# Interferon-induced human MxA GTPase blocks nuclear import of Thogoto virus nucleocapsids

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**ABSTRACT** Interferon-induced human MxA protein belongs to the dynamin superfamily of large GTPases. It exhibits antiviral activity against a variety of RNA viruses, including Thogoto virus, an influenza virus-like orthomyxovirus transmitted by ticks. Here, we report that MxA blocks the transport of Thogoto virus nucleocapsids into the nucleus, thereby preventing transcription of the viral genome. This interaction can be abolished by a mAb that neutralizes the antiviral activity of MxA. Our results reveal an antiviral mechanism whereby an interferon-induced protein traps the incoming virus and interferes with proper transport of the viral genome to its ultimate target compartment within the infected cell.

In most cases, interferons (IFNs) mediate their antiviral action by influencing viral transcription or translation, leading to reduced viral protein synthesis (1). In some cases, late multiplication steps such as virus assembly and budding seem to be affected (2). In contrast, inhibition of intracellular trafficking events that take place early in infection have not been implicated in the antiviral action of IFNs. This is surprising because virus multiplication relies heavily on the transport machinery of the host cell (3). Therefore, specific transport processes would seem to provide excellent targets for interference with viral life cycles.

A key component of the IFN-mediated action against viruses is the human MxA protein. MxA belongs to the newly defined dynamin superfamily of high molecular weight GTPases (4) that play important roles in transport processes, such as endocytosis (5), intracellular vesicle transport (6), and cell plate formation in plants (7). MxA protein is induced exclusively by type I ($\alpha/\beta$) IFNs (8), accumulates in the cytoplasm of cells (9, 10), and interferes with the multiplication and spread of certain orthomyxoviruses (11–13), rhabdoviruses (14), paramyxoviruses (15–17), and bunyaviruses (18, 19). Transfected cells (11, 12, 18) and transgenic mice (13) expressing MxA acquire a high degree of antiviral resistance, demonstrating that MxA is a powerful antiviral agent. In humans, synthesis of MxA is induced during acute viral infections and may thus protect humans from severe disease (20, 21). However, the mechanisms by which MxA is able to inhibit such a diverse group of viruses is far from being understood.

In contrast to most other GTPases, large GTPases seem to act as mechanochemical enzymes rather than as molecular switches (22). For example, dynamin self-assembles around tubular membrane invaginations (23) and is able to constrict and sever membranes into separate vesicles (24). It has been proposed that the ability to form helical arrays around tubular templates might be a functional link between all dynamin-like large GTPases (25). In fact, purified recombinant MxA protein also forms aggregates of $\approx$30 molecules (26) that adopt a helical structure in solution (G.K., U. Aebi, and O.H., unpublished results). Furthermore, C-shaped and ring-like structures have been described for mouse Mx1 protein (27), supporting

the idea that Mx proteins might inhibit viruses by a dynamin-like force-generating mechanism. Thus, MxA might recognize and wrap around viral nucleocapsids that, in case of orthomyxoviruses, represent tubular structures with a diameter of 10–15 nm (25, 28). To demonstrate a potential physical interaction of MxA with viral nucleocapsids, we developed an *in vitro* cosedimentation assay (29). We used nucleocapsids of Thogoto virus (THOV) for these experiments, because this influenza-like virus represents the most MxA-susceptible virus known to date (12). The assay is based on three elements, namely highly active MxA GTPases as effector molecules, viral nucleocapsids as targets, and nonhydrolyzable GTP-$\gamma$S as stabilizing factor. We could show that MxA associates in the GTP-bound form with the nucleocapsids by binding to the nucleoprotein (NP) component (30). We could further show that this interaction is mediated by domains in the C-terminal moiety of MxA and can be inhibited by mAb 2C12 directed against a C-terminal epitope (30).

Here, we determined whether MxA would serve a similar function *in vivo*, by following the fate of the infecting virus in the living cell. By using microinjection experiments, we demonstrate that MxA keeps the viral nucleocapsids in the cytoplasm of cells, thereby preventing the virus from entering the nucleus and from transcribing its genome. This transport block can be released by mAb 2C12. We further show that this MxA-specific antibody neutralizes the antiviral activity of MxA when microinjected into the cytoplasm of living cells. We propose that MxA protein can work by specifically recognizing incoming viral nucleocapsids in the cytoplasm of the infected cell, thereby blocking their transport into the cell nucleus.

