Analysis of LaCrosse Virus S mRNA 5' Termini in Infected Mosquito Cells and *Aedes triseriatus* Mosquitoes

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Nucleotide sequences were determined for the 5' termini of La Crosse virus (LAC) S segment mRNA from persistently infected mosquito cell cultures (C6/36 from *Aedes albopictus*) and embryos (*Aedes triseriatus*). LAC primes transcription of its mRNA with "scavenged" 5' caps and adjacent oligonucleotides from host mRNAs, and these non-virus-encoded 5'-terminal extensions are heterogeneous in infected mammalian cells. The nature of mosquito host-derived primers has not been previously investigated. During early C6/36 cell infection, LAC mRNA 5'-terminal sequences were heterogeneous, but variability decreased as infection persisted. One predominant sequence, 5' CCACTCGCCACT (sequence 1), was observed throughout C6/36 cell infection but was more prevalent after 15 days postinfection. This LAC mRNA 5'-terminal sequence comprised 81% of the scavenged host oligonucleotides from vertically infected *A. triseriatus* eggs during embryogenesis. As these embryos progressed in the dormant overwintering stage (diapause), the predominant scavenged sequence became 5' AGGAAAAGATGGT (sequence 2), and sequence 1 became less prevalent. As the eggs emerged from diapause, the LAC mRNA 5' termini were more variable; 33% had sequence 1, and the remainder were heterogeneous. In post-diapausing eggs, 100% of viral mRNAs had sequence 1 at their 5' termini. Molecular analyses thus revealed continuous but selective LAC cap scavenging during persistent C6/36 cell infection and during embryogenesis and diapause in *A. triseriatus* eggs. The variety of host-derived sequences was limited in both biosynthetically active (embryonating) and dormant (diapausing) eggs.

La Crosse virus (LAC) is a member of the *Bunyavirus* genus of the *Bunyaviridae* (8). LAC is transmitted by *Aedes triseriatus* mosquitoes and is the most important cause of arboviral encephalitis in the United States (7). LAC replicates in both mammalian and insect cell cultures. Infection of mammalian cells is cytopathic, leading to cell lysis and death, whereas in mosquito cells, it is noncytopathic and becomes persistent after 24 h (30). LAC also causes a persistent, nonpathogenic infection in its natural vector, *A. triseriatus*. The virus can be transovarially (TO) transmitted to mosquito embryos. These mosquitoes remain persistently infected throughout development, and emerging adult females can immediately transmit the virus to vertebrates. Remarkably, in northern regions, where *A. triseriatus* embryos survive adverse climatic conditions in a dormant state known as diapause, TO-acquired infection persists through the winter in diapausing embryos (1). Little is known of the molecular events which allow the survival of either rapidly developing or diapausing mosquito embryos while maintaining a persistent virus infection.

The LAC RNA genome has negative polarity and consists of three segments, small (S), medium (M), and large (L) (14, 31). These encode nucleocapsid and nonstructural proteins (6, 17), glycoproteins (19), and polymerase (15), respectively. The virion-associated polymerase transcribes mRNA from viral genomic RNA templates (4, 13, 16, 22, 28). Like influenza

viruses (25), LAC transcription requires host mRNA primers. The viral polymerase contains a methylated cap-dependent endonuclease activity that presumably cleaves capped 10- to 18-nucleotide segments from the 5' termini of cellular mRNAs (cap scavenging) to prime viral transcription initiation (24, 28). In mammalian and plant cells, these nontemplated sequences are heterogeneous (2, 5, 16, 23, 27, 34). The LAC S mRNAs isolated from infected *A. albopictus* C6/36 cells were also shown to have 10 to 18 nontemplated 5'-terminal nucleotides, but it was not determined if the scavenged sequences were heterogeneous (20).

