Human MxA protein is an interferon-induced member of the dynamin superfamily of large GTPases. MxA inhibits the multiplication of several RNA viruses, including Thogoto virus, an influenza virus-like orthomyxovirus transmitted by ticks. Previous studies have indicated that GTP binding is required for antiviral activity, but the mechanism of action is still unknown. Here, we have used an in vitro cosedimentation assay to demonstrate, for the first time, a GTP-dependent interaction between MxA GTPase and a viral target structure. The assay is based on highly active MxA GTPase as effector molecules, Thogoto virus nucleocapsids as viral targets, and guanosine 5′-O-(3-thiotriphosphate) (GTPγS) as a stabilizing factor. We show that MxA tightly interacts with viral nucleocapsids by binding to the nucleoprotein component. This interaction requires the presence of GTPγS and is mediated by domains in the carboxyl-terminal moiety of MxA. We propose that GTP-bound MxA adopts an antivirally active conformation that allows interaction with viral nucleocapsids, thereby impairing their normal function.

Human MxA, a 76-kDa GTPase, belongs to the newly defined dynamin superfamily of high molecular mass GTPases found in yeast, plant, and animal cells (1). These large GTPases play key roles in fundamental cellular processes, such as endocytosis (2); intracellular vesicle transport (3); cell plate formation in plants (4); and in the case of Mx proteins, resistance to virus infection (5). MxA is induced exclusively by type I (α/β) interferons (6), accumulates in the cytoplasm of cells (7), and interferes with the multiplication of distinct RNA viruses. Work with both transfected cells (8–10) and MxA transgenic mice (11) demonstrated that MxA is a powerful antiviral agent. In man, synthesis of MxA is induced during acute viral infections and may thus protect humans from severe disease (12, 13).

GTP binding seems to be crucial for antiviral activity because mutations within the N-terminal GTP-binding domain destroy the antiviral activity (14, 15). In addition, the C-terminal part of MxA seems to play an important role because the coexpression of an antivirally inactive C-terminal fragment of MxA interferes with wild-type MxA activity in a dominant-negative manner (15). Furthermore, a single amino acid exchange in the C terminus affects the antiviral specificity of wild-type MxA. This substitution results in loss of activity against vesicular stomatitis virus (VSV) while maintaining wild-type activity against influenza A virus (FLUAV) and Thogoto virus (THOV), a tick-borne orthomyxovirus (9, 16).

It has been shown that cytoplasmic MxA inhibits primary transcription of VSV and measles virus, an early step in the viral life cycle that occurs in the cytoplasm of infected cells (17, 18). In contrast, FLUAV transcribes its genome in the cell nucleus and is blocked by MxA at a later step after primary transcription that is still unknown (8). However, MxA is able to block primary transcription of FLUAV when translocated to the nucleus by a foreign nuclear translocation signal (16). Moreover, recombinant MxA is capable of inhibiting the transcriptional activity of purified viral nucleocapsids in vitro (19). Nevertheless and despite much effort, biochemical data demonstrating a physical interaction with viral components are still missing, and viral target structures are presently unknown.

Most GTPases act as molecular switches, with the GTP-bound form usually representing the active state (20). Members of the dynamin superfamily may be unique among GTPases because they seem to behave as mechanochemical enzymes rather than as molecular switches (21, 22). It has been shown that the GTP-bound conformation of dynamin self-assembles around tubular membrane invaginations (23) and that GTP hydrolysis leads to constriction and vesiculation of dynamin-coated tubes (22). Moreover, it has been proposed that the ability to form helical arrays around tubular templates might be a functional link between all dynamin-like GTPases (21). In fact, MxA also forms aggregates of ~30 molecules (24) that adopt a helical structure in solution, and C-shaped and ring-like structures have been described for mouse Mx1 protein (25).

Biochemical studies revealed that MxA has an intrinsic GTPase activity characteristic of large GTPases and that a high percentage of MxA molecules may be complexed with GTP in vivo (24). We reasoned that it is GTP-bound MxA that represents the antivirally active form and that interacts with viral targets. Therefore, we performed binding studies in the presence of the non-hydrolyzable GTP analogue GTPγS. For this approach, we used THOV because this virus represents the most MxA-sensitive virus known to date.

