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REVIEW

Membrane occupation and recognition nexus (MORN) motif controls protein localization and function

Jinrun Zhou, Honghong Liu, Yushuang Lin and Jing Zhao

Shandong Provincial Key Laboratory of Animal Cells and Developmental Biology, School of Life Science, Shandong University, Qingdao, China

Correspondence
J. Zhao, Shandong Provincial Key Laboratory of Animal Cells and Developmental Biology, School of Life Science, Shandong University, Qingdao 266237, China
Tel: +86 531 88371718
E-mail: jingzhao@sdu.edu.cn

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The membrane occupation and recognition nexus (MORN) motif was first defined in 2000, when it was identified in the junctophilin protein family. Dozens of studies have been published ever since, mainly focusing on the function of a given MORN motif-containing protein in parasites, plants or animal cells. Proteins with MORN motifs are not only expressed in most animal and plant cell types, but also significantly differ in their intracellular localization, suggesting that the MORN motifs may fulfill multiple physiological functions. Recent studies have found that MORN motif-containing proteins junctophilin-1/2 and MORN3 play a role in cardiac hypertrophy, skeletal muscle fiber stability and cancer. Hence, MORN motif-containing proteins may be exploited to develop improved treatments for various pathological conditions, such as cardiovascular diseases. Here, we review current research on MORN motif-containing proteins in different organisms and provide both ideas and approaches for follow-up exploration of their functions and applications.

Keywords: animals; cancer; electrostatic interactions; hydrophilicity; MORN motif; parasites; plants; post-translational modification; spatial conformation

The membrane occupation and recognition nexus (MORN) motif was first identified as a special sequence of 14 amino acids (Y-Q/E-G-E/Q-T-X-N-G-K-X-H-G-Y-G) in 2000 [1], even though most published work primarily concerning *Trypanosoma brucei* MORN1 (TbMORN1) and MORN4 has confirmed that a single MORN repeat consists of 23 amino acids with a highly-conserved GxG sequence at residues 12–14 [2-4]. MORN motifs can be found in various positions [5-7] and many MORN motif-containing proteins have been reported in plants, animals and protists [8]. The MORN motifs vary in number (from 2 to 20) in different proteins and are involved in many biological functions [8-12]. However, how different MORN motifs impact the functional properties of MORN motif-containing proteins is poorly understood.

One important feature of the MORN motif is its membrane-targeting function, which has been widely verified [1,6]. For example, plant phosphatidylinositol kinases (PIPKs) contain MORN motifs and can localize to the plasma membrane or other biomembranes, whereas PIPKs from animals and yeast, which have lost the MORN motifs, lack the membrane-location ability [6]. Junctophilin (JPH)2 was initially discovered as a structural protein holding a membrane anchoring domain (the MORN motif) that connects T-tubules with the sarcoplasmic reticulum (SR) membrane [1]. Recently, JPH2 was shown to contain additional regulatory domains that extend the role of JPH2 beyond that of a structural protein: the MORN domain was hydrolyzed after stress, thereby releasing regulatory domain of JPH2 from the membrane, which then translocated into the nucleus to transduce mechanical information into transcriptional reprogramming in the stressed heart [13]. In HeLa and skeletal muscle cells, JPH1 also localizes at the membranes *via* MORN motif-mediated interactions with phosphatidylinositol-4, 5-bisphosphate (PIP2) [14]. The above-mentioned

examples illustrate the role of the MORN motifs in mediating membrane localization of proteins.

Proteins containing MORN motifs are found on the plasma membrane and biomembranes of several organelles, including chloroplast and endoplasmic reticulum, etc. The different location is vital for MORN motif-containing proteins to exert different functions. Here, we reviewed the studies of MORN motif-containing proteins in various organisms and tissues over the past 20 years, analyzed and discussed controversial issues, which will provide a basis for future studies on MORN motif-containing proteins.

Relationship between the structure and function of MORN motif-containing proteins

How MORN motif-containing proteins bind to membranes has long been a point of controversy. Ma *et al*. [6] confirmed that the MORN motifs in *Oryza sativa* phosphatidylinositol 4-phosphate 5-kinase 1 (OsPIPK1) were connected in two ways: closely connected or connected with a joint sequence (large stretches of intervening sequence), with the interaction of OsPIPK1 with target components on the plasma membrane (PM) depending on the number and/or sequence of MORN motifs. To clarify whether the number of MORN motifs directly affects the protein's localization and even function, we evaluated the location and function of proteins with different numbers of MORN motifs (Table 1). The results showed that proteins with different numbers of MORN motifs have huge differences in localization and function. There appears to be no direct relationship with the number of motifs. It is worth noting that the endoplasmic reticulum (ER)/-SR-PM junction did not correlate with the number of motifs in JPH2, but depended on *S*-palmitoylation in MORN motifs [15]. Does the differential MORN sequence determine the function and localization of a MORN motif-containing protein? Along with the noted studies, the importance of the MORN motifs and the joint sequence attracted more attention. JPH2 was reported to have multiple localizations and functions, although its exact role and mechanism in plasma membrane binding remain unknown [15-19]. Here, we took JPH2 as an example to analyze the possibility and reasons why different sequences affect the location and function of MORN proteins. We mainly studied the influences of MORN motifs and the joint sequence on the post-translational modifications and structures of MORN proteins. First, we observed the amino acids homology of JPH2 in 15 species. The results showed that the homology of JPH2 was significantly different among these species (56.1–99.4%) (Fig. 1A). We selected three species (human, mouse and rat) commonly used in experiments to analyze the homology among MORN motifs (motif1-motif8) in JPH2. The results showed that the homology of the MORN motifs among these three species was 92.4–98.4%, and the amino acid positions of these differences mainly existed in the joint sequence (Fig. 1B). Subsequent prediction of the post-translational modification site in JPH2 showed that there are various differential modification sites in the MORN motifs and the joint sequence (Fig. 1C), which suggested that differential modification might be the main factor affecting JPH2 location and functions. Among them, *S*-palmitoylation in JPH2 has been reported to play a key role in regulating SR contacts with the plasma membrane [15,20]. To reveal the popular roles of palmitoylation, we also analyzed the palmitoylation in other MORN proteins. It is predicted that the MORN motifs and joint sequence in OsPIPK1 both contain typical palmitoylation modification sites (motif7: C183 and C194; motif9: C321; joint sequence: C301; https://prosite.expasy.org; CSS-PALM 4.0; http://csspalm.biocuckoo.org). Whether the palmitoylation modification determines the localization and function of OsPIPK1 deserves further verification. Moreover, palmitoylation sites exist in the MORN motifs and the joint sequence of JPH1 (motif4: C101; motif8: C318; joint sequence: C264 and C267), at the same time as existing in MORN motifs of JPH2 (motif1: C15 and C29; motif8: C328). The latest reports showed that two of the proposed palmitoylation sites of JPH2 are buried, and the remaining site is at the interface with the CaV1.1 peptide, which also suggested that another mechanism is responsible for the membrane target function [19]. We speculate that the post-translational modification (not just palmitoylation) of the MORN motifs and the joint sequence may jointly affect the biological function of the MORN proteins.

