Many viruses subvert the cellular interferon (IFN) system with so-called IFN antagonists. Bunyamwera virus (BUNV) belongs to the family Bunyaviridae and is transmitted by arthropods. We have recently identified the nonstructural protein NSs of BUNV as a virulence factor that inhibits IFN-β gene expression in the mammalian host. Here, we demonstrate that NSs targets the RNA polymerase II (RNAP II) complex. The C-terminal domain (CTD) of RNAP II consists of 52 repeats of the consensus sequence YSPTSPS. Phosphorylation at serine 5 is required for efficient initiation of transcription, and subsequent phosphorylation at serine 2 is required for mRNA elongation and 3‘-end processing. In BUNV-infected mammalian cells, serine 5 phosphorylation occurred normally. Furthermore, RNAP II was able to bind to the IFN-β gene promoter as revealed by chromatin immunoprecipitation analysis, indicating that the initiation of transcription was not disturbed by NSs. However, NSs prevented CTD phosphorylation at serine 2, suggesting a block in transition from initiation to elongation. Surprisingly, no interference with CTD phosphorylation was observed in insect cells. Our results indicate that BUNV uses an unconventional mechanism to block IFN synthesis in the mammalian host by directly dysregulating RNAP II. Moreover, by inducing a general transcriptional block, NSs may contribute to the lytic infection observed in mammalian cells as opposed to persistent infection in the insect host.

α/β interferons (IFN-α/β)1 play a key role in host defense against invading viruses (1). The induction of IFN-β gene expression is best understood for IFN-β. The IFN-β promoter has binding sites for the stress-activated transcription factors NF-κB and AP-1 and for the IFN regulatory factors IRF-3 and IRF-7. These factors normally reside in the cytoplasm. Upon activation by virus infection or double-stranded RNA, they move to the nucleus and associate with RNA polymerase II (RNAP II) to initiate transcription of the IFN-β gene (2, 3).

During the transcription cycle, the C-terminal domain (CTD) of the large subunit of RNAP II is reversibly phosphorylated (4). In mammalian cells, the CTD is composed of 52 repeats of the consensus sequence YSPTSPS. RNAP II containing unphosphorylated CTD is recruited to the promoter (5), whereas the CTD hyperphosphorylated form is involved in active transcription (6). Phosphorylation occurs at two sites within the heptapeptide repeat, at serine 5 and serine 2. Serine 5 phosphorylation is confined to promoter regions and is necessary for the initiation of transcription, whereas serine 2 phosphorylation is important for mRNA elongation and 3‘-end processing (7–11).

Viruses have evolved numerous mechanisms for evading the IFN system. Specific viral gene products have been identified that inhibit either IFN synthesis, IFN signaling, or IFN action and are therefore referred to as IFN antagonists (2, 3, 12). Bunyamwera virus (BUNV) is a member of the family Bunyaviridae, representing a large group of negative strand RNA viruses that are mainly transmitted by arthropods (13). Several bunyaviruses cause encephalitis or hemorrhagic fevers in humans, e.g. the Hantaan virus, the Sin Nombre virus, the Rift Valley fever virus, the La Crosse virus, and the Crimean-Congo hemorrhagic fever virus (14). Like other arboviruses, BUNV can replicate both in mammals and insects. Depending on the host, however, the outcome of infection is different. In mammalian cells, infection is lytic and causes host cell shutoff and cell death. In insect cells, however, infection is non-lytolytic and leads to long-term viral persistence (15–17).

We have demonstrated previously that the nonstructural NSs protein of BUNV acts as an IFN antagonist (18). A mutant virus with a deleted NSs gene (BUNdelNSs virus) was generated (19) and found to be highly attenuated in immunocompetent mice. However, in mice with a defective IFN system this NSs-deficient virus was as virulent as the wild-type virus (wt BUNV). Further analyses revealed that NSs inhibited transcriptional activation of the IFN-β gene without interfering with the activation of IRF-3 (18, 20). These findings suggested that NSs blocks a transcriptional step downstream of IRF-3. Here, we further investigated the mechanism and show that NSs inhibits a basic process in host cell gene transcription by targeting the CTD of RNAP II.