Here, we show for the first time an association of MxA with viral proteins. In its GTP-bound form, MxA associates with the nucleocapsids of THOV by binding to the nucleoprotein (NP) component. We show that this interaction is mediated by do...
main(s) in the carboxy-terminal moiety of MxA and can be prevented by a monoclonal antibody directed against this region. These results suggest that the binding of GTP to MxA induces an active conformation of the carboxy-terminal domain. Furthermore, we suggest that MxA works by recognizing and wrapping around incoming nucleocapsids in the cytoplasm of the infected cell, thereby inactivating their function.

EXPERIMENTAL PROCEDURES

Cells and Viruses—Parental Swiss mouse 3T3 cells and cells stably transfected and constitutively expressing wild-type human MxA (clone 4.5.15) were the same as described previously (26). Cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum with or without 0.5 mg/ml genetin (G418).

3T3 cells were infected with THOV strain Sia126 (27) or Dhori virus (DHOV) strain DHO/India/1313/61 (28) with an input multiplicity of infection of 10 and incubated for 16 h. For metabolic labeling of viral proteins, cells were incubated for the last 4 h in Dulbecco’s modified Eagle’s medium minus methionine and 50 μCi/ml [35S]methionine.

Expression and Purification of Recombinant Proteins—The NP of THOV was synthesized in Escherichia coli and purified by means of an N-terminal His tag. Using polymerase chain reaction, a Smal site was added at positions 14–19 of the NP cDNA insert of the plasmid pBR-L3 (29). The plasmid was made single-stranded and inserted into the NP into the blunt BamHI site of the procaryotic expression vector pQE9 (QIAGEN, Inc., Hilden, Germany), yielding the plasmid pHisNP. All MxA mutants (see Fig. 1A) used in coimmunoprecipitation experiments derive from the plasmid pHis-MxA, which contains the His-tagged MxA cDNA (14, 15, 30).

Histidine-tagged proteins were produced in E. coli M15 after induction with isopropyl-β-D-thiogalactopyranoside and were isolated using Ni2+ chelate agarose chromatography as described (14). NP was further purified using heparin-Sepharose (Amersham Pharmacia Biotech) in 20 mM Tris, pH 8.0. It eluted from the column as a sharp peak at 0.7 M NaCl.

Coimmunoprecipitation—Cell extracts were prepared in 50 mM Tris, 0.1% Nonidet P-40, 5 mM MgCl2, and 0.5 mM dithiothreitol, pH 7.5. After separation of the cell debris (15 min at 12,000 × g), the supernatants were used for coimmunoprecipitation. First, lysates of MxA-expressing cells or the E. coli-expressed MxA mutants were mixed with a polyclonal rabbit anti-MxA antiserum. The mixture was then added to protein A-Sepharose beads using polyclonal anti-MxA antibodies (31–33). The immunoprecipitated proteins, cells were incubated for the last 4 h in Dulbecco’s modified Eagle’s medium minus methionine and 50 μCi/ml [35S]methionine.

RESULTS

MxA Interacts with the NP of THOV—To search for viral target proteins of MxA, the following strategy was used. Wild-type and mutant forms of MxA (Fig. 1A) were immobilized on protein A-Sepharose beads using polyclonal anti-MxA antibodies. These MxA-loaded beads were subsequently used to coprecipitate proteins from lysates of THOV-infected cells. To allow detection of putative interaction partners, newly synthesized proteins were radioactively labeled by incubating the infected cells with [35S]methionine. Fig. 1B shows that wild-type MxA synthesized in transected 3T3 cells, but not a control preparation without MxA, was able to precipitate a [35S]-labeled viral protein from THOV-infected cells (lanes 1 and 2). The radiolabeled protein had an estimated molecular mass of 52 kDa and was identified as the NP of THOV by immunoprecipitation with monospecific anti-NP antibodies (Fig. 1D). The same viral protein was precipitated by wild-type MxA produced in E. coli (Fig. 1B, lane 3). To determine which region of MxA was critical for NP binding, we produced various truncated forms of MxA in E. coli, as schematically depicted in Fig. 1A. The presence of wild-type (76 kDa) and mutant forms of MxA in the coprecipitation reactions was confirmed by Western blot analysis demonstrating bands of the expected sizes in each reaction sample (Fig. 1C). A truncated form of MxA lacking the last 90 amino acids (A572–662) had lost the capacity to precipitate NP (Fig. 1B, lane 4). Likewise, a MxA mutant with a central deletion of 275 amino acids (A301–576) was unable to precipitate NP (Fig. 1B, lane 5). Most significantly, however, a truncated form consisting of only the C-terminal half of MxA (A572–662) was able to coprecipitate NP, albeit at seemingly lower efficiency as compared with the wild type (Fig. 1B, lane 6). Taken together, these experiments suggest that NP is the viral target recognized by MxA and that the domains responsible for recognition reside in the C-terminal moiety of MxA.