The necessity for cap scavenging could be a factor in maintaining persistent infections in mosquito embryonic tissue. If the availability of host mRNA were limited, viral replication would be down-regulated and adverse effects of infection on embryos would be minimized. Unavailability of host mRNA and other macromolecules needed for viral replication seems particularly likely in diapausing embryos, in which biosynthetic activity is greatly reduced (32). Our previous studies showed that LAC replicates continuously in metabolically active ovaries of mosquitoes stimulated by repeated blood meals; however, LAC mRNA and replicative-form RNA are greatly reduced or undetectable in quiescent ovaries (11). In these studies, we tested the hypothesis that LAC cap scavenging is a factor in maintaining viral persistence in developing mosquitoes by comparing sequences of the 5' termini of LAC mRNA in persistently infected *A. albopictus* C6/36 cell cultures and TO-infected *A. triseriatus* eggs during embryogenesis and diapause.

MATERIALS AND METHODS

Virus. LAC was isolated from the brain of a patient with LAC encephalitis (33). Stock virus was prepared in BHK-21 cell cultures, and the titer was determined by plaque assays in Vero cells (10).

Mosquito embryogenesis and diapause. LAC-infected mosquito embryos were obtained from a TOT virus-positive colony (Bluff strain) of *A. triseriatus* mos-

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quitoes, which originated from materials collected in La Crosse, Wis. (9). Eggs were collected on wooden tongue blades (OBs) within 4 h after oviposition, and the day of oviposition was designated day 0. The eggs were held at 20 to 23°C under 80% relative humidity in a 16-h-light, 8-h-dark cycle for 10 days for embryogenesis, and samples were removed for RNA extraction every 2 days. Embryonated eggs were induced into diapause by reducing the photoperiod to a 10-h-light, 14-h-dark cycle. In December 1994, approximately 2 weeks after diapause induction, the eggs were placed outdoors in a natural setting in Madison, Wis. Every 5 weeks, two OBs were returned to Colorado State University and processed immediately. Eggs from one OB were hatched to determine diapause and filial infection rates, and RNA was extracted from eggs on the other OB (9, 26).

A. albopictus C6/36 cell infection. C6/36 cells (21), grown at 28°C in L-15 medium containing 5% fetal bovine serum, were infected with LAC at a multiplicity of infection of 10. At predetermined times postinfection (p.i.), one 25-cm² flask was removed, and the cells were scraped from the flask, centrifuged to a pellet, and stored at -70°C.

RNA extraction. Total RNA was extracted from C6/36 cells, embryonating and diapausing eggs, and adult mosquitoes with acid-guanidinium thiocyanate-phenol-chloroform (12). The cells were lysed by trituration in 4 M guanidinium thiocyanate-25 mM sodium citrate-0.5% sodium lauroyl sarcosine-0.1 M 2-mercaptoethanol. RNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1) in 0.2 M sodium acetate (pH 4.8), precipitated in isopropanol at -20°C, and stored in sterile diethylpyrocarbonate-treated H₂O at -70°C.

cDNA synthesis, PCR amplification, and cloning of LAC S mRNA 5' termini. The LNR primer, 5' GGAAGCTGATGCCAAATTCTG, identical to nucleotides 763 to 785 of the LAC S genome segment, was used for LAC S mRNA reverse transcription (35). An aliquot (2 to 5 µg) of total cellular RNA was mixed with 15 pmol of the LNR primer. The samples were incubated at 70°C for 10 min, cooled to 20°C, and reverse transcribed at 40°C for 1 h in buffer containing 25 mM Tris-HCl (pH 7.3), 75 mM KCl, 3 mM MgCl₂, 20 mM dithiothreitol, and 1.5 U of reverse transcriptase (Superscript II; Gibco BRL). Oligo(dC) tails were added to the 3' ends of the cDNA with terminal deoxytransferase as specified by the supplier (Gibco BRL). The reaction mixtures were incubated at 37°C for 30 min. Oligo(dC)-tailed cDNA was amplified by PCR with an oligo(dG) forward primer (5' GACATCGAAAGGGGGGGGGGG) and a reverse primer identical to nucleotides 231 to 245 of the LAC S genome (5' CGCGAGAGACACGG TTGGCC). The samples were incubated at 92°C for 1 min, 55°C for 30 s, and 72°C for 2 min for 40 cycles followed by a final extension at 72°C for 7 min. The products were analyzed by electrophoresis on a 1.5% agarose gel.

