Proteolytic Cleavage of the p65-RelA Subunit of NF-κB during Poliovirus Infection*

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Activation of NF-κB during viral infection is one of the critical elements in innate immune response. Several virus-specific factors, such as double-stranded RNA, can trigger host defense mechanisms by inducing NF-κB-mediated expression of cytokines and interferons. Early stages of poliovirus infection are also associated with degradation of IκBα and translocation of NF-κB into the nucleus. However, at later stages of poliovirus replication the p65-RelA component of the NF-κB complex undergoes a specific cleavage that coincides with the onset of intensive poliovirus protein synthesis and the appearance of the activity of poliovirus protease 3C. Indeed, the p65-RelA amino acid sequence contains the recognition site for 3C, and recombinant protein 3C was shown to be capable of proteolytic cleavage of p65-RelA, generating truncated product similar to that observed during poliovirus infection. Cleavage of p65-RelA occurs during replication of ECHO-1 and rhinovirus 14, suggesting that inactivation of NF-κB function by proteolytic cleavage of p65-RelA is the common mechanism by which picornaviruses suppress the innate immune response.

The innate immune response to viral and bacterial infections involves the production of immune molecules, including cytokines, interferons, chemokines, and major histocompatibility complex proteins that act in combination to suppress infectious agents (1). Expression of the genes encoding the majority of these proteins is regulated by NF-κB. NF-κB activation was reported during many viral infections, such as cytomegalovirus, human immunodeficiency virus, rhinovirus, and measles virus (2–4). Viruses can activate NF-κB by inducing NF-κB-dependent expression of cytokines and interferons, and play a role in the termination codon to the 3′-end of protein 3C cDNA, the polyhistidine tag-coding sequence, and the termination codon to the 3′-end of protein 3C cDNA were added by PCR. The sequencing proved the integrity of the tagged protein 3C cDNA molecules. The ability of the vectors to express proteins was tested by Western blotting.

NF-κB Electrophoretic Mobility Shift Assay—The protocol for these experiments was described in our previous publication (19). The cytoplasmic and nuclear protein extracts were purified from 5 × 10⁶ HeLa cells, according to the Dignam protocol (20), after 1, 2, 3, 4, and 6 h of poliovirus infection. The NF-κB activation by TNF was initiated at 1 h prior to protein purification.

Immunoblotting—Total protein extracts from 6 × 10⁶ HeLa and HeLaBcl-2 cells were prepared in 0.3 ml of radioimmunoprecipitation assay buffer (150 mM NaCl, 1% SDS, 10 mM Tris, pH 8.0, 1% sodium deoxycholate, 1% Nonidet P-40) with a protease inhibitor mixture (Sigma). The nuclear and cytoplasmic protein extracts from 6 × 10⁶ HeLa cells were purified according to the Dignam protocol (20). Nylon filters with proteins were incubated either with primary rabbit polyclonal anti-p65 (C terminus), primary rabbit polyclonal anti-p65 (N terminus), anti-IkBα (C terminus), anti-p50 antibodies, anti-RelB-2 antibodies (Santa Cruz Biotechnology), rabbit polyclonal anti-keratin 18

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antibodies (21), mouse monoclonal anti-caspase-3 antibodies (Santa Cruz Biotechnology), or mouse monoclonal anti-RelA antibodies (22). After extensive washing in phosphate-buffered saline, the immune complexes were visualized by enhanced chemiluminescence ECL (PerkinElmer Life Sciences). Anti-actin goat polyclonal antibodies (Santa Cruz Biotechnology) were used to control the protein loading in the gel. Horseradish peroxidase-conjugated secondary anti-rat, anti-rabbit, anti-goat, and anti-mouse antibodies were purchased from Santa Cruz Biotechnology.