MxA Binds to Nucleocapsids of THOV—NP is the most abundant component of viral nucleocapsid, also referred to as viral...
Interaction of GTP-bound MxA with Viral Nucleocapsids

A

| Protein       | Lane | MxA/NP association |
|---------------|------|--------------------|
| MxA           | 1    | Yes                |
| Δ572-662      | 2    | No                 |
| Δ301-576      | 3    | No                 |
| Δ1-362        | 4    | Yes                |
| MxA, Δ572-662 | 5    |                     |
| MxA, Δ301-576 | 6    |                     |

B

1. Control
2. MxA
3. MxA
4. Δ572-662
5. Δ301-576
6. Δ1-362

C

1. Control
2. MxA
3. Δ572-662
4. Δ301-576
5. Δ1-362

D

1. Control
2. MxA
3. Δ572-662
4. Δ301-576
5. Δ1-362

Fig. 1. The carboxyl-terminal moiety of MxA interacts with the NP of THOV. A, schematic diagram of wild-type and mutant MxA proteins purified from E. coli and used in coprecipitation assays. MxA represents the wild-type protein. The consensus tripartite motif for guanine nucleotide binding at the N terminus is represented by three open bars. Hatched boxes near the C terminus indicate two putative leucine zippers. The region recognized by monoclonal antibody 2C12 is indicated. Deletion mutant Δ572–662 lacks the last 90 amino acids of MxA, deletion mutant Δ301–576 lacks a central fragment of 278 amino acids, and deletion mutant Δ1–362 consists of the C-terminal half of the protein. B, wild-type and mutant MxA proteins were immobilized on protein A-Sepharose beads and incubated with 35S-labeled extracts of THOV-infected cells. Coprecipitated metabolically labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis (10%) and autoradiography. Lane 1, No MxA; lane 2, wild-type MxA produced in E. coli; lane 4, MxA mutant Δ572–662; lane 5, MxA mutant Δ301–576; lane 6, MxA mutant Δ1–362. C, Western blot analysis of the immobilized proteins used in B demonstrated the presence of wild-type or mutant MxA proteins in the coprecipitation samples. MxA was detected by a polyclonal mouse antiserum against E. coli-expressed wild-type MxA. This antibody detected also a contaminating protein of unknown nature (lane 4, upper band). Molecular mass markers are indicated. D, the identity of the coprecipitated 35S-labeled NP was confirmed by immunoprecipitation with anti-NP antibodies. 35S-labeled THOV NP coprecipitated with beads containing no MxA (lane 1), or wild-type MxA (lane 2) was detected by autoradiography. The coprecipitated protein was reisolated from the MxA immunocomplex and immunoprecipitated with monoclonal antibody mAb2 specific for NP (lane 4), with monoclonal antibody mAb10 directed against the viral glycoprotein (GP; lane 5), or with a polyclonal anti-THOV antisera (THOV; lane 6). Lane 3 shows the control without antibody.

ribonucleoprotein complexes (vRNPs). We therefore investigated whether MxA would recognize NP in these viral structures. Binding was assessed in a cosedimentation assay that enabled us to test the capacity of MxA to cosediment with THOV RNPs during ultracentrifugation in a discontinuous glycerol gradient. A gradient system was established that allowed us to distinguish between free MxA and MxA bound to vRNPs. Lysates were prepared from cells expressing recombinant MxA (26) and were then mixed with lysates obtained from either THOV-infected or uninfected control cells. The mixture was separated by glycerol gradient centrifugation, and fractions of the gradient were analyzed by immunoblotting with antibodies to MxA and to the NP of THOV. The NP-specific bands allowed easy identification of fractions containing vRNPs. As shown in Fig. 2A, MxA alone was not able to penetrate into the gradient beyond fractions 5 and 6. Instead, MxA stayed on top of the glycerol layer. The nucleocapsids present in lysates of THOV-infected cells sedimented as a broad peak into fractions of high density exhibiting a sedimentation behavior that was very distinct from that of free MxA (data not shown). When MxA-containing lysates were mixed with lysates of infected cells, an altered distribution of MxA was observed. MxA now cosedimented with vRNPs to fractions of higher density, suggesting a tight association of MxA with these viral structures. In addition, we were able to demonstrate cosedimentation of MxA with purified vRNPs isolated from THOV particles (data not shown).

