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Cessation of Reentry of Simian Virus 40 DNA into Replication and Its Simultaneous Appearance in Nucleoprotein Complexes of the Maturation Pathway

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Newly synthesized SV40 DNA is used as a template for further DNA synthesis (reenters replication) or as a substrate in the assembly of virions (maturation pathway). The time courses of reentry into replication and progression along the maturation pathway were both determined on identical samples. DNA, synthesized during a 20-min pulse, reentered replication over a period of several hours and then was removed from the pool of molecules available for replication. The cessation of reentry coincided with the maturation of this DNA from the chromatin form to previrion and virion forms. More reentry and less maturation was observed at 24 h postinfection than at 42 h postinfection. The data are consistent with the hypothesis that the factor(s) responsible for cessation of reentry is also responsible for initiation of the maturation pathway.

The steps in the maturation (encapsidation) pathway of simian virus 40 (SV40) are currently under investigation in several laboratories (1, 3, 4, 6-8, 11). New extraction procedures have revealed that (i) the majority of newly synthesized SV40 DNA is encapsidated and that (ii) several species of nucleoprotein complexes (NPCs) can be isolated (1, 3, 6-8). Pulse-chase experiments suggest that labeled SV40 DNA is converted from chromatin (NPC-I) to previrions (NPC-II) to mature virions (1, 3, 6-8). NPC-I is composed of DNA and cellular histones H1, H2a, H2b, H3, and H4 (3, 7). It is used for biosynthetic processes and has associated with it SV40 T antigen (13, 19, 23), the DNA-binding protein involved in initiation of viral DNA synthesis (14, 24, 25, 27) and regulation of viral transcription (18, 20, 26), and cellular proteins involved in these processes (for examples, see references 5, 15). NPC-II is a heterogeneous set of complexes grouped together because they sediment more rapidly than NPC-I under neutral conditions and yet are salt labile. NPC-II is composed of DNA, histones with a higher degree of acetylation than NPC-I, and viral structural proteins (3, 4, 11). Mature virions are salt stable and contain DNA, all histones except H1, and viral structural proteins (3, 12, 16). The order of addition of viral proteins to an NPC has not been determined.

Although these studies shed light on the steps involved in virus assembly, little is known of the factor(s) determining the fate of newly synthesized DNA. Roman and Dulbecco (22) showed that newly synthesized polyoma DNA can serve as a template for further DNA synthesis (reenter replication) over a period of 2 to 3 h and then is removed from the pool of molecules available for replication. Green and Brooks (9) reported similar kinetics of reentry of newly synthesized SV40 DNA into replication. Recently, Roman reported that polyoma DNA labeled at 20 h postinfection (p.i.) reentered replication at a greater rate than molecules labeled at 28 h p.i. and that a greater proportion of molecules labeled at 20 h p.i. reentered replication than at 28 h p.i. (21). These data support the hypothesis of Roman and Dulbecco that the rate of reentry is a reflection of the availability of proteins required for initiation of DNA synthesis (22). Regardless of the time p.i. examined, however, progeny molecules appeared to be removed from the pool available for replication about 3 h after their initial synthesis. A role for maturation proteins was postulated by Roman and Dulbecco (22) and Roman (21). Similarly, Garber et al. (8) and Baumgartner et al. (1) compared their determinations on the rate of encapsidation of DNA with the published data on the kinetics of reentry of DNA into replication (9, 21, 22), and postulated an association between the two pathways.

The research reported here was conducted to determine, on identical samples, the kinetics of reentry of newly synthesized DNA into replication and the kinetics of maturation of this same DNA to establish the temporal relationship of

the two pathways to each other. Since Roman had previously reported that the kinetics of reentry were dependent upon the time in the infectious cycle examined (21), the kinetics of the two pathways were also determined at 24 and 42 h p.i. Cessation of reentry into replication of DNA labeled during a 20-min pulse was found to coincide with the maturation of this DNA from chromatin to previrions and virions. The rate and extent of reentry decreased with time p.i. whereas the rate of encapsidation increased.

