Domains Mediating Intramolecular Folding and Oligomerization of MxA GTPase*

Beate Schumacher and Peter Staeheli‡

From the Abteilung Virologie, Institut für Medizinische Mikrobiologie und Hygiene, University of Freiburg, 79008 Freiburg, Germany

MxA is an interferon-induced GTPase of human cells that inhibits the multiplication of several RNA viruses by a still poorly understood mechanism. Previous biochemical studies indicated that the C terminus of MxA folds back to form a functional GTP-binding pocket, and that an internal fragment contains a domain required for oligomerization. Using the yeast two-hybrid system, we now have mapped these domains. MxA sequences located downstream of amino acid 564 were found to strongly interact with an internal domain that includes amino acids 372 to 540. This interaction was abolished by mutating phenylalanine 382 or leucine 612, which is part of a leucine zipper motif. Neither the C-terminal nor the internal MxA fragments formed homo-oligomers. Using a mammalian nuclear transport assay that can detect protein-protein interactions, we further found that full-length MxA forms complexes with MxA fragments that include amino acids 372 to 540. This interaction was not observed when phenylalanine 382 was exchanged for alanine or arginine. Furthermore, interaction of two full-length MxA molecules occurred only if at least one of them carried a functional C-terminal leucine zipper motif. These results suggest that C-terminal back-folding and oligomerization are two alternative outcomes of the same type of interaction between the C-terminal and the internal domains of MxA. Intramolecular interaction is believed to result in the formation of MxA monomers, whereas intermolecular interaction may induce the formation of large MxA oligomers.

MxA is a 76-kDa protein that accumulates in the cytoplasm of many types of human cells in response to exposure to type I interferon (1–3). It is an antiviral protein that can act in the absence of other interferon-induced proteins. Constitutive expression of MxA in cell lines from mice, monkeys or humans mediates a high degree of resistance to infection with influenza A virus (4), Thogotovirus (5), and several other RNA viruses (4, 6–10). Furthermore, transgenic mice constitutively expressing MxA resist challenge infections with Thogotovirus (11). Because overexpression of MxA can induce apoptosis in Hep3B cells, and because it is abundantly expressed in lymphoblastoid cell lines from Fanconi anemia patients (12), it was suggested that MxA may contribute to the pathological losses of hematopoietic cells in this disease.

The primary structures and biochemical properties indicate that Mx proteins are members of the superfamily of large GTPases, which includes dynamin (13) and yeast pVPS-1 (14). Characteristic properties of these enzymes are that they bind GTP with low affinity and perform multiple cycles of GTP hydrolysis in the absence of nucleotide exchange factors (15, 16). Cells expressing MxA variants with mutations in the GTP-binding consensus element do not exhibit enhanced virus resistance (17), suggesting that GTP-binding is required for biological activity of Mx proteins. In vitro transcription experiments with vesicular stomatitis virus nucleocapsids showed that purified MxA can inhibit this viral multiplication step (18). Because inhibition was also observed when GTP-γS was the only guanine nucleotide present in the reaction mixtures, it was concluded that binding but not hydrolysis of GTP is essential for Mx activity (18).

Several attempts were made to define functional domains of Mx proteins. The N-terminal moiety of MxA contains a consensustripartite GTP-binding element (1) that is required for GTPase and antiviral activity (16, 17). The C-terminal region which includes a leucine zipper motif seems to serve several different functions. It was shown to contain the antiviral effector domain (19) and a domain that determines antiviral specificity (20). Surprisingly, this region also contains a domain which is required for binding and hydrolysis of GTP (21). Limited proteolysis of purified MxA resulted in a N-terminal 60-kDa and a C-terminal 10-kDa fragment, which remained complexed and, together, exhibited high GTPase activity (21). Although the sequences involved in the formation of this complex had not been defined, it was hypothesized that the C terminus of MxA might fold back to form the GTP-binding pocket (21).