Next, we wanted to know whether this association was virus-specific. We have previously shown that MxA is an efficient inhibitor of THOV, but is inactive against DHov, another tick-borne orthomyxovirus (9). Therefore, we tested whether MxA would similarly bind to DHov nucleocapsids. Fig. 2A shows that there was no cosedimentation of MxA with vRNPs of DHov. The nucleocapsids present in lysates of DHov-infected cells penetrated deeply into the gradient like THOV nucleocapsids. In contrast, MxA remained on top of the gradient as in gradients with uninfected control cell lysates, indicating a failure to interact. These findings suggest that the capacity to interact in our in vitro test system and the ability to block viral multiplication in vivo are most likely two facets of the same phenomenon.

Cosedimentation of MxA with vRNPs Depends on GTPγS—The initial experiments were performed in the presence of the non-hydrolyzable GTP analogue GTPγS (Fig. 2A). Previous studies have shown that GTP binding is critical for the antiviral action of Mx proteins (14, 15, 19). Therefore, it was conceivable that GTP was required for proper interaction of MxA with vRNPs. To address this question, cosedimentation assays of MxA with THOV nucleocapsids were performed in the presence or absence of GTPγS. Clearly, MxA interacted with vRNPs only in the presence of GTPγS (Fig. 2B, panel b). No interaction occurred in the absence of this nucleotide analogue or in the presence of GDPγS (Fig. 2B, panels a and c). In the presence of GTPγS, the RNPs moved deeper into the gradient than in the absence of GTPγS, as indicated by the position of the NP-
specific bands. This shift of RNPs to higher density suggests that large and stable complexes between MxA and nucleocapsids were formed. We also tested hydrolyzable GTP and GDP nucleotides. In both cases, MxA did not show an interaction with vRNPs (data not shown), indicating that the non-hydrolyzable GTP analogue most likely stabilized an interactive conformation of MxA.

**Monoclonal Antibody 2C12 Prevents the Association of MxA with vRNPs**—We used monoclonal antibody 2C12 to abrogate cosedimentation of MxA with vRNPs. This antibody is known to recognize a conserved epitope on rodent and human Mx proteins (37, 38) and to neutralize the antiviral effect of murine Mx1 protein (39). In addition, this antibody is also able to neutralize the antiviral activity of human MxA against THOV when microinjected into the cytoplasm of MxA-expressing cells (data not shown). Therefore, we tested whether this antibody would also prevent the interaction of MxA with vRNPs. Lysates of MxA-expressing cells were preincubated with antibody 2C12 and then mixed with lysates of THOV-infected cells and subsequently subjected to glycerol gradient centrifugation. Under these conditions, MxA did not cosediment with the nucleocapsids, indicating that preincubation with antibody 2C12 prevented the interaction of MxA with the viral target structure (Fig. 3A). In contrast, addition of a non-Mx-specific antibody (h38III) had no effect (Fig. 3C). Preincubation of THOV lysates with a monoclonal antibody directed against the NP of THOV likewise prevented the MxA-vRNP interaction (Fig. 3B), whereas addition of a monoclonal antibody directed against the THOV glycoprotein (Fig. 3D) was without effect. These results support the idea that cosedimentation was mediated by a specific interaction between MxA and the NP component of vRNPs.

**RNA-bound NP Is the Binding Partner of MxA**—In addition to NP, vRNPs consist of genomic vRNA and the three viral polymerase subunits. The results of the coprecipitation assay (Fig. 1) and the antibody inhibition experiment (Fig. 3B) suggested NP as the binding partner of MxA. To further elucidate the role of NP in the cosedimentation assay, we constructed artificial RNPs consisting only of genomic RNA and recombinant NP of THOV. NP containing six extra histidine residues at the N terminus was produced in *E. coli*, isolated by Ni\(^{2+}\) affinity chromatography, and further purified by heparin column chromatography (see “Experimental Procedures”). The pure recombinant NP had an estimated molecular mass of 52 kDa and exhibited RNA-binding capacity (29). The genomic vRNA preparation from purified virions consisted of a mixture of all six genomic RNA segments varying in length from 900 to 2200 nucleotides (29). Synthetic RNPs were produced by incubating vRNA with the purified NP in an appropriate binding buffer. Interestingly, these artificial RNPs had a similar sedimentation behavior in the glycerol gradient as authentic RNPs extracted from infected cells (Fig. 4B). More important, MxA cosedimented with these structures and reached the bottom of the gradient together with these artificial RNPs (Fig. 4B). In the absence of artificial RNPs, MxA remained at the top of the gradient, as expected (Fig. 4A).

