JOURNAL OF VIROLOGY, Feb. 1975, p. 433-437

Vol. 15, No. 2
Printed in U.S.A.

Posttranscriptional Selection of Simian Virus 40-Specific RNA

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Received for publication 17 October 1974

Analysis of the viral-specific RNA in simian virus 40(SV40)-infected monkey kidney cells indicated the extensive transcription of both DNA strands. These symmetrically transcribed sequences were localized in the nucleus of infected cells, whereas only the "true" early and late SV40 transcripts were found in the cytoplasm. These results suggest that selective posttranscriptional degradation and/or transport occurs after transcription of the viral DNA. On the basis of hybridization experiments with cytoplasmic RNA and the separated strands of the SV40 *Hin* fragments, the early SV40 region appears to include all of *Hin* fragments A, H, I, and B (48% of the genome), whereas the late region is represented in *Hin* fragments C, D, E, K, F, J, and G (52% of the genome).

The lytic cycle of simian virus 40 (SV40) in monkey kidney cells is characterized by early and late phases. Prior to viral DNA synthesis, viral RNA complementary to the minus (E) strand of SV40 DNA can be detected; subsequent to viral DNA replication, late SV40-specific RNA complementary to the plus (L) strand is synthesized (10, 12, 14, 19). From hybridization studies employing ^{32}P-labeled separated strands of the 11 *Haemophilus influenzae* fragments of SV40 DNA, and total infected cellular RNA, a transcriptional map of the SV40 genome was constructed. The template for "early" RNA synthesis was shown to extend from 0.26 to 0.59 map units on the minus strand; the late region of the viral genome encompassed sequences present in two-thirds of the plus DNA strand (0.59 to 0.26 map units) (13). Similar findings were subsequently obtained by others (20, 22). Whereas these studies indicated the general positions of the early and late regions in SV40 DNA, some of the experimental results suggested that viral DNA transcription was a complex process.

The significant reaction (35 to 46%) between total late lytic cellular RNA and the plus strands of *Hin* fragments H and I (which are situated in the middle of the early gene region) was difficult to explain, as was the inability to saturate either strand of *Hin* H or I with late lytic total cellular RNA. These results, as well as the findings of Patch et al. (17), raised the possibility that the early template was discontinuous and contained sequences that were expressed after viral DNA synthesis. The low, yet significant levels of annealing between (i) early SV40 RNA and the plus strands of all *Hin* fragments (6 to 21%), (ii) between late RNA and the plus strands of the early fragments H and I (35 and 46%), and (iii) between late RNA and the minus strands of the late *Hin* fragments (7 to 46%) suggested that extensive symmetrical transcription of SV40 DNA occurred in vivo as originally proposed by Aloni (1, 2).

In the present study we have examined the viral-specific RNA present in the nucleus and the cytoplasm of SV40-infected cells. Our results indicate that the early gene region encompasses nearly one-half of the viral DNA. We have also detected the presence of large amounts of stable, symmetrically transcribed viral RNA which is confined to the nucleus of infected cells. In addition, we present evidence to suggest that modulation of viral gene activity may involve the selective degradation or transport of specific SV40 RNA molecules from the nucleus to the cytoplasm; this mechanism does not appear to rely solely on the degradation of "anti-sense" transcripts within the nucleus.

As in our previous experiments (13), we hybridized the separated strands of ^{32}P-labeled *Hin* fragments of SV40 DNA (specific activity, 3 $\times$ 10^5 to 6 $\times$ 10^5 counts/min per μg) to RNA, isolated from BSC-1 cells 48 h after infection (multiplicity of infection, 20 to 50 PFU/cell) with SV40 (strain 776). Infected cell nuclear and cytoplasmic RNAs were prepared after treatment of cells with 0.5% NP40 as previously described (18). The cytoplasmic fraction contained approximately four to six times more

RNA than the nuclear fraction. Reaction mixtures containing 0.5 to 2.0 ng of the separated strands of either fragmented ^{32}P-labeled SV40 DNA or SV40 *Hin* fragments, and varying concentrations of late lytic cytoplasmic or nuclear RNA from SV40-infected BSC-1 cells, were incubated for 36 h at 68 C in 1.0 M NaCl, 0.02 M phosphate buffer. The percent of ^{32}P-labeled DNA in hybrid molecules was then assayed by hydroxyapatite chromatography (12).

