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Characterization of Ribonucleoprotein Complexes Containing an Abundant Polyadenylated Nuclear RNA Encoded by Kaposi's Sarcoma-Associated Herpesvirus (Human Herpesvirus 8)

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Infection with Kaposi's sarcoma-associated herpesvirus (KSHV) (also called human herpesvirus 8) is strongly linked to all forms of Kaposi's sarcoma. We have previously identified two polyadenylated KSHV transcripts that are actively transcribed in Kaposi's sarcoma (KS) tumors and in KSHV-infected B-lymphoma cells. One of these RNAs (termed T1.1 or *nut-1* RNA) is a 1.1-kb transcript present in a subpopulation of KS tumor cells. This RNA is localized to the nucleus of infected cells and has no open reading frames longer than 62 codons, suggesting that it may not function as an mRNA in vivo. Here we demonstrate that *nut-1* RNA is a lytic-cycle gene product that is found in high-molecular-weight ribonucleoprotein complexes in infected cell nuclei. The transcript lacks the trimethylguanosine (TMG) cap found in many U-like small nuclear RNAs, but a subpopulation of *nut-1* RNAs can associate with Sm protein-containing small nuclear ribonucleoproteins, as judged by immunoprecipitation analyses using monoclonal anti-Sm and anti-TMG antibodies. This interaction does not require other viral gene products, and deletion of the sole candidate Sm binding site on *nut-1* RNA does not ablate this association. This finding suggests an indirect interaction with Sm-containing structures, and models for such associations are presented.

Kaposi's sarcoma (KS) is now widely recognized as the leading neoplasm of patients with AIDS (1a). Initially, KS was described as a rare and largely indolent neoplasm affecting elderly individuals of Mediterranean and Eastern European origin. It has subsequently been found to be much more common in some parts of Africa and has also been identified, though less frequently, among immunosuppressed organ transplant recipients (1a). These human immunodeficiency virus (HIV)-negative forms of KS are rare in the United States, where AIDS-related KS predominates. But while HIV infection substantially increases KS risk, HIV alone can not fully account for the etiology of KS. First, KS can certainly arise in HIV-uninfected hosts, as exemplified by its classical and African endemic forms. Second, the HIV infection model cannot explain the large differences in KS risk among different subgroups of AIDS patients; for example, over 20% of homosexual men with AIDS develop KS, while less than 1 to 3% of AIDS patients who acquired HIV from blood products will be similarly afflicted (1a, 2). These and other data have suggested the involvement of a sexually transmitted agent or cofactor other than HIV in the etiology of KS.

By using PCR-based representational difference analysis, Chang et al. (7) found that DNA sequences of a novel human herpesvirus, termed KS-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8), are regularly associated with AIDS-related KS tumors. Subsequent work has established that KSHV sequences are also present in all HIV-negative KS tissues tested (4, 8, 25, 28), that infection precedes tumor development (32), and that infection in vivo is targeted to spindle cells (3, 29), thought to be the central cells in KS pathogenesis. Moreover, recent seroepidemiologic studies indicate that KSHV infection is sexually transmitted and that infection with this agent tracks strikingly with the risk for KS development (9, 16). Thus, evidence is mounting that KSHV is the agent predicted by the epidemiology of KS.

In addition to its linkage to KS, KSHV/HHV8 sequences are also found in a minor subset of AIDS-related B-cell lymphomas, the body cavity-based lymphomas (BCBL) or primary effusion lymphomas (6). We have recently shown that a B-cell line (BCBL-1) derived from a patient with BCBL (27) is latently infected with KSHV/HHV8 and that lytic KSHV/HHV8 replication can be induced in these cells by the addition of phorbol ester to the medium (27). This BCBL-1 cell line provides a system for studying in detail the molecular biology of latent and lytic KSHV replication.

Central to understanding the role of viral infection in KSHV-associated disorders is the characterization of the program of viral gene expression in infected cells. We have recently identified and characterized two highly abundant, polyadenylated viral transcripts found in KS tumors and BCBL-1 cells (33). One of these, a 1.1-kb RNA (initially referred to as T1.1 RNA), is present only in a small percentage of the spindle-shaped tumor cells, with a copy number estimated to be at least 10,000 per cell. Moreover, this RNA was found only in the nuclei of those cells (29). Examination of its coding structure showed only small open reading frames, with the 5′ most one being only 37 amino acids long (33). Taken together, these findings suggested that T1.1 is not a coding mRNA in vivo. In this study, we show that this RNA is expressed during lytic viral replication and that it exists in the nucleus as a high-molecular weight (ribonucleoprotein [RNP]) complex; we also provide an initial characterization of these complexes. The results reveal an unusual association with cellular Sm proteins and suggest that the biology of this RNA species is quite distinct from that

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of other herpesvirus nuclear transcripts. We propose the name *nut-1* (nuclear transcript 1) for this unusual RNA.