## MATERIALS AND METHODS

**Cells and Viruses.** Parental Swiss mouse 3T3 cells and stably transfected cells constitutively expressing wild-type human MxA protein (clone 4.5.15) were the same as described in ref. 14. Cells were grown in DMEM containing 10% fetal calf serum with or without 0.5 mg of geneticin (G418) per ml. 3T3 cells were infected with THOV strain SiAr 126 (31) with a multiplicity of infection of 10 and incubated for 16 h.

**Analysis of Primary Transcription.** To examine the activity of the incoming viral polymerase complex, defined as primary transcription, cells were treated with cycloheximide (CHX) throughout the experiment as described by Pavlovic *et al.* (11). CHX (50 $\mu$g/ml) was added to the cultures 45 min before infection. Then, the cells were infected with 20 plaque-forming units of THOV per cell for 7 h. Total RNA was prepared and RNA samples (20 $\mu$g of RNA per lane) were determined by Northern blot analysis. Plus-sense transcripts of segment 1 (PB2 mRNA), segment 5 (NP mRNA), and segment 6 (putative M mRNA) were detected by using radiolabeled negative-strand RNA transcripts as hybridization probes, exactly as

Abbreviations: CHX, cycloheximide; IFN, interferon; NP, nucleoprotein; THOV, Thogoto virus; vRNPs, viral ribonucleoprotein complexes.
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Cell Biology: Kochs and Haller

*Proc. Natl. Acad. Sci. USA* 96 (1999)     2083

described (11). The blot was stripped and rehybridized with a radiolabeled cDNA probe that detects glyceraldehyde-3-phosphate dehydrogenase transcripts (32).

**Microinjection of Antibody.** The monoclonal Mx-specific antibody 2C12 (9) and a mouse polyclonal antibody directed against influenza A virus [strain A/Hong Kong/1/68(H3N2)] were purified by protein A-Sepharose chromatography (Pharmacia). The antibodies were dialyzed against PBS and concentrated to 0.4 $\mu$g/$\mu$l. For microinjection, cells were seeded onto glass coverslips. Antibodies were injected into the cytoplasm of cells with a 5242 Eppendorf microinjector. After incubation for 8 h at 37°C under 5% $CO_2$, cells were infected with 50 plaque forming units per cell of THOV for 16 h. The cells were fixed with 3% paraformaldehyde and analyzed by double immunofluorescence. Viral antigens were labeled with a guinea pig antiserum, kindly provided by Jones and Nuttall (33). Finally, cells were stained with tetramethylrhodamine isothiocyanate-conjugated goat anti-guinea pig antibodies and with dichlorotriazyl amino fluorescein-conjugated goat anti-mouse antibodies (Dianova, Hamburg, Germany) that recognize the microinjected antibodies.

**Preparation and Microinjection of Viral Ribonucleoprotein Complexes (vRNPs).** Viral particles of THOV were isolated from supernatants of THOV-infected baby hamster kidney BHK-21 cells and purified by sequential centrifugation steps as described (34). For preparation of vRNPs, purified virus was lysed according to the method described by Seong and Brownlee in the presence of Nonidet P-40, Triton X-100, and lysolecithine (35). The RNPs were separated from viral glycoprotein and matrix protein by a subsequent glycerol gradient centrifugation step, and the fractions were analyzed by SDS/PAGE. For microinjection, the RNP fractions were pooled, diluted four times in microinjection buffer (10 mM Tris/120 mM KCl, pH 8.0), and concentrated at 120,000 × g for 2 h at 4°C in an SW50.1 rotor (Beckman Instruments). The pellet was resuspended in microinjection buffer to a concentration of 1–2 $\mu$g/$\mu$l. Microinjection of the vRNPs was performed as described for the antibodies. After injection, the cells were incubated in culture medium containing 50 $\mu$g/ml CHX for 2 h before fixation with 3% paraformaldehyde. Viral proteins were detected with mAb MAb2 directed against THOV nucleoprotein (36). To visualize MxA protein, a polyclonal rabbit antiserum was used (37). Cells were then stained with tetramethylrhodamine isothiocyanate- and dichlorotriazyl aminofluorescein-conjugated goat anti-mouse and anti-rabbit antibodies. The cells were analyzed with a Leica TCS[4D] confocal laser scanning microscope.