EXPERIMENTAL PROCEDURES

Cells and Viruses—Simian VeroE6 cells and human 293 cells were grown at 37 °C in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Aedes albopictus C6/36 cells were maintained at 33 °C in L-15 medium (Invitrogen) supplemented with 5% fetal calf serum and 10% tryptose phosphate broth (Difco). The wt BUNV and the BUNdelNSs virus stocks used in this study were plaque-purified in BHK-21 cells, and working stocks were grown in VeroE6 cells as described (21).
Antibodies—Monoclonal antibodies H5 and H14 (Covance) specifically recognize phosphoserine 2 and 5, respectively, on the CTD (22). Rabbit polyclonal antibody N-20 (Santa Cruz Biotechnologies) recognizes the N terminus of the large subunit of RNAP II. Rabbit polyclonal serum against the N protein of BUNV has been described previously (23). Mouse monoclonal antibody M2 (Sigma) and a rabbit polyclonal antiserum (Sigma) were used to detect the FLAG epitope. Rabbit polyclonal antibody FL-425 (Santa Cruz Biotechnologies) recognizes IFN-β. Cy2- and Cy3-conjugated goat anti-rat (Alexis) were used as secondary antibodies, and mouse monoclonal antibody B-10 (Santa Cruz Biotechnologies), which recognizes protein kinase R, was used as an IgG control.

Plasmids—The firefly luciferase (FF-Luc) reporter constructs for measuring IFN-β promoter activation (p-125Luc) and NF-κB activity (p55A2-Luc) were kindly provided by Takashi Fujita, Tokyo, Japan (24). The FF-Luc reporter plasmid for AP-1 activity was purchased from Stratagene. The FF-Luc plasmid containing the Herpes virus thymidine kinase promoter was kindly provided by Stephen Goodbourn, London, United Kingdom (25). Plasmid pRL-SV40 (Promega) contains the Renilla luciferase gene under control of the constitutive simian virus 40 promoter. The plasmid constructs pCDNA-CTRL (expressing a short fragment of the human MxA protein), pCDNA-FLAG-CTRL, pCDNA-FLAG-BUNV Ns, pCDNA-FLAG-BUNV NSs, and pCMV-BUNV NSs (23) contain the appropriate cDNA sequences under the control of the CMV immediate early promoter (pCDNA backbone) and the T7 promoter (pCMV backbone), respectively.

Reporter Assays—Subconfluent monolayers of 293 cells were transfected with 0.5 μg luciferase reporter plasmid and 0.5 μg expression plasmid in 200 μl of OptiMEM (Invitrogen) containing 2 μl of D-30 (Eurogentec). After 5 h at 37 °C, the liposome-DNA mixture was removed, and cells were treated with a specific inducer (10 μg of the synthetic double-stranded RNA poly(I-C) (Sigma) prepared with 10 μl of D-30 liposomes, 50 μg/ml tumor necrosis factor α (Sigma), or 1 μM N7-benzyl-cAMP (Calbiochem)) or left untreated. At 24 h post-transfection, cells were harvested and lysed in 200 μl of reporter lysis buffer (Promega). An aliquot of 20 μl of lysate was used to measure luciferase activities as described by the manufacturer (Promega).

Chromatin Immunoprecipitation Assays—Cells were lysed in radioimmunoprecipitation assay buffer (50 mM Tris, pH 7.6, 150 mM NaCl, and 1% Nonidet P-40) containing protease inhibitors (Complete, Roche Applied Science) and phosphatase inhibitors (phosphatase inhibitor mixture II, Calbiochem). A total of 10 μg protein was separated on a 5% polyacrylamide gel by electrophoresis and blotted onto an Immobilon-P membrane (Millipore) following the manufacturer’s instructions (Bio-Rad). The membrane was incubated with a 1:500 dilution of mouse antibodies H5 or H14 or rabbit anti-BUNV N serum. Protein bands were visualized using the ECL Plus method (Amersham Biosciences).

