with Ran and was not localized to the microtubule-organizing center [2]. However, among ten members of the Ran-binding protein family, RanBPM and RanBP10 do not acquire the function of nuclear trafficking and does not show interaction with Ran *in vivo* [3]. Ubiquitous expression of RanBPM in different tissues and cell lines shows the conserved role of RanBPM in different organisms [4,5]. However, we can speculate that different tissues might express different forms of RanBPM and it interacts with different binding partners exhibiting diverse cellular functions.

The RanBPM protein contains multiple conserved domains that provide potential protein–protein interaction sites, such as an N-terminus proline rich domain (PRD), spore lysis A and ryanoine receptor (SPRY) domain, a lissencephaly type-I-like homology (LisH) motif, a carboxy-terminal to LisH (CTLH) motif and a CT11-RanBPM (CRA) motif at the C terminus of RanBPM (Fig. 1) [6]. The SPRY domain was identified in *Dictyostelium* as dual-specificity kinase splA and in ryanodine receptor, which was proposed to be a protein–protein interaction domain [7]. The SPRY domain of RanBPM contains less-conserved 64-residue region termed the PRY domain followed by the highly conserved core SPRY domain [8]. The LisH domain is mostly found in proteins that are involved in regulation of microtubule cytoskeleton. The function of CTLH domain is not well studied. The CRA domain was found to be a protein–protein interaction domain of crown eukaryotes, and it was identified as a domain interacting with the fragile X mental retardation protein (FMRP) [9]. A nuclear localization signal (NLS) sequence is located within the 635–649 amino acids (aa) of the CRA domain. The detailed domain information of RanBPM is illustrated in Fig. 1.

Recent studies have demonstrated the association of RanBPM with a broad spectrum of proteins. A previous review on RanBPM discusses its origin, structure and its function in both the immune and nervous systems [6]. To date, approximately 45 interactors for RanBPM and its regulatory biological functions have been reported. Thus, in this article we focus on the recent studies on RanBPM and its interacting proteins regulating diverse cellular functions and its impact on various diseases. Additionally, we emphasize the importance of post-translational modification of RanBPM and the possibility of developing RanBPM as a potential drug therapeutic for various diseases.

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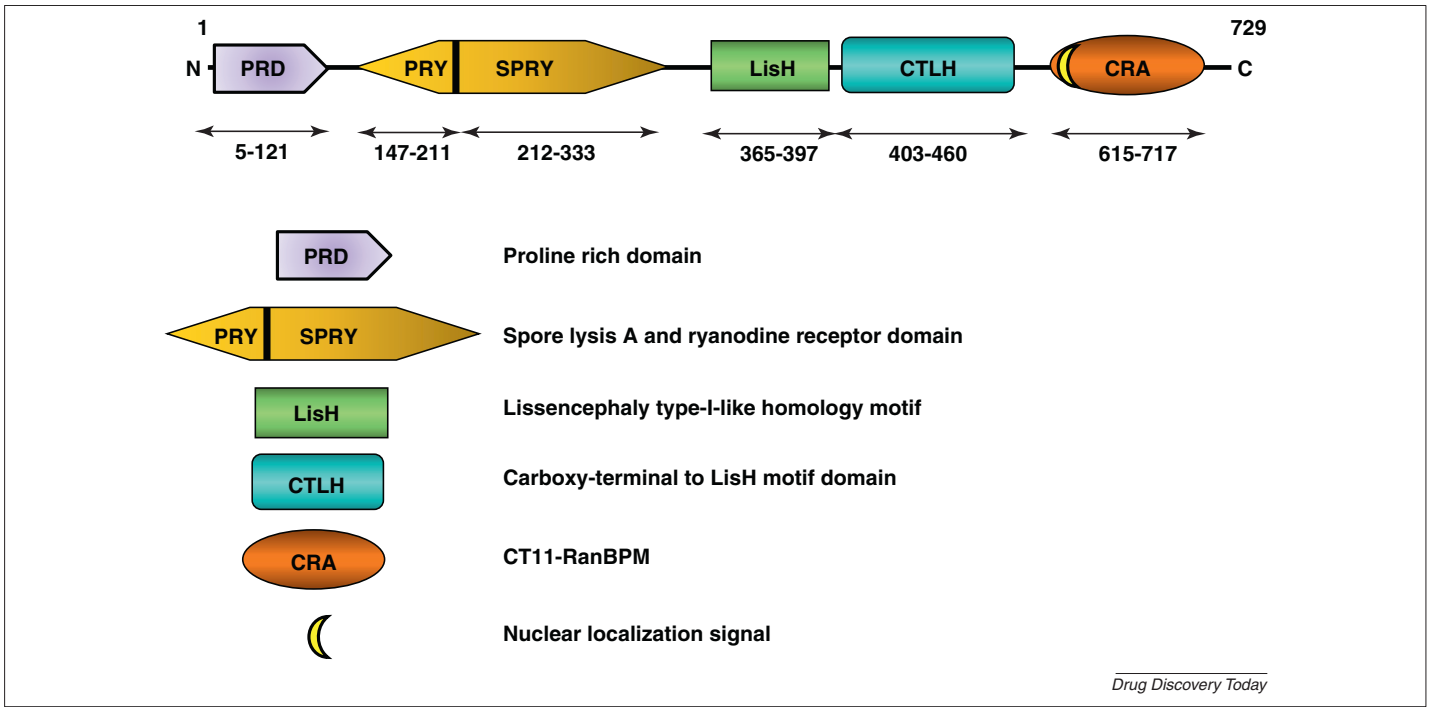

