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Efficiency of Processing of Viral RNA During the Early and Late Phases of Productive Infection by Polyoma Virus

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The efficiency of processing of polyoma viral RNA and of its export from nucleus to cytoplasm was measured in primary mouse kidney cells by comparing the initial rates of incorporation of [^{3}H]uridine into cytoplasmic and nuclear viral RNA. Appropriate methods of cell fractionation were chosen to maximize yields of cytoplasmic RNA and to minimize leakage of nuclear RNA. Incorporation of [^{3}H]uridine into cellular 4S RNA in the cytoplasm was followed to monitor pool equilibration and maintenance of an excess of radioactive precursor throughout the experimental period. During the early phase of infection (9 to 11 h, in the presence of 5-fluorodeoxyuridine), viral RNA was rapidly and efficiently exported from nucleus to cytoplasm. Viral RNA appeared in the cytoplasm within 6 min of its synthesis; greater than half of the viral RNA synthesized in the nucleus was exported to the cytoplasm. In contrast, during the late phase of infection (28 to 30 h), viral RNA was exported more slowly, appearing in the cytoplasm 12 to 20 min after its synthesis, and much less efficiently—only 5% of late nuclear transcripts was exported. The poor efficiency of processing of late viral RNA may be, in part, a result of (i) the presence in nuclear transcripts of non-mRNA sequences which are removed during processing; (ii) the presence in nuclear transcripts of multiple copies of mRNA sequences, only one of which is incorporated into mature mRNA; and (iii) inefficient polyadenylation of viral nuclear RNA.

The pattern of transcription of polyoma viral DNA undergoes a striking change between the early and late phases of productive infection. Transcription during the early phase is mostly limited to the one-half of the E strand of viral DNA which codes for viral mRNA's (5, 13). During the late phase, in contrast, transcription is most active on the L strand (11), and all regions of this strand are nearly equally represented in late RNA (6, 7, 13).

The major early nuclear transcript of the E strand is only slightly larger than E-strand cytoplasmic mRNA's (5). On the other hand, late nuclear transcripts of the L strand are made as "giant" molecules up to 15 to 20 kilobases in length (three to four times the length of the viral genome) (4). These giant RNAs contain tandem repeats of the entire nucleotide sequence of the L strand of viral DNA (2). However, late cytoplasmic mRNA's derived from the L strand are only one-fourth to two-fifths the length of the viral genome (9, 14), but contain reiterated 5'-proximal "leader" sequences (15).

These findings suggested that the processing of late, L-strand transcripts may differ in important ways from the processing of early, E-strand transcripts. In particular, if each of the giant L-strand transcripts gives rise to only a single mRNA molecule, only a fraction of late nuclear RNA might be conserved during mRNA maturation steps. The present experiments were undertaken to determine the relative efficiency with which early and late viral transcripts are processed into the corresponding mRNA's and exported to the cytoplasm.

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MATERIALS AND METHODS

Infection and labeling of cells. Primary mouse kidney cells were infected with plaque-purified polyoma virus at a multiplicity of 20 to 40 PFU/cell. For early RNA, cells were incubated with 6×10^{-5} M 5-fluoro-2'-deoxyuridine (FUdR) starting at 2 h after infection, and labeling began at 9 h after infection, as described elsewhere (5). For late RNA, FUdR was omitted, and labeling began 28 to 30 h after infection. Cells were labeled at 37°C with 400 to 500 μCi of [5-^{3}H]uridine in 1 ml of reinforced Eagle medium per 88-mm petri dish (two to four petri dishes per sample). Labeling was terminated by washing with ice-cold 140 mM NaCl-5 mM KCl-1 mM Na_2HPO_4-25 mM Tris-hydrochloride (pH 7.4). In other experiments (data not shown), labeling of late RNA was carried out in the presence of FUdR. Incorporation of [^{3}H]uridine into viral RNA in nucleus and cytoplasm was similar to that found (see below) in the absence of FUdR, indicating that FUdR does not seriously alter viral mRNA metabolism.