## RESULTS

**Cytoplasmic MxA Protein Inhibits a Nuclear Replication Step of THOV.** THOV, like all other orthomyxoviruses, needs to have its RNA genome transported into the nucleus for transcription and replication (38). By using an *in vitro* cosedimentation assay, we have recently shown that MxA protein specifically binds to THOV nucleocapsids (29, 30). We reasoned that, if MxA behaved in the same way *in vivo*, it might associate with the incoming nucleocapsids in the cytoplasm of infected cells. A possible consequence of such an interaction in infected cells could be that MxA keeps the nucleocapsids out of the nucleus, thereby preventing primary transcription. Alternatively, MxA might cotranslocate together with the nucleocapsids into the nucleus and interfere with primary transcription of the viral genome. Primary transcription of the viral genome takes place in the absence of host cell protein synthesis, because it is catalyzed by the incoming virion polymerase. The extent of primary transcription can be determined by measuring the accumulation of viral mRNAs in infected cells that are treated with the protein synthesis inhibitor CHX. To test whether primary transcription of THOV was indeed

affected by MxA, we measured this replication step in infected cells with or without MxA. Parallel cultures of MxA-expressing cells or control cells were infected with a high input multiplicity of THOV in the presence of CHX. Total RNA was prepared 7 h after infection and determined by Northern blot analysis. To specifically detect viral primary transcripts, the blots were hybridized with radiolabeled *in vitro*-transcribed negative-sense RNA probes derived from the PB2, NP, and M genes of THOV. Fig. 1 shows that infected control (neo) cells contained detectable amounts of primary transcripts. In cells expressing MxA, primary transcripts of PB2, NP, and M were below the level of detection, indicating that MxA interfered with viral transcript accumulation.
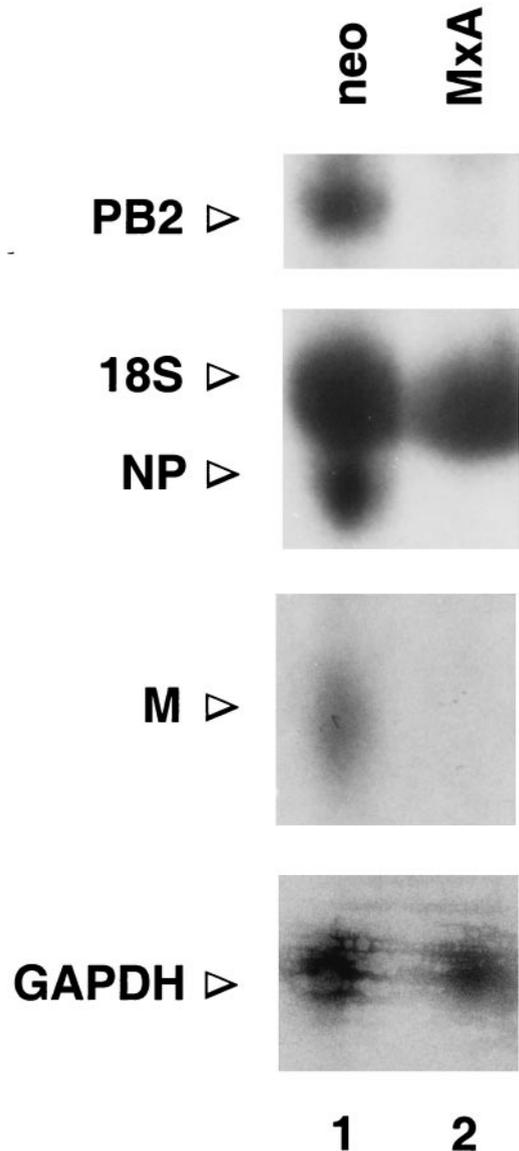


Fig. 1. Detection of primary THOV transcripts by Northern blot analysis. Parallel cultures of MxA-expressing 3T3 cells (MxA, lane 2) or control cells (neo, lane 1) (14) were infected with 20 plaque forming units of THOV (31) per cell in the presence of 50 $\mu$g/ml CHX. Total RNA was prepared 7 h after infection, and samples of 20 $\mu$g of RNA were loaded into each lane. Northern blots were hybridized with radiolabeled, negative-sense RNA probes derived from the PB2, NP, and M genes of THOV (34), as indicated. For the detection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts, blots were analyzed with a specific, nick-translated cDNA probe. Blots were exposed to x-ray films to detect the radioactive signals. The position of the cross-hybridizing 18S rRNA is indicated.