MATERIALS AND METHODS

Cells. The cell line BCBL-1 was established in vitro from a malignant effusion as described elsewhere (27). BCBL-1 cells were grown in suspension culture in RPMI 1640 medium supplemented with 10% fetal calf serum. The cells were split about twice a week. Induction of BCBL-1 cells with tetradecanoyl phorbol acetate (TPA) was done as previously described (27). For treatment of BCBL-1 cells with phosphonoformic acid (PFA), cells were grown in medium containing 750 μM PFA for approximately 3 weeks before TPA induction. 293T cells were grown in Dulbecco's modified essential medium supplemented with 10% fetal bovine serum and were passaged every 3 days.

Cell transfection and preparation of total cell extract. 293T cells were transfected by the calcium phosphate precipitation method as previously described (14). One day after cells in a 10-cm-diameter dish were exposed to DNA precipitates, they were washed twice with phosphate-buffered saline and fresh medium was added. The cells were then incubated at 37°C for 2 to 3 days before being harvested for preparation of total cell extract. To prepare cell extracts, BCBL-1 cells or 293T cells transfected with plasmids were rinsed once with phosphate-buffered saline and once with cold Tris-buffered saline (40 mM Tris-Cl [pH 7.4], 150 mM NaCl). The cells were then resuspended to approximately 5×10^6 per ml in NET-2 buffer (50 mM Tris-Cl [pH 7.4], 150 mM NaCl, 0.05% Nonidet P-40) at 4°C and sonicated three times for a total of 1 min. Cellular debris was removed by centrifugation, and total cell extract was aliquoted and stored at −70°C.

Immunoprecipitation and Northern blot analysis. About 250 μl of total cell extract precleared with protein A-Sepharose beads was incubated with 5 μl of the indicated antibody (~1 to 2 μg) for 1 to 2 h at 4°C with gentle shaking. Twenty-five microliters of protein A-Sepharose slurry (1:1) in NET-2 buffer was then added to the extract, which was incubated for another hour at 4°C. The anti-Sm monoclonal antibody Y12 was generously provided by Joan Steitz, Yale University. Antihemagglutinin (anti-HA) (12CA5), anti-cMyc, and anti-trimethylguanosine (anti-TMG) monoclonal antibodies were purchased from commercial sources. After incubation, the extract was centrifuged at maximum speed for 5 s, and the beads were washed four to five times with 1 ml of NET-2 buffer. After the final wash, 300 μl of NET-2, 20 μg of yeast tRNA, 30 μl of 10% sodium dodecyl sulfate, and 300 μl of phenol-chloroform mixture was added to the beads, which were warmed to 37°C and vortexed occasionally for 15 min. The aqueous phase was transferred to a new tube containing 40 μl of 3 M sodium acetate and precipitated with ethanol at −70°C for 30 min. After centrifugation, RNA pellet was washed once with 70% ethanol and vacuum dried. For Northern blot analysis, RNA samples were separated on a 1.4% formaldehyde-containing agarose gel, transferred to a nylon filter, and hybridized with radiolabeled probes. Gene-specific probes were made by random labeling by using a Rediprime random primer labeling kit from Amersham.

Velocity sedimentation in glycerol gradient and fraction analysis. Two hundred microliters of BCBL-1 total extract or RNA isolated from such extract was loaded onto a 10 to 40% linear glycerol gradient in NET-2 buffer. The gradient was centrifuged in an SW50.1 rotor for 10 h at 25,000 rpm at 4°C. After centrifugation, the gradient was fractionated at about 300 μl per fraction; each fraction was extracted with phenol-chloroform and precipitated with ethanol. RNA samples from those fractions were then resolved on a formaldehyde-containing agarose gel and hybridized with a T1.1- or U1-specific probe.