Studies have shown that the hydrophilicity and electrostatic interactions of amino acids in the MORN motifs may bind and aggregate PIP2 or affect the conformation of MORN proteins, such as the U-shape in MORN4 and the V-shape in *Toxoplasma gondii* MORN1 and *Plasmodium falciparum* MORN1 [2,3,19,21]. Accordingly, we analyzed the differences in the secondary structure of the JPH2 motifs (motif1 to motif8) in human, rat, and mouse. First, hydrophilicity analysis showed that human and rat JPH2 exhibited low hydrophilicity and hydrophobicity at sites 185–195 and no hydrophilicity and low hydrophilicity at sites 225–235, whereas mouse showed high hydrophilicity at

Table 1. Localization and function of proteins with different membrane occupation and recognition nexus (MORN) motif copies.

Number of MORN motifs	Name	Localization/target protein	Function	References
Motif 2	MOPT	Sperm	Dynamic regulation of acrosome biogenesis during late spermiogenesis	Choi *et al.* [12]
Motif 3	MORN3; ARC3; SET7	P53; PARC6; Sam68	Combine and modify P53 to exert anti-tumor function; promote the remodeling of the Z ring during chloroplast division; interacts with Sam68 and methylates it	Liang *et al.* [11]; Chen *et al.* [27]; Vasileva *et al.* [63]
Motif 4	RTP	NINAC, myosin-IIIa; Axonal	Enhance the association of the complex with membranes, or facilitate association with scaffolding and actin-based structures; participates in axonal degeneration	Mecklenburg *et al.* [35]; Bhattacharya *et al.* [5]
Motif 5	MORN5	Maxillary/mandibular/ frontonasal/cranial	Craniofacial development (BMP and TGFβ pathways)	Cela *et al.* [58]
Motif 7	MSAP; AtPIPK1	Sperm; PIP2	Development and differentiation of sperm flagella; regulates both the function and distribution of the enzyme that is sensitive to the lipid environment	Ju *et al.* [56]; Im *et al.* [4]
Motif 8	JPH1; JPH2; JPH3; JPH4; ALS2	Sarcoplasmic reticulum/transverse tubule; junctional membrane; Chromosome 16q24.2/mouse primary islets; Target of miR-205/ ER-PM junctional areas; Rab17/Brain and the spinal cord	Supports the stability at triads in adult skeletal muscle fibers; important regulator of the LTCC/cardiac SK channels; cardiotoxicity; Huntington disease-like 2 (HDL2)/glucose-stimulated insulin secretion; candidate tumor suppressor gene in endometrioid endometrial adenocarcinoma/contributor to the inflammatory pain mechanisms; Rab17-associated endosomal trafficking and maturation/motor neuron diseases related protein	Rossi *et al.* [14]; Fan *et al.* [46]; Krause *et al.* [50]; Zhu *et al.* [42]; Hu *et al.* [49]; Hogea *et al.* [51]; Chung *et al.* [52]; Ono *et al.* [60]; Miceli *et al.* [61]
Motif 9	OsPIPK1; PpPIPK1	PA, PI4P, PIP2; PA, PIP2	Subcellular localization and phospholipid-binding; aggregate PIP2 and promote product activation	Ma *et al.* [6]; Mikami *et al.* [22]
Motif 15	MORN1; TbMORN1; TgMORN1	IMC, Centrocone; Flagellar pocket; Basal and leading edge of the IMC, centrosome and the apical end of *T. gondii*	The dynamic component of the *Toxoplasma gondii* cell division apparatus; facilitates protein entry into the flagellar pocket of *Trypanosoma brucei*, parasite cytokinesis	Gubbels *et al.* [9]; Morriswood *et al.* [33]; Heaslip *et al.* [29]

both sites (Fig. 2A, green boxes). A comparison of secondary structure also found significant differences in human JPH2 motifs compared to mouse and rat ones, including α-helix, β-sheet and T-turn, etc. (Fig. 2A, red box). It is worth noting that the differences in secondary structure and hydrophilicity in JPH2 motifs are mainly concentrated in the joint sequence (human, 152–290; rat, 152–284; mouse, 152–284). These results suggest that, except for post-translational modifications, the properties of differential amino acid side chain residues in MORN motif proteins are also an important factor affecting the localization and function of MORN proteins.