FIGURE 1

RanBPM shows functional domain regions, including proline rich domain, splA and Ryr domain (212–333 aa), a lissencephaly type-I-like homology motif (365–397 aa), a carboxy-terminal to LisH motif domain (403–460 aa), a CT11-RanBPM (CRA motif) (615–717 aa) and a nuclear localization signal (635–649 aa).

The role of RanBPM as a modulator

RanBPM as a protein stabilizer

RanBPM was characterized as an activator of apoptotic pathways in HeLa cells [10]. The overexpression of RanBPM activates caspase-3 activity and induces cell death, whereas knockdown of RanBPM increases cell survival in response to irradiation (IR) treatment. Depletion of endogenous RanBPM was associated with the decrease of mitochondria-associated Bax and increased levels of Bcl-2. Upon IR treatment, RanBPM was found to translocate from the nucleus to the cytoplasm, suggesting that RanBPM is a DNA damage-activated factor regulating the intrinsic cell death pathway [10]. RanBPM being a proapoptotic protein binds to and modulates the function and stability of several proteins that regulate apoptosis. p73 is a p53-related nuclear transcription factor, and participates in cell cycle regulation and induction of apoptosis [11]. The stability and function of the p73 protein are modulated by a physical interaction with RanBPM [12]. Further, it was demonstrated that RanBPM interaction is specific to p73α but not with p53. The binding of RanBPM with p73 results in the nuclear translocation of cytoplasmic RanBPM and stabilizes p73 protein by preventing its degradation through the ubiquitin–proteasome pathway (UPP) As a functional consequence, RanBPM enhances the transcriptional activity of p73α and subsequently increases the proapoptotic function of p73α [12].

Recently, we showed that RanBPM interacts and modulates the stability and biological functions of oncoprotein mammalian lethal giant larvae-1 (Mgl-1) [13]. A lethal giant larvae (Lgl), one of the cytoskeletal complex proteins, has been identified in establishing polarized epithelia, cell proliferation, differentiation and tissue organization [14,15]. RanBPM was found to inhibit Mgl-1-mediated cell proliferation and cell migration, suggesting that RanBPM modulates the tumor suppressor activity of Mgl-1 [13]. RanBPM increases the Mgl-1 protein level in a dose-dependent manner and extends its half-life by preventing Mgl-1 protein degradation [13]. Taken together, the function of RanBPM on p73α and Mgl-1 indicates a novel role of RanBPM as a protein stabilizer. Interestingly, RanBPM is associated with USP11 a deubiquitinating enzyme (DUB) which is generally involved in preventing ubiquitination of targeted proteins [16]. Therefore, one can speculate that RanBPM promotes deubiquitination of p73α or Mgl-1 by recruiting USP11 to p73α or Mgl-1. Since, the expression of N-terminal fragment of RanBPM was elevated in Alzheimer's disease (AD), DUBs might have a crucial role in stabilizing RanBPM protein and driving the amyloid cascade in AD [17].

RanBPM in Ca^{2+} channel signaling

RanBPM has a key role in the modulation of $Ca_v3.1$ T-type Ca^{2+} channel-mediated signaling pathways [18]. The elevated level of RanBPM was found to augment T-type Ca^{2+} currents in HEK293/$Ca_v3.1$ cells. RanBPM can bind to the cytoplasmic intracellular loop between transmembrane domains I and II of $Ca_v3.1$ channels and increase $Ca_v3.1$ currents by hyperpolarizing shift in the activation curve. RanBPM enhances current density by increasing the expression of channels at the plasma membranes. The activation of protein kinase C (PKC) leads to the inhibition of $Ca_v3.1$ currents, whereas binding of RanBPM to the C1 loop of $Ca_v3.1\alpha1$ subunit abolishes this inhibitory effect [18]. These results indicate the significant role of RanBPM as a regulator of the $Ca_v3.1$ channel-mediated signaling pathways. It has been reported that the endogenous RanBPM is associated with brain-specific protein $p42^{IP4}$ *in vivo* [19]. Galvita *et al.* clearly demonstrated $p42^{IP4}$ localization predominantly at the inner membrane and in the inter membrane

space of mitochondria and interacts with 2′,3′-cyclic nucleotide 3′-phosphodiesterase in mitochondria [20,21]. Based on these observations, authors hypothesized that $p42^{IP4}$ is involved in the calcium transport or permeability transition pore function in mitochondria. It is possible that RanBPM acts as a scaffolding protein for $p42^{IP4}$ to regulate $p42^{IP4}$-mediated signaling pathways by determining the composition of the RanBPM/$p42^{IP4}$ complexes in different cellular compartments.

