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Cellular proteins specifically bind single- and double-stranded DNA and RNA from the initiation site of a transcript that crosses the origin of DNA replication of herpes simplex virus 1

(RNA-binding proteins/DNA-binding proteins/RNA protection)

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ABSTRACT **The small-component origins of herpes simplex virus 1 DNA synthesis are transcribed late in infection by an RNA with heterogeneous initiation sites ≈290–360 base pairs from the origins. We report that cellular proteins react with a labeled RNA probe representing the 5′ terminus of a subset of this RNA but not with the complementary strand of this RNA. The proteins form two complexes. Complex 2 was formed by all nuclear extracts tested, whereas complex 1 was invariably formed by proteins present only in nuclear extracts of mock-infected cells. Complex 1 protects a contiguous stretch of 40 nucleotides of the labeled RNA probe from nuclease degradation. Formation of complex 1 was competitively inhibited in a sequence-specific fashion by single-stranded RNA and DNA and by double-stranded RNA and DNA. The protein(s) forming complex 1 is, thus, quite distinct from known nucleic acid-binding proteins in that they recognize a specific nucleotide sequence, irrespective of the nature (single- and double-stranded RNA and DNA) of the nucleic acid. We conclude the following: (*i*) the proteins forming complex 1 and 2 are probably different, (*ii*) complex 1 is neither required throughout infection for viral replication nor able to hinder viral replication in cells in culture, and (*iii*) cells susceptible to infection encode one or more proteins that recognize specific sequences in single-stranded nucleic acids; either these proteins impart a compatible conformation on single-stranded nucleic acids with the conformation of the same strand in the double-stranded nucleic acid, or these proteins confer a specific, distinct conformation to both single-stranded and double-stranded nucleic acids.**

The herpes simplex virus (HSV) genome encodes at least 73 open reading frames likely to specify proteins (1–3). The HSV 1 (HSV-1) genes form at least three groups, α, β, and γ, the expression of which is coordinately regulated and sequentially ordered in a cascade fashion (4, 5). The α genes are the first set to be expressed after infection, and the transcription of these genes does not require *de novo* protein synthesis. Expression of β genes requires functional α proteins, whereas the expression of γ genes requires both functional α proteins and, to a variable degree, viral DNA synthesis. The viral genome (Fig. 1*A*), ≈152 kilobase (kb) pairs in length, consists of two covalently linked components (8). The long component consists of the "quasi-unique" sequence U_L flanked by the inverted repeats *ab* and *b′a′*, whereas the unique sequences of the U_s component are flanked by the inverted repeat sequences *a′c′* and *ca*.

The inverted repeat sequences of the short component are of particular interest because each encodes an origin of DNA synthesis (ori_s) sandwiched between the promoter domain of the *α4* gene, which is located entirely in the repeat sequence, and the identical promoters of the *α22* and *α47* genes, which have coding domains located in the unique sequence (9, 10). Recently this laboratory reported that the region encodes two RNAs (7, 11). One RNA, ori_sRNA2, detected late in infection, begins downstream from the transcription initiation sites of *α22/α47* genes (Fig. 1*B*), runs antisense to the mRNAs of these genes across ori_s, and is 3′ coterminal with the *α4* gene mRNA. The second RNA, ori_sRNA1, synthesized early in infection, is 5′ coterminal with ori_sRNA2 but terminates at or near the initiation site of the *α47* gene. The function of these transcripts is not yet clear, but we have suggested (7) that, as in other systems, transcription of the DNA replication origin may serve to stimulate or regulate the onset of DNA synthesis. It is noteworthy that attempts to delete both ori_s regions or to ablate by insertional mutagenesis the transcribed domains of the ori_sRNAs were not successful (J. Hubenthal-Voss and B.R., unpublished data).

The possibility that ori_sRNA plays a role in regulating DNA synthesis stimulated an interest to characterize cis-acting sequences and trans-acting factors that control the ori_s transcription unit. In this paper we show that RNA representing the DNA sequences around the transcription initiation site of the ori_sRNAs forms a sequence-specific complex with a cellular protein designated as the RNA–protein complex 1 (RPC-1) and that this activity may be regulated by HSV-1 infection.

MATERIALS AND METHODS

Viruses and Cells. The properties and propagation of HSV-1 strain F [HSV-1(F)], HSV-2 strain G [HSV-2(G)], and the deletion mutant R3631 were described elsewhere (6, 12, 13). HeLa (American Type Culture Collection) HEp.2 (M.A. Bioproducts), baby hamster kidney cells (Human Genetic Mutant Cell Repository, Camden, NJ) rabbit skin cells (American Type Culture Collection), and 143 cell lines (from C. Croce, Wistar Institute) were propagated as described (14–16).