RESULTS

***nut-1* (T1.1) RNA is expressed during lytic growth.** Our previous analysis of *nut-1* (T1.1) expression in KS tumors by in situ hybridization showed that the RNA was confined to a small subpopulation of spindle cells and that these cells were expressing transcripts for known late herpesviral genes (e.g., major capsid protein) (29). This finding suggested that *nut-1* is likely a lytic cycle gene. To better characterize the regulation of *nut-1* RNA synthesis, we examined expression of this transcript by Northern blotting in BCBL-1 cells following induction from latency with TPA. As shown in Fig. 1, although *nut-1* RNA is readily detectable prior to TPA addition, levels of this RNA rise 20- to 30-fold following induction of lytic growth (lanes 1 and 2). When induction from latency is carried out in cells treated with PFA, a known inhibitor of KSHV DNA synthesis (15a), a similar increase in *nut-1* production was observed (lanes 3 and 4); under these conditions, late mRNA production is strongly inhibited (16a). These results show that *nut-1* is expressed during the lytic cycle, with the characteristics of either an immediate-early or delayed-early gene. Presumably,

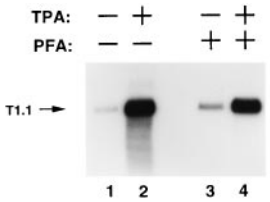

FIG. 1. T1.1/*nut-1* is expressed during viral lytic replication. Total RNA (10 μg in each lane) was extracted from uninduced BCBL-1 cells (lane 1), TPA-induced BCBL-1 cells (lane 2), PFA-treated BCBL-1 cells (lane 3), and TPA-induced PFA-treated BCBL-1 cells (lane 4). PFA treatment was done as described in Materials and Methods. TPA induction was performed essentially as described previously (27). Cells were incubated in medium containing 20 ng of TPA per ml for 24 h before being harvested for RNA extraction by the RNAzol method (Tel-Test). RNA was resolved on a 1.4% formaldehyde-agarose gel, transferred to a nylon filter, and hybridized with a T1.1-specific probe.

the background level of *nut-1* RNA present in the culture prior to TPA addition reflects the spontaneous level of lytic reactivation known to occur in these cells (27). Consistent with this observation, in situ hybridization studies (28a) show that prior to induction, only 1 to 2% of BCBL-1 cells display this RNA, a number in close accord with the known percentage of virus-producing cells prior to TPA treatment, as judged by electron microscopy (1).

***nut-1* RNA is present in a high-molecular-weight complex.** To examine the state of *nut-1* RNA in BCBL-1 cells, we fractionated BCBL-1 cell extracts (prepared by sonication of uninduced cells) on a 10 to 40% linear glycerol gradient. After centrifugation, RNA isolated from each fraction of this gradient was analyzed by Northern blot hybridization with *nut-1*-specific probe. As shown in Fig. 2A, *nut-1* RNA was present in a complex sedimenting around 40S; a subpopulation of *nut-1* transcripts sedimented even more rapidly (top panel). As a control, the same filter was hybridized with probe specific for the cellular small nuclear RNA (snRNA) U1, which is known to be assembled into a small nuclear RNP (snRNP); as expected from previous work (17, 19, 24), the U1 snRNP sediments around 10S under similar conditions (Fig. 2A, bottom panel). No change in the pattern of *nut-1* RNA sedimentation was seen after TPA treatment (data not shown). When the extract was deproteinized by phenol-chloroform treatment, the RNA sedimented closer to the top of the gradient (Fig. 2B), indicating that the observed complexes were RNPs.

Association of *nut-1* RNA with Sm proteins. Sm proteins are the common protein components of cellular snRNPs containing spliceosomal U RNAs (11). Y12, a monoclonal antibody directed against Sm, is able to efficiently and specifically precipitate these snRNPs (18, 20). Because the related gammaherpesvirus herpesvirus saimiri (HVS) encodes small U-like nuclear RNAs (HVS U-like RNAs [HSURs]) that are associated with Sm proteins (18, 19), we were interested in the possibility that Sm proteins also complex with *nut-1* RNA. However, we note that *nut-1* differs dramatically from cellular or viral U RNAs both in size (*nut-1* is much larger) and in the presence of a poly(A) tail (absent in U RNAs). To determine if *nut-1* is associated with the spliceosomal snRNPs, total BCBL-1 cell extracts prepared by sonication were subjected to immunoprecipitation with Y12 antibody, followed by Northern blot analysis using a *nut-1*-specific probe. In parallel, two other mouse monoclonal antibodies, anti-HA and anti-cMyc, were tested as specificity controls. As shown in Fig. 3, only Y12 is able to precipitate *nut-1* (T1.1) RNA from the BCBL-1 extract (lane 2), while no detectable amounts of *nut-1* were precipitated under the same conditions by either the anti-HA or