**MxA Blocks Nuclear Import of Viral Nucleocapsids.** To demonstrate the fate of viral nucleocapsids in living MxA-expressing cells, we microinjected purified nucleocapsids into the cytoplasm of MxA or control (neo) cells and investigated their translocation into the nucleus. The subcellular localization of the injected particles was determined by confocal laser scanning microscopy by using NP- and MxA-specific antibodies for indirect double immunofluorescence. To selectively stain microinjected nucleocapsids, cells were kept in the presence of CHX to block *de novo* viral protein synthesis. Fig. 2 shows that the microinjected virion nucleocapsids almost quantitatively moved into the nucleus of control cells, leaving the cytoplasm virtually unstained 2 h after microinjection. In contrast, the viral nucleocapsids remained in the cytoplasm of cells expressing MxA. Superimposition of the signals for MxA and NP suggested that the microinjected nucleocapsids colocalized with the cytosolic MxA protein, most likely by forming complexes. The microinjected nucleocapsids remained in the cytoplasm for up to 8 h after injection (data not shown).

**MxA-Specific Antibody Releases Import Block.** To demonstrate the specificity of the observed nucleocapsid translocation block, we tried to reverse this inhibition by microinjecting MxA-specific antibody 2C12 into the cytoplasm along with nucleocapsids. The antibody 2C12 recognizes a conserved C-terminal epitope on rodent and human Mx proteins (9, 39) and is able to prevent the interaction of MxA with nucleocapsids *in vitro* (30). The prediction was that antibody 2C12 should likewise release the MxA-mediated translocation block *in vivo*. Therefore, MxA-expressing cells were microinjected with both nucleocapsids and antibody 2C12 and were kept for 2 h in the presence of CHX. Cells were then analyzed for the presence of microinjected 2C12 antibody and for the localization of viral

nucleocapsids by immunofluorescence staining (Fig. 3*A*). The results clearly showed that the microinjected nucleocapsids were now imported into the nucleus, whereas the comicroinjected antibody stayed in the cytoplasm. These results indicated that antibody 2C12 interfered with the cytoplasmic retention of the nucleocapsids by MxA and allowed their unhindered transport into the nucleus. We concluded that the import machinery in MxA-expressing cells was intact and that MxA protein kept the viral nucleocapsids in the cytoplasm.

