Extent of Transcription of the E Strand of Polyoma Virus DNA During the Early Phase of Productive Infection

NICHOLAS H. ACHESON* AND FRANÇOISE MIÉVILLE

Department of Virology, Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland

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Early polyoma virus-specific RNA, in nuclei and cytoplasm of cells labeled with [³H]uridine, was analyzed by hybridization with filter-bound *Hpa* II fragments of polyoma DNA. About 40% of labeled cytoplasmic virus-specific RNA hybridized with *Hpa* II fragment 2, which represents about 40% of the region coding for E-strand mRNA's; less than 5% hybridized with fragments 1 or 3, which lie outside this region. A somewhat lower proportion (about 30%) of labeled nuclear virus-specific RNA hybridized with fragment 2, and a small but significant fraction (7 to 14%) hybridized with fragments 1 and 3. About two-thirds of the nuclear RNA which hybridized to fragment 1 was complementary to the E strand, and one-third was complementary to the L strand. Results did not vary greatly in samples labeled for periods of from 15 min to 3 h. The major species of pulse-labeled nuclear polyoma-specific RNA sedimented at 22S and thus is slightly larger than the 19S cytoplasmic mRNA. These results show that most early nuclear RNA (~75%) is transcribed from the region of the E strand, which codes for early mRNA's, and that there is probably a site at which transcription is terminated at the end of this region. However, a small amount of early nuclear RNA (~15%) is transcribed from the remainder of the E strand, perhaps by readthrough of this termination signal. In addition, there is a small amount of transcription from the L strand, whose significance is unclear. Neither the L-strand transcripts nor the nonmessenger E-strand transcripts are transported to the cytoplasm.

The growth cycle of polyoma virus (and of the analogous monkey virus simian virus 40 [SV40]) is conveniently divided into an early phase, which lasts until the beginning of viral DNA synthesis, and a late phase (for review, see 1). Early virus-specific RNA is by definition transcribed from parental viral DNA; only a small fraction (<0.01%) of RNA synthesized during the early phase is virus specific (8, 23, 40). Partly for this reason, little is known of the primary products of early transcription.

Early cytoplasmic mRNA sediments at 19S in aqueous sucrose gradients (40) and represents sequences copied from approximately one-half of the E strand of polyoma DNA (7, 26, 27). There may be at least two different early mRNA's, each containing sequences from most of this region (called the "early region") but lacking different sets of intervening sequences, as has recently been found with SV40 (9). We previously found that early nuclear RNA contains the same sequences as early cytoplasmic RNA—that is, sequences complementary to about one-half of the E strand but to none of the L strand of viral DNA (7). This result suggested that early primary transcripts may be similar in size and sequence content to the early

mRNA which is exported to the cytoplasm (in contrast, most late primary transcripts of the L strand of viral DNA are up to several times the genome length; 2, 3). However, these results were obtained by hybridization of unlabeled nuclear RNA to labeled separated strands of polyoma DNA, a technique which gives information on the steady-state distribution of RNA sequences rather than the sequences present in nascent transcripts. It could not be ruled out that all E-strand sequences are transcribed, followed by rapid degradation within the nucleus of non-mRNA sequences.

In the present study, cells were pulse-labeled with [³H]uridine during the early phase of infection, and nuclear RNA was hybridized to an excess of unlabeled restriction endonuclease fragments of polyoma DNA, which lie either wholly within the region coding for early mRNA's or wholly outside this region. By this means the relative rate of synthesis of RNA from coding and noncoding regions of the E strand can be more directly measured. The results show that most early nuclear RNA labeled for periods as short as 15 min is complementary to the region of the E strand coding for early mRNA's. However, a low level of transcripts

from the noncoding region of the E strand, as well as from most of the L strand, can also be detected in pulse-labeled nuclear RNA.