**Fig. 1.** Poliovirus infection stimulates translocation of NF-κB into the nucleus. A, electrophoretic mobility shift assay of protein extracts from the cytoplasm and the nuclei of poliovirus-infected HeLa cells. 10 μg of protein extracts from cytoplasm (1–5) and from the nuclei (6–11) were used for assay with a 32P-labeled NF-κB-specific oligonucleotide probe. Arrows show the position of the p65/p50 dimers. B, Western blotting analysis is shown of the protein extracts from the cytoplasm and the nuclei of poliovirus-infected HeLa cells. 10 μg of cytoplasmic (1–5) and nuclear (6–10) protein extracts were analyzed with anti-p65 antibodies specific to the C terminus of the protein. Control for poliovirus infection was done with anti-protein 3A antibodies. These antibodies recognize poliovirus proteins 3AB and 3A. Coomassie blue staining was used as a protein loading control. C, the activation of NF-κB by poliovirus is accompanied by IκBα degradation. Western blotting analysis of the cytoplasmic protein extracts from poliovirus-infected HeLa cells with anti-IκBα antibodies. IκBα degradation between 2 and 3 h postinfection coincided with the time of NF-κB activation. Western blotting with anti-actin antibodies was used as a protein loading control. D, Western blotting analysis is shown of the cytoplasmic protein extracts from TNF/CHI-treated and TNF-treated HeLa cells with anti-IκBα antibodies. IκBα reappearance after degradation because of the blockade of translation by CHI.

**Fig. 2.** Proteolytic cleavage of p65-RelA during poliovirus infection. A, poliovirus infection stimulated degradation of p65-RelA and a slow decline of p50. Western blotting analysis is shown of the cytoplasmic proteins from poliovirus-infected HeLa cells with antibodies specific for the C terminus of p65-RelA and with anti-p50 antibodies. B, cleavage of the p65-RelA C terminus during poliovirus infection. Western blotting analysis is shown of the protein extracts from panel A with antibodies specific for the N terminus of p65-RelA. The truncated form of p65-RelA (p65dC) accumulated between 2 and 6 h postinfection. Western blotting with anti-protein 3A antibodies was used as a control of protease 3C activity. Poliovirus proteins 3AB and 3A are the products of protease 3C activity. Cleavage of the p65-RelA protein coincided with the appearance of protease 3C-specific cleavage of poliovirus polyprotein. C, Coomassie blue staining of the gel from panels A and B was used as a protein loading control. Poliovirus infection did not stimulate significant protein degradation up to 6 h postinfection. D, schematic diagram indicating the positions of cleavage sites for caspases and protease 3C on p65-RelA protein. The positions of cleavage sites for caspase-3 (445) and -6 (465) are marked by bold italic and for protease 3C (480) by bold on the sequence of the human p65-RelA protein.

**Polyspecific Affinity Protein Purification and Protease 3C Assay—** The polyspecific-containing protease 3C was affinity purified on nickel-nitrotriacetic acid beads (Dynabeads). 3 × 10⁷ HeLa cells were infected by T7 RNA polymerase-expressing vaccinia virus for 2 h and then transfected with expression vector that contained a FLAG-3C-His. The cells were collected on ice and resuspended in 500 μl of lysis buffer (50 mM sodium phosphate buffer, pH 7.5, 50 mM NaCl, 10 mM imidazole, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin). The cytoplasmic protein extract was purified according to the Dignam protocol (20).
and was fractionated on nickel-nitritotriacetic acid beads according to the manufacturer's protocol (Dynal). The proteins from the beads were analyzed by immunoblotting. In vitro cleavage assay was performed with affinity-purified protease FLAG-3C-His. 100 ng of column-purified protease 3C was incubated for 30 min at 37 °C with 20 μg of cytoplasmic protein extract from HeLa cells in cleavage buffer (100 mM NaCl, 5 mM MgCl₂, and 10 mM HEPES-KOH, pH 7.4). The reaction was terminated by addition of loading buffer, and proteins were separated by SDS-PAGE.

RESULTS

Poliovirus Infection Leads to Activation of NF-κB—The effect of poliovirus infection on NF-κB function was analyzed by monitoring translocation of the NF-κB complex into the nucleus using electrophoretic mobility shift assay (Fig. 1A) and Western immunoblotting (Fig. 1B). According to both assays, the poliovirus infection stimulated translocation into the nucleus of the NF-κB complex at 2–3 h postinfection. The translocation of NF-κB into the nucleus was accompanied by the degradation of IκBα (Fig. 1C). The scale of NF-κB activation in poliovirus-infected HeLa cells was similar to that caused by TNF treatment (Fig. 1A). However, the reappearance of IκBα in TNF-treated cells was not observed in poliovirus-infected cells, presumably as a result of poliovirus-mediated block of cap-dependent translation (Fig. 1, C and D).

