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Analysis of Putative RNase P RNA from Orthopoxviruses

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A putative RNase P RNA gene in camelpox virus, one of the orthopoxviruses, was cloned and transcribed *in vitro*. No RNase P activity could be detected *in vitro* from camelpox virus RNase P RNA alone, or by addition of the *Escherichia coli* RNase P protein subunit to reaction mixtures. Camelpox virus RNase P RNA reconstituted *in vitro* with camel or HeLa cell extracts, which were pre-treated with micrococcal nuclease to degrade the endogenous RNase P RNA, showed no RNase P activity. Vaccinia virus, another orthopoxvirus, showed no RNase P activity in vaccinia-infected HeLa cells, even though transcription of the vaccinia RNase P RNA could be identified in the cells by both Northern blot and RNase protection assay. Camelpox virus RNase P RNA inhibited an endogenous HeLa RNase P activity by 20% in our assays. The 5 S RNA showed no significant inhibition in this assay.

Keywords: RNase P RNA subunit; RNase P cleavage activity; tRNA; orthopoxviruses

Ribonuclease P activity is ubiquitous in Bacteria, Archaea, and Eucarya.[1,2] Recently, Li *et al*. successfully developed a simpler procedure to quickly retrieve DNA sequence encoding the RNA subunit of RNase P from microbial genomes[3] in which no RNase P RNA had been predicted previously. One sequence was found in camelpox virus (an orthopoxvirus) that could be folded into a putative RNase P RNA. The sequence had 98% homology with all other orthopoxviruses, including vaccinia and smallpox virus.[3] No rRNA or tRNA were found in orthopoxviruses, and the putative RNase P RNA is not a homolog of RNase MRP RNA.[3]

Cloning and transcription *in vitro*

A sequence of camelpox virus was found to be similar in structure and conserved sequences to RNase P RNA[3] and could be folded into a putative RNase P RNA (Figure 1). A DNA fragment that encoded this putative camelpox virus RNase P RNA (CPVRPR) was synthesized and inserted into pBT7 vector[4] to generate the pBT7D-CPVRPR plasmid. The CPVRPR gene was under the control of a T7 promoter and the insert sequence was verified experimentally. The first nucleotide in this sequence was chosen to be the first one (shown in Figure 1), although there is no proof that this is the first nucleotide of the putative RNA transcript of the gene. The putative CPVRPR was also transcribed *in vitro* by T7 RNA polymerase (data not shown).

RNase P activity of camelpox virus RNase P RNA

CPVRPR in buffers that contained 100 mM $MgCl_2$ showed no obvious cleavage activity on *Escherichia coli* precursor to $tRNA^{Tyr}$ (pTyr). An attempt was made to reconstitute CPVRPR *in vitro* with *E. coli* RNase P protein subunit C5.[5] No cleavage activity was detected during a 60 min incubation. Some other precursor tRNAs (*E. coli* precursor to $tRNA^{Phe}$ (pPhe) and human precursor to $tRNA^{fMet}$ (pfMet)) were also used in an assay *in vitro* but no cleavage activity was observed from CPVRPR (data not shown).

RNase P activity of camelpox virus RNase P RNA in a camel cell extract

Camel fibroblast cell S16 extract[6–8] was prepared for reconstitution of RNase P *in vitro*. Incubation of

Abbreviations used: CPVRPR, camelpox virus RNase P RNA; MN, micrococcal nuclease; pTyr, precursor to $tRNA^{Tyr}$ from *E. coli*; pPhe, precursor to $tRNA^{Phe}$ from *E. coli*; pfMet, precursor to $tRNA^{fMet}$ from human cells.

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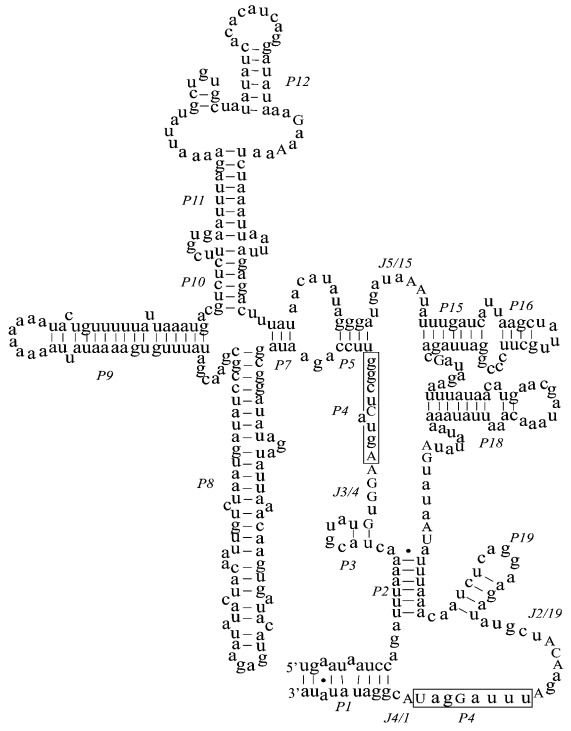