MATERIALS AND METHODS

Labeling, cell fractionation, and extraction of RNA. Primary mouse kidney cell cultures (10^7 cells per 88-mm petri dish) were infected with plaque-purified polyoma virus at a multiplicity of 20 to 40 PFU/cell. Two hours after infection, the virus suspension was removed, and cultures were washed once and then incubated at 37°C with reinforced Eagle medium containing 6×10^{-5} M 5-fluoro-2'-deoxyuridine (FUDR) (36). Cells were labeled by incubation with 500 μ Ci of [5- 3 H]uridine per ml (25 Ci/mmol; Amersham) in medium containing FUDR, 1 ml/petri dish. Labeling was terminated at 10 to 10.5 h after infection by washing with cold 25 mM Tris-hydrochloride (pH 7.4)–140 mM NaCl–5 mM KCl–1 mM Na_2HPO_4 , followed by cold 10 mM triethanolamine (pH 8.5)–10 mM NaCl–1.5 mM MgCl_2 (35). Cells were then scraped into the same buffer and lysed by addition of Nonidet P-40 (Shell) to a 0.5% final concentration. Nuclei were sedimented at $1,500 \times g$ for 3 min, the supernatant cytoplasmic extract was decanted, and the nuclear pellet was resuspended in the same buffer. RNA was extracted from both nuclei and cytoplasm by addition of 1 volume of 10 mM triethanolamine (pH 8.5)–100 mM NaCl–10 mM EDTA, containing 4% triisopropyl-naphthalenesulfonate (Serva) (17, 34), and 2 volumes of phenol–chloroform–isoamyl alcohol (50:50:1). After 2 to 4 min of vigorous shaking at room temperature, the aqueous phase was separated by centrifugation and reextracted two more times with an equal volume of phenol–chloroform–isoamyl alcohol. Nucleic acids were precipitated with ethanol, redissolved, reprecipitated two more times, and then incubated at 0°C for 30 min with 10 μ g of DNase I per ml (RNase-free; Worthington Biochemicals Corp.) in 10 mM sodium acetate (pH 5.1)–100 mM NaCl–2 mM MnCl_2 . RNA was reextracted with phenol–chloroform in the presence of 1% sodium dodecyl sulfate (SDS) and ethanol precipitated two times.

Preparation of restriction endonuclease fragments of polyoma DNA and separation of strands of fragments. Polyoma DNA was isolated by Hirt extraction from cells infected at 0.1 PFU/cell. The Hirt supernatant was phenol extracted, and superhelical form I DNA was purified by CsCl-ethidium bromide centrifugation. After dialysis, DNA was digested with 1 U of *Hpa* II (New England BioLabs) per μ g of DNA for 2 h at 37°C in 10 mM triethanolamine (pH 7.4)–5 mM NaCl–10 mM MgCl_2 –1 M dithioerythritol–50 μ g of bovine serum albumin per ml. After addition of 1/9 volume of 90% glycerol–1% SDS–0.1% bromophenol blue, 50 μ g of DNA was loaded onto a 1.5% agarose (Sigma) gel (12.5 by 11 by 0.25 cm) made in 90 mM Tris-borate (pH 8.3) and subjected to electrophoresis at 8 V/cm for 2.25 h at room temperature. DNA was located by staining with ethidium bromide (10 μ g/ml for 5 min), and the bands corresponding to *Hpa* II fragments 1 to 4 were cut out. Gel slices were fragmented by passage through a syringe and incubated in 5 to 10 volumes of 50 mM Tris-hydrochloride

(pH 7.9)–150 mM NaCl–10 mM EDTA–0.1% SDS at 37°C overnight. Gel fragments were pelleted by centrifugation at $300,000 \times g$ for 30 min, and DNA in the supernatant was adsorbed to a column of DEAE-cellulose in the same buffer. After extensive washing, DNA was eluted with 1 M NaCl (11). At this stage, purified fragments were contaminated with neighboring fragments at levels of 1 to 5%. Thus, individual fragments were repurified by a second cycle of electrophoresis, elution, and DEAE-cellulose adsorption. Final recovery was about 10% of input DNA. Contamination with other *Hpa* II fragments was less than 1%.

