The human MxA protein is an interferon-induced large GTPase with antiviral activity against a wide range of viruses, including influenza viruses. Recent structural data demonstrated that MxA oligomerizes into multimeric filamentous or ring-like structures by virtue of its stalk domain. Here, we show that negatively charged lipid membranes support MxA self-assembly. Like dynamin, MxA assembled around spherical liposomes inducing liposome tubulation. Cryo-transmission electron microscopy revealed that MxA oligomers around liposomes have a “T-bar” shape similar to dynamin. Moreover, biochemical assays indicated that the unstructured L4 loop of the MxA stalk serves as the lipid-binding moiety, and mutational analysis of L4 revealed that a stretch of four lysine residues is critical for binding. The orientation of the MxA molecule within the membrane-associated oligomer is in agreement with the proposed topology of MxA oligomers based on crystallographic data. Although oligomerization of wild-type MxA around liposomes led to the creation of helically decorated tubes similar to those formed by dynamin, this lipid interaction did not stimulate GTPase activity, in sharp contrast to the assembly-stimulated nucleotide hydrolysis observed with dynamin. Moreover, MxA readily self-assembles into rings at physiological conditions, as opposed to dynamin which self-assembles only at low salt conditions or onto lipids. Thus, the present results indicate that the oligomeric structures formed by MxA critically differ from those of dynamin.

Human MxA protein is a dynamin-like GTPase with a molecular mass of 76 kDa (1). Members of the dynamin superfamily have important roles in endocytosis, vesicle transport, antiviral resistance, and many other cellular functions (2, 3). MxA has intrinsic antiviral activity against many different viruses, including highly pathogenic influenza A and bunyaviruses (4). Its expression is strictly controlled by type I (α/β) and type III (α) interferons (IFN) (5). MxA accumulates in the cytoplasm of IFN-stimulated cells and recognizes viral nucleocapsids during infection thereby blocking a critical step for virus replication (6, 7).

Previous sequence alignments and biochemical analysis showed that MxA has a multidomain structure similar to dynamin and other dynamin-like proteins (Fig. 1) (1, 8). It features an N-terminal GTP-binding domain (G domain; residues 1–341) that binds and hydrolyzes GTP, a central middle domain (MD; residues 366–533), and a C-terminal GTPase effector domain (GED; residues 572–662) known to interact with the MD and G domain by intramolecular backfolding (9–11). Dynamin has a similar domain structure, but it contains two additional regions, namely a pleckstrin homology (PH) domain involved in lipid binding (12) and a proline-rich domain known to mediate protein-protein interactions (13) (Fig. 1). Recent crystallographic data revealed that the architecture of MxA and dynamin is more complex and consists of three distinct entities that are composed of discrete subunits of the classical domains (1, 14). These are (i) the globular G domain, (ii) the bundle-signaling element consisting of the C-terminal portion of the GED and two helices connected to the G domain (15) and (iii) an elongated stalk structure formed by the MD and the N-terminal part of the GED (8). Interestingly, the MxA stalk contains a 40-amino acid long loop L4 (residues 533–572) which is located at equivalent position to the PH domain of dynamin (Fig. 1) (8). MxA stalks assemble in a criss-cross fashion into stable dimers and tetramers which further oligomerize into extended multimers (8). This arrangement guarantees that the G domains are positioned at one side of the MxA filaments whereas the L4 loops are found at the opposite side. The positioning of L4 implies that this unstructured loop may serve as a docking site for membranes or viral targets.

A hallmark of dynamin family members is their association with lipid templates and the assembly-stimulated GTP hydrolysis. The MxA stalk contains a 40-amino acid long loop L4 (residues 533–572) which is located at equivalent position to the PH domain of dynamin (Fig. 1) (8). MxA stalks assemble in a criss-cross fashion into stable dimers and tetramers which further oligomerize into extended multimers (8). This arrangement guarantees that the G domains are positioned at one side of the MxA filaments whereas the L4 loops are found at the opposite side. The positioning of L4 implies that this unstructured loop may serve as a docking site for membranes or viral targets.
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Plasmid Construction—To introduce single amino acid exchanges from lysine to glutamic acid into loop L4 of the full-length MxA, site-directed mutagenesis was performed. The constructs were cloned into the pQE9 expression vector using BamHI and HindIII restriction sites for further purification and production.