Cell fractionation and extraction of RNA.

Three different lysis solutions were tested for their effect on yield and purity of nuclear and cytoplasmic RNA (see below). These are designated: buffer A, 250 mM sucrose–25 mM NaCl–5 mM $MgCl_2$–10 mM triethanolamine hydrochloride (pH 7.4)–1% Nonidet P-40 (9); buffer B, same as buffer A plus 0.5% sodium deoxycholate; and buffer C, 10 mM NaCl–1.5 mM $MgCl_2$–10 mM triethanolamine hydrochloride (pH 8.5)–1% Nonidet P-40 (5, 22). Cells were washed in ice-cold buffer lacking detergents, then scraped in 1 to 2 ml per petri dish of buffer plus detergent, and pipetted gently to disperse clumps. Nuclei were pelleted at 1,500 × *g* for 3 min or 10,000 × *g* for 10 min. The supernatant cytoplasmic extract was decanted, and the nuclear pellet was resuspended in the same buffer. RNA was extracted from cytoplasmic extracts either with 1% sodium dodecyl sulfate (24) or with 2% triisopropylnaphthalenesulfonate (TNS) (20) plus 1 volume of phenol-chloroform-isoamyl alcohol (50:50:1), as described previously (5, 9). Nuclear RNA was extracted either with 1% sodium dodecyl sulfate-phenol at 65°C (4) or with 2% TNS-phenol-chloroform-isoamyl alcohol at room temperature. Nucleic acids were ethanol precipitated and treated with DNase as previously described (5).

DNA-RNA hybridization; normalization of results. Nitrocellulose filters containing 0.2 μg of polyoma DNA per 3.5 mm^2 were prepared as described (5). Hybridizations of early RNA samples, which contained 0.005% to 0.02% of radioactivity as polyoma-specific RNA, were carried out in 50% formamide–2× SSC (SSC, 0.15 M NaCl–0.015 M sodium citrate) at 37°C as described previously (5). Hybridization of late RNA samples, which contained 1 to 10% of radioactivity as polyoma-specific RNA, were carried out in 4× SSC at 65°C as described elsewhere (4). From 50 to 150 μg of early RNA, or 1 to 2 μg of late RNA, was hybridized with 0.4 μg of viral DNA in a volume of 100 μl. Under these conditions, viral DNA is in excess over viral RNA, and hybridization is nearly exhaustive (unpublished data).

Values of counts per minute hybridized per microgram of RNA in the hybridization mixtures were multiplied by the total micrograms of RNA (nuclear or cytoplasmic) in each sample to give the total virus-specific counts per minute in the sample. To correct for variable recovery of RNA from sample to sample, these values were normalized by dividing by the sum of the micrograms of nuclear RNA plus the micrograms of cytoplasmic RNA in the sample. The result, multiplied by 100, yields the counts per minute hybridized per 100 μg of total (nuclear plus cytoplasmic) RNA.

RESULTS

The efficiency of processing of a nuclear transcript into a mature, cytoplasmic mRNA can be assessed by comparing the rate of incorporation of a radioactive precursor (e.g., [^{3}H]uridine) into the cytoplasmic mRNA with the corresponding rate of incorporation into the nuclear transcript (16, 19, 25). The ratio of these two rates should be equal to the fraction of nuclear RNA which is successfully processed and exported to the cytoplasm as mRNA. However, the significance of the results of such experiments depends on the control of a number of experimental conditions, including (i) purity of nuclear and cytoplasmic fractions, (ii) rapid achievement of constant specific activity of nucleotide pools, and (iii) presence of an excess of [^{3}H]uridine throughout the experiment. These points will now be considered in detail.