Next, we tried to neutralize the antiviral effect of MxA in intact cells that were exposed to infectious virus rather than to microinjected nucleocapsids. mAb 2C12 was microinjected into the cytoplasm of MxA-expressing 3T3 cells, and the microinjected cells were infected 8 h later with THOV. Likewise, cells were microinjected with a control antibody reacting with an unrelated antigen. At 16 h postinfection, cells were fixed and processed for indirect double-immunofluorescence to localize injected antibodies and to analyze accumulation of viral proteins. Both microinjected antibodies, namely Mx-specific antibody 2C12 (Fig. 3*Ba*) and the unrelated control antibody (Fig. 3*Bb*), were found in the cytoplasm of injected cells. Cells injected with mAb 2C12 had lost their antiviral state, and viral proteins were produced, indicating that the antiviral activity of MxA protein was neutralized (Fig. 3*Bc*). By contrast, cells injected with the control antibody remained protected against THOV, showing that the antiviral effect of MxA was still in place (Fig. 3*Bd*). These findings nicely complement the microinjection data and support the view that human MxA protein affects the incoming nucleocapsids during the natural course of infection as it recognizes and immobilizes nucleocapsids after microinjection.
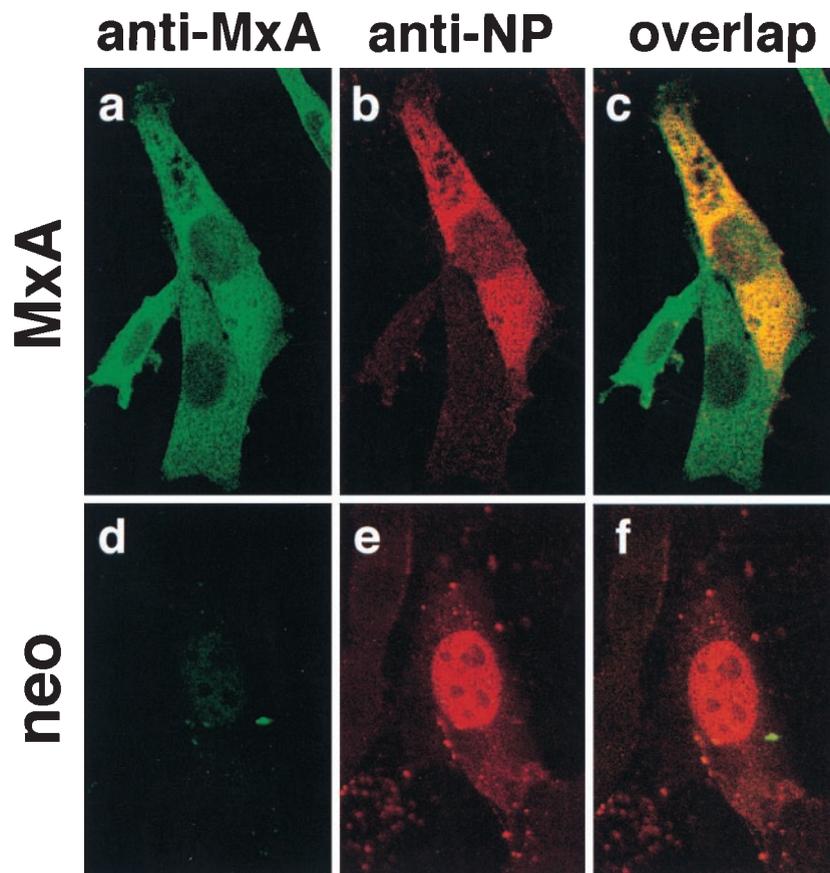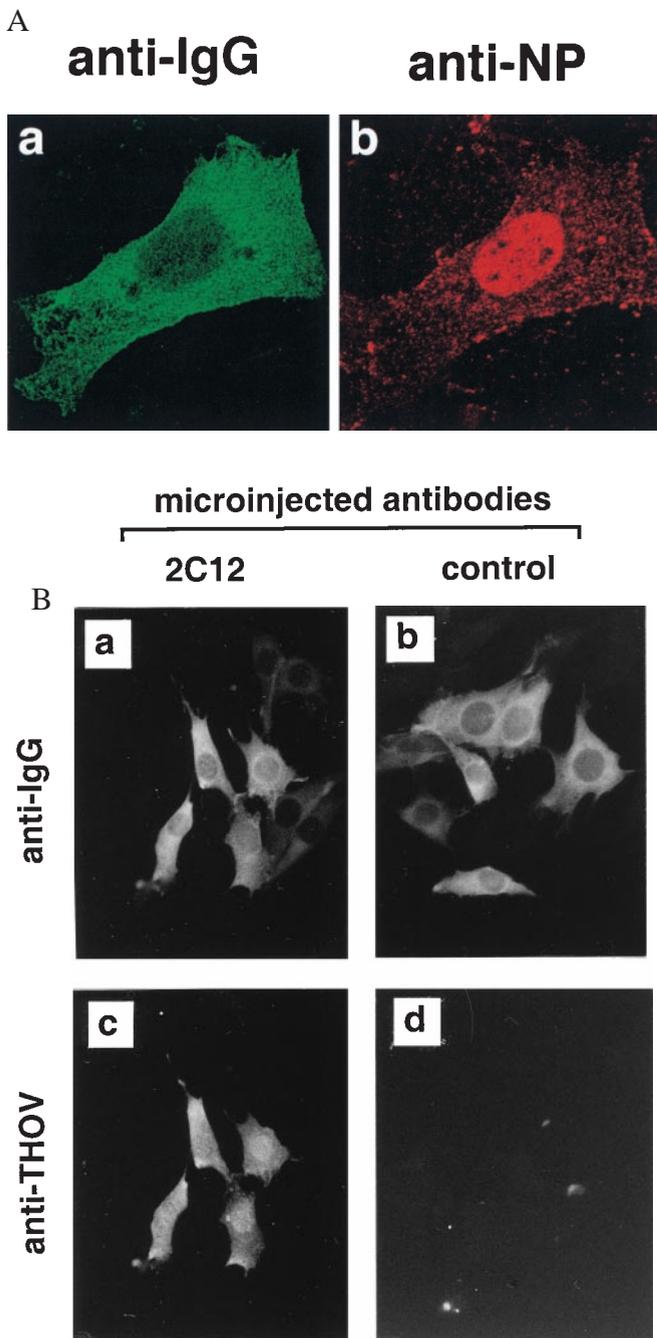