RanBPM in developing cells

RanBPM was recognized as an essential gene for normal gonad development in both genders [22]. RanBPM$^{-/-}$ male mice showed severe atrophy of the gonads leading to significant decrease in spermatogonia proliferation during postnatal development. Similarly, female RanBPM$^{-/-}$ mice also showed premature ovarian failure due to marked germ cell loss at the end of prophase I indicating its key role in both spermatogenesis and oogenesis [22].

RanBPM has a crucial role in myotube development. RanBPM acts as a ligand for the Rho-guanine nucleotide exchange factor (Rho-GEF) domain of obscurin regulating myofibrillogenesis [23]. The Rho-GEF domain abolishes the assembly of titin integration at the Z-disk during developing skeletal myotubes resulting in the instability of the Z-disk and the A/I junction. During myofibrillogenesis, the Rho-GEF domain of obscurin appears in striations as early as 72 hours, whereas the appearance of RanBPM was delayed but both proteins were colocalized by day 5 in culture. Initially, Rho-GEF domain and RanBPM are associated with Z-disks and at the later stages of development; they are colocalized at the I-bands and M-bands in developing myotubes. The myonuclear labeling for RanBPM was reduced in the developing skeletal myotubes, suggesting that RanBPM in the nucleus might influence the early stages of myofibrillogenesis. Later, the expression of Rho-GEF domain of obscurin and RanBPM was detected in M-bands, Z-disks and Z-I junctions of adult skeletal muscles. Further, both the Rho-GEF domain and RanBPM were found to interact with the N-terminal region of titin and specifically inhibit the organization of titin at the Z-disk of developing skeletal muscle [23]. Thus, RanBPM along with Rho-GEF domain of obscurin regulates the assembly of titin during the formation of the Z-disk and A/I junction.

In *Drosophila*, RanBPM consists of two isoforms: short RanBPM expressed in all somatic and germline cells of the ovary and the long RanBPM specifically confined in the germline stem cell (GSC) niche. The two isoforms of RanBPM were expressed in the GSC niche of the ovary. The long isoform is specifically enriched in the CpCs and terminal filament of the GSC niche. Loss of the long isoform from the niche causes defects in the regulation of niche capacity and adhesion. Thus, niche-specific long isoform of RanBPM is involved in the regulation of cell shape, size and organization of the GSC niche [24].

RanBPM in cell polarity and cell morphology

RanBPM interacts with numerous cell surface receptors and cell adhesion proteins to have a major role in regulating cell shape, arrangement, polarity and cell proliferation. RanBPM interacts with Citron kinase (CITK), a key protein that is polarized to the ventricular surface during cell division [25]. The RanBPM is crucial for the polarization of CITK to the ventricular/apical surface in cells during mitosis. It was also reported that the functional interaction between RanBPM and muskelin is needed for the activity of muskelin in cell spreading and the distribution of muskelin between cytoplasm and nucleus of the cell [26]. Similar to muskelin, RanBPM-depleted cells also showed alterations in F-actin distributions and adherent cells showed protrusive morphologies. Thus, nuclear muskelin might function through the RanBPM complex to regulate cellular morphology. We demonstrated RanBPM as a novel interaction partner for the Mgl-1 [13]. Lgl functions in concert with Disc large (Dlg) and scribble protein, primarily involved in maintenance of basolateral membrane domain and basal protein targeting. Lgl also functions in a Par6–aPKC protein complex that is crucial for the apical membrane domain [27]. Therefore, we propose that RanBPM acts as a modulator for Mgl-1-mediated maintenance of basolateral membrane domain or apical membrane domain.

The role of RanBPM in transcriptional activity

RanBPM was found to interact with several transcriptional factors resulting in induction or repression of the transcriptional activity of its interacting proteins. RanBPM interacts with multiple domains of the androgen receptor (AR), a ligand-dependent transcription factor [4]. AR belongs to a large steroid receptor family, which includes progesterone, mineralocorticoid and glucocorticoid receptors (GRs). RanBPM specifically enhances AR transactivation in a ligand-dependent fashion. Similar results were observed with GR activity, whereas the estrogen receptor activity remained unchanged [4]. Recently, RanBP10 was found to enhance AR transactivation as a homo-oligomer or a hetero-oligomer with RanBPM [28]. The N-terminal polyglutaminated region of RanBPM interacts with the thyroid hormone receptor (TR), a member of nuclear receptor superfamily and stimulates TR transactivation in a ligand-independent manner [29]. RanBPM interacts with integrin lymphocyte function-associated antigen-1 (LFA-1), but the interaction is not only restricted to β1 and β2 integrins. Several lymphocyte surface receptors also showed interaction with RanBPM. The RanBPM along with the stimulation through LFA-1 significantly enhances the promoter activity resulting in the induction of activator protein-1 (AP-1) dependent transcription [30]. RanBPM interacts with MET tyrosine kinase protein and showed increased hepatocyte growth factor-dependent cell migration in cancer cell lines [5]. Because MET overexpression has been found in several human cancers, the overexpression of RanBPM augmenting the MET signaling could be the cause of cancers. Additionally, RanBPM acts as an adaptor protein for MET to recruit SOS to stimulate Ras/Erk activation [5].