The Original TA Cloning Kit (Invitrogen) was used to insert PCR products into the pCR II vector, and INValphaF' One Shot *E. coli* cells (Invitrogen) were transformed with the ligated plasmids.

Sequence determination. Nucleotide sequences of cloned cDNA were determined by use of the *fml* DNA sequencing system (Promega) with the T7 promoter primer. Sequences were read manually from the autoradiographs into the SeqAid II 3.6 computer program (3, 29) and aligned with the LAC S mRNA sequence obtained from GenBank (6).

Northern blot analysis. Total RNA was extracted from heads, midguts, malpighian tubules, ovaries, and the remainder (carcasses) of uninfected adult female *A. triseriatus* mosquitoes; from whole uninfected adult male mosquitoes; and from uninfected diapausing and nondiapausing mosquito embryos. From each of these samples, 3 µg of RNA was denatured and separated by electrophoresis in a 1.3% agarose gel containing 0.66 M formaldehyde and MOPS buffer [0.2 M 3-(*N*-morpholino)propanesulfonic acid, 0.05 M sodium acetate, 1 mM EDTA (pH 7.0)]. RNA was transferred to a nylon membrane (GeneScreen; Dupont) and cross-linked by UV irradiation.

Blots were prehybridized in 7% sodium dodecyl sulfate-250 mM disodium phosphate (pH 7.0) for 30 min at 35°C. Probes complementary to sequence 1 (3' AGTGGCGAGTGG) and sequence 2 (3' ACCATCTTTCCT) were synthesized (Macromolecular Resources, Colorado State University) and end labeled with [γ -³²P]ATP and T4 DNA polynucleotide kinase. A 50-ng portion of one of the labeled probes was added to the hybridization buffer, and the mixture was incubated for 4 h at 35°C (T_m = -22°C for the complement of sequence 1, T_m = -14°C for the complement of sequence 2). The blots were washed twice in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 35°C and exposed to X-ray film for 16 or 96 h (with intensifying screens) at -70°C.

RESULTS

5'-terminal sequences of LAC S segment mRNA from persistently infected C6/36 (*A. albopictus*) cells. Every 3 days, from 3 to 36 days p.i., cDNA clones were prepared from the 5' termini of LAC S segment mRNA in persistently infected C6/36 cells. Five clones were randomly selected at each time point for sequence determination. The host-derived termini ranged from 4 to 18 nucleotides and had an overall G+C content of 64% (Fig. 1). At least one cDNA sample at each time point had the non-virus-encoded sequence 5' CCACTC

LAC S mRNA	-----+1	AGTAGTGTACTCCACTTGAATACTTTG
Day 3	aggaaaagttaggt	AGTAGTGTACTCCACTTGAATACTTTG
	cgggtgtgcggg	GTAGTGTACTCCACTTGAATACTTTT
	ccttttctctc	AGTAGTGTACTCCACTTGAATACTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG
Day 6	ccttttctccagt	AGTAGTGTACTCCACTTGAATACTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (4)
Day 9	ccttttctccagt	AGTAGTGTACTCCACTTGAATACTTTG
	ccttttctccagt	AGTAGTGTACTCCACTTGAATACTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (3)
Day 12	ctggcggggggt	AGTAGTGTACTCCACTTGAATACTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (4)
Day 15	cgattcttttgcgt	GTAGTGTACCCCACTTGAATACTTTG
	tgaagacagacg	AGTAGTGTACTCCACTTGAATACTTTG
	tgct	AGTAGTGTACTCCACTTGAATACTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (2)
Day 18	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (5)
Day 21	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (5)
Day 24	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (5)
Day 27	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (5)
Day 30	atcagttccg	AGCAGTGGACTCCACTTGAATACTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (4)
Day 33	agcgcagagcggt	GTAGTGTACCCCACTTGAATACTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (4)
Day 36	ccttttctccagt	AGTAGTGTACTCCACTTGAATACTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACTTTG (4)

FIG. 1. Host-derived sequences at the 5' termini of LAC S mRNAs from infected C6/36 cells. The terminal 5' nucleotide of the genomic templated LAC S mRNA is at the +1 position. The addition of 4 to 18 nonviral nucleotides on LAC S mRNA 5' termini is shown to the left of the LAC S 5' terminal nucleotide. The number of clones with the same sequence at each time point is shown in parentheses.