Fig. 2.    MxA inhibits nuclear import of viral nucleocapsids. MxA-expressing (MxA) and control (neo) 3T3 cells were microinjected with purified viral nucleocapsids and incubated for 2 h in the presence of 50 μg/ml CHX. Cells were then analyzed by indirect double-immunofluorescence with anti-MxA (*a* and *d*; green) and anti-NP (*b* and *e*; red) antibodies. Panels at the right (*c* and *f*) show the superimposition of two images. Colocalization of NP (red) and MxA (green) results in yellow staining.

Cell Biology: Kochs and Haller

*Proc. Natl. Acad. Sci. USA* 96 (1999) 2085

## A



## anti-IgG  anti-NP

## B

### microinjected antibodies

2C12  control



with newly synthesized nucleocapsids and prevents their re-import into the nucleus (41). Here, we demonstrate that an IFN-induced cellular protein, MxA, recognizes incoming viral nucleocapsids in the cytoplasm and blocks their normal movement into the nucleus.

MxA protein belongs to the growing superfamily of large GTPases that include, among other members, dynamin (42) and yeast Vps1p (6). Characteristically, these enzymes are involved in intracellular trafficking. They seem to act as force-generating molecules ("molecular motors") rather than molecular switches, as do most other GTPases (22). Dynamin can self-assemble into rings and stacks of interconnected rings in solution (43). Mx proteins of mice and humans also self-assemble into multimeric structures that resemble the C-shaped and ring-shaped polymers of dynamin (ref. 27 and G.K., U. Aebi, and O.H., unpublished observation). It is known that dynamin can bind, constrict, and sever membranes into individual vesicles (22, 24). Here, we have presented evidence that MxA recognizes viral nucleocapsids and blocks their normal movement into the nucleus (Fig. 4). How exactly MxA prevents nuclear transport at the molecular level remains to be determined. Presumably, multimers of MxA wrap around incoming nucleocapsids, thereby covering karyophilic signals that are normally exposed. Alternatively, MxA may direct nucleocapsids to alternative sites in the cytoplasm where they are immobilized and subsequently degraded. It remains to be seen whether the present mechanism of MxA action is unique for THOV or universally applicable to all MxA-susceptible viruses. MxA inhibits primary transcription of vesicular stomatitis virus (14) and measles virus (15). However, both of these viruses transcribe their genomes in the cytoplasm, and it is presently not clear whether transport processes are critically involved. Influenza A viruses are much less sensitive to MxA-mediated inhibition than THOV (12, 13, 44) and seem to be blocked at a later multiplication step that is as yet poorly characterized (11).

Circumstantial evidence indicates that cell-type specific factors may influence the antiviral activity of MxA *in vivo* (15, 45, 46). Therefore, it is conceivable that the ability of MxA to bind viral nucleocapsids is also modulated to different degrees by as yet unknown host cell factors. Alternatively, MxA may be a multifunctional protein mediating several distinct antiviral mechanisms. In any case, the present results reveal an antiviral principle of IFN action that aborts viral infection at an early

