Vol. 107, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 30, 1982 Pages 470-480

STRUCTURE OF ADENOVIRUS NUCLEOPROTEIN CORE STUDIED BY CIRCULAR DICHROISM AND SELECTIVE RADIOCHEMICAL LABELING

PIERRE BOULANGER[+] AND MARIE-HENRIETTE LOUCHEUX-LEFEVBRE[::]

[+]*Unité de Virologie Moléculaire de l'INSERM, U. 233, Place de Verdun, 59045 Lille Cédex*
[::]*Institut de Recherches sur le Cancer de Lille, BP 311, 59020 Lille Cédex, France*

Received May 25, 1982

ABSTRACT.- The circular dichroic (CD) spectra of adenovirus DNA were compared within the intact virion and in deoxycholate and sarcosyl cores. It was found that the viral DNA was in highly condensed and packed conformation in both intact virus particles and deoxycholate cores, as in chromatin nucleosomes. Minor but significant CD spectral differences with eucaryotic chromatin indicated that the adenovirus nucleosome-like structures had specific conformational features. Adenovirus DNA only complexed with protein VII in sarcosyl cores elicited a CD spectrum reminiscent of a relaxed DNA, suggesting that the adenovirus sarcosyl cores were no longer organized in nucleosome subunits.

In parallel to CD analysis, the nature of the bonds involved in core structure was investigated by *in vitro* selective labeling of some critical amino acids. ^{14}C-dansyl was thus used to probe the accessibility of lysyl and tyrosyl residues in adenovirus core proteins. The data suggested that core protein V was associated with protein VII mainly via hydrophobic bonds, and had a major role in the maintainance of the nucleosomal structure of the viral core.

INTRODUCTION

The capsid of human adenovirus type 2 contains an internal core, consisting of a single linear DNA duplex, with a molecular weight (MW) of 25×10^6 (1) and at least two core proteins, numbered V and VII (2-5). It is generally accepted that about 180 copies of core protein V (MW 48,500 (2)), and 1070 copies of the arginine-rich protein VII (MW 18,500 (2)) are present within the virion (3-6).

However, conflicting results have been published concerning the nucleosome-like organization of the adenovirus core (6-10). Moreover, adenovirus cores can be isolated after various treatments of the virus particles, such as heat shock (11), acetone (12), deoxycholate at 56°C (13), pyridine (14) and sarcosyl (5). The polypeptide composition, and hence the physical and biochemical properties of the cores, vary with the procedure used. Deoxy-

0006-291X/82/140470-11$01.00/0

cholate and pyridine cores contain both core proteins V and VII (5, 15), whereas sarcosyl and acetone cores lack protein V (5, 16).

In the present study, the conformational structure of viral DNA within intact virion and within adenovirus cores of different protein contents was investigated by two different approaches, biophysical and biochemical. The circular dichroism (CD) data were correlated with selective labeling of basic and aromatic amino acid residues of capsid and core proteins.

MATERIALS AND METHODS

Cells and virus.- KB cells were grown in suspension culture at 3 x 10^5 cells/ml in Eagle's medium supplemented with 5 % horse serum. Wild-type of human adenovirus type 2 was propagated on KB cells, and was extracted and purified in CsCl gradient as previously described (17).

Preparation of viral material for dichroic studies.- Dialysis of a virus particle suspension isolated in CsCl gradient against a buffer of low ionic strength is known to provoke aggregation and precipitation of virions, and also the loss of vertex subunits of the virus icosahedron (18). In order to avoid such an alteration of the biological material in low salt medium, the virus band at density 1.345, freshly obtained from a self-generating CsCl gradient, was diluted to 1:10 (v/v) with 0.01 M Tris-HCl, pH 7.8, and analyzed as such in the dichrograph, or further treated with detergents (deoxycholate or sarcosyl). This virus material, usually contained 0.3 to 0.6 mg/ml protein, in 0.27 M CsCl, 0.01 M Tris-HCl, pH 7.8.