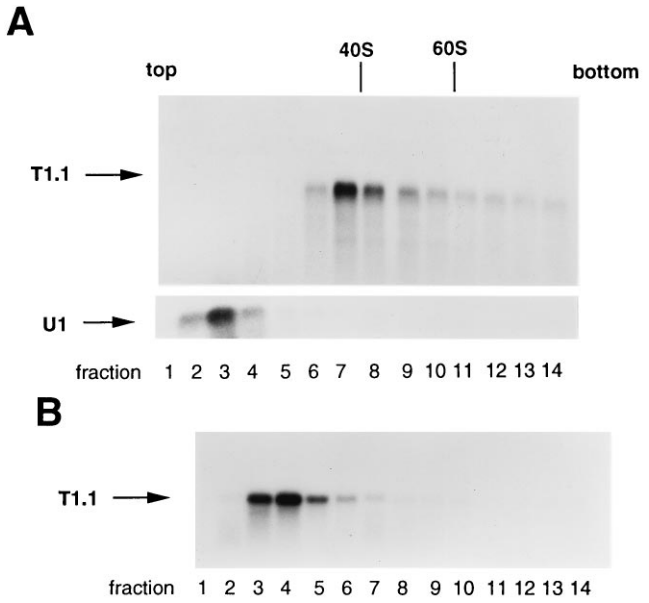

FIG. 2. Velocity sedimentation of a *nut-1*-containing complex in a glycerol gradient. (A) Two hundred fifty microliters of BCBL-1 total cell extract was centrifuged through a 10 to 40% linear glycerol gradient prepared in NET-2 buffer. Centrifugation was carried out in an SW50.1 rotor at 25,000 rpm for 10 h at 4°C. Fractions (300 μl each) were extracted with phenol-chloroform, and RNA was precipitated with ethanol. RNA samples were then analyzed by Northern blot hybridization with either a T1.1/*nut-1*-specific (top) or U1-specific (bottom) probe. (B) Deproteinized BCBL-1 extract was sedimented through a 10 to 40% glycerol gradient under the same conditions as for panel A, followed by Northern blot analysis. Two hundred microliters of BCBL-1 extract was extracted with RNAzol (Tel-Test) according to the manufacturer's instructions; total RNA was precipitated with ethanol and then redissolved in 250 μl of NET-2 buffer before being applied to the gradient.

anti-cMyc antibody (lanes 3 and 4). Importantly, however, this immunoprecipitation is not quantitative; in Fig. 3, 20 times more BCBL-1 extract was used in the Y12 immunoprecipitation (lane 2) than in the total RNA control (lane 1), and titration experiments with increased amounts of Y12 did not noticeably increase the precipitation of *nut-1* (data not shown). These observations indicate that only a small portion (less than 5%) of this RNA was precipitable by Y12, a situation which differs markedly from the case of the HSURs, which are nearly quantitatively precipitable with Y12 (18, 19). This finding suggests that *nut-1* is not itself directly assembled into an Sm-containing snRNP but rather associates with Sm proteins more

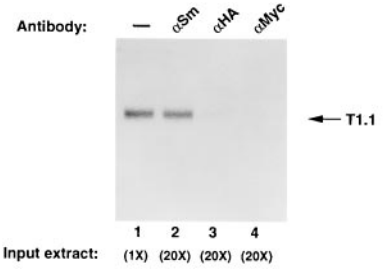

FIG. 3. Immunoprecipitation of BCBL-1 total cell extracts. Two hundred microliters of BCBL-1 extract prepared by sonication was immunoprecipitated with an anti-Sm (lane 2), anti-HA (lane 3), or anti-cMyc (lane 4) mouse monoclonal antibody. RNAs precipitated in the immunoprecipitation were separated on a 1.4% formaldehyde-agarose gel, transferred to a nylon filter, and hybridized with a *nut-1* (T1.1)-specific probe. Lane 1 is the total RNA extracted from 10 μl of the BCBL-1 cell extract prior to immunoprecipitation.