Figure 1. Structure of of orthopoxvirus (camelpox virus) RNase P RNA. Conserved nucleotides are in capital letters. Boxed nucleotides are in a pseudo-knot. Construction of DNA plasmids: three primer oligonucleotide sets, CP1T (5′-CTGAATAATCCAGATTTAAAACTACGTATGTGG-AAGTACTCGGTTCCAGAATAGCGGATATAGATATT-AAACAAGTGATACATGAGAATATACTACAATTGTC-TAATGATATCCGCGAACGATATTTGTGAAAATATT-AAAAAAAAA-3′)/CP2B (5′-ACAGTATTTTTTTTTAAT-ATTTTCACAAATATCGTTCGCGGATATCATTAGACA-ATTGTAGTATATTCTCATGTATCACTTGTTTAATATCT-ATATCCGCTATTCTGGAACCGAGTACTTCCACATAC-GTAGTTTTAAATCTGGATTATTCAGGTAC-3′), CP3T (5′-TACTGTTTTTATTAAATGACGTCTCTTCGTGAATTTA-GAAAATTATGCTGTGCTATATATCACACATCAGGAT-ATAAAGAAAAATCTAAATTAATTAGAGACTTTATA-ACATATAGGGA-3′)/CP4B (5′-TTATCATCCCTATATG-TTATAAAGTCTCTAATTAATTTAGATTTTTCTTTATA-TCCTGATGTGTGATATATAGCACAGCATAATTTTCT-AAATTCACGAAGAGACGTCATTTAATAAAA-3′), and CP5T (5′-TGATAAATATTTGATCATTAAGCTATTGCTT-CCCGGATTAGACGATAGAATTTATAACATGAACGA-TAAACAAATTATAAAAATATATAGTATAATATTTAAA-CAATCTCAGGAAGATATGCTACAAGATTTAGGATA-CGGATATATAA-3′)/CP6B (5′-AGCTTTATATATCCGT-ATCCTAAATCTTGTAGCATATCTTCCTGAGATTGTTT-AAATATTATACTATATATTTTTATAATTTGTTTATCGT-TCATGTTATAAATTCTATCGTCTAATCCGGGAAGCA-ATAGCTTAATGATCAAATAT-3′) were each denatured for 5 min at 95 °C, and each pair annealed by cooling to room temperature in annealing buffer (10 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 30 mM NaCl). These three annealed primer oligonucleotide sets were ligated to produce a DNA fragment that contains the full-length putative CPVRPR gene. This DNA fragment was amplified by PCR with primer oligonuclotides CPVRPRF (5′-AAA*GGTACC*T-GAATAATCCAGATTTAAAAC-3′) with a KpnI site (in italics) and CPVRPRR (5′-AAA*AAGCTT*TATATATCCG-TATCCTAAATC-3′) with a HindIII site (in italics). The amplified fragment was purified, digested with KpnI and HindIII, and inserted into the KpnI and HindIII sites of the pBT7 vector, to generate plasmid pBT7-CPVRPR. After digestion with StuI and KpnI, pBT7-CPVRPR was further treated with Klenow enzyme and ligated to generate pBT7D-CPVRPR. Oligonucleotides were synthesized by the Keck Facility at Yale University. Every plasmid was confirmed by restriction enzyme digestion and by DNA sequencing. DNA sequencing was performed according to the protocol described by the USB Corporation Sequencing Kit. Restriction and modification enzymes were obtained from New England Biolabs.

CPVRPR and camel fibroblast cell S16 extract was carried out through the use of pPhe that was used as substrate for RNase P in buffer H (C. Guerrier-Takada, unpublished results). The camel cell extract itself demonstrated RNase P cleavage activity (Figure 2(a), lane 1) and 2 pmol of CPVRPR added to the camel cell extract demonstrated no additional RNase P activity (Figure 2(a), lane 2). However, the CPVRPR showed a 20–25% reduction of RNase P cleavage activity (Figure 2). In addition, reduction of the amount of CPVRPR from 2 pmol did not lower the cleavage activity of the attempted reconstitution *in vitro* of CPVRPR and the camel cell extract (Figure 2(b), left panel). When camel cell extract was varied from 1 μg downwards, the activity of CPVRPR reconstituted *in vitro* with camel cell extract diminished dramatically (Figure 2(b), right panel). These results show that any RNase P activity of CPVRPR could not be distinguished from the endogenous camel cell RNase P enzyme but that CPVRPR was an effective inhibitor of the cellular RNase P activity.