Adenovirus cores and DNA.- Two types of nucleoprotein cores were analyzed : deoxycholate and sarcosyl cores. Dilutions of adenovirus preparations (at 0.3 to 0.6 mg/ml protein in 0.27 M CsCl) were brought to 0.5 % sodium deoxycholate at room temperature and analyzed in thermostatized cell at temperatures ranging from 25° to 56°C. 56°C has been found to be critical for the capsid integrity in 0.5 % deoxycholate (13, 15). Adenovirus sarcosyl cores were prepared by treatment of the virus suspension with 0.7 % sarcosyl for 30 min at room temperature (5), and analyzed in the dichrograph at 25°C. Adenovirus DNA was obtained by treatment of adenovirus particles with sarcosyl (0.5 %, w/v) and pronase (1 mg/ml) for 3 h at 37°C, followed by centrifugation in self-generating CsCl gradient, in a Beckman SW 50-1 rotor, at 45 krpm for 72 h at 4°C. The concentrations of DNA were determined spectrometrically, using E_{260} = 6600 cm^{-1} mol^{-1} for a nucleotide residue.

Circular dichroism (CD) analysis.- The CD spectra were recorded using a Jobin-Yvon dichrograph Mark III in thermostatized cell of 0.01 cm path-length. The CD spectra of each sample were recorded at selected temperatures between 250 and 300 nm, the domain in which the ellipticity depends upon the conformation of the DNA. The baseline for each buffer was recorded before and after the measurement of each corresponding sample, and automatically subtracted. The ellipticity was expressed as mean residual molar ellipticity [θ] in degree.cm^2.$dmol^{-1}$. The mean residue weight was 330 for a nucleotide.

Dansylation of virion proteins.- ^{14}C-labeled dimethylamino naphtalene sulfonyl chloride (dansyl chloride, DNS-Cl) solution in acetone (104 mCi/mmol, 0.5 mCi/ml) was purchased from the Commissariat à l'Energie Atomique (Saclay, France). ^{14}C-DNS-Cl was reacted with four types of virus samples : (i) Intact virus particle suspension at 0.3 to 0.6 mg/ml in 0.27 M CsCl (native virion) ; (ii) virus treated with 0.5 % deoxycholate at 56°C for 90 sec (DOC-disrupted virion) ; (iii) virus treated with 0.7 % sarcosyl for 30 min at 25°C (sarcosyl-disrupted virion) ; (iv) control disintegrated virus obtained by heating with 2 % SDS for 2 min at 100°C (SDS-disrupted virion).

50 µl of virus sample was mixed with 50 µl of 0.1 M ammonium bicarbonate, pH 8.9, and incubated with 10 µl of ^{14}C-DNS-Cl in acetone for 20 h at room temperature and in the dark. Virus material was then precipitated by addition of 15 µl of 100 % (w/v) trichloroacetic acid (TCA) and washed twice with 5 % cold TCA and once with 0.1 % cold TCA followed by cold ethanol. ^{14}C-DNS-labeled virus was kept dried until further analysis. The degree of labeling of each virus protein was determined by analytical SDS-polyacrylamide gel electrophoresis and the accessibility of ε-amino groups of lysine and of phenolic hydroxyl groups of tyrosine to dansylation in virion was determined by two-dimensional thin-layer chromatography of hydrolyzed samples.

Identification of dansylated amino acid residues.- Since most of the adenovirus proteins are N-acetylated (19), ^{14}C-DNS will react with the available side chains of certain amino acids such as lysine and tyrosine, and yield mainly ^{14}C-DNS-ε-lysine and ^{14}C-DNS-O-tyrosine, detectable in acidic or proteolytic hydrolysates.

DNS-imidazolium-histidine being unstable, the histidyl residues will escape this type of investigation. Total proteolysis of ^{14}C-DNS-labeled material was preferred to acidic hydrolysis, in order to minimize the degradation of dansylated amino acids into DNS-NH_2. ^{14}C-DNS-labeled virus samples were denatured with 0.2 % SDS at 100°C for 2 min and hydrolyzed by pronase (Calbiochem, 0.1 mg/ml) at 37°C for 24 h. The samples were heated again at 100°C for 5 min to inactivate the pronase and hydrolysis was achieved by aminopeptidase M (Boehringer, 0.1 mg/ml) for an additional 24 h at 37°C. Hydrolysates were dried in a vacuum dessicator before thin-layer chromatography.

