The Antiviral Dynamin Family Member, MxA, Tubulates Lipids and Localizes to the Smooth Endoplasmic Reticulum*

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Mx proteins are induced by type I interferon and inhibit a broad range of viruses by undefined mechanisms. They are included within the dynamin family of large GTPases, which are involved in vesicle trafficking and share common biophysical features. These properties include the propensity to self-assemble, an affinity for lipids, and the ability to tubulate membranes. In this report we establish that human MxA, despite sharing only 30% homology with conventional dynamin, possesses many of these properties. We demonstrate for the first time that MxA self-assembles into rings that tubulate lipids in vitro, and associates with a specific membrane compartment in cells, the smooth endoplasmic reticulum.

Cells respond to type I interferons by up-regulating the expression of more than 50 different proteins and, as a result, can inhibit the replication of many RNA and DNA viruses. Mx proteins in particular are rapidly induced to high levels after interferon treatment and may be responsible for the bulk of the antiviral effect of interferon (for reviews, see Ref. 1). Several Mx proteins have been shown to inhibit the replication of a diverse set of viruses. However, the mechanism by which this occurs is not clearly defined and varies depending on the Mx protein, virus, and cell type. Human MxA is one of the best characterized proteins of this group and has antiviral activity against a number of viruses including vesicular stomatitis virus, influenza virus (2), hepatitis B virus (3), and Thogoto virus (4).

Mx proteins are members of the dynamin family of large GTPases. Dynamins work in membrane trafficking and remodeling events throughout the cell (for reviews, see Refs. 5 and 6). A common property of dynamins is the propensity to self-assemble into ring structures of a characteristic size (7, 8), which is thought to be crucial for membrane remodeling function (9–11). Equally vital to their role within the cell is the ability to bind, tubulate, and in some cases, vesiculate membranes (12, 13). These characteristics are consistent with the role of dynamins in membrane fission processes. Dynamin family members display distinct cellular locations that often provide insight into function (5, 6). For example, conventional dynamin can be found at the plasma membrane and Golgi and is involved in the scission of both endocytic and secretory vesicles (14–23). MxA has been reported to oligomerize into large, undefined complexes (24–28), but how this relates to function is not clear, because an assembly-deficient protein was still able to inhibit Thogoto virus replication (24, 27, 29). Thogoto virus appears to be the most sensitive to the actions of MxA (4), and the inhibitory mechanism is the most clearly described. It has been demonstrated that MxA blocks the nuclear import of Thogoto virus nucleocapsids (30), a step essential for its replication in the nucleus. It was proposed that MxA wraps around the incoming viral nucleocapsids, perhaps masking a nuclear localization signal and preventing entry into the nucleus. MxA must block other viruses in a somewhat different manner, however, because not all of the viruses it inhibits have a nuclear replication step. In general, MxA appears to interfere with steps involving viral transcription. However, neither the precise mechanisms of interference nor the roles of MxA assembly and GTP hydrolysis in antiviral activity are currently understood.

To further define the mechanism of MxA antiviral activity, we tested if this large GTPase shares certain properties with conventional dynamin in vitro despite modest sequence homology. We examined and defined the nature of MxA self-assembly and tested its ability to bind and modify the shape of added lipids. Here we demonstrate that, like other dynamin family members, recombinant His6-tagged MxA can assemble into very regular rod and ring structures as visualized by EM and that MxA can bind and tubulate lipids in vitro. To test whether these properties are directed toward specific organelles, we conducted extensive light and electron microscopic imaging of cells expressing wild-type or mutant MxA protein. By light microscopy we found MxA associates with smooth ER but not rough ER endosomes, mitochondria, microtubules, or actin, without any obvious defect by either light or electron microscopy. Expression of the MxA mutant however, demonstrated a substantial proliferation of the smooth ER as visualized by EM. Taken together these findings provide compelling evidence that the Mx family of proteins possesses many biophysical properties of traditional dynamin proteins and may act to inhibit viral replication through alterations in membrane organization or traffic.