GCCACT (sequence 1). Between days 3 and 15, the sequences were relatively heterogeneous, with 11 of 25 cDNAs having various sequences different from sequence 1. From days 18 to 36, however, over 90% of the cDNAs sampled (32 of 35) were initiated by sequence 1. All of the cDNAs that had sequence 1 lacked the 5' A of the virus-templated mRNA sequence and had a T-to-C transition at position +11. Of the 13 heterogeneous primer sequences, 8 had 3'-terminal ...GT or ...AGT.

5'-terminal sequences of LAC S segment mRNA from transovarially infected *A. triseriatus* eggs during embryogenesis. During embryogenesis, nucleotide sequences of LAC mRNA termini were determined at 2-day intervals from 0 until 10 days after oviposition (Fig. 2). At each time point, five cDNA clones were randomly selected for sequence determination. The non-virus-encoded sequences ranged in length from 9 to 13 nucleotides and had an overall G+C content of 64% (Fig. 2). Throughout embryogenesis, the same 5' terminus as had been predominant in *A. albopictus* cells, sequence 1, was the most prevalent mRNA primer; 81% of the sequences analyzed were 5' CCACTCGCCACT. This motif comprised 60 to 100% of the terminal sequences at each time point. All sequence 1-primed mRNAs lacked the virus-encoded 5' A and had the T-to-C transition at position +11.

5'-terminal sequences of LAC S segment mRNA from transovarially infected *A. triseriatus* embryos during diapause. Nucleotide sequences of 5' termini of LAC mRNA from diapausing mosquito embryos were determined. Eggs were induced into diapause, held under natural conditions of photoperiod and temperature to maintain diapause, and sampled at 5-week intervals.

LAC S mRNA	-----+1	AGTAGTGTACTCCACTTGAATACCTTT
Day 0	ctcgtcgccgg	TAGTGTGACCCACTTGAATACCTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG (4)
Day 2	ctcgaattcttg	AGTAGTGTACCCCACTTGAATACCTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG (4)
Day 4	ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG (5)
Day 6	atcttttgcgtgt	AGTAGTGTACTCCACTTGAATACCTTTG
	ctttttctcc	GTAGTGTACTCCACTTGAATACCTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG (3)
Day 8	aggaaaagtaggt	AGTAGTGTACTCCACTTGAATACCTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG (5)
Day 10	aggaaaagtaggt	GTAGTGTACCCCACTTGAATACCTTTG
	ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG (4)

FIG. 2. Host-derived sequences at the 5' termini of LAC S mRNAs from TO-infected *A. triseriatus* during embryogenesis. The terminal 5' nucleotide of the genomic templated LAC S mRNA is at the +1 position. The addition of 9 to 13 nonviral nucleotides on LAC S mRNA 5' termini is shown to the left of the LAC S 5'-terminal nucleotide. The number of clones with the same sequence at each time point is shown in parentheses.

The non-virus-encoded sequences ranged from 9 to 15 nucleotides and had an overall G+C content of 49% (Fig. 3). During the first 17 weeks, 100% of the eggs remained in diapause (reference 26 and data not shown). During the period of 100% diapause, the host-derived mRNA termini underwent a transition from 60% sequence 1 and 20% sequence 2 (5'AG GAAAAGATGGT) at 7 weeks to 20% sequence 1 and 40% sequence 2 at 12 weeks to 0% sequence 1 and 60% sequence 2 at 15 weeks.

At 22 weeks, 35% of the eggs had emerged from diapause and none of the mRNAs sampled had sequence 2 primers while 33% (two of six) had sequence 1 primers (Fig. 3). At 27 weeks, when 89% of the eggs had emerged from diapause, 100% of mRNAs analyzed had sequence 1 primers. Sequence 2 appeared to be diapause specific, whereas sequence 1 appeared to be present at all stages and prevalent in metabolically active mosquito cells.