A characteristic feature of Mx proteins is that they form large oligomers (16, 17, 19, 22, 23). Biochemical work with the mouse Mx1 protein suggested that a domain in the N-terminal moiety located between the first and the second motif of the tripartite GTP-binding element mediates oligomerization (23). However, results from another study indicated that the leucine zipper motif near the C terminus of Mx1 was both necessary and sufficient for this activity (22). This discrepancy is probably because of technical difficulties resulting from the high tendency of most Mx fragments to form huge aggregates (21). More recent work with dominant negative MxA mutants demonstrated that a newly developed nuclear transport assay may permit the mapping of the interaction domains (19). It further showed that a fragment comprising amino acids 359 to 572 associated with full-length MxA, indicating that oligomerization is controlled by a domain in this region (19).

Here, we employed the yeast two-hybrid system and the nuclear transport assay to define sequences that are involved in the intramolecular folding and oligomerization of MxA. Our results indicate that back-folding of the C terminus and oli-
Domain Structure of MxA

The C Terminus of MxA Interacts with an MxA Domain Located Downstream of the GTP-binding Motif—Limited proteolysis of MxA yields a large N-terminal and a small C-terminal fragment that form a stable GTPase-active complex (21), indicating that the C terminus of MxA may fold back to interact with the GTP-binding motif in the N-terminal moiety of the molecule thus giving rise to a GTPase-active conformation. To analyze the proposed intramolecular interactions, we employed the yeast two-hybrid system. A C-terminal fragment of MxA comprising amino acids 564 to 662 was fused to the DNA-binding domain of GAL4 and coexpressed with various MxA fragments fused to the GAL4 activation domain in the yeast reporter strain CG-1945. Interactions between the fusion proteins should result in complex formation and transcriptional activation of the HIS3 reporter gene. Using this approach, we were able to demonstrate a physical interaction between the C-terminal fragment of MxA encoded by plasmid BD-MxA(564–662) and the remainder of the molecule encoded by plasmid AD-MxA(1–573) (Fig. 1). This interaction appeared to be strong because yeasts carrying these plasmids rapidly formed dense cell patches on selective medium. Interestingly, we could not detect an interaction between the C-terminal fragment and the N-terminal half of MxA, which harbors the tripartite GTP-binding motif: yeasts carrying the corresponding combination of plasmids did not grow on selective medium (Fig. 1).

To identify the interaction-competent domain, AD-Mxa(564–662) was used as a bait to screen an AD-fusion library of random MxA fragments ranging in size from approximately 100 to 300 amino acids. These partially overlapping interacting clones were isolated, and the shortest wild-type clone coding for AD-MxA(372–540) was isolated by screening a library of random MxA fragments with BD-MxA(546–662) as bait. The formation of dense (+), sparse (+) or no (−) cell patches on selective medium was recorded. This analysis allowed rating of the interactions of the partner proteins in question. L612K, F382RA, and L389R indicate amino acid substitutions that were introduced into the various MxA fusion proteins.

Results

Expression Plasmids for Yeasts—MxA cDNA fragments were fused with their 5′-ends either to the DNA-binding domain (BD)1 of GAL4 in vectors pGAD424 or to the activation domain (AD) of GAL4 in the vectors pGBT or pAS2 (CLONTECH, Heidelberg, Germany). Suitable cDNA fragments were generated by polymerase chain reaction. To generate a fusion library of random Mxa fragments, the Mxa cDNA was sheared by sonication and fragments of approximately 300–1,000 base pairs were blunted by T4 DNA polymerase and ligated into the Smal-digested vector pGAD424. The resulting plasmid library was amplified before the DNA was used for the transfection experiments. Deletion mutants of selected library plasmids were created by exonuclease III treatment of the BamHI- and PstI-digested fusion plasmids, and by religation after blunting with nucleases S1 and Klenow polymerase. Mutants Mxa(F382R) and MxA(F382A) were generated by replacing the arginine codon CGT or by the alanine codon GCT. In Mxa(L389R), the codon CTC for leucine 389 was converted to CGC for arginine. The mutations were introduced with the QuickChange site-directed mutagenesis kit (Stratagene, Heidelberg, Germany). All mutations were confirmed by sequencing. A cDNA encoding MxA(L612K) (provided by J. Pavlovic, Zu¨rich) was subcloned into yeast expression vectors by standard procedures. All plasmids, except BD-Mxa(1–362) which was excluded from this study, could not induce colony formation on selective medium when introduced into yeasts together with an irrelevant hybrid protein, indicating that they were devoid of cryptic DNA binding and transcriptional activation sites.