Double Immunofluorescence Assays—Cells were lysed in radioimmunoprecipitation assay buffer (50 mM Tris, pH 7.6, 150 mM NaCl, and 1% Nonidet P-40) containing protease inhibitors (Complete, Roche Applied Science). An aliquot of 20 μl of 10 mg/ml protease K was added and allowed to incubate for 1 h at 45 °C. DNA was extracted with phenol/chloroform, ethanol-precipitated using 50 μg of glycogen (Roche Applied Science) as carrier, and dissolved in 20 μl of H2O. Input and precipitated DNAs were analyzed by PCR using primers specific for the human IFN-β promoter from nucleotides −138 to −19 (26).

**RESULTS**

NSs Represses Reporter Gene Expression in a Promoter-independent Manner—We investigated the effect of NSs on RNAP II transcription using reporter assays. Cells in parallel dishes were transfected with an NSs-expressing cDNA construct together with various reporter plasmids containing the FF-Luc or Renilla luciferase genes under control of different inducible or constitutively active promoters. To activate the inducible promoters, cells were treated with either double-stranded RNA (IFN-β promoter), TNF-α (NF-κB-inducible promoter), or cyclic AMP (AP-1-inducible promoter). After overnight incubation, luciferase activity was determined as a measure of promoter stimulation. As expected, NSs inhibited induction of the IFN-β promoter, whereas a control construct (CTRL) encoding a polypeptide of a similar size as that of NSs had no effect (Fig. 1A, columns 1 and 2). Interestingly, NF-κB- and AP-1-inducible promoter activity was similarly blocked by NSs (Fig. 1A, columns 3–6). Surprisingly, the constitutively active thymidine kinase promoter and the simian virus 40 immediate early promoter were also inhibited (Fig. 1B), indicating that NSs had a general effect on cellular transcription. It should be noted that, in these experiments, the expression of NSs was under the control of the CMV immediate early promoter. This resulted in almost undetectable levels of NSs (Fig. 1C, right panel), presumably due to autocrine self-inhibition. In contrast, the CTRL protein was expressed from the same vector to high amounts, as expected (Fig. 1C, left panel). Thus, even small amounts of NSs seem to be able to repress transcription from cellular or viral RNAP II promoters.

NSs Targets the C-terminal Domain of the Mammalian RNA Polymerase II—CTD phosphorylation plays a central role in the regulation of RNAP II (4, 11). We therefore investigated whether NSs would affect the phosphorylation of the CTD heptapeptide at serine 5 or serine 2. Cells were infected with either the wt BUNV or the NSs-deficient BUNdelNSs virus or were left uninfected. At 6 h after infection, cells were lysed and subjected to Western blot analysis using phosphorylation state-specific antibodies. Fig. 2A (panel 1) shows that infection with either wt BUNV or BUNdelNSs did not change CTD phosphorylation on serine 5. By contrast, phosphorylation on serine 2 was strongly reduced in wt BUNV-infected cells but not in BUNdelNSs-infected cells (Fig. 2A, panel 2). We also determined the overall amount of RNAP II in infected cells by using an antiserum that recognizes the N terminus of the RNAP II large subunit. As demonstrated in Fig. 2A (panel 3), infection with wt BUNV shifted the distribution of the RNAP II CTD from the hyper- to the hypophosphorylated state (Fig. 2A, Ila and Iia, respectively), in agreement with the observed reduction in CTD phosphorylation. The overall levels of RNAP II, however, were comparable between cells infected with either virus. Immunostaining for the viral N protein showed that the
A reporter plasmids together with expression plasmids for BUNV NSs or a polypeptide of similar size (CTRL). NSs or CTRL expression was mediated by the CMV immediate early promoter. A, inhibition of inducible promoters expressing the FF-Luc reporter gene. The IFN-β promoter was stimulated by double-stranded RNA treatment (columns 1 and 2), the NF-κB-inducible promoter was stimulated with TNF-α (columns 3 and 4), and the AP-1-inducible promoter was stimulated with 6-benzacyl-cAMP (columns 5 and 6) as described under “Experimental Procedures.” B, inhibition of constitutive promoters expressing the FF-Luc (columns 1 and 2) or Renilla Luc (columns 3 and 4) reporter genes. Herpes simplex virus thymidine kinase (TK) promoter activity (columns 1 and 2) or simian virus 40 (SV40) immediate early promoter activity (columns 3 and 4) were analyzed. In all experiments, luciferase activities of cells transfected with the CTRL expression plasmids were set to 100%. Mean values and S.D. from three independent experiments are shown. C, expression of the FLAG-tagged CTRL protein and BUNV NSs. VeroE6 cells were transfected with expression plasmids for FLAG-CTRL (left panel) or FLAG-BUNV NSs (right panel) and analyzed 24 h later by indirect immunofluorescence using antibodies directed against the FLAG portion of the fusion proteins (green). To visualize cell nuclei, chromosomal DNA was counterstained with propidium iodide (red).

