curred about 12,000 to 10,000 years ago, peaking about 11,600 years ago. He noted (*47*) that the accelerated rise in sea level was on the order of decimeters per year. Present day surging or collapse of polar ice masses (or both) and the consequent rapid rise in sea level could be beyond the capacity of humans to easily adjust to it. Thus, the combined negative thermal effects on animal life and the flooding associated with melting of the ice caps signal potential catastrophy ahead if the increasing atmospheric content of human-generated CO_2 is not brought under control.

A critical problem for humans is to avoid arriving inadvertently at a critical threshold that might trigger an abrupt accelerated warming of the climate beyond their capacity to control, or to adapt to, it. The duration of such a "greenhouse" would, in human terms, last an interminable period, and its impact on life would be incalculable. Animals today are generally adapted to relatively cool conditions, as were faunas prior to the terminal Mesozoic extinctions. A sudden climatic warming could potentially impose on us conditions comparable to those that terminated a geologic era.

References and Notes

1. D. I. Axelrod and H. P. Bailey, *Evolution* **22**, 595 (1967).
2. J. L. Cutbill and B. M. Funnell, in *The Fossil Record*, W. B. Harland, C. H. Holland, M. R. House, N. F. Hughes, A. B. Reynolds, M. J. S. Rudwick, G. E. Satterthwaite, L. B. H. Tarlo, E. C. Willey, Eds. (Geological Society of London, London, 1967), p. 796.
3. T. Worsley, in *Studies in Paleo-oceanography*, W. W. Hay, Ed. (S.E.P.M. Spec. Pub. 20, Tulsa, Okla., 1974).
4. D. A. Russell, in *Syllogeus* (National Museum of Canada, Ottawa, 1976).
5. R. B. Cowles, *Am. Nat.* **79**, 160 (1945).
6. National Academy of Sciences, Energy and Climate Panel Report, *Phys. Today* **30**, 17 (October 1977).
7. E. H. Colbert, *The Age of Reptiles* (Norton, New York, 1965).
8. D. A. Russell, in *The Cretaceous Systems in the Western Interior of North America*, W. G. E. Caldwell, Ed. (Geological Association of Canada Special Paper No. 13, 1975), pp. 132–133.
9. J. M. Hancock, in *The Fossil Record*, W. B. Harland, C. M. Holland, M. R. House, A. B. Reynolds, M. J. S. Rudwick, G. E. Satterthwaite, L. B. H. Tarlo, E. C. Willey, Eds. (Geological Society of London, London, 1967), p. 96.
10. E. H. Colbert, R. B. Cowles, C. M. Bogert, *Bull. Am. Mus. Nat. Hist.* **86**, 360 (1946).
11. R. B. Cowles, *Am. Nat.* **74**, 542 (1940).
12. H. Tappan, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **4**, 200 (1968).
13. N. J. Shackleton, in *The Fate of Fossil Fuel CO_2 in the Oceans*, N. R. Andersen and A. Malahoff, Eds. (Plenum, New York, 1977), p. 401.
14. P. M. Kroopnick, S. V. Margolis, C. S. Wong, in *ibid.*, p. 295.
15. W. S. Broecker, in *Carbon and the Biosphere*, G. M. Woodwell and E. V. Pecan, Eds. (U.S. Atomic Energy Commission, Washington, D.C., 1973), p. 44.
16. E. Voight, *Geol. Rundsch.* **54**, 270 (1964).
17. C. A. Wicher, *Geol. Jahrb.* **68**, 7 (1953).
18. J. A. Jeletzky, *Geol. Jahrb. Beih.* **1**, 142 (1951).
19. R. B. Cowles and C. M. Mitchell, *Bull. Am. Mus. Nat. Hist.* **83**, 288 (1944).
20. R. B. Cowles, *Ecology* **22**, 125 (1941).
21. L. C. Cole, *ibid.* **24**, 94 (1943).
22. R. B. Cowles, *Am. Nat.* **79**, 561 (1945).
23. H. A. Lowenstam and S. Epstein, in *20th International Geological Congress, Mexico* (1959), pp. 65–76; H. A. Lowenstam, in *Problems in Paleoclimatology*, A. E. M. Narin, Ed. (Interscience, New York, 1964), pp. 227–252.
24. R. B. Cowles and G. L. Burleson, *Am. Nat.* **79**, 417 (1945).
25. G. C. Packard, C. R. Tracy, J. J. Roth, *Biol. Rev.* **52**, 72 (1977).
26. H. K. Erben, *Abh. Akad. Wiss. Lit. Math.-Naturwiss. Kl.* **6**, 191 (1972).
27. H. R. Bustard, K. Simkiss, N. K. Jenkins, *J. Zool.* **158**, 311 (1969); N. K. Jenkins, *Comp. Biochem. Physiol.* **A51**, 891 (1975).
28. A. T. Leighton and W. L. Beane, personal communication.
29. H. S. Siegel, in *Adaptation of Domestic Animals*, E. S. E. Hafez, Ed. (Lea & Febiger, Philadelphia, 1968), p. 301.
30. A. J. Desmond, *The Hot Blooded Dinosaurs* (Blond & Briggs, London, 1975), p. 196.
31. N. F. Hughes, *Palaeobiology of Angiosperm Origins* (Cambridge Univ. Press, London 1976), p. 36.
32. C. D. Keeling (unpublished data), cited in *Man's Impact on the Climate*, W. H. Matthews, W. W. Kellogg, G. D. Robinson, Eds. (MIT Press, Cambridge, Mass., 1971), p. 23.
33. M. N. Bramlette, *Science* **148**, 1696 (1965).
34. R. H. Whittaker and G. E. Likens, in *Carbon and the Biosphere*, G. M. Woodwell and E. V. Pecan, Eds. (U.S. Atomic Energy Commission, Washington, D.C., 1973), p. 283.
35. S. Manabe and R. T. Wetherald, *J. Atmos. Sci.* **24**, 786 (1967).
36. R. Revelle, in *Man's Impact on the Climate*, W. H. Matthews, W. W. Kellogg, G. D. Robinson, Eds. (MIT Press, Cambridge, Mass., 1971), p. 282.
37. W. S. Broecker, in *The Late Cenozoic Glacial Ages*, K. K. Turekian, Ed. (Yale Univ. Press, New Haven, Conn., 1971), p. 246.
38. L. Machta, in *Carbon and the Biosphere*, G. M. Woodwell and E. V. Pecan, Eds. (U.S. Atomic Energy Commission, Washington, D.C., 1973), p. 26.
39. J. M. Mitchell, Jr., in *Global Effects of Environmental Pollution*, S. F. Singer, Ed. (Springer-Verlag, New York, 1968), p. 145.
40. P. E. Damon and S. M. Kunen, *Science* **193**, 447 (1976).
41. R. M. Koerner, *ibid.* **196**, 15 (1977).
42. S. Manabe and R. T. Wetherald, *J. Atmos. Sci.* **32**, 3 (1975).
43. K. K. Turekian, *Oceans* (Prentice-Hall, Englewood Cliffs, N.J., 1968), p. 80.
44. E. Eriksson, *J. Geophys. Res.* **68**, 3873 (1963).
45. W. S. Broecker, *Science* **189**, 460 (1975).
46. C. Emiliani, quoted by F. Warshofsky, *Reader's Digest* **111**, 133 (1977).
47. C. Emiliani, S. Gartner, B. Lidz, K. Eldridge, D. K. Elvey, T. C. Huang, J. J. Stipp, M. F. Swanson, *Science* **189**, 1083 (1975). Arguments against Emiliani's concept of a rapidly melting Laurentian ice cap, and the time of occurrence of the melting are by H. E. Wright Jr., and J. Stein, W. R. Farrand and E. B. Evenson, and J. T. Andrews, *Science* **193**, 1268 (1976). Emiliani's rebuttal occurs in *ibid.*, p. 1270.