Expression Plasmids for Mammalian Cells—Plasmids pHMG-FLAG-MxA and pHMG-FLAG-MxA(359–662) have been described (19). Plasmids encoding mutants of FLAG-Mxa were either constructed as described (19) or were generated by simple replacement of wild-type sequences in pHMG-FLAG-Mxa by appropriate cDNA fragments carrying the desired mutations.

Yeast Two-hybrid System—Yeast two-hybrid assays were performed according to the protocol provided with the Matchmaker system (CLONTECH, Heidelberg, Germany). Briefly, pAS- or pGBT- and pGAD-Mxa fusion plasmids were co-transfected into the yeast reporter strain CG-1945 by the lithium acetate method, and the transformants were selected on SD minimal medium plates lacking tryptophan and leucine. To test for expression of the HIS3 reporter gene, the transformants were plated on agar medium lacking tryptophan, leucine, and histidine. To reduce background growth, the selective medium was recorded. This analysis allowed rating of the interactions of the partner proteins in question. L612K, F382RA, and L389R indicate amino acid substitutions that were introduced into the various MxA fusion proteins.

Materials and Methods

Cells—Swiss mouse 3T3 cells were grown in Dulbecco’s modified essential medium containing 10% fetal calf serum.

Expression Plasmids for Yeasts—Mxa cDNA fragments were fused with their 5′-ends either to the DNA-binding domain (BD)1 of GAL4 in vector pGAD424 or to the activation domain (AD) of GAL4 in the vectors pGBT or pAS2 (CLONTECH, Heidelberg, Germany). Suitable cDNA fragments were generated by polymerase chain reaction. To generate a fusion library of random Mxa fragments, the Mxa cDNA was sheared by sonication and fragments of approximately 300–1,000 base pairs were blunted by T4 DNA polymerase and ligated into the Smal-digested vector pGAD424. The resulting plasmid library was amplified before the DNA was used for the transfection experiments. Deletion mutants of selected library plasmids were created by exonuclease III treatment of the BamHI- and PstI-digested fusion plasmids, and by religation after blunting with nucleases S1 and Klenow polymerase. Mutants Mxa(F382R) and MxA(F382A) were generated by replacing the arginine codon CGT or by the alanine codon GCT. In Mxa(L389R), the codon CTC for leucine 389 was converted to CGC for arginine. The mutations were introduced with the QuickChange site-directed mutagenesis kit (Stratagene, Heidelberg, Germany). All mutants were confirmed by sequencing. A cDNA encoding MxA(L612K) (provided by J. Pavlovic, Zu¨rich) was subcloned into yeast expression vectors by standard procedures. All plasmids, except BD-Mxa(1–362) which was excluded from this study, could not induce colony formation on selective medium when introduced into yeasts together with an irrelevant hybrid protein, indicating that they were devoid of cryptic DNA binding and transcriptional activation sites.

Expression Plasmids for Mammalian Cells—Plasmids pHMG-FLAG-MxA and pHMG-FLAG-MxA(359–662) have been described (19). Plasmids encoding mutants of FLAG-Mxa were either constructed as described (19) or were generated by simple replacement of wild-type sequences in pHMG-FLAG-Mxa by appropriate cDNA fragments carrying the desired mutations.