Fig. 1. Effect of NSs on inducible and constitutive promoters. Human 293 cells were transfected with different promoter-luciferase reporter plasmids together with expression plasmids for BUNV NSs or a polypeptide of similar size (CTRL). NSs or CTRL expression was mediated by the CMV immediate early promoter. A, inhibition of inducible promoters expressing the FF-Luc reporter gene. The IFN-β promoter was stimulated by double-stranded RNA treatment (columns 1 and 2), the NF-κB-inducible promoter was stimulated with TNF-α (columns 3 and 4), and the AP-1-inducible promoter was stimulated with 6-benzacyl-cAMP (columns 5 and 6) as described under “Experimental Procedures.” B, inhibition of constitutive promoters expressing the FF-Luc (columns 1 and 2) or Renilla Luc (columns 3 and 4) reporter genes. Herpes simplex virus thymidine kinase (TK) promoter activity (columns 1 and 2) or simian virus 40 (SV40) immediate early promoter activity (columns 3 and 4) were analyzed. In all experiments, luciferase activities of cells transfected with the CTRL expression plasmids were set to 100%. Mean values and S.D. from three independent experiments are shown. C, expression of the FLAG-tagged CTRL protein and BUNV NSs. VeroE6 cells were transfected with expression plasmids for FLAG-CTRL (left panel) or FLAG-BUNV NSs (right panel) and analyzed 24 h later by indirect immunofluorescence using antibodies directed against the FLAG portion of the fusion proteins (green). To visualize cell nuclei, chromosomal DNA was counterstained with propidium iodide (red).

Cell cultures were infected with similar efficiencies, irrespective of whether the wild-type or the mutant virus was used (Fig. 2A, panel 4). In summary, these data demonstrate that infection with wt BUNV reduces CTD serine 2 phosphorylation without affecting CTD serine 5 phosphorylation or the overall levels of RNAP II. To examine this finding at the single-cell level, we performed double immunofluorescence analyses using the phosphorylation state-specific antibodies. Again, no major changes were detected for CTD phosphoserine 5 (Fig. 2B, upper panel), whereas the phosphoserine 2-specific signal disappeared in wild-type BUNV-infected cells but not in cells infected with the delNSs mutant (Fig. 2B, lower panel).

The only difference between wt BUNV and the BUNdelNSs virus is their capacity to express NSs. We therefore argued that NSs was most likely responsible for the observed effect on CTD phosphorylation. To verify this hypothesis, we expressed recombinant NSs from appropriate plasmids. To bypass the suppressive effect of NSs on RNAP II-driven promoters (see Fig. 1C), we used a T7 system for NSs expression. Cells were transfected with a FLAG-NSs construct containing a T7 promoter, infected with an MVA-T7 helper virus, and fixed and stained at 6 h post-infection. Fig. 3A shows that T7-mediated expression of NSs indeed resulted in much higher protein levels than did CMV-driven expression, which is in agreement with our hypothesis that NSs inhibits the RNAP II. Furthermore, in cells expressing FLAG-NSs the phosphorylation of both serine 5 (Fig. 3, panels 1–3) and serine 2 (Fig. 3, panels 4–6) was barely detectable, whereas the surrounding untransfected cells were not affected. By contrast, parallel immunostainings with an antiserum that recognizes the N terminus of the RNAP II large subunit did not detect any major changes in RNAP II levels (Fig. 3, panels 7–9), indicating that NSs does not simply induce RNAP II degradation (compare Fig. 2A).

