Supplementary Data

Materials and Methods

In vitro SV40 DNA replication

Reaction mixtures (25 μl) contained 0.5 μg SV40 large T antigen, 0.85 μg replication protein A (RPA), 0.5 μg pSLVD (1), 20 mM Hepes-NaOH, pH 7.4, 2 mM DTT, 3 mM ATP, 2 mM $MgCl_2$, 80 mM potassium acetate, and bovine serum albumin (0.2 mg/ml). After incubation at 37°C for 20 min, reaction mixtures were adjusted to 50 μl by adding 100 μg HeLa S100, 20 mM Hepes-NaOH, pH 7.4, 2 mM DTT, 12 mM CP, 1.25 μg CPK, 0.1 mM GTP/CTP/UTP, 0.1 mM dGTP/dCTP/dTTP, 10 μM dATP, and 20 μCi α-^{32}P-dATP (3,000 Ci/mmol). After incubation at 37°C for an additional 1.5 hr, de novo synthesized SV40 DNA was analyzed by agarose gel electrophoresis as follows. For agarose gel analysis, reaction mixtures were treated with SDS (0.5%) and proteinase K (0.25 mg/ml) and incubated for 30 min at 37°C. DNA was then isolated by conventional phenol/chloroform extraction followed by ethanol precipitation. Isolated DNA was treated with DpnI (10 units/reaction) and RNase A (0.2 μg/reaction) for 3 hr in the presence of 0.1 M NaCl. DNA was then resolved on a 0.7% agarose gel in 1X TAE, visualized by autoradiography, and quantified using a PhosphorImager.

Results

SV40 DNA replication was reconstituted using SV40 large T antigen, replication protein A (RPA), and pSLVD (1), and de novo synthesized SV40 DNA was analyzed by agarose gel electrophoresis, as described in Materials and Methods. As summarized in Supplementary

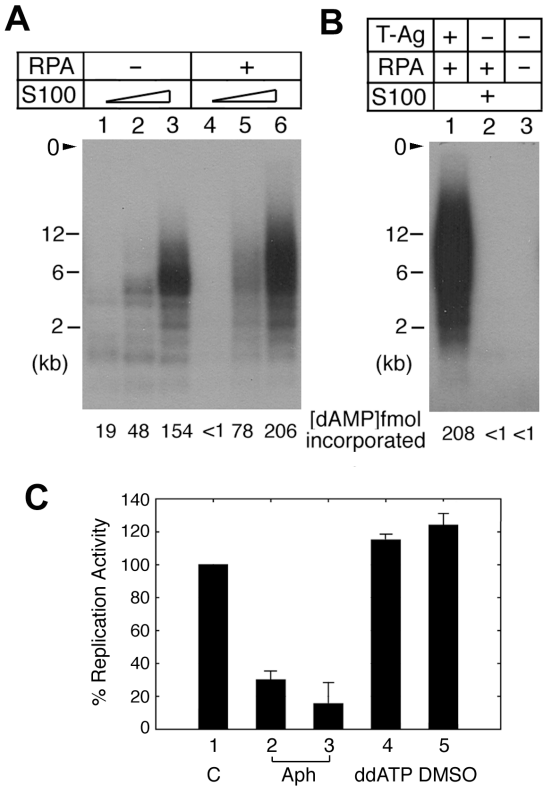
Fig. 1A, increasing amounts (20-80 μg) of S100 gradually stimulated SV40 DNA replication (lanes 1-3), which was further enhanced by the presence of RPA (lanes 4-6). Also, there were no detectable levels of SV40 DNA replication when SV40 large T antigen was omitted (Supplementary Fig. 1B). As expected (2), treatment with aphidicolin (20-100 μg/ml) severely inhibited SV40 DNA replication (Supplementary Fig. 1C, lanes 2-3), while the presence of excess ddATP (0.5 mM) over dATP (10 μM) did not affect SV40 DNA replication (Supplementary Fig. 1C, lane 4).

Figure Legend

Supplementary Figure 1. Effect of DNA polymerase inhibitors on SV40 DNA replication. (**A**) SV40 DNA replication was measured in the absence (lanes 1-3) or presence (lanes 4-6) of replication protein A (RPA or HeLa SSB) (0.85 mg), as described in Methods. Amounts of S100 added were as follows: lanes 1 & 4, 20 mg; lanes 2 & 5, 40 mg; lanes 3 & 6, 80 mg. (**B**) In vitro reactions for SV40 DNA replication were performed with the indicated replication factors and replication products were visualized by autoradiography and quantified by PhosphorImager. (**C**) Duplicate sets of reactions for SV40 DNA replication were carried out using the standard reaction mixture with the following modification. When the reaction mixtures were supplemented with S100 fraction after pre-incubation, aphidicolin (Aph, 20 or 100 mg/ml, lanes 2-3), ddATP (0.5 mM, lane 4) or DMSO (4%, lane 5) was also included. In the control reaction (lane 1), 100% represents the incorporation of 143 fmol a-^{32}P-dAMP into high molecular weight DNA.

References

1. Lee, S.H., Eki, T. and Hurwitz, J. (1989) Synthesis of DNA containing the simian virus 40 origin of replication by the combined action of DNA polymerases alpha and delta. *Proc Natl Acad Sci U S A*, **86**, 7361-7365.
2. Weiser, T., Gassmann, M., Thommes, P., Ferrari, E., Hafkemeyer, P. and Hubscher, U. (1991) Biochemical and functional comparison of DNA polymerases alpha, delta, and epsilon from calf thymus. *J Biol Chem*, **266**, 10420-10428.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