The 5' CCACTCGCCACT sequence is associated with a specific host cellular RNA species. To determine if the scav-

Diapause					
Date	Time	%		+1	
Jan	7 weeks	100	-----	AGTAGTGTACTCCACTTGAATACCTTTG	
			aggaaaagtaggt	GTAGTGTACCCCACTTGAATACCTTTG	
			tctctcttacc	AGTAGTGTACCCCACTTGAATACCTTTG	
			ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG (3)	
Feb	12 weeks	100	aggaaaagtaggt	GTAGTGTACCCCACTTGAATACCTTTG	(2)
			ctggcggggt	GTAGTGTACCCCACTTGAATACCTTTG	
			tctcggtctt	AGTAGTGTACTCCACTTGAATACCTTTG	
			ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG	
Mar	17 weeks	100	aggaaaagtaggt	AGTAGTGTACTCCACTTGAATACCTTTG	(3)
			cgtggctttccct	GTAGTGTACCCCACTTGAATACCTTTG	
			aaactggctttccct	GTAGTGTACCCCACTTGAATACCTTTG	
Apr	22 weeks	65	ctttttccagt	AGTAGTGTACTCCACTTGAATACCTTTG	
			agccactgccc	GTAGTGTACCCCACTTGAATACCTTTG	
			tcacggcgct	AGTAGTGTACTCCACTTGAATACCTTTG	
			ccactcgcttct	GTAGTGTACCCCACTTGAATACCTTTG	
			ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG (2)	
May	27 weeks	11	ccactcgccac	AGTAGTGTACCCCACTTGAATACCTTTG	
			ccactcgccact	GTAGTGTACCCCACTTGAATACCTTTG (4)	

FIG. 3. Host-derived sequences at the LAC S mRNA 5' termini from TO-infected *A. triseriatus* diapausing eggs. The terminal 5' nucleotide of the genomic templated LAC S mRNA is at the +1 position. The addition of 9 to 15 nonviral nucleotides on LAC S mRNA 5' termini is shown to the left of the LAC S 5'-terminal nucleotide. The number of clones with the same sequence at each time point is shown in parentheses.

FIG. 4. Hybridization of a sequence 1-specific probe to RNA from uninfected mosquitoes. Total RNA was isolated from mosquito tissues, and 3 µg from each extract was loaded in a lane of a 1.3% agarose gel, separated by electrophoresis, and transferred to a nylon membrane. The blot was hybridized with a ³²P-labeled probe complementary to sequence 1. Samples loaded in lanes 1 to 8, respectively, were from adult female heads, midguts, malpighian tubules, ovaries, carcasses; whole male mosquitoes; and nondiapausing and diapausing *A. triseriatus* embryos (7 and 27 weeks), respectively. Arrowheads indicate, from top to bottom, the positions of the 2.1-, 0.8-, and <0.24-kb RNA hybrids. The horizontal bars indicate the positions of 2.4-, 1.4-, and 0.24-kb size markers (top to bottom). Exposure was for 4 days.

enged sequences 1 and 2 were part of specific host RNA species or were found at the termini of a variety of host mRNAs, Northern blot analyses were performed. Total RNA from uninfected *A. triseriatus* diapausing and nondiapausing embryos, whole male mosquitoes, and various organs of female mosquitoes were separated by gel electrophoresis, blotted, and hybridized with sequence 1- or sequence 2-specific probes. Figure 4 shows that the sequence 1 probe detected bands at 2.1, 0.8, and <0.24 kb in variable amounts in all samples. The sequence 2 probe detected the 2.1-kb band in all samples (data not shown). Northern blot hybridization analysis of twice-selected poly(A)⁺ RNA from diapausing and nondiapausing embryos, with a sequence 1-specific probe, detected only the band at 0.8 kb (data not shown). It was concluded that hybridization to the 2.1- and >0.24-kb bands by both probes was nonspecific. The sequence 1-containing 5' terminus appeared to be part of a single size class of host mRNA which occurred in all tissues of embryonic and adult mosquitoes but was more abundant in diapausing embryos.