Yeast Two-hybrid System—Yeast two-hybrid assays were performed according to the protocol provided with the Matchmaker system (CLONTECH, Heidelberg, Germany). Briefly, pAS- or pGBT- and pGAD-Mxa fusion plasmids were co-transfected into the yeast reporter strain CG-1945 by the lithium acetate method, and the transformants were selected on SD minimal medium plates lacking tryptophan and leucine. To test for expression of the HIS3 reporter gene, the transformants were plated on agar medium lacking tryptophan, leucine, and histidine. To reduce background growth, the selective medium was supplemented with 3 mT3-aminotriazole, and the plates were incubated at 30 °C for 3–5 days to allow colony formation.

Transfection of 3T3 cells—Transient co-transfections with expression constructs for T-Mxa and FLAG-tagged Mxa were done by the standard calcium phosphate co-precipitation method (24).

Immunofluorescence Analysis—The subcellular distribution of Mxa was determined using a polyclonal rabbit antisera against purified, histidine-tagged Mxa protein expressed in Escherichia coli and a tetramethylrhodamine isothiocyanate-conjugated goat anti-rabbit antibody (Dianova, Hamburg, Germany). Flagged Mxa was stained with the monoclonal anti-FLAG antibody M2 (Intega Biosciences, Fernandez, Germany) and a fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Dianova, Hamburg, Germany).

Interaction partners Interaction

1 The abbreviations used are: BD, binding domain; AD, activation domain.

FIG. 1. A C-terminal domain of Mxa interacts with an Mxa domain located between residues 372 and 540. The yeast reporter strain CG-1945 was co-transformed with a plasmid that encodes a fusion protein consisting of the DNA-binding domain of GAL4 and the Mxa fragment comprising amino acids 564 to 662 (black bars) and a tetanus toxin fragment that encodes fusion proteins consisting of the activation domain of GAL4 and various Mxa fragments (striped bars) comprising the indicated amino acids. Interactions of the fusion proteins were assessed by monitoring the ability of the co-transformants to form colonies on SD minimal medium lacking tryptophan, leucine, and histidine. The interaction clone that codes for AD-Mxa(372–540) was isolated by screening a library of random Mxa fragments with BD-Mxa(546–662) as bait. The formation of dense (+), sparse (+) or no (−) cell patches on selective medium was recorded. This analysis allowed rating of the interactions of the partner proteins in question. L612K, F382RA, and L389R indicate amino acid substitutions that were introduced into the various Mxa fusion proteins.
AD-MxA(418–544) resulted in loss of interaction with the C-terminal fragment: yeasts carrying these plasmids could not form colonies on selective agar medium (Fig. 1). To define more precisely the critical amino acids that mediate the contact between the C-terminal and the internal MxA domain, we mutated single amino acids in the two interacting polypeptides. A leucine zipper motif extending from amino acid 598 to 619 is present in the C-terminal fragment of MxA (22). We therefore replaced leucine 612 of BD-MxA(564–662) by lysine (L612K). The mutated fusion protein was no longer able to activate HIS3 expression when co-expressed with AD-MxA(372–540) (Fig. 1), indicating that the leucine zipper motif plays a role in the back-folding process. The interactive internal MxA fragment contains a heptad repeat of hydrophobic amino acids that includes Leu-375, Phe-382, Leu-389, Val-396, and Leu-403. This motif is highly conserved in all known Mx proteins. Computer analysis (program: COILS, version 2.2; matrix: MTDIK) (25) revealed a low probability of an α-helical conformation of the corresponding sequence in MxA. Exchanging phenylalanine 382 for arginine (F382R) or alanine (F382A) abolished the interaction between BD-MxA(372–540) and the C-terminal fragment (Fig. 1). The interaction was not inhibited when leucine 389 was changed to arginine (L389R): yeast colonies appeared on selective medium (Fig. 1), suggesting that phenylalanine 382 rather than the whole heptad repeat is involved in the interaction with the C-terminal MxA fragment.