Expression and Purification of MxA—Recombinant MxA and mutant forms of MxA protein were expressed in *Escherichia coli* M15 using pQE-9 vector (Qiagen) as an N-terminal His-tagged protein as described (22, 23). Cell lysates were incubated with nickel-nitrilotriacetic acid-agarose (Qiagen) in buffer A (50 mM Tris, pH 8.0, 400 mM NaCl, 5 mM MgCl₂, 7 mM β-mercaptoethanol, 2 mM imidazole). His-tagged MxA was eluted using 250 mM imidazole in buffer A. For further purification, His-MxA was loaded on a Superdex 200 (1.6 × 60 cm) column and eluted in HCB500 buffer (20 mM Hepes, pH 7.5, 500 mM NaCl, 2 mM MgCl₂, 1 mM DTT). Aliquots of purified protein were stored at −70 °C.

Purification of Dynamin—Dynamin was extracted from rat brains using its affinity to SH3 domains as described previously (26). The pGEX vector (Amersham Biosciences), encoding amphiphysin-2 SH3 domain fused to glutathione S-transferase, was used for expression of SH3-GST in *E. coli* BL21 cells for expression. The cell pellet was lysed in buffer B (50 mM Tris, pH 8.0, 300 mM NaCl, 5 mM EDTA, 0.05% Nonidet P-40) by sonication. Cell lysates were incubated with glutathione-Sepharose beads (GE Healthcare) to prepare an SH3 affinity column. Rat brains were homogenized in buffer C (20 mM Hepes, pH 7.4, 150 mM NaCl, 0.1% Triton X-100). The homogenate was incubated with the SH3-GST-loaded beads, dynamin was eluted with 2 ml of buffer D (20 mM Pipes, pH 6.2, 1.2 mM NaCl, 10 mM CaCl₂) and dialyzed overnight against 400 ml of buffer E (20 mM HEPES, pH 7.4, 100 mM NaCl, 10% glycerol). Aliquots of purified dynamin were stored at −70 °C.

GTP Hydrolysis—MxA protein (60 μg/ml) and dynamin (160 μg/ml) were incubated in 50 μl of HCB150 (20 mM Hepes, pH 7.5, 1 mM MgCl₂, 1 mM DTT, 150 mM NaCl) with 1 mM GTP, 200 nCi of [α-32P]GTP, and 100 nM AMP-PNP as described (23). At various time points reactions were terminated by mixing with the same volume of 2 mM EDTA in 0.5% SDS. Radio-labeled GDP was separated from the GTP substrate by chromatography on PEI-cellulose coated plates (Merck) with 1 M LiCl and 1 M acetic acid. Radioactive spots were detected by autoradiography and quantified using MacBAS software from Fuji.

Liposome Preparation—Phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE) (10 mg/ml) (from Avanti Polar lipids, obtained in chloroform) and Folch fraction of total bovine brain lipids (TBLs) (Sigma) dissolved in chloroform to 10 mg/ml, were dried under a stream of nitrogen and kept under vacuum for at least 2 h. Then the lipids were resuspended to a final concentration of 2 mg/ml in HCB of the indicated salt concentration. To ensure the integrity of the liposomes, each preparation was osmotically balanced for the salt concentration used in the different experiments. To generate liposomes of uniform size, the lipid solution was extruded 21 times through a 400-nm polycarbonate membrane (Avanti Polar Lipids).