The annealing of cytoplasmic late lytic RNA to fragmented ^{32}P-labeled plus and minus SV40 DNA strands is shown in the first panel of Fig. 1. From the plateau values obtained, we conclude that 45 to 50% of the minus DNA strand and 50 to 55% of the plus DNA strand is complementary to stable species of cytoplasmic viral-specific RNA. Furthermore, the kinetics of these reactions suggest that RNA homologous to the plus strand of SV40 DNA is significantly more abundant in the cytoplasm (30- to 50-fold) than is the RNA homologous to the minus DNA strand (see also ref. 11). Panels A through K (Fig. 1) depict the kinetics of hybridization of cytoplasmic late lytic SV40 RNA with the plus and minus strands of each of the 11 SV40 *Hin* fragments. Late cytoplasmic RNA readily saturates the plus strands of *Hin* fragments C, D, E, F, G, J, and K under the hybridization conditions employed. The reaction of late cytoplasmic RNA with the plus strand of fragments *Hin* A, B, H, and I, on the other hand, is very low and may reflect a low level of contaminating nuclear RNA in the cytoplasmic extract. Since the late functions are represented on the plus strand (12, 19) of SV40 DNA, the contiguous *Hin* fragments C, D, E, F, G, J, and K comprise the late region of the viral genome, as was previously reported (13, 20). The minus strands of *Hin* fragments A, H, I, and B react almost completely with cytoplasmic SV40 RNA, indicating that these fragments comprise the early gene region. No significant hybridization was detected to the minus strands of *Hin* fragments C, D, E, F, G, J, and K. The kinetics of hybridization of late cytoplasmic RNA to the minus strands of *Hin* fragments A, H, I, and B also confirm our previous observation that early transcripts are present in significantly lower concentrations than the late viral RNA sequences.

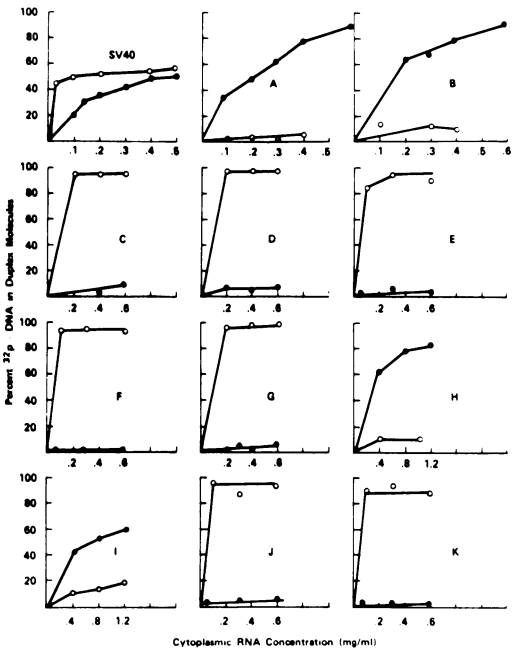

FIG. 1. *Hybridization of late lytic cytoplasmic RNA to the separated strands of SV40 DNA. Increasing amounts of cytoplasmic RNA from BSC-1 cells obtained 48 h after infection with SV40 were incubated with small amounts (0.5 to 2.0 ng) of ^{32}P-labeled fragments of either the plus* (○) *or minus* (●) *SV40 DNA strand. After 36 h, the reaction mixtures were analyzed by hydroxyapatite chromatography to determine the percent DNA in hybrid molecules. The first panel shows the results of hybridization to fragments of the entire plus or minus DNA strand. Panels A-K show the reactions with the separated strands of each of the 11 SV40 Hin fragments.*