Taken together, these data suggest that NSs represses RNAP II (but not T7 polymerase) by down-regulating the CTD phosphorylation. The main target appears to be phosphoserine 2. However, phosphoserine 5 was also affected in cells overexpressing recombinant NSs. In line with this, wt BUNV but not BUNdelNSs inhibited both serine 5 and serine 2 phosphorylation late in infection (data not shown).

Subcellular Localization of NSs—In the T7 RNA polymerase-mediated high expression system, NSs was found to accumulate mainly in the nucleus (Fig. 3), whereas in the CMV promoter-mediated low expression system, NSs was detected in the cytoplasm (Fig. 1C). To investigate a possible relationship between NSs expression levels and intracellular localization, we monitored the subcellular distribution of NSs at different time points after the onset of expression by using the T7 system. Cells were transfected with the T7-FLAG-NSs construct, infected with MVA-T7 helper virus, and fixed and stained at 2...
and 6 h post-infection. At 2 h after the onset of expression, NSs formed a punctate pattern in the cytoplasm (Fig. 4, left panel) similar to the pattern observed with CMV promoter-driven expression (see Fig. 1C). In addition, minor amounts of NSs were localized in the nucleus, as indicated by a faint nuclear staining. At 6 h after the onset of expression, NSs was mainly nuclear, and the cytoplasm showed a faint and more uniform staining (Fig. 4, right panel). This staining pattern was also observed at later time points (data not shown). Because NSs in the CMV promoter-driven expression system was cytoplasmic even 24 h after transfection, we conclude that the subcellular localization of NSs depends on the expression levels, i.e. low amounts of NSs result in a mainly cytoplasmic, punctate pattern, whereas higher amounts localize to the nucleus.

**CTD Phosphorylation in Insect Cells**—Most bunyaviruses have the ability to replicate both in mammals and in arthropods (13). Infection of mammalian cells is lytic and results in a shutoff of host cell protein synthesis, whereas insect cells are persistently infected without deleterious effects on cell function (15–17). This difference in the outcome of infection is not easily explained, especially because NSs is expressed in BUNV-infected mosquito cells (17). Therefore, we investigated whether...
NSs also affects CTD phosphorylation in insect cells. C6/36 mosquito cells were infected for 24 h, and CTD serine phosphorylation was investigated by immunofluorescence analyses. No apparent difference in serine 5 phosphorylation was observed between wt BUNV-infected and uninfected cells (Fig. 5, panels 1 and 2). Similarly, CTD serine 2 phosphorylation was unaffected (Fig. 5, panels 4 and 5). Infection with the BUNdelNSs virus also did not impair CTD phosphorylation, as expected (Fig. 5, panels 3 and 6). These results imply that NSs does not target the CTD of RNAP II in insect cells, allowing cell survival and persistent infection.

Promoter Binding by RNAP II—Current models of RNAP II transcription postulate that CTD phosphorylation at serine 5 is confined to promoter regions and is necessary for initiating mRNA synthesis (8, 9, 11). Subsequent phosphorylation at serine 2 is required for mRNA elongation and 3′-end processing. We determined the promoter binding capacity of RNAP II in infected cells and suggest that NSs affects a step after RNAP II has bound to the promoter. We also tested whether the bound RNAP II was phosphorylated at serine 2. As expected, in wt BUNV-infected cells no PCR signal was detected. In contrast, a strong signal was detectable in cells infected with the NSs-deficient mutant, indicating serine 2-phosphorylation of RNAP II (Fig. 6A, lane delNSs).