Protease K Digestion—Purified MxA was diluted in HCB150 to a protein concentration of 180 μg/ml in a total volume of 50 μl. When indicated, preformed TBL liposomes were added at a concentration of 1 mg/ml. Then the protein mixture was incubated for 20 min at 20 °C. Proteinase K was added to a final concentration of 8 μg/ml, and the mixture was further incubated for 5 min at 20 °C. The digestion was stopped by adding 15 μl of 4-fold SDS-sample buffer. Samples were analyzed by SDS-PAGE and subsequent Western blotting using antibodies specific for MxA (M143) and the histidine tag (Sigma).

Sedimentation Assay—Purified MxA was diluted in HCB of the indicated NaCl concentrations to a protein concentration of 180 μg/ml in a total volume of 50 μl. Preformed liposomes were added at a concentration of 1 mg/ml. Then the protein-lipid mixture was incubated for 20 min at 20 °C and centrifuged at 100,000 × g for 20 min at 20 °C in a TLA-55 rotor (Beckman Instruments). Supernatants (50 μl) were mixed with 15 μl of 4-fold SDS-sample buffer, and pellets were resuspended in 65 μl of SDS-sample buffer (29). Supernatants (20 μl) and pellets (20 μl) were analyzed by SDS-PAGE, stained with Coomassie Brilliant Blue, and the protein bands were quantified using the Tytitzyme software of The Discovery series (Bio-Rad).

Negative Stain TEM—MxA was diluted in HCB of the indicated NaCl concentration to a protein concentration of 250 μg/ml and incubated for 2 h at 25 °C or 16 h at 4 °C. Then, a 400-mesh carbon-coated grid was placed on a 20-μl sample drop for 2 min at room temperature and blotted with a filter paper to remove excess solution. The sample was chemically stained by placing the grid on a 20-μl drop of 2% uranyl acetate for 2 s and blooting, then placing the grid on another 20-μl drop of 2% uranyl acetate for 2 min, followed by blotting and air-drying. Specimens were examined in a Philips CM120 TEM.
operating at 120 kV. Images were recorded digitally on a Gatan MultiScan 791 camera using the DigitalMicrograph software (Gatan, U.K.).

Cryo-TEM—Specimens were prepared manually in a controlled environment vitrification system (CEVS) or using the commercial environmentally controlled automated Vitrobot (FEI). In both devices, cryo-TEM samples were prepared at a controlled temperature and at saturated air humidity. A 6-μl drop of the solution was placed on a 200-mesh TEM copper grid (Ted-Pella) covered with a perforated carbon film. The drop was blotted, manually in the CEVS and automatically in the Vitrobot, and the sample was plunged into liquid ethane (−183 °C) to form a vitrified specimen, then transferred to liquid nitrogen (−196 °C) for storage. Vitrified specimens were examined in a Tecnai T12 G2 TEM (FEI) or in a Philips CM120 at 120 kV, at temperatures below −175 °C. Images were recorded digitally on a Gatan UltraScan 1000 2k × 2k or Gatan MultiScan 791 cooled CCD camera using DigitalMicrograph (Gatan) in the low dose imaging mode to minimize beam exposure and electron beam radiation damage, as described previously (30).

RESULTS

MxA Self-assembly into Rings and Filamentous Structures—We first studied the effect of salt concentration and temperature on MxA self-assembly by electron microscopy. MxA formed ring-like structures in the presence of 25 mM and 100 mM NaCl at 25 °C (Fig. 2, a and b) that disassembled at 300 mM salt concentration (Fig. 2c). The rings of oligomerized MxA had a diameter of about 50 nm, similar to rings created by dynamin in low salt (19) and by DLP1 (31).

We used a salt concentration of 100 mM NaCl to analyze the MxA structures in more detail by cryo-TEM in the next set of experiments. Incubation of MxA at 25 °C resulted in the formation of ring-like structures (Fig. 2e) whereas at 4 °C MxA oligomerized into long filamentous structures with a diameter of about 10 nm and a length of about 500–1000 nm (Fig. 2d). The assembly of MxA into rings or long filaments was reversible. A shift to lower temperature led to the conversion of the ring structures into long filaments and vice versa (data not shown). Higher magnification of the cryo-TEM images revealed that the rings and filaments contained two parallel sets of electron-dense structures that together form the more compact structures (Fig. 2, f and g, arrowheads). These results support the recent model of two parallel chains of MxA molecules in the oligomer, based on the crystal structure of the MxA stalk region (8).