The expression of RanBPM influences the activity of Rta, regulating transcription of the Epstein–Barr virus (EBV) lytic genes and the lytic cycle, indicating RanBPM as a viral protein regulator [31]. Transcription factor Rta acts alone or along with Zta to activate several EBV lytic genes [32,33]. Sumoylation of Rta enhances its activity to complete the EBV lytic development in an efficient way. RanBPM promotes Rta sumoylation by interacting with Ubc9, which indicates the participation of RanBPM in EBV lytic activation [31]. Raf kinase is the upstream activator of MEK1/2, which is involved in phosphorylation and activation of ERK1/2. The interaction between RanBPM and Raf kinase does not involve the inhibition of the kinase activity [34]. Additionally,

RanBPM interacts with Axl, Sky transmembrane receptor tyrosine kinases [35] and homeodomain-interacting protein kinase 2 (HIPK2) [36], but functional data have been limited by the lack of functional studies.

RanBPM also exhibited negative regulation, the interaction between RanBPM and Mirk/Dirk1B results in the inhibition of Mirk functions. RanBPM was found to inhibit minibrain kinases, including the function of Mirk as a transcriptional coactivator leading to reduced cell migration [37]. CD39, a prototypic member of the NTPDase family forms a complex with the SPRY domain of RanBPM. This association substantially downregulates the NTPDase activity of CD39 [38]. Taken together, RanBPM acts as a potent coactivator for several receptors regulating various transcriptional activities.

The role of RanBPM in neurological functions

Earlier studies highlighted the importance of RanBPM interaction with proteins that are involved in neurological function. Neurotrophins (NTs) are essential for proper development and maturation of the nervous system [39]. The biological function of NTs depends on tropomyosin-related kinases (Trk) receptor and pan-neurotrophin receptor 75 kDa (p75NTR). RanBPM was shown to interact with both TrkA [40] and p75NTR [41]. Activation of Trk receptors eventually leads to activation of transcription factors, which alter gene expression [42]. It was reported that nerve growth factor (NGF) was associated with the nuclear factor of activated T cells (NFAT) transcription factor in the signaling pathways through TrkA receptor [43]. RanBPM interacts with the TrkA receptor and inhibits NGF-mediated NFAT-dependent transcription, which suggests the role of RanBPM as a novel tyrosin kinase inhibitor [40]. Thus, RanBPM interaction with TrkA might alter the phosphorylation status of activation loop residing in the tyrosine kinase domain of the TrkA receptor, which is essential for NFAT activation. In addition, RanBPM is associated with TrkB, a receptor tyrosine kinase for brain-derived neurotrophic factor (BDNF) [44]. Expression of RanBPM significantly regulates BDNF-mediated TrkB downstream signaling, mainly the mitogen-activated protein kinase (MAPK) and Akt pathways. RanBPM promotes BDNF-induced neuronal morphogenesis and also enhances BDNF-mediated protection from serum deprivation-induced apoptosis [44]. Considering RanBPM as a novel interacting protein of TrkA and TrkB, a detailed investigation on RanBPM-mediated signaling during neuronal survival and differentiation and morphogenesis is essential in developing therapeutics for neuronal disorders.

It has been demonstrated that RanBPM function is required in the *Drosophila* nervous system for larval feeding, light-induced changes in locomotion and growth [45]. RanBPM mutants showed reduced proliferation, but did not show major disruption in the nervous system development. RanBPM showed high expression in the kenyon cells of the larval mushroom body, and its expression is sufficient to rescue the response to light and feeding phenotypes. RanBPM interacts with the FMRP and inhibits the RNA-binding ability of FMRP [45]. Thus, RanBPM alters FMRP RNA binding either by directly competing for RNA-binding sites in FMRP or by modulating structural changes of FMRP, which might directly contribute to the development of fragile X syndrome.