MATERIALS AND METHODS

Cells and virus. BSC-1 cells, a gift from M. Singer, were grown in Dulbecco modified Eagle medium containing 10% calf serum and 50 μg of gentamycin per ml. SV40, a gift from W. Eckhart, was plaque purified three times and propagated at a low (0.05) multiplicity of infection. Virus stocks were prepared from infected cells by three cycles of freezing and thawing followed by low speed centrifugation and they were stored at −20°C.

Labeling and extraction of viral DNA and NPCs. Confluent BSC-1 cells were infected with SV40 at multiplicity of infection of 10 PFU per cell. At different times after infection, the cells were rinsed two times with TD buffer (150 mM NaCl, 5 mM KCl, 0.7 mM Na_3HPO_4, and 20 mM Tris, pH 7.4), and labeled for 20 min with 75 or 150 μCi of [^{3}H]thymidine (specific activity, 40 to 60 Ci/mmol) per ml in Dulbecco modified Eagle medium containing 2% serum and 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.0). Cells were then rinsed twice with TD buffer, covered with Dulbecco modified Eagle medium containing 10^{-6} M thymidine, 2% serum and 25 mM HEPES for 30 min, and then chased with bromodeoxyuridine (BUdR) chase medium [5×10^{-5} M 5-bromodeoxyuridine, 2×10^{-5} M 5-fluorodeoxyuridine (28), 9×10^{-6} M deoxycytidine (2, 17), 2% serum, and 25 mM HEPES in Dulbecco modified Eagle medium].

After various lengths of chase, DNA was extracted from half of the plates by the method of Hirt (10). The Hirt supernatant was used to determine the kinetics of reentry.

NPCs were extracted from infected cells by the method of Fernandez-Munoz et al. (7). Cells were rinsed twice with cold TBS buffer (TD buffer containing 0.9 mM CaCl and 1 mM $MgCl_2$) and scraped into cold hypotonic buffer (25 mM Tris, pH 7.9, 1 mM $MgCl_2$, and 0.4 mM $CaCl_2$). The cells were broken by homogenization (30 strokes) in a tightly fitting glass Dounce homogenizer. The lysates were centrifuged at 2,000 rpm for 5 min. The NPC-containing supernatant was used to determine the kinetics of maturation. Similar results were obtained if the cells were rinsed with TD buffer or extracted with hypotonic buffer containing 0.5 M dithiothreitol and phenylmethylsulfonylfluoride.

The efficiency of extraction of ^{3}H-labeled DNA in the form of NPCs was determined by comparing the total ^{3}H counts per minute (cpm) in unfractionated NPCs to the total ^{3}H cpm in DNA extracted by the method of Hirt. The efficiency of extraction of NPCs was approximately 70% that of DNA. During the 6-h chase, no decrease in the ^{3}H cpm was observed, suggesting that essentially all of the prelabeled DNA remained cell associated.

Analysis of viral DNA. Form I DNA was separated from form II and replicative-intermediate DNAs by layering Hirt supernatants onto 5 to 20% alkaline sucrose gradients containing 0.75 M NaCl, 1 mM EDTA, and 0.3 M NaOH (pH 12.6). The samples were centrifuged in an SW41 Ti rotor at 39,000 rpm for 2.6 h. Fractions were dripped from the bottom of the centrifuge tubes, and the radioactivity in a sample was determined after precipitation with 5% trichloroacetic acid. Fractions containing form I were pooled, neutralized, and mixed with CsCl in TE buffer (10 mM Tris, pH 7.4, 1 mM EDTA) to give a final density of 1.72 g/ml. The samples were centrifuged in a type 40 rotor at 33,000 rpm for 48 h to separate form I DNA with BUdR substitution in one strand (HL DNA) from unsubstituted form I DNA (LL DNA). The percentage of ^{3}H-labeled HL form I DNA [(HL DNA cpm + HL DNA cpm + LL DNA cpm) × 100] was determined.