The reaction of late lytic nuclear RNA with the separated plus or minus strand fragments of SV40 DNA is shown in the first panel of Fig. 2. Nearly all of the plus DNA strand (85% as measured by hydroxyapatite) is transcribed into a stable species of nuclear RNA; this is confirmed by the extensive reactions between nuclear RNA and the plus strands of all 11 *Hin* fragments (Fig. 2A-K). Hybridization between nuclear RNA and the minus strand of SV40 DNA was less extensive (40 to 50%; first panel, Fig. 2); the reaction observed can be localized as hybridization between the nuclear RNA and the minus strands of *Hin* fragments A, B, H, I, and C. The lack of significant reaction between nuclear RNA and the minus strands of the other *Hin* fragments (Fig. 2) could reflect possible RNA-RNA interactions between "anti-late" RNA species and the more abundant true late sequences, effectively reducing the reaction with the radiolabeled DNA probe to subdetectable levels.

The results of these experiments lead to several conclusions regarding the in vivo transcription of SV40 DNA: (i) late in the lytic cycle, there is extensive transcription of anti-

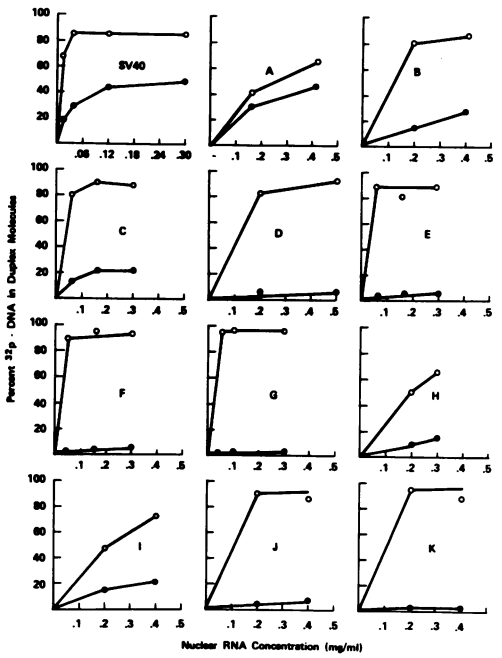

FIG. 2. *Hybridization of late lytic nuclear RNA to the separated plus* (○) *and minus* (●) *strands of* ^{32}*P-labeled SV40 DNA fragments. (See Fig. 1 and text for details.) The first panel shows the results of hybridization to fragments of the entire plus or minus DNA strands. Panels A-K represent similar reactions with the separated strands of each of the 11 SV40 Hin fragments.*

sense sequences on the plus (late) DNA strand and perhaps the minus (early) DNA strand in the nucleus of SV40-infected cells. This result is in agreement with the findings of Aloni (1, 2), who showed that a large portion of pulse-labeled SV40-specific RNA self-anneals. (ii) The SV40-specific RNA present in the cytoplasm of infected cells subsequent to viral DNA synthesis is complementary to the minus strands of *Hin* fragments A, H, I, and B and the plus strands of fragments C, D, E, F, G, J, and K only (Fig. 3), in agreement with earlier analyses of polysomal (20) or cytoplasmic (11) SV40 RNA. Findings (i) and (ii) suggest that processing and/or selection occurs, controlling the RNA species which appear in the cytoplasm. The kinetics of hybridization between nuclear RNA and the plus and minus strands of *Hin* fragments A, H, I, and B indicate that "anti-early" RNA is present in significantly greater concentrations than "true" early viral RNA transcripts in the nucleus (Fig. 2). If viral gene expression were only controlled at the translational level, one would expect to find viral-specific RNA complementary to the plus strands of *Hin* fragments A, H, I, and B (anti-early RNA) in both the nucleus and cytoplasm. Our results show that anti-early SV40 RNA is present only in the nucleus of infected cells. This suggests the existence of a selective, posttranscriptional control mechanism which ensures that only the true early and late RNA transcripts appear as the stable, abundant class of cytoplasmic viral RNA. The presence of large amounts of anti-sense RNA in the nucleus of infected cells indicates that little, if any, degradation of this RNA occurs within the nucleus. Rapid digestion of anti-sense SV40-specific RNA could occur at the nuclear membrane or within the cytoplasm, however. Alternatively, regulation could involve the selective transport of true early and late RNA transcripts from the nucleus to the cytoplasm or the selective retention of anti-sense RNA species within the nucleus. It should be pointed out that we have not necessarily demonstrated a precursor-product relationship between the RNA molecules containing the anti-sense sequences and those true early and late SV40 transcripts which eventually become associated with the polysomes. The possibility exists, for example, that sense and anti-sense viral RNA species are each transcribed as discrete classes of RNA. If only the true early and late SV40 RNAs are transported into the cytoplasm and enter polyribosomes, regulation could be effected without significant alteration of the primary RNA transcript. The possibility that polyadenylation could in some way be responsible for the selection of those