Preparation of Cellular Extracts. Small-scale nuclear extracts were prepared as described by Lee *et al.* (17) from HeLa cells infected with 10 plaque-forming units per cell and harvested 20 hr after infection.

***In Vitro* Transcription.** Plasmid templates were prepared and transcribed *in vitro* with SP6 and T7 RNA polymerases as recommended by Promega Biotec except that all RNAs were capped during synthesis using the cap analog GppG as described (37). Labeled probes were synthesized using 42.5

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Abbreviations: HSV-1, herpes simplex virus 1; nt, nucleotide(s); ssRNA and ssDNA, single-stranded RNA and DNA; dsRNA and dsDNA, double-stranded RNA and DNA; ori_s, origin of DNA synthesis in the small component of HSV-1 DNA; ori_sRNA, RNA transcribed across ori_s; RPC, RNA–protein complex that protects the binding site from degradation by nucleases; HSV-1(F), HSV-1 strain F; HSV-2(G), HSV-2 strain G.

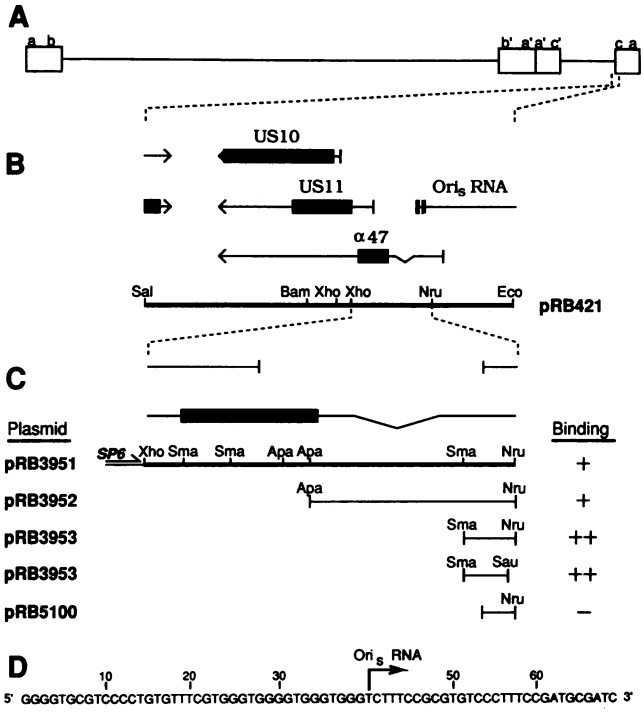

FIG. 1. Sequence arrangement of the HSV-1 genome and the position and binding ability of fragments. (*A*) Schematic diagram of the HSV-1 genome (prototype arrangement). (*B*) Expansion of the domain of the S component cloned as a *Sal* I and *Eco*RI fragment in pRB421 (6). Vertical bars, transcription initiation sites; thin lines, transcribed noncoding domain; and solid boxes, coding domains of open reading frames *US10*, *US11*, and *α47*. The vertical bars of the ori$_S$RNA reflect heterogeneity in the transcription initiation site. The far right vertical bar represents the 5′ terminus of the RNA cloned as cDNA (7). ori$_S$RNA and α47 RNA initiate within the inverted repeat sequence *c*, whereas US10 and US11 RNAs initiate within the unique sequence U_S. (*C*) Restriction map of the 650-base pair (bp) HSV-1 *Xho* I–*Nru* I fragment cloned in pRB3951 and the position of the viral sequences subcloned in pRB3952 and pRB3953 and of the ori$_S$ cDNA cloned in pRB5100, all cloned in pGEM-3Z by standard techniques. Binding ability of the transcripts derived from the viral sequences in each subclone (relative to the transcript of mutant 3951) is indicated at right. (*D*) HSV-1 template sequence (from *Sau*-linearized pRB3953) for the smallest RNA probe that still supports efficient binding of RPC-1 and RPC-2. Arrow, reported 5′ end of the cDNA copy of ori$_S$RNA.

μCi (1 Ci = 37 GBq) of [^{32}P]CTP in a 10-μl synthesis reaction supplemented with 50 μM unlabeled CTP.

Binding Assays. The labeled RNA probe was treated with 5 μg of nuclear-extract protein in 7 μl of binding buffer [200 mM NaCl/20 mM Tris, pH 7.9, at 4°C/2 mM $MgCl_2$/0.5 mM dithiothreitol/0.2 mM EDTA/0.2 mM phenylmethylsulfonyl fluoride/20% (vol/vol) glycerol] for 20 min at room temperature and then digested for 10 min with 10 units of RNase T1. The mixture was allowed to react for another 10 min after adding heparin (5 mg/ml). One microliter of loading dye (100% glycerol/0.4% bromphenol blue/0.4% xylene cyanole FF) was then added, and samples were applied to a nondenaturing 4% acrylamide gel poured and run in 0.5× TBE (45 mM Tris/45 mM boric acid/1.25 mM EDTA).