^{14}C-DNS-labeled amino acids were redissolved in 20 % pyridine-2 % acetic acid and spotted onto 5 x 5 cm polyamide sheet in the following solvents. First dimension : 1.5 % formic acid. Second dimension : benzene-acetic acid (90:10, v/v). A third migration in the same dimension was performed in ethyl acetate-methanol-acetic acid (20:1:1, v/v) in order to separate DNS-O-tyrosine from DNS-OH. After being dried, the thin-layer plates were autoradiographed. The spots corresponding to ^{14}C-DNS-O-tyrosine and ^{14}C-DNS-ε-lysine were cut off the plate and radioactivity counted in a liquid scintillation spectrometer with OCS fluid (Amersham) as scintillator.

Analytical polyacrylamide gel electrophoresis.- ^{14}C-DNS-labeled virus material was denatured by heating at 100°C for 2 min in SDS-containing sample buffer (62.5 mM Tris-HCl buffer, pH 6.8, containing 4 % SDS, 10 % 2-mercaptoethanol, 6 M urea and 0.005 % bromophenol blue as marker). ^{14}C-DNS-labeled virus polypeptides were analyzed in SDS-containing 15.5 % polyacrylamide slab gel (acrylamide : bisacrylamide ratio of 50:0.235) in the discontinuous buffer system described by Laemmli (20). The gels were stained with Serva Blue R (Serva), dried under vacuum and autoradiographed on Kodak Royal-X-Omat S film.

Protein.- Protein was determined by the method of Lowry *et al.* (21) using bovine serum albumin as standard.

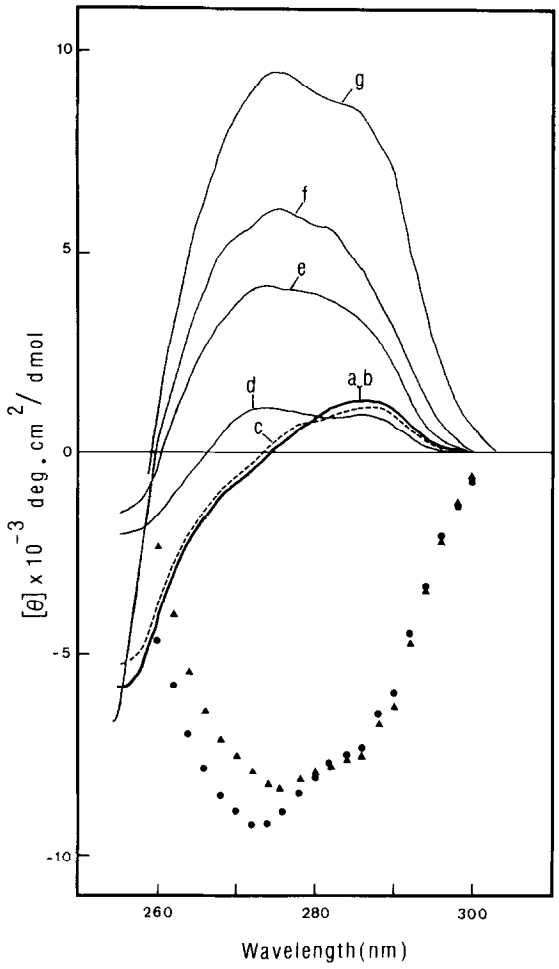

Figure 1.- CD spectra of intact and disrupted adenovirus particle in the wavelength range of DNA (250-300 nm). Curves : (a) intact adenovirus ; (b) virion treated with 0.5 % deoxycholate (DOC) at 25°C ; (c) virion treated with DOC at 37°C ; (d) virion treated with DOC at 56°C ; (e) virion treated with 0.7 % sarcosyl at 25°C ; (f) virion denatured with 2 % SDS for 2 min at 100°C ; (g) protein-free adenovirus DNA obtained after sarcosyl pronase-treatment of virions, analyzed in 0.27 M CsCl, 0.01 M Tris-HCl, pH 7.8, as samples (a-f). Each ellipticity value corresponds to the automatic computerized averaging of 4 measures at each wavelength. The difference spectra are as follows : (●) intact virion minus protein-free DNA (g) ; (▲) deoxycholate core minus protein-free DNA (g).

RESULTS

Circular dichroism (CD) of adenovirus DNA in intact and disrupted particles

The CD spectrum of the adenovirus DNA within the intact virus particles showed a positive maximum of 1270 deg.cm^2.dmol^{-1} at 285 nm, a value for ellipticity which corresponds to a highly condensed DNA, such as in chromatin. However, the negative maximum of about - 200 deg.cm^2.dmol^{-1} at 294 nm, expected for a DNA in eucaryotic chromatin (22) was not observed (Fig. 1a).