To diminish the effect of endogenous RNase P enzyme, camel cell S16 extract was treated with five units of micrococcal nuclease (MN) at 37 °C for 30 min with 1 mM Ca^{2+} to degrade endogenous RNA molecules,[9] followed by addition of EGTA (20 mM final concentration) to inactivate the MN activity. Extra CPVRPR was reconstituted with the MN-treated S16 extract by incubating at 37 °C for 30 min and then RNase P activity was assayed. Even with the extra amount of CPVRPR (Figure 2(c)), no additional RNase P activity was detected from the MN-treated camel cell S16 extract. Northern blot analysis (Figure 2(d)) showed that 20 mM EGTA did inactivate the remaining MN activity. CPVRPR was added to the RNase P-inactivated camel cell S16 extract during RNase P reconstitution. A possible catalytic function of CPVRPR was assayed in a 30 min reaction.

RNase P assay of orthopoxvirus RNase P RNA in infected HeLa cells

With pPhe as a substrate, both HeLa and vaccinia-infected HeLa cells (a gift of Bernard Moss, NIH/NIAID) showed the same RNase P cleavage activity with no CPVRPR added (Figure 3(a), lane 1). The activity of vaccinia RNase P could not to be distinguished from HeLa RNase P enzyme in the vaccinia-infected HeLa cells. Furthermore, the RNase P cleavage activity showed no increase with CPVRPR