Furthermore, we observed the characteristics of the tertiary structures of JPH2. Similarly, we found that the joint sequence in human JPH2 shows obvious differences in 3D structure compared to mouse and rat ones (shown in the red box), which is the main reason for the difference in 3D structure in these three species (Fig. 2B, green box). Meanwhile, MORN motifs form a continuously repeating β-sheet backbone, which is consistent with a recent report by Yang *et al.* [19] on the crystal structure of JPH2. Interestingly, unlike the monomeric structure of JPH2 obtained by Yang *et al.* [19], we found that two complete human JPH2 motifs depend on the C-terminus to form a centrosymmetric

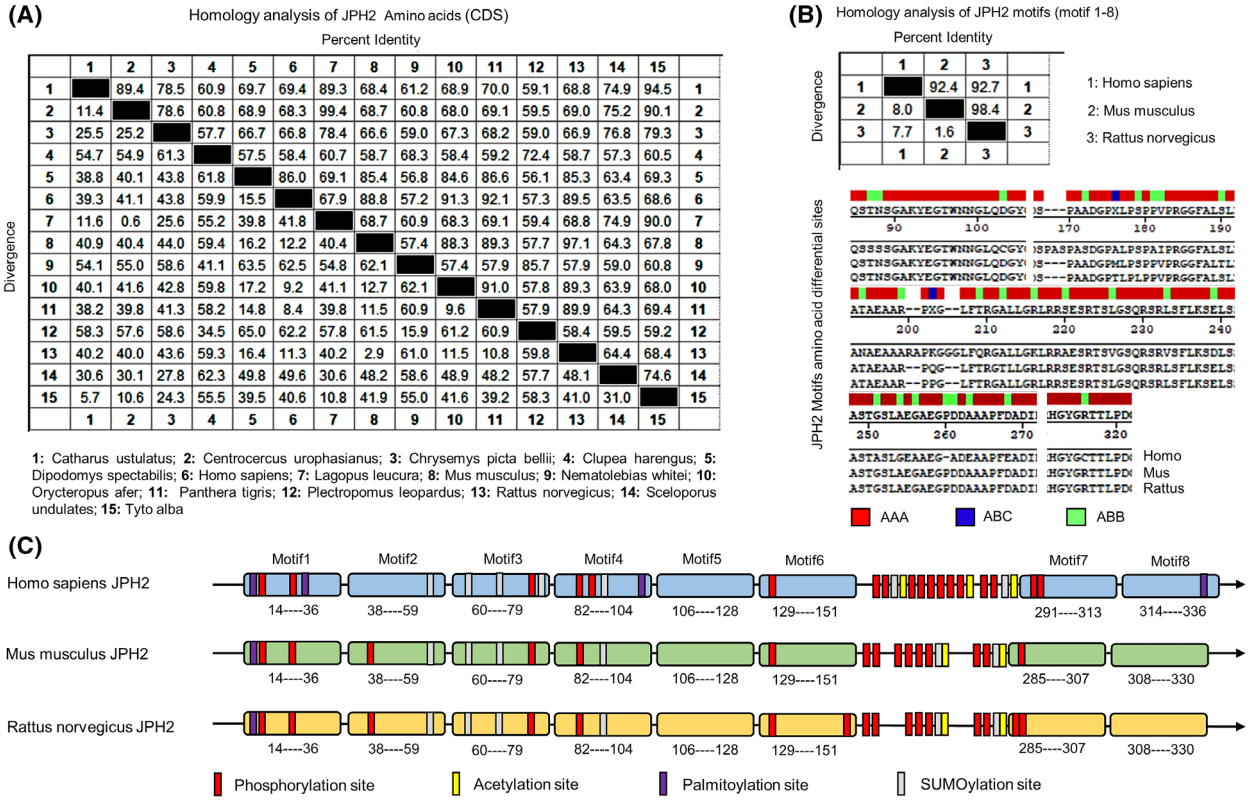

Fig. 1. Homology analysis and amino acid composition of the membrane occupation and recognition nexus (MORN) motif-containing protein JPH2. (A) Homology analysis of JPH2 amino acids between different species; the intersection of the horizontal and vertical in the table indicates the homology difference between the two species. The percentage above the black box shows the percent identify and the value of percent identify that is closer to 100 indicates high homology between the two species. The percentages below the black box show divergence and the value of divergence that is closer to 100 indicates the great homology difference between the two species. (*Homo sapiens* Junctophilin 2, NM_020433.5; *Mus musculus* junctophilin 2, NM_021566.2; *Rattus norvegicus* Junctophilin 2, NM_001037974. *Panthera tigris* Junctophilin 2, XM_042980345.1; *Tyto alba* Junctophilin 2, XM_0330036802; *Chrysemys picta* bellii Junctophilin 2, XM_005304651.4; *Chrysemys picta* bellii Junctophilin 2, XM_005304651.4; *Lagopus leucura* Junctophilin 2, XM_042883644.1; *Centrocercus urophasianus* Junctophilin 2, XM_042832394.1; *Orycteropus afer* Junctophilin 2, XM_007938414.2; *Nematolebias whitei* Junctophilin 2, XM_037684646.1; *Catharus ustulatus* Junctophilin 2, XM_033075331. *Clupea harengus* Junctophilin 2, XM_012835061.3; *Dipodomys spectabilis* Junctophilin 2, XM_042694602.1; *Plectropomus leopardus* Junctophilin 2, XM_042491345.1; *Sceloporus undulatus* junctophilin 2, XM_042461502) (MEGALIGN; DNASTAR, Inc., Madison, WI, USA). (B) Comparison of the amino acids composition of human, mouse and rat JPH2 MORN motifs. The homology differences are shown with percent identity and divergence. All different sites between the three species are shown by amino acid arrangement. AAA, three species are the same; ABB, one species is different; ABC, three species are different (MEGALIGN). (C) Analysis of post-translational modification sites of human, mouse and rat JPH2 MORN motifs. (https://prosite.expasy.org; CSS-PALM 4.0 software; GPS-PAIL; http://pail.biocuckoo.org; GPS-SUMO 1.0; http://sumosp.biocuckoo.org).