DISCUSSION

Sequence heterogeneity has been demonstrated for the host cell-derived 5' termini of mRNAs from members of the *Bunyaviridae* propagated in several mammalian and plant systems (2, 5, 13, 16, 18, 21, 23, 24, 27, 34). However, no studies have reported the nature of *Bunyavirus* transcripts in their natural arthropod vector. The unique ability of LAC to vertically infect *A. triseriatus* mosquito embryos, coupled with the requisite cap-scavenging mechanism for LAC transcription, led to the hypothesis that coregulation of host and viral transcription may play a role in the apparent lack of viral cytopathogenicity in TO-infected embryos.

Sequence analysis of LAC S mRNA 5' termini revealed that LAC carries out cap scavenging during persistent infection of both mosquito cell cultures and TO-infected embryos but is more selective than during lytic mammalian cell infection. Early after infection of *A. albopictus* C6/36 cultured cells (through day 15 p.i.), the scavenged sequences were relatively varied: 58% were sequence 1, and 42% were heterogeneous (Fig. 1). From days 18 through 36 p.i., however, 91% of the

primers were sequence 1. Sequence 1 also predominated in TO-infected *A. triseriatus* eggs during embryonation, comprising 81% of host-derived primers and suggesting that the 5' CCACTCGCCACT motif was part of a conserved mosquito mRNA and was not species specific. However, after infected embryos were induced into diapause, the most common primer for viral mRNA gradually shifted to become sequence 2. This transition suggested not only that 5' AGGAAAAGTAGGT was derived from a diapause-specific mRNA but also that continuous viral transcription occurred in embryos rather than stabilization of transcripts acquired during oogenesis. Since no direct evidence was obtained for mRNA turnover, however, this observation will require further quantitative studies.

The selective scavenging of mosquito mRNA sequences for viral transcription initiation could be due to the specificity of the viral endonuclease or to the relative abundance of mRNAs containing sequence 1 in metabolically active mosquito tissue and sequence 2 during diapause. Restriction of viral cap scavenging to abundantly synthesized or stable host sequences would minimize deleterious effects of viral replication and allow the maintenance of viral persistence. The Northern blot analysis suggested that sequence 1 was part of a single size class of mRNA, present in all mosquito tissues but more abundant in embryos. Stringency of hybridization conditions did not allow specific hybridization of sequence 2. There have been few observations of mRNA populations present in mosquito embryos. Wattam and Christensen (36) demonstrated a variety of mRNAs which changed with the sex and stage of development in larval, pupal, and adult *A. aegypti* mosquitoes. Additional studies will be required to examine this point and determine whether the LAC primers were selected from a varied mRNA population and if sequence 2 is indeed a marker for diapause induction or maintenance in *A. triseriatus*.

Certain bunyaviruses show a strict sequence specificity for the 3'-terminal nucleotide(s) of the host-derived primer (34). Our studies revealed a preference for 3'-terminal T in mosquito cells (105 of 116 sequences examined). In addition, sequence 2 and a majority of the heterogeneous sequences in C6/36 cells had . . .GT or . . .AGT at their 3' termini, complementary to the 3'-terminal repeats of viral RNA (Fig. 1). Primers with 3'-terminal T (U) would allow the prime-and-realign mechanism of transcription initiation proposed for Hantaan virus replication (18) with initial alignment of the primer at either the +3 or +6 position on the template. It is noteworthy, although not uncommon among bunyaviruses, that the virus-encoded mRNA consistently lacks A at the +1 position when primed by sequence 1. It is also interesting that the sequence 5' CCACT, which is directly repeated at the 5' and 3' ends of the predominant primer, occurs at positions +12 to +16 in the virus-encoded mRNA.

These studies suggest that LAC viral transcription and thus replication are continuous in persistently infected mosquito cell culture and metabolically active as well as dormant mosquito embryos. They also suggest that selection of a limited variety of host-derived transcription primers may play a role in viral persistence and vertical transmission in the absence of deleterious effects on the mosquito. Further investigation will be needed to quantitate host and viral mRNAs during critical stages of development and to test the translatability of viral mRNA derived from persistent infections.

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