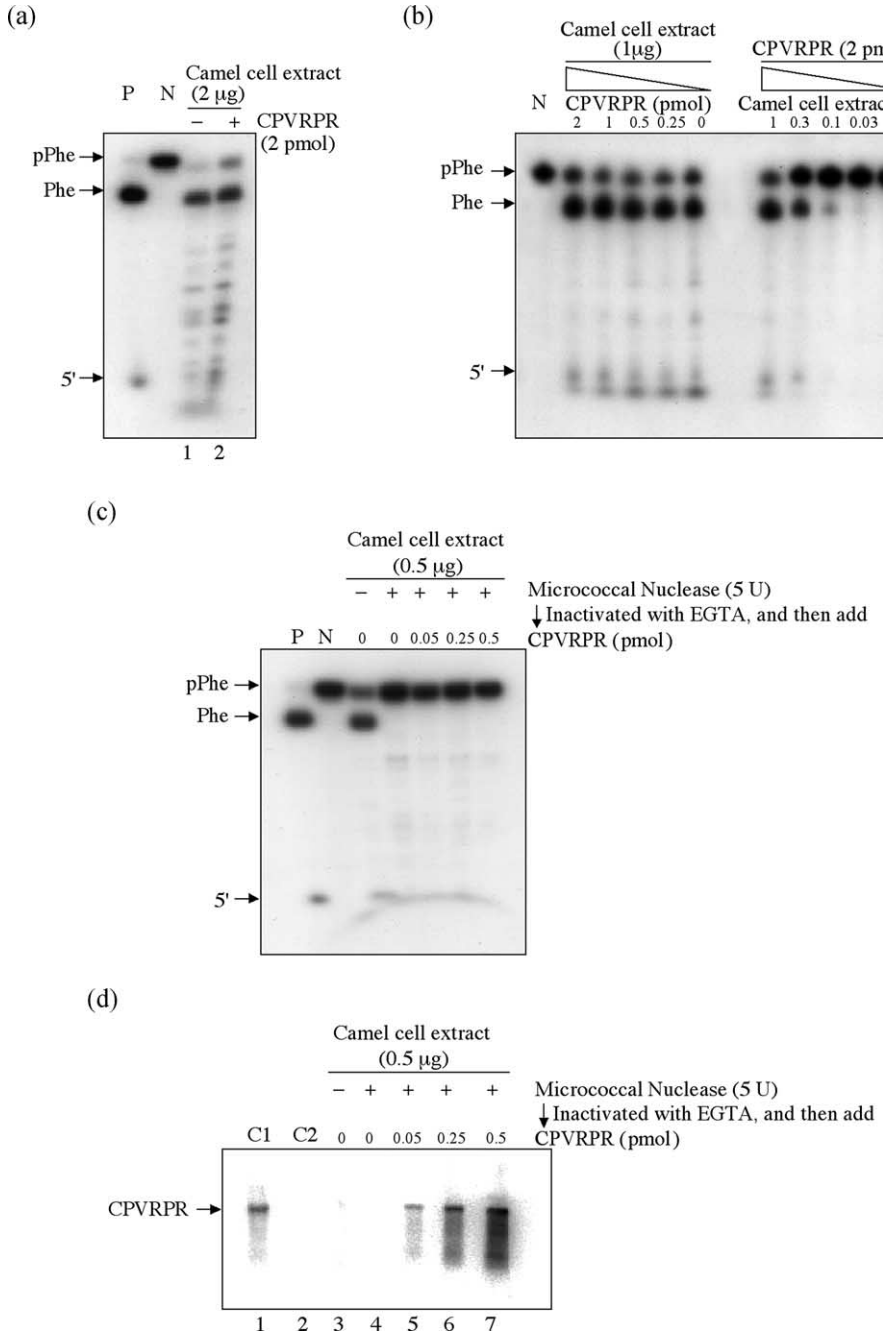

Figure 2. RNase P activity of reconstitution *in vitro* of CPVRPR with camel cell extract. Camel cells were treated with lysis buffer (10 mM Tris–HCl (pH 8.0), 2.5 mM $MgCl_2$, 5 mM KCl, 1 mM DTT, 0.5 mM Pefabloc), passed through a 25 gauge needle 10–12 times, and centrifuged at 16,000***g***. The supernatants (S16 extract) were used for RNase P assays, which were performed in buffer H (10 mM Tris–HCl (pH 8.5), 100 mM KCl, 2 mM $MgCl_2$ and 0.1% (v/v) Triton X-100) with labeled precursor $tRNA^{Phe}$ (pPhe) at 37 °C for 30 min in a volume of 10 μl. Transcription *in vitro*: putative RNase P RNA from camelpox virus was transcribed by T7 RNA polymerase from plasmid pBT7D-CPVRPR, linearized first by HindIII. The transcription reactions were carried out as described,[5] and transcripts were purified using Sephadex G-50 Quick Spin columns (Roche Applied Science). Human RNase P RNA subunit H1 RNA was generated by transcription *in vitro* using T7 RNA polymerase. *E. coli* RNase P RNA subunit M1 was generated by transcription *in vitro* using T7 RNA polymerase. The substrate RNAs, *E. coli* precursor $tRNA^{Tyr}$ (pTyr), *E. coli* precursor $tRNA^{Phe}$ (pPhe), and human precursor $tRNA^{fMet}$ (pfMet) were transcribed *in vitro* with [α-^{32}P]GTP, purified on an 8 M urea/5% (w/v) polyacrylamide gel, and used at a final concentration of 100 nM (2000 cpm per reaction). Radiochemicals were obtained from Amersham. The 5′ at the left-hand-side of gels indicates the 5′ fragment of a precursor tRNA separated by RNase P from the entire precursor tRNA molecule. (a) *E. coli* RNase P holoenzyme was used as a positive control (lane P). Labeled pPhe was used as a negative control (lane N). Samples were electrophoresed in an 8% polyacrylamide/8 M urea gel, and exposed to Kodak BioMax film. The camel fibroblast cell line was from ATCC (catalog number, CRL-2276). (b) Dose-dependent cleavage. Camel cell S16 extracts (1 μg) with different amounts of CPVRPR (from left to right, 2, 1, 0.5, 0.25, and 0 pmol, respectively) transcribed *in vitro* were used for the RNase P assay, shown in the left panel. CPVRPR (2 pmol)