The complementary strands of *Hpa* II fragments 1 and 2 were separated by electrophoresis of alkali-denatured *Hpa* II-digested polyoma DNA on a composite agarose-acrylamide gel by the following procedure. After *Hpa* II digestion, 50 μ g of DNA was phenol extracted and ethanol precipitated and then resuspended in 100 μ l of distilled water. The solution was adjusted to 50 mM NaOH, incubated at 37°C for 5 min, and then transferred to an ice-water bath. The mixture was made 10% in glycerol (final volume, 200 μ l) and layered onto a 2.5% acrylamide–0.125% bisacrylamide–0.5% agarose gel of the aforementioned dimensions in 45 mM Tris-borate (pH 8.3). Electrophoresis was at 17 V/cm (30 mA) for 4 to 5 h at 4°C. Under these conditions (but not at room temperature), the bulk of the DNA remains denatured, and the separated strands of a fragment migrate at about one-half the rate of the native double-stranded DNA fragment (Fig. 1). This is advantageous for isolation of pure separated strands; in agarose-acrylamide gels made in Tris-acetate buffer, or in agarose gels made in either Tris-borate or Tris-acetate buffer, the separated strands run slightly ahead of, or nearly at the same position as, the renatured fragment, making purification more difficult. Gel slices containing individual strands were sandwiched between bungs of glass wool in a 10-ml plastic pipette, and DNA was eluted by electrophoresis in Tris-borate buffer (3 hr, 150 V) into a dialysis tubing slipped onto the pipette tip. Carrier *Escherichia coli* tRNA (25 μ g/ml) was included in the elution buffer within the dialysis sac. The eluate was phenol extracted and ethanol precipitated. Analysis of the purified separated strands of *Hpa* II fragments 1 and 2 is shown in Fig. 2.

Purified DNA fragments, or intact polyoma DNA (further purified by alkaline sedimentation) were loaded onto Selectron BA-85 nitrocellulose filters after denaturation by boiling in 0.1 $\times$ SSC (SSC = 0.15 M NaCl plus 0.015 M sodium citrate) and dilution into cold 6 $\times$ SSC. Purified separated strands were loaded onto filters without prior denaturation to allow the small amount of double-stranded DNA fragment (the result of renaturation with some contaminating complementary strand; see Fig. 2) to pass through the filter. After baking at 80°C for 2 h, filters were cut into 3.5-mm squares, each containing an amount of DNA corresponding to 0.1 to 0.2 μ g of intact polyoma DNA.

Hybridization. RNA was dissolved in 50% formamide–2 $\times$ SSC–10 mM triethanolamine (pH 7.4)–0.1% SDS; and 100- μ l volumes were incubated in 300- μ l capped polyethylene tubes with two DNA-containing filters and one or two blank filters or filters containing λ phage or SV40 DNA. After 4 days at 37°C (33),

FIG. 1. Separation of strands of *Hpa* II fragments of polyoma DNA by electrophoresis on composite agarose-polyacrylamide gels. See text for details. (A) Analytical gel showing the position of the separated strands and the renatured DNA fragments. Load was 0.5 μ g of DNA onto a 0.5-cm-wide slot. Electrophoresis was at 16 V/cm for 1.75 h at 4°C. (B) Vertical slice cut from a preparative gel. Load was 46 μ g of DNA onto a 12-cm-wide slot. Electrophoresis was at 16 V/cm for 4.75 h at 4°C. Numbers refer to the *Hpa* II fragments, S (slow) and F (fast) refer to the separated strands of a given fragment, and N (native) refers to the double-stranded fragment.

filters were rinsed three times in 2 $\times$ SSC, incubated in 10 μ g of RNase A per ml for 1 h at room temperature in 2 $\times$ SSC, rinsed three more times in 2 $\times$ SSC, dried, and counted in a toluene-based scintillation fluid. All hybridizations were done in duplicate (total of four DNA-containing filters per sample). Filters were counted three times, 10 min each, in an Intertechnique SL-30 scintillation counter with a machine background of 2.5 cpm in a narrow tritium channel. Levels of hybridization were calculated by subtracting twice the counts per minute of the blank filter from the sum of

the counts per minute on the two DNA-containing filters.