MATERIALS AND METHODS

Plasmid Construction—The following primers were used to amplify MxA from the plasmid pBS-T/MxA, a generous gift from Dr. G. Kochs: 5'-GTACAGGGATCCATGGTTGTTTCCGAAGTG and 5'-GTACAACCTCGAGACCGGGGAACTGGGCAAGCCG-3’ or 5’-GTAGACGCTAGGGCCACCATGTTGTTTCCGAAGTG and 5’-GTACCAAGATCCAGTTTACCCGGGAACTGGGCAAGCCG-3’. The gene was cloned into the

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1 The abbreviations used are: EM, electron microscopy; ER, endoplasmic reticulum; AMF-R, autocrine motility factor receptor; GTP-γS, guanosine 5’-3-O-(thio)triphosphate; PH domain, pleckstrin homology domain.
pQE80 plasmid (Qiagen) for protein expression in bacteria and into pCR3.1 (Invitrogen) for mammalian cells. To make the MxA K83A mutant, the primers 5′-GACCAGGCTGGGCGCTAGCTCCGTGGAG and its reverse were used. Human interferon β (Sigma) was used at 1000 units/ml.

Cells and Antibodies—HeLa cells were grown in Dulbecco’s modified Eagle’s medium plus 10% fetal bovine serum. Hep3B cells were grown in minimum Eagle’s medium supplemented with 10% fetal bovine serum and 2 mM glutamine. Cells were transfected using GeneJammer (Qiagen) according to the manufacturer’s protocol. Rabbits were immunized with the keyhole limpet hemocyanin-conjugated peptide, LLLNLG-DATVAQKNPGSVA, and the anti-MxA antibody, MxA (20–37), was purified and characterized as described previously for other antibodies (22). The AMF-R antibody was a generous gift from Dr. I. R. Nabi. For AMF-R staining, cells were fixed for 10 min at −20°C with an 80%/20% methanol/acetone solution. The remaining immunofluorescence methodology was carried out as published elsewhere (22).

Protein Expression and Purification— Cultures of M15(pREP4) Escherichia coli (Qiagen) transformed with pQE80-MxA were induced overnight at 25 °C with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside. The bacteria were pelleted, resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5 mM MgCl₂, 10 mM mercaptoethanol, 10% glycerol, 2 mM imidazole, 0.1% Triton X-100), and sonicated. The cell debris was removed by centrifugation at 10000 × g, and the supernatant was incubated with ProBond resin (Invitrogen) with agitation. The resin was washed with lysis buffer and then twice each with wash buffer 1 (50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5 mM MgCl₂, 10 mM mercaptoethanol, 10% glycerol, 20 mM imidazole, 0.1% Triton X-100) and wash buffer 2 (20 mM Tris-HCl, pH 8.0, 100 mM KCl, 5 mM MgCl₂, 20% glycerol, 20 mM imidazole, 0.1% Triton X-100). The protein was eluted in elution buffer (20 mM Tris-HCl, pH 8.0, 100 mM KCl, 5 mM MgCl₂, 20% glycerol, 250 mM imidazole, 0.1% Triton X-100), concentrated, and dialyzed overnight at 4 °C against the appropriate buffer. Assembly assays, lipid binding and tubulation, and electron microscopy of negatively stained proteins were performed as described elsewhere (31).

RESULTS

MxA Self-assembles into Rings and Rods—To better understand the mechanisms by which MxA mediates viral resistance, we utilized purified recombinant MxA using biochemical assays already established for dynamin. One property of dynamin family members is the ability to homo-oligomerize. MxA has been reported to form large multimers when purified from tissue or expressed in bacteria (24–28); however, the nature of these structures is undefined. In an attempt to further elucidate the nature of Mx oligomerization, we expressed His-tagged MxA in E. coli and analyzed the sedimentation properties of the purified protein. This sedimentation assay has been used to estimate the relative amount of assembled protein for dynamin (7, 8, 32) and the dynamin-like protein, DLP1 (31). In the absence of nucleotide, ~34% of MxA is in the pellet fraction in a buffer containing 150 mM KCl, indicating that a substantial amount of MxA is oligomeric (Fig. 1a). Under similar conditions, dynamin 1 has been found to be predominantly soluble (8, 32). To test the nature of oligomerization, we varied the salt concentration in the sedimentation experiments. We found that
pellet, whereas in higher salt conditions (300 mM) more than enhanced the formation of MxA oligomers in all salt conditions with results found for another dynamin-related protein, dynamin and DLP1 (31). Furthermore, we found that GTP consistently enhanced the formation of MxA oligomers in all salt conditions (Fig. 1a). This is in agreement with an earlier report that recombinant murine Mx1 formed larger aggregates in the presence of GTP based on negatively stained purified protein (33), as well as a very recent paper published while this manuscript was in review (34). High concentrations of GTP cause conventional dynamin to disassemble. In summary, MxA self-assembles through an ionic interaction that is enhanced in the presence of guanine nucleotides.