Degradation of p65-RelA at Later Stages of Poliovirus Infection—Poliovirus-induced accumulation of the NF-κB complex in the nucleus occurred between 2 and 3 h after infection and was followed by the degradation of p65-RelA and the appearance of a shorter fragment. These events were consistent with proteolytic cleavage of the protein (Fig. 1B and Fig. 2A). Because the truncation of the p65-RelA structure could be functionally significant, we followed this observation in more detail. Western blot analysis of p65-RelA with antibodies specific for the C terminus of the protein showed a rapid and profound decrease of p65-RelA in the cytoplasm of infected cells between 2 and 4 h after infection and complete disappearance from the cytoplasm and the nucleus by 6 h after infection (Fig. 1B and Fig. 2A). Antibodies specific for the N terminus of p65-RelA revealed the accumulation of a shorter product, named p65dC (Fig. 2B). The total protein levels did not show a significant decline even at late stages of poliovirus infection (Fig. 2C).

These results indicate that p65-RelA undergoes proteolytic cleavage during poliovirus infection resulting in removal of the C-terminal fragment of the protein containing a transactivation domain. In contrast to p65-RelA subunit, the amounts of the p50 component of the NF-κB complex only slightly decreased during infection, probably as a result of general protein synthesis shutdown (Fig. 2A), with no indications of protein truncation.

Truncation of p65-RelA Is Not the Result of Caspase-mediated Cleavage—It was previously shown that caspase-3 and caspase-6, activated during apoptosis, could cleave p65-RelA close to the C terminus (23–25). This cleavage results in creation of the products that fail to act as the transactivators and turn into the inhibitors of transcription (25).

According to previous reports, enterovirus infection does not involve the appearance of the markers of apoptosis (26); on the contrary, it was shown to be associated with the suppression of apoptosis (27). However, we examined the possibility that p65dC in poliovirus-infected cells appears as a result of caspase-mediated cleavage (28, 29). We analyzed whether poliovirus infection stimulates activation of caspase-3 from its inactive form (30) and the appearance of the products of caspase-mediated cleavage of keratin 18 (21) and lamin A (31) in infected cells. In contrast to apoptotic cells, these proteins did not show apoptosis-specific cleavage in poliovirus-infected cells (Fig. 3, A–C), indicating a lack of caspase activation.

Lack of involvement of apoptotic mechanisms in p65-RelA degradation was also evident from the results of analysis of HeLaBcl-2, the poliovirus-infected variant of HeLa cells expressing anti-apoptotic protein Bcl-2 (32). These cells became resistant to Fas-mediated apoptosis (Fig. 4A) and did not show apoptosis-specific cleavage of p65-RelA (Fig. 4B). At the same time, they were as susceptible to poliovirus infection as the original HeLa cells (Fig. 4A), including the poliovirus-specific cleavage of p65-RelA (Fig. 4B). The pattern of p65-RelA cleavage in HeLaBcl-2 poliovirus-infected cells was not changed (Fig. 4C).

Finally, we examined the effect of the pan caspase inhibitor z-VAD-fmk on p65-RelA cleavage during poliovirus infection. Although z-VAD-fmk protected HeLa cells from Fas-induced apoptosis and from apoptosis-specific cleavage of p65-RelA (Fig. 5A), it had no effect on poliovirus infection and on poliovirus-specific cleavage of p65-RelA (Fig. 5A). Moreover, the cleavage products of p65-RelA in poliovirus-infected HeLa cells and in apoptotic HeLa cells had different molecular masses (Fig. 5B). All these observations indicate that generation of p65dC during poliovirus infection has no relation to apoptosis and caspases and is likely to be carried out by virus-specific proteins.

p65-RelA Is Cleaved by Virus Protease 3C during Poliovirus Infection—The poliovirus genome encodes two proteases, 2A and 3C (33, 34), both capable of targeting cellular proteins (35, 36). A search for picornavirus protease cleavage sites using...
NetPicornaRNA web-based software (37) revealed the amino acid sequence specific for the protease 3C cleavage close to the C terminus of the p65-RelA protein (Fig. 2D). The size of the poliovirus-specific truncated form of p53-RelA, p65dC, was consistent with that expected from the protease 3C cleavage site on p65-RelA (Fig. 2D). Moreover, the initiation of cleavage of p65-RelA during infection coincided with the appearance of 3C protease activity detected by cleavage of the poliovirus polyepitope (Fig. 2, A and B).