Primer Extension. The analyses were done as described by Lee and Luse (18) with 100,000 cpm of end-labeled 20-mer oligonucleotide primer and 20 μg of total RNA from infected or uninfected cells, except that hybridizing was done by slow cooling after denaturing at 70°C and the RNA primer was extended by Moloney murine leukemia virus reverse transcriptase.

Preparation of Nucleic Acid Competitors. The competitors were prepared as follows: Specific single-stranded RNA (ssRNA) competitor was transcribed *in vitro* from *Rsa* I-linearized pRB3953 (Fig. 1*C*) by using conditions for preparative synthesis of RNA. Specific single-stranded DNA (ssDNA) competitor was synthesized on an Applied Biosystems 380B DNA synthesizer. The sequence of this competitor was the same as that shown in Fig. 1*D*. Both types of single-stranded competitor were purified on denaturing polyacrylamide gels. Specific double-stranded DNA (dsDNA) was prepared by synthesizing the complement to the single-stranded competitor and hybridizing equimolar amounts (3.25 μg of each) of the two strands in 10 μl of binding assay buffer at 62°C for 17 hr. To remove residual ssDNA, the hybridized oligonucleotides were diluted 10-fold in S1 buffer [200 mM NaCl/50 mM sodium acetate (pH 4.5)/1 mM $ZnSO_4$/0.5% glycerol] and digested with 5 units of S1 nuclease per μg of DNA at 37°C for 30 min. DNA was purified by phenol extraction and ethanol precipitation. Specific double-stranded RNA (dsRNA) competitor was prepared by transcribing RNA from both strands of pRB3953, hybridizing them as described above, and removing residual ssRNA by digestion with a mixture of RNase A (40 μg/ml) and RNase T1 (2 μg/ml). The RNA was then purified by phenol extraction and ethanol precipitation. Total RNA from uninfected Vero cells was used as nonspecific ssRNA competitor. M13mp18 DNA (United States Biochemical) and *Bst*EII-cut phage λ DNA (New England Biolabs) were used as nonspecific ss- and dsDNA competitors. Nonspecific dsRNA competitors were prepared by hybridizing RNA homopolymers (Pharmacia). Poly(A) and poly(U) were hybridized at a 1:5 mass ratio and then digested with RNase A to remove residual single-stranded poly(U). Poly(G) and poly(C) were hybridized at a 1:1 ratio and digested with a mixture of RNases A and T1. All competitors were measured at A_{260}.

RESULTS

HeLa Cells Contain a Sequence-Specific HSV-1 RNA-Binding Activity. A modification of the method of Konarska and Sharp (19) was used to test for specific binding of uninfected and HSV-1-infected HeLa cell proteins to synthetic transcripts of HSV-1 DNA. In this assay HSV-1 sequences cloned in the pGEM-3Z plasmid were transcribed with SP6 or T7 polymerase in the presence of [^{32}P]CTP. Approximately 0.5 ng (50,000 cpm) of the uniformly labeled probe RNAs were treated with nuclear extracts (5 μg) of infected or uninfected cells for 20 min at room temperature. To eliminate nonspecifically bound probe, the mixture was digested for 10 min with 10 units of RNase T1 and then exposed to heparin (5 mg/ml) for an additional 10 min before electrophoresis in a nondenaturing 4% acrylamide gel.

Fig. 2*A* shows that the labeled RNA probe formed two complexes that partially protected it from digestion by nucleases. The RNA–protein complex (RPC) designated no. 2 was formed by all nuclear extracts, whereas that designated no. 1 (RPC-1) was formed by extracts of R3631-, HSV-2(G)-, and mock-infected cells but not by extracts of HSV-1(F)-infected cells. The following observations are relevant to these results: The RPC-2 complex was formed uniformly by all extracts tested. The RPC-1 complex was formed by two independently derived extracts of HSV-2(G)-infected cells, all extracts of mock-infected cells tested, and extracts of three deletion mutants lacking the *α47* and *US11* genes. The RPC-1 complex was absent in five independently derived extracts of HeLa cells infected with HSV-1(F) but was present in some extracts of infected HeLa cells and in all extracts of cells other than HeLa cells (e.g., baby hamster kidney, HEp-2, 143, and rabbit skin cell lines) infected with HSV-1(F). Preliminary experiments suggest that in experi-