MxA Oligomerization Results in Liposome Tubulation—MxA has been shown to associate with membranes of the smooth endoplasmic reticulum (17) and with liposomes in vitro (16). To study association of MxA with lipid membranes, sedimentation assays in the absence and presence of liposomes were carried out (22). Low salt concentrations led to accumulation of MxA into the pellet fraction, whereas at 300 mM salt buffer, MxA remained mostly in the supernatant (Fig. 3A). In the presence of liposomes, MxA co-sedimented with the liposomes even at 300 mM salt, suggesting an association with the lipid structures (Fig. 3B). This association was further studied by TEM (Fig. 3C). Clearly, MxA bound to spherical liposomes and transformed them into tubes with a diameter of about 50 nm (Fig. 3C, a and b). Evidently, the tubes were decorated by MxA in a highly ordered helical pattern as shown by cryo-TEM for MxA with TBL (Fig. 3Cc) as well as with PS at 100 mN (Fig. 3Cd) and 300 mM NaCl (Fig. 3Ce). Furthermore, MxA structures on the surface of lipid tubes strikingly resembled the dynamin oligomers formed under identical conditions (Fig. 3Cf).

MxA Oligomerization on Lipid Bilayers Does Not Enhance GTP Hydrolysis—As in the case of dynamin, MxA shows protein concentration-dependent cooperative activation of GTP
hydrolysis in solution (8, 23). For dynamin and dynamin-like proteins, it was shown previously that oligomerization on liposomes enhances GTP hydrolysis most likely by favoring intermolecular G domain-G domain contacts (15, 26–28). Surprisingly, incubation of MxA with TBL liposomes did not result in enhanced GTP hydrolysis (Fig. 4A). To verify that this lack of stimulation was not an artifact of the assay system, control experiments were performed. Cross-linking of MxA by monoclonal antibody 2C12 but not antibody M143 (see Fig. 8a) is known to stimulate GTP hydrolysis (32). In the present experiment, incubation of MxA with the 2C12 antibody stimulated GTP hydrolysis about 3-fold as demonstrated by an increase of the turnover number from 31.2/min to 79.4/min (Fig. 4A). This increase is in the expected range (23, 32) and demonstrates the intrinsic ability of MxA for enhanced GTP hydrolysis under the present assay conditions. Incubation of dynamin with liposomes led to about 20-fold increase of its GTPase activity (Fig. 4B). The basic GTP turnover number of 5.3/min of our dynamin preparation is in the range of previously published dynamin activities (18, 24) and is about 6-fold lower than the basal activity of MxA under identical assay conditions. The difference in the liposome-activated GTP hydrolysis between MxA and dynamin implies that the lipid-bound oligomeric structures of these two dynamin family members differ in an as yet unknown way.