Although in infected cells the IFN-β gene is inactive, we detected CTD-phosphorylated RNAP II bound to the IFN-β promoter region (Fig. 6A, panels 1 and 2, compare lanes m and delNSs). Binding of fully phosphorylated RNAP II to the inactive IFN-β promoter was also observed in human A549 cells and in mouse embryo fibroblasts (data not shown), ruling out cell-specific effects. To elucidate the differences between the uninduced and induced state of the IFN-β promoter, we determined promoter binding of the IFN-specific transcription factor IRF-3, again using ChIP assays. In uninfected cells, IRF-3 did not bind to the IFN-β promoter (Fig. 6B, panel 1). By contrast, an IRF-3-specific signal was obtained in cells infected with either bunyavirus (Fig. 6B, panel 1). This result is in agreement with our previous finding that IRF-3 activation is not affected by NSs (20). Taken together, these data suggest that RNAP II can be fully phosphorylated and recruited to the IFN-β promoter even in the absence of virus infection but requires IRF-3 for efficient transcriptional activity. Moreover, NSs affects RNAP II after it has bound to the promoter.

**DISCUSSION**

In this study, we demonstrate that the IFN antagonist NSs of BUNV profoundly impairs the function of the mammalian RNAP II. NSs targets a step in the cellular transcription cycle that occurs after the RNAP II has bound to the promoter. NSs inhibits phosphorylation of CTD residue serine 2 but not of...
CTD serine 5. As a consequence, RNAP II function is disturbed, leading to the down-regulation of host cell mRNA synthesis.

CTD phosphorylation is required for the eukaryotic RNAP II to overcome rate-limiting steps in transcription. Serine 5 phosphorylation initiates transcription of a short mRNA fragment and recruitment of a capping enzyme (9), whereas the subsequent phosphorylation at serine 2 is important for mRNA elongation and 3′-end processing (7, 8, 10, 11). Serine 5 phosphorylation is mainly mediated by cyclin-dependent kinase 7, a subunit of the general transcription factor TFIH2 (9). The main kinase responsible for CTD serine 2 phosphorylation is called P-TEFb, a complex consisting of cyclin-dependent kinase 9 and cyclin T (27). Recently, TLK-1 has been identified as a further serine 2-specific CTD kinase that is essential for transcription (28). After termination of transcription, dephosphorylation of both serine 5 and serine 2 is necessary to recycle the RNAP II. Three CTD phosphatases have been identified to date, namely FCP1 (29) and PPI (30), which dephosphorylate both serine 5 and serine 2 indiscriminately, and SCPP1, which preferentially dephosphorylates serine 5 (31). Given the preferred interference of NSs with phosphorylation at serine 2, it is more likely that NSs acts by deregulating one of the serine 2 kinases rather than by activating a CTD phosphatase. It is also conceivable that NSs influences some component of the RNAP II complex such that transcription does not proceed to the step at which CTD serine 2 phosphorylation would occur. Because NSs is located in the cytoplasm early after the onset of expression (see Fig. 4), it is possible that NSs sequesters a nuclear factor necessary for CTD serine 2 phosphorylation. Experiments are under way to explore the exact molecular mechanism and to identify the cellular interaction partners of NSs.

It is becoming increasingly clear that CTD phosphorylation represents an important step in the regulation of host gene expression. For example, cellular regulators like granulin (32), hnRNP (33), PIE-1 (34), and the immunosuppressive glucocorticoid receptor (35) specifically inhibit CTD phosphorylation. This leads to a repression of RNAP II elongation. On the other hand, the transactivator protein Tat of human immunodeficiency virus has the opposite effect and stimulates phosphorylation of CTD serine 2, thus promoting RNAP II elongation (36, 37). Similarly, heat shock (38) and chemical RNAP II inhibitors (39) increase CTD serine 2 phosphorylation. Our data indicate that the bunyaviruses also take advantage of the regulatory role of the CTD.