The DNA released from virus particles by low concentration of sarcosyl (0.7 %) and still complexed with protein VII (5) elicited a positive maximum of 4150 deg.cm^2.dmol^{-1} at 275 nm (Fig. 1e).

Adenovirus DNA deproteinized by SDS-treatment of the particle had a maximal ellipticity of 6090 deg.cm^2.dmol^{-1} at 275 nm (Fig. 1f), a value significantly lower than that usually observed for protein free DNA (22-24), and that found for sarcosyl pronase-treated DNA analyzed in the same ionic conditions (9460 deg.cm^2.dmol^{-1} at 275 nm, with a shoulder of 8330 at 285 nm ; Fig. 1g). It is known that adenovirus DNA obtained after treatment with detergents without protease still remains associated with a terminal protein covalently bound to both 5' termini (25).

The CD spectrum of virus core DNA between 250 and 300 nm remained unchanged in the presence of 0.5 % deoxycholate at 25, 37, and up to 50°C, when compared with that of intact virus (Fig. 1). At 56°C, a shoulder of 1090 deg. cm^2.dmol^{-1} appeared near 272 nm and the value of the maximum at 286 nm decreased slightly to 910 deg.cm^2.dmol^{-1}. The curve affected therefore the shape of a plateau between 285 and 272 nm and might be interpreted as a linear combination of the two spectra defined above : the CD spectrum of a highly constrained DNA and that of a relaxed DNA. This was confirmed by difference CD spectra calculated between the protein-free DNA spectrum (Fig. 1f) and the spectrum of deoxycholate nucleoprotein core (Fig. 1d). The negative band exhibited at 275 nm, was characteristic for ψ DNA (23). The difference CD spectrum calculated by substracting free DNA from intact virion showed a negative maximum at 272 nm, which did not correspond to the ψ type band (23), and could imply a contribution of capsid proteins in DNA conformation.

Accessibility of lysine and tyrosine side chains in adenovirus core proteins

In vitro dansylation of lysyl and tyrosyl residues was used to determine the amount of free ε-amino groups and phenolic hydroxyl groups, non-engaged in DNA-protein or protein-protein interactions. As shown in Table 1, in intact virion the ^{14}C-DNS tagged mainly the lysine ε-amino groups (98.7 %), suggesting that most of the phenolic rings were deeply buried in hydrophobic domains, or engaged. Complete denaturation of the adenovirus proteins by treatment with SDS at 100°C rendered more tyrosines accessible to dansylation. However, the dansylation of aromatic residues was still incomplete if it is considered that lysine and tyrosine are equally represented in the whole virion (26). The reorganization of proteins induced by SDS (27) might explain

Table 1 .- *Accessibility of lysines and tyrosines in intact and disrupted adenovirion*[a].

Adenovirion[b]	^{14}C-DNS-label in		Per cent label in	
	lysine[c]	tyrosine[c]	lysine	tyrosine
Intact	3214	42	98.7	1.3
Deoxycholate-treated	4117	299	95.3	4.7
Sarcosyl-treated	3436	448	88.6	11.4
SDS-denatured	6320	1498	80.8	19.2

[a]The accessibility of ε-amino groups of lysines and phenolic hydroxyl groups of tyrosines is determined by reaction with ^{14}C-labeled dansyl chloride, followed by total enzymatic hydrolysis and thin-layer chromatography of DNS-ε-lysine and DNS-O-tyrosine, as described under Materials and Methods.

[b]Amino acid composition reveals that lysyl and tyrosyl residues are equally respresented in the whole virion : 4.4 % of each (26).

[c]Results are given as cpm and represent the average of three different experiments. The radioactivity in DNS-ε-lysine and DNS-O-tyrosine was determined by cutting the polyacrylamide sheet around the corresponding spots and counting in liquid scintillation spectrometer.