RanBPM interacts with plexin-A and mediates semaphorin3A signaling, which is involved in axonal growth [46]. RanBPM interacts with L1, a neural cell adhesion molecule which is involved in various developmental processes, such as neurite outgrowth, axon fasciculation, myelination and migration of neuronal precursors [47]. Mutations in the L1 gene cause several X-linked disorders, such as X-linked hydrocephalus, agenesis of the corpus callosum, spastic paraplegia type-I and Mental retardation, Aphasia, Shuffling gait, and Adducted thumbs (MASA) syndrome [48]. L1 activates the ERK pathway for neurite outgrowth [49]. The overexpression of RanBPM decreased L1-induced ERK activation leading to partial inhibition of neurite outgrowth in cerebellar neurons, suggesting that RanBPM serves as an adaptor in L1-mediated signaling in neurite growth [47]. RanBPM is highly expressed in brain and retina, confined to the inner plexiform layers and binds to metabotropic glutamate (mGlu) receptors. Interaction between RanBPM and most of the isoforms of mGlu receptors suggests that RanBPM might be involved in the regulation of synaptic signal transduction [50]. RanBPM interacts with brain-specific protein $p42^{IP4}$, and this interaction is controlled by the D-Ins $(1,3,4,5)P_4$, a natural specific ligand for $p42^{IP4}$. D-Ins $(1,3,4,5)P_4$ has a highly stereospecific and concentration-dependent inhibitory effect on this interaction. RanBPM and $p42^{IP4}$ were found to colocalize both in the nucleus and cytoplasm, but prominently in the cytoplasm of the cells. RanBPM might act as a modulator of $p42^{IP4}$ function to regulate synaptic plasticity, actin cytoskeleton remodeling and MAPK cascade [19]. RanBPM also interacts with calbindin D_{28K} [51] and TAF4 to regulate neuritogenesis in neural stem cells [52]. TAF4 has been implicated in neurodegeneration owing to the interaction with CAG-repeat containing factors, such as Huntingtin [53]. Such interactions result in aberrations in CREB- and Sp1-mediated transcription signaling pathways and subsequently results in Huntington's disease [54]. Thus, the interaction between RanBPM and TAF4 might enhance TAF4 and suppress CREB activity resulting in suppression of neuronal differentiation, which contributes to the pathology of Huntington's disease.

The fundamental pathological hallmark of AD is the accumulation of amyloid β (Aβ) protein. RanBPM increases beta-site APP cleaving enzyme-1 (BACE1) cleavage of amyloid precursor protein (APP) and Aβ generation both in non-neuronal and neuronal cell lines [55]. The SPRY–LisH domains of RanBPM interact with BACE1, APP and lipoprotein receptor-related protein (LRP) and increases Aβ generation. RanBPM has a crucial role in the enhancement of APP interaction with LRP and BACE1, and serves as a bridge to stabilize these multiprotein complexes. Knockdown of RanBPM significantly reduces β-secretase cleavage and Aβ level, which strongly suggests that RanBPM has a novel role as a potential therapeutic target for AD [55]. Recently, the same group reported that the N-terminal proteolytically processed form of RanBPM, which has a lower molecular weight, was found to be expressed highly in AD brains. The processed form of RanBPM having LisH dimerization domain was more potent than the full-length RanBPM in increasing β-secretase processing of APP, indicating that its expression level is crucial in driving the amyloid cascade in AD [17]. Accumulated data signify that RanBPM has a crucial role in the modulation of neuronal protein functions to maintain cell homeostasis.

The role of RanBPM in cell cycle

RanBPM is associated with CITK to regulate the progression of neuronal precursors [25]. CITK has an important role in neurogenic mitoses, and its mutation leads to severe primary

TABLE 1

List of RanBPM interactors, the methods used to investigate interaction, type of the interaction and cellular colocalization are summarized

Binding proteins	Methods	Interaction	Cellular colocalization	Refs
p73	Y2H, IP, GST	Endo/Exo	Nucleus	[12]
FMRP	Y2H, IP	Endo/Exo	Cytoplasm	[45]
Mgl-1	Y2H, IP, GST	Endo/Exo	ND	[13]
MOP	Y2H, IP	Exo	Cell membrane	[65]
Ubc9	IP	Exo	Nucleus	[31]
BACE1	IP	Exo	ND	[55]
APP	IP	Endo/Exo	ND	[55]
LRP	Y2H, IP	Endo/Exo	ND	[55]
TrkB	IP	Endo/Exo	ND	[44]
Mirk/Dirk1B	Y2H, IP, GST	Endo/Exo	ND	[37]
Raf kinase	Y2H	Exo	ND	[34]
L1	Y2H, IP, GST	Endo/Exo	Plasma membrane	[47]
PBGD	Y2H, IP	Endo/Exo	Nucleus	[59]
$Ca_v3.1\alpha1$	Y2H, IP	Endo	Cell membrane	[18]
CD39	Y2H, IP	Endo/Exo	Cell membrane	[38]
Integrin LFA-1	Y2H, IP, GST	Endo/Exo	Cell membrane	[30]
AR	IP	Exo	Cytoplasm	[4]
TR	Y2H, IP, GST	Endo/Exo	ND	[29]
MET	Y2H, IP, GST	Exo	Nucleus/cytoplasm	[5]
Rta	Y2H, IP, GST	Endo/Exo	Nucleus	[31]
TrkA	Y2H, IP, GST	Endo/Exox	ND	[40]
Plexin-A	Y2H, IP	Endo/Exo	Cytoplasm	[46]
TAF4	Y2H	Exo	Nucleus	[52]
Titin	GST	Exo	ND	[23]
CITK	Y2H, IP	Endo/Exo	Cytoplasm	[25]
Muskelin	Y2H, IP	Exo	Cytoplasm/nucleus	[26]
USP11	Y2H, IP	Endo	Nucleus	[16]
Plk	Y3H	Exo	ND	[63]
$CDK11^{p46}$	Y2H, IP, GST	Exo	Nucleus	[64]
Twa1	Y2H, IP, GST	Endo/Exo	Nucleus	[56]
ARMC8α	IP, GST	Exo	Nucleus/cytoplasm	[57]
ARMC8β	IP, GST	Exo	Nucleus/cytoplasm	[57]
p48EMLP	IP, GST	Exo	Nucleus/cytoplasm	[57]
p44CTLH	IP, GST	Exo	Nucleus/cytoplasm	[57]
$p42^{IP4}$	Y2H, IP, GST	Endo/Exo	Cytoplasm/nucleus	[19]
Dectin-1	Y2H, IP, GST	Endo/Exo	Cytoplasm	[68]
YPEL5	Y2H, IP	Endo/Exo	ND	[58]
Ax1	Y2H, IP	Endo/Exo	ND	[35]
Sky	Y2H, IP	Endo/Exo	ND	[35]
mGlu	Y2H, GST	Exo	Nucleus	[50]
Calbindin D_{28K}	Y2H, IP	Endo	ND	[51]
p75NTR	Y2H, IP	Exo	ND	[41]
HIPK2	Y2H, IP	Exo	Nucleus	[36]