RESULTS

To ensure that in the following experiments predominantly early polyoma-specific RNA was synthesized, FUDR (an inhibitor of DNA synthesis) was present throughout the period after infection (36), and cells were harvested no later than 10 to 10.5 h after infection. Under these conditions, less than 0.01% of total [3 H]uridine incorporation into infected cell RNA is virus specific. Obviously, the hybridization assay used to detect virus-specific RNA must have a very low background, which can be achieved by hybridization of RNA to nitrocellulose filter-bound DNA in the presence of formamide, followed by RNase treatment of the filters (18, 30, 33). The method used here (see Materials and Methods) resulted in nonspecific binding of 1 to 5 cpm of [3 H]RNA per 10⁶ cpm in a sample (0.0001 to 0.0005% of input cpm) to filters containing no DNA or SV40 or λ phage DNA.

It is also important for these experiments that the method of cell fractionation used results in nuclear and cytoplasmic fractions which are relatively free of cross-contamination. The methods used (35; see Materials and Methods) result in intact, high-molecular-weight nuclear and cytoplasmic RNA in high yield, with minimal (<4%) nuclear contamination of the cytoplasmic extract and low (8 to 16%) cytoplasmic contamination of nuclei (P.-E. Montandon and N. Acheson, unpublished data).

Use of *Hpa* II fragments of polyoma DNA for analysis of pulse-labeled RNA. *Hpa* II fragments 2 and 4 lie wholly within the region which codes for early mRNA's (the "early" region) on polyoma DNA, and *Hpa* II fragments 1 and 3 lie in the "late" region (Fig. 3; 21, 26, 27). It was therefore convenient to use these DNA fragments as probes for the detection of transcripts from the early and late regions in pulse-labeled, early nuclear RNA.

To test the ability of the filter-bound *Hpa* II fragments to hybridize to complementary RNA, DNA-containing filters were incubated with late polyoma-specific RNA from cells labeled for 20 min with [3 H]uridine (Table 1). It is known that greater than 90% of late polyoma-specific RNA is copied from the L strand of viral DNA (15) and that all sequences of the L strand are transcribed (2, 7, 26). One would therefore expect DNA fragments to hybridize with an amount of late RNA proportional to their fractional length of the polyoma genome. Table 1 shows that this is roughly so for fragments 1 to 3 but that fragment 4 hybridizes with significantly less late RNA than expected from its size. The reason for

FIG. 2. Analytical agarose-polyacrylamide gel electrophoresis of purified strands of *Hpa* II fragments 1 and 2 cut from a gel similar to that shown in Fig. 1B and electrophoretically eluted. Electrophoresis was at 16 V/cm for 3 h at 4°C. Slots 1 and 6 were loaded with partly denatured, *Hpa* II-cleaved polyoma DNA as markers of the positions of the native and denatured fragments. Slots 2 to 5 were loaded with samples of, respectively, purified strands 1S, 1F, 2S, and 2F. A small amount of renatured fragment 1 or 2, revealing contamination with the complementary strand, can be seen in each track.

this is unknown; it may be that RNA complementary to fragment 4 is present in reduced amounts in 20'-labeled RNA. Alternatively, it

may be that part of fragment 4 is not stably bound to the filter due to its small size and is eluted during the hybridization. Because of this

FIG. 3. Map of the polyoma genome, oriented with the origin of replication (O_R) at 12 o'clock, the early region (which codes for early mRNA's complementary to the E strand) on the left, and the late region (which codes for late mRNA's complementary to the L strand) on the right. The positions of the eight DNA fragments produced by cleavage with the enzyme *Hpa* II, and of the two subfragments produced by cleavage of fragment *Hpa* II-1 with the enzyme *Hind* II, are shown. Data are from Griffin et al. (21), Crawford et al. (12), Kamen et al. (26), and Folk et al. (16).

TABLE 1. Ability of purified *Hpa* II fragments of polyoma DNA to hybridize with pulse-labeled late RNA^a

DNA fragment	% of polyoma DNA length ^b	cpm of [³ H] RNA hybridized ^c	(cpm hybridized/cpm hybridized to polyoma DNA) × 100
1	27.3	162	26
2	21.4	153	25
3	16.8	141	23
4	13.2	55	9
Polyoma DNA	100	620	100

^a DNA bound to nitrocellulose filters was hybridized with total cellular RNA extracted from cells labeled with 500 μ Ci of [³H]uridine per petri dish for 20 min at 28 h after infection with polyoma virus.