To check whether poliovirus-specific protease 3C can induce proteolytic cleavage of p65-RelA, we generated recombinant protein 3C with a FLAG tag on its N terminus and a His tag on its C terminus expressed from the T7 RNA polymerase/vaccinia virus expression system. FLAG-3C-His purified by affinity chromatography on Ni beads was incubated in vitro with cytoplasmic protein extract from HeLa cells. Western immunoblotting with the antibodies specific to the N terminus of p65-RelA showed the cleavage of p65-RelA protein in the protein extract incubated with protease 3C (Fig. 5C) similar to that occurring during poliovirus infection.

Infection with ECHO-1 and Rhino-14 Viruses Stimulates p65-RelA Cleavage—Is the ability to stimulate p65-RelA proteolytic cleavage unique for the poliovirus infection, or does it occur during infection by other picornaviruses? To address this question, we analyzed the status of p65-RelA during infection with ECHO-1 and rhino-14 viruses. Rhinovirus was especially interesting because activation of NF-κB was previously reported for this virus infection (13). The patterns of p65-RelA cleavage were very similar during poliovirus and ECHO-1 virus infections (Fig. 6, A and B). Rhinovirus 14 infection also
stimulated p65-RelA cleavage in HeLa cells, but the cleavage started later than in poliovirus-infected cells, consistent with the slower growth cycle of this virus (Fig. 6, C and D).

**DISCUSSION**

Organisms react to infection through the innate immune response. It starts from the activation of NF-κB; as a result, the expression of genes encoding pro-inflammatory cytokines is stimulated, including TNF, interleukin 6, interleukin 8, chemokines, and adhesion molecules (38). These molecules are involved in the immunity process by recruiting the immune cells to the site of infection. NF-κB is also a component of the activation of the important anti-viral cytokine, interferon-β (39). The expression of MHCI protein genes is regulated by the slower growth cycle of this virus (Fig. 6).

RNA-containing viruses activate the NF-κB-specific response and interferon synthesis via activation of Toll-like receptor 3 by dsRNA (7, 42). Activation of NF-κB was described for several picornaviruses, including rhinovirus and TME virus infections (10, 13). In this study, we report that poliovirus infection also initiates NF-κB activation that takes place between 2 and 3 h after infection. This is in contrast with rhinovirus and TME virus infections, which initiate NF-κB activation after infection either later (8 h) or earlier (30 min) (10, 13).

The time of poliovirus-specific activation of NF-κB correlated well with the time of dsRNA synthesis (43), suggesting it may be the trigger for NF-κB activation. As happens with NF-κB activation by many other factors, such as cytokines and dsRNA (44), the poliovirus-specific activation of NF-κB was preceded by the degradation of IκBα (Fig. 1C), indicating that it goes through the conventional mechanism.

To counteract the effect of NF-κB, some viruses develop activities that interfere with virus-specific NF-κB activation. For instance, NS1 protein of the influenza A virus prevents the activation of NF-κB (45). The African swine fever virus has two homologues of IκB with potent anti-inflammatory effect (46). Adenovirus E1A protein affects TNF-specific NF-κB stimulation (47). Finally, many members of the Pozivirus genus encode the proteins that interfere with the positive regulation of NF-κB activation (11, 48). All these examples show that anti-inflammatory activities are common among viruses of different origin and that these activities are important for viral infection.

In this communication, we report a new mechanism used by picornaviruses to mitigate the NF-κB-dependent innate immune response. This mechanism consists of rapid and efficient proteolytic cleavage of the p65-RelA component of the NF-κB complex by the virus-encoded protease 3C. Similar proteolytic cleavage of the C terminus of p65-RelA was shown to inactivate the protein (25). This cleavage of p65-RelA starts ~3 h after poliovirus infection. After 4 h of infection, most of the p65-RelA molecules in poliovirus-infected cells are truncated. The beginning of the degradation coincides with a time of intensive poliovirus protein synthesis and does not involve caspases (Figs. 3A and 5A). The ability of protease 3C to cleave p65-RelA may explain the pro-apoptotic activity of this protease described previously (49), because the activation of NF-κB in the majority of cases has a strong anti-apoptotic effect (50). Based on these data, we suggest that picornaviruses developed the mechanism of p65-RelA degradation. This mechanism is especially important for them because the inhibition of cellular mRNA translation destroys the IκB-dependent regulatory loop in infected cells (Fig. 1C), thus abrogating the natural mechanism of negative regulation of NF-κB response. The proteolytic cleavage of p65-RelA resolves this problem and limits the time of NF-κB activation to less than 2 h in HeLa cells.