Separation of nuclei and cytoplasm. Three methods for nucleus-cytoplasm separation (see above), using (i) an isotonic sucrose (pH 7.4) buffer with the detergent Nonidet P-40 (method A), (ii) the same buffer with both Nonidet P-40 and sodium deoxycholate (method B), and (iii) a hypotonic (pH 8.5) buffer with Nonidet P-40 (method C), were tested for yield of cytoplasmic RNA and leakage of nuclear RNA (Table 1). Yield was measured by the fraction of the total cellular RNA (determined by absorbance at 260 nm) in the cytoplasmic extract. In primary mouse kidney cells we estimated (by quantitation of 18S rRNA in nuclear and cytoplasmic fractions and assumption that all 18S rRNA is cytoplasmic) that nuclei contain about 15% of total cellular RNA and cytoplasm contains about 85% (P.-E. Montandon and N. H. Acheson, unpublished data). Thus, with buffer A, 42/85ths, or approximately 50% (in other experiments up to 70%), of the cytoplasmic RNA is present in the cytoplasmic fraction; with buffer C, 80 to 90% of the cytoplasmic RNA is present in this fraction.

Nuclear leakage was estimated by the fraction of labeled cellular RNA sedimenting faster than 10S, or by the fraction of labeled late viral RNA, present in cytoplasmic extracts after a 10- or 15-

TABLE 1. *Yield and purity of cytoplasmic RNA prepared by three different methods*[a]

Lysis buffer[b]	% Nucleic acid or radioactivity in cytoplasmic fraction			
	Nucleic acid[c]	[^{3}H]RNA	[^{3}H]-RNA ≥ 10S	^{3}H-labeled viral RNA
A	42	4,6	0.8	0.9
B	54	5.8	1.1	2.5
C	67	7.8	2.4	5.6

[a] Cells infected 28 to 30 h previously with polyoma virus were incubated with 500 μCi of [^{3}H]uridine per ml for 15 min. Cytoplasmic and nuclear fractions were prepared, and nucleic acids were extracted by the TNS-phenol-chloroform method and further processed as described in the text.

[b] For composition of buffers, see the text.

[c] Determined by optical density at 260 nm. These values vary by about 10% within a given experiment and by up to 20% between experiments.

min labeling period with [^{3}H]uridine (Table 1 and Fig. 1). This is based on knowledge that few RNAs other than tRNA's enter the cytoplasm within 15 min after their synthesis (23, 24, 28) and that little late polyoma viral RNA enters the cytoplasm by this time (see below). However, these values should be considered maximal estimates of nuclear leakage, since some mRNA or viral RNAs may enter the cytoplasm more rapidly. Method A gave rise to minimal nuclear leakage (Table 1); with method B, nuclear leakage was slightly higher (especially when estimated by presence of viral RNA). With method C (see also Fig. 1), the level of nuclear leakage was significantly higher; three times as much labeled RNA larger than 10S, and six times as much viral RNA, was in the cytoplasmic extract compared with that of method A, although the yield of total cytoplasmic RNA was only 60% greater.

During the early phase of polyoma virus infection, approximately equal amounts of labeled viral RNA are present in nuclei and cytoplasm after labeling periods of 20 to 120 min (5, and see below). In this case, method C is most appropriate, since yields of cytoplasmic RNA are high and nuclear leakage is moderate. But during the late phase, most viral RNA, even after long labeling periods, remains in the nucleus (see below); here, use of method A is called for since otherwise leakage of viral RNA from the nucleus could account for a significant fraction of the viral RNA present in cytoplasmic extracts during short labeling periods.