In some experiments, the same protocol was used to determine the percentage of labeled HL DNA in the slowly sedimenting DNA (form II, RI, cellular) of the initial alkaline sucrose gradient. When mentioned, the percentage of labeled HL DNA in the Hirt supernatant was also determined in CsCl.

Analysis of SV40 NPCs. The NPC supernatants were layered onto 10 to 40% sucrose gradients containing 2 mM Tris (pH 7.4) and spun in an SW41 Ti rotor at 37,000 rpm for 90 min. Fractions were dripped from the bottom and processed as described above. The percentages of NPC-I and of NPC-II+V were determined by the formulas %(NPC-I) = (NPC-I cpm + total NPCs cpm) × 100 and %(NPC-II+V) = (NPC-II+V cpm + total NPCs cpm) × 100, respectively. Fractions from the NPC-II+V region of the gradient were pooled, and salt-labile NPC-II was separated from salt-stable virions by density gradient equilibrium centrifugation in CsCl (ρ = 1.34 g/ml in TBS). The samples were spun in a type 40 rotor at 33,000 rpm for 40 h. Fractions were dripped from the bottom and processed as described above. The percentage of virions was calculated by the formula (V cpm + NPC-II+V cpm) × %(NPC-II+V). Similarly, the percentage of NPC-II was equal to (NPC-II cpm + NPC-II+V cpm) × %(NPC-II+V). Where noted, the different NPCs were pooled, and the DNA was extracted by the method of Hirt (10) and subjected to density gradient equilibrium centrifugation as described above to determine the percentage of HL DNA in each NPC.

RESULTS

Location of NPCs. Several laboratories have reported the sedimentation and buoyant density characteristics of chromatin (NPC-I), previrions (NPC-II), and virions (1, 3, 6–8). Initial experiments were conducted to locate these complexes in sucrose and CsCl gradients. SV40-infected BSC-1 cells were labeled for 20 min with [^{3}H]-thymidine at 33 h p.i. NPCs were extracted, at

the end of the pulse or after a 6-h chase with 10^{-6} M thymidine, and separated on 10 to 40% sucrose gradients (Figure 1A and 1B, respectively). With a 12-min pulse, only the slower sedimenting species of NPC was labeled (data not shown). The labeling kinetics and location of this species in the gradient identify it as NPC-I (1, 3, 6–8). The more rapidly sedimenting species contained NPC-II+V. This was verified by pooling the fractions (Fig. 1A and B) and subjecting the complexes to equilibrium density gradient centrifugation in CsCl (ρ = 1.34 g/ml) (Fig. 1C and D). Whereas the NPCs located in the NPC-II+V region after a 20-min pulse were predominantly salt-labile NPC-II, approximately 50% were salt stable after a 6-h chase. The sucrose gradients were used to quantitate the distribution of label between NPC-I and NPC-II+V. When necessary, NPC-II and virions were further separated on CsCl. It should be noted that, on the CsCl gradients, a new NPC was detected which had a greater buoyant density than mature virions. This complex was separable from both NPC-II and mature virions and appeared to be an intermediate in the maturation pathway and not a breakdown product of virions