Under conditions in which dynamin self-assembles, the resulting multimers can be visualized as rings and stacks of rings by EM after negative staining (7). Using this same technique, we found that MxA also formed rings (Fig. 1, c and d) as well as rod-like structures. The rings had inner and outer diameters of ~25 and 35 nm, respectively, which is significantly smaller than rings formed by conventional dynamin (30–50 nm) or DLP1 (30–40 nm) (31) that were viewed together during the same experiments. The rods varied in length but were generally 50 nm long and 5 nm wide. In some instances the rods were curved and occasionally looped back upon themselves. These assembly data clearly demonstrate a fundamental similarity to conventional dynamin, in that MxA oligomerizes in a salt- and nucleotide-dependent manner into ring- and rod-shaped structures. Lower salt conditions favor both MxA and dynamin assembly, whereas nucleotides promote MxA assembly. At the same time, the GTP-induced aggregation implies that MxA may have unique oligomerization or hydrolysis characteristics.

**MxA binds Lipids**—Another distinct property of dynamins is the propensity to associate with membranes (12, 13, 35). Conventional dynamin contains a pleckstrin homology (PH) domain, which mediates binding to phosphoinositides within the membrane (36–39). Recently however, DLP1 was also found to bind lipids, despite the lack of a PH domain (31). These findings prompted us to examine whether recombinant MxA, which also lacks a PH domain, could associate with lipids as well. To this end, we mixed purified MxA with liposomal membrane and tested whether MxA would co-sediment with the lipid. MxA alone when centrifuged at a low speed (10,000 × g), did not pellet, but remained in the supernatant (Fig. 2, −PS). Adding nucleotide to the assay had little effect on the ability of MxA alone to pellet, indicating that assembled protein alone is not sedimentable under these conditions. However, the addition of phosphatidylserine to the recombinant protein caused ~50% of MxA to pellet with the lipid (Fig. 2, +PS). The presence of GTP or GTP•S did not increase MxA pelleting further, suggesting that the MxA-membrane interaction is nucleotide-independent. These results show that, like dynamin, MxA has the ability to associate and pellet with lipids. As for DLP1, this interaction does not depend on an obvious PH domain.

**MxA Tubulates Lipids—**Dynamin has been described as a molecular pinchase (6, 9) in light of the elegant in vitro data showing the ability of dynamin to deform purified liposomes into tubules (12, 13, 40) and the subsequent vesiculation of the tubules upon the addition of GTP (12). Likewise, DLP1 tubulates membranes in vitro as well as in cells (31). Given that MxA self-assembles into rings and binds lipids, we tested whether this dynamin family member also remodels membranes in vitro. Synthetic liposomes were prepared from phosphatidylserine, and recombinant MxA was added to the liposomes in the presence or absence of nucleotide. The mixture was spotted onto EM grids and negatively stained as described previously (31). As shown in Fig. 3, b–d, MxA constricted the liposomes into long, often branched tubules. Although tubules were visible in the absence and presence of nucleotide, no tubules were seen in the absence of MxA, consistent with the observations made in the lipid binding assay (Fig. 3a). These tubules were electron dense in the central core, like lipid tubules bound by dynamin and DLP1. Upon examination of higher magnification micrographs, striations were visible along the membrane (Fig. 3d). Preparations that did not include GTP also exhibited tubulated lipid structures although striations could not be resolved, suggesting that the nucleotide-bound MxA rings may undergo a conformational change into a higher ordered structure making them more readily visible. These structures presumably represent assembled MxA wrapped around the liposome. This is the first evidence that an Mx protein cannot only associate with lipids but is able to deform and tubulate membranes in vitro.