Higher Order Structure of Simian Virus 40 Chromatin

Histone H1 is responsible for the condensation of intranuclear viral DNA-protein complexes.

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Ingrid Eicken, Walter Keller

The DNA in the nuclei of all eukaryotic cells is packaged by association with histone proteins into chromatin fibers consisting of a linear array of particles termed ν-bodies (*1*), nucleosomes (*2*), or platysomes (*3*). Each nucleosome contains about 140 base pairs of DNA complexed with a pair of each of the four histone types H2A, H2B, H3, and H4 (*4, 5*). The nucleosomes are connected by DNA linkers varying in length between about 30 and 70 base pairs, depending on the source of the tissue investigated (*4*). The fifth type of histone, H1, is not part of the nucleosome proper and is probably bound to the linker regions between adjacent particles (*6, 7*). The DNA helix is coiled around the outside of the nucleosomes, giving rise to a flexibly jointed chain of repeating units (*5, 8*). Chromatin fibers vary in appearance in the electron microscope, depending on the conditions employed during preparation. When nuclei are lysed on the specimen grid at very low ionic strength, the nucleosomes are well separated and alternate with segments of uncoiled DNA (*1, 2, 9*); this structure is referred to as the "beads-on-a-string" conformation. Frequently, however, chromatin fibers prepared by gentle methods and spread at low ionic strength are in a less extended state and

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look like linear arrays of nucleosomes that are in close contact (*10–12*). This type of close-packed chain of particles has been called the nucleofilament (*11*).

The nucleofilament may be the lowest level of a hierarchical series of higher-order packing modes of chromatin, which culminate in the mitotic chromosome (*13, 14*). There are indications that, in the interphase nucleus, the chromatin may normally not be unraveled to the nucleofilament state, but may maintain a higher state of organization (*11, 15, 16*). For example, chromatin fibers visualized in the presence of small amounts of divalent cations or moderate concentrations of monovalent salts have a diameter of 200 to 300 Å (*17, 18*) or three times greater than that of the primary nucleofilament. These "thick fibers" are thought to be formed by higher-order coiling (*11, 16*) or folding (*12*) of the nucleofilament and may represent the native state of the bulk of the chromatin in the interphase nucleus. Like the 100-Å nucleofilament, thick fibers can show either fairly uniform contours in the electron microscope (*13*) or else reveal a more globular periodicity (*12, 19, 20*), depending on experimental conditions. Histone H1 has been implicated in the maintenance of this higher order structure of chromatin (*4, 5, 7, 12, 21, 22*).

To study the conditions required for the formation and maintenance of higher order chromatin structure we have employed viral DNA-protein complexes isolated from the nuclei of cultured cells infected with simian virus 40 (SV40). The genome of SV40 can be regarded as a unique extrachromosomal replicon and transcription unit. Both replication and transcription of the viral DNA is accomplished with enzymes provided by the host cells (*23*). Because of its genetic simplicity, SV40 has served as a model for many studies on the molecular details of DNA replication and transcription (*23*). Moreover, the viral DNA is always associated with cellular proteins in the form of a nucleoprotein complex (*24–26*), whose main protein components are histones that are furnished by the host cells (*27*). Under certain conditions of isolation, the viral complexes contain a full complement of the intranucleosomal histones H2A, H2B, H3, and H4, as well as histone H1 (*28*). Such viral chromatin has properties that in many ways resemble those of the much larger and infinitely more complex chromatin of mammalian cells. When visualized by electron microscopy, SV40 chromatin exhibits a beaded-string conformation (*28–32*). In fact, one of the earliest demonstrations of the nucleosome structure of chromatin came from Griffith's work (*29*) on the ultrastructure of viral chromatin. Again with SV40 nucleoprotein complexes it was possible to show that the supercoiling of closed circular DNA (*33*) is a consequence of the association of histones in the form of nucleosomes (*30*).

Summary. Simian virus 40 nucleoprotein complexes undergo an ionic strength–dependent structural transition. At moderate ionic strength they contain histone H1 as well as the nucleosomal histones and have a compact conformation with globular subunits 190 angstroms in diameter. At high ionic strength histone H1 is released, and the structure unfolds into chains with an average of 24 nucleosomes. The extended viral chromatin converts to the compact form by the addition of histone H1. Transcriptionally active simian virus 40 chromatin undergoes the same structural transitions. The higher order structure of viral chromatin may be analogous to the compact state of cellular chromatin fibers observed at physiological ionic strength.

In this article we show that SV40 chromatin is highly condensed in its native state. The removal of histone H1 from viral chromatin by treatment with high concentrations of salt leads to the conversion of the compact state to an extended beaded-string conformation. Such H1-depleted nucleoprotein complexes can be brought back to a compact state by addition of purified histone H1 in vitro; this demonstrates the decisive role of histone H1 in the formation and maintenance of the higher order structure of chromatin. While our experiments were being carried out, similar studies were reported by Christiansen and Griffith and by Varshavsky *et al.* (*34*).

In solutions of extremely low ionic strength, the compact H1-containing SV40 nucleoprotein assumes an extended conformation similar to that of chromatin depleted of histone H1. This unfolding can be fully reversed by restoring the original salt concentration.