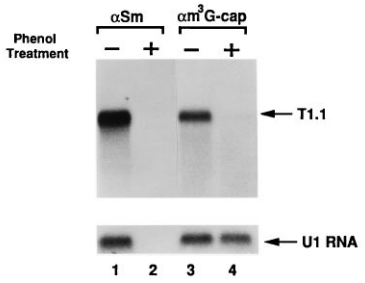

FIG. 4. Immunoprecipitation of BCBL-1 total cell extract with anti-TMG antibody. Cell extracts were extracted with phenol-chloroform (lanes 2 and 4) or without such treatment (lanes 1 and 3) before immunoprecipitation. Immunoprecipitation was performed as described in Materials and Methods, using either an anti-Sm (lanes 1 and 2) or anti-m^3G (lane 3 and 4) antibody. RNA precipitated was analyzed by Northern blot analysis with a *nut-1* (T1.1)-specific (top) or U1-specific (bottom) probe.

indirectly; further evidence in favor of this view will be presented below. The low precipitation efficiency could be attributed to either (i) low-affinity interactions between *nut-1* RNPs and snRNPs which are disrupted under the conditions of extraction and immunoprecipitation or (ii) a low percentage of *nut-1* transcripts that are actually associated with snRNPs (see below).

No hypermethylated guanosine cap structure at the 5′ end of *nut-1*. All RNAs that are known to be assembled into Sm snRNPs (e.g., cellular U RNAs and HSURs) contain a 5′ TMG cap structure (11, 18). Binding of Sm proteins to snRNAs occurs in the cytoplasm and is required for hypermethylation of their 5′ cap structure by a cytosolic methylase (22, 26). Transport of newly assembled snRNPs to the nucleus requires the Sm proteins and, at least in some cases, the TMG cap. To determine if *nut-1* also contains a TMG cap at the 5′ end, total BCBL-1 extract was immunoprecipitated with an anti-TMG monoclonal antibody, either before or after deproteinization by phenol treatment. As shown in Fig. 4, *nut-1* was precipitated from the extract before phenol treatment but not after the treatment (top panel, lanes 3 and 4). Similar results were obtained in parallel immunoprecipitation reactions using the anti-Sm antibody Y12 (top panel, lanes 1 and 2). As a control for the efficacy of the anti-TMG immunoprecipitation, the same filter was hybridized with an U1-specific probe. As shown in the lower panel, U1 snRNA was precipitated at comparable efficiency by the anti-TMG antibody before or after phenol treatment (lanes 3 and 4) but was precipitated only before phenol treatment by the anti-Sm antibody (lower panel, lanes 1 and 2). This result indicates that unlike cellular U snRNAs or HSURs, *nut-1* RNA does not contain a hypermethylated guanosine cap structure at the 5′ end. As binding of Sm proteins triggers 5′ cap hypermethylation of U RNAs (22, 26), this observation is consistent with the interpretation that *nut-1* RNA does not bind directly with Sm proteins. The precipitation of some *nut-1* RNA by the anti-TMG antibody (Fig. 4, lane 3) confirms that prior to deproteinization, T1.1 RNPs can associate with snRNPs containing RNAs with TMG caps.

***nut-1* (T1.1) coimmunoprecipitates with snRNPs in the absence of other viral gene products.** To determine if *nut-1* RNA is able to interact with Sm protein-containing snRNPs in the absence of other virally encoded products, we examined cells expressing recombinant *nut-1* RNA alone. First we isolated the *nut-1* genomic locus from a genomic DNA library prepared from a pulmonary KS lesion (33). This genomic clone (pBS-T1.1g) contains the entire *nut-1* gene as well as flanking sequences that provide transcription initiation and polyadenyla-