**MxA Localizes to the Smooth ER**—Emerging data from many laboratories indicate that dynamin family members function at distinct cytoplasmic locations, with the precise intracellular localization of each dynamin providing information about its function (5, 6). Despite intense investigation, however, MxA has not been localized to a specific intracellular site or structure. Previously, MxA has been reported as being spread throughout the cytoplasm in a granular or punctate pattern (41), but no organelle association has been described to date. Hep3B cells were treated with interferon, fixed, and labeled with anti-MxA antibodies. Immunofluorescence images revealed MxA was distributed in a linear punctate pattern, suggesting that it may be associated with an intracellular structure. After exploring markers for multiple organelles and cytoskeletal elements, we did not observe a significant colocalization with microtubules, actin, rough ER, endosomes, or mitochondria. We did observe, however, that a large portion of MxA colocalized with one organelle marker, the autocrine motility factor receptor (AMF-R; Fig. 4). This protein has been reported to cycle between the plasma membrane and the smooth ER (42–44). Fig. 4 shows the partial overlap between the immunofluorescence patterns of AMF-R and MxA in the human hepatocyte line, Hep3B. Crude subcellular fractionation (Fig. 4d) of these cells supports our immunofluorescence data as much of the MxA is in the cytosolic fraction (S100), in addition to the microsomal fraction (P100) where AMF-R is found. Based on these data, we hypothesize that MxA may interfere with viral replication at the smooth ER.

**Expression of a GTPase-defective Mutant Results in Smooth ER Expansion**—In an attempt to characterize the function of MxA at the smooth ER, we constructed and expressed the GTPase-defective mutant, MxA K83A. The corresponding dom-
inant negative mutation in other dynamin proteins has provided functional insights into how these enzymes participate in a variety of distinct cell processes, largely through the induction of gross morphological defects in specific organelle compartments such as coated pits, the Golgi apparatus, and mitochondria. As in other dynamin family members, substitution of a conserved lysine to an alanine leads to a lower affinity binding of GTP and a reduced rate of hydrolysis (41). This mutant is expected to differ from wild-type MxA protein incubated with GTP. The resulting protein, MxA K83A, also has no antiviral activity (41), suggesting that the conservation of the GTP binding elements is important for function. When we expressed the MxA K83A mutant in mammalian cells, we observed a distribution pattern similar to that of wild-type MxA by immunofluorescence, although the puncta tended to be slightly larger (41) (data not shown). To ascertain whether the expression of this mutant affects an intracellular structure, we examined the transfected HeLa cells by electron microscopy. The expression of MxA K83A resulted in a dramatic expansion of a membranous compartment (Fig. 5, b–d) relative to control cells (Fig. 5a). The membranous structures were tubular and appeared to come into and out of the plane of section (Fig. 5, c and d), suggesting a highly reticular, fenestrated organelle spread throughout the cytoplasm. The morphology of the membranes is very similar to that of rough ER, yet they are devoid of ribosomes. Expression of the wild-type construct resulted in no obvious change in organelle morphology. These ultrastructural observations support our immunofluorescence data that MxA associates with the smooth ER, suggesting that MxA may somehow modulate the size and complexity of this organelle. In addition to these changes, we also observed an accumulation of caveola-like structures in HeLa cells expressing MxA K83A (Fig. 5e). It has been reported that this cell type has few

![Fig. 3. MxA tubulates membranes in vitro.](image)

MxA remodels spherical phosphotidylserine liposomes into tubules. a, negatively stained liposomes are spherical and of various sizes in the absence of MxA. b–d, the liposomes are deformed into branched tubules upon addition of His6-tagged MxA and GTP. Higher magnification reveals striations along tubulated liposomes (arrows in d). Scale bars represent 500 (a), 200 (b and c), and 50 nm (d).

![Fig. 4. MxA colocalizes with a smooth ER marker.](image)

a–c, Hep3B cells were treated with IFN-β and prepared for indirect immunofluorescence to detect MxA (a and b) and AMF-R (a' and b'). Extensive co-localization is evident in a' and b' and at higher magnification (c) d, HeLa cells expressing MxA were fractionated by centrifugation into 1,000 × g (P1), 13,000 × g (P13), and 100,000 × g (P100) pellets and cytosol (S100). A Western blot of SDS-PAGE-separated proteins with anti-MxA or AMF-R antibodies is shown. The scale bars in a and b insets represent 10 μm.
caveolae (45), and we have also found this to be true in control cells or in those expressing wild-type MxA. Expression of the mutant MxA K83A, however, appears to increase the number of caveolae without obviously increasing Cav1 levels (data not shown). The significance of this alteration is currently unclear. We also observed the expansion of the smooth ER and proliferation of caveolae in CV-1 cells expressing MxA K83A (data not shown), indicating this alteration in organelle morphology is not a cell type-specific phenomenon. The expansion of the smooth ER upon expression of an MxA mutant may suggest that wild-type MxA is involved in a constriction or fission event associated with this organelle.