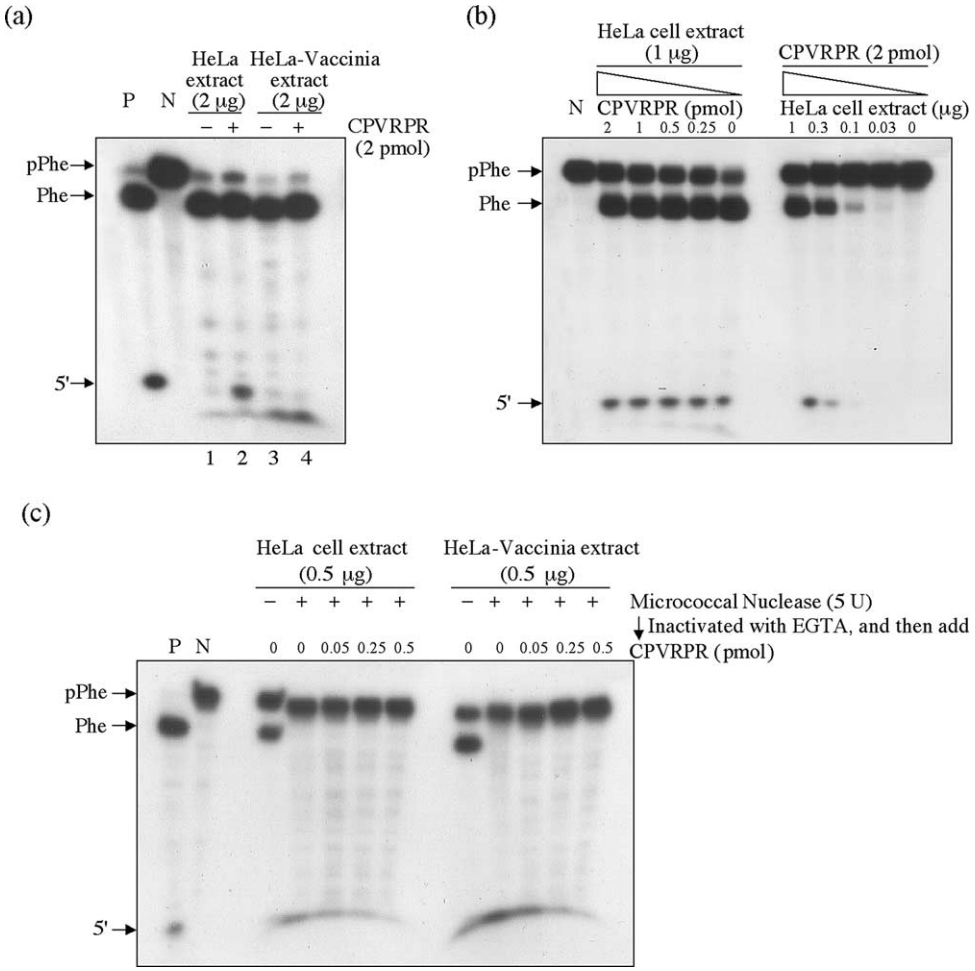

Figure 3. RNase P activity of orthopoxvirus (vaccinia)-infected HeLa cells. Vaccinia-infected HeLa cells (2×10^7) and control HeLa cells (2×10^7) were harvested by centrifugation and washed with phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4 (pH 7.0)), a gift from Dr Bernard Moss of NIH/NIAID/HeLa and vaccinia-infected HeLa cells were treated with lysis buffer (10 mM Tris–HCl (pH 8.0), 2.5 mM $MgCl_2$, 5 mM KCl, 1 mM DTT, 0.5 mM Pefabloc), passed through 25 gauge needle 10–12 times, and centrifuged at 16,000*g*. The supernatants (S16 extract) were used for RNase P assays, which were performed in buffer H (10 mM Tris–HCl (pH 8.5), 100 mM KCl, 2 mM $MgCl_2$, 0.1% Triton X-100) with labeled precursor $tRNA^{Phe}$ (pPhe) at 37 °C for 30 min. (a) *E. coli* RNase P holoenzyme was used as a positive control (lane P), and labeled pPhe alone was used as a negative control (lane N). RNase P reactions were in 10 μl. Samples were then electrophoresed in an 8% polyacrylamide/8 M urea gel, and exposed to Kodak BioMax film. RNase P activity of HeLa cell extract with (lanes 2 and 4) and without (lanes 1 and 3) CPVRPR (2 pmol). (b) Dose-dependent cleavage. HeLa S16 extracts (1 μg) with different amounts of CPVRPR (from left to right, 2, 1, 0.5, 0.25, and 0 pmol, respectively) transcribed *in vitro* were used for RNase P assay, shown in the left panel. CPVRPR (2 pmol), transcribed *in vitro* with different amounts of HeLa S16 extracts (from left to right, 1, 0.3, 0.1, 0.03, and 0 μg, respectively) were used for the RNase P assay, shown in the right panel. (c) Micrococcal nuclease treatment. HeLa and vaccinia-infected HeLa S16 extracts were first treated with five units of micrococcal nuclease with 1 mM Ca^{2+} at 37 °C for 30 min, inactivated with 20 mM EGTA, and then used for the RNase P assay by adding different amounts of CPVRPR (from left to right, 0, 0.05, 0.25, and 0.5 μg, respectively).

transcribed *in vitro* with different amounts of camel cell S16 extracts (from left to right, 1, 0.3, 0.1, 0.03, and 0 μg, respectively) were used for the RNase P assay, shown in the right panel. RNase P activity was calculated using radioactivity measured by a Phosphorimager. The extent of reactions was calculated using Image Gauge (V3.3). (c) Micrococcal nuclease treatment. Camel cell S16 extract was treated with five units of micrococcal nuclease with 1 mM Ca^{2+} at 37 °C for 30 min, inactivated with 20 mM EGTA, and then reconstituted with different amounts of CPVRPR (from left to right, 0, 0.05, 0.25, and 0.5 μg, respectively) by incubation at 37 °C for 30 min. The reconstituted samples were finally used for RNase P assay. (d) Northern blot analysis was performed with probe 1 (5′-ACTTGTTTAA-TATCTATATCCGCTATTCTGGAACCGA-3′). CPVRPR (0.05 pmol) was used as a positive control (C1, lane 1), and 0.05 pmol of CPVRPR treated with five units of micrococcal nuclease was used as a negative control (C2, lane 2). RNAs were prepared from those reconstituted samples described in (c), separated on agarose gel, transferred to nylon membrane, hybridized with 5′-end labeled probe against CPVRPR, and exposed to Kodak BioMax film. Micrococcal nuclease was from Roche Diagnostics.