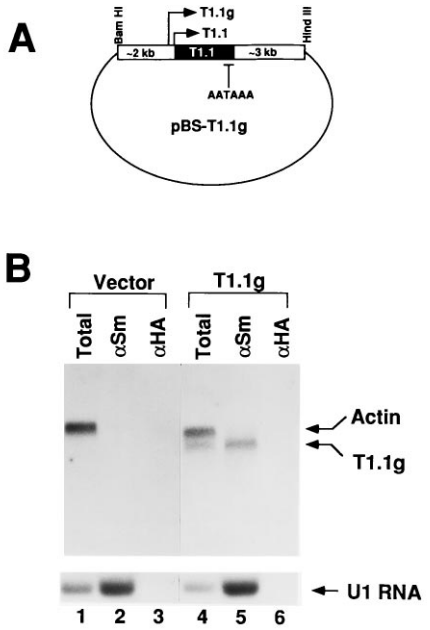

FIG. 5. Expression and immunoprecipitation of T1.1g RNA in transfected 293T cells. (A) pBS-T1.1g, the genomic clone containing T1.1 (*nut-1*) and its flanking sequences, was isolated from a genomic DNA library prepared from a pulmonary KS lesion (33) and inserted in pBlueScript vector at *Bam*HI and *Hin*dIII sites. The T1.1 arrow indicates the *nut-1* transcription initiation site in KS tumors as well as in BCBL-1 cells. The T1.1g arrow indicates the transcription initiation site in KS tumors as well as in BCBL-1 cells. The T1.1g arrow indicates the transcription initiation site in 293T cells transfected with pBS-T1.1g, which is approximately 200 bp upstream of the authentic initiation site. (B) Immunoprecipitation of 293T cell extracts transfected with pBlueScript (vector) (lanes 1 to 3) or with pBS-T1.1g (lane 4 to 6) with an anti-Sm (lanes 2 and 5) or anti-HA (lanes 3 and 6) antibody. About 250 μl of total cell extract was used for immunoprecipitation, and RNA precipitated was analyzed by Northern blot hybridization with T1.1- and actin-specific probes (top) or with a U1-specific probe (bottom). Total RNA controls (lanes 1 and 4) were extracted from about 10 μl of the total cell extract.

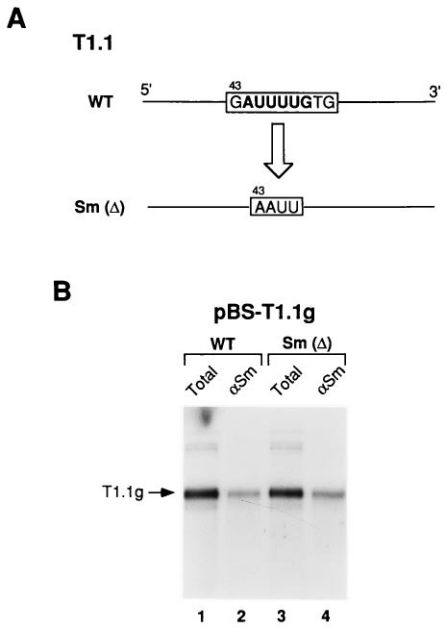

FIG. 6. Deletion of the potential Sm-binding site in T1.1g does not affect association with Sm snRNPs. (A) Sequence alteration of the putative Sm-binding site in T1.1. The sequence change was introduced by site-directed mutagenesis in pBS-T1.1g and was confirmed by sequencing. Boldface letters represent the consensus Sm-binding motif found in *nut-1*. (B) Immunoprecipitation of 293T cell extracts transfected with pBS-T1.1g (wild type [WT]; lanes 1 and 2) or with pBS-T1.1-Sm(Δ) (lanes 3 and 4). Immunoprecipitation was done as described in Materials and Methods with 200 μl of the total cell extract as indicated. Total RNA controls were prepared from about 10 μl of the cell extracts (lanes 1 and 3).

tion signals (Fig. 5A). pBS-T1.1g was then introduced into 293T cells by transfection, and RNA prepared posttransfection was examined by Northern blotting. This revealed a transcript of approximately 1.3 kb (rather than 1.1 kb). The larger size of this product was due to transcription initiation at a site further upstream of the T1.1 initiation site, as determined by primer extension analysis (data not shown). The mechanism of this ectopic initiation and its significance in the KSHV/HHV8 replication cycle is not clear. Nevertheless, this transcript behaves similarly to the endogenous T1.1/*nut-1* in terms of its nuclear localization (33); to distinguish it from the authentic *nut-1*, we will refer this transcript as T1.1g (genomic) in this study. Three days after 293T cells were transfected with pBS-T1.1g, cells were harvested and total cell extract was prepared as usual. The extract was then immunoprecipitated with the anti-Sm antibody and the precipitated RNA was analyzed by Northern blot hybridization with T1.1- and actin-specific probes (Fig. 5B, top panel). As expected, when 293T cells were transfected with the vector (pBlueScript) alone, no *nut-1* expression was detected (lane 1) and therefore no *nut-1*-specific RNA was precipitated by either the anti-Sm or anti-HA antibody (lanes 2 and 3). In contrast, when cells were transfected with pBS-T1.1g, a T1.1-specific RNA was easily detected (lane 4), and this RNA was immunoprecipitated by the anti-Sm antibody (lane 5) but not by the anti-HA antibody (lane 6). As a control for the specificity of these precipitations, we note that actin mRNA, despite its high abundance in these cells, was not precipitated by either antibody (lanes 2, 3, 5, and 6). These results demonstrate that T1.1g RNA can be specifically precipitated by the anti-Sm antibody in the absence of other virally encoded products. As in Fig. 3, over 20-fold more extract was used for anti-Sm precipitation (lane 5) than for total RNA extraction (lane 4), indicating that, as in BCBL-1 cells, the ratio of precipitable T1.1g in 293 cell extract was about 5% of the total T.1.1g RNA expressed. In contrast, over 50% of U1 RNA was precipitated by the antibody under the same conditions (bottom panel, lanes 1 and 2 or 4 and 5), suggesting that immunoprecipitation of the Sm snRNPs was close to quantitative.