**DISCUSSION**

In the present study, we have demonstrated that MxA, despite limited sequence homology, shares several important properties with conventional dynamin. These characteristics include the propensity to self-assemble into defined structures and the ability to bind and tubulate lipids. This observation, and those of a very recent paper published as our manuscript was being reviewed (34), is the first demonstration that MxA forms oligomeric structures having a morphology similar to other dynamin family members. Furthermore, the observation that MxA not only binds lipids but can tubulate them in vitro, much like conventional dynamin, is a completely novel finding for Mx studies to date. Additionally, we provide the first evidence that MxA associates with a specific organelle, namely the smooth ER, and may be involved in the remodeling of this membrane compartment.

We initiated this study with the hypothesis that purified recombinant MxA might possess characteristics similar to those of conventional dynamin despite sharing only ~30% sequence homology. Most of this homology resides in the GTPase N terminus with the remaining portion of the molecule sharing little identity with dynamins. That a dynamin-related protein with relatively low sequence homology to other family members might still behave as a conventional dynamin is not without precedent. Yoon et al. (31) demonstrated that DLP1, which is only about 35% homologous to conventional dynamin, can assemble into rings and is able to bind and tubulate lipids. MxA previously had been found to oligomerize in vitro (24–28).
Using His<sub>6</sub>-tagged recombinant protein, we also found that MxA assembles in a nucleotide- and salt-dependent manner. At low salt, much of the protein is an assembled, pelletable form, whereas at higher salt conditions, MxA tends to be more soluble. The modest salt effects suggest a small ionic component to the self-assembly of MxA. We also found that under the conditions tested, both GTP and GTP·y·S promote assembly. This is unlike other dynamin family members and seems to be attributable to or caused by the high GTP hydrolysis activity reported for MxA (46–48). Dynamin hydrolyzes GTP rapidly, which is thought to induce disassembly (11). We thus considered the possibility that the recombinant protein was simply inactive. However, given that our His<sub>6</sub>-tagged protein retained high GTPase activity (data not shown), was >95% pure as estimated by Coomassie staining of SDS-PAGE, and formed defined structures as visualized by EM, we believe the MxA preparations used for this study are indeed active.

At the moment, the significance of the increased assembly in the presence of GTP is not clear, although this observation is consistent with a previous report finding that mouse Mx1 undergoes GTP-dependent aggregation (33). It has been suggested that MxA may require cofactors within the cell (49), and it is thus possible that, in vivo, MxA has a GTPase activating protein. However, the high GTP hydrolysis rates of purified protein reported elsewhere suggest otherwise. Additionally, it has been reported that an MxA mutant (L612K) can function as a GTPase-defective monomer to block Thogoto virus replication (24, 27, 29), implying that neither the ability to assemble nor its GTPase activity is integral to the function of MxA. The authors of this paper hypothesized that the assembled form of MxA may be a mechanism to store the inactive protein. This would be unprecedented in the dynamin family and would make the conservation of assembly properties and enzymatic activity puzzling. Collectively, these data suggest that MxA can exist as a much more stable oligomer and that GTP hydrolysis might play a different role in assembly relative to conventional dynamin. We also found that GTP·y·S enhanced assembly, which is consistent with other dynamin family members.