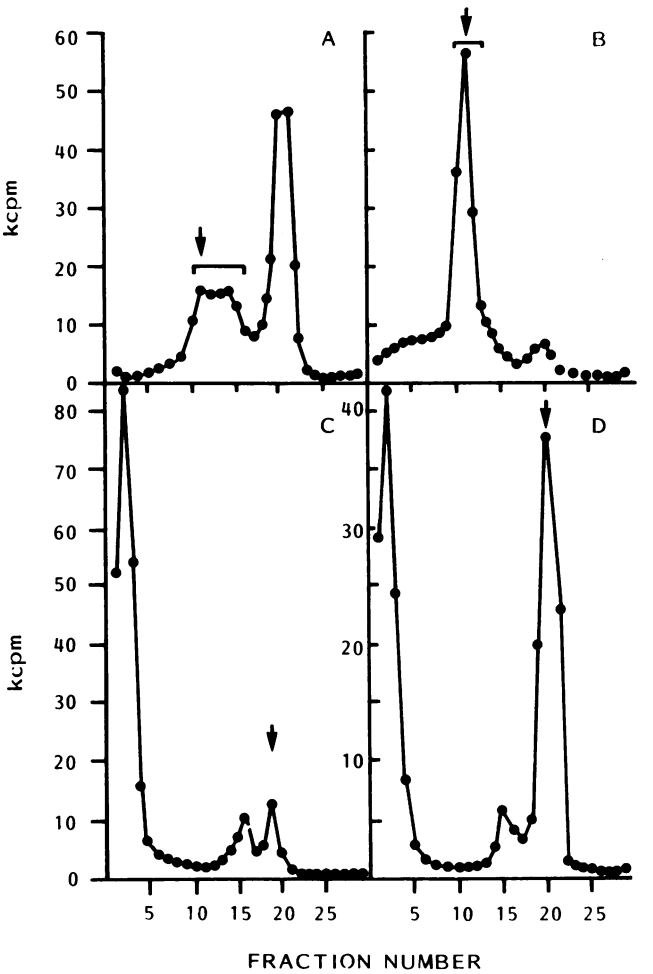

FIG. 1. *Separation of NPCs on sucrose and CsCl gradients. SV40-infected BSC-1 cells were labeled for 20 min at 33 h p.i. with 75 μCi of [^{3}H]thymidine per ml (ca. 40 to 60 Ci/mmol). NPCs were extracted from some cells after the pulse (A and C), from others after a 6-h chase in 10^{-6} M thymidine (B and D). NPC-containing supernatants were sedimented on 10 to 40% sucrose gradients containing 2 mM Tris (pH 7.4) (A and B). Gradients were centrifuged for 90 min at 37,000 rpm in an SW41 Ti rotor. Sedimentation is from right to left. Fractions were pooled as shown and banded to equilibrium in CsCl (ρ = 1.34 g/ml) in a type 40 rotor at 33,000 rpm for 40 h (C and D). The arrow represents the position of the majority of complexes labeled in a 12-h pulse. The density of this material is 1.34 g/ml.*

(data not shown); it was named NPC-III. In the quantitation of the percentage of newly synthesized DNA in chromatin, previrions, and virions, the cpm in NPC-III were added to NPC-II, since both are previrions.

Effect of BUdR substitution on encapsidation. The protocol for following reentry of newly synthesized DNA into replication involves the use of BUdR. Thus, it was necessary to determine the effect of BUdR on the process of encapsidation. At 36 h p.i., 4 plates of SV40-infected BSC-1 cells were labeled for 20 min with [^{3}H]thymidine and chased with 10^{-6} M thymidine for 30 min. NPCs were extracted from two plates. A third was rinsed and covered with Dulbecco modified Eagle medium containing BUdR chase medium. The remaining plate was rinsed, and Dulbecco modified Eagle medium with 10^{-6} M thymidine was added. NPCs were extracted from the latter two plates 6 h later. The percentage of NPC-II+V extracted from all four plates was determined on 10 to 40% sucrose gradients. BUdR substitution did not affect the extent of accumulation of NPC-II+V during the 6-h chase (Table 1). These two species of NPCs were further separated on CsCl. Virions accumulated to a similar extent under the two chase conditions (Table 1).