**Structure of Lipid-associated MxA Oligomers**—The present cryo-TEM analysis of lipid-bound MxA revealed a “T-bar”-shaped structure (Fig. 5A, a and b) that is very similar to the appearance of lipid-bound dynamin (Fig. 3Cf). The same tubulation of liposomes was found with MxA(T103A), a mutant molecular G domain-G domain contacts (15, 26–28). For dynamin and dynamin-like proteins, it was shown previously that oligomerization on liposomes enhances GTP hydrolysis most likely by favoring intermolecular G domain-G domain contacts (15, 26–28). Surprisingly, incubation of MxA with TBL liposomes did not result in enhanced GTP hydrolysis (Fig. 4A). To verify that this lack of stimulation was not an artifact of the assay system, control experiments were performed. Cross-linking of MxA by monoclonal antibody 2C12 but not antibody M143 (see Fig. 8a) is known to stimulate GTP hydrolysis (32). In the present experiment, incubation of MxA with the 2C12 antibody stimulated GTP hydrolysis about 3-fold as demonstrated by an increase of the turnover number from 31.2/min to 79.4/min (Fig. 4A). This increase is in the expected range (23, 32) and demonstrates the intrinsic ability of MxA for enhanced GTP hydrolysis under the present assay conditions. Incubation of dynamin with liposomes led to about 20-fold increase of its GTPase activity (Fig. 4B). The basic GTP turnover number of 5.3/min of our dynamin preparation is in the range of previously published dynamin activities (18, 24) and is about 6-fold lower than the basal activity of MxA under identical assay conditions. The difference in the liposome-activated GTP hydrolysis between MxA and dynamin implies that the lipid-bound oligomeric structures of these two dynamin family members differ in an as yet unknown way.
with a single amino acid exchange within the GTP-binding site that inactivates GTPase activity (33) (Fig. 5A, c and d).

To clarify the orientation of the MxA monomers within these membrane-associated structures, we made use of two proteinase K cleavage sites in full-length MxA. The first cleavage occurs after an N-terminal asparagine at position 40. It removes the N-terminal His tag thereby reduces the molecular mass of the protein to 72 kDa (p72) (Fig. 5B). The second cleavage takes place after a C-terminal phenylalanine at position 563 in the L4 loop resulting in two fragments of 60 and 10 kDa (p60 and p10, respectively) (8, 9, 34). The full-length MxA and the p72 and p60 cleavage products were detected by the monoclonal antibody M143 recognizing a central epitope of MxA (see Fig. 8a) (32). Preincubation of MxA with TBL liposomes and subsequent incubation with proteinase K resulted in partial protection of the L4 loop from cleavage but did not prevent the loss of the His tag. Protection of the L4 cleavage site by lipid association could be reversed when the lipid bilayer was destroyed by Triton X-100 (Fig. 5C). Theoretically, protection from proteinase K digestion could be due to nonspecific oligomerization of MxA in the presence of lipid membranes rather than to lipid binding. To exclude this possibility, we took advantage of the fact that GTPγS, a nonhydrolyzable GTP analog, promotes MxA oligomerization in the absence of lipid membranes (8, 22). We therefore studied the proteolytic digestion of L4 in the presence of GTPγS. Incubation with ATP and GDP were used as controls. GTPγS-induced oligomerization of MxA did not prevent proteinase K digestion, whereas preincubation with liposomes partially affected the cleavage of MxA, as expected (Fig. 5D). These findings support the view of an MxA ring structure in which the L4 loops contact the lipid membrane and are thereby protected from proteolytic attack.