^b From reference 20.

^c Input RNA, 18,400 cpm.

uncertainty, fragment 4 was not used further.

Table 2 shows that about 40% of early polyoma-specific cytoplasmic RNA hybridized to fragment 2, which represents 42% of the early region of polyoma DNA (27); less than 5% hybridized to fragments 1 and 3, which are outside the early region. These results confirm previous findings (7, 26) that only mRNA's from the early half of the genome are present in the cytoplasm during the early phase of infection. In addition, this experiment shows that the preparations of fragments 1 and 3 used were not contaminated with significant amounts of fragment 2.

Hybridization of ³H-labeled early nuclear RNA with *Hpa* II fragments of polyoma DNA. Table 3 shows that the proportion of pulse-labeled early nuclear RNA which hybridized with fragment 2 was substantially higher than that which hybridized with fragment 1 or 3 for labeling periods between 15 and 180 min. This implies that most transcription is limited to the early region of polyoma DNA. If all regions of the E strand were transcribed with the same efficiency, then pulse-labeled early nuclear RNA should hybridize almost equally well with fragments from both the early and late regions, as does pulse-labeled late nuclear RNA (see Table 1). This is clearly not the case.

However, a low level of transcription from the late region can be detected. Fragments 1 and 3 hybridized with a significantly higher proportion of early polyoma-specific nuclear RNA (Table 3) than cytoplasmic RNA (Table 2). This is particularly evident in samples labeled for longer times. The relative amount of labeled nuclear RNA which hybridized to these fragments (5 to 10 cpm above background in the experiments shown in Table 3) was significantly different from the relative amount of labeled cytoplasmic RNA which hybridized to the same fragments in at least two independent experiments for each labeling period. Fragment 2, on the other hand, hybridized with a significantly lower proportion of early nuclear than early cytoplasmic RNA. Thus, early nuclear RNA contains a low level of sequences transcribed from the late region,

TABLE 2. Hybridization of ³H-labeled early cytoplasmic RNA to *Hpa* II fragments of polyoma DNA^a

Labeling period (min)	Relative hybridization ^b of RNA to DNA fragment:			Input RNA (cpm)	cpm hybridized to whole polyoma DNA
	1	2	3		
30	5	39	3	0.20×10^6	63
60	5	42	3	0.57×10^6	113
180	3	39	2	0.91×10^6	121

^a RNA was extracted from cytoplasm of cells labeled with 500 μ Ci of [³H]uridine per petri dish for the time shown, the end of the labeling period being 10 h after infection. Infected cells were incubated in the presence of 6×10^{-5} M FUDR before and during the labeling period. RNA was hybridized to DNA bound to nitrocellulose filters. Samples labeled for different times shown here and in following tables were usually derived from independent experiments; thus, the ratio of virus-specific counts per minute to total input counts per minute is not strictly comparable from one sample to the next.

^b Hybridization levels are expressed as (counts per minute hybridized to DNA fragment/counts per minute hybridized to whole polyoma DNA) × 100.

TABLE 3. Hybridization of ³H-labeled early nuclear RNA to Hpa II fragments of polyoma DNA^a

Labeling period (min)	Relative hybridization of RNA to DNA fragment:			Input RNA (cpm)	cpm hybridized to whole polyoma DNA
	1	2	3		
15	8	26	3	0.81×10^6	58
30	10	25	7	1.42×10^6	72
60	14	29	11	2.27×10^6	69
180	13	31	8	1.53×10^6	67

^a RNA was extracted from nuclei of cells infected and labeled as in footnote a, Table 2. Nuclear RNA was derived from the same experiment as cytoplasmic RNA, labeled for the corresponding time, and used for hybridizations as shown in Table 2. Hybridization levels are expressed as in Table 2.

which are not efficiently transported to the cytoplasm.