Comparison of the kinetics of maturation and kinetics of reentry. To directly compare the time course of maturation to that of reentry, at 33 h p.i., SV40-infected BSC-1 cells were pulse-labeled for 20 min with [^{3}H]thymidine (75 μCi/ml), chased for 30 min with 10^{-6} M thymidine, and chased again with the BUdR chase medium for different lengths of time. The kinetics of maturation of this pulse-labeled DNA was determined by plotting the percentages of labeled NPC-I, NPC-II, and virions versus BUdR chase time (Fig. 2). At the end of the pulse and 30-min chase with 10^{-6} M thymidine (T = 0), the majority of [^{3}H]DNA was in NPC-I; very little was in virions. As the BUdR chase time increased, the percentage of [^{3}H]DNA in NPC-I decreased, with the rate of decrease being greatest during the first 2 to 3 h after initiation of the BUdR chase. There was a concomitant increase in the fraction of [^{3}H]DNA appearing in virions. The fraction of [^{3}H]DNA in NPC-II remained relatively constant during the chase period (Fig. 2). The level of the NPC-II pool varied from experiment to experiment (compare Fig. 2A and B; similar variations in levels of NPCs have been seen by others [6]). Within any one experiment, the level of NPC-II was the same in the presence or absence of dithiothreitol and phenylmethylsulfonylfluoride. Therefore, it seems unlikely that this variation is due to breakdown of virions.

TABLE 1. *Effect of BUdR on the extent of encapsidation of viral chromatin*

Chase medium	%(NPC-II+V)[a] at:		%(V)[b] at:	
	0[c]	6	0	6
BUdR	42	89	7	45
10^{-6} M thymidine	43	91	8	46

[a] The percent of NPC-II+V was determined from separation on 10 to 40% sucrose by using the formula (NPC-II+V cpm + total cpm of NPCs) × 100.

[b] The percent of virions (V) was determined after the separation of NPC-II from V by CsCl density equilibrium centrifugation by using the formula %(NPC-II+V) × (V cpm + NPC-II+V cpm).

[c] Chase time in hours. SV40-infected BSC-1 cells were labeled for 20 min with [^{3}H]thymidine and chased for 30 min with 10^{-6} M thymidine. NPCs were either extracted at this time (0) or after a 6-h chase in the medium shown (6).

To determine the reentry kinetics for newly synthesized DNA during the BUdR chase, DNA was extracted by the Hirt method, form I DNA was isolated on alkaline sucrose gradients, and HL DNA and LL DNA were separated by CsCl density equilibrium gradient centrifugation. ^{3}H-labeled HL DNA represented DNA that replicated during the short ^{3}H pulse and was subsequently used as template for further DNA synthesis (reentered replication). The percentage of HL DNA plotted versus chase time with BUdR showed the kinetics of reentry of the pulse-labeled DNA into replication (Fig. 3). It should be noted that only one round of reentry was detected by the protocol since form I DNA with BUdR substitution in both strands (HH DNA) would not be labeled. LL DNA reentered replication (was converted to HL DNA) over a period of 2 to 3 h. After this length of chase, either the pulse-labeled DNA ceased to reenter replication or the rate at which it reentered greatly decreased.

Also plotted in Fig. 3, for direct comparison with the reentry kinetics, is the rate at which [^{3}H]DNA left the NPC-I pool, calculated from the data in Fig. 2. A good correlation may be seen between the time that pulse-labeled DNA was removed from the pool of molecules available for replication and the time it left the NPC-I pool.

The NPC data presented in Fig. 2 represent the fate of all pulse-labeled DNA, whereas the reentry data in Fig. 3 represent only the fate of form I DNA (containing 85 to 89% of the ^{3}H cpm) in a Hirt supernatant. The reentry kinetics was, therefore, determined directly on a sample of a Hirt supernatant and in parallel on the form

