Adenovirus infection elevates levels of cellular topoisomerase I

(covalent DNA-enzyme complex/adenovirus early region 1A/eukaryotic gene regulation)

KUAN-CHIH CHOW* AND GEORGE D. PEARSON†

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331

Communicated by Harold J. Evans, November 28, 1984

ABSTRACT We have developed a specific, sensitive, and quantitative assay for topoisomerase I, which is based on the formation of a covalent enzyme-DNA intermediate. Our assay measures the quantitative transfer of ^{32}P radioactivity from ^{32}P -labeled DNA to topoisomerase I. Since ^{32}P -labeled topoisomerase molecules are resolved by NaDodSO₄/PAGE, HeLa topoisomerase I (100 kDa) and calf thymus topoisomerase I (82 kDa) can be quantitatively assayed in the same reaction mixture. The assay can detect at least 0.3 ng (3 fmol) of topoisomerase I. We have used our assay to measure the levels of topoisomerase I activity in crude extracts of nuclei prepared from uninfected, adenovirus-infected, and adenovirus-transformed human cells. The evidence suggests that an adenovirus early gene product, presumably a protein encoded in early region 1A (E1A), increases cellular topoisomerase I activity at least 10-fold. Immunoblotting analysis with antiserum against calf thymus topoisomerase I shows that the increase in activity is due to an increase in the amount of enzyme.

Topoisomerases alter the topological properties of DNA molecules (1, 2). Type I topoisomerases change the linking number of covalently closed circular DNA in steps of 1 by introducing single-strand breaks (3), and type II topoisomerases, in steps of 2 by catalyzing double-strand breaks (4–6). We have developed a specific, sensitive, and quantitative assay for topoisomerase I based on the formation of a covalent enzyme-DNA intermediate (7–14). In the case of eukaryotic type I topoisomerases, the cycle of strand breakage and reunion proceeds through an energy-conserving intermediate in which the linkage is between a tyrosine residue on the enzyme (14) and a 3'-phosphoryl residue at the cleavage site (7, 11–13). Our assay measures the transfer of ^{32}P from ^{32}P -labeled DNA to topoisomerase I. Since ^{32}P -labeled topoisomerase molecules are identified by NaDodSO₄/PAGE, HeLa topoisomerase I (100 kDa) and calf thymus topoisomerase I (82 kDa) can be quantitatively assayed in the same reaction mixture. The assay can detect at least 0.3 ng (3 fmol) of topoisomerase I.

We have used our assay to measure the levels of topoisomerase I activity in crude extracts of nuclei prepared from uninfected, adenovirus-infected, and adenovirus-transformed human cells. The evidence, although circumstantial, suggests that an adenovirus early gene product, presumably a protein encoded in early region 1A (E1A), increases cellular topoisomerase I activity at least 10-fold. Further analysis by immunoblotting shows that the increase in activity corresponds to an increase in the amount of topoisomerase I. E1A expression facilitates the efficient transcription of other adenovirus genes (15–17), activates the transcription of the human heat-shock gene (18, 19) but not cellular genes in general, stimulates the transcription of genes newly introduced into cells by transfection (20, 21) or viral infection (22), and eliminates the *cis*-requirement for enhancers on transfected

genes (20, 21). It is tempting to conclude that, as in the case of the human heat-shock gene (18, 19), E1A expression increases cellular topoisomerase I expression at the level of transcription.

MATERIALS AND METHODS

Materials. *Escherichia coli* DNA polymerase I (Klenow fragment) was from New England Biolabs, DNase I was from Worthington, and Pronase was from Calbiochem-Behring. The synthetic 15-base primer, dNTPs, and ddCTP were from P-L Biochemicals. [α - ^{32}P]dCTP (5000 Ci/mmol; 1 Ci = 37 GBq) and ^{125}I -labeled protein A (100 $\mu\text{Ci}/\mu\text{g}$, about 4000 Ci/mmol) were from New England Nuclear. Nitrocellulose membranes (pore size, 0.22 μm) were obtained from Schleicher & Schuell. Bovine serum albumin (IgG-free) was from Sigma. L. F. Liu generously provided calf thymus and HeLa type I topoisomerases and antiserum against calf thymus topoisomerase I.