dimer, whereas the JPH2 motifs in rat and mouse only contain a single helical structure (Fig. 2B). Because the crystal structures of JPH2 were performed under high ionic strength, which is required to prevent aggregation and precipitation, and because JPH1 and JPH2 fusion proteins (containing joint sequence) can form homo- and heterodimers in the cytosolic region [14], it is still possible that dimerization occurs under physiological conditions.

We should further emphasize the importance of joint sequence in MORN proteins. The join sequence in *Physcomitrella patens* PIPK1 (PpPIPK1) was reported to regulate its enzymatic activities [22]. In 2021, the joining region in JPH2 was proposed to be the necessary region for its binding with the L-type calcium channel (LTCC) because mutation of the joint sequence impairs the binding [17]. Using ITC experiments and the crystal structure, Yang *et al.* [19] reported that this interaction involves the first three MORN repeats of JPH2, but not the joint sequence. Although the latest studies direct addressed the interaction, the results showing that mutation of the joint sequence impaired the binding

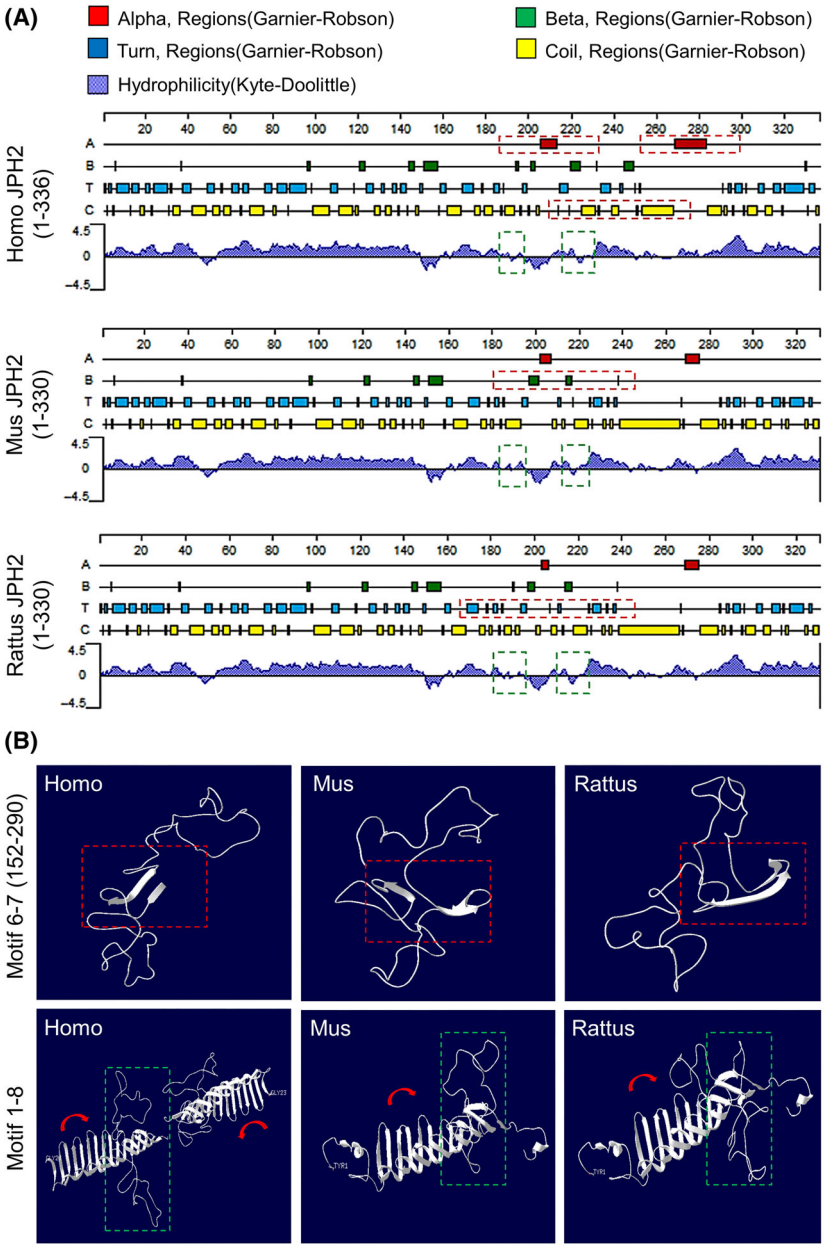

Fig. 2. Prediction of secondary structure and three-dimensional structure of human, rat and mouse JPH2. (A) Prediction of the secondary structure of human, rat and mouse JPH2 (motif1 to motif8). The differences in the secondary structure are marked with red boxes and the difference in hydrophobicity are marked with green boxes (PROTEAN, https://www.dnastar.com/). (B) Three-dimensional structure prediction of joint sequence and membrane occupation and recognition nexus domain of human, mouse and rat JPH2. The differences in joint sequence and JPH2 motifs are marked with red dotted boxes and green dotted boxes. Red arrows show the spatial orientation of the 3D conformation (N-terminal to C-terminal; https://swissmodel.expasy.org/interactive/TJkbL7/models; SPDBV; https://spdbv.unil.ch).

reflect the importance of the joint sequence. Further exploration of non-MORN motif regions, such as the joint sequence, will help us to obtain different insights about the MORN proteins.