Endo: endogenous; Exo: exogenous; GST: glutathione S-transferase pull down; IP: immunoprecipitation; ND: not determined; Y2H: yeast two hybrid.

microcephaly, disrupted mitoses, failed cytokinesis and cell death in neuronal precursors during the development of the central nervous system [25]. During the development of the neocortex, RanBPM was found to localize at the adherens junctions. RanBPM interacts with CITK and has a novel role in the progression of neocortical precursors through M-phase at the ventricular surface. Depletion of endogenous RanBPM enhances the number of mitotic cells and reduces number of cells entering cytokinesis at the surface of the neocortical ventricular zone. Taken together, RanBPM has a key role in polarizing CITK during mitosis to the VZ surface, and accelerates the progression of mitosis in the development of neuroepithelia [25].

Based on the RanBPM interactors and its reported functions we have predicted that RanBPM might form different complexes in different subcellular localizations, which might be involved in the regulation of the cell cycle process. RanBPM was found to be involved in a protein complex with a 20S complex [2] and further both muskelin and two-hybrid associated protein No. 1 (Twa1) were reported to participate in the same complex with RanBPM [56]. Another group characterized six more proteins interacting with RanBPM, such as muskelin, armadillo repeat containing 8α (ARMC8α), ARMC8β, 48 kD erythroblast macrophage-like protein (p48EMLP), 44 kD protein coding for CTLH motif (p44CTLH), and Twa1 from HEK293 cells. Furthermore, they identified LisH/CTLH motifs in RanBPM, muskelin, p48EMLP and p44CTLH, which are generally present in proteins involved in microtubule dynamics, cell migration, nucleokinesis and chromosome segregation [57]. RanBPM is associated with YPEL5, which is a member of the YPEL gene family that is highly conserved in the eukaryotic species that are involved in a cell division-related function [58]. RanBPM was shown to interact with porphobilinogen deaminase (PBGD), which is a component of the condensed chromatin of metaphase and anaphase cells. During cell differentiation with butyrate, the level of PBGD along with Ran and its nucleotide exchange factor RCC1 was significantly declined. Thus, it is possible that the interaction between PBGD and RanBPM might regulate the nuclear transport mechanism of PBGD [59]. Detailed information on the method used to identify interactors of RanBPM, type of interaction, and its subcellular colocalization are summarized in Table 1. This provides us with information on RanBPM complexes in different cellular environments and its regulation on various cellular processes.

Post-translational modifications of RanBPM

RanBPM is a relatively stable protein, with an estimated half-life of 99 min [16]. RanBPM was reported to undergo ubiquitination through the UPP [16]. Post-translational modification of intracellular proteins by ubiquitinating and DUBs has a key regulatory role in multiple cellular processes [60,61]. DUBs reverse the ubiquitination process by removing the ubiquitin from conjugated target proteins [62]. It has been demonstrated that ubiquitin-specific protease USP11 binds specifically to RanBPM and inhibits its