**Oligomerization of MxA Is Controlled by Domains in the C-terminal Moiety**—Mx proteins can form oligomers both in vivo and in vitro (16, 17, 19, 22, 23). Two different oligomerization domains have been proposed for the murine Mx1 protein. Melen et al. (22) demonstrated the importance of a leucine zipper motif near the C terminus, whereas Nakayama et al. (23) defined a self-assembly motif in the N-terminal moiety of Mx1. For the human MxA protein, a region in the C-terminal moiety comprising amino acids 359 to 572 was found to contain an interaction domain that plays a critical role for oligomerization (19). We therefore tested whether the oligomerization domain of human MxA protein could be further characterized with the help of the yeast two-hybrid system. Co-expression of cDNAs for BD-MxA and AD-MxA fusion proteins in the yeast strain CG-1945 caused strong induction of the HIS3 indicator gene (Fig. 2), indicating that complex formation of full-length MxA molecules indeed occurred in this artificial system. Surprisingly, neither the C-terminal domain of MxA comprising amino acids 564 to 662 nor a C-terminally truncated MxA fragment comprising amino acids 1 to 573 were able to form homo-oligomers in this system (Fig. 2). By contrast, the complete C-terminal half of MxA did (Fig. 2), indicating that the interactive domain is located in this part of the molecule. However, because colony formation of yeasts carrying these plasmids was rather slow, this result also showed that the N-terminal half of MxA contributes to optimal complex formation. Interestingly, the MxA fragment comprising amino acids 372 to 540 did not interact with itself (Fig. 2), suggesting that the interactive domain is very large or possibly of bipartite nature.

To define the interactive domain, we used full-length BD-MxA as a bait to screen our AD-fusion library of random MxA fragments for interacting molecules. This screen yielded a single interactive recombinant plasmid that coded for amino acids 379 to 662 of MxA (Fig. 3). Several deletion mutants of this plasmid were prepared and tested individually. N-terminal deletion of 26 amino acids (AD-MxA(405–662)) as well as C-terminal deletion of 63 amino acids (AD-MxA(372–559)) both destroyed the ability of this MxA fragment to interact with BD-MxA (Fig. 3). Similarly, all fragments with larger deletions failed to interact with full-length MxA (data not shown). Unexpectedly, MxA(1–573), which lacks the C-terminal 99 amino acids, also failed to show interaction with wild-type MxA in this system (Fig. 3). This latter result clearly contradicted previous findings by Ponten et al. (19), who had demonstrated that MxA(1–573) can associate with wild-type MxA in mammalian cells. It thus appeared that the yeast two-hybrid system was unable to detect this particular interaction.

**MxA Forms Complexes with an Internal MxA Fragment in Mouse 3T3 Cells**—To further define the internal MxA domain involved in oligomerization, we employed the nuclear translocation assay previously established by Ponten et al. (19). In this assay, MxA fragments are tagged with a FLAG peptide and expressed in 3T3 cells either alone or together with T-MxA, a nuclear variant of wild-type MxA that has a foreign nuclear localization signal attached to its N terminus (20, 26). In this system, specific interactions with T-MxA result in nuclear transfer of otherwise cytoplasmic MxA fragments, which can easily be monitored by immunofluorescence using an antibody directed against the FLAG peptide. As demonstrated previously (19), the C-terminal MxA fragment comprising amino acids 362 to 662 efficiently accumulated in the nucleus when co-expressed with T-MxA (Fig. 4), indicating that it strongly interacted with full-length MxA. The fragment MxA(372–540), which contains a domain involved in the back-folding of the C terminus, was also transferred to the nucleus by T-MxA in this assay (Fig. 4). Nuclear transfer of this MxA fragment was abolished when the mutation F382A was introduced (Fig. 4).
Because this same mutation also abolished the interaction with the C-terminal MxA fragment (see Fig. 1), this result suggested that back-folding of the C terminus and oligomerization are mechanistically related processes.