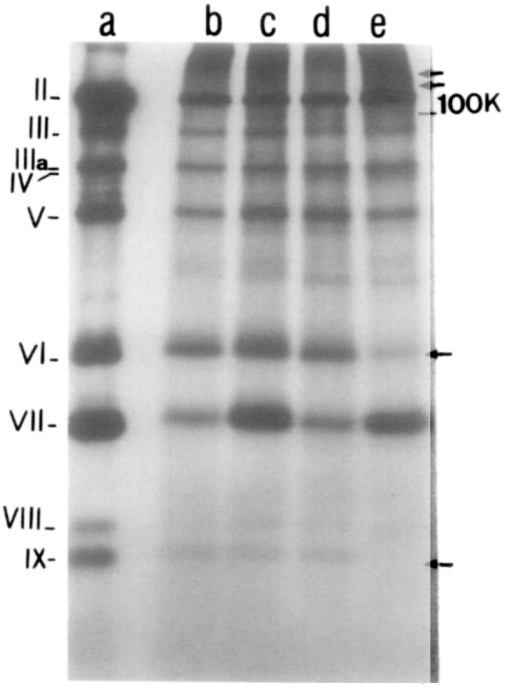

Figure 2.- SDS-polyacrylamide gel analysis of ^{14}C-DNS-labeled adenovirus polypeptides in intact and disrupted particles. (a) control virion labeled *in vivo* with ^{14}C-valine ; (b) ^{14}C-DNS-labeled intact adenovirus ; (c) adenovirus labeled with ^{14}C-DNS after treatment with 0.5 % deoxycholate at 56°C for 90 sec ; (d) adenovirus labeled with ^{14}C-DNS after disruption with 0.7 % sarcosyl ; (e) adenovirus heated at 100°C for 2 min in the presence of 2 % SDS and labeled with ^{14}C-DNS.

Note that ^{14}C-DNS label in the protein bands VI and IX decreased significantly in SDS-denatured sample (e), whereas labeled high molecular weight (MW) material appeared on top of the gel, over the hexon band (polypeptide II). These high MW bands likely corresponded to strongly dansylated polypeptides II, VI and IX, which are neighbors in the adenovirus capsid (4), and which could associate in ternary complexes, via hydrophobic bonds resisting the 0.1 % SDS-environment present within the gel.

the fact that certain tyrosines remain inaccessible. Sarcosyl treatment of the virion seemed to unmask preferentially tyrosines (Table 1).

The amount of ^{14}C-DNS label in the different polypeptide bands of intact or disrupted adenovirus was determined by SDS-polyacrylamide gel electrophoresis. As shown in Figure 2, intact virus was poorly labeled in core proteins V and VII and in hexon, as compared with control virus labeled *in vivo* with ^{14}C-valine (Fig. 2a). No significant difference was observed in ^{14}C-DNS-labeled polypeptides of intact (Fig. 2b) and sarcosyl-treated virus (Fig. 2d). On the contrary, SDS-treated adenovirus was heavily labeled on the hexon and protein VII (Fig. 2e). In deoxycholate-treated virion (Fig. 2c), the core protein VII appeared as the only band whose label increased, compared with intact virion, and revealed as strongly labeled as SDS-denatured VII (Fig. 2e) Correlated with the data of Table 1, this suggested that deoxycholate treat-

ment had a major denaturing and unfolding effect on protein VII, resulting mainly in unmasking of lysines. Since protein VII was still associated with virus DNA in deoxycholate core, this also suggested that all the lysines contained in VII (28) are not required to bind the protein with the DNA or that other types of bonds are involved (e.g. arginyl bonds) or both.

DISCUSSION

Despite a number of biochemical and biophysical studies (10, 28-31), the nucleosome-like organization of the adenovirus core still remains a matter of controversy. By using micrococcal nuclease as a biochemical probe for chromatin structure, it has been first claimed that pentonless virus particles and pyridine cores presented a nucleosome-like protection pattern resembling that of eucaryotic chromatin (6), a result which has not been confirmed by others (7). Based on the absence of detectable nucleosome repeat pattern typical of cell chromatin, the hypothesis has been recently formulated that the intranuclear adenovirus DNA late in infection has a nucleosome subunit organization similar to that of virion core DNA, but different from that of cellular chromatin (8, 9). The discrepancies observed in the literature prompted us to investigate the conformational structure of the "native" core, i.e. the nucleoprotein core contained within the intact adenovirus particle, by using methods which do not alter the structure of the virus capsid, such as circular dichroism (CD) analysis. The DNA spectrum of native cores was then compared with cores released by two different procedures of virus disruption : deoxycholate and sarcosyl.