Labeling conditions. For the rate of accumulation of [^{3}H]uridine into RNA to be a valid indication of the rates of synthesis, export, and degradation of RNAs, the specific radioactivities of nucleotide pools must remain approximately constant throughout the experiment. This can be conveniently controlled by measuring the rate of labeling of cellular 4S RNA in the cytoplasm. Since 4S RNA can be considered stable over the period (2 h) of these experiments and is rapidly exported to the cytoplasm after its synthesis (1, 17, 18), incorporation of [^{3}H]uridine

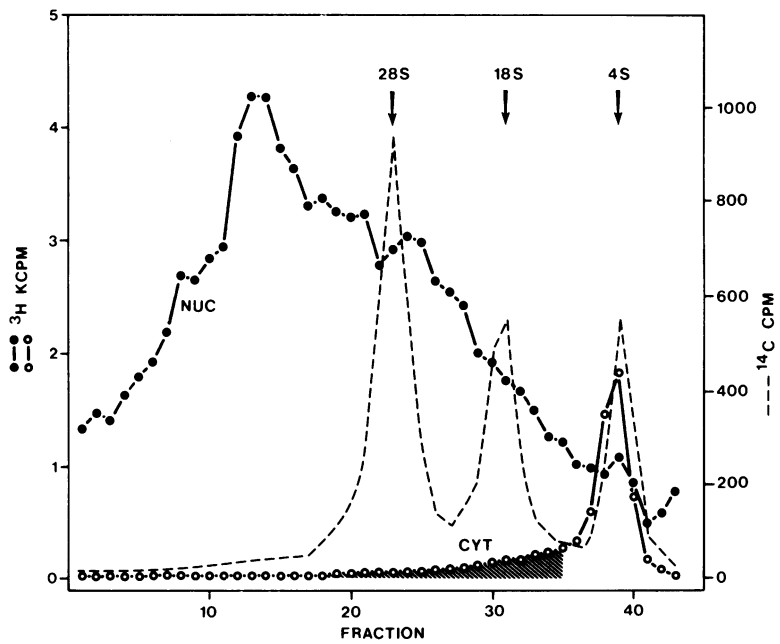

FIG. 1. *Size distribution of pulse-labeled nuclear and cytoplasmic RNAs prepared after lysis with buffer C. Polyoma virus-infected cells were labeled, and nucleic acids were prepared from nuclear and cytoplasmic extracts as described in footnote a, Table 1. Samples representing equal proportions of nuclear or cytoplasmic RNA were mixed with ^{14}C-labeled mouse kidney cell RNA and were separately sedimented on 15 to 30% (wt/wt) sucrose gradients in 50 mM LiCl–0.5% sodium dodecyl sulfate–10 mM triethanolamine hydrochloride (pH 7.4) in the SW60 rotor at 55,000 rpm and 20° C for 2 h. ^{3}H and ^{14}C counts per minute (corrected for background and isotope overlap) in gradient fractions were determined by spotting samples on filter paper disks, washing with 5% trichloroacetic acid, and measuring radioactivity in a scintillation counter. The gradient profiles were aligned so that the ^{14}C-labeled RNA peaks coincide (only one of the ^{14}C profiles is shown). Symbols: ●, nuclear RNA; ○, cytoplasmic RNA; – – –, ^{14}C marker RNA. Cytoplasmic RNA which sediments faster than 10S is shown by shading.*

into cytoplasmic 4S RNA should occur at a constant rate, once constant pool specific radioactivities are achieved. Figure 2 shows that, after a short lag (3 to 6 min in various experiments), incorporation of [^{3}H]uridine into 4S RNA was constant under the conditions used for at least 2 h. Thus, pool equilibration was relatively rapid, and exogenous [^{3}H]uridine was in excess throughout this period.

Figure 2 also shows that the synthesis, processing, and export of cellular rRNA appeared normal in cells labeled with high concentrations of [^{3}H]uridine late during polyoma virus infection. The 18S rRNA began to arrive in the cytoplasm 20 to 30 min after the addition of label, and 28S rRNA began to arrive between 45 and 60 min. These times of appearance are similar to those found, for example, in HeLa cells (21).

Export of early viral RNA. Cells were labeled with [^{3}H]uridine for periods of from 15 min to 2 h during the early phase of productive infection. RNA was extracted from nuclear and cytoplasmic fractions prepared by method C, and the RNA samples were hybridized with an excess of polyoma viral DNA bound to nitrocellulose filters. Figure 3 shows the accumulation of [^{3}H]uridine in viral RNAs as a function of labeling time. By extrapolation of the initial parts of the curves to the abscissa, it can be estimated that cytoplasmic viral RNA begins to be labeled about 6 min after nuclear viral RNA. This lag defines the minimum time required for processing and export of viral RNA to the cytoplasm subsequent to its synthesis.