DNA. M13mp8 (23), mKM4 (24), and mKC96 (25) have been described. Phage were grown in *E. coli* JM103. Single-stranded circular DNA was purified by electrophoresis in a horizontal 0.8% agarose gel.

Labeling the DNA. The hybridization reaction mixture (30 μl) contained 10 μg of mKM4 DNA, 10 ng of primer, 70 mM NaCl, 17 mM Tris Cl (pH 7.5), 10 mM MgCl₂, and 0.7 mM EDTA. The solution was heated at 100°C for 5 min and then cooled to room temperature over a period of 45 min. Primer extension was carried out in a reaction mixture (50 μl) containing 10 μg of mKM4 DNA hybridized to 10 ng of primer; 75 mM NaCl; 15 mM Tris Cl (pH 7.5); 11 mM MgCl₂; 9 mM dithiothreitol; 0.4 mM EDTA; 19 μM each dATP, dGTP, and dTTP; 1 μM [α - ^{32}P]dCTP (250 μCi); and 5 units of *E. coli* DNA polymerase I (Klenow fragment). After incubating the solution for 15 min at 37°C, the dCTP concentration was adjusted to 50 μM and incubation was continued for another 15 min; 600–2000 nucleotides were polymerized under these conditions. The reaction then was heated for 5 min at 65°C, the volume of the solution was adjusted to 100 μl with STE buffer (100 mM NaCl/10 mM Tris Cl, pH 7.5/1 mM EDTA), and ^{32}P -labeled DNA was separated from unincorporated nucleotides by chromatography on Sephadex G-75. DNA was precipitated with ethanol and dissolved at a concentration of 250 $\mu\text{g}/\text{ml}$ in TE buffer (10 mM Tris Cl, pH 7.5/1 mM EDTA).

Topoisomerase Reaction. Nuclear and cytoplasmic extracts were prepared from uninfected and adenovirus-infected HeLa cells and from the adenovirus-transformed human embryonic kidney cell line 293 as described (25). The protein concentration in the nuclear extracts ranged from 4 to 6 mg/ml, and the concentration in the cytoplasmic extracts varied from 14 to 17 mg/ml. The standard reaction mixture contained 30 mM NaCl, 50 mM Hepes (pH 7.5), 5 mM

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: E1A and E1B, adenovirus early regions 1A and 1B.
*Present address: Department of Cell and Molecular Biology, Rockefeller University, New York, NY 10021.

†To whom reprint requests should be addressed.

MgCl₂, 1 mM dithiothreitol, 100 μ M ddCTP, 1 mM ATP, 2 μ l of nuclear extract (8–12 μ g of protein), and 1.25 μ g of DNA substrate in a total volume of 10 μ l. Some reaction mixtures contained 2 μ l of cytoplasmic extract (28–34 μ g of protein) or purified topoisomerase I instead of nuclear extract. After incubation for 30 min at 37°C, DNase I was added at a concentration of 100 μ g/ml, and the incubation was continued for 10 min. The reaction was stopped by adding an equal volume of double-strength Laemmli sample buffer (26) and heating at 100°C for 5 min. Samples digested with Pronase prior to gel electrophoresis were first inactivated by heating for 5 min at 65°C after DNase I treatment, adjusted to 0.1% NaDodSO₄, and then incubated with Pronase at a concentration of 1 mg/ml for 1 hr at 37°C.

Gel Electrophoresis. Gel electrophoresis was carried out at 120 V for 2 hr in 10% polyacrylamide gels containing 0.1% NaDodSO₄ (10 cm wide $\times$ 8 cm long $\times$ 0.8 mm thick). The stacking gel contained 6% polyacrylamide and 0.1% NaDodSO₄. The electrophoresis buffer was 50 mM Tris borate, pH 8.3/1 mM EDTA/0.1% NaDodSO₄. After fixation with 50% (vol/vol) methanol and staining with AgNO₃, the gels were dried and autoradiographed. Autoradiograms were scanned with a Zeineh SL-504-XL soft-laser densitometer and band intensities were integrated by using an electrophoresis reporting integrator program (Biomed).