In vitro formation of the primary nucleofilament by reconstituting closed cir-

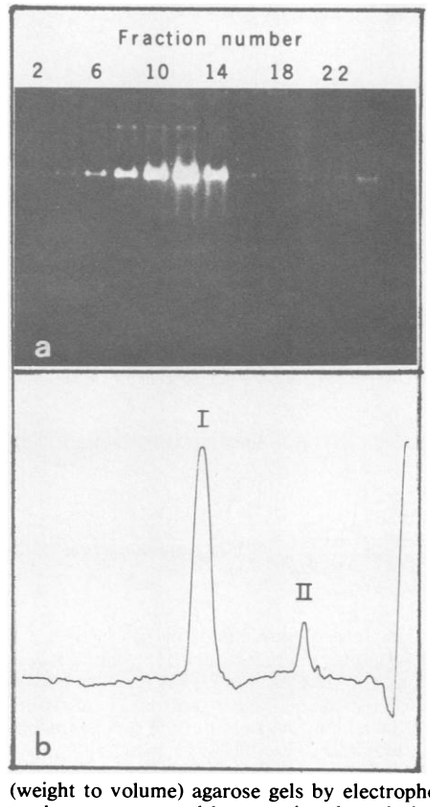


Fig. 1. Agarose gel electrophoresis of the DNA after sucrose gradient centrifugation of SV40 chromatin. Subconfluent African green monkey kidney cells (CV-1) were grown in petri dishes and infected at a multiplicity of approximately 10 pfu/cell with plaque-purified SV40 virus, strain 776 (*38*). The cultures were kept at 37°C for 1 hour, and 10 ml of culture medium containing 5 percent fetal calf serum was then added. After 28 to 38 hours (in some cases after 24 hours), the cells were harvested and nuclei were prepared (*39*). The SV40 chromatin was eluted from the nuclei (*40*), and the suspension was centrifuged for 10 minutes at 10,000 rev/min (Sorvall SS34 rotor). The SV40 nucleoprotein complexes were recovered in the supernatant. Samples (1 ml) of nuclear extract were layered on sucrose gradients (5 to 20 percent) prepared in the following buffer: 0.02*M* tris-HCl, *p*H 7.8; 0.05*M* $(NH_4)_2SO_4$; NaCl, varying from 0.05*M* to 0.6*M* as indicated in the legends of the appropriate figures; 1 m*M* dithiothreitol; and 1 m*M* EDTA. The gradients were centrifuged for either 2 hours or 3 hours at 39,000 rev/min at 4°C (SW40 rotor). Fractions (approximately 0.5 ml) were collected from the bottom of the centrifuge tubes. To quantify the amount of viral DNA in fractions from sucrose gradients, portions (25 μl) were mixed with 25 μl of 10 percent sodium dodecyl sulfate, 0.05*M* EDTA, 0.02 percent bromophenol blue, and 20 percent glycerol; they were incubated at 37°C for 10 minutes and analyzed on 1.4 percent (weight to volume) agarose gels by electrophoresis (*55*). The amount of DNA in various gel tracks was measured by scanning the gel photographs with a Joyce-Loebl densitometer and comparing the peak heights with those of a reference DNA sample on the same gel. (a) Photograph of DNA, stained with ethidium bromide, in fractions of a sucrose gradient, containing 0.15*M* sodium chloride, subjected to electrophoresis for 2 hours. (b) A densitometer scan of slot No. 10 of the gel shown in (a). The gradient fractions with the peak of SV40 chromatin contained viral DNA exclusively, most of which was in the supercoiled form I (*33*).

cular, relaxed DNA with the four nucleosomal histones H2A, H2B, H2, and H4 requires a change in the topological winding number (*33*) or linking number (*35, 36*) of the DNA (*30*). This change is effected by transient interruptions of the covalently closed phosphodiester backbone resulting from the presence of DNA-relaxing enzyme (*37*) in the reconstitution mixture. We will show below that the transition from the compact to the extended form of SV40 chromatin is not accompanied by a change in the topological winding number of its DNA.

Our experiments also suggest that transcriptionally active complexes of SV40 DNA and protein are in a compact higher order state similar to that of transcriptionally inactive SV40 chromatin.

Isolation of SV40 Chromatin

CV-1 cells, an established line of African green monkey kidney cells, grown in culture were infected at a multiplicity of about 10 plaque-forming units of SV40 virus, strain 776 (*38*). Between 28 and 38 hours later, cells were scraped from the plates and collected by low-speed centrifugation. Nuclei were isolated (*39*), and the viral nucleoprotein complexes were selectively eluted (*40*). Nuclear extracts were centrifuged through linear sucrose gradients at various ionic strengths as described below. To monitor the amount and the distribution of SV40 chromatin in gradient fractions, samples were analyzed by agarose gel electrophoresis (Fig. 1, legend). After sucrose gradient centrifugation, the SV40 chromatin is of sufficient purity to be visualized in the electron microscope. The complexes contain only SV40 DNA of which 80 percent is in the supercoiled (form I) state after dissociation of the proteins by treatment with sodium dodecyl sulfate (Fig. 1). The amount of viral