Fig. 3.  mAb 2C12 abrogates import block. (*A*) MxA-expressing 3T3 cells were microinjected with a mixture of viral nucleocapsids and antibody 2C12 (9). Microinjected cells were kept for 2 h in the presence of CHX and then analyzed for the localization of the antibody (*a*; green) or the viral NP protein (*b*; red). (*B*) MxA-expressing 3T3 cells were microinjected with either antibody 2C12 (*a* and *c*) or an unrelated control antibody (*b* and *d*), and were infected 8 h later with THOV at a multiplicity of 50 per cell. Cells were fixed 16 h later and analyzed for viral protein synthesis by double-immunofluorescence labeling using a hyperimmune anti-THOV antiserum (*c* and *d*). The microinjected antibodies were detected with the help of a fluorescein-conjugated goat anti-mouse IgG antibody (*a* and *b*).
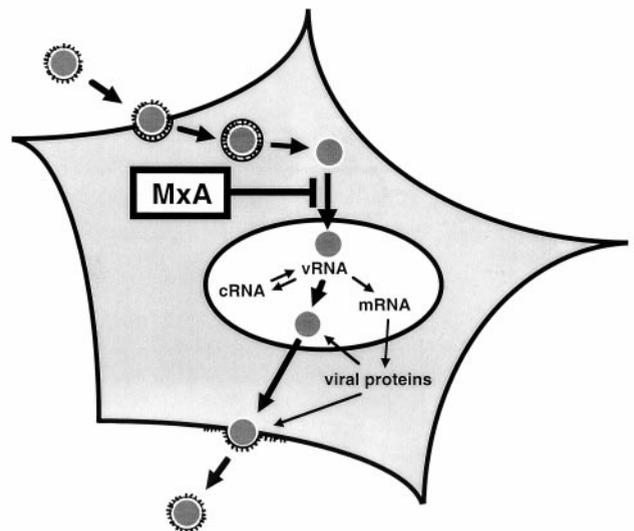
## DISCUSSION

Specific transport processes provide excellent targets for interference with the viral life cycle. Intracellular transport of viral nucleocapsids is often regulated by viral proteins, such as the matrix protein in case of influenza viruses (40). Late in infection, the matrix protein of influenza viruses associates



Fig. 4.  MxA-mediated antiviral effect of IFN. MxA binds to THOV nucleocapsids upon entry into the cytosol and prevents their transport to the nucleus. As a consequence, primary transcription of the viral genome cannot take place.

stage of infection. Preventing proper intracellular transport and, as a consequence, inhibition of early viral gene expression may be an excellent safeguard against the rapid evolution of resistant virus variants that might otherwise overcome this host defense mechanism.