MxA Binds to Negatively Charged Phospholipids—To determine the influence of the lipid composition on MxA binding, liposomes generated from synthetic phospholipids PE or PC were compared with negatively charged PS- and TBL-based liposomes. Sedimentation of MxA at high salt in the presence of PS and TBL was comparable whereas liposomes generated from PE or PC did not induce MxA sedimentation (Fig. 6A). Thus, MxA was found to have a preference for negatively charged phospholipids. TEM experiments were consistent with the sedimentation assay and showed that MxA assembled onto negatively charged PS liposomes, whereas no assembly was detected on PC liposomes at high salt (Fig. 6B). The membranes of the endoplasmic reticulum compartment which is the site of MxA accumulation within IFN-treated cells (16, 17) show a high content of PC (64%) but only limited amounts of PS (4%) (35, 36). We therefore tested MxA association to liposomes formed by various proportion of PS versus PC in TEM analysis. We found a steadily increasing association of MxA (Fig. 6C) and increasing tendency for tube formation (Fig. 6B, a and c) in the presence of increasing PS concentration in the lipid mixture with a single amino acid exchange within the GTP-binding site that inactivates GTPase activity (33) (Fig. 5A, c and d).
confirming the preference of MxA for negatively charged lipids. This was also evident in the proteinase K protection assay. Incubation of MxA with TBL or PS liposomes prevented cleavage of the L4 loop, whereas PE and PC had no protective effect (Fig. 7A). Furthermore, binding of MxA to PS suggested that positively charged residues in the L4 loop are involved in binding to the negatively charged phospholipids. We therefore analyzed single amino acid exchanges from lysine to glutamic acid within a stretch of four lysine residues (position 554 to 557) within L4. In each case the exchange from a basic to an acidic residue prevented co-sedimentation of MxA with the liposomes (Fig. 7D). In parallel, we tested the ability of the L4 mutants to self-assemble in the presence of GTPyS, to exclude the possibility that the mutations in the flexible loop L4 pro-
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FIGURE 8. Structural model of MxA oligomers associated with liposomes. a, linear structure of human MxA with the N-terminal G domain and the C-terminal part forming the stalk. The recombinant protein is N-terminal fused to a His tag (His). Arrowheads indicate the positions of two proteinase K cleavage sites (PK). A red bar marks the position of loop L4 (position 533–572). The positions of the antibody binding sites for anti-His and anti-Mx antibodies, M143 and 2C12, are indicated by Y. b, hypothetical structure of a MxA monomer (side view) consisting of the MxA stalk fused to the G domain of dynamin. The red line indicates the position of loop L4. c, front view of an MxA oligomer associated with the membrane via loop L4 (red line), with the G domains sticking out in opposite directions leading to the typical T-bar structure. d, schematic side view of an MxA oligomeric ring structure associated with a lipid tubule (modified according to Ref. 8).

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voked general defects in the structure of the MxA stalk. All the mutants were found in the pellet fraction in the presence of GTPγS, whereas they did not sediment in the absence of the nucleotide, similar to wild-type MxA (Fig. 7, B and C). In summary, these data indicate a preference of MxA for negatively charged lipid surfaces which is most likely recognized by positively charged amino acids in its L4 loop.

DISCUSSION

Here, we investigated MxA self-assembly onto lipid bilayers under controlled near physiological conditions and analyzed their structure by cryo-TEM. Furthermore, we characterized lipid binding and nucleotide hydrolysis by biochemical approaches. The present data support and extend recent conclusions on the architecture of the MxA GTPase based on its crystal structure (8). Importantly, the results reveal that the unstructured L4 loop protruding from the compact stalk serves as the membrane binding domain, analogous to the PH domain of dynamin (Figs. 1 and 8, a and b). Our results also demonstrate that self-assembly onto lipid bilayers does not stimulate GTP hydrolysis, in sharp contrast to dynamin. We conclude that MxA assemblies on membranes critically differ from those of dynamin although both have almost identical appearance in cryo-TEM images.

In a first set of experiments we studied MxA oligomers in the absence of liposomes. Depending on the incubation conditions, rings or linear filaments were detected, as described previously (22). Interestingly, MxA readily self-assembled into rings at physiological salt conditions, as opposed to dynamin, which self-assembles only at low salt concentrations or in the presence of lipids (37, 38). Detailed examination with the higher resolution cryo-TEM technique revealed that each ring or filament consists of a bundle of two parallel thin electron-dense structures (Fig. 2). This composition of two thin strands is compatible with the appearance of two parallel chains of MxA molecules in which the globular G domains stick out on both sides of the central stalk bundle forming the backbone of the long polymer as suggested from the structure of MxA stalk oligomers within the protein crystals (8).

In the presence of liposomes, MxA self-assembled into highly organized rings or helical structures that tubulated the vesicles (Fig. 3). The liposome-associated MxA oligomers showed a characteristic T-bar shape in the front views (Fig. 5A). This feature is consistent with the current model of MxA oligomers. The criss-cross arrangements of the stalks can be modeled into ring-like structures with the globular G domains pointing out of the rings into opposite directions, giving rise to the typical T-bar appearance (Fig. 8C) (8).