To test whether the transcripts from the late region covered part or all of this region, nuclear RNA was hybridized with fragment 1a, derived by cleavage of fragment 1 with restriction endonuclease *Hind*II (16). This fragment covers only the middle one-half of the late region (Fig. 3). Nine percent of early nuclear virus-specific RNA labeled for 30 min hybridized with this fragment, compared with 10% which hybridized with intact fragment 1. This shows that there is a low but detectable level of transcripts complementary to most, and perhaps all, of the late region in early nuclear RNA.

Strand specificity of transcripts from the late region. To determine whether RNA from the late region is transcribed from the E or the L strand of polyoma DNA, the strands of fragments 1 and 2 were separated (see Materials and Methods) and bound to nitrocellulose filters. Hybridization of filter-bound DNA strands with pulse-labeled late nuclear RNA (transcribed predominantly from the L strand) showed that the slow component of fragment 1 and the fast component of fragment 2 correspond to the L strand of polyoma DNA (Table 4). These data also show that there is little cross-contamination between the strands and that the L strands hybridize an amount of pulse-labeled RNA roughly proportional to their fractional length of polyoma DNA.

Table 5 shows that neither strand of fragment 1 hybridized with a significant fraction of early cytoplasmic RNA and that strand 2E hybridized with early cytoplasmic RNA in proportion to its fractional length of the early region. Thus, the filter-bound separated strands were free of significant contamination by other fragments.

Labeled early nuclear RNA was hybridized

with the separated strands of these fragments. The results (Table 6) show that transcripts from the late region are complementary to both DNA strands, although E-strand transcripts appear to be more abundant. The sum of the amounts of early nuclear RNA which hybridized to the two strands of fragment 1 (12% of total polyoma-specific RNA) is nearly the same as the total RNA which hybridized to complete fragment 1 (Table 3), as expected.

Table 6 also shows that early nuclear RNA contains a low level of transcripts from the L strand of fragment 2, as well as the expected abundant transcripts from the E strand. This suggests that there is an equivalent level of L-strand transcription from all regions of the genome.

Size of pulse-labeled early nuclear and cytoplasmic RNA. RNA labeled for 30 min with [³H]uridine was heat denatured in 80% formamide and sedimented on aqueous sucrose gradients. Portions of each fraction were hybridized with filter-bound polyoma DNA to determine the size distribution of virus-specific RNA (Fig. 4). Polyoma-specific cytoplasmic RNA (Fig. 4A) sedimented as a narrow peak at 19S with reference to mouse rRNA, a finding in agreement with previous reports (40), plus a shoulder in the region of 5-15S. A very low level of virus-specific RNA sedimented in the region between 20S and 30S, and no detectable RNA sedimented more rapidly than 33S.

The major peak in polyoma-specific nuclear RNA, in contrast, reproducibly sedimented at 22S when compared with the positions of 18S and 28S rRNA (Fig. 4B). A shoulder in the

TABLE 4. Identification of E and L strands of Hpa II fragments 1 and 2 by hybridization with pulse-labeled late nuclear RNA^a

DNA fragment	Strand	cpm of [³ H] RNA hybridized ^b	Identification of strand ^c	Ratio cpm hybridized to L strand/cpm hybridized to E strand
1	Slow	289	L	32
	Fast	9	E	
2	Slow	11	E	25
	Fast	274	L	

^a DNA bound to nitrocellulose filters was hybridized with RNA extracted from nuclei of cells labeled with 300 μCi of [³H]uridine per ml for 20 min at 29 h after infection.

^b Input RNA, 22,000 cpm; RNA hybridized to whole polyoma DNA, 1,047 cpm.

^c The DNA strand to which most late nuclear RNA hybridizes is identified as the L strand (15).

TABLE 5. Hybridization of ^3H -labeled early cytoplasmic RNA to separated strands of *Hpa* II fragments of polyoma DNA^a

Labeling period (min)	Relative hybridization of RNA to DNA fragment:				Input RNA (cpm)	cpm hybridized to whole polyoma DNA
	1		2			
	E strand	L strand	E strand	L strand		
30	2	2	38	0.7	0.14×10^6	68
180	3	2	37	0.3	3.59×10^6	246

^a RNA was extracted from cytoplasm of cells infected and labeled as in footnote a, Table 2. Hybridization levels are expressed as in Table 2.