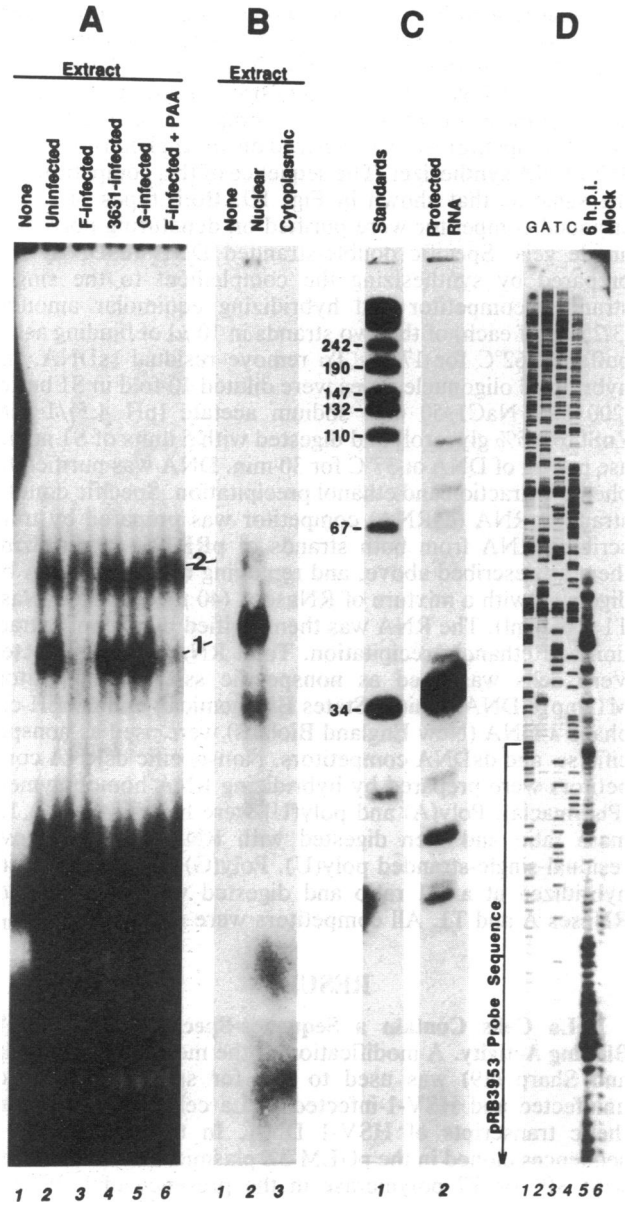

FIG. 2. Autoradiographic image of RPC-1 and RPC-2 bands (*A* and *B*) of domains protected from nuclease digestion in RPC-1 (*C*) and of the products of primer extension of ori$_s$RNA subjected to electrophoresis along with a sequencing ladder (*D*). (*A*) The 710-nt SP6 RNA transcript of pRB3951 linearized at the polylinker *Eco*RI site was treated with nuclear extracts as shown. F, HSV-1(*F*); 3631, deletion mutant R3631 lacking the *α47* gene (6, 20); G, HSV-2(G); PAA, phosphono acetic acid; 1, RPC-1; and 2, RPC-2. (*B*) The SP6 RNA transcript of pRB3953 linearized with *Sau*3A was treated with nuclear and cytoplasmic extract of uninfected HeLa cells. (*C*) The portion of RNA probe described in *B* and protected by RPC-1 from nuclease digestion was extracted from a nondenaturing gel and subjected to electrophoresis along with size markers (*Msp* I fragments of pGEM-3Z) in a 12% denaturing acrylamide gel as described in text. (*D*) The products of primer extension of ori$_s$RNA prepared as described in text were electrophoretically separated in denaturing gels along with the sequencing ladder of the HSV-1 sequence in pRB3951 by using the same primer as that used in the primer extension of ori$_s$RNA. The primer sequence was 5′-GGATCGG-GATCGCATCGGAA-3′. 6 h.p.i., 6 hr after infection.

ments in which HSV-1(F)-infected cells failed to form the RPC-1 complex, the loss of activity was from products of the *α47* gene rather than those of the *US11* gene. Thus, the RPC-1 activity was lost in HSV-1(F)-infected cells that had been treated with phosphonoacetic acid to prevent expression of *US11*, a late gene (Fig. 2*A*, lane 6). Also, incubation of HSV-1(F) and of mutant R3631-infected cells at 39°C resulted in loss of RPC-1 activity in cells infected with the wild-type virus but not in cells infected with the deletion mutant. HSV-1(F), like many wild-type HSV-1 strains characterized by a history of minimal passage in the laboratory, is temperature sensitive. At 39°C, the wild-type and the deletion mutants derived from it predominantly express α proteins and fail to express β and γ genes (21). Because viruses that cause loss of RPC-1 and those that do not are equally viable, it seems that RPC-1 activity is neither required for, nor detrimental to, virus multiplication in culture.