The rate of labeling of nuclear viral RNA declined after about 30 min of labeling, whereas that of cytoplasmic viral RNA remained constant up to 2 h. This suggests that the bulk of early nuclear viral RNA has a relatively short lifetime (on the order of 30 min or less). This short lifetime could result from rapid degradation in the nucleus, or rapid and efficient export of viral RNA from the nucleus, or both. That the decline in labeling is not due to an exhaustion of exogenous [^{3}H]uridine has been documented earlier. Neither is the decline caused by a decrease in the rate of synthesis of nuclear viral RNA, for cells labeled for 15 min at the

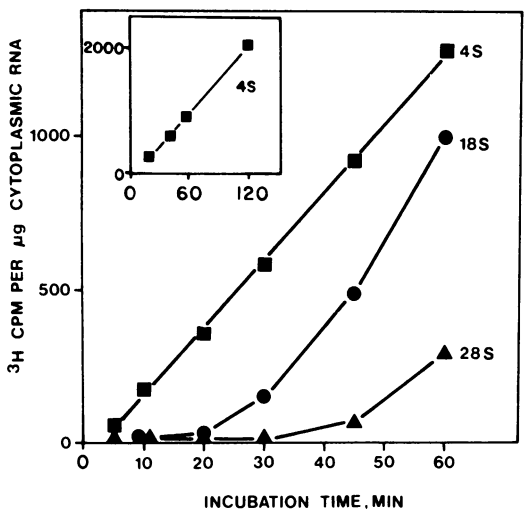

FIG. 2. *Incorporation of [^{3}H]uridine into cellular 4S, 18S, and 28S RNA in the cytoplasm during the late phase of infection. Cells were labeled with 500 µCi of [^{3}H]uridine per ml for the times shown beginning 28 h after infection. Nuclei and cytoplasm were separated after lysis with buffer C; nucleic acids were extracted from the cytoplasm by the TNS-phenol-chloroform method. A sample of each RNA preparation was mixed with ^{14}C-labeled mouse kidney cell RNA and sedimented on 15 to 30% (wt/wt) sucrose gradients in 50 mM LiCl-0.5% sodium dodecyl sulfate-10 mM triethanolamine hydrochloride (pH 7.4) in the SW60 rotor at 59,000 rpm and 20°C for 3.25 h. ^{3}H counts per minute in peaks corresponding to 4S, 18S, and 28S RNA were summed for each gradient and are expressed as counts per minute per microgram of cytoplasmic RNA loaded on the gradient. Symbols: ■, 4S RNA; ●, 18S RNA; ▲, 28S RNA. Inset: results from a separate experiment.*

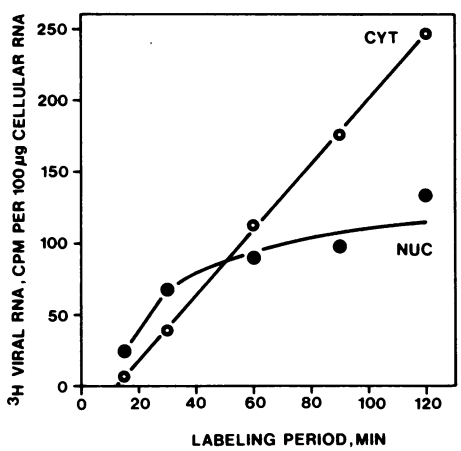

FIG. 3. *Incorporation of [^{3}H]uridine into polyoma viral RNA in the nucleus and cytoplasm during the early phase of infection. Cells were labeled with 500 µCi of [^{3}H]uridine for the times shown, beginning 9 h after infection. From 2 h after infection and during the labeling period, 6×10^{-5} M FUdR was present continuously. Nuclear and cytoplasmic fractions were prepared after lysis with buffer C; nucleic acids were extracted by the TNS-phenol-chloroform method. Hybridization, washing and counting of filters, and normalization of hybrid counts per minute were carried out as described in the text. Symbols: ●, nuclear viral RNA; ○, cytoplasmic viral RNA.*

same time after infection (9 h), or 1 or 2 h later, incorporated approximately the same amount of radioactivity into nuclear viral RNA (data not shown).