Here, we discuss the relationship between the structure and function of MORN proteins. It was found that the differential sequences in MORN motifs rather than the number comprised the key factor affecting the localization and function. It is worth noting that the joint sequence also plays an important role in regulating the localization and function of MORN proteins compared to the MORN motifs. MORN motifs and the joint sequence may collectively affect the biological function of MORN proteins through post-translational modifications, hydrophobic or electrostatic interactions, etc.

Role of the MORN motif-containing proteins in plants

MORN motifs were commonly found in PIPKs with different members in a variety of plants [23]. OsPIPK1 is a rice protein containing nine MORN motifs, Ma *et al.* [24] revealed the widespread distribution of OsPIPK1 in rice roots, stems, leaves and flowers, and

reported that the MORN motifs in OsPIPK1 were key structures regulating the signal transduction related to flowering genes and rice heading. To determine whether the presence of MORN motifs in plants is the key to mediate different subcellular localization of PIPK proteins (animal and yeast PIPKs proteins lack MORN motifs), Ma *et al.* [6] conducted a further study and confirmed that MORN motifs could target PS in a fusion protein by systematic multiple enzymes targeting analysis. Furthermore, deletion studies verified that MORN motifs in OsPIPK1, together with a 104 amino acid joint sequence, were involved in the regulation of subcellular localization of the plasma membrane or nucleus.

In previous studies, the MORN motif has been widely reported to be related to membrane targeting without showing enzymatic activity. In a study conducted in tobacco cells, using *Arabidopsis thaliana* PIPK1 (AtPIPK1) to investigate the potential function of the N-terminal MORN motifs, it was found that the MORN motifs in AtPIPK1 binds to phosphatidic acid (PA) and PIP2 and increase enzyme activity by aggregating PIP2 and promoting product activation [4]. Consistent with this report, the MORN motifs in OsPIPK1 could strongly bind to PA and relatively slightly to phosphatidylinositol 4-phosphate (PI4P) and PIP2 [6]. However, Stenzel *et al.* [25] and Mikami *et al.* [22] demonstrated a contrary result in AtPIP5K3 and PpPIPK1. They reported that the extreme C-terminal, joint sequence and N-terminal region, but not the MORN motif, were found to modulate their catalytic activity [22,25]. These reports are consistent with our prediction that the joint sequence could affect the functions of MORN proteins.

Except for PIPKs, Shimada *et al.* [26] found that fission factor accumulation and replication of chloroplasts 3 (ARC3) was crucial in the process of chloroplast division. The phosphatidylinositol 4-phosphate 5-kinase homologous region of ARC3 does not have a catalytic domain but contains the MORN motifs. Further studies confirmed that ARC3 protein regulates chloroplast protein accumulation and replication through endosymbiosis with prokaryotic cell division protein FtsZ gene during chloroplast evolution. Similarly, Chen and Cao [27] also reported that the MORN motifs in ARC3 can mediate the interaction of ARC3-PARC6 (paralog of ARC6) and prevent the interaction of ARC3-FtsZ, which promotes the remodeling of Z ring during chloroplast division. In addition, Lee *et al.* [7] reported the regulatory effect of brassica rapa MORN motif protein (BrMORN) in *Arabidopsis*. This reveals that BrMORN may locate on the plastid envelope to increase the growth rate of *Arabidopsis* plants, the elongation of hypocotyls, the size of vegetative organs and seed yield by promoting cell enlargement. Finally, Liang *et al.* [28] demonstrated that MORN protein embryo-defective 1211 mutant (EMB1211) in *Arabidopsis* plays an important role in embryogenesis and chloroplast biogenesis by constructing an embryo-defective mutant.

In conclusion, we found that the MORN motif affects the localization and function of MORN proteins in plants by the regulation of plasma membrane binding and enzymatic activation (Fig. 3). Phosphatidylinositol kinases proteins can rely on MORN motifs to regulate rice heading through peptide lipid binding (OsPIPK1), or binding PA or PIP2 to increase kinase activity in tobacco cells (AtPIPK1). In ARC3 and BrMORN, the MORN motif is an important unit that affects chloroplast division and cell proliferation in *Arabidopsis*, which regulates plant growth and seed yield. Furthermore, in AtPIP5K3 and PpPIPK1, non-MORN motif regions also modulate their catalytic activity. Therefore, the MORN motif is not the only unit that determines the function of the MORN protein. Further exploration of non-MORN motif regions, such as the joint sequence, as well as the N-terminus and C-terminus, will help us to reveal the reasons why MORN proteins exert different functions in plants.

Role of the MORN motif-containing proteins in parasites

MORN motif-containing proteins have also been reported to be involved in the regulation of many

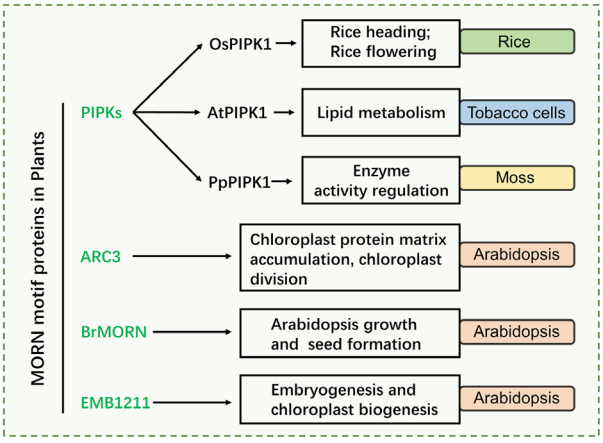

Fig. 3. The role of membrane occupation and recognition nexus (MORN) motif-containing proteins in plants. The described functions involving the different MORN proteins are shown in black boxes and the plant species in which MORN proteins have been characterized are represented by different colored blocks.