Binding Activity Is Confined to the Nucleus. Although the RPC activity was first detected in nuclear extracts, the possibility existed that this activity was caused by a contaminating cytosol protein. Comparison of the activity of 5 μg of protein from nuclear extract with that of the postnuclear supernatant fluid made during the extraction (Fig. 2*B*) indicated that binding activity is confined to the cell nucleus, inasmuch as the protein from the postnuclear supernatant fluid failed to protect the probe.

Binding Activity Protects ≈40 Nucleotides (nt) of RNA Sequence from T1. In this series of experiments, the binding assay was done with a high-specific-activity probe (5×10^5 cpm of 1.3×10^9 cpm/μg), and the RPC-1 band visualized by autoradiography of the wet gel was excised. RNA was eluted by soaking, resolved on a 12% denaturing acrylamide gel, and visualized by autoradiography of the dried gel. Most of the protected label was found in a single band, corresponding in its mobility to an RNA ≈40 nt in length (Fig. 2*C*, lane 2).

Binding Site Maps to the Region of the ori$_s$RNA 5′ End. The HSV-1 sequences represented in pRB3951 contain portions of three genes—*α47*, *US11*, and *US10*—and one transcribed sequence (ori$_s$RNA). Only the ori$_s$RNA is transcribed from the same strand as the RNA probe used in these experiments. To more precisely locate the RPC-1 binding site, we constructed subclones of pRB3951 that contained fewer HSV-1 sequences upstream of the ori$_s$RNA initiation site. Two subclones (Fig. 1*C*) were produced by deleting sequences between the vector *Hin*dIII site and the most distal *Apa* I site (pRB3952) or the most distal *Sma* I site (pRB3953). In addition, the 5′ end of an ori$_s$RNA cDNA clone (7) was transcribed to the *Nru* I site and tested for binding. This cDNA clone has been shown to be close to full length (7).

Results of the binding assays are summarized in Fig. 1*C*. Probes synthesized with SP6 polymerase from pRB3952 and pRB3953 (both linearized at the vector *Eco*RI site) could form RPC-1 and -2. A 3′ truncation of the pRB3953 probe produced by linearizing template with *Sau*3A also bound, narrowing the binding site for both complexes to 69 nt and placing the site very near to, or within, ori$_s$RNA (Fig. 1*D*). Transcript of the ori$_s$RNA cDNA linearized at the *Nru* I site, however, failed to form either complex.

The failure of the transcript of the ori$_s$ cDNA to bind suggested that either the binding site was not contained within ori$_s$RNA or that the cDNA template was not completely full length. To test these alternatives, a 20-mer oligonucleotide primer (Fig. 2*D*), which hybridizes to ori$_s$RNA 17 nt downstream of the putative initiation site, was end-labeled and used to prime cDNA synthesis by Moloney murine leukemia virus reverse transcriptase. Total RNAs from HSV-1-infected Vero cells isolated at 6 hr after infection (lane 5) and from uninfected cells (lane 6) were used as templates for the extensions. The extension products were separated on an 8% acrylamide squencing gel. The products of a dideoxynucleotide chain-termination sequencing reaction, primed with the same primer, were separated in adjacent lanes (lanes 1–4) on the same gel to allow precise localization of the ori$_s$RNA 5′ end. The products of primer extension on ori$_s$RNA (lane 5) are heterogeneous, indicating that there are several initiation sites

for this transcript. This result was to be expected, because the ori$_s$RNA template does not have a TATA box. The most abundant extension product corresponds to the 5′ end of the ori$_s$ cDNA reported previously (7). However, as many as half of the transcripts initiate farther upstream of the major site, and most of these transcripts initiate farther upstream than the 5′ end of the pRB3953 transcript and must contain the binding site.

Nucleotide Sequence Specificity of the Binding Reaction. To test the specificity of the formation of RPC-1, we tested the ability of ss- and dsDNA, and ss- and dsRNA (Fig. 3) to compete for binding with the ssRNA probe. Single- and double-stranded competitors containing the binding sequence were prepared as described. Varying amounts of ssRNA, ssDNA, and dsDNA were mixed with labeled RNA probe and then subjected to the binding assay. In addition to these specific competitors, nonsequence-specific competitors of all types were also tested, including total uninfected cell RNA and RNA homopolymers (data not shown) M13mp18 ssDNA, phage λ dsDNA, and dsRNA homopolymers. All nucleic acids that contained the specific binding sequence could compete for binding at levels comparable to that of the ssRNA (Fig. 3 *A–C*). The levels of each required to compete ≈50% of probe binding had only a 4-fold range, varying from ≈0.04 pmol for ssDNA to ≈0.16 pmol for dsDNA. The best competitor was not ssRNA but was, rather, ssDNA, followed by dsRNA, ssRNA, and dsDNA. Nonsequence-specific competitors were competitive only when present at great mass excess, indicating that competition from the binding-site-containing probes was sequence specific. The greater apparent affinity of the binding activity for specific dsRNA than for ssRNA might be due either to a more beneficial presentation of determinants present in the ssRNA probe or to recognition of the complementary strand itself. Though we cannot rule out the latter hypothesis, labeled probe synthesized from pRB3951 with T7 RNA polymerase rather than SP6 polymerase, forms no specific complexes with nuclear extract protein (data not shown). It should be noted that the source of specific dsDNA competitor significantly affected its ability to compete. dsDNA competitor prepared as an 87-bp *Ava* I fragment of pRB3951 competed only ≈1/16th as well as the synthetic dsDNAs (data not shown).