These positive results encouraged us to use *in vitro* synthesized RNA molecules instead of RNA extracted from virions. First, we generated an 81-nucleotide-long model RNA that contained the complementary terminal sequences of segment 5 of influenza A virus. It has been shown that this molecule is able to form a double-stranded panhandle structure similar to the genomic influenza viral RNA segments (35). Indeed, preincubation of this model RNA with recombinant NP of THOV resulted in the formation of artificial RNPs capable of dragging MxA into the gradient (Fig. 4C). It was conceivable that this
effect was dependent on the panhandle structure of the viral RNA molecules. To further investigate this assumption, we synthesized a linear 2200-nucleotide-long RNA molecule that was predicted not to contain such a secondary structure. This RNA was then used as backbone to build up artificial RNPs. Again, MxA coprecipitated with such RNPs, and GTP\(\gamma\)S was necessary for interaction (Fig. 4, D and E). These findings clearly demonstrated that MxA interacted with the RNA-bound NP of THOV and that binding was independent of RNA secondary structure.

DISCUSSION

Specificity of vRNP Recognition—Previous attempts to demonstrate a physical interaction of MxA with target structures of FLUAV or VSV have invariably failed. Here, we used THOV because this orthomyxovirus shows an extraordinarily high sensitivity to the inhibitory effect of MxA. In cell culture, virus titers are on average 1,000,000-fold lower in MxA-expressing cells than in similarly infected control cells (9). By comparison, FLUAV and VSV titers are reduced at best 100–1000-fold in the presence of MxA (26). Accordingly, MxA transgenic mice were found to be completely resistant to infection with THOV, but showed only partial resistance to infection with FLUAV or VSV (11). We believe that our ability to identify THOV nucleocapsids as targets for MxA is a direct reflection of the extreme MxA sensitivity of THOV. Consistent with this reasoning is the finding that nucleocapsids of DHOV were not recognized by MxA (Fig. 2A). DHOV belongs to the same genus of tick-transmitted orthomyxoviruses as THOV (40). However, DHOV is insensitive to inhibition by MxA (9). Sequence comparisons of the NP genes of these two viruses showed that the degree of amino acid sequence similarity is only 43% (29). It may therefore well be that the target domain for MxA is not conserved among tick-borne orthomyxoviruses.

We also investigated the cosedimentation behavior of MxA with nucleocapsids of FLUAV and VSV. In both cases, no clear cosedimentation was observed.\(^3\) A possible explanation is that binding of GTP-bound MxA to vRNPs must be of great strength in order to be detected in our \textit{in vitro} cosedimentation assay and that weaker interactions are not readily demonstrable, although they may still be sufficient for mediating resistance \textit{in vivo}. It is, of course, also possible that MxA recognizes different target structures in THOV, FLUAV, and VSV. Finally, we cannot exclude a cooperative effect of additional host cell factors since all our experiments were done in the presence of cellular extracts. In fact, there is circumstantial evidence that cell type-specific factors may modulate the antiviral specificity of MxA action (18, 41).

Nature of the Viral Target Structure—The vRNPs of THOV
contain five components, namely genomic RNA, NP, and the RNA-dependent RNA polymerase complex consisting of three subunits PA, PB1, and PB2. NP is the major protein component of the vRNP structure (42). Previous studies with FLUA and mouse Mx1 protein pointed to the viral polymerase as the target of Mx action because high level expression of reconstituted PB2 could abolish the nuclear Mx1 block in infected cells, resulting in virus growth (43, 44). However, a similar effect with human MxA was not reported. Moreover, all attempts to demonstrate a biochemical interaction of mouse Mx1 or human MxA with PB2 were unsuccessful (44). Here, we have demonstrated that artificial RNPs consisting only of RNA and viral NP are sufficient to interact with MxA (Fig. 4). This clearly indicates that, at least for THOV, the polymerase subunits are not the primary target of MxA within the RNP structure. RNA is also excluded because MxA has no RNA-binding capacity.