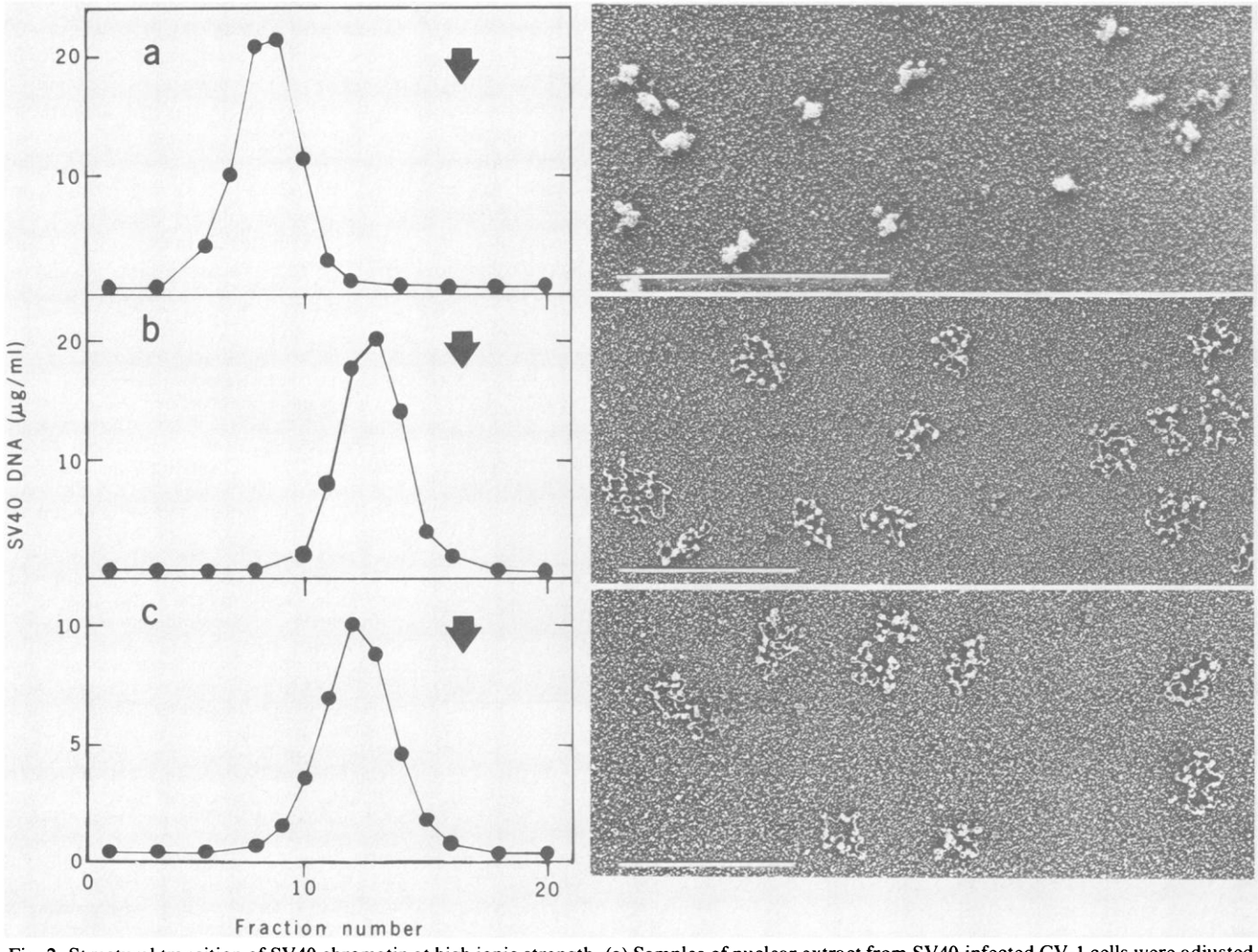


Fig. 2. Structural transition of SV40 chromatin at high ionic strength. (a) Samples of nuclear extract from SV40-infected CV-1 cells were adjusted to 0.1*M* sodium chloride and centrifuged in sucrose gradients containing 0.1*M* sodium chloride. The SV40 chromatin complexes which sedimented at 70*S* (the arrow indicates the 21*S* position of ^{3}H-labeled SV40 DNA I, which served as sedimentation marker in separate gradients), revealed a highly compact globular configuration when examined under the electron microscope. (b) When the nucleoprotein complexes were isolated and centrifuged in the presence of 0.6*M* sodium chloride, a shift of the sedimentation rate to 40*S* was observed. The compact globular form of the SV40 chromatin converted under these high salt conditions to an open structure with a characteristic beads-on-a-string appearance. (c) This nucleosomal arrangement of the SV40 chromatin was preserved when the "high-salt" complexes were extensively dialyzed against 0.1*M* sodium chloride. The sedimentation rate of this complex was approximately 50*S* when resedimented in sucrose gradients containing 0.1*M* sucrose. For the electron microscopy samples (1 to 5 μg of DNA per milliliter) were fixed with glutaraldehyde (final concentration 0.2 percent) for 15 minutes at 4°C (*29*). Small drops of the solution were placed, for 1 or 2 minutes, on glow-discharged carbon-coated copper grids. The excess liquid was removed by filter paper, and the grids were washed twice face-down on 100-μl drops of double-distilled water (placed on Parafilm) for 10 minutes. The samples were stained with uranyl acetate (*53*), rinsed in ethanol, and dried in air. Rotary shadow-casting was performed with platin-palladium (80 : 20) at an angle of 8°. Electron micrographs were taken with a Zeiss electron microscope (EM 10A) at 40 kV and magnifications of 20,000 to 35,000. The magnification indicator was routinely controlled by comparison with a grating replica. (Bar is 0.5 μm, in each case.)

DNA throughout the gradient fractions was quantified by densitometry of gel photographs (Fig. 1b) (*41*).

Structural Transition at High Ionic Strength

There is a consistent correlation between the sedimentation rate and the appearance under the electron microscope of SV40 chromatin. Both properties depend on the ionic strength of the solutions in which the nucleoprotein complexes are kept.

At moderate salt concentrations, 0.1*M* sodium chloride, SV40 complexes sedimented at approximately 70*S*, compared with protein-free SV40 marker DNA, which sediments at 21*S* (Fig. 2a). Increasing the ionic strength in the region from 0.2 to 0.7 led to a gradual decline of the sedimentation rate. At an ionic strength of 0.7, the complexes sedimented at approximately 40*S* (Fig. 2b), and the sedimentation coefficient did not decrease with further increase of the salt concentration, up to an ionic strength of 0.95. At still higher salt concentrations, the sedimentation coefficient dropped to 21*S* as a result of the release of all histones from the DNA (results not shown). Electron microscopy of SV40 chromatin centrifuged through gradients containing moderate and high concentrations of sodium chloride revealed that nucleoprotein complexes isolated at the lower salt concentration were highly compact, whereas those exposed to high salt consisted of open rings with the characteristic beaded-string morphology of chains of nucleosomes (Fig. 2). Thus, the lower sedimentation rate at high ionic strength was due to the opening of a condensed higher order structure of SV40 chromatin.

When the peak fractions of SV40 chromatin from gradients with high salt concentrations were brought back to the original low ionic strength by dialysis and centrifuged in low salt gradients, the nucleoprotein complexes remained slow-sedimenting and retained their extended conformation (Fig. 2c). This result suggested that SV40 chromatin during centrifugation in high salt had lost a component required for the maintenance of the compact conformation. The transition from the compact form to the extended configuration began at an ionic strength of 0.3 and was complete between 0.6 and 0.7 (Fig. 3). At this salt concentration, histone H1 is selectively dissociated from cellular chromatin (*42*) and, as is demonstrated below, it is indeed histone H1 which is responsible both for the formation and the maintenance of the compact state of SV40 chromatin.