RESULTS

Assay of Topoisomerase I. The strategy for assay of topoisomerase I is detailed in Fig. 1. The assay measures the transfer of ³²P radioactivity from ³²P-labeled DNA to topoisomerase I. Central to the scheme is the observation that eukaryotic topoisomerase I forms a covalent intermediate with DNA where the linkage is between a tyrosine residue on the enzyme (14) and a 3'-phosphoryl group at the cleavage site (7, 11–13). The covalent intermediate can be trapped by digesting DNA with DNase I. After NaDodSO₄/PAGE, the resulting ³²P-labeled topoisomerase I molecules can be detected by autoradiography.

Fig. 2 shows that a single radioactive band was detected when ³²P-labeled DNA was incubated with crude nuclear ex-

FIG. 2. Assay of topoisomerase I activity in nuclear extracts from uninfected (lane 1) and adenovirus-infected HeLa cells (lane 2).

tracts (25, 27, 28) prepared from uninfected or adenovirus-infected HeLa cells. The radioactive band is a polypeptide, since it was completely eliminated by subsequent Pronase, trypsin, or chymotrypsin digestion (not shown). As discussed below, the protein is identical to the 100-kDa HeLa topoisomerase I. Topoisomerase I was labeled from ³²P-labeled DNA directly, since incubation of [α -³²P]dCTP and unlabeled DNA with nuclear extracts did not label the enzyme (also see ref. 29). Neither the sequence of the ³²P-labeled DNA substrate (e.g., M13mp8, mKM4, or mKC96) nor the specific [α -³²P]dNTP used to synthesize the substrate had more than a marginal effect (less than 2-fold) on the labeling of topoisomerase I. Topoisomerase I was optimally labeled in reaction mixtures containing a total NaCl concentration of 60 mM (the nuclear extract contributes 30 mM NaCl), although activity could be detected in concentrations of NaCl up to 150 mM. Added ATP was not required, but 1 mM ATP marginally stimulated the reaction (less than 2-fold). We estimate that the endogenous ATP concentration in the reaction mixture is less than 0.2 μ M (24, 30).

FIG. 1. Strategy for measuring the transfer of ³²P radioactivity from substrate DNA to topoisomerase I. (A) Radioactive substrate DNA (thick line) was synthesized on single-stranded circular M13mp8 or mKM4 (thin line) by elongating a 15-base primer (open bar) with the Klenow fragment of DNA polymerase I, using [α -³²P]dCTP and unlabeled dGTP, dATP, and dTTP. The structure of the DNA within the dashed box is detailed in B. (B) Topoisomerase I (topo I) forms a covalent intermediate with radioactive substrate DNA during the reaction. The linkage is between a tyrosine residue on the enzyme (14) and a 3'-phosphoryl residue at the cleavage site (7, 11–13). The covalent intermediate can be trapped by digesting DNA with DNase I. (C) ³²P-labeled topoisomerase I molecules differing in size can be resolved by NaDodSO₄/PAGE. The diagram shows the pattern expected if HeLa topoisomerase I (100 kDa) and calf thymus topoisomerase I (82 kDa) are assayed singly or together.

Fig. 2 also shows that the 100-kDa topoisomerase I band was labeled to a 10-fold greater extent in the reaction with nuclear extract from adenovirus-infected cells even though the total protein concentration in both extracts was identical. This difference was not due to leakage during the isolation of nuclei, since no topoisomerase I activity was observed in cytoplasmic extracts from either adenovirus-infected or uninfected HeLa cells (data not shown). Since the activity of purified HeLa topoisomerase I could be detected when added to either cytoplasmic extract (data not shown), the cytoplasmic extracts did not contain an inhibitor of the enzyme. Moreover, the nuclear extract from uninfected cells did not contain an enzyme inhibitor (see Fig. 6).