Why would picornaviruses benefit from the specific suppression of NF-κB if they have a more general mechanism suppressing cap-dependent translation that should affect NF-κB-mediated protein expression? Indeed, poliovirus-infected cells fail to restore their IκB levels (Fig. 2). However, some of the cellular mRNAs for NF-κB-responsive genes, unlike IκBo mRNA, can be efficiently translated regardless of poliovirus infection (51). These include such well characterized NF-κB targets as interleukin 6, interleukin 8, and c-Myc (51). Hence, poliovirus protein 2A-mediated inhibition of host translation is not enough to completely shut down the innate immune response.

Poliovirus is known to inhibit not only translation but also the transcription of cellular genes acting against RNA polymerase II (52–54) or specific transcription factors, such as cAMP-response element-binding protein (15, 55). In this article, we report a new target for poliovirus-specific inhibition of cellular gene transcription, p65-RelA protein, the critical component of the NF-κB complex.

The cleavage of the C terminus of p65-RelA can be also detected in cells infected by other picornaviruses such as ECHO-1 and rhinovirus 14, thus reflecting the common nature of the discovered phenomenon. Together with previously described activities (inhibition of translation, transcription, nuclear shuttling, protein trafficking, and receptor presentation), this mechanism is used by the virus to modify cellular processes to its own benefit and is served as part of a viral strategy.
of effective replication and abrogation of defense mechanisms of the host.