When His<sub>6</sub>-MxA was negatively stained and examined by EM, we observed rings and rods at physiological salt concentrations. Dynamin forms rings and stacks of rings (7, 8), and there is one report of it assuming a rod-like structure (40). The rings have been determined to comprise a tetramer of tetramers using analytical centrifugation (50). The precise composition of MxA oligomers has not been determined, although based on in vitro cross-linking and gel exclusion chromatography estimates, MxA may form a complex of 3 to more than 30 individual proteins (25, 48). The MxA rings we observe have a 35-nm outer diameter, making them slightly smaller in size than those of conventional dynamin (50 nm in diameter) and making it likely that these may be trimers of MxA. This diameter is significantly smaller than the 60-nm rings reported recently for MxA in another study (34). The dimensions of our MxA rings are based on direct measurements and on structural comparisons of these smaller ring structures with those formed by conventional dynamin (Dyn2) and the dynamin-like protein (DLP1) made during the same experiment. Because the MxA ring structures published by Kochs et al. (34) do appear larger in size than our preparations, we believe such differences are due to variations in buffer and salt conditions used in the two studies. In addition to rings, we also found many rod-like structures in MxA preparations. Although the nature of the rods is not currently understood, strikingly similar structures have been reported in the assembly of septin multimers. Septins are GTPases originally identified as important for the yeast cell budding cycle (51) but are now known to participate in cytokinesis and secretion in mammalian cells (52). Septins are not dynamin family members, but it is intriguing that another class of GTPases also functioning in membrane remodeling processes forms rod-like structures in vitro.

Unlike conventional dynamin, MxA does not contain a PH domain. However, because DLP1 can associate with membranes in the absence of a defined lipid-binding domain (31), we examined whether MxA could also bind lipids. By lipid-protein co-sedimentation assays, we clearly demonstrated that MxA can associate with lipid in vitro (Fig. 2). In light of these novel data, we then examined whether MxA could remodel the lipid. The addition of MxA to spherical phosphotidylserine liposomes resulted in the formation of long, often branched tubules. This strongly suggests that MxA, like other dynamin family members, plays a role in membrane remodeling in vivo. How this relates to its antiviral function is not presently clear. It is possible that MxA sequesters key viral components in a membranous compartment or that it disrupts membrane trafficking events in the cell on which a virus depends. The lipid association appears to be independent of nucleotide, implying that MxA does not cycle on and off membranes like small GTPases. It is equally possible that other cellular components are required for proper regulation of MxA assembly and membrane binding in vivo.

Finally, we show that MxA colocalizes with a smooth ER marker by immunofluorescence and subcellular fractionation. We examined numerous immunofluorescent markers for organelles such as mitochondria, the rough ER, the intermediate compartment, endosomes, and caveolae, and found little colocalization. We also looked at the actin and microtubule cytoskeletons and found little overlap with these structures despite the often linear punctate pattern of MxA immunofluorescence staining. After extensive searching, we found significant colocalization with an antibody against AMF-R, a protein found at the smooth ER.

The immunofluorescence data are supported by electron microscopic examination of cells expressing a mutant MxA, in which we observe an expansion of a smooth membranous compartment, presumably the smooth ER. It is currently unclear how a mutant MxA might induce this proliferation, although the mutant protein need not increase its association with smooth ER membranes directly. Rather, the MxA K83A mutant protein may have a significantly altered binding to smooth ER-associated membrane proteins or to signaling molecules leading to organelle proliferation. As compared with the functions of other dynamin family members, MxA may be involved in membrane trafficking to or from the smooth ER, so that expressing a nonfunctional MxA mutant disrupts the normal equilibrium of membrane flow, resulting in an expanded smooth ER compartment. From a virological point of view, this was somewhat unexpected because it is not an organelle generally thought to be associated with virus replication. SV40, however, is one example of a virus that is known to traffic through the smooth ER (53). After entering the cell via caveolae, SV40 proceeds through caveosomes to the smooth ER and finally enters the nucleus where it replicates (54). In the current study, a concomitant increase of both the smooth ER and caveolae in cells expressing a GTPase-defective MxA is intriguing, given that SV40 traffics through both organelles. However, there are currently no reports of MxA inhibiting SV40 replication. Accordingly, we found that MxA did not block the ability of SV40 to enter the nucleus as assessed by large T antigen expression. This was by no means a complete study, however, and therefore we cannot rule out some effect of MxA on SV40 replication.

The present study demonstrates that MxA is a true member...
of the dynamin family and shares more than just a short consensus sequence. Our data suggest that despite the low overall sequence homology among themselves, dynamin family members share key characteristics. These common properties directly related to the function of conventional dynamin, the self-assembly, lipid binding, and tubulation by MxA, in combination with its smooth ER localization as presented in this study, provide substantial insight for future studies into how this protein confers antiviral resistance.

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