Quantitation of Topoisomerase I. Purified calf thymus topoisomerase I (M_r 82,000) was reacted with ^{32}P -labeled DNA substrate. Fig. 3 shows that when the calf thymus enzyme was assayed alone (lanes 8–12), only the two highest concentrations of the serial dilution showed activity. However, when the purified enzyme was diluted into reaction mixtures containing nuclear extract from adenovirus-infected cells, activity could easily be detected down to the lowest concentration (lanes 3–7). The extent of labeling of the 82-kDa topoisomerase I in each reaction was determined by quantitative densitometry. Fig. 4 shows that the labeling was stoichiometric; the intensity of each band was linearly proportional to the amount of added enzyme. The labeling of topoisomerase I was constant with time of incubation (data not shown) and likely reflects the instantaneous fraction of enzyme in the covalent intermediate state during the overall nicking-closing reaction (31). This fraction corresponds to 4% of the enzyme molecules for rat liver topoisomerase I (11). Fig. 3 also shows that calf thymus topoisomerase I, even when added in 300-fold molar excess, did not interfere with the activity of the endogenous HeLa topoisomerase I. Quantitative densitometry established that the intensity of each 100-kDa HeLa enzyme band in lanes 3–7 was identical to the intensity of that band in lane 2 where no calf thymus enzyme had been added. Interestingly, human topoisomerase I was labeled to the same extent in nuclear extracts from cells of the adenovirus-transformed human cell line 293 (32) as the HeLa enzyme in nuclear extracts from adenovirus-

FIG. 4. The transfer of ^{32}P radioactivity from substrate DNA to topoisomerase I is stoichiometric. Radioactive calf thymus topoisomerase I was quantitated (lanes 3–7, Fig. 3) by using a soft-laser scanning densitometer. The integrated intensity of each 82-kDa band is plotted against the amount of enzyme in the assay mixture.

infected cells (compare lanes 1 and 2). This result will be discussed below.

The relative amounts of topoisomerase I in nuclear extracts from uninfected and adenovirus-infected HeLa cells were independently measured by an immunoblotting assay (33, 34). Nuclear extracts were fractionated by polyacrylamide gel electrophoresis, and the proteins were transferred from the gel to a nitrocellulose membrane. HeLa topoisomerase I was detected by using cross-reacting antiserum against calf thymus topoisomerase I followed by ^{125}I -labeled protein A. A 100-kDa band was visualized in each nuclear extract (Fig. 5). Quantitative densitometry revealed that the band in the extract from adenovirus-infected cells was 10-fold more intense than the band in the nuclear extract from

FIG. 3. Assay of purified calf thymus topoisomerase I activity in the presence (lanes 3–7) or absence (lanes 8–12) of nuclear extract from adenovirus-infected HeLa cells (INF). Reactions contained 1800 ng (lanes 3 and 8), 1500 ng (lanes 4 and 9), 750 ng (lanes 5 and 10), 150 ng (lanes 6 and 11), or 75 ng (lanes 7 and 12) of the 82-kDa calf thymus topoisomerase I. Lane 1: nuclear extract from line 293 cells (adenovirus-transformed human cells). Lane 2: nuclear extract from adenovirus-infected HeLa cells. In some of the lanes material migrating more slowly than topoisomerase I is incompletely digested DNA or DNA-enzyme complex. Minor bands migrating faster are proteolytic products of topoisomerase I.

FIG. 5. Quantitation of topoisomerase I levels by immunoblotting with antiserum against calf thymus topoisomerase I. After electrophoresis, gels were soaked for 1 hr in 10 mM Tris Cl, pH 7.4/50 mM NaCl/2 mM EDTA/0.1 mM dithiothreitol/4 M urea. Proteins were transferred from gels to nitrocellulose membranes by immersion for 24 hr in the same buffer lacking urea. After transfer, membranes were soaked for 2 hr in P_i/NaCl (10 mM sodium phosphate buffer, pH 7.4/100 mM NaCl) with 5% bovine serum albumin and then rinsed with P_i/NaCl alone. Membranes were incubated overnight at 4°C with antiserum against calf thymus topoisomerase I (diluted 1:500 into P_i/NaCl containing 5% bovine serum albumin), washed twice with P_i/NaCl containing 0.05% Nonidet P-40, and rinsed once with P_i/NaCl alone. Immunocomplexes were reacted with protein A by incubating membranes at 4°C for 4 hr with ^{125}I -labeled protein A ($\approx 10^5$ cpm/ml in P_i/NaCl containing 5% bovine serum albumin). After two rinses with P_i/NaCl containing 0.05% Nonidet P-40 and one rinse with P_i/NaCl alone, membranes were dried and autoradiographed. Lane 1: nuclear extract from uninfected HeLa cells. Lane 2: nuclear extract from adenovirus-infected HeLa cells.