The present data strongly suggest that MxA binds to the RNP structure by recognizing its major protein component, NP. A monoclonal antibody directed against NP prevented vRNPs from associating with GTP-bound MxA, whereas a monoclonal antibody against the envelope glycoprotein had no effect (Fig. 3). More importantly, MxA was able to coprecipitate NP from lysates of THOV-infected cells (Fig. 1). A truncated form of MxA lacking only 90 amino acids at the carboxyl terminus had lost this capacity, indicating that the observed interaction of NP with wild-type MxA was significant and not merely due to nonspecific binding. To obtain functional evidence for the role of NP, we have tried to neutralize the antiviral effect by expressing recombinant NP in MxA-containing cells. However, high level expression of NP proved to be toxic to the cells, and reliable results were not obtained. It remains to be seen whether MxA interacts with free NP or preferentially with NP integrated in RNPs. The coprecipitation assay used did not allow us to differentiate between these possibilities.

Importance of the Carboxyl-terminal Domain for Viral Target Recognition—To define the interactive domains of MxA, we took advantage of the availability of the broadly Mx-reactive monoclonal antibody 2C12 (37). We demonstrated that antibody 2C12 is capable of preventing the recognition of THOV nucleocapsids by MxA (Fig. 3). We have mapped the binding site of this antibody to an internal domain comprising amino acids 363–574 that is located in the C-terminal half of MxA (Fig. 1A; data not shown). This domain corresponds to the rat Mx3 protein domain containing the 2C12 epitope recently reported by Johannes et al. (38). Direct evidence for the presence of interactive domain(s) within the C-terminal half of MxA arises from the present finding that a C-terminal fragment was sufficient to coprecipitate THOV NP, whereas truncated forms of MxA with deletions in the C-terminal moiety had lost this function (Fig. 1B). Taken together, our results support the idea that the interactive structure of MxA is formed by sequences in the internal domain carrying the 2C12 epitope and sequences in the extreme C-terminal part. Both domains are seemingly also involved in the formation of a proper GTP-binding pocket and in oligomerization (30, 45). Our present results are supported by previous work using quite different approaches. Experiments with dominant-negative mutant forms of MxA showed that sequences in the C-terminal moiety were necessary and sufficient for interference with wild-type function (15). The C-terminal region of MxA (Fig. 1A) contains two highly conserved leucine zipper repeats (46). Leucine repeats form amphipathic α-helices that are known to promote protein-protein interactions (47). Targeted mutations impairing the amphipathic character of these helices destroy the antiviral activity of mouse Mx1 protein (48, 49). More convincingly, a single amino acid exchange within the distal leucine zipper motif changes the antiviral specificity of MxA such that the mutant protein lost antiviral activity against VSV, but maintained wild-type activity against FLUA and THOV (9, 16). The importance of the C-terminal region for the antiviral activity was also suggested by comparing the amino acid sequences of the inactive rat Mx3 and active rat Mx2 proteins (50). Substitutions of single amino acids in the C-terminal region of rat Mx3 led to gain of antiviral function in the protein (38). In summary, these findings indicate that the C-terminal half may expose domains that interact directly with viral target structures, although recognition of interposed cellular molecules can presently not be excluded. The cosedimentation assay presented here will be helpful in the search for such additional factors.

Crucial Role of GTP Binding for Antiviral Activity—Previous studies indicated that an intact GTP-binding domain is required for antiviral activity of MxA (14, 15). We show here that GTP binding is necessary and sufficient for the association of MxA with THOV RNPs (Fig. 2B). Most likely, binding of GTP leads to a conformational change of the molecule that allows specific recognition of viral targets, such as RNPs of THOV (Fig. 5). In addition, GTP binding and association with target structures may favor MxA-MxA interactions and stabilize the complex. Similar interactions have been observed with dynamin, another member of the superfamily of large GTPases. Dynamin is essential for receptor-mediated endocytosis and synaptic vesicle recycling (2). It is believed that dynamin forms a collar around the necks of clathrin-coated pits and helps to bud off vesicles from the plasma membrane (23). Dynamin can self-assemble into rings and stacks of interconnected rings in solution (51, 52). In the presence of GTPyS, long ring-like structures form around tubular membrane invaginations of ~25 nm in diameter (23). Recently, Sweitzer and Hinshaw (22) directly demonstrated that purified dynamin binds to lipid bilayers and forms helical tubes that constrict and generate vesicles upon GTP addition. These data suggest that dynamin does not behave as a molecular switch, but as a force-generat-

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