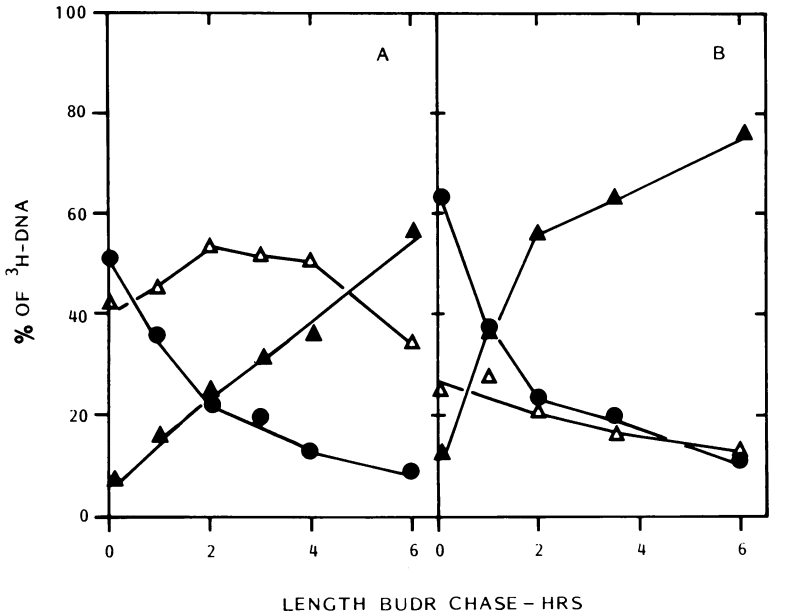

FIG. 2. *Relationship between the length of BUdR chase and percentage of [^{3}H]DNA in NPC-I, NPC-II, and virions. (A and B) Results of representative experiments. NPCs were isolated as described in the text. Symbols: ●, percentage of NPC-I; △, percentage of NPC-II; ▲, percentage of virions.*

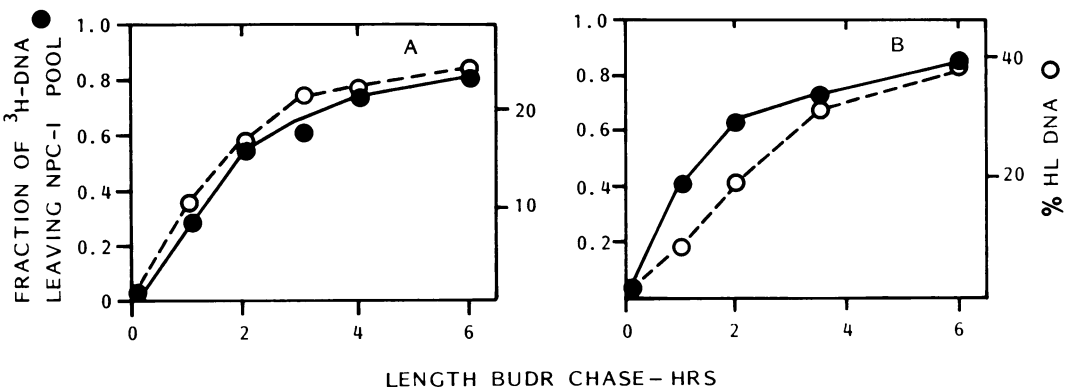

FIG. 3. *Relationship between the fraction of the initial [^{3}H]DNA in NPC-I leaving the NPC-I pool and the percentage of ^{3}H-labeled HL DNA (kinetics of reentry) with increasing chase time in BUdR. (A and B) Data from the same experiments shown in Fig. 2A and B. To calculate the fraction of [^{3}H]DNA leaving the NPC-I pool during the BUdR chase, the total percentage of NPC-I at the time BUdR was added (e.g., 51% at T = 0; Fig. 2A) was set equal to 1.0. The fraction of [^{3}H]DNA leaving the NPC-I pool is determined by the formula 1 − [%(NPC-I$_{T=X}$) ÷ %(NPC-I$_{T=0}$)], in which X and 0 are the lengths of the BUdR chase. The percentage of ^{3}H-labeled HL DNA was determined after separation of form I HL and LL DNA molecules by CsCl density equilibrium centrifugation as described in the text.*

I DNA isolated from that supernatant. The kinetics of reentry was qualitatively similar to that determined for form I DNA (Table 2). The percentage of HL DNA, however, was lower than that found when only form I DNA was analyzed. This was expected, since the non-form I DNA isolated on alkaline sucrose and analyzed on CsCl contained only ^{3}H-labeled LL DNA (data not shown).