Deletion of a potential Sm-binding site in T1.1g does not abolish its association with Sm snRNPs. Cellular U RNAs and the HSURs bind directly to Sm proteins via a *cis*-acting element in the RNA, the Sm-binding site (21, 23). Sm-binding sites contain a highly conserved sequence motif $A(U)_nG$ ($n > 3$) which is often found in a single-stranded region of the molecule (5, 12). A search of the entire sequence of *nut-1* identified a single potential Sm-binding sequence (AUUUUG) near the 5′ terminus of the RNA. To determine if this putative site can initiate binding with Sm proteins, we deleted this Sm consensus sequence in pBS-T1.1g by using site-directed mutagenesis as shown in Fig. 6A. The resulting mutant construct [pBS-T1.1g-Sm(Δ)] was then transfected into 293T cells, and total cell extract was prepared from the transfected cells and subjected to immunoprecipitation with anti-Sm antibody Y12. As shown in Fig. 6B, RNA derived from pBS-T1.1g-Sm(Δ) was precipitated by Y12 with an efficiency comparable to that derived from the wild-type T1.1g construct (compare lanes 2 and

4). This result indicates that the putative Sm-binding site in *nut-1* is not involved in binding with Sm snRNPs and is also consistent with our earlier observation that *nut-1* RNA does not have a hypermethylated 5′ cap structure, a structure created after direct binding of the Sm proteins. Therefore, it is likely that *nut-1* interacts with Sm snRNPs through a mechanism that is different from the one used by cellular snRNAs or viral HSURs (see below).

DISCUSSION

These data show that T1.1/*nut-1* RNA of KSHV is a lytic cycle gene product that is found in the nuclei of infected cells in large (≥40S) and possibly heterogeneous RNP particles. Although these particles do not themselves contain classical Sm proteins directly bound to T1.1 RNA, they (or, more likely, a subset of them) appear to be able to interact with Sm protein-containing snRNPs. (Of course, we cannot exclude the formal possibility that novel proteins bearing Sm epitopes but with noncanonical RNA binding properties are present in *nut-1* RNPs).

These properties sharply distinguish *nut-1* RNA from other herpesvirus-encoded nuclear transcripts. Abundant, nuclear, virus-specific RNAs are well described in the two other well-studied gammaherpesviruses, HVS and Epstein-Barr virus (EBV); in addition, the principal viral transcripts (latency-associated transcripts [LATs]) in neurons latently infected by herpes simplex type 1 are also nuclear. As previously mentioned, HVS encodes several abundant small (76- to 143-nucleotide [nt]) U-like RNAs that are found in Sm protein-containing snRNPs (18, 19), just like their cellular U RNA cognates. However, HSURs are nonpolyadenylated, TMG capped, and much smaller than *nut-1*. EBV produces two small (165- to 169-nt) EBV-encoded RNAs (EBERs); unlike *nut-1*, which has all the hallmarks of a typical polymerase II transcript (33), EBERs are produced by RNA polymerase III (15). EBERs are also found in RNPs in infected cells; EBER-associated proteins include the autoantigen La and ribosomal protein L22. Herpes simplex virus type 1 LAT was originally described as a nuclear, nonpolyadenylated, stable RNA, but this likely corresponds to sequences excised from a larger polyadenylated transcript of uncertain function (30, 34). HSURs, EBERs, and LATs are all produced in latently infected cells. By contrast, *nut-1* expression is primarily observed in productively infected cells. It thus seems likely that the biology of *nut-1* RNA differs substantially from that of each of the above-mentioned RNAs, which is perhaps not surprising, since the HSURs, EBERs and LATs themselves appear to have little in common with one another.