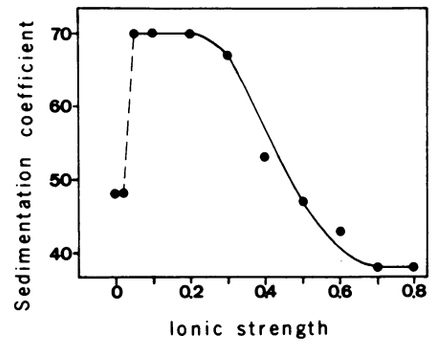


Fig. 3. Dependence of sedimentation rate of SV40 chromatin on ionic strength. The peak position of a series of sucrose gradient centrifugations containing increasing concentrations of sodium chloride are plotted as a function of ionic strength. The two gradients at low ionic strength (to the left of the dotted line) were prepared in 1 m*M* tris-HCl, *p*H 8.4, and 1 m*M* EDTA; or in 10 m*M* tris-HCl, *p*H 8.4, and 1 m*M* EDTA, respectively.

Proteins in SV40 Chromatin

The proteins associated with the different forms of SV40 chromatin were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 4). Purified histone fractions and the proteins of SV40 virions as references are shown in Fig. 4a. The polypeptide patterns obtained from individual fractions of sucrose gradients containing either moderate (μ = 0.2) or high (μ = 0.7) salt concentrations are shown in Fig. 4, b and c. In the regions of the gradients containing SV40 chromatin, a number of prominent polypeptide bands could be distinguished, including bands corresponding to the viral capsid proteins VP1, VP2, and VP3, and bands representing the nucleosomal histones H2A, H2B, H3, and H4. The viral capsid polypeptides did not cosediment precisely with the DNA-protein complexes; they probably originated from empty capsids that sedimented to a similar position in the gradients as the viral chromatin and varied in their relative amounts among different preparations (*43*). As was expected, capsid bands were very faint in complexes isolated relatively early (24 hours) after infection and became more intense in complexes isolated at later times in the infectious cycle (33 to 38 hours). By contrast, the histone bands corresponded in their intensity and location to the distribution of viral DNA measured in the same gradient fractions. Compact SV40 chromatin sedimenting at 70*S* contained, in addition to the proteins just described, a doublet band, which migrated in the sodium dodecyl sulfate-polyacrylamide gel

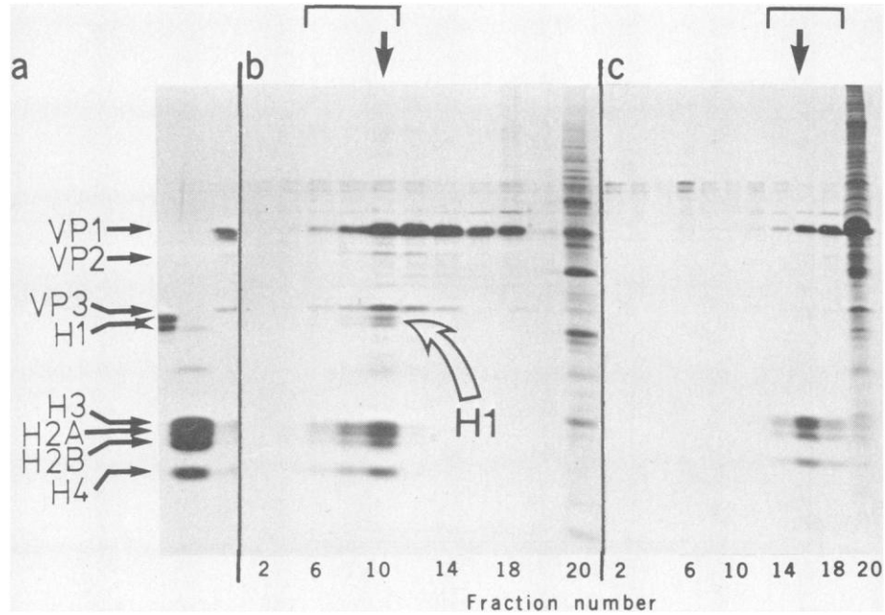


Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the proteins in SV40 chromatin. (a) The first three channels (from left to right) show the position of marker proteins: histone H1; histones H3, H2A, H2B, and H4; purified SV40 virions. (b) These channels show the proteins from every second fraction of the sucrose gradient shown in Fig. 2a. (c) The proteins from corresponding fractions of the gradient shown in Fig. 2c. The arrows and brackets at the top indicate the peak positions of SV40 DNA in the gradients. Electrophoresis was carried out as described (*54*).

to the characteristic position of a histone H1 marker (Fig. 4b). This double band was missing from extended 40*S* complexes treated with high salt (Fig. 4c). The presence of histone H1 in compact complexes and its absence in extended SV40 chromatin represents the most conspicuous difference in the polypeptide electrophoretic patterns of these two types of viral nucleoprotein.

In Vitro–Condensation of H1-Depleted SV40 Chromatin

As was shown above, the unfolding of the condensed form of SV40 chromatin during sedimentation at high ionic strength is accompanied by the disappearance of histone H1 from the viral nucleoprotein. To test the role of histone H1 in the maintenance of higher order structure, H1-depleted SV40 chromatin was mixed with increasing amounts of purified histone H1, dialyzed, centrifuged through sucrose gradients, and examined in the electron miscroscope (Fig. 5). In the absence of added histone H1, the complexes were slow sedimenting and showed an extended conformation (Fig. 5a). The addition of histone H1 caused the unfolded nucleoprotein complexes to contract. When the concentration of added histone H1 was sufficiently high, the reconstituted complexes sedimented at the same rate and exhibited the same compact globular shape as did native SV40 chromatin (Fig. 5c). When the concentration of histone H1 in the reconstitution mixture was subsaturating, the complexes sedimented with rates intermediate between fully extended and fully condensed forms (Fig. 5b). Electron microscopy of such complexes revealed their partially condensed appearance (Fig. 5b); they were composed of globules that were larger than nucleosomes and of smaller beads the size of nucleosomes. Both sedimentation analysis and electron microscopy indicated that partially reconstituted complexes formed unimodal populations, implying that the condensation reaction proceeds in a noncooperative fashion.