DISCUSSION

Although proteins capable of binding RNA or RNA and DNA have been reported, to our knowledge, there have been no reports of protein specifically binding HSV-1 RNA. Here we report proteins forming two sequence-specific complexes, RPC-1 and RPC-2. RPC-2 was formed by extracts of infected and mock-infected cells, whereas RPC-1 was invariably formed by mock-infected cell extracts, and its activity was undetected in some infected cell extracts. Inasmuch as RPC-1 was absent in some HeLa cell stocks infected with HSV-1(F), the data suggest that RPC-1 and RPC-2 contain different proteins. The observation that the RPC-1 activity was lost in extracts of some HSV-1-infected, but not in HSV-2-infected, cells suggests that this activity is not essential for viral multiplication in cells in culture. We have noted the irreproducibility of turn-off of RPC-1 activity, primarily because this criterion differentiates RPC-1 from RPC-2. The loss of host nucleic acid-binding activity after infection is not new; this laboratory reported such loss in host DNA-binding activity very rapidly after infection and found that this loss could not be attributed to simple turnover of the protein concomitant with the shut-down of host protein synthesis (22). Although RPC-1 activity is not turned off in all HSV-infected cells, the differences between viruses cannot be ascribed to differences in general shut-down of host macromolecular synthesis.

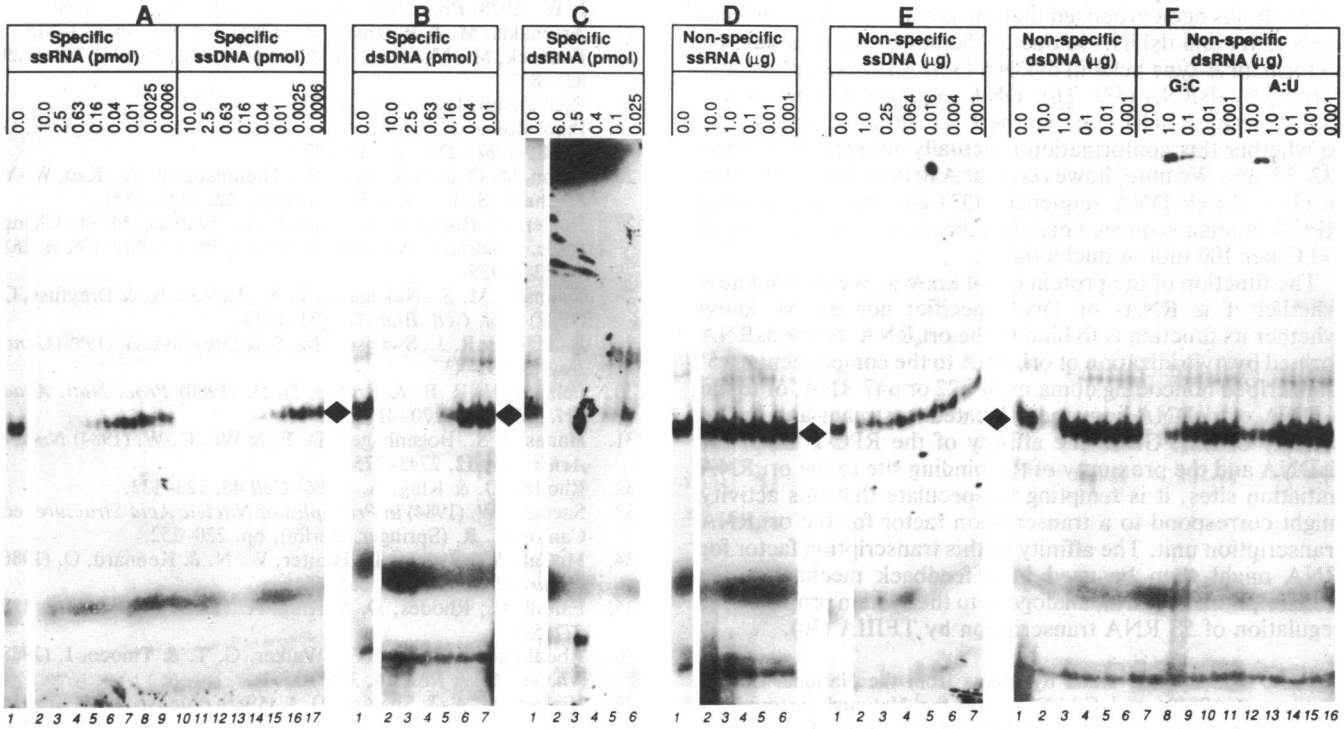

FIG. 3. Autoradiographic images of RPCs formed by the 100-nt RNA probe derived from SP6 transcription of pRB3953 with nuclear extracts of mock-infected cells and various nucleic acid competitors. The plasmid was linearized at the polylinker *Rsa* I site. The reaction mixtures were as described, except that probe and competitor nucleic acids were mixed before adding 2.5 μg of nuclear extract protein. (*A*) Competition with specific ssRNA and ssDNA. (*B*) Competition with specific dsDNA probe. (*C*) Competition with specific dsRNA. (*D*) Competition with nonspecific ssRNA. (*E*) Competition with nonspecific ssDNA. (*F*) Competition with nonspecific dsDNA and dsRNA. The RPC-1 band is identified by a solid diamond.

HSV-1(F) cannot be differentiated from mutant R3631 virus with respect to shut-down of host protein synthesis and RNA turnover; these functions are expressed even more rapidly in HSV-2(G)-infected cells (23).

The protein forming the RPC-1 complex binds nucleic acids in a sequence-specific fashion. This protein protects ≈40 nt of the RNA probe—a rather large footprint, about the same size as that left by mRNA splicing complexes (19). The observation that ss- and dsRNAs and ss- and dsDNAs can compete for RPC-1 in sequence-specific fashion and within a narrow range of molar concentrations has two implications. (*i*) Determinants of recognition are likely to be the bases rather than the backbone. (*ii*) Either the protein imparts on single-stranded nucleic acids a conformation compatible with that of the same strand in the double-stranded conformation, or it confers a specific, distinct conformation to both single-stranded and double-stranded nucleic acids.