physiological processes in parasitic organisms. During bud division of *Toxoplasma gondii*, Gubbels *et al.* [9] reported that MORN1 is localized at the top and back end of the inner membrane complex (IMC) to regulate nuclear division and cell budding by forming a ring structure and interacting with the centrocone. Similarly, Heaslip *et al.* [29] revealed that MORN1 deletion grossly affects the structure of *T. gondii* basic complex, which also displayed defects in cytokinesis, confirming that MORN1 is required for maintaining the structural integrity of the parasite posterior end. Except for *T. gondii*, MORN1 is highly conserved in apicomplexan parasites. MORN1 localizes to the basal complex of the IMC and this structure can be observed across the Apicomplexa, which is involved in both asexual and sexual development [30]. Knockdown or mutation of MORN1 leads to the apical complexes and the impaired separation of daughter cells, resulting in the development of a two-headed parasite [31].

TbMORN1 is part of a multi-protein complex called the hook complex that contains MORN motifs and binds around the neck of the flagella. TbMORN1 and TbCentrin4 together define a hairpin structure acting as an essential component of the cytoskeletal component in trypanosome cells [32]. Morriswood [33] analyzed the phenotypic effect of TbMORN1 depletion in mammalian *Trypanosoma brucei* infection. It was revealed that TbMORN1 is involved in regulating cargo entry to the flagellar pocket of *T. brucei*, thus providing a link between the cytoskeleton and the endomembrane system [33]. MORN protein Cp-P34 deposits in sporozoite gliding trails as a surface antigen of Cryptosporidium in a form similar to virulence factors, which can be used as immune targets for parasite treatment [34]. Although the ciliate *Tetrahymena thermophila* is not a parasite, it expresses 129 MORN protein-coding genes [8]. Habicht *et al.* [8] analyzed the arrangement of MORN motifs using the lipid coverage test and protein liquid chromatography. When arranged in tandem, the MORN motif acts as a general membrane-binding domain in different proteins. For example, the MORN motif plays a key role in communication between two complementary cells and fusing during sexual reproduction in ciliates.

Here, we summarize the research on MORN motif proteins in existing parasites. In apicomplexan parasites, MORN1 maintains the structural integrity of the parasite rear end and is involved in the division and proliferation of *T. gondii* by localizing to the IMC. Loss of MORN1 results in daughter cell budding and developmental disorder. Moreover, Cp-P34 is a surface antigen of Cryptosporidium and can be used as an immune target for parasite therapy. Furthermore, TbMORN1 is located around the neck of the flagellum and facilitates the entry of proteins into the flagellar pocket and is involved in endocytosis (Fig. 4). MORN proteins can be localized in the different plasma membranes and dynamic components of parasites that affect various physiological processes (division reproduction and immune response, etc.) by mediating protein transport and regulating the connection between the cytoskeleton and the endomembrane system, and so on.

Role of MORN-containing proteins in animals

The role of MORN motif-containing proteins in *Drosophila*

Drosophila retinophilin (RTP) contains four MORN motifs and is one of the ocular enriched proteins, being expressed in the retina and central nervous system [35,36]. Mecklenburg *et al.* [37] found that RTP

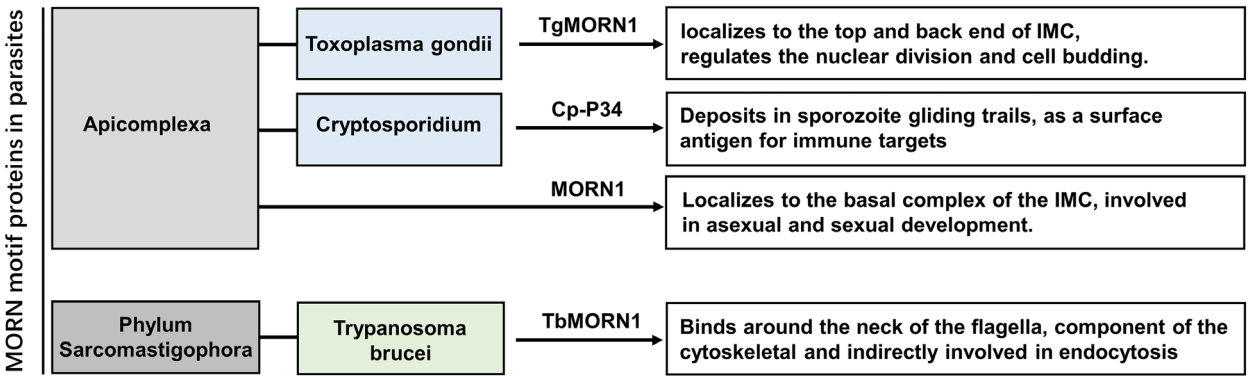

Fig. 4. The role of membrane occupation and recognition nexus (MORN) motif-containing proteins in parasites. Black boxes show the described functions of different MORN proteins and the species of parasites in which MORN proteins have been characterized are represented by different colored blocks.

localization requires MORN repeats, and N-terminal and C-terminal domains in microvilli aggregator organelles, whereas the extended planar topology of MORN repeats may allow RTP to organize and stabilize phospholipids and other components on the membrane surface. Furthermore, it was revealed that myosin III encoded by neither inactivation nor afterpotential C (NINAC) can form a complex with RTP to regulate photosensitivity in *Drosophila*. Finally, they also verified the interaction between the RTP homologous protein MORN4 and the NINAC homologous protein myosin-IIIa in COS7 cells, revealing the key role of MORN proteins in regulating myosin dynamics and behavior. Bhattacharya *et al*. [5] also investigated the role of RTP protein in axonal degeneration induced by paclitaxel in *Drosophila* larvae. It was found that the normal axonal degeneration triggered by traumatic and toxic axonal injury is inhibited after RTP knockout because RTP is an important component of axonal degeneration. Moreover, MORN4 has the same function as RTP after axon swelling but before axonal rupture in mouse embryonic dorsal root ganglion neurons.