The results of the reconstitution experiments demonstrate that histone H1 is both necessary and sufficient to induce and to maintain the higher order compaction of SV40 chromatin. Results very similar to these have also been obtained by Griffith and Christiansen (*34*). A contraction of extended SV40 nucleoprotein complexes after the addition of histone H1 has been observed by Bellard *et al.* (*44*); Renz *et al.* (*12*) have described an increase in sedimentation rate of H1-depleted chromatin fragments after the addition of purified histone H1. The molecular details of the H1-binding reaction re-

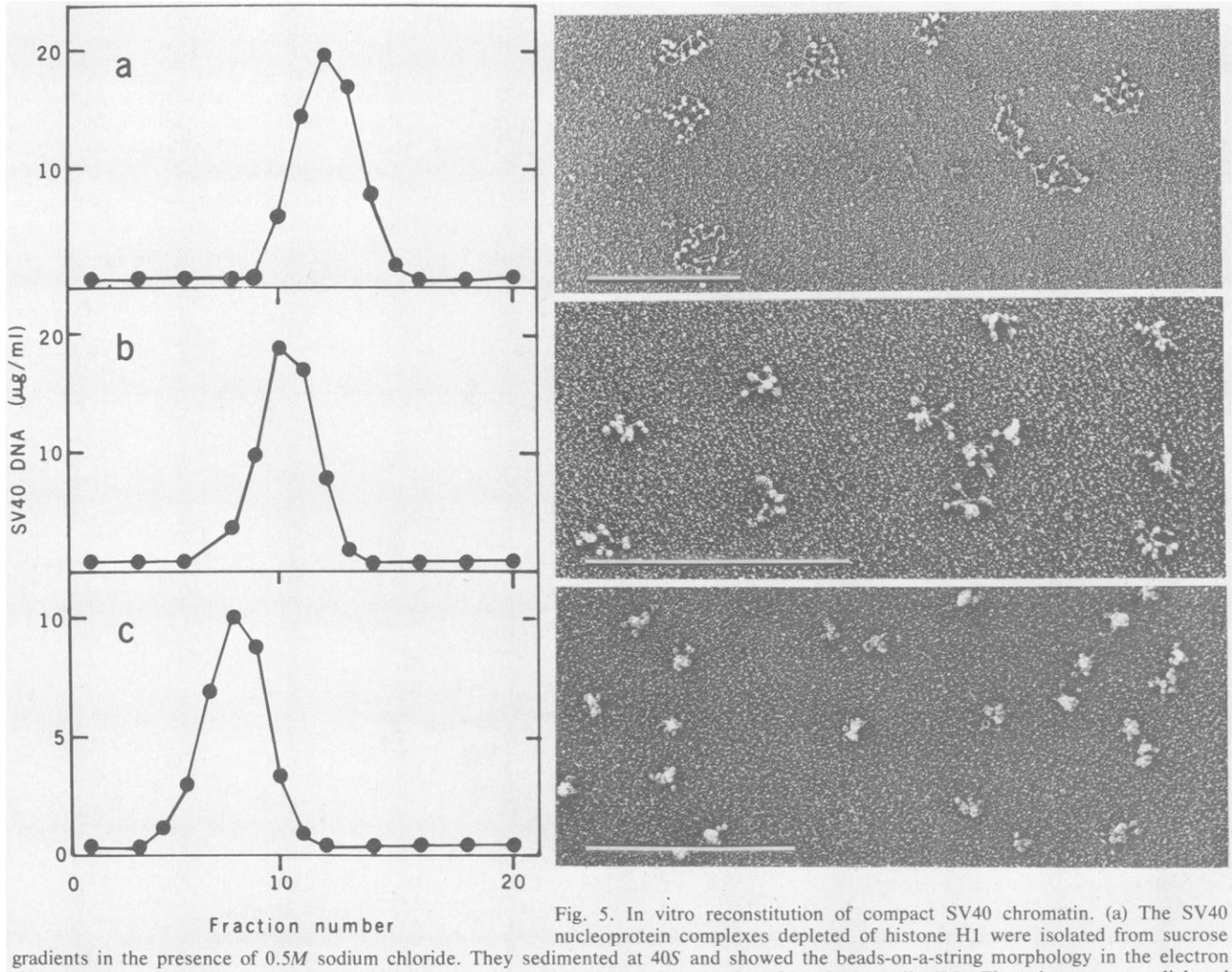


Fig. 5. In vitro reconstitution of compact SV40 chromatin. (a) The SV40 nucleoprotein complexes depleted of histone H1 were isolated from sucrose gradients in the presence of 0.5*M* sodium chloride. They sedimented at 40*S* and showed the beads-on-a-string morphology in the electron microscope. Portions from the peak fractions were mixed with histone H1 prepared from HeLa cells (*56*). The mixtures were dialyzed overnight against 0.15*M* sodium chloride, 0.01*M* tris-HCl, *p*H 7.8, and 1 m*M* EDTA, and subsequently centrifuged through sucrose gradients containing this buffer. Reconstitution with histone H1 caused the extended nucleoprotein complexes to contract resulting in an increase of the sedimentation rate and the appearance of a compact globular structure in the electron microscope. The concentration of histone H1 in the reconstitution mixture was (b) 10 μg/ml and (c) 30 μg/ml; the concentration of DNA was 50 μg/ml. (Bar is 0.5 μm in each case.)

main to be elucidated. As far as we can tell, the condensation is specific for histone H1. Other proteins that we have tested (bovine serum albumin, histones H2A, H2B, H3, and H4) had no effect on the structure of H1-depleted SV40 chromatin. The addition of basic proteins such as lysozyme or polylysine resulted in a nonspecific clumping of the material.

Structural Transition at Low Ionic Strength

At an initial phase of our study we noticed that compact SV40 nucleoprotein complexes converted spontaneously into the extended beaded-string form when they were diluted in water and kept for a few minutes at room temperature prior to fixation and processing for electron microscopy (*32*). The compact conformation is unstable at low salt concentrations (Fig. 6). Compact globular SV40 chromatin sedimenting at 70*S* at moderate ionic strength (0.2) was dialyzed against buffer of very low ionic strength and sedimented in a sucrose gradient prepared in the same buffer (Fig. 6). Such complexes sedimented more slowly than control samples (Fig. 6b) and exhibited the extended beaded-string morphology typical of SV40 chromatin that has lost histone H1. However, unlike H1-depleted complexes, the complexes that became unfolded in low salt could be fully restored to their original compact conformation simply by raising the salt concentration (Fig. 6c). This implies that histone H1 remained bound to the viral chromatin at low ionic strength. Protein analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis confirmed this assumption (data not shown).

The cause for the unfolding of higher order structure at low ionic strength is not known. Again, histone H1 may be implicated. As has been discussed by Renz and co-workers (*12*), the unfolding of compact chromatin at low ionic strength can be correlated with a change from a cooperative to a random binding mode of histone H1, as has been observed with complexes of protein-free DNA and histone H1 (*45*). The change in binding mode could then be the underlying cause for the inability of histone H1 to form cross-links with adjacent nucleosomes.