TABLE 6. Hybridization of ^3H -labeled early nuclear RNA to separated strands of *Hpa* II fragments of polyoma DNA^a

Labeling period (min)	Relative hybridization of RNA to DNA fragment:				Input RNA (cpm)	cpm hybridized to whole polyoma DNA
	1		2			
	E strand	L strand	E strand	L strand		
30	7	5	33	5	1.39×10^6	118
180	8	4	25	3	1.76×10^6	116

^a RNA was extracted from nuclei of cells infected and labeled as in footnote a, Table 2. Hybridization levels are expressed as in Table 2.

region of 5-15S was also present. More notably, on the high-molecular-weight side, a significant fraction of polyoma-specific RNA sedimented between 25S and 40S.

These results are consistent with the fragment hybridizations already presented, which suggested that most early nuclear RNA is transcribed only from the early region and, therefore, should be the same size as, or slightly larger than, the cytoplasmic mRNA. The 25-40S RNA in the nucleus may represent the rarer transcripts which read through the entire E strand, as well as those which apparently are copies of the entire L strand.

DISCUSSION

The results presented in this paper are most simply explained by the model illustrated in Fig. 5. First, it is clear that the E strand is the template for the bulk of early transcription. This is supported by results in Tables 3 and 6, which show that pulse-labeled transcripts complementary to fragment 2E are substantially more abundant than transcripts complementary to fragment 1L or 2L. The ratio of E- to L-strand transcription can be estimated as approximately 7:1 from the proportion of labeled RNA which hybridizes with fragment 2E compared with that which hybridizes with fragment 1L or 2L (Table 6). (Since the absolute numbers of counts per minute hybridized are low, this ratio should not be considered very precise.) This ratio is indicated by the relative number of E- and L-strand transcripts drawn in Fig. 5.

Second, most E-strand transcripts are initiated at the beginning of the early region (near the *Hpa* II 5-3 junction) or slightly upstream from this point. This follows because (i) the entire early region must be transcribed to produce early mRNA's (26, 27), and (ii) regions of the DNA upstream from the beginning of the early region, i.e., fragments 3 and 1a, are transcribed at a very low level compared with fragments from the early region (Table 3; see Results).

Third, most E-strand transcripts must terminate near the end of the early region. This is based on the relative proportion of pulse-labeled nuclear RNA which hybridizes with fragment 2E compared with that which hybridizes with fragment 1E (Table 6; see also Table 3). From these data it can be estimated that for every five E-strand transcripts from the early region, there is about one from the late region, which suggests that transcription terminates with an efficiency of about 80%.

The major early nuclear RNA labeled during a 30-min pulse is slightly larger (22S) than early cytoplasmic mRNA (19S); if the 22S RNA represents the major primary transcript of the E strand, its size is consistent with a transcript which initiates near the beginning and terminates near the end of the early region (55% of 5,400 nucleotides = 3,000 nucleotides, or ca. 10^6 molecular weight). The difference in size between polyoma nuclear and cytoplasmic early RNAs could arise by elimination of some internal sequences ("splicing"); SV40 E-strand

FIG. 4. Sedimentation profile of early nuclear and cytoplasmic polyoma-specific RNA labeled for 30 min with [^3H]uridine. Cells were labeled with 500 μCi of [^3H]uridine per petri dish in 1 ml from 10 to 10.5 h after infection. FUDR, 6×10^{-5} M, was present from 2 h after infection until harvest. After extraction and DNase treatment, RNA samples were denatured by heating at 65°C for 3 min in 80% formamide-0.5% SSC and then ethanol precipitated along with total mouse kidney cell RNA labeled for 24 h with [^{14}C]uridine, as a sedimentation marker. Samples were resuspended in 300 μl of 10 mM triethanolamine (pH 7.4)-50 mM LiCl-0.1% SDS and layered onto 13.5-ml 15 to 30% (wt/wt) sucrose gradients in the same buffer. Sedimentation was for 5.5 h at 40,000 rpm in a Spinco SW40 rotor at 20°C . Total ^3H and ^{14}C radioactivity in samples of each fraction was measured by spotting on filter paper disks, which were washed in 5% trichloroacetic acid, rinsed in ethanol, dried, and counted. The remainder of each fraction was ethanol precipitated in the presence of 10 μg of yeast tRNA, resuspended in 100 μl of 50% formamide-2% SSC-0.1% SDS, and hybridized with two nitrocellulose filters containing 0.2 μg of polyoma DNA each, plus one blank filter. Filters were RNase treated, and ^3H radioactivity in polyoma-specific RNA ($\bullet$) was determined as in the text. Total ^3H ($\circ$) and ^{14}C (---) cpm, corrected for counting overlaps and background, are shown as counts per minute per fraction hybridized. (A) Cytoplasmic RNA: 330 μg of RNA containing 0.73×10^6 cpm was loaded. (B) Nuclear RNA: 234 μg of RNA containing 8.1×10^6 cpm was loaded.