The role of MORN motif-containing proteins in mammals

JPHs are the most studied MORN motif-containing proteins in mammals. During development, JPH2 knockout caused mice to die of embryonic cardiac arrest, and induced abnormal Ca^{2+} signaling in cardiomyocytes [38]. JPH2 also affects heart functions in adults. It was reported that JPH2 participated in hypertrophic cardiomyopathy by participating in intracellular calcium release, myocardial contractility and myocardial proliferation [39-41]. First, cyclophosphamide can increase the m6A levels of JPH2 mRNA, resulting in the reduction of JPH2 expression levels and dysregulated calcium signaling, which induces cardiotoxicity [42]. Then, Reynolds *et al*. found that restoring JPH2 levels can maintain heart function in mice with early heart failure during JPH2 gene therapy [43]. The molecular mechanisms studies confirmed that the MORN motif in JPH2 is assumed to mediate its membrane attachment by binding to membrane lipids or proteins in SR [1,15,38,44]. In a recent study, JPH2 was found to interact with t-tubule, which can help LTCC recruit to t-tubule. The interaction between the junction area of LTCC and JPH2 promotes the assembly of the doublet and maintains the normal Ca^{2+}-induced Ca^{2+} release of cardiomyocytes [17,45]. Furthermore, JPH2 can tightly bind to the t-tubule and SR, which prevents the loss of the t-tubule and suppresses the abnormal SR Ca^{2+} leakage related to the contraction failure after transverse aortic constriction [43]. Furthermore, studies of the association of JPH2 and small-conductance calcium-activated potassium channel 2 (SK2) channels in mouse heart and HEK293 cells also found that JPH2 directly interacted with SK2 channels via the MORN motifs in its N-terminus, and inhibition of JPH2 significantly reduced the density of $I_{K,Ca}$ and Ca^{2+} transient amplitude [46].

Moreover, JPH1 and JPH2 stabilize the junctional membrane by bridging the sarcolemmal and SR membranes, which recruit LTCCs to the junctional membrane through physical interaction and ensure robust excitation–contraction coupling at triads in skeletal muscle [47]. JPH1 was also found to immobilize on the membrane of SR through the C-terminal transmembrane domain and bind to the t-tubular membrane through the cytoplasmic N-terminal MORN domain. Further studies revealed that JPH1 can interact with several proteins in the excitation–contraction coupling, and play a role in the recruitment and stability of adult skeletal muscle fiber triads [14]. Finally, deletion of JPH3 in mouse islets impairs glucose-stimulated insulin secretion through the changes in Ca^{2+} transient amplitudes and ER-mitochondrial contacts [48]. Furthermore, JPH3 was also reported as a novel tumor suppressor gene in colorectal and gastric tumors that promotes mitochondrial-mediated apoptosis [49] and a CAG/CTG repeat expansion in JPH3 gene on chromosome 16q24.2 causes Huntington disease-like 2 (HDL2) [50]. JPH4 maintained junctional nanodomain Ca^{2+} signaling as an important mechanism of inflammatory pain [51]. JPH4 appears to be a miR-205 target, as well as a candidate tumor suppressor gene in endometrioid endometrial adenocarcinoma [52].

Except for JPHs, other MORN motif proteins have also been reported to be specifically distributed in mouse testis and germ cells [53]. MORN2 (MOPT) is a newly discovered MORN protein in mouse testis that has been shown to play an important role in the dynamic regulation of acrosome biogenesis during the later stages of spermatogenesis [2]. Moreover, mouse MORN motif protein meichroacidin (MCA) was cloned from the cDNA library and compared with carp MORN motif sperm-specific axonal protein (MSAP) and human MCA. It was also revealed that the MORN motifs may be an important part of MCA in regulating sperm flagella development [54], which was consistent with subsequent research on human MCA by Shetty *et al*. [55] and carp MSAP by Ju and Huang [56]. Futhermore, MORN3 was widely

distributed in mouse testis, which localized to the acrosome of germ cells throughout spermatogenesis and interacted with meiotically expressed gene 1 and regulated spermatogenesis [57]. MORN5 can be expressed on both sides of the original oral cavity within the maxillary region. The variants of MORN5 were associated with an increased risk of non-syndromic cleft lip with or without cleft palate [58]. The amyotrophic lateral sclerosis type 2 (ALS2) gene was known as a guanine nucleotide exchange factor for Rab5, which plays a crucial role in the regulation of Rab17-associated endosomal trafficking and maturation [59,60]. Mutations and dysfunction of ALS2 are also closely associated with neurodegenerative diseases and motor neuron diseases [61,62].