The C Terminus of MxA Is Important for Oligomerization—If Mx oligomerization and back-folding of the C terminus were mechanistically related, mutations in the leucine zipper motif near the C terminus of MxA should abolish both types of interactions. Because our yeast two-hybrid experiments (Fig. 1) had indicated that back-folding of the C-terminal fragment was affected by the L612K mutation, we introduced this amino acid exchange into full-length MxA and tested whether the resulting mutant fusion protein would still interact with BD-MxA. This was indeed the case (Fig. 5). However, when the L612K mutation was introduced in both the AD-MxA and the BD-MxA fusion proteins, the interaction could no longer be detected. Yeasts expressing the two mutants proteins were no longer able to grow on selective medium (Fig. 5). These results were compatible with a concept that suggests that the C-terminal fragment of MxA might either recognize the internal interactive domain of the same molecule (back-folding) or a second MxA molecule (oligomerization) as indicated in the model shown in Fig. 7.

This concept predicts that mutations in the internal interactive domain should also abolish oligomerization, provided they are present on both partner molecules. Because Phe-382 is part of the internal domain that mediates back-folding of the C-terminal fragment, we tested whether altering Phe-382 has a negative effect. Fig. 5 shows that this was not the case and suggested that either additional weak interaction domains are present in the N terminus of MxA, which compensated the effect of the F382A mutation, or that the overall structure of the internal interaction domain is affected more drastically by the F382A mutation in the context of the minimal fragment than in the context of the full-length MxA molecule.

To further evaluate the concept outlined in Fig. 7, we tested the proposed role of the C-terminal leucine zipper motif for MxA oligomerization with the help of a modified MxA nuclear transfer assay. To do this, we constructed two mutant forms of T-MxA with defective leucine zipper motifs. T-MxA(1–573) lacks the C-terminal 99 amino acids, whereas T-MxA(L612K) has leucine 612 exchanged by lysine. T-MxA, T-MxA(1–573) and T-MxA(L612K) all accumulated in the nucleus of 3T3 cells when expressed alone (data not shown). All three forms of T-MxA were able to transfer co-expressed flagged wild-type MxA to the nucleus (Fig. 6), although the efficacies of nuclear transfer by T-MxA(1–573) and T-MxA(L612K) were reduced. Additional transfections were performed to test whether the T-MxA variants would still mediate nuclear transfer of flagged MxA carrying the L612K mutation. Wild-type T-MxA could perform this task, whereas T-MxA(1–573) and T-MxA(L612K) both failed to transport MxA(L612K) into the nucleus (Fig. 6). These results thus demonstrated that interaction was only possible if at least one of the two interacting partners possessed an intact leucine zipper motif.

DISCUSSION

In this report we identified two interaction-competent domains in the MxA protein. We found that a first domain, which is located in the C-terminal 99 amino acids is able to specifically contact a second domain, which is located between amino acids 372 and 540. This constellation predicts the existence of monomeric and multimeric forms of MxA in which the two domains participate in the formation of either intramolecular or intermolecular interactions as outlined in Fig. 7.

Previous work with mouse Mx1 protein suggested that a leucine zipper motif located near the C terminus is responsible for oligomerization (22). Here we present evidence that the corresponding motif near the C terminus of MxA is required but not sufficient for oligomerization. In contrast to the results reported for mouse Mx1, the MxA domain, which carries the leucine zipper motif, cannot associate with the same motif on a second MxA molecule. Rather, the interaction target of the C-terminal domain is an internal MxA domain. Evidence that the leucine zipper motif is of importance for this interaction came from experiments with the MxA mutant L612K, which has a leucine residue of the motif replaced by lysine. As expected, if the model in Fig. 7 was correct, full-length MxA(L612K) still interacted with wild-type MxA presumably because the intact C-terminal domain of the wild-type molecule made contact with the unchanged internal domain of the mutant molecule, although it could no longer interact with MxA mutants carrying either the same defect or lacking part of the C-terminal domain.