uninfected cells. This result correlates elevated topoisomerase I activity with an increase in the number of enzyme molecules (also see Fig. 6).

We have used purified calf thymus enzyme (or purified HeLa enzyme, see below) as an internal standard to calculate the number of topoisomerase I molecules in nuclear extracts. The enzyme concentration in uninfected HeLa cells is 0.6 ng per 10^5 cells or 4×10^4 molecules per cell, and in adenovirus-infected HeLa cells or line 293 cells, it is 6 ng per 10^5 cells or 4×10^5 molecules per cell. These values fall within the estimates previously calculated for HeLa cells, rat liver cells, and chicken erythrocytes (35). We estimate that the assay can detect at least 0.3 ng (3 fmol) of topoisomerase I.

Identification of Topoisomerase I. Topoisomerase I activity is heat-sensitive. Nuclear extracts from uninfected and adenovirus-infected HeLa cells were incubated at 60°C for various periods of time prior to assay for topoisomerase I activity. Endogenous topoisomerase I was totally inactivated by heating either extract for 10 min or longer (data not shown). To demonstrate that the enzyme in nuclear extracts was identical to HeLa topoisomerase I, we diluted purified HeLa topoisomerase I into heat-inactivated nuclear extracts from uninfected HeLa cells and then assayed the topoisomerase activity. The results are shown in Fig. 6. Purified HeLa topoisomerase I appeared as a 100-kDa band (lanes 3 and 4) which comigrated in the gel with the endogenous activity in the control extract (lane 1). Even the pattern of minor labeled bands, presumably proteolytic degradation products of HeLa topoisomerase I, was identical in all lanes. Partial peptide mapping with trypsin or chymotrypsin (not shown) further identified the endogenous topoisomerase I as HeLa topoisomerase I. Fig. 6 also shows that the labeling of purified HeLa topoisomerase I was stoichiometric (compare lanes 3 and 4), since twice as much enzyme gave twice the intensity when analyzed by quantitative densitometry. This confirms the results of the immunoblotting assay. Purified HeLa enzyme could not be detected (lane 2) when assayed alone at high dilution. The high protein concentration contributed by nuclear or cytoplasmic extracts from uninfected and adenovirus-infected HeLa cells, even if heat-inactivated, presumably stabilizes both calf thymus and HeLa enzymes equally effectively when these enzymes are assayed at high dilution (Figs. 3 and 6; unpublished results). We cannot exclude the possibility, however, that the extracts provide heat-stable stimulatory factors such as histone H1 or high mobility group proteins (36). Thus, it is important to determine whether or not the assay is quantitative in reaction mixtures lacking nuclear or cytoplasmic extracts.

Elevation of Topoisomerase I Levels. Several observations suggest that an early adenovirus gene, presumably E1A, ele-

vates the level of topoisomerase I in infected cells. Topoisomerase I activity was increased in adenovirus-infected HeLa cells in both the absence and the presence of 10 mM hydroxyurea (data not shown). Hydroxyurea treatment blocks adenovirus DNA replication and late-gene expression but allows early-gene expression, including expression of all the genes required for viral DNA replication (27). Moreover, HeLa topoisomerase I activity increased by 6 hr after infection (data not shown), before the onset of late-gene expression. Finally, we found high levels of topoisomerase I activity in line 293 cells (Fig. 3), adenovirus-transformed human cells that express adenovirus E1A and E1B genes (37).