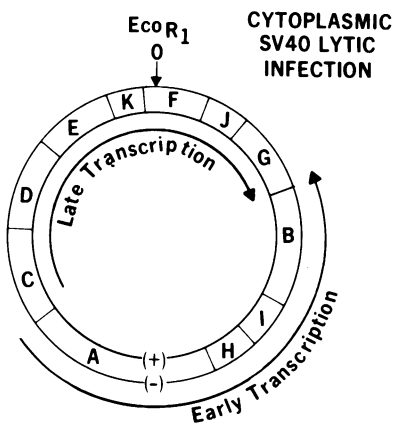

FIG. 3. *A map of the SV40 genome indicating the locations of the early and late gene regions based on the results of the present study. It should be noted that the junctions of the early and late templates are very close to, if not coincident with, the Hin AC and Hin BG junctions. Arrows indicate the direction of transcription (see ref. 13 and 20).*

transcripts eventually transported to the cytoplasm is presently under investigation. (iii) It is clear from the above results that the early region of SV40 DNA is larger than was previously thought (13, 20). On the basis of annealing experiments with cytoplasmic SV40 RNA (Fig. 1), it encompasses sequences corresponding to most, if not all, of *Hin* fragments A, H, I, and B (approximately 48% of the genome extending from 0.175 to 0.655 [Fig. 3]). A comparable proportion of polyoma DNA is expressed prior to the onset of viral DNA synthesis (9). The locations of the early and late gene regions on the physical map of SV40 DNA are in good agreement with results obtained by Dhar et al., who have sequenced terminal nucleotides of viral-specific RNA (6, 7). Our previous under-estimate of the early gene region most likely resulted from our use of total cellular rather than cytoplasmic RNA in nucleic acid hybridization assays (13). The substantial quantity of stable, SV40-specific RNA, which is complementary to the plus (late) strand of early *Hin* fragments (and which we now know is confined to the nucleus [Fig. 1 and 2]) can readily form RNA-RNA duplex structures with "true early" RNA. Such interaction would reduce the amount of true early RNA available for reaction with the ^{32}P-labeled DNA probe and lead to a low estimate of the early gene region. In our previous study we were also concerned with our inability to fully saturate *Hin* fragments H, and particularly I, with lytic RNAs (13). This now appears to be due to the contamination of fragments *Hin* H and particularly *Hin* I with significant amounts of fragment *Hin* J (unpublished data). This contamination can be largely eliminated by a second cycle of polyacrylamide gel electrophoresis, but there is still some difficulty in fully saturating *Hin* I with lytic RNA. Whereas our present results indicate no discontinuity in the early region of the viral genome, we cannot rule out the possibility of a small silent region. The present estimate of the early gene region is 46 to 49% of the genome, and this segment of DNA provides sufficient coding capacity for a protein of 70,000 to 100,000 daltons. This is of particular interest in light of recent studies on the isolation and characterization of SV40 T antigen (3, 5, 15, 16, 21); some of these studies suggest that T antigen is a viral protein in this size range (3, 5, 21).

This study provides a more precise location of the early and late regions of the SV40 genome. The proximal junction of these segments lies very close to the *Hin* AC junction and, therefore, very close to the initiation site for DNA replication (4, 8). Whereas the relationship between these sites is presently unknown, it seems likely that their proximity may have some functional significance.

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