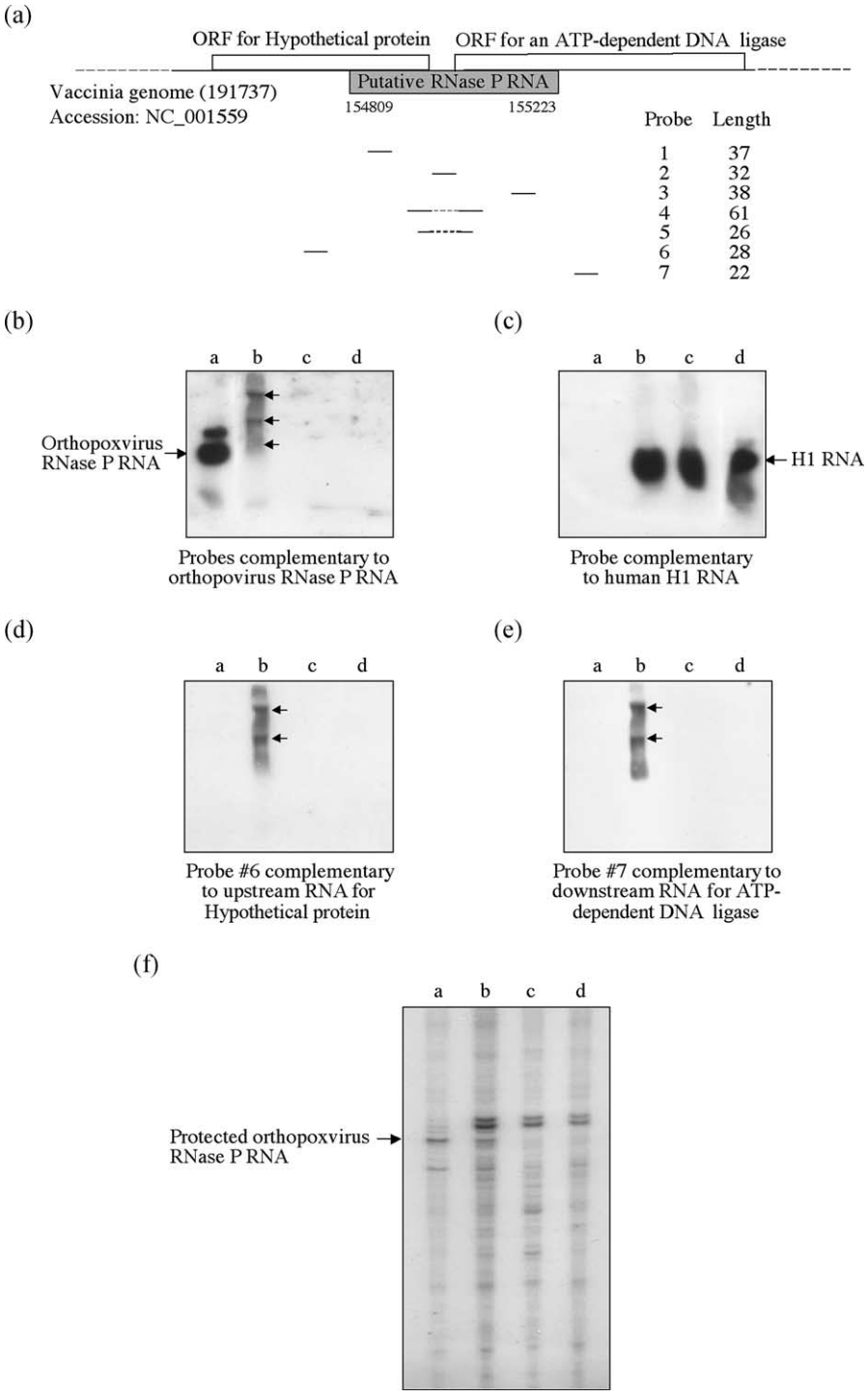

Figure 4. Expression analysis of vaccinia RNase P RNA in orthopoxvirus (vaccinia)-infected HeLa cells. (a) Schematic of the RNase P RNA gene covering an intergenic region between an overlapped upstream ORF region (for hypothetical protein) and overlapped downstream (for an ATP-dependent DNA ligase) ORF regions. The first nucleotide of putative RNase P RNA is in +1 frameshift according to the upstream ORF. Five antisense oligonucleotides (1 to 5) complementary to orthopoxvirus RNase P RNA were individually used for Northern blotting analysis. Probe 1 (5′-ACTTGTTTAATATCTATATCCGCTATTCTGGAACCGA-3′) overlaps with the upstream ORF. Probe 2 (5′-TTAA-TAAAAAMAGTATTTTTTTTTAATATTTT-3′, M=C or A, nucleotide of RNase P RNA at this site is different between camelpox virus and vaccinia) contains only the intergenic region. Probe 3 (5′-GAGATTGTTTAAATATTATACTATA-TATTTTTATAATT-3′) overlaps with the downstream ORF. Probes 4 (5′-CACAGCATAATTTTCTAAATTCACGAAR-AGACGTCATTCACAAATATCGTTCGCGGATATC-3′, R=A or G, nucleotide of RNase P RNA at this site is different between camelpox virus and vaccinia) and 5 (5′-GAARAGACGTCATTCACAAATATCGT-3′, R=A or G, nucleotide of RNase P RNA at this site is different between camelpox virus and vaccinia) contained different parts of upstream and downstream ORFs, but missing (shown as Figure 5(a), broken line) the intergenic region. Oligonucleotide 6 (5′-CAATCGATAATTTCATTGTCGTCTAATG-3′) is complementary to the upstream RNA for hypothetical protein,