Transcription of bunyaviruses is dependent on host cell mRNAs. In a process called cap snatching, the viral L polymerase cleaves short, capped oligonucleotides from the 5′-end of host cell mRNAs and uses them as primers (13). It appears puzzling as to why, by inhibiting RNAP II, bunyaviruses would cut themselves off from the supply of such an important substrate. However, studies with the related La Crosse bunyavirus have shown that viral RNA synthesis occurs unhindered in the presence of the RNAP II inhibitor actinomycin D (40). Thus, RNAP II activity is dispensable for bunyaviruses, and the pool of cytoplasmic mRNAs is sufficient to support bunyavirus transcription. Orthomyxoviruses such as the influenza A virus, by contrast, cleave their capped primers from nascent cellular mRNAs. They replicate in the nucleus and are dependent on ongoing host cell transcription (41). This may explain why the IFN antagonist NS1 of the influenza A virus blocks IFN-β transcription at specific pre- and post-transcriptional levels (42–48) instead of targeting RNAP II itself.

The shutoff induced by bunyaviruses appears to be caused by multiple factors. In the nucleus, NSs inhibits the transcriptional activity of RNAP II by interfering with CTD phosphorylation. In the cytoplasm, degradation of cellular capped mRNAs by cap snatching further contributes to the depletion of mRNAs (40). For this reason, a residual shutoff is still detectable in cells infected with the NSs-deficient mutant BUNdelNSs (19). The inhibition of translation by NSs shown in vitro (49) possibly completes the host cell shutoff. In insect cells, bunyavirus infection is non-cytolytic persistent (50), and no apparent shut-off occurs (17). Our data suggest that NSs is not functional in insect cells (Fig. 4). This may contribute to the persistent infection and indicates that the target of NSs is specific for mammalian cells. Interestingly, the C terminus of the mammalian CTD contains 10 unique amino acids that are absent in non-vertebrates (51). Thus, basic differences in CTD regulation appear to exist between mammalian and insect cells, which are exploited by BUNV to regulate its infection cycle.

Despite having a broad effect on mammalian transcription, the biological function of NSs appears to be suppression of IFN synthesis, because NSs confers only a growth advantage for the virus in IFN-competent animals (18). A similar situation has been described for the nonstructural NSs protein of Rift Valley Fever virus, which has no sequence similarity to the BUNV NSs. The NSs of the Rift Valley Fever virus suppresses IFN gene expression by interacting with the p44 subunit of the essential transcription factor TFIH2, thereby preventing the assembly of TFIH (52, 54). Furthermore, the matrix protein of the vesicular stomatitis virus is a potent host cell shutoff factor that inhibits the basal transcription factor TFIIID (55), impairs the nuclear/cytoplasmic transport of RNAs and proteins (56), and inactivates translation factors (57). As is the case with NSs, the biological significance of vesicular stomatitis virus matrix protein-mediated shutoff is to suppress the IFN system (58, 59). Similarly, subversion of the IFN system is assumed to be the reason for the shutoff of general transcription and translation in picornavirus-infected cells (60). Thus, viral IFN antagonists need not necessarily block the IFN signaling chain in a direct manner but may simply interfere with general gene expression. In contrast, viruses such as the influenza A virus, which depend on RNAP II activity for their productive replication, cannot afford to impair general transcription and, therefore, use alternative strategies.

An outstanding feature of IFN-α/β genes is the absence of introns (1). Considering the fact that viral IFN antagonists often interfere with basal transcription (BUNV NSs, vesicular stomatitis virus matrix protein, and picornaviruses) or RNA splicing (the influenza A virus NS1), it is tempting to speculate that the short primary transcripts of the IFN genes are the result of an evolutionary race. IFN genes may have lost their introns to optimize the escape from viral inhibitors of mRNA synthesis. In line with this, it has been shown that IFN-stimulated promoters can bypass the need for the TATA-binding protein. The TATA-binding protein is normally necessary for general transcription, and picornaviruses induce TATA-binding protein degradation to impair a host cell shutoff (61). IFN-stimulated gene expression, however, is an exception because it can proceed without the TATA-binding protein (53). This finding illustrates that viruses and the host cell transcription machinery indeed are in continuous competition.

Acknowledgments—We thank Takashi Fujita and Stephen Goodbourn for providing reporter plasmids and Alain Kohl, Georg Kochs, and Peter Staeheli for critically reading the manuscript.

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