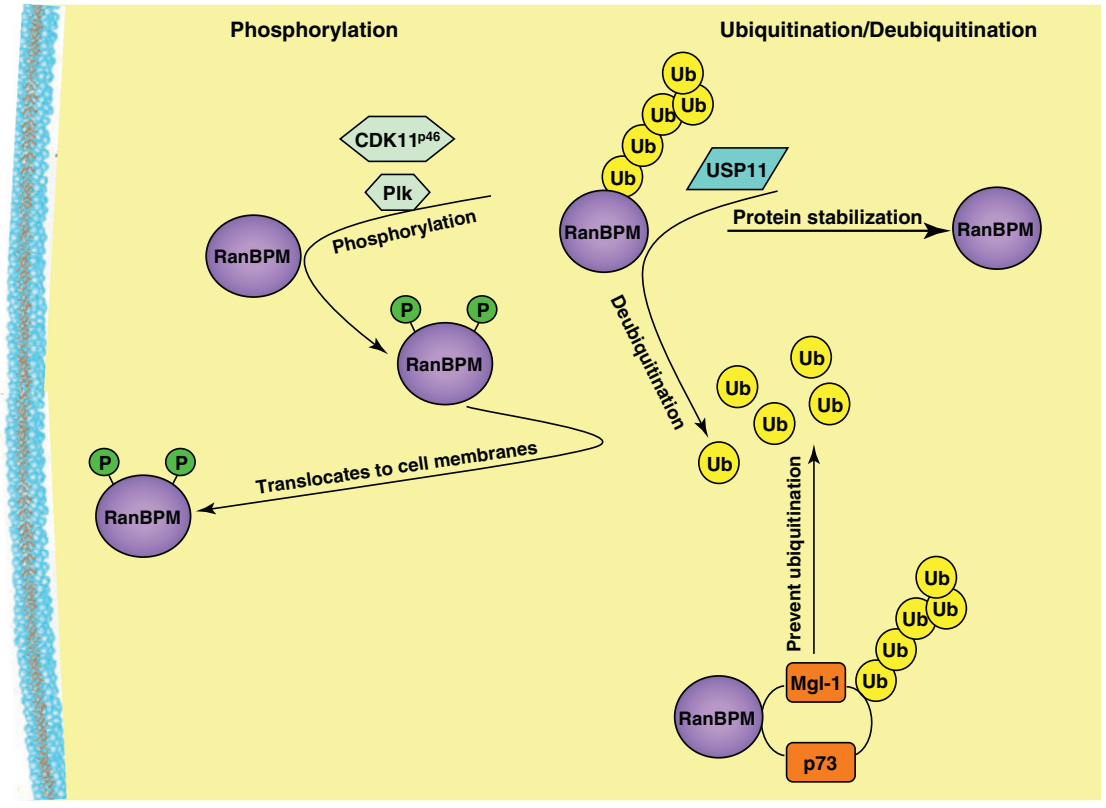

FIGURE 2

Post-translational modification of RanBPM. Phosphorylation of RanBPM regulating its localization and ubiquitination/deubiquitination of RanBPM altering its protein stability to regulate RanBPM-mediated biological functions in the cells. *Abbreivations*: Ub: ubiquitin; P: phosphate group; RanBPM: Ran binding protein microtubule-organizing center; USP11: ubiquitin-specific protease 11; $CDK11^{p46}$: cyclin-dependent kinase 11(p46); Plk: polo-like kinase; Mgl-1: mammalian lethal giant larvae-1.

ubiquitination and protein degradation, and thereby contributes to its stability by increasing its half-life [16].

RanBPM is a phosphoprotein and its phosphorylation is regulated by stress stimuli, such as ultraviolet (UV) and osmotic shock [30]. The expression of endogenous RanBPM is widely distributed in cell lines and tissues exhibiting different phosphorylation states. Most of the unphosphorylated RanBPM is readily soluble in the cytoplasm, whereas phosphorylated forms are enriched in the cell membrane [30]. Thus, the phosphorylation state of RanBPM has a significant role in determining its localization in the cells. Depending on the location of endogenous RanBPM, it has a crucial role in interacting with either cytosolic proteins or membrane proteins, which are listed in Table 1, to regulate several cellular activities for maintaining cell homeostasis. Phosphorylation of RanBPM by the kinases, such as polo-like kinase (Plk) and cyclin-dependent kinase 11(p46) ($CDK11^{p46}$) [63,64] might also regulate the protein level of RanBPM subsequently inhibiting or activating its activity. Likewise, ubiquitination and deubiquitination synergistically regulate protein degradation of RanBPM [16]. Thus, regulation of protein level of RanBPM by ubiquitination and deubiquitination system has a significant role in switching on and off several signaling pathways. Approximately 100 DUBs exist in the human proteome. In this context, one can speculate that more than one DUB might interact with RanBPM and regulate its biological functions. Therefore, to understand the diverse functions of RanBPM, identification of substrate specific ubiquitin ligases and DUBs for RanBPM is necessary. Finally, we have illustrated the phosphorylation, ubiquitination and deubiquitination of RanBPM and its importance on its localization and stability in Fig. 2.

Concluding remarks

Recently, much progress has been made in understanding the diverse role of RanBPM in regulating various cellular functions. RanBPM can interact with several signaling molecules including kinases, transcription factors, membrane receptors, adhesion molecules, structural proteins, oncoproteins, cell cycle regulators and neurological factors leading to the regulation of various signaling pathways in the cellular system. RanBPM regulates these proteins in either an inhibitory or inducing manner to maintain cellular homeostasis. The existence of several RanBPM binding partners and domains essential for such interactions and its regulatory functions are illustrated in Table 2.

RanBPM increases expression of tumor suppressor proteins, such as p73 and Mgl-1 resulting in cell cycle arrest and apoptosis in malignant cells [12,13]. Additionally, RanBPM was found to activate caspase-3 and subsequent cell death and apoptosis [10]. Overexpression of RanBPM was found to increase the Bax:Bcl-2 ratio in cancerous cells [10]. This suggests that RanBPM can be used to develop small molecules which can be administered to activate several tumor suppressing pathways in malignant cells. The capacity to trigger the apoptotic pathways might enable RanBPM small molecules to lower the apoptotic threshold and ultimately render malignant cells more susceptible to cytotoxic agents during cancer therapy.