1. Samuel, C. E. (1991) *Virology* **183,** 1–11.
2. Staeheli, P. (1990) *Adv. Virus Res.* **38,** 147–200.
3. Whittaker, G. R. & Helenius, A. (1998) *Virology* **246,** 1–23.
4. Staeheli, P., Pitossi, F. & Pavlovic, J. (1993) *Trends Cell Biol.* **3,** 268–272.
5. Urrutia, R., Henley, J. R., Cook, T. & McNiven, M. A. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 377–384.
6. Rothman, J. H., Raymond, C. K., Gilbert, T., O'Hara, P. J. & Stevens, T. H. (1990) *Cell* **61,** 1063–1074.
7. Gu, X. & Verma, D. (1996) *EMBO J.* **15,** 695–704.
8. Simon, A., Fah, J., Haller, O. & Staeheli, P. (1991) *J. Virol.* **65,** 968–971.
9. Staeheli, P., Dreiding, P., Haller, O. & Lindenmann, J. (1985) *J. Biol. Chem.* **260,** 1821–1825.
10. Aebi, M., Fäh, J., Hurt, N., Samuel, C. E., Thomis, D., Bazzigher, L., Pavlovic, J., Haller, O. & Staeheli, P. (1989) *Mol. Cell Biol.* **9,** 5062–5072.
11. Pavlovic, J., Haller, O. & Staeheli, P. (1992) *J. Virol.* **66,** 2564–2569.
12. Frese, M., Kochs, G., Meier-Dieter, U., Siebler, J. & Haller, O. (1995) *J. Virol.* **69,** 3904–3909.
13. Pavlovic, J., Arzet, H. A., Hefti, H. P., Frese, M., Rost, D., Ernst, B., Kolb, E., Staeheli, P. & Haller, O. (1995) *J. Virol.* **69,** 4506–4510.
14. Pavlovic, J., Zürcher, T., Haller, O. & Staeheli, P. (1990) *J. Virol.* **64,** 3370–3375.
15. Schneider-Schaulies, S., Schneider-Schaulies, J., Schuster, A., Bayer, M., Pavlovic, J. & ter Meulen, V. (1994) *J. Virol.* **68,** 6910–6917.
16. Schnorr, J. J., Schneider-Schaulies, S., Simon-Jödicke, A., Pavlovic, J., Horisberger, M. A. & ter Meulen, V. (1993) *J. Virol.* **67,** 4760–4768.
17. Zhao, H., De, B. P., Das, T. & Banerjee, A. K. (1996) *Virology* **220,** 330–338.
18. Frese, M., Kochs, G., Feldmann, H., Hertkorn, C. & Haller, O. (1996) *J. Virol.* **70,** 915–923.
19. Kanerva, M., Melen, K., Vaheri, A. & Julkunen, I. (1996) *Virology* **224,** 55–62.
20. Von Wussow, P., Jakschies, D., Block, B., Tschechne, B., Schedel, I., Horisberger, M. A., Hochkeppel, H. K. & Deicher, H. (1990) *AIDS* **4,** 119–124.
21. Roers, A., Hochkeppel, H. K., Horisberger, M. A., Hovanessian, A. & Haller, O. (1994) *J. Infect. Dis.* **169,** 807–813.
22. McNiven, M. A. (1998) *Cell* **94,** 151–154.
23. Takei, K., McPherson, P. S., Schmid, S. L. & De Camilli, P. (1995) *Nature (London)* **374,** 186–190.
24. Sweitzer, S. M. & Hinshaw, J. E. (1998) *Cell* **93,** 1021–1029.
25. Kelly, R. B. (1995) *Nature (London)* **374,** 116–117.
26. Richter, M. F., Schwemmle, M., Herrmann, C., Wittinghofer, A. & Staeheli, P. (1995) *J. Biol. Chem.* **270,** 13512–13517.
27. Nakayama, M., Yazaki, K., Kusano, A., Nagata, K., Hanai, N. & Ishihama, A. (1993) *J. Biol. Chem.* **268,** 15033–15038.
28. Arnheiter, H., Frese, M., Kambadur, R., Meier, E. & Haller, O. (1996) *Curr. Top. Microbiol. Immunol.* **206,** 119–147.
29. Kochs, G., Trost, M., Janzen, Ch. & Haller, O. (1998) *Methods Companion Methods Enzymol.* **15,** 255–263.
30. Kochs, G. & Haller, O. (1999) *J. Biol. Chem.* **274,** in press.
31. Albanese, M., Bruno-Smiraglia, C., Di Cuonzo, G., Lavagnino, A. & Srihongse, S. (1972) *Acta Virol.* **16,** 267.
32. Panabieres, F., Piechaczyk, M., Rainer, B., Dani, C., Fort, P., Riaad, S., Marty, L., Imbach, J. L., Jeanteur, P. & Blanchard, J. M. (1984) *Biochem. Biophys. Res. Commun.* **118,** 767–773.
33. Jones, J. D. & Nuttall, P. A. (1989) *Virus Res.* **14,** 129–140.
34. Weber, F., Haller, O. & Kochs, G. (1996) *J. Virol.* **70,** 8361–8367.
35. Seong, B. L. & Brownlee, G. G. (1992) *Virology.* **186,** 247–260.
36. Portela, A., Jones, L. D. & Nuttall, P. (1992) *J. Gen. Virol.* **73,** 2823–2830.
37. Ponten, A., Sick, C., Weeber, M., Haller, O. & Kochs, G. (1997) *J. Virol.* **71,** 2591–2599.
38. Whittaker, G., Bui, M. & Helenius, A. (1996) *Trends Cell Biol.* **6,** 67–71.
39. Johannes, L., Kambadur, R., Lee-Hellmich, H., Hodghinson, C. A., Arnheiter, H. & Meier, E. (1997) *J. Virol.* **71,** 9792–9795.
40. Martin, K. & Helenius, A. (1991) *Cell* **67,** 117–130.
41. Whittaker, G., Bui, M. & Helenius, A. (1996) *J. Virol.* **70,** 2743–2756.
42. Warnock, D. E. & Schmid, S. L. (1996) *BioEssays* **18,** 885–893.
43. Hinshaw, J. E. & Schmid, S. L. (1995) *Nature (London)* **374,** 190–192.
44. Haller, O., Frese, M. & Kochs, G. (1998) *Rev. Sci. Tech. Off. Int. Epizoot.* **17,** 220–230.
45. Horisberger, M. A. (1992) *J. Virol.* **66,** 4705–4709.
46. Landis, H., Simon-Jödicke, A., Klöti, A., Di Paolo, C., Schnorr, J., Schneider-Schaulies, S., Hefti, H. P. & Pavlovic, J. (1998) *J. Virol.* **72,** 1516–1522.