The protein composition of the RNPs bearing *nut-1* RNA is not yet known. Clearly, however, they do not contain classical Sm antigens directly bound to the RNA. However, at least a fraction of them are specifically associated with Sm protein-containing structures, as judged by coimmunoprecipitation of *nut-1* with Sm proteins. Since comparable amounts of T1.1 RNA can also be precipitated with anti-TMG cap monoclonal antibodies, and since *nut-1* RNA itself is nonreactive with these antibodies, it appears that *nut-1*-positive RNPs can interact with Sm antigen-containing RNPs. How such interactions occur is a matter for further experimentation. In principle, protein-protein, RNA-protein, or RNA-RNA interactions (or some combination thereof) could be involved. Since the key spliceosomal snRNPs contain Sm proteins, and since modulation of RNA splicing is known to occur in some lytic herpesvirus infections, we explored the possibility that base-pairing interactions between *nut-1* and the cellular U RNAs exist. A computer-directed search for sequence relationships between T1.1 RNA and cellular U RNAs showed several short regions of partial complementarity between *nut-1* and the main U RNAs (U1, U2, U4, U5, and U6); none, however, targeted a region of these RNAs of known functional importance.

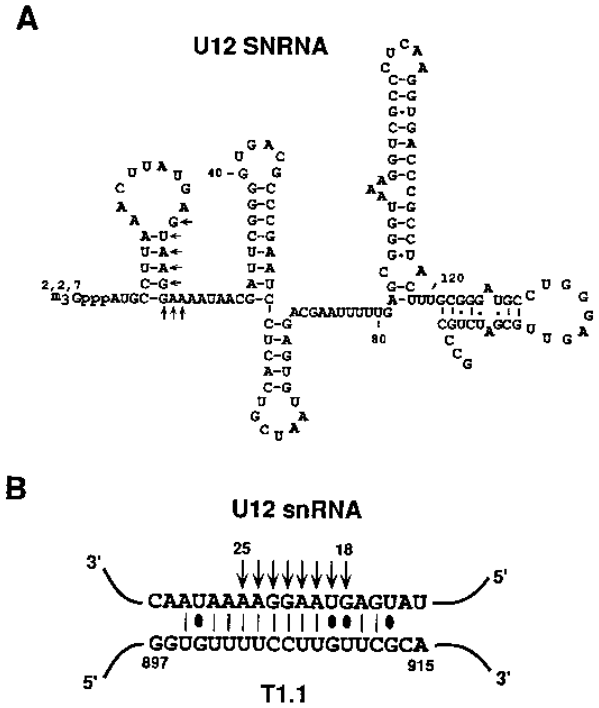

FIG. 7. Potential base pairing between U12 snRNA and *nut-1* (T1.1). (A) Primary and secondary structures of U12 snRNA (31). Nucleotides indicated with arrows are involved in base pairing with the 3′ conserved branch site sequence of the P120 intron, a member of the AT-AC minor intron subset (31). (B) Putative base pairing between *nut-1* and the U12 snRNA sequence implicated in annealing with the 3′ branch site sequence of the P120 intron. Nucleotides indicated with arrows are the same as in panel A.

However, we did identify a striking complementarity between nt 899 to 913 of T1.1 (*nut-1*) and nt 15 to 29 of the cellular RNA U12 (Fig. 7B). U12 is a minor U RNA that is assembled into a Sm protein-containing snRNP; such snRNPs exist both singly and in association with another snRNP containing U11 RNA (24, 31). Although the functions of U11 and U12 have long been obscure, recent data identify them as key components of a parallel splicing pathway that operates on introns which contain AU and AC at their 5′ and 3′ boundaries (rather than the canonical GU and AG) (13, 31). U12 nt 18 to 25 are complementary to a region surrounding the branch point of such introns; base pairing between U12 and this region has been directly demonstrated experimentally and leads to bulging of the branchpoint A residue (13, 31). Intron mutations that disrupt this base pairing inhibit splicing, and compensatory U12 mutations that restore complementarity with the intron restore splicing (13). Remarkably, the region of *nut-1* cited above is exactly complementary to the region of U12 critical to this base pairing. In fact, it is more complementary to it than is the best-studied AT/AC intron (31), and base pairing with *nut-1* bulges no nucleotide in this region. Thus, *nut-1* has the potential to be an inhibitor of U12-mediated splicing events, a possibility that is now being tested. How such an inhibition would relate to virus replication is unclear. However, we note that evidence exists suggesting that the U11-U12 system might regulate certain more conventional (GT/AG)

splicing reactions as well (10), so that inhibition of this pathway might have more widespread inhibitory effects on host gene expression than would be expected from blockade of AT/AC splicing alone.

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