The ratio of the initial rates of incorporation into cytoplasmic and nuclear viral RNA is a measure of the fraction of nuclear viral RNA sequences which is exported to the cytoplasm (see, e.g., 16, 19, 25). Examination of Fig. 3 shows that this ratio was close to 1; thus, a large fraction of the early viral RNA synthesized in the nucleus was exported to the cytoplasm. This suggests that the decline in the rate of labeling of nuclear RNA is primarily due to the efficient export of viral RNA from the nucleus to the cytoplasm.

The constant rate of accumulation of label in cytoplasmic viral RNA shows that this RNA is stable over the duration of the experiment; its half-life can be estimated to be substantially greater than 2 h.

Export of late viral RNA. Figure 4 shows the accumulation of [^{3}H]uridine in viral RNA in the nucleus and cytoplasm during the late phase of productive infection. These curves are strikingly different from those for early RNA (Fig. 3). First, extrapolation of the curves to the abscissa shows that there was a lag of about 13 min between the synthesis of RNA in the nucleus and its export to the cytoplasm. In two additional experiments, this lag was 12 and 21 min. Thus, it would appear that the delay between the synthesis of RNA and its appearance in the cytoplasm is significantly longer in the late phase than in the early phase.

Second, the rate of incorporation of label into late nuclear viral RNA decreased much more slowly than that of early nuclear viral RNA. This suggests that late nuclear RNA is less efficiently exported or is degraded less rapidly or both than is early RNA. A comparison of the data for late nuclear RNA with theoretical curves for the labeling of RNAs with half-lives of 0.5, 1, or 2 h is shown in Fig. 5. The experimental data best match the curve for RNA with a 1-h half-life.

Third, the slope of the accumulation curve for cytoplasmic viral RNA is about 25-fold smaller than that for nuclear viral RNA (note the 20-fold scale difference for the curve for cytoplasmic RNA plotted as a dashed line in Fig. 4). This means that only a very small fraction of late nuclear RNA was exported to the cytoplasm. The ratio of the two slopes in this experiment (0.036) must be corrected for the significant contamination of the nuclear fraction with cytoplasmic RNA, since method A was used for nucleus-cytoplasm separation. In this experiment, an

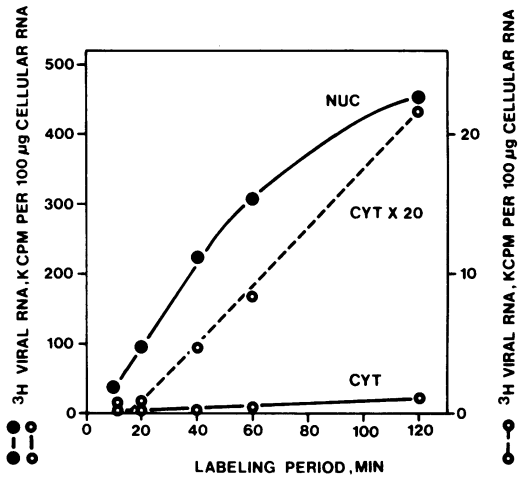

FIG. 4. *Incorporation of [^{3}H]uridine into viral RNA in the nucleus and cytoplasm during the late phase of infection. Cells were labeled with 400 µCi of [^{3}H]uridine for the times shown, beginning 28 h after infection. Nuclei and cytoplasm were separated after lysis with buffer A; nucleic acids were extracted by the hot phenol method (nuclei) or by the sodium dodecyl sulfate-phenol-chloroform method (cytoplasm). Hybridization, washing and counting of filters, and normalization of hybrid counts per minute were carried out as described in the text. Symbols: ●, nuclear RNA; O——O, cytoplasmic RNA (left-hand ordinate); O- - -O, cytoplasmic RNA (right-hand ordinate).*