Morphology of SV40 Chromatin Observed During Unfolding

The finding that unfolding of compact SV40 chromatin on dilution into low ionic strength conditions was time-dependent offered an opportunity to examine intermediate forms occurring in this structural transition. Samples of native

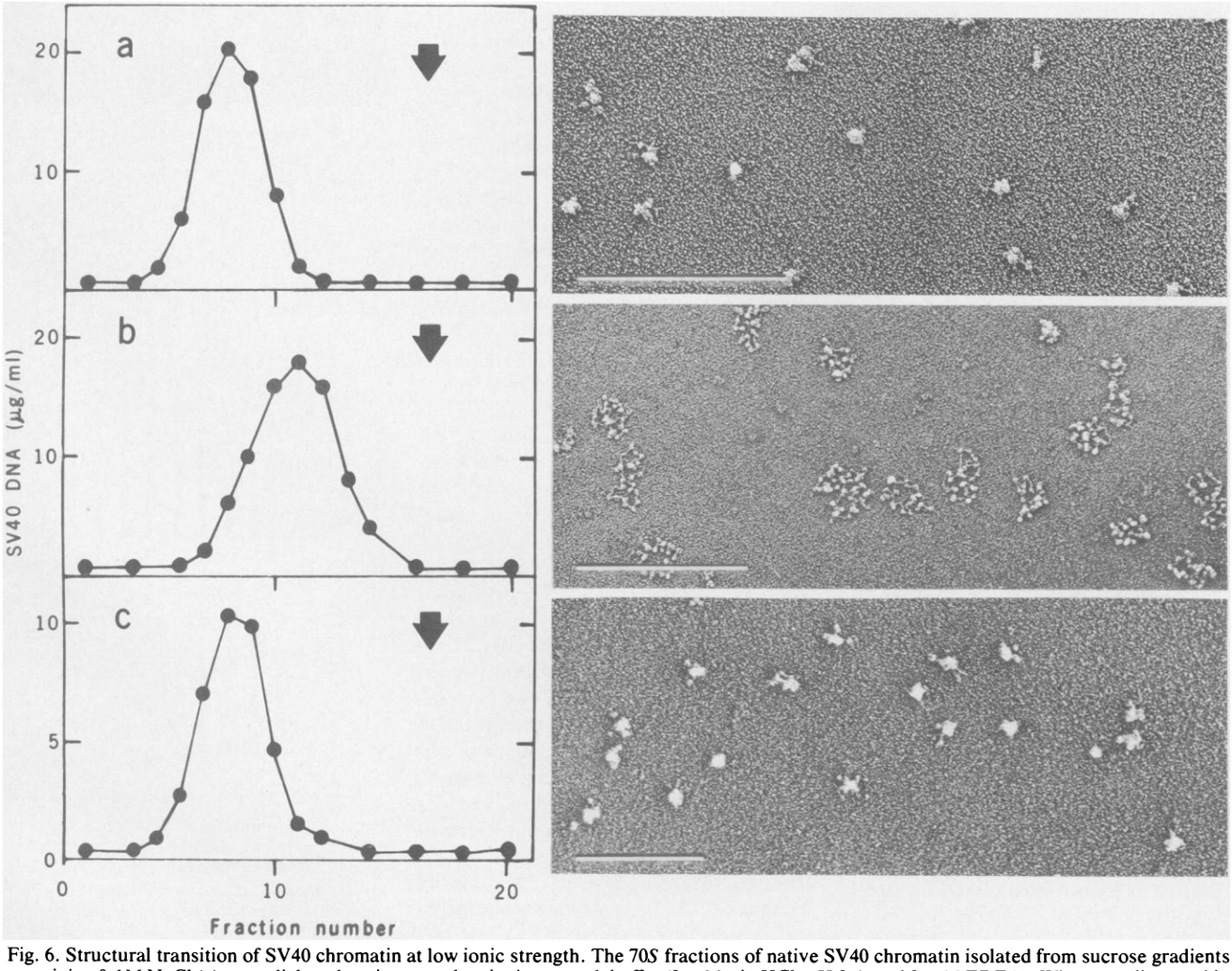


Fig. 6. Structural transition of SV40 chromatin at low ionic strength. The 70*S* fractions of native SV40 chromatin isolated from sucrose gradients containing 0.1*M* NaCl (a) were dialyzed against very low ionic strength buffer (2 m*M* tris-HCl, *p*H 8.4, and 2 m*M* EDTA). When resedimented in sucrose gradients (same ionic strength as dialysis buffer) the sedimentation rate shifted (a) from 70*S* to (b) a lower value. (c) In contrast to H1-depleted SV40 chromatin (compare Fig. 5), the compact globular morphology as well as the 70*S* sedimentation rate could be restored simply by raising the salt concentration in the sample to 0.1*M* sodium chloride. (Bar is 0.5 μm in each case.)

SV40 chromatin (see Fig. 2a) were diluted 20-fold with water, kept for various times at room temperature, and then fixed and spread for electron microscopy. The resulting preparations often contained on one and the same specimen grid a spectrum of forms ranging from fully condensed to completely extended.

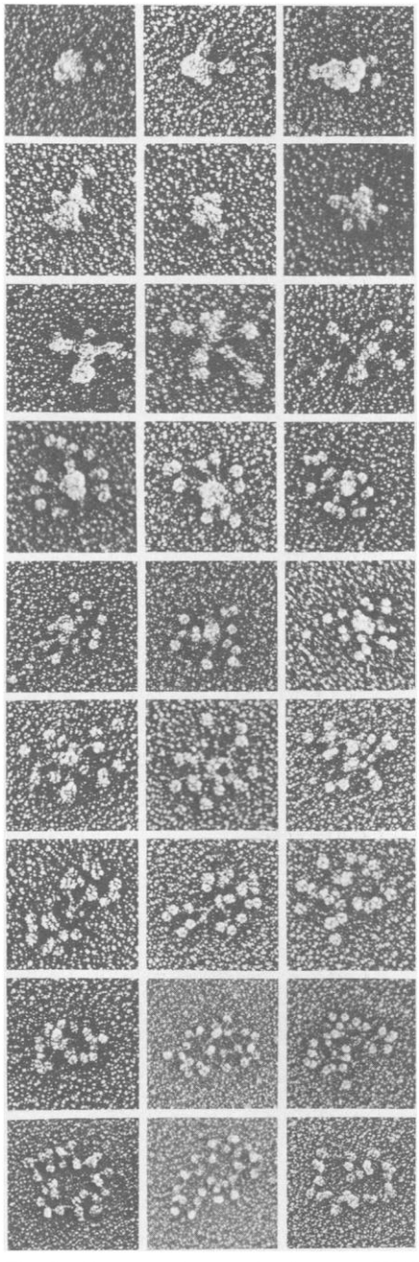


Fig. 7. Electron micrograph of native SV40 chromatin complexes diluted 20-fold with water and kept at room temperature (10 minutes) before spreading. Compact globular structures, beaded-string complexes, and intermediates can be observed. Selected examples of different degrees of unfolding are shown and illustrate the transition of the compact globular native structure to the beaded appearance of SV40 chromatin. In each row, complexes at a similar stage of unfolding are grouped together.