DISCUSSION

Several topoisomerase I assays based on the formation of a covalent DNA-enzyme intermediate have already been developed (11–13, 35, 38). Our assay, which measures the transfer of ^{32}P radioactivity from ^{32}P -labeled DNA to topoisomerase I (Fig. 1), is (i) simple: topoisomerase I activity can be measured in crude nuclear extracts; (ii) specific: topoisomerase I is the only protein labeled in nuclear extracts (Fig. 2); (iii) sensitive: at least 0.3 ng (3 fmol) of topoisomerase I can be detected; and (iv) quantitative: topoisomerase I is labeled in proportion to enzyme concentration (Figs. 3, 4, and 6). Since topoisomerase I molecules of different sizes can be quantitatively assayed together (Fig. 3), we have used purified 82-kDa calf thymus topoisomerase I to standardize reactions. This assay should be useful for measuring topoisomerase I activity in a wide range of eukaryotic cells. We recently have assayed topoisomerase I in nuclear extracts from Chinese hamster ovary (CHO) cells (unpublished results).

We have measured the levels of topoisomerase I activity in crude nuclear extracts from uninfected and adenovirus-infected HeLa cells (Fig. 2) and from cells of the adenovirus-transformed human cell line 293 (Fig. 3). The available evidence, although circumstantial, suggests that an adenovirus early gene product, presumably a protein encoded in the E1A region, elevates cellular topoisomerase I activity by at least 10-fold. Immunoblotting analysis with antiserum against calf thymus topoisomerase I demonstrates that the increase in activity is the consequence of an increase in the amount of enzyme (Fig. 5). The E1A region is the first to be transcribed upon adenovirus infection, and efficient transcription of the remaining viral genes requires one of the E1A gene products (15–17). E1A expression also induces the transcription of the human heat-shock gene (18, 19), activates the transcription of exogenous genes in *trans* (20–22), and overcomes the *cis*-requirement for enhancer or activator sequences in transient-expression assays (20, 21). Although we favor the view that, as in the case of the human heat-shock gene, E1A expression elevates cellular topoisomerase I activity at the level of transcription, a direct test awaits the cloning of the topoisomerase I gene.

In vitro adenovirus DNA replication requires three virus-encoded proteins (adenovirus DNA binding protein, adenovirus preterminal protein, and adenovirus DNA polymerase) as well as two cellular proteins (nuclear factor I and nuclear factor II) (39). Nuclear factor II, which contains a DNA topoisomerase I-like activity, facilitates the completion of full-length adenovirus DNA strands (40). In fact, the requirement for nuclear factor II can be totally satisfied by purified HeLa or calf thymus topoisomerase I but not *E. coli* topoisomerase I (40). The increase in topoisomerase I activity in adenovirus-infected cells may, therefore, represent an example of viral induction of a cellular factor that is required for viral DNA replication.

We thank Kate Mathews for constructing mKM4 and for excellent technical assistance. Mr. Terry Johnson helped with certain experi-

FIG. 6. Assay of purified HeLa topoisomerase I activity in the presence (lanes 3 and 4) or absence (lane 2) of heat-inactivated nuclear extract from uninfected HeLa cells. Reaction mixtures contained 50 ng (lane 3) or 100 ng (lanes 2 and 4) of the 100-kDa HeLa topoisomerase I. Lane 1: overexposed autoradiogram of reaction mixture with nuclear extract from uninfected HeLa cells. The minor bands are proteolytic products of topoisomerase I.

ments. Dr. Leroy Liu generously provided purified HeLa and calf thymus type I topoisomerases as well as antiserum directed against calf thymus topoisomerase I. G.D.P. was the recipient of a Faculty Research Award (FRA-236) from the American Cancer Society. This work was supported by a grant from the Medical Research Foundation of Oregon, by Biomedical Sciences Support Grant RR07079 administered through the Oregon State University Research Council, and by National Cancer Institute Grant CA17699.