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REFERENCES

1. Guidotti, L. G., and Chisari, F. V. (2001) Annu. Rev. Immunol. 19, 65–91
2. Demarchi, F., Gutierrez, M. L., and Giacone, M. (1999) J. Virol. 73, 7080–7086
3. Helin, E., Matikainen, S., Julkunen, I., Heino, J., Hyytiä, and Vainio, P. (2002) Arch. Virol. 147, 1721–1732
4. Kowalik, T. F., Wing, B., Haskill, J. S., Azizkhan, J. C., Baldwin, A. S., Jr., and Bender, A. (2000) J. Virol. 74, 5534–5541
5. Kowalik, T. F., Wing, B., and Haskill, J. S. (2001) J. Virol. 75, 9921–9928
6. Kowalik, T. F., Wing, B., and Haskill, J. S. (2002) J. Virol. 76, 9042–9046
7. Pahl, H. L., and Beauerle, P. A. (1995) J. Virol. 69, 1480–1484
8. Beieck, K., Lien, E., Klage, I. M., Avot, E., Schneider-Schaulies, J., Duprex, W. P., Wagner, H., Kirschning, C. J., T., M. E., and Schneider-Schaulies, S. (2002) J. Virol. 76, 8729–8736
9. Palma, J. P., Kwon, D., Clippetto, N. A., and Kim, B. S. (2003) J. Virol. 77, 6322–6331
10. Gill, J., Rupars, J., Alcami, J., and Esteban, M. (2001) J. Gen. Virol. 82, 3027–3034
11. Taddeo, B., Luo, T. R., Zhang, W., and Roizman, B. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 12408–12413
12. Taddeo, B., Luo, T. R., Zhang, W., and Roizman, B. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 7551–7556
13. Papi, A., and Johnston, S. L. (1999) J. Biol. Chem. 274, 2538–2544
14. Neuzil, J., Schroder, A., von Hundelshausen, P., Zernecke, A., Weber, T., Gellert, N., and T., M. E. (2001) Biochemistry 40, 6866–6872
15. Vezza, B., Scallabini, M., Giacci, M., Ross, R., and Raines, E. W. (1999) Nat. Cell Biol. 1, 227–233
16. Belov, G. A., Romanova, L. I., Tolskaya, E. A., Kolesnikova, M. S., Lazebnik, Y. A., and Agol, V. I. (2003) J. Virol. 77, 45–56
17. Agol, V. I., Belov, G. A., Bains, R., Egger, D., Kolesnikova, M. S., Romanova, L. I., Sladkov, A. V., and Tolskaya, E. A. (2000) J. Virol. 74, 5534–5541
18. Campanella, M., de Jong, A. S., Lanke, K. W., Melchers, W. J., Willems, P. H., Pinto, P., Rizzuto, R., and van Kuppevelt, T. F. (2004) J. Biol. Chem. 279, 18440–18450
19. Tolskaya, E. A., Romanova, L. I., Kolesnikova, M. S., Ivanivkova, T. A., Smirnova, E. A., Raikhlin, N. T., and Agol, V. I. (1995) J. Virol. 69, 1181–1189
20. Karin, M. W., Srinivasan, A., Foster, L. M., Testa, M. P., Ord, T., Noller, D., Wang, H. G., Reed, J. C., Breeden, D. E., and Kayalar, C. (1997) J. Neurosci. Res. 48, 168–180
21. Lazebnik, Y. A., Takahashi, A., Mair, R. D., Goldman, R. D., Foigier, G. R., Kaufmann, S. H., and Earnshaw, W. C. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9042–9046
22. Sory, C., and Adams, J. M. (2002) Nat. Rev. Cancer 2, 647–656
23. Nicklin, M. J., Krausslich, H. G., Toyoda, H., Dunn, J. J., and Wimmer, E. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4002–4006
24. Haynes, L. M., Moore, D. D., Kurt-Jones, E. A., Finberg, R. W., Anderson, L. J., and Tripp, R. A. (2001) J. Virol. 75, 10730–10737
25. Blom, N., Hansen, J., Blaa, D., and Brunak, S. (1996) Protein Sci. 5, 2203–2216
26. Mogensen, T. H., and Paludan, S. R. (2001) Microbiol. Mol. Biol. Rev. 65, 131–150
27. Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H., and Schreiber, R. D. (1998) Annu. Rev. Biochem. 67, 277–284
28. Martz, L., Boot, A. J., Mahabhar, G., Zantiema, A., and van der Eb, A. J. (1992) Cell Immunol. 145, 56–65
29. Bose, S., Kar, N., Maitra, R., DiDonato, J. A., and Banerjee, A. K. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 10890–10895
30. Haynes, L. M., Moore, D. D., Kurt-Jones, E. A., Pinberg, R. W., Anderson, L. J., and Tripp, R. A. (2001) J. Virol. 75, 10730–10737
31. Pfefferkorn, E. R. (1975) J. Med. Biol. 53, 337–341
32. Karin, M. (1999) Oncogene 18, 6867–6874
33. Wang, X., Li, M., Zheng, H., Muster, T., Palese, P., and Garcia-Sastre, A. (2000) J. Virol. 74, 11566–11573
34. Tait, S. W., Reid, E. B., Greaves, D. R., Wileman, T. E., and Powell, P. P. (2006) J. Biol. Chem. 281, 34656–34664
35. Shaor, R., Hu, M. C., Zhou, B. P., Lin, S. Y., Chiao, P. J., von Linden, R. H., Spohn, B., and Hung, M. C. (1999) J. Biol. Chem. 274, 21495–21498
36. Oep, K. L., and Pickup, D. J. (2001) Virology 298, 175–187
37. Calandria, C., Irozun, A., Bacev, A., and Carrasco, L. (2004) Virus Res. 104, 39–49
38. Karin, M., Cao, Y., Greten, F. R., and L., Z. W. (2002) Nat. Rev. Cancer 2, 301–310
39. Johannes, G., Carter, M. S., Eisen, M. B., Brown, P. O., and Sarnow, P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13118–13123
40. Yalamanchili, P., Panerjee, R., and Dasgupta, A. (1997) J. Virol. 71, 6831–6836
41. Sharma, R., Raychaudhuri, S., and Dasgupta, A. (2004) Virology 320, 195–205
42. Clark, M. E., Lieberman, P. M., Berk, A. J., and Dasgupta, A. (1997) Mol. Cell. Biol. 17, 1232–1237
43. Yalamanchili, P., Dutta, U., and Dasgupta, A. (1997) J. Virol. 71, 1220–1226