Distribution of ^{3}H-labeled HL DNA in NPC-I, NPC-II, and virions. To determine more precisely the relationship of reentry to maturation, the percentages of ^{3}H-labeled HL DNA in NPC-I and in NPC-II+V were deter-

mined. SV40-infected cells were pulse-labeled and chased for various lengths of time as described above. NPC-I and NPC-II+V were separated on 10 to 40% sucrose gradients, and the percentage of HL DNA was determined on each pool. In parallel, the percentage of HL DNA in form I isolated from a Hirt supernatant was determined. Figure 4 shows the accumulation of HL DNA in NPC-I, NPC-II+V, and form I. ^{3}H-labeled HL DNA accumulated in NPC-I for a period of 2 to 3 h then ceased to accumulate, a pattern qualitatively similar to the kinetics of reentry determined on form I DNA. ^{3}H-labeled HL DNA accumulated more slowly and to a lesser extent in NPC-II+V. It is possible to calculate the reentry kinetics curve for total NPCs by taking into account the relative contribution of each NPC pool to the total NPCs extracted at each time. This curve is very similar to that found by analyzing form I DNA (Fig. 4). Thus, the kinetics of reentry established by analysis of form I DNA is directly comparable to the kinetics of maturation.

Comparison of the kinetics of reentry and maturation at 24 h p.i. versus 42 h p.i. Roman (21) demonstrated that the kinetics of reentry of polyoma DNA was dependent upon the time in the infectious cycle that was examined. Early in the replication cycle, reentry occurred more rapidly and to a greater extent than at later times. If the cessation of reentry is related to a step in the maturation pathway, the kinetics of maturation should also be different at different times. Therefore, the kinetics of reentry and maturation were determined at 24 and 42 h p.i. (Table 3). More extensive reentry was seen at 24 h p.i. than at 42 h p.i. When cells were harvested after a 20-min pulse and 30-min chase (T = 0), the percentage of the NPC-II+V at 42 h p.i. was twice that seen at 24 h p.i. Therefore, pulse-labeled DNA proceeds along the maturation pathway more rapidly later in infection.

TABLE 2. *Kinetics of reentry determined on isolated form I DNA or unfractionated Hirt supernatants*[a]

HL DNA source	% of HL DNA at:		
	0[b]	2	6
Form I	0	17	26
Hirt supernatant	0	15	24

[a] At 36 h p.i., SV40-infected cells were pulse-labeled with [^{3}H]thymidine, chased with 10^{-6} M thymidine, and chased again for different lengths of time with a BUdR chase mixture. DNA was isolated by the Hirt method (10). Either a sample from the Hirt supernatant was directly analyzed by CsCl density equilibrium centrifugation, or form I DNA isolated from the Hirt supernatant was analyzed.

[b] Chase time in hours.

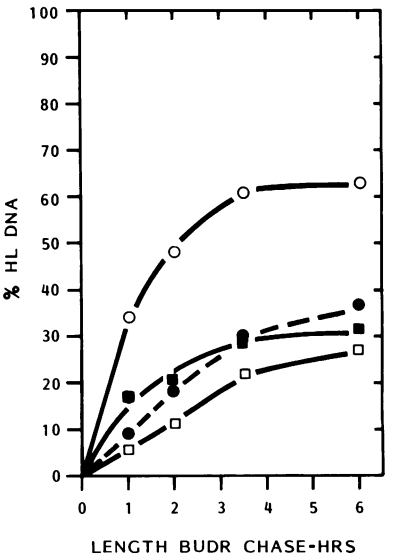

FIG. 4. *Percentage of ^{3}H-labeled HL DNA in NPC-I, NPC-II+V, total NPC, and form I DNA. Pools of NPC-I and NPC-II+V, as shown in Fig. 1, were deproteinized, put into CsCl gradients (ρ = 1.72 g/ml) and centrifuged for 48 h at 33,000 rpm in a type 40 rotor to separate ^{3}H-labeled HL DNA from ^{3}H-labeled LL DNA. Symbols: ○, Percentage of HL DNA in NPC-I; □, percentage of HL DNA in NPC-II+V; ●, percentage of HL DNA in form I; ■, calculated percentage of HL DNA in total NPC. The percentage of HL DNA in total NPC at each time point was calculated by the formula [%(HL DNA in NPC-I) × (NPC-I cpm ÷ total NPC cpm)] + [%(HL DNA in NPC-II+V) × (NPC-II+V cpm ÷ total NPC)].*