It is important to note that the internal interaction domain of MxA that we defined here is not related to the proposed oligomerization domain of mouse Mx1 (23), which was mapped to the N-terminal moiety. Our results indicate rather that the N-terminal moiety of MxA does not actively participate in complex formation. Little is known about the structural motifs present in the internal MxA domain, which are important for the interaction with the C-terminal domain. Here, we experi-
The leucine zipper motif near the C terminus of MxA is required for oligomerization. cDNAs for flagged wild-type or mutant MxA were co-expressed in 3T3 cells with constructs encoding T-MxA or T-MxA variants with either a C-terminal truncation (T-MxA(1–573)) or a mutation in the leucine zipper motif (T-MxA(L612K)). Nuclear transport of the flagged proteins was determined as in Fig. 4.

Our studies also revealed that the various methods that can be used to measure molecular interactions in vitro may yield conflicting results under certain conditions. For example, using the yeast two-hybrid system it was not possible to map the boundaries of the internal domain of MxA that interacts with full-length MxA (Fig. 3), whereas such studies were feasible with the eukaryotic nuclear translocation assay (Fig. 4). From theoretical considerations it appears that this intermolecular interaction may be difficult to detect because of competition by the intramolecular interaction between the C-terminal and internal domains in the wild-type molecule. It is possible, therefore, that the bulky fusion proteins used in the yeast two-hybrid system performed less well in this respect than the much smaller molecules used in the nuclear translocation assay.

The results reported here support a previous hypothesis (21) that the C terminus of MxA folds back to constitute the functional GTPase domain. Surprisingly, however, the major touch-down site of the C-terminal domain is not located in the immediate vicinity of the tripartite GTP-binding motif, as we had speculated (21); rather, it is located more than 120 amino acids further downstream. Although unexpected, this constellation might be advantageous and may allow greater flexibility of the different MxA domains involved in binding and hydrolysis of GTP. This particular structural property may also explain why an MxA mutant that lacks the internal interaction domain (MxA(A301–576)) retained about 10% of wild-type GTPase activity (21). In this mutant, there is probably no need for the C-terminal domain to fold back because the large internal deletion may fortuitously move the critical C-terminal domain into the immediate vicinity of the GTP-binding motif so that the resulting structure may resemble the GTP-binding pocket of full-length MxA.

The overall structure of MxA as defined in this study clearly resembles that of dynamin. Recent experiments with dynamin (15) indicated that, like in the case of MxA (21), limited proteolysis yields a large N-terminal and a small C-terminal fragment that remain complexed via noncovalent bonds. Furthermore, backfolding of a C-terminal domain is believed to be essential for GTP hydrolysis of dynamin (15), although the relevant interaction domains of dynamin have not yet been mapped. From our work with MxA it seems likely that the internal interaction domain of dynamin may also be located downstream of the GTP-binding element and that back-folding and oligomerization of dynamin may also involve intra- and intermolecular interactions as illustrated for MxA in Fig. 7.

An interesting aspect of this work is that we identified an MxA mutant that no longer forms oligomers. Because the importance of oligomerization for GTPase and antiviral activity of MxA has not yet been elucidated, mutant MxA(L612K) is a precious tool for further studies along these lines. Preliminary results indicate that MxA(L612K) exhibits substantial antiviral activity when expressed in mouse 3T3 cells, although it lacks detectable GTPase activity.2

Acknowledgments—We thank Jovan Pavlovic for helpful discussions and for providing a construct encoding MxA(L612K), Georg Kochs, Martin Schwenmke, Kirsten Weinig, and Otto Haller for comments on the manuscript and for providing various constructs encoding N-terminally flagged MxA, and Annette Ohnemus for excellent technical assistance.