To our knowledge, the protein or proteins forming the RPC-1 complex are the only known nucleic acid-binding proteins that combine versatility in the type of nucleic acid bound with a high degree of specificity. Ability to bind both ssRNA and ssDNA is not unusual among RNA-binding proteins. Thus, *Escherichia coli rho* termination factor (24), most of the heterogeneous nuclear RNP core proteins (25), and the pre-rRNA binding protein nucleolin (26, 27) all bind ssDNA with high affinity. These proteins are all members of a family of RNA-binding proteins that contain the so-called ribonucleoprotein consensus sequence first described by Swanson *et al.* (28). For a recent list of such proteins see Bandziulis *et al.* (29). One "zinc-finger" DNA binding protein, TFIIIA, has been reported to bind sequence specifically to both dsDNA and ssRNA (30, 31) and to bind with high affinity, but no specificity, to ssDNA (31). Thus, TFIIIA shows some of the versatility of RPC-1 protein but does not have the same degree of sequence specificity on some nucleic acids. It has been proposed that the ability of TFIIIA to bind both RNA and dsDNA is due to the ability of its target site to form an A-type helix in dsDNA (32). A helices are usually formed by dsRNA (33). This DNA sequence has been crystallized as an A helix (34), but there is conflicting evidence as to whether this conformation is actually adopted in solution (32, 35, 36). We note, however, that A helices have been seen in G + C-rich DNA sequences (33) and that the minimal RPC-1 binding sequence matches this description (65 mol of G+C per 100 mol of nucleotides).

The function of the protein is not known. We do not know whether it is RNA- or DNA-specific; nor do we know whether its function is to bind to the ori$_s$RNA, to the dsRNA formed by hybridization of ori$_s$RNA to the complementary 5′ transcribed noncoding domains of α22 or α47 RNA, or to the ssDNA or dsDNA sequence, located upstream and in the vicinity of ori$_s$. Given the affinity of the RPC-1 factor for dsDNA and the proximity of the binding site to the ori$_s$RNA initiation sites, it is tempting to speculate that this activity might correspond to a transcription factor for the ori$_s$RNA transcription unit. The affinity of this transcription factor for RNA might then be used in a feedback mechanism for transcriptional control, analogous to the system proposed for regulation of 5S RNA transcription by TFIIIA (30).