Identification of RanBPM as a regulator of several signaling pathways during neuronal survival, differentiation, neuromuscular junction, neuritogenesis, morphogenesis and synaptic signal

TABLE 2

List of RanBPM interactors, RanBPM domains involved in the interactions and its regulatory functions, which are manually extracted from the literature, are summarized

Interactors	Regulatory action	RanBPM domains	Refs
Modulator/protein stabilizer			
p73	Protein stability and transactivation	ND	[12]
FMRP	Modulates FMRP binding to RNA	CRA	[45]
Mgl-1	Protein stability and tumor suppressing	SPRY	[13]
MOP	Regulates receptor internalization	ND	[65]
Ubc9	EBV lytic activation	ND	[31]
BACE1	APP processing and Aβ generation	SPRY/LisH	[55]
APP	APP processing and Aβ generation	SPRY/LisH	[55]
LRP	APP processing and Aβ generation	SPRY/LisH	[55]
Transcription activity			
TrkB	Kinase signaling	ND	[44]
Mirk/Dirk1B	Kinase signaling	ND	[37]
Raf kinase	Kinase signaling	ND	[34]
L1	Kinase signaling	SPRY	[47]
PBGD	Nuclear trafficking	ND	[59]
$Ca_v3.1\alpha1$	Channel-mediated signaling	ND	[18]
CD39	Nucleotide-mediated signaling	SPRY	[38]
Integrin LFA-1	Transactivation	ND	[30]
AR	Transactivation	ND	[4]
TR	Transactivation	CTLH	[29]
MET	Transactivation	SPRY	[5]
Rta	Transactivation	ND	[31]
TrkA	Transactivation	SPRY	[40]
Neurological functions			
Plexin-A	Inhibits axonal outgrowth	PRD/SPRY/LisH	[46]
TAF4	Neuritogenesis	ND	[52]
Cell cycle			
Titin	Formation of Z-disk and A/I junction	ND	[23]
CITK	Cell polarity	ND	[25]
Muskelin	Cell morphology	ND	[26]
Post-translational modification			
USP11	Deubiquitinates RanBPM	SPRY	[16]
Plk	Phosphorylates RanBPM	ND	[63]
$CDK11^{p46}$	Phosphorylates RanBPM	SPRY	[64]
Unknown functions			
Twa1	Unknown	ND	[56]
ARMC8α	Unknown	ND	[57]
ARMC8β	Unknown	ND	[57]
p48EMLP	Unknown	ND	[57]
p44CTLH	Unknown	ND	[57]
$p42^{IP4}$	Unknown	SPRY	[19]
Dectin-1 isoform E	Unknown	ND	[68]
YPEL5	Unknown	SPRY	[58]
Ax1	Unknown	ND	[35]
Sky	Unknown	ND	[35]
mGlu	Unknown	CTLH	[50]
Calbindin D_{28K}	Unknown	ND	[51]
p75NTR	Unknown	ND	[41]
HIPK2	Unknown	ND	[36]

ND: not determined.

transduction [44–47,50] indicates a potential new mechanism for developing therapeutics that can lead to rapid and specific modulation of neuronal physiology. Indeed, RanBPM was recognized as a potential therapeutic target for AD as knockdown of endogenous expression of RanBPM decreases β-secretase cleavage and Aβ levels [55]. The high expression level of RanBPM indicates that it is crucial in driving the amyloid cascade in AD [17]. A promising alternative to targeting RanBPM protein turnover itself is a valid option for AD treatment. DUBs, which are responsible for the removal of ubiquitin, act as an additional level of control over the RanBPM protein degradation. RanBPM protein degradation was prevented by USP11 resulting in the protein stabilization [16]. Remarkable recent developments on inhibitors with specific action against DUB targets, demonstrated the feasibility of selective targeting of DUBs. Therefore, an attempt to develop specific USP11 inhibitors would facilitate controlled endogenous expression of RanBPM, which might be potentially useful for the treatment of AD.

RanBPM binds to the Mu opioid receptor (MOP) and inhibits the agonist-induced receptor internalization without altering MOP signaling through adenylyl cyclase [65]. MOPs are transducers of the pharmacological effects of most clinically important opioid drugs [66]. RanBPM was also shown to bind to several mGlu receptors [50], which have been implicated in modulating morphine and related opioid drug effects [67]. A strategy to identify the specific site of interaction between RanBPM and MOP or mGlu receptors would provide novel targets for therapeutic interventions to selectively modulate MOP signal transduction.

Additionally, extensive investigation on post-translational modifications of RanBPM, such as ubiquitination, sumoylation, neddylation, ISGylation, deubiquitination, phosphorylation and acetylation is required to understand more about its regulatory mechanism. Because these post-translational modifications can regulate localization, functions and expression level of RanBPM, one can clearly understand where, when and under which biological circumstances RanBPM has particular interaction with its interacting partners to perform specific task in the cells. Taken together, RanBPM has an orchestrated role in coordinating homeostasis and contributes to manifestation of physiological processes in health and diseases.

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