1. Cozzarelli, N. R. (1980) *Science* **207**, 953-960.
2. Gellert, M. (1981) *Annu. Rev. Biochem.* **50**, 879-910.
3. Brown, P. O. & Cozzarelli, N. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 843-847.
4. Brown, P. O. & Cozzarelli, N. R. (1979) *Science* **206**, 1081-1083.
5. Liu, L. F., Liu, C.-C. & Alberts, B. M. (1980) *Cell* **19**, 697-707.
6. Mizuuchi, K., Fisher, L. M., O'Dea, M. H. & Gellert, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1847-1851.
7. Champoux, J. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3800-3804.
8. Champoux, J. J. (1978) *J. Mol. Biol.* **118**, 441-446.
9. Depew, R. E., Liu, L. F. & Wang, J. C. (1978) *J. Biol. Chem.* **253**, 511-518.
10. Liu, L. F. & Wang, J. C. (1979) *J. Biol. Chem.* **254**, 11082-11088.
11. Been, M. D. & Champoux, J. J. (1980) *Nucleic Acids Res.* **8**, 6129-6142.
12. Been, M. D. & Champoux, J. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2883-2887.
13. Prell, B. & Vosberg, H. P. (1980) *Eur. J. Biochem.* **108**, 389-398.
14. Champoux, J. J. (1981) *J. Biol. Chem.* **256**, 4805-4809.
15. Jones, N. & Shenk, T. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3665-3669.
16. Berk, A. J., Lee, F., Harrison, T., Williams, J. & Sharp, P. A. (1979) *Cell* **17**, 935-944.
17. Nevins, J. R. (1981) *Cell* **26**, 213-220.
18. Nevins, J. R. (1982) *Cell* **29**, 913-919.
19. Kao, H.-T. & Nevins, J. R. (1983) *Mol. Cell. Biol.* **3**, 2058-2065.
20. Green, M. R., Treisman, R. & Maniatis, T. (1983) *Cell* **35**, 137-148.
21. Treisman, R., Green, M. R. & Maniatis, T. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7428-7432.
22. Gaynor, R. B., Hillman, D. & Berk, A. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1193-1197.
23. Messing, J. (1981) in *Third Cleveland Symposium on Macromolecules: Recombinant DNA*, ed. Walton, A. (Elsevier, Amsterdam), pp. 143-153.
24. Chow, K.-C. & Pearson, G. D. (1984) *Nucleic Acids Res.* **12**, 1489-1500.
25. Pearson, G. D., Chow, K.-C., Enns, R. E., Ahern, K. G., Corden, J. L. & Harpst, J. A. (1983) *Gene* **23**, 293-305.
26. Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685.
27. Challberg, M. D. & Kelly, T. J., Jr. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 655-659.
28. Kaplan, L. M., Ariga, H., Hurwitz, J. & Horwitz, M. S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5534-5538.
29. Tamanoi, F. & Stillman, B. W. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2221-2225.
30. De Jong, P. J., Kwant, M. M., van Driel, W., Jansz, H. S. & van der Vliet, P. C. (1983) *Virology* **124**, 45-58.
31. Champoux, J. J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3488-3491.
32. Graham, F. L., Smiley, J., Russell, W. C. & Nairn, R. (1977) *J. Gen. Virol.* **36**, 59-72.
33. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350-4354.
34. Jahn, R., Schiebeler, W. & Greengard, P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1684-1687.
35. Tricoli, J. V. & Kowalski, D. (1983) *Biochemistry* **22**, 2025-2031.
36. Tse, Y.-C., Javaherian, K. & Wang, J. C. (1984) *Arch. Biochem. Biophys.* **231**, 169-174.
37. Harrison, T., Graham, F. & Williams, J. (1977) *Virology* **77**, 319-329.
38. Liu, L. F., Rowe, T. C., Yang, L., Tewey, K. M. & Chen, G. L. (1983) *J. Biol. Chem.* **258**, 15365-15370.
39. Lichy, J. H., Nagata, K., Friefeld, B. R., Enomoto, T., Field, J., Guggenheimer, R. A., Ikeda, J.-E., Horwitz, M. S. & Hurwitz, J. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 731-740.
40. Nagata, K., Guggenheimer, R. A. & Hurwitz, J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4266-4270.