mRNA's are known to be spliced (9). The 5' or 3' ends of the 22S RNA may also be trimmed during the process of mRNA maturation. It is not certain that the 22S RNA represents the

FIG. 5. Model of early transcription of polyoma DNA. The viral DNA, shown as a thick double circle partitioned into the eight *Hpa* II fragments, is oriented with the origin of DNA replication at 12 o'clock, the early region on the left, and the late region on the right. Transcripts are shown as lines beginning with a dot and terminating with an arrowhead. The sites of initiation and termination of transcription are not precisely known; this is indicated by the dashed lines. The relative rates of transcription of the E and L strands, and of different regions thereof, are indicated by the number of transcripts shown corresponding to each region.

primary transcript, since it could be a cleavage product of a larger transcript; however, the relative scarcity of E-strand transcripts from fragments outside the early region (see above), even in RNA labeled for periods as short as 15 min, argues against this.

The model shown in Fig. 5 also shows the rare L-strand transcripts continuing around the entire L strand, as they do during the late phase of infection (2, 3). This is based on the hybridization of detectable levels of labeled nuclear RNA with both fragments 1L and 2L (Table 6).

An alternative to the model presented in Fig. 5 would be that all E-strand sequences are transcribed with the same efficiency (i.e., no termination takes place), but nonmessenger sequences are unstable. In this case, the half-life of these sequences would have to be very short (on the order of 5 min), since even in cells labeled for 15 min RNA complementary to the late region is scarce. The data in Table 3, which show that RNA labeled for 3 h contains at least as much

polyoma-specific RNA complementary to fragments from the late region as does RNA labeled for shorter times, do not support the concept that nonmessenger transcripts have such a short lifetime.

The results of this paper confirm and extend our previous observations (7) that most early nuclear RNA is complementary to only one-half of the E strand of polyoma DNA. Others have suggested that all sequences of the E strand are transcribed with equal efficiency, during either the early phase (26) or the late phase (5, 26) of polyoma infection. As we have previously pointed out (1, 7), the results of Kamen et al. (26) may be due to a perturbation of early transcription caused by the presence of cytosine arabinoside, which is known to be incorporated into growing DNA chains (31, 32). The results of Aloni and Locker (5), Aloni (4), and Kamen et al. (26) do not, in fact, show that all E-strand sequences are transcribed with equal efficiency during the late phase of virus infection, but only that some transcripts of the entire E strand are made, which the present results (limited to the early phase) confirm. It is not yet known whether the extent of E-strand transcription differs during the early and late phases.

The presence of low levels of L-strand transcription during the early phase of polyoma infection, shown here to occur *in vivo*, has also been detected by *in vitro* labeling of SV40 transcription complexes (14). Its significance, however, remains difficult to assess. For transcription to be initiated on the L strand, it may be sufficient that the DNA template contain a single-stranded region or a single-stranded nick (19). If this were the case, any partially degraded DNA present either in the infecting virus or as a result of attack by cellular nucleases might give rise to L-strand transcripts. Alternatively, transcription might be initiated on the L strand of intact DNA at a low efficiency compared with initiation on the E strand. The relatively high efficiency of L-strand transcription during the late phase would then be a result of changes in the structure of the template due to DNA replication (19, 39) or to modulation of E- or L-strand transcription by early viral proteins (6, 10, 28, 38).

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