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1. McGeoch, D. J., Dalrymple, M. A., Davison, A. J., Dolan, A., Frame, M. C., McNab, D., Perry, L. J., Scott, J. E. & Taylor, P. (1988) *J. Gen. Virol.* **69**, 1531–1574.
2. Ackermann, M., Chou, J., Sarmiento, M., Lerner, R. A. & Roizman, B. (1986) *J. Virol.* **58**, 843–850.
3. Chou, J. & Roizman, B. (1986) *J. Virol.* **57**, 629–637.
4. Honess, R. W. & Roizman, B. (1974) *J. Virol.* **14**, 8–19.
5. Honess, R. W. & Roizman, B. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1276–1280.
6. Mavromara-Nazos, P., Ackermann, M. & Roizman, B. (1986) *J. Virol.* **60**, 807–812.
7. Hubenthal-Voss, J., Starr, L. & Roizman, B. (1987) *J. Virol.* **61**, 3349–3355.
8. Roizman, B. (1979) *Cell* **16**, 481–494.
9. Mocarski, E. S. & Roizman, B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5626–5630.
10. Stow, N. D. (1982) *EMBO J.* **1**, 863–867.
11. Hubenthal Voss, J. & Roizman, B. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8454–8458.
12. Ejercito, P. M., Kieff, E. D. & Roizman, B. (1968) *J. Gen. Virol.* **2**, 357–364.
13. Roizman, B. & Spear, P. G. (1968) *J. Virol.* **2**, 83–84.
14. Kristie, T. M. & Roizman, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3218–3222.
15. Post, L. E. & Roizman, B. (1981) *Cell* **25**, 227–232.
16. Arsenakis, M., Hubenthal-Voss, J., Campadelli-Fiume, G., Pereira, L. & Roizman, B. (1986) *J. Virol.* **60**, 674–682.
17. Lee, K. A. W., Bindereif, A. & Green, M. R. (1988) *Gene Anal. Tech.* **5**, 22–31.
18. Lee, D. C. & Luse, D. S. (1982) *FOCUS* (Bethesda Res. Lab., Gaithersburg, MD), Vol. 4, pp. 1–3.
19. Konarska, M. M. & Sharp, P. A. (1986) *Cell* **46**, 845–855.
20. Mavromara-Nazos, P., Silver, S. D., Hubenthal-Voss, J., McKnight, J. L. C. & Roizman, B. (1986) *Virology* **149**, 152–164.
21. Knipe, D. M., Ruyechan, W. T., Roizman, B. & Halliburton, I. W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3896–3900.
22. Arsenakis, M. & Roizman, B. (1984) *J. Virol.* **49**, 813–818.
23. Fenwick, M., Morse, L. S. & Roizman, B. (1979) *J. Virol.* **29**, 825–827.
24. Richardson, J. P. (1982) *J. Biol. Chem.* **257**, 5760–5766.
25. Pinol-Roma, S., Choi, Y. D., Matunis, M. J. & Dreyfuss, G. (1988) *Genes Dev.* **2**, 215–227.
26. Olson, M. O. J., Rivers, Z. M., Thompson, B. A., Kao, W.-Y. & Chase, S. T. (1983) *Biochemistry* **22**, 3345–3351.
27. Bugler, B., Bourbon, H., Lapeyre, B., Wallace, M. O., Chang, J.-H., Amalric, F. & Olson, M. O. J. (1987) *J. Biol. Chem.* **262**, 10922–10925.
28. Swanson, M. S., Nakagawa, T. Y., LeVan, K. & Dreyfuss, G. (1987) *Mol. Cell. Biol.* **7**, 1731–1739.
29. Bandziulis, R. J., Swanson, M. S. & Dreyfuss, G. (1989) *Genes Dev.* **3**, 431–437.
30. Pelham, H. R. B. & Brown, D. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4170–4174.
31. Hanas, J. S., Bogenhagen, D. F. & Wu, C.-W. (1984) *Nucleic Acids Res.* **12**, 2745–2758.
32. Rhodes, D. & Klug, A. (1986) *Cell* **46**, 123–132.
33. Saenger, W. (1984) in *Principles of Nucleic Acid Structure*, ed. Cantor, C. R. (Springer, Berlin), pp. 220–252.
34. McCall, M., Brown, T., Hunter, W. N. & Kennard, O. (1986) *Nature (London)* **322**, 661–664.
35. Fairall, L., Rhodes, D. & Klug, A. (1986) *J. Mol. Biol.* **192**, 577–591.
36. Aboul-ela, F., Varani, G., Walker, G. T. & Tinoco, I. (1988) *Nucleic Acids Res.* **16**, 3559–3572.
37. Nielsen, D. A. & Shapiro, D. J. (1986) *Nucleic Acids Res.* **14**, 5936.