Individual complexes, selected from such samples, are shown in Fig. 7.

The native nucleoprotein complexes are represented by a highly compact structure with an average diameter of approximately 300 Å (Fig. 8). Although no details of an internal substructure can be made out, it is nevertheless clear that compact complexes are made up of large globular subunits. These globules are bigger than nucleosomes and have an average diameter of 190 Å. The number of large globules per complex could not accurately be determined because of the extremely close packing. The minimum estimate is four, but there may be up to eight large globules in a compact complex.

Attempts to visualize the internal structure of the large globules by negative staining methods have not been successful. Occasionally, one could see nucleosome-sized small beads at the edges of compact complexes; however, the interior composition of the large globules remained obscure. We assume that the individuality of the nucleosomes forming the structural elements of the large globules is maintained, a notion that is supported by the results of Renz and coworkers (*12*) and of Olins (*20*). These workers were able to demonstrate the presence of close-packed nucleosomes in the interior of thick chromatin fibers.

At the other end of the spectrum of structures observed by electron microscopy are the completely unfolded nucleoprotein complexes which simply consist of circular strings with beads of nucleosomes (Fig. 7). As is evident from a comparison with the morphology of H1-depleted complexes (Figs. 2 and 5), the two types are very similar in appearance. There is, however, one important difference, and that is the number of nucleosomes found per complex. As is shown in Fig. 9, the average number of nucleosomes in complexes unfolded by dilution in water was 24. By contrast, H1-depleted SV40 chromatin had only 21 nucleosomes per complex, as had also been reported by others (*29, 31, 44*). We presume that the two types of complexes in fact do contain the same number of nucleosomes; H1-depleted complexes may, however, tend to lose some of their nucleosomes upon spreading for electron microscopy. Otherwise, it would be difficult to explain the fact that H1-depleted complexes can be restored to their original compact form simply by adding histone H1. The length of SV40 DNA puts an upper limit to the number of nucleosomes that can be accommodated in one complex. The biochemical repeat unit of SV40 chromatin contains approximately 200 base pairs of DNA (*44*) and the total length of its DNA is 5200 base pairs (*23*). Therefore, the number of nucleosomes per nucleoprotein complex cannot exceed 26. Indeed, we did not find any SV40 complexes with more than 26 nucleosomes. Our results imply that SV40 chromatin in its native form is fully covered by nucleosomes and does not contain any free stretches of DNA. Inspection of extended SV40 chromatin af-

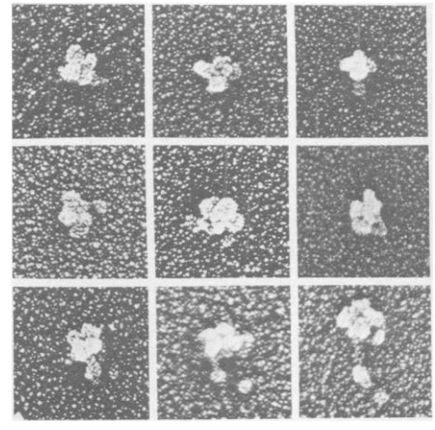


Fig. 8. Native SV40 nucleoprotein complexes. The native SV40 chromatin appears as a compact globular structure with an average diameter of 300 Å.

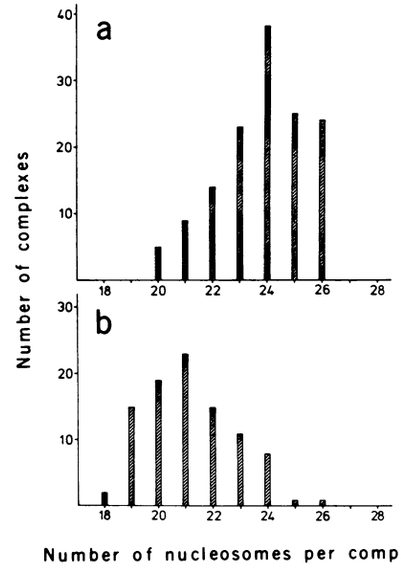


Fig. 9. Distribution of the number of nucleosomes (a) in native SV40 chromatin unfolded by dilution into low ionic strength and (b) in H1-depleted nucleoprotein complexes. The medians and the confidence intervals were determined by computer. They were 24 (24/24) in (a) and 21 (21/21) in (b). The medians are indicated in the following notation: M (l/u) where M stands for the median and l and u for the lower and upper bound of the 5 percent confidence interval for the median.

ter negative staining confirmed this conclusion (data not shown).

Partially unfolded nucleoprotein complexes are composed of both large globules and of small nucleosomes (Fig. 7). Frequently, cross-shaped or star-shaped conformations were observed. Although no precise details can be deduced from such structures, there are discrete domains of condensation where a number of nucleosomes are rearranged such that larger subunits, the globules, are formed. These globules may be equivalent to the "superbeads" described to occur in nuclear chromatin (*12*). One possible mechanism for the condensation could be the helical coiling of the nucleofilament into a toroidal superhelix as shown in a highly schematic manner in Fig. 10. After further condensation, the individual turns or loops of this superhelix would appear in the electron microscope as globules. This model is consistent with the concept of Finch and Klug (*11*), who postulate the organization of the primary nucleofilament into a helical superstructure, so-called solenoids. Alternatively, the globules in condensed SV40 chromatin could simply be formed by clustered arrays of densely packed nucleosomes.

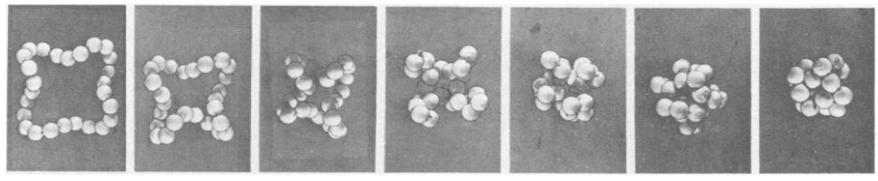