In the light of our knowledge of the structural features and physical properties of the chromatin (22, 32, 33), the CD data thus obtained might be interpreted as follows. (i) Adenovirus DNA within the intact virus particle is in a highly condensed structural conformation similar to that of nucleosomal DNA. (ii) However, the absence of shoulder near 275 nm and of negative maximum at 294 nm, two signals characteristic for eucaryotic chromatin (22), suggests that the structure of the adenovirus core within the virus particle differs significantly from that of the chromatin. This result would thus confirm recent observations (7-9). (iii) Adenovirus DNA only complexed with protein VII in the sarcosyl core behaves as long DNA in relaxed conformation (22). (iv) At the opposite, the DNA in deoxycholate core, which is associated with both proteins V and VII has an ellipticity magnitude corresponding to that of a DNA in a collapsed conformation. In addition, the negative band at 275 nm obtained in difference CD spectrum between protein-free DNA and deoxycholate core is consistent with the existence of ψ DNA, as in eucaryotic chromatin (23). (v) Nevertheless, in the deoxycholate core, a signi-

ficant fraction of the DNA molecule appears as relaxed DNA, giving its CD spectrum the profile of the long chromatin, i.e. nucleosomes plus linkers (22). (vi) Lastly, the difference in molar ellipticity between protein-free DNA and viral DNA still bound with its terminal protein suggests that this protein, which circularizes the viral DNA molecule (25), also affects its global conformation, and hence its dichroic signal.

The CD spectrum of the sarcosyl core, which lacks protein V, is therefore completely different from that of the histone H1-depleted chromatin (22). The CD data would rather indicate that the adenovirus DNA-protein VII complex in sarcosyl core is not organized in nucleosomes, but that the DNA complexed with core proteins V and VII, as in the intact virion or deoxycholate-released core, affects a nucleosome-like structure. This would suggest that adenovirus core protein V has a role fundamentally different from that played by histone H1 which is thought to bind to linker DNA.

It has been proposed that the interactions between histones and DNA involve ionic bonds between basic amino acid residues in histones and acidic phosphate groups in DNA (32, 33). Thus, interaction between adenovirus core protein VII with DNA *in vitro* has been shown to be sensitive to ionic environments (34). It was therefore of interest to determine the degree of involvement of lysines and also of tyrosines in the interaction of core proteins with DNA in intact virion and in cores released by deoxycholate or sarcosyl : non-engaged lysines and tyrosines would be accessible to dansylation. Unfortunately, arginyl residues escape this type of investigation which does not signify that arginines are not involved in such interactions.

Considering the data summarized in Table 1 and the results of ^{14}C-DNS polypeptide labeling shown in Fig. 2c, it might be assumed that deoxycholate unmasked mainly lysyl residues of the core protein VII, either engaged in bonds with DNA or proteins, or deeply buried. However, in spite of the deoxycholate-induced structural alterations of core protein VII, the presence of protein V seemed to be sufficient to maintain the nucleosome-like structure of the deoxycholate core. Sarcosyl treatment of the virion, which releases the core protein V from its complex with core protein VII and DNA, did not significantly enhance the ^{14}C-DNS-labeling of any virion protein, except core protein V, but enhanced the accessibility of the bulk of tyrosines (Table 1). This suggested that core proteins V and VII are associated via hydrophobic bonds, in which at least some tyrosines participate.

Taken together, the CD and dansylation data suggest that adenovirus protein V plays a crucial role in maintaining the integrity of the adenovirus nucleosome-like structures existing in the virion core. Isola-

tion of adenovirus mutants carrying a thermosensitive lesion on either one or the other core protein is now in progress in our laboratory to elucidate the respective role of proteins V and VII in the chromatin-like structure of the adenovirus core.

ACKNOWLEDGEMENTS.- This work was supported by funds from the CNRS (ERA 225), the INSERM (U. 124 and U. 233), the DGRST (Contrat 80.7.0290) and the Université du Droit et de la Santé de Lille. We acknowledge with thanks the skilful technical assistance of Claude Denis.