average of 59% of the total RNA was in the cytoplasmic fraction, which means that only about 59/85ths or 69% of cytoplasmic RNA was in the cytoplasmic fraction. The corrected efficiency of the export of RNA from nucleus to cytoplasm was thus 0.036/0.69, or 0.052. Thus, only about 5% of late viral RNA synthesized in the nucleus was exported to the cytoplasm. In two additional experiments, the fraction of viral RNA exported to the cytoplasm was 5% and 6% (data not shown).

As for early cytoplasmic RNA, the accumulation curve for late cytoplasmic RNA was linear over the 2-h period of this experiment, suggesting that late mRNA's also have half-lives substantially longer than 2 h.

DISCUSSION

These experiments show that early polyoma viral RNA is rapidly and efficiently processed and transported to the cytoplasm. The lag between synthesis of early RNA and its appearance in the cytoplasm was about 6 min. A large fraction of viral RNA labeled in the nucleus was transported to the cytoplasm, as shown by two results. (i) The initial slopes of the curves de-

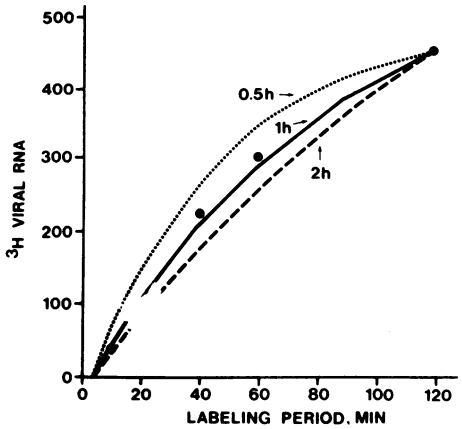

FIG. 5. *Estimation of the average half-life of late nuclear viral RNA. Curves were drawn by calculating the accumulated radioactivity incorporated into RNA at 10, 20, 40, 60, 90, and 120 min after the beginning of labeling by use of the formula* $A/A_\infty = 1 - e^{-\ln 2(1/T_{1/2})t}$ *(12), (i) assuming labeling effectively begins at 4 min on the time scale shown (to take into account the observed lag in labeling due to slow pool equilibration), (ii) setting* A/A_∞ *at 120 min equal to the observed incorporation into late nuclear viral RNA at 120 min in the experiment described in Fig. 3 and 4), using half-lives* ($T_{1/2}$) *of 0.5 h (·····), 1 h (——), and 2 h (-----). The experimentally determined points (●) are from Fig. 4.*

scribing the accumulation of [^{3}H]uridine into nuclear and cytoplasmic RNA are nearly the same; the ratio of these slopes is proportional to the fraction of nuclear RNA exported to the cytoplasm. (ii) The rate of accumulation of [^{3}H]uridine into nuclear RNA began to decline after about 30 min; this would be expected if most viral RNA leaves the nucleus after a short delay.

Hybridization of pulse-labeled early nuclear RNA with specific fragments of polyoma DNA has shown (5) that about three-fourths of early transcripts are derived from that portion of the E strand which codes for viral mRNA's (13). The one-fourth of early transcripts derived from other regions of the E strand, or from the L strand, is not transported to the cytoplasm (5). Thus, if all E-strand mRNA sequences synthesized in the nucleus were successfully processed and exported, approximately three-fourths of the labeled nuclear RNA should appear in the cytoplasm. The present results suggest that this is indeed the case. However, the following experimental uncertainties limit the accuracy of this conclusion. (i) Since RNA is exported rapidly, the initial rate of labeling of nuclear RNA may be somewhat higher than that measured by the slope of the curve between 15 and 30 min, by which time some labeled viral RNA has already been exported. (ii) The relatively low levels of hybridizable viral RNA, as well as variability in the recovery of nuclear and cytoplasmic RNA from one sample to the next, introduce imprecision in the determination of the levels of labeled viral RNA at each point. In spite of these uncertainties, it is clear that a large fraction of early nuclear RNA, probably more than 50%, was exported to the cytoplasm.