Finally, MORN motif proteins have been reported to affect the occurrence of cancer through post-translational modifications and other mechanisms. MORN3 was reported as a potential regulator of P53. It can interact with E3 ubiquitin-protein ligase mdm2 to accelerate P53 ubiquitination degradation or interact with deacetylase sirtuin-1 to promote the acetylation-dependent anti-tumor activity of P53 [11]. Furthermore, the MORN protein histone-lysine *N*-methyltransferase 7/9 (SET7/9) was shown to regulate the cell cycle and apoptosis of colon cancer [63] by interacting with KH domain-containing, RNA-binding, signal transduction-associated protein 1 (Sam68) through its MORN domain. Maintaining a high level of Sam68/SET7/9 co-expression can improve the survival of colon cancer patients. Another study also confirmed that the N-terminal MORN domain of SET7/9 is the key structure for its interaction with ribosomal protein eL42 and methylation modification to regulate protein synthesis [64].

Here, we have briefly summarized the role of MORN motif in animals by taking RTP, JPHs, MOPT, ALS2, MORN3 and SET7/9 as objects. MORN proteins are widely distributed in different organs and tissues, which are involved in many physiological processes, such as cardiac hypertrophy, cardiotoxicity, heart failure, spermatogenesis and neurological diseases, etc. First, MORN proteins participated in heart disease by interacting with phospholipids and proteins to mediate the connection between organelles and membranes (SR and PM, ER–PM, ER–mitochondria, etc.), which regulate the homeostasis of the Ca^{2+} channel and SK2 channel. Second, MORN proteins also regulate spermatogenesis and neuronal functional integrity by participating in protein transport and endosomal transport. Moreover, MORN proteins can also affect the proliferation of tumor cells by participating in post-translational modification or binding to miRNAs (Fig. 5).

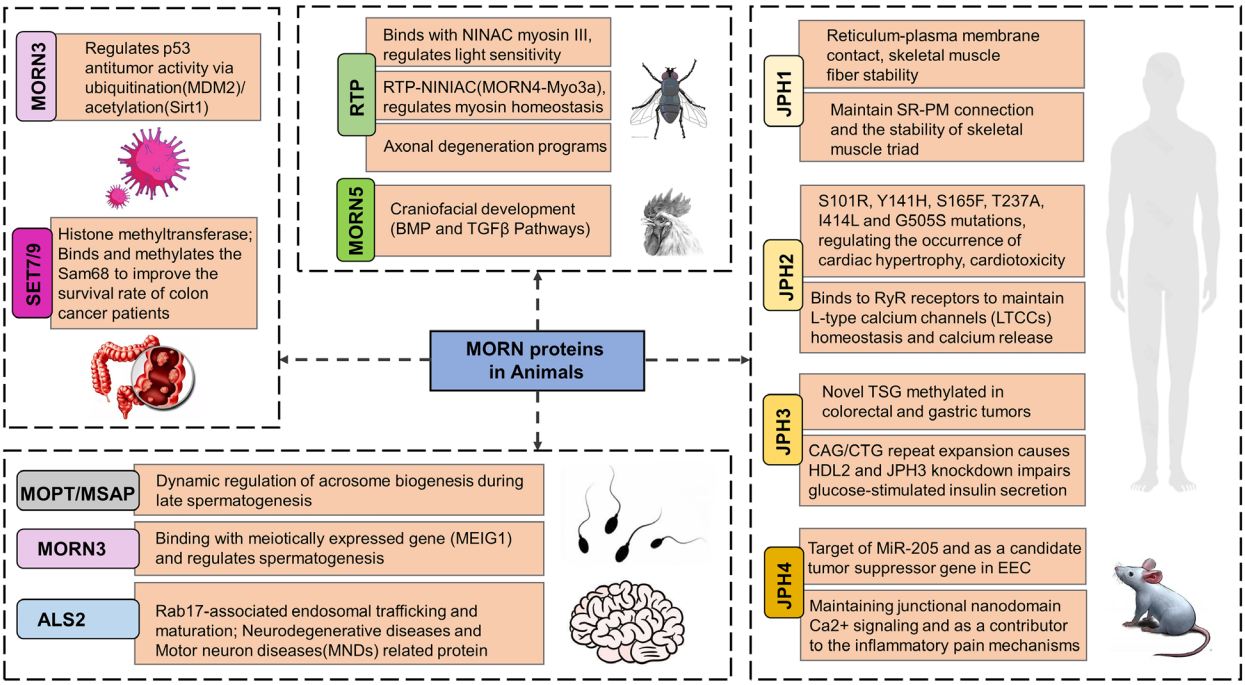

Fig. 5. The role of membrane occupation and recognition nexus (MORN) motif-containing proteins in animals. Different color blocks represent different MORN proteins, and the orange boxes show the described functions of different MORN proteins in animals.

Conclusions

We have reviewed recent research on MORN motif-containing proteins. The MORN motif consists of 23 amino acids, including tyrosine, glutamine and others, and is present in variable copies within the sequence of many functionally distinct proteins. Moreover, the MORN motif exerts a variety of functions in different tissues and different species by influencing protein-membrane and protein–protein interactions, as well as post-translational modifications. This indicates that the MORN motif is an important structural unit involved in the regulation of a wide array of biological processes, such as development, cell proliferation and survival. Emerging evidence regarding the MORN motifs impacts on protein localization and function brings up new ideas and questions warranting future research. One such idea is that it is the sequence rather than the number of copies of a MORN motif that regulates the localization and function of MORN motif-containing proteins. Second, post-translational modifications along with the properties of the side chain residues of a MORN motif may be important factors that determine the function of MORN motif-containing proteins. Of course, the contribution of non-MORN motif regions to the localization and function of MORN motif-containing proteins cannot be ignored. Third, addition or deletion of MORN motifs through gene editing or recombination may be an effective means of targeting MORN motif-containing proteins on certain tissues/proteins and allowing them to perform important biological functions.

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Author contributions

JZ designed the study. JZ and HL wrote the paper. JZ and YL checked and improved the manuscript. All authors read and approved the final version of the manuscript submitted for publication.

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