TABLE 3. *Kinetics of reentry and maturation of DNA pulse-labeled at 24 and 42 h p.i.*

Time p.i. (h)	% of HL DNA at:			% of NPC-II+V at:		
	0[a]	2	6	0	2	6
24	0	28	45	25	51	80
42	0	16	31	50	64	80

[a] Chase time in hours.

The percentage of NPC-II+V was the same (80%) after a 6-h BUdR chase at the two times; the 20% of [^{3}H]DNA which remained in NPC-I may be either unavailable for packaging or inefficiently packaged.

DISCUSSION

Experiments were undertaken to determine whether the cessation of reentry of newly synthesized DNA into replication coincided with the progression of that DNA along the maturation pathway. SV40-infected BSC-1 cells were labeled for a short period with [^{3}H]thymidine and then chased with BUdR. The use of newly

synthesized DNA as the template for further DNA synthesis (reentry into replication) was measured by the conversion of ^{3}H-labeled LL DNA to ^{3}H-labeled HL DNA (Fig. 3, Table 3). In parallel, its progression along the maturation pathway was monitored by determining the percentage of ^{3}H cpm in different NPCs (Fig. 2 and 3; Table 3). The time course of appearance of [^{3}H]DNA in NPC-I, NPC-II, and virions (Fig. 2) was consistent with previous reports that NPC-I progressed to virions through the intermediate NPC-II (1, 3, 6–8).

Two facts emerge from this study. First, cessation of reentry and maturation are closely linked in time. There do not appear to be factors that terminate reentry for any significant period of time before maturation. Cessation of reentry coincides with the maturation of DNA from NPC-I to NPC-II or virions (Fig. 3). The time of cessation of reentry and of the conversion of NPC-I to NPC-II+V varies somewhat from experiment to experiment, but the two are always coincident (Fig. 3). Second, newly synthesized DNA becomes encapsidated more rapidly late in the infectious cycle. This is consistent with the observation that the rate and extent of reentry decreases with time p.i. (Table 3). After a 6-h BUdR chase, 80% of the pulse-labeled DNA has matured to NPC-II+V, whether the experiment was initiated at 24 or 42 h p.i. (Table 3). However, when the pool of NPC-II+V was separated on CsCl, 24 and 59% of the [^{3}H]DNA was in the virion fraction at 24 and 42 h p.i., respectively (data not shown). This suggests that not only is there a slower progression of NPC-I to NPC-II+V early in infection but also that the progression from NPC-II to virions is slower.

We propose that the fate of a newly synthesized SV40 molecule is determined by whether it is bound first by a protein or proteins involved in replication (or transcription) or by a protein or proteins involved in maturation. This competition, between the binding of large T antigen (and perhaps other proteins involved in DNA or RNA synthesis) and a structural protein(s), could be affected by the relative abundance of early versus late proteins. The affinity of different proteins for the NPC-I template would also affect the outcome of the competition. Coca-Prados et al. (4) recently proposed that whether a DNA molecule was used for replication or maturation might depend on the extent of acetylation of histones, with structural proteins having a higher affinity for histones with a greater level of acetylation.

In summary, the data presented suggest a close correlation between removal of DNA from the pool of molecules available for replication and progression of this same DNA along the maturation pathway. The simultaneous analysis of the kinetics of reentry and maturation, using several mutants of SV40 defective in known viral proteins, should result in a determination of viral proteins involved in one or both of these processes and reveal essential details of their interdependence.

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