added (Figure 3(a), lane 2). A considerable reduction (about 20%) of RNase P cleavage activity was found by adding an extra 2 pmol of CPVRPR to both HeLa and vaccinia-infected HeLa cells (see also Figures 2(a) and 3(a)). There was no activity change when 5 S RNA was added as a control at similar concentrations (data not shown). Inhibition by CPVRPR was at least 100-fold more efficient than bulk tRNA was for a human KB cell extract.[10]

Reduction of CPVRPR from 2 pmol did not reduce HeLa RNase P cleavage activity *in vitro* (Figure 3(b), left panel). When the HeLa extract was reduced from 1 μg downwards, the activity of CPVRPR *in vitro* reconstituted with HeLa extract diminished dramatically (Figure 3(b), right panel). These results suggested that the high RNase P cleavage activity in both HeLa and vaccinia-infected HeLa cells was not caused by orthopoxvirus RNase P, but from the HeLa RNase P itself.

To eliminate the effect of endogenous HeLa RNase P enzyme on reconstitution *in vitro* of CPVRP with the HeLa RNase P protein component, HeLa and vaccinia-infected HeLa cell extracts were also treated with five units of MN at 37 °C for 30 min with 1 mM Ca^{2+} to degrade endogenous RNA molecules.[9] This was followed by addition of EGTA (to 20 mM final concentration) to inactivate the remaining MN activity. Extra CPVRPR was reconstituted with the MN-treated S16 extract by incubation at 37 °C for 30 min, and then used for the RNase P assay. As shown in Figure 3(c), even with the extra amount of CPVRPR, no additional RNase P activity was detected in this reconstitution system. EGTA could inactivate the remaining MN activity. The CPVRPR added to RNase P-inactivated cell extract was intact during reconstitution (see Figure 2(d) for the Northern blot experiment).

Endogenous expression of orthopoxvirus RNase P RNA in vaccinia-infected HeLa cells

Sequence analysis showed that the vaccinia RNase P RNA gene overlaps with an upstream open reading frame (ORF) for a hypothetical protein and a downstream ORF for ATP-dependent DNA ligase (Figure 4(a), top panel).[12–14] The first nucleotide of putative RNase P RNA, taken to be the nucleotide that was identified in the gene search mechanism,[3] is a one nucleotide frame shift (+1) removed in relation to the upstream ORF. These aspects of sequence organization were similar in the camelpox virus RNase P RNA gene.[14] With five different oligonucleotides (1 to 5; Figure 4(a), bottom panel), the same expression of vaccinia RNase P RNA in vaccinia-infected HeLa cells was obtained in Northern blots. The Northern blot result with end-labeled oligonucleotide 1 is shown in Figure 4(b). CPVRPR (lane a) showed a major lower band and a minor higher band. The higher band is the dimer of CPVRPR. Two specific RNA bands with higher molecular masses and a weak band with a similar molecular mass to that of CPVRPR were identified in vaccinia-infected HeLa cell extract (lane b), but not in the HeLa cell extract (lane c) or H1 RNA alone (lane d). H1 RNA expression was examined in both vaccina-infected HeLa extract (Figure 4(c), lane b) and HeLa extract (Figure 4(c), lane c), and compared with H1 RNA as a marker (Figure 4(c), lane d).