2 B. Schumacher and P. Staeheli, unpublished results.
REFERENCES

1. Horisberger, M. A. (1992) J. Virol. 66, 4705–4709
2. Aebi, M., Fah, J., Hurt, N., Samuel, C. E., Thomis, D., Bazzigher, L., Pavlovic, J., Haller, O., and Staeheli, P. (1989) Mol. Cell. Biol. 9, 5062–5072
3. Staeheli, P., and Haller, O. (1985) Mol. Cell. Biol. 5, 2150–2153
4. Pavlovic, J., Zürcher, T., Haller, O., and Staeheli, P. (1990) J. Virol. 64, 3370–3375
5. Frese, M., Kochs, G., Meier-Dieter, U., Siebler, J., and Haller, O. (1995) J. Virol. 69, 3904–3909
6. Frese, M., Kochs, G., Feldmann, H., Hertkorn, C., and Haller, O. (1996) J. Virol. 70, 915–923
7. Schnorr, J. J., Schneider-Schaulies, S., Simon Jodicke, A., Pavlovic, J., Horisberger, M. A., and ter Meulen, V. (1993) J. Virol. 67, 4760–4768
8. Schneider-Schaulies, S., Schneider-Schaulies, J., Schuster, A., Bayer, M., Pavlovic, J., and ter Meulen, V. (1994) J. Virol. 68, 6910–6917
9. Landis, H., Simon-Jodicke, A., Klotz, A., Di Paolo, C., Schnorr, J., Schneider-Schaulies, S., Hefti, H. P., and Pavlovic, J. (1998) J. Virol. 72, 1516–1522
10. Zhao, H., De, B. P., Das, T., and Banerjee, A. K. (1996) Virology 220, 330–338
11. Pavlovic, J., Arzet, H. A., Hefti, H. P., Frese, M., Rost, D., Ernst, B., Kelb, E., Staeheli, P., and Haller, O. (1995) J. Virol. 69, 4506–4510
12. Li, Y., and Youssoufian, H. (1997) J. Clin. Invest. 100, 2873–2880
13. Obar, R. A., Collins, C. A., Hammarback, J. A., Shpetner, H. S., and Vallee, R. B. (1990) Nature 347, 256–261
14. Rothman, J. H., Raymond, C. K., Gilbert, T., O’Hara, J., and Stevens, T. H. (1990) Cell 61, 1063–1074
15. Warnock, D. E., Terlecky, L. J., and Schmid, S. L. (1995) EMBO J. 14, 1322–1328
16. Richter, M. F., Schwemmle, M., Herrmann, C., Wittinghofer, A., and Staeheli, P. (1995) J. Biol. Chem. 270, 13512–13517
17. Pitosi, F., Blank, A., Schroder, A., Schwarz, A., Hussi, P., Schwemmle, M., Pavlovic, J., and Staeheli, P. (1995) J. Virol. 67, 6726–6732
18. Schwemmle, M., Weining, K. C., Richter, M. F., Schumacher, B., and Staeheli, P. (1994) Virology 206, 545–554
19. Ponten, A., Sick, C., Weber, M., Haller, O., and Kochs, G. (1997) J. Virol. 71, 2591–2599
20. Zürcher, T., Pavlovic, J., and Staeheli, P. (1992) EMBO J. 11, 1657–1661
21. Schwemmle, M., Richter, M., Herrmann, C., Nassar, N., and Staeheli, P. (1995) J. Biol. Chem. 270, 13518–13523
22. Melen, K., Ronni, T., Breni, B., Krug, R. M., von Bonsdorff, C. H., and Julkunen, I. (1992) J. Biol. Chem. 267, 25898–25907
23. Nakayama, M., Yazaki, K., Kusano, A., Nagata, K., Hanai, N., and Ishihama, A. (1993) J. Biol. Chem. 268, 15033–15038
24. Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., and Struhl, K. (1992) Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York
25. Lupas, A., Van Dyke, M., and Stock, J. (1991) Science 252, 1162–1164
26. Zürcher, T., Pavlovic, J., and Staeheli, P. (1992) J. Virol. 66, 5059–5066