Late polyoma viral RNA is processed and exported somewhat more slowly and much less efficiently than early RNA. The lag between the synthesis of late RNA and its appearance in the cytoplasm was 12 to 20 min in different experiments. Only about 5% of the RNA synthesized in the nucleus was exported to the cytoplasm. If the remaining 95% of the nuclear viral RNA were stable, the rate of incorporation of label should remain nearly constant with time (as does that of cellular 4S RNA). Instead, the accumulation curve indicates that this RNA decayed, with an average half-life of about 1 h; however, it is possible that there are multiple components of different stabilities in this RNA (see, e.g., 10).

The half-life of late polyoma mRNA has been estimated to be greater than 10 h (26). Although the present experiments were not designed to measure the lifetimes of viral mRNA, the results indicate that both early and late viral mRNA's have half-lives substantially greater than 2 h.

Chiu et al. (10) measured the rate and efficiency of processing of late simian virus 40 RNA by using a pulse-chase technique. They found that radioactive viral RNA began to appear in the cytoplasm by 10 to 15 min after the beginning of labeling, a finding similar to those with polyoma virus reported here. However, about 35% of the simian virus 40 RNA labeled during a 5-min period was transported to the cytoplasm during the following 30 to 60 min. Thus, processing of simian virus 40 late RNA appears to be much more efficient than that of polyoma late RNA.

Why is processing and export of late polyoma RNA so inefficient? First, all L-strand sequences are transcribed in the nucleus in roughly equimolar amounts (6, 7, 13), but only RNAs derived from the late region (14), which accounts for about one-half of the L strand, are transformed into mRNA's (7, 13). Furthermore, the most abundant L-strand mRNA (16S) represents only about one-fourth of L-strand sequences. Thus, even if processing were 100% efficient, only one-fourth to one-half of L-strand transcripts would be conserved in mature mRNA's.

Second, many L-strand transcripts in the nu-

cleus contain multiple, tandemly repeated copies of the sequences in the entire L strand (2), whereas L-strand mRNA's contain only one "body" sequence attached to multiple, short leader sequences (15). It is likely that the mRNA's are derived from the nuclear transcripts by a series of splicing reactions which results in the incorporation of only one of the several mRNA body sequences, but all of the leader sequences in a given transcript, into the mature mRNA (3, 15). This would result in the loss of mRNA sequences, as well as non-mRNA sequences, during RNA processing.

It has been estimated that polyoma L-strand mRNA's contain an average of 4 leader sequence repeats per molecule (15, 29). If this were the case in mRNA's analyzed in the present experiments, it would be nearly sufficient to explain the 5% conversation of nuclear L-strand sequences; only one-quarter of mRNA sequences, or 6 to 12% of total L-strand sequences, would be conserved if every transcript were successfully processed.

However, analysis of nascent viral RNA chains in my laboratory (unpublished data) suggests that the average L-strand transcript contains only two copies of each mRNA sequence; if this were the case, one-half of mRNA sequences, or 12 to 25% of total L-strand sequences, should be conserved. Since conservation is significantly less than this, it may be that not all nuclear viral transcripts are successfully processed. There is evidence that only a fraction of polyoma viral RNA is polyadenylated (8, 27); polyadenylation may well be a prerequisite to further processing. Further experiments correlating the multiplicity of leader sequence repeats in mRNA's with nuclear RNA size and with efficiency of polyadenylation in the same experimental system will be needed to clear up these issues.

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