The MxA GTPase specifically interacts with lipid surfaces although it lacks a PH domain (16, 39 and present results). In dynamin, the PH domain is responsible for lipid interaction and is located between the MD and the GED (40). In MxA, the unstructured loop L4 is located at an equivalent position (residues 533–572) (Fig. 1) (8). This loop is highly variable (41) and is accessible to proteolytic digestion at glutamine 564 by proteinase K (9). Here, we demonstrate that liposome binding of full-length MxA protects the L4 loop from cleavage, suggesting that the loop is contacting the lipid bilayer and may represent the lipid binding part of the MxA stalk (Figs. 5C and 8, C and d). In contrast, a second, N-terminal cleavage site that is located at the opposite side of the stalk was susceptible to proteinase K digestion. Of note, MxA seems to bind preferentially to liposomes formed by negatively charged phospholipids, suggesting that positively charged residues within the L4 loop are responsible for lipid binding, as described for dynamin (38). Therefore, we propose that a stretch of four positively charged lysines within L4 (residues 554–557) might be critical for this interaction. Because substitutions of all four lysine residues affected the ability of MxA to self-assemble in the presence of GTPγS,7 we introduced single amino acid exchanges from lysine to glutamic acid. All of the resulting mutant proteins showed a strong reduction in liposome binding without affecting their capacity to oligomerize in the presence of GTPγS. We therefore conclude that the loop L4 is directly involved in lipid binding and represents the functional equivalent of the PH domain of dynamin, although the two domains show no sequence similarity.

Cooperative stimulation of GTP hydrolysis is a characteristic feature of dynamin family members. Surprisingly, the assembly of MxA onto liposomes did not change GTP hydrolysis whereas liposome binding of dynamin increased nucleotide hydrolysis about 20-fold, as expected (Fig. 4) (26, 28, 42). Intracellular membranes are the active sites of dynamin action. Assembly-stimulated GTPase activity induced by oligomerization around liposomes is required for dynamin membrane constriction and fission activity (20, 43). The role of lipid binding for MxA is less clear (16, 17). We have previously proposed that oligomer formation in association with intracellular membranes might protect MxA from proteolytic degradation (44). Some MxA

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molecules would be expected to dissociate from these membrane-associated pools and form stable tetramers to serve antiviral functions, whereas the majority of membrane-associated MxA molecules might stay in a latent, nonactivated state. The lack of assembly-stimulated GTPase activity of lipid-bound MxA observed here is in agreement with such a scenario. This functional difference between MxA and dynamin must be based on structural characteristics of the liposome-associated oligomers. In dynamin, co-operativity was shown to be a consequence of a direct contact between the G domains (15). It was postulated that in the dynamin tetramer the G domains stick out in opposite directions and do not get in contact with each other. Only when assembled into helices around lipid tubes, the G domains of neighboring turns would be able to interact and cause the cooperative stimulation of GTP hydrolysis (40). Cryo-TEM images and three-dimensional reconstructions revealed close inter-turn contacts between the supposed G domains of neighboring turns, supporting this scenario (21, 45). The present cryo-TEM images show that the morphology of lipid-bound MxA (Figs. 3 and 5) and lipid-bound dynamin are similar. Interestingly, we recently demonstrated that a monomeric form of MxA with a single amino acid substitution (M527D) in interface 2 of the stalk showed a concentration-dependent increase in GTPase activity, suggesting that self-assembly of wild-type MxA restricts GTP hydrolysis (8). The observed increase in hydrolysis was presumably due to free interactions of the MxA monomers allowing frequent G-G domain contacts. In contrast, oligomerization of MxA onto liposomes and their tubulation might not provide sufficiently close contacts between G domains of neighboring rings to stimulate hydrolysis. Further structural analysis of full-length MxA crystals or three-dimensional reconstructions based on our cryo-TEM images may help to solve this question. Finally, it is conceivable that post-translational modifications as well as interactions with cellular or viral factors may modulate the enzymatic and antiviral activity of Mx GTPases (1).

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