and oligonucleotide 7 (5′-ATCTCTTGGACGGATTTCTGTG-3′) is complementary to downstream RNA for ATP-dependent DNA ligase. Oligonucleotide BM21 (5′-AATGGGCGGAGGAGAGTGGTC-3′) is complementary to human H1 RNA. All oligonucleotides were end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP. (b) Northern blot analysis to detect the exogenous expression of orthopoxvirus RNase P RNA in orthopoxvirus (vaccinia)-infected HeLa cells. CPVRPR transcribed *in vitro* was used as a positive control (lane a). Vaccinia-infected HeLa total RNAs and HeLa total RNAs were separated in lane b and lane c, respectively. H1 RNA transcribed *in vitro* (lane d) was used as a negative control. RNA samples were separated on agarose gel, transferred to nylon membrane, hybrized with 5′-end labeled antisense oligonucleotides (1 to 5, respectively) complementary to orthopoxvirus RNase P RNA, and exposed to Kodak BioMax film. Similar hybridization results were obtained with all antisense oligonucleotides 1 to 5, and only the hybridization result with antisense oligonucleotide 1 is shown here. (c) Northern blot analysis to detect the endogenous expression of human RNase P RNA. The same nylon membrane used in (b) was stripped with 0.1% (w/v) SDS, hybrized with 5′-end labeled antisense oligonucleotide complementary to H1 RNA, and exposed to Kodak BioMax film. (d) Northern blot analysis with the same nylon membrane after stripping with 0.1% SDS, hybrized with 5′-end labeled antisense oligonucleotide 6 complementary to the RNA transcript of upstream ORF for hypothetical protein, and exposed to Kodak BioMax film. (e) Northern blot analysis with the same nylon membrane after stripping with 0.1% SDS, hybridized with 5′-end labeled antisense oligonucleotide 7 complementary to the RNA transcript of downstream ORF for an ATP-dependent DNA ligase, and exposed to Kodak BioMax film. (f) An RNase protection assay[11] was performed to detect the expression of orthopoxvirus RNase P RNA in orthopoxvirus (vaccinia)-infected HeLa cells. For the RNase protection assay, a DNA fragment was generated by annealing of primer oligonucleotides RPAFP (5′-CCTTTTATAATTTGTTTATCGTTCATGTTATAAATTCTATCGTCTAATCCGGGAAGCAATAGCTTAATGATCAAA-TATTTATCATCCCTATA-3′) and RPARP (5′- AGCTTATAGGGATGATAAATATTTGATCATTAAGCTATTGCTTCCCG-GATTAGACGATAGAATTTATAACATGAACGATAAACAAATTATAAAAGG-3′) with the method described in the legend to Figure 1, and inserted into the StuI and HindIII sites of pBT7 vector, to generate the plasmid pBT7-RPAP. To produce the RNase protection assay probe, plasmid pBT7-RPAP was transcribed with T7 RNA polymerase with [α-^{32}P] UTP from the appropriate plasmid pBT7-RPAP, which had been linearized by HindIII. RNA samples were protected with internally labeled probe against orthopoxvirus RNase P RNA, electrophoresed in an 8% polyacrylamide/8 M urea gel, and exposed to Kodak BioMax film. CPVRPR that was "protected" by an oligonucleotide probe was used as a positive control (lane a). "Protected" vaccinia-infected HeLa cell total RNAs and HeLa cell total RNAs were separated in lanes b and lane c, respectively. "Protected" H1 RNA transcribed *in vitro* (lane d) was used as a negative control.

Since the vaccinia RNase P RNA gene overlaps with an upstream and a downstream ORF, and specific vaccinia RNase P RNA migrates with a higher molecular mass, other antisense oligonucleotides 6, complementary to upstream RNA, and 7, complementary to downstream RNA, were used individually for further Northern blot analysis. Two RNA bands with a higher molecular mass than mature camelpox RNA were also hybridized with both antisense oligonucleotides 6 (Figure 4(d)) and 7 (Figure 4(e)), suggesting that the vaccinia RNase P RNA might be transcribed together with both the upstream and downstream genes.

An RNase protection assay was performed to further confirm the vaccinia RNase P RNA transcription in vaccina-infected HeLa extract.[11] As shown in Figure 4(f), one distinct RNA band (around 70 nt) was protected by a specific probe against orthopoxvirus RNase P RNA in vaccina-infected HeLa cell extract (lane b) and positive control RNA (lane a, CPVRPR), but not in the HeLa cell extract (lane c) or the negative control RNA (lane d, H1 RNA).

Conclusion

We report the cloning and functional analysis of the putative RNase P RNA gene from camelpoxvirus, an orthopox virus. No RNase P activity could be detected *in vitro* from CPVRPR alone, or after the addition of the RNase P protein subunit from *E. coli* or a crude extract from camel cells. When using vaccinia virus-infected HeLa cells, vaccinia RNase P activity was not identified, even though the transcription of the exogenous, putative RNase P RNA subunit of vaccinia was confirmed by Northern blot and RNase protection assay.

Probes complementary to upstream RNA for a hypothetical protein or downstream RNA for ATP-dependent DNA ligase were chosen individually for Northern blot analysis. Higher molecular mass RNA bands were detected (Figure 4(d) and (e)). This result suggested that the transcripts for the upstream hypothetical protein, for vaccinia RNase P RNA and for downstream ATP-dependent DNA ligase were transcribed together as a cluster of genes. In vaccinia, some larger RNAs were also identified spanning two or more adjacent genes.[12,13]

We show that camelpox virus RNase P RNA subunit added into HeLa extract could inhibit the endogenous HeLa RNase P activity by 20% or more. This inhibition from virus RNase P RNA might down-regulate the host protein synthesis by retarding the activity of host RNase P and accelerate virus infection. It is also possible that there are unknown substrates for camelpox putative RNase P RNA that either stimulate camelpox replication during their biosynthesis or, as indicated above, inhibit HeLa cell resistance to this virus when they are intact.

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