REFERENCES

1. Green, M., Piña, M., Kimes, R.C., Wensink, P.C., MacHattie, L.A. and Thomas, C.A. Jr. (1967) *Proc. Natl. Acad. Sci. USA* 57, 1302-1309.
2. Anderson, C.W., Baum, P.R. and Gesteland, R.F. (1973) *J. Virol.* 12, 241-252.
3. Everitt, E., Sundquist, B., Pettersson, U. and Philipson, L. (1973) *J. Virol.* 5, 742-753.
4. Everitt, E., Lutter, L. and Philipson, L. (1975) *Virology* 67, 197-208.
5. Brown, D.T., Westphal, M., Burlingham, B.T., Winterhoff, U. and Doerfler, W. (1975) *J. Virol.* 16, 366-387.
6. Corden, J., Engelking, H.M. and Pearson, G.D. (1976) *Proc. Natl. Acad. Sci. USA* 73, 401-404.
7. Tate, V.E. and Philipson, L. (1979) *Nucleic Acids Res.* 6, 2769-2785.
8. Brown, M. and Weber, J. (1980) *Virology* 107, 306-310.
9. Daniell, E., Groff, D.E. and Fedor, M.J. (1981) *Molec. Cell. Biol.* 1, 1094-1105.
10. Nermut, M.V. (1979) *Arch. Virol.* 62, 101-116.
11. Russell, W.C., Valentine, R.C. and Pereira, H.G. (1967) *J. Gen. Virol.* 1, 509-522.
12. Laver, W.G., Pereira, H.G., Russell, W.C. and Valentine, R.C. (1968) *J. Mol. Biol.* 37, 379-386.
13. Russell, W.C., McIntosh, K. and Skehel, J.J. (1971) *J. Gen. Virol.* 11, 35-46.
14. Prage, L., Pettersson, U., Höglund, S., Lonberg-Holm, K. and Philipson, L. (1970) *Virology* 42, 341-358.
15. Boulanger, P.A., Lemay, P., Blair, G.E. and Russell, W.C. (1979) *J. Gen. Virol.* 44, 783-800.
16. Tate, V., Richards, B. and Pardon, J. (1975) in *In vitro Translation of Viral Genomes* (Haenni, A.L. and Beaud, G., eds.), pp. 75-82, INSERM, Paris.
17. D'Halluin, J.C., Martin, G.R., Torpier, G. and Boulanger, P.A. (1978) *J. Virol.* 26, 357-363.
18. Laver, W.G., Wrigley, N.G. and Pereira, H.G. (1969) *Virology* 39, 599-604.
19. Jörnvall, H., Ohlsson, H. and Philipson, L. (1974) *Biochem. Biophys. Res. Commun.* 56, 304-310.
20. Laemmli, U.K. (1970) *Nature (Lond.)* 227, 680-685.
21. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) *J. Biol. Chem.* 193, 265-275.
22. Watanabe, K. and Iso, K. (1981) *J. Mol. Biol.* 151, 143-163.
23. Cowman, M.K. and Fasman, G.D. (1978) *Proc. Natl. Acad. Sci. USA* 75, 4759-4763.
24. Chan, A., Kilkuskie, R. and Hanlon, S. (1979) *Biochemistry* 18, 84-91.
25. Rekosh, D.M.K., Russell, W.C., Bellet, A.J.D. and Robinson, A.J. (1977) *Cell* 11, 283-295.
26. Polasa, M. and Green, M. (1967) *Virology* 31, 565-567.

27. Boulanger, P. and Loucheux, M.H. (1972) *Biochem. Biophys. Res. Commun.* 47, 194-201.
28. Sung, M.T., Lischwe, M.A., Richards, J.C. and Hosokawa, K. (1977) *J. Biol. Chem.* 252, 4981-4987.
29. Harpst, J.A., Ennever, J.F. and Russell, W.C. (1977) *Nucleic Acids Res.* 4, 477-490.
30. Nermut, M.V. (1980) *Arch. Virol.* 64, 175-196.
31. Sato, K. and Hosokawa, K. (1981) *Biochem. Biophys. Res. Commun.* 101, 1318-1323.
32. Kornberg, R.D. (1977) *Ann. Rev. Biochem.* 46, 931-954.
33. Felsenfeld, G. (1978) *Nature (Lond.)* 271, 115-122.
34. Black, B.C. and Center, M.S. (1979) *Nucleic Acids Res.* 6, 2331-2353.