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# Amphiphysin 2 (BIN1) in physiology and diseases

Ivana Prokic · Belinda S. Cowling · Jocelyn Laporte

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**Abstract** Amphiphysin 2, also named bridging integrator-1 (BIN1) or SH3P9, has been recently implicated in rare and common diseases affecting different tissues and physiological functions. BIN1 downregulation is linked to cancer progression and also correlates with ventricular cardiomyopathy and arrhythmia preceding heart failure. Increased BIN1 expression is linked to increased susceptibility for late-onset Alzheimer's disease. In addition, altered splicing may account for the muscle component of myotonic dystrophies, while recessive germinal mutations cause centronuclear myopathy. Despite undoubtedly underlining the relevance of BIN1 in human diseases, the molecular and cellular bases leading to such different diseases are unclear at present. BIN1 is a key regulator of endocytosis and membrane recycling, cytoskeleton regulation, DNA repair, cell cycle progression, and apoptosis. In light of the recent findings on the molecular, cellular, and physiological roles of BIN1, we discuss potential pathological mechanisms and highlight common disease pathways and also tissue-specific regulation. Next challenges will be to validate BIN1 both as a prognostic marker for the related diseases and as a potential therapeutic target.

**Keywords** BIN1 · Centronuclear myopathy · Myotonic dystrophy · Alzheimer's disease · Cancer · c-MYC · Dynamin

## Introduction

Amphiphysin 2, also named BIN1 (MYC box-dependent interacting protein-1 or bridging integrator-1) or SH3P9, is an ubiquitously expressed nucleocytoplasmic protein that was identified as a binding partner of the transcription factor MYC and in parallel as an interactor of a tyrosine kinase SRC SH3 ligand peptide.

BIN1 is a key regulator of different cellular functions, including endocytosis and membrane recycling, cytoskeleton regulation, DNA repair, cell cycle progression, and apoptosis. BIN1 alteration is of strong medical relevance as it was linked to cancer progression, several myopathies, heart failure, and late-onset Alzheimer's disease. Here, we focus on the different roles of BIN1 in specific tissues, in relation to BIN1-associated human diseases.

## *BIN1* gene organization and expression

*BIN1* is located on human chromosome 2q14 and mouse 18q32. The human and mouse coding sequences have high DNA and protein sequence homology (89 and 95 %, respectively) [1]. Human Bin1/amphiphysin 2 and amphiphysin 1 are members of the amphiphysin family. Amphiphysin 1 and 2 share 49 % amino acid sequence homology [2, 3]. Amphiphysin 1 is a neuronal protein involved in synaptic vesicle endocytosis [4]. Orthologues have been studied in *Caenorhabditis elegans*, drosophila, and zebrafish. The amphiphysins are structurally related to yeast RVS 167, which is involved in endocytosis [5] and is a negative regulator of the cell cycle [6].

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BIN1 is ubiquitously expressed with highest expression in skeletal muscle and brain [7, 8]. The regulatory sequence in the promoter region of human BIN1 encompasses predicted myogenin/myoD, Sp1, and serum response factor (SRF) binding sites, providing a molecular explanation for the high expression of BIN1 in striated muscle [1]. Sp1 and SRF sites are conserved in mouse, whereas the myogenin/myoD sites are substituted by Mef2 and Tef sites [1]. Moreover, in both human and mouse, a strong NF- $\kappa$ B binding site is conserved [1]. Additionally, E2F1 and c-MYC were found to modulate transcription of BIN1 [9, 10].

The *BIN1* gene has 20 exons which can be spliced into multiple isoforms (Fig. 1). Exons 7, 13, 14, 15, and 16 are brain specific [8, 11, 12]; whereas, exon 11 is a muscle-specific exon [1, 13, 14]. Mis-splicing of exons 7 and 11 was identified in muscles from myotonic dystrophy patients [15], while exon 13 is often mis-spliced in cancer resulting in lack of c-MYC binding [16]. Isoforms 1–7 are expressed in brain. The muscle-specific isoform is isoform 8; whereas, isoforms 9 and 10 are ubiquitously expressed.

#### BIN1 domain organization and protein regulation

BIN1 has several distinctive protein domains (Fig. 1). The N-BAR (BIN-amphiphysin/Rvs) domain encoded by exons 1 to 10 is ubiquitously expressed, binds lipid membranes in a dimer conformation, and has a role in generating and sensing membrane curvature [17, 18]. The N-terminal amphipathic helix inserts into the membrane and promotes curvature, while the positively charged surface of the BAR domain interacts with the negatively charged membrane to sense and maintain the curvature [18]. Dimerization of the BAR domain is a prerequisite for this function. The BAR domain is involved in homo or hetero dimerization (with amphiphysin 1) [19, 20], and the deletion of *AMPH1* lead to loss of both AMPH1 and BIN1 in brain [21].

The phosphoinositide (PI) binding motif is a short polybasic sequence encoded by the muscle exon 11 [8, 13, 22]. The PI motif increases BIN1 affinity towards the negatively charged lipids PtdIns (4, 5) P<sub>2</sub> [23] and/or PtdIns3P and PtdIns5P [15]. This motif may potentially target BIN1 to membrane compartments such as T-tubules, muscle-specific plasma membrane invaginations. Additionally, it could provide a muscle-specific regulation of BIN1 conformation through binding to the SH3 domain and phosphoinositides [24].

The clathrin and AP2 (CLAP) binding domain encoded by exons 13–16 is found only in brain isoforms [8, 11, 25], and it is responsible for binding to endocytic proteins clathrin and AP2 [19].

The Myc-binding domain (MBD) is encoded by exons 17 and 18 [7]. Alternative splicing of exon 17 leads to the loss of this interaction [26] [13].

The Src homology 3 (SH3) domain encoded by the last two exons (19 and 20) is present in all isoforms. SH3 domains bind

proline-rich motifs [27]. The SH3 domains of amphiphysin 1 and 2 differ from other SH3 domains due to a large patch of negative electrostatic potential and an unusually extended n-Src loop [3].

BIN1 functions are regulated through specific exon splicing. Open and closed conformations of BIN1 have been suggested to be regulated by inclusion of the PI motif and integrity of the SH3 domain [24, 28]. In isoform 8, the BAR+PI domains bind the SH3 domain intramolecularly and compete with SH3 interactors, and PtdIns (4, 5) P<sub>2</sub> binding to the PI motif induces the open conformation and interaction with protein effectors as dynamin on PtdIns (4, 5) P<sub>2</sub> enriched membranes [24]. BIN1 mutations causing centronuclear myopathy and found in the SH3 domain affect BIN1 conformation and binding to dynamin [22, 28]. Differential splicing of exon 7 in the BAR domain also modulates protein-protein interaction with dynamin [12]. In addition, BIN1 may also be regulated by phosphorylation [29, 30].

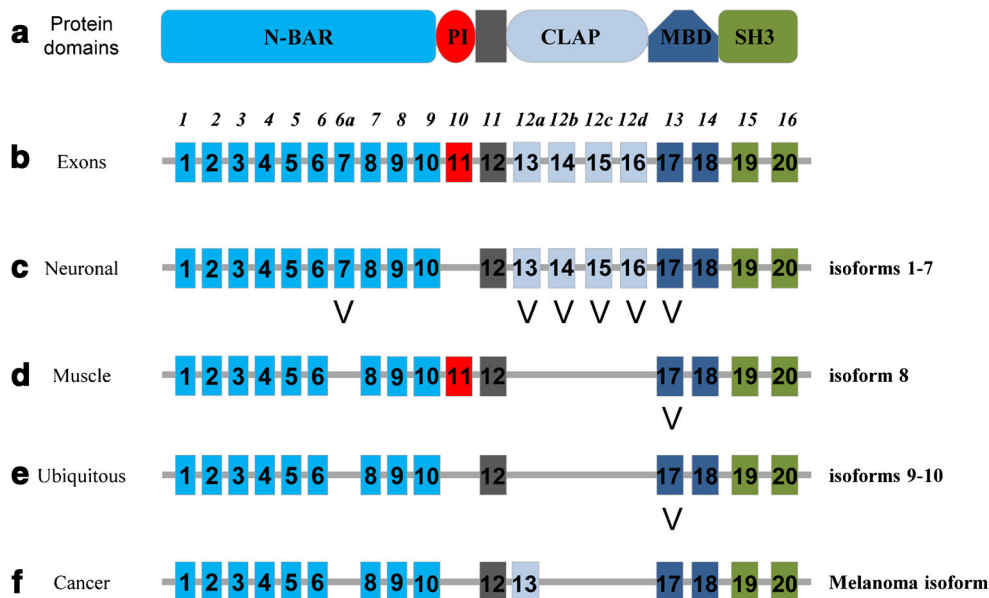
#### *BIN1* cellular functions

##### *Membrane trafficking and membrane remodeling*

The most characterized function of amphiphysins is in endocytosis. Amphiphysins are involved in endocytosis in neuronal and non-neuronal cells [4, 31–33] through interaction with several proteins associated with clathrin-coated pits: dynamin [31], AP2 adaptor complexes [31, 33], clathrin [25, 34], synaptojanin [35], and endophilin/SH3GL2 [36, 37] (Table 1). Noteworthy, dephosphorylation of amphiphysin 1 and BIN1 by calcineurin is necessary to induce endocytosis [29]. Moreover, exogenous expression of the amphiphysin SH3 domain alone inhibits dynamin-dependent transferrin uptake [3]. The mutual exclusivity for clathrin or dynamin binding may be correlated to their non-overlapping sites at the clathrin-coated pit [34]. Thus, it is believed that amphiphysins orchestrate the sequential recruitment and action of effectors in parallel to membrane deformation, at sites of membrane fission [38].

However, knockdown of *BIN1* in HeLa cells or knockout (KO) in MEF cells did not alter significantly transferrin uptake, but increased the intracellular transferrin levels due to defects in transferrin receptor recycling [39, 40]. Similarly, the only *C. elegans* orthologue named AMPH-1 colocalizes and directly interacts with RME-1 (dynamin-like protein and EHD orthologue) to initiate endosome tubulation and membrane recycling [39]. Thus, depending on the context, amphiphysins control endocytosis and/or recycling (Fig. 2). Functional interaction of EHD1 and BIN1 is further sustained by the finding that EHD1 mediates vesicle trafficking and development or maintenance of T-tubules in muscle; EHD1 loss impairs T-tubule structure and BIN1 localization [41].

The role of BIN1 in membrane trafficking reflects its ability to regulate membrane remodeling. In vitro studies have



**Fig. 1** *BIN1* functional domains and tissue-specific isoforms. **a** Protein domains of *BIN1*: The *N-BAR* domain is involved in membrane binding and tubulation; The *PI* motif is skeletal muscle-specific, and it is involved in phosphoinositides binding; The *PS* motif is a Proline-Serine rich region encoded by exon 12 and present in all isoforms; The *CLAP* domain is involved in clathrin and AP2 binding and is only present in neuronal

isoforms; The *MBD* domain binds c-Myc, and it is involved in its regulation; and the *SH3* domain interacts with Proline-rich domains found in proteins such as dynamin 2. **b** Gene organization of *BIN1*. The main nomenclature used is from NCBI and in italic is the nomenclature by Weschler-Reya et al.1997. Transcript isoforms found in brain (**c**), skeletal muscle (**d**), most of other tissues (**e**), and in melanoma (**f**). Alternative exons are indicated

shown that the N-BAR domain of amphiphysin and endophilin binds and evaginates lipid membranes into narrow tubules [42–44]. Exogenously expressed amphiphysin 1 or the brain isoform of BIN1 in Chinese hamster ovary (CHO) cells exhibit diffused and cytosolic distribution. Conversely, overexpression of BIN1 muscle isoform 8 in CHO and C2C12 muscle cells leads to its strong concentration at the cell surface and formation of numerous narrow tubules that are prevented by cholesterol depletion. Electron microscopy (EM) and correlative microscopy identified that these membrane tubules are connected to the plasma membrane [23, 45], resembling the T-tubules found in skeletal muscle. Deletion of the PI motif strongly decreases membrane tubulation [15, 46, 47], implying that the PI motif promotes BIN1-dependent membrane tubulation. Overall, a tight regulation of BIN1 controls the balance between membrane fission and trafficking versus membrane tubulation, most probably in a tissue-specific manner.

#### Cytoskeleton network

Several studies have suggested a potential role for BIN1 in regulating the actin cytoskeleton. In particular, amphiphysin 1 directly binds N-WASP [48] and regulates actin polymerization during phagocytosis [49]. Moreover, the yeast amphiphysin orthologue RVS167 is involved in actin dynamics regulation in response to environmental signals [6, 50].

BIN1 may also modulate the microtubule (MT) network through binding via its BAR domain to the coiled-coil region of CLIP170, a plus-end protein involved in MT stability [46]. BIN1 membrane tubulation in HeLa cells is sensitive to nocodazole (MT depolymerizing agent), and depletion of CLIP170 decreased BIN1 tubulation capacity [46]. Moreover, in human neuroblastoma cells and in mouse brain, BIN1 colocalizes and interacts with TAU, a microtubule-associated protein promoting microtubule assembly and stabilization and implicated in Alzheimer's disease (AD) [51]. While mechanistic data are needed, these studies support the hypothesis that BIN1 coordinates membrane and cytoskeleton remodeling.

#### DNA repair, cell cycle, and apoptosis

In addition to cytosolic functions, BIN1 has several roles in the nucleus (Fig. 2). BIN1 isoform 9 that lacks the PI motif and maintains a functional MYC-binding domain (without exon 11 and with exon 17) was reported to localize mainly in the nucleus of proliferating cultured cells [26, 52]. Indeed, the MBD of BIN1 interacts with the MB1 domain of c-MYC, a transcription factor with the basic helix-loop-helix-leucine zipper (bHLH-LZ) structure that plays a central role in cell growth, apoptosis, and malignancy [7]. Only isoforms of BIN1 which localize in the nucleus can activate programmed cell death [16, 53].

**Table 1** Amphiphysin 2 (BIN1) interactors and regulated functions

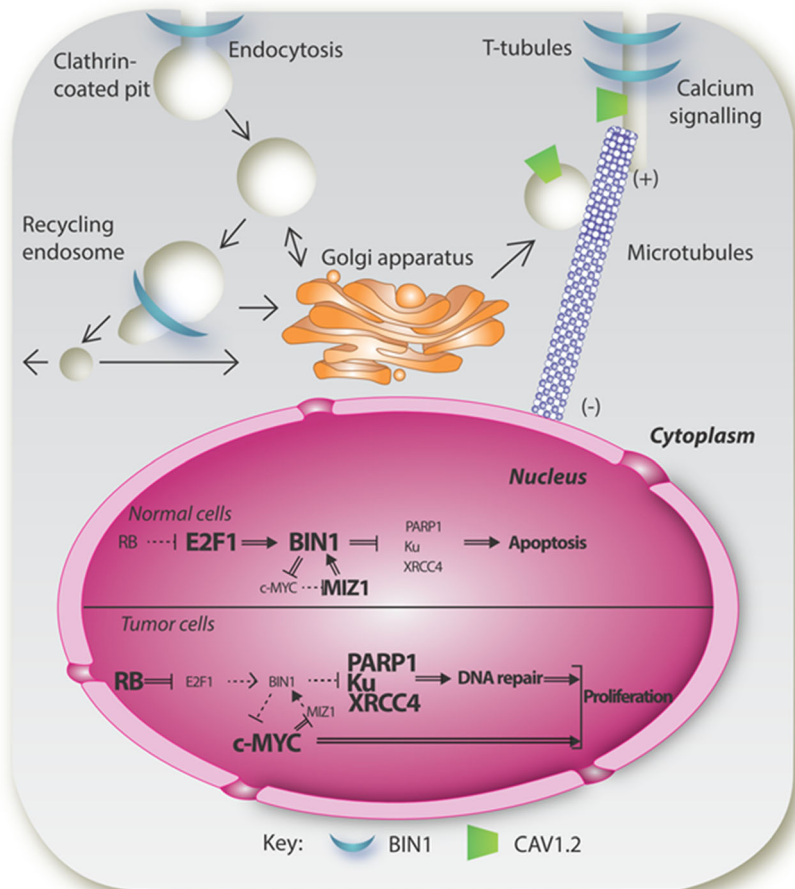
NCBI gene name	Protein	Interaction site	Function	Reference
BIN1	Amphiphysin 2	BAR+PI with SH3; BAR with BAR	Membrane remodeling	Kojima et al. 2004 [24]; Royer et al. 2013 [28]; Ramjaun et al. 1999 [99]
MTM1	Myotubularin	BAR+SH3	Membrane remodeling	Royer et al. 2013 [28]
AMPH1	Amphiphysin 1	BAR	Endocytosis	Wigge et al. 1997 [33]
AP1	AP-1, adaptin	CLAP	Endocytosis	Huser et al. 2013 [100]
AP2	AP-2, adaptin	CLAP	Endocytosis	Wigge et al. 1997 [33]
CLTC	Clathrin heavy chain	CLAP	Endocytosis	Ramjaun et al. 1998 [19]
DNM1	Dynamin 1	SH3	Endocytosis	Grabs et al. 1997 [101]
DNM2	Dynamin 2	SH3	Endocytosis	Dong et al. 2000 [102]; Kojima et al. 2004 [24]
RIN2	Ras and Rab interactor 2	SH3	Endocytosis	Kajiho et al. 2003 [103]
RIN3	Ras and Rab interactor 3	SH3	Endocytosis	Kajiho et al. 2003 [103]
SH3GL2	Endophilin A1	SH3	Endocytosis	Micheva et al. 1997 [36]
SH3GLB1	Endophilin B1	SH3	Endocytosis	Micheva et al. 1997 [36]
SNX4	Sorting nexin 4	SH3	Endocytosis	Leprince et al. 2003 [104]
SYNJ1	Synaptojanin 1	SH3	Endocytosis	McPherson et al. 1996 [35]
SOS1	Son of sevenless homolog 1		Endocytosis, signal transduction	Leprince et al. 1997 [2]
SOS2	Son of sevenless homolog 2		Endocytosis, signal transduction	Leprince et al. 1997 [2]
CDK5	Cyclin-dependent kinase 5		Signal transduction	Fernando et al. 2009 [81]
PLD1	Phospholipase D1		Signal transduction	Lee et al. 2000 [105]
PLD2	Phospholipase D2		Signal transduction	Lee et al. 2000 [105]
PP3CA	Calcineurin		Signal transduction	Cousin et al. 2001 [106]
ITGB1	Integrin alpha 3 beta 1		Cellular adhesion	Wixler et al. 1999 [107]
PTK2	FAK, focal adhesion kinase	SH3	Cellular adhesion	Messina et al. 2003 [108]
CLIP1	CLIP170, CAP-Gly domain-containing linker protein	BAR	Cytoskeleton	Meunier et al. 2009 [46]
CACNA1C	CAV1.2, voltage-dependent calcium channel		Channel transport	Hong et al. 2010 [85]
ACTA1	Actin alpha 1		Sarcomere assembly	Fernando et al. 2009 [81]
MHC	Myosin heavy chain		Sarcomere assembly	Fernando et al. 2009 [81]
TTN	Titin		Sarcomere assembly	Fernando et al. 2009 [81]
KU	Ku	BAR	DNA repair	Ramalingam et al. 2007 [59]
PARP1	Poly (ADP-ribose) polymerase 1	BAR	DNA repair	Pyndiah et al. 2011 [10]
XRCC4	X-ray repair cross-complementing protein 4		DNA repair	Grelle et al. 2006 [60]
ABL1	c-ABL	SH3	Tumor suppressor	Kadlec et al. 1997 [56]
MYC	c-MYC	MBD	Tumor suppressor	Sakamuro et al. 1996 [7]
MYCN	N-MYC	MBD	Tumor suppressor	Hogarty et al. 2000 [69]

Interestingly, overexpression of BIN1 in malignant but not in non-malignant cells induced apoptosis [53, 54]. BIN1 can inhibit cell transformation in a caspase-independent cell death process through both Myc-dependent and Myc-independent mechanisms [55]. The cell death program engaged by BIN1 is susceptible to the serine protease inhibitor AEBSF and to inhibition by the SV40 large T antigen [53]. In addition, BIN1 interacts with the proto-oncogene c-ABL, and BIN1 overexpression results in morphological transformation of NIH 3T3 fibroblasts in a c-ABL-dependent manner [56].

Moreover, BIN1 is part of the transforming pathway induced by the adenovirus gene product E1A. E1A can inactivate the tumor suppressor retinoblastoma protein (RB), leading to the decreased release of the DNA-binding factor E2F1 and the subsequent failure in activation of BIN1 transcription [9, 57], which again leads to the decreased inhibition of c-MYC and potentially impaired cell cycle control. Interestingly, while BIN1 interacts with and inhibits c-MYC [10], BIN1 may also impact directly on the activation of c-MYC target genes.



**Fig. 2** BIN1 cellular roles and proposed mechanisms of linked diseases. BIN1 is implicated in membrane remodeling for intracellular trafficking (*top left*), or for T-tubule maturation or maintenance (*top right*). BIN1 also directly regulates *c-MYC* nuclear function and proteins implicated in DNA repair (*bottom*), leading to a tight balance between proliferation and apoptosis



Additionally, in transformed cells, *c-MYC* can repress *BIN1* transcription which further facilitates cell transformation [10].

Other studies suggested that BIN1 plays an important role in DNA repair by binding and inhibiting poly (ADP-ribose) polymerase (PARP1), a key component of the base excision repair pathway, via the BAR domain [10, 58]. Depletion of endogenous BIN1 abolishes cisplatin-induced cell death via activation of *c-MYC* and PARP1 [10]. *BIN1* downregulation also reduces cell sensitivity to the DNA-damaging chemotherapeutic agents etoposide and doxorubicin [10]. BIN1-dependent decrease of intrinsic PARP1 activity may sensitize cancer cells to DNA damage [10]. In addition, BIN1 interacts with two other proteins important for DNA repair in the non-homologous end-joining (NHEJ) pathway, Ku and XRCC4 [59, 60], strengthening a role for BIN1 as a regulator of DNA repair.

Additional evidence of a role for BIN1 in cell cycle regulation came from studies of the fission yeast homologue *hob1* (homologue of *Bin1*). *Hob1p* was shown to be dispensable for actin organization and endocytosis but mutants were hypersensitive to starvation or genotoxic stress [61]. In particular, *hob1* mutants were more susceptible to DNA damage and failed to undergo G1

arrest after DNA damage [61]. BIN1 but not amphiphysin 1 rescued this phenotype, confirming an evolutionary conserved role for BIN1 in cell cycle regulation [61].

#### Physiological functions of *BIN1* and physiopathology of related diseases

##### *BIN1* and cancer

BIN1 expression is reduced or altered in several cancer types including breast, colon, prostate and lung cancers, hepatocarcinoma, and neuroblastoma [62–65]. Loss of heterozygosity or mis-splicing of BIN1 also correlates with cancer prognosis and increased metastasis [16, 65–67].

In a mosaic *Bin1*-null mouse, mammary gland specific *Bin1* deletion delays the differentiation of the glandular ductal network during pregnancy but does not increase tumor susceptibility [62]. Nevertheless, when tumor formation is initiated by the carcinogen DMBA, mice lacking *Bin1* developed more aggressive tumors which are characterized by an increase in proliferation, survival, and motility [62].

When present at a normal level, BIN1 can inhibit MYC-dependent transformation and tumor growth [7, 68]. BIN1 also regulates another member of MYC family of oncogenes, N-Myc [69], which contains the N-terminal domain and MYC box 1 needed for the interaction with BIN1 [7]. N-Myc is highly expressed in neurons and overexpressed in aggressive neuroblastoma [70, 71]. In 6/8 neuroblastoma cell lines overexpressing N-Myc, BIN1 is strongly downregulated, while its overexpression in these cells leads to apoptosis [72].

Decrease of *BIN1* may contribute to malignant development via the loss of processes required for terminal differentiation (through downregulation of p21 and p53), and by contributing to Myc deregulation and loss of control over NHEJ repair. Cisplatin-based chemotherapy is a commonly used approach for the various human malignancies; however, many cancer patients respond poorly to this treatment [73]. *BIN1* downregulation was shown to increase resistance to this therapy, most likely through loss of control over the DNA repair mechanism [10]. Quantification of *BIN1* expression may thus be an important marker to predict cisplatin sensitivity. Additionally, increasing the expression of *BIN1* may be a novel therapeutic strategy for treatment of cisplatin-resistant cancers [74].

In addition to the nuclear functions discussed above, BIN1 could be implicated in tumor cell migration and invasion through cytoskeleton and membrane remodeling. Altogether, it appears that *BIN1* decrease does not strongly increase the risk of tumor formation but favors tumor progression [75].

### *BIN1 in skeletal muscle and myopathies*

BIN1 is implicated in two myopathies: centronuclear myopathy (CNM) and myotonic dystrophy (DM). Germline mutations cause autosomal recessive centronuclear myopathy (ARCNM), a rare congenital disease associated with non-progressive muscle weakness with onset at birth or infancy [22, 47, 76]. Patient muscle presents histologically with increased centralized nuclei and fiber atrophy not linked to excessive muscle regeneration [47, 77]. No history of cancer has been noted in patients, and potential cardiac involvement was reported in only one patient [22, 47, 76, 78]. To date, the following homozygous mutations have been reported: missense changes in the amphipathic helix and BAR domain (K35N, D151N, and R154C) [22, 76], stop codon mutations in the last exon truncating the SH3 domain [22, 78], and a mutation in the exon 11 donor splice site leading to exon 11 skipping [47]. While BIN1 expression appears not altered, mutations in N-BAR or skipping of the PI motif strongly decreases the membrane tubulation properties of BIN1, while the SH3 truncations impair both intramolecular binding and binding to dynamin 2. Noteworthy, dynamin 2 is a GTPase implicated in cytoskeleton and membrane remodeling and mutated in autosomal dominant CNM [79].

Myotonic dystrophies are multisystemic diseases characterized by progressive muscle wasting, myotonia, cataracts, and heart conduction defects. Congenital myotonic dystrophy (CDM1) and myotonic dystrophy of type 1 (DM1) or of type 2 (DM2) are caused by the expression of mutant RNAs containing expanded CUG or CCUG repeats that sequester splicing factors as muscleblind-like-1 (MBNL1), resulting in aberrant splicing of other mRNAs, including *BIN1*. In DM patient muscles, the *BIN1* embryonic isoform containing exon 7 and lacking exon 11 is persistently expressed [15]. As centronuclear myopathy and myotonic dystrophy share several clinical and histopathological features such as muscle weakness and centralized myonuclei, alteration of BIN1 represents a potential common molecular mechanism.

*BIN1* is highly expressed in skeletal muscles, where expression increases during in vitro differentiation of muscle cells, and *Bin1* knockdown inhibits myoblast fusion and differentiation [7, 8, 23, 26]. The inclusion of the muscle-specific PI motif during muscle differentiation and its importance for membrane tubulation also indicate a significant role for BIN1 in membrane remodeling in muscles [8, 26]. In addition, BIN1 localizes to the triad, the membrane structure sustaining the excitation-contraction (EC) coupling machinery [8].

Several studies in animal models revealed the physiological importance of BIN1 in skeletal muscles. *Drosophila* with a deletion of the unique amphiphysin orthologue (*amph*) is viable but flightless and generally sluggish [44]. *Drosophila* AMPH is localized on the postsynaptic membrane of the neuromuscular junction and on the T-tubule network, and adult *amph* mutants have severe defects in T-tubule organization and EC coupling, without any strong defects in synaptic vesicle endocytosis or recycling, suggesting *Drosophila* AMPH is functionally closer to mammalian BIN1 than to AMPH1 [44]. Concordantly, knockdown of *Bin1* in adult mouse flexor digitorum brevis (FDB) muscle leads to alteration of T-tubule structure and  $\text{Ca}^{2+}$  levels [80]. Similarly, U7-antisense-induced exon 11 skipping in mouse tibialis anterior induces a defect in T-tubule organization and DHPR distribution and a decrease in muscle strength while muscle mass and fiber size are unaffected [15]. Recently, a BIN1 canine model was reported with a spontaneous mutation affecting exon 11 splicing, reducing the overall BIN1 expression and resulting in the inherited myopathy of Great Danes, a late onset and very progressive myopathy [47]. A high number of centrally located nuclei was detected, reminiscent of CNM, associated with abnormal sarcolemmal invaginations and structural defects in T-tubules [47]. T-tubule defects and altered localization of T-tubule markers (RYR1, DHPR, and CAV3) are also noted in patients with CNM [14, 47]. Taking together the molecular data indicating BIN1 is a membrane tubulating protein and the consistent data from animal models and patients, it appears BIN1 is essential in the positioning and remodeling of T-tubules. BIN1 may also have additional roles in skeletal muscle, for example in sarcomere organization, as suggested through

transgenic overexpression of the BIN1 SH3 domain in mice [81], or in autophagosome maturation [47].

#### *BIN1 in heart and cardiac failure*

Decreased BIN1 expression was observed in patients with ventricular arrhythmia. Plasma BIN1 level appears correlated with disease progression and advanced heart failure in patients, although these recent findings await confirmation [82, 83]. Moreover, BIN1 mRNA and protein expression was significantly decreased in failing cardiomyocytes [84]. BIN1 also localizes to T-tubules in cardiac muscle and knockdown in cardiomyocytes reduces the distribution of DHPR (CAV1.2) to the T-tubules and delays calcium influx [85]. Thus, in addition to a structural role at the T-tubules, BIN1 may regulate DHPR transport to the T-tubules.

Preliminary data sustained a physiological role for BIN1 in heart function. Injection of a *bin1* antisense morpholino in zebrafish induces a strong cardiac phenotype, with altered calcium transient and contractility and a significant decrease in heart rate [84]. Complete KO of *Bin1* in mice is perinatally lethal [40]. An increased thickness and occlusion of both ventricular chambers was identified, suggesting mice die from ventricular cardiomyopathy. As genetic decrease of *Bin1* specifically in mouse skeletal muscle also leads to perinatal death (Prokic et al, unpublished), the contribution of the cardiac phenotype to survival remains to be investigated.

#### *BIN1 in brain and Alzheimer's disease*

Recently, large scale genome-wide association studies (GWAS) linked *BIN1* to late-onset Alzheimer's disease (LOAD) [86–93]. Alzheimer's disease (AD) is the most common cause of dementia, affecting 13 % of the population older than 65 years of age and 30–50 % of the population older than 80 years. LOAD is characterized by a large number of senile plaques and neurofibrillary tangles in the brain [94]. *BIN1* is the second most important susceptibility locus for LOAD after apolipoprotein E (*APOE*) (<http://www.alzgene.org/>) [95]. SNPs upstream of *BIN1* correlate with a higher risk to develop LOAD, and to increased *BIN1* transcript levels [51, 95]. Altered expression of *BIN1* was demonstrated in aging mice, transgenic AD models, and AD brains. Whether BIN1 may be a biomarker or a therapeutic target for Alzheimer's disease remains to be investigated. Mammalian models to confirm the implication of BIN1 in Alzheimer's disease and investigate the normal role of BIN1 in mammalian brain are required.

BIN1 was shown to interact with the microtubule-associated protein TAU (MAPT) in drosophila, and while altered *amph* expression did not modify the A $\beta$ 42-induced rough eye and neurodegeneration phenotype, loss of *amph* was able to suppress TAU-induced neurotoxicity [51]. The authors hypothesized BIN1 overexpression could disrupt vesicle transport at the

synapse, promoting TAU sequestration at the membrane. Importantly, these results associate BIN1 modulation of the AD pathogenesis with TAU signaling. BIN1 and several proteins recently linked to AD appear interconnected through multiple interacting proteins and share related functions on membrane trafficking, sustaining a potential central role of BIN1 in AD [96]. A recent study linked PtdIns3P, a regulator of membrane trafficking, with AD [97]. Interestingly, PtdIns3P is also implicated in CNM as the PtdIns3P phosphatase myotubularin is mutated in X-linked CNM [98]. These findings suggest a PtdIns3P-BIN1 signaling pathway is common to both CNM (muscle) and AD (brain).

#### Conclusion and pending questions

BIN1 is linked to several human diseases affecting different tissues. Downregulation is linked to increased cancer progression and may correlate with ventricular cardiomyopathy and arrhythmia preceding heart failure, while increased expression increases susceptibility to develop late-onset Alzheimer's disease. Altered splicing may account for the muscle compound of the multisystemic phenotypes in myotonic dystrophies, and germline mutations cause congenital centronuclear myopathy. While it undoubtedly underlines the medical importance of BIN1 in human diseases, the molecular and cellular bases leading to such different diseases linked to alterations in BIN1 are unclear at present and represent an exciting area of future research. A more precise clarification of BIN1 isoforms, tissue-specific regulation, and interactors will be needed, together with the generation of mammalian models to test the pathological hypotheses summarized in this review. Future translational research should aim to validate BIN1 as a prognostic marker for the related diseases and as a potential therapeutic target.

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**Conflict of interest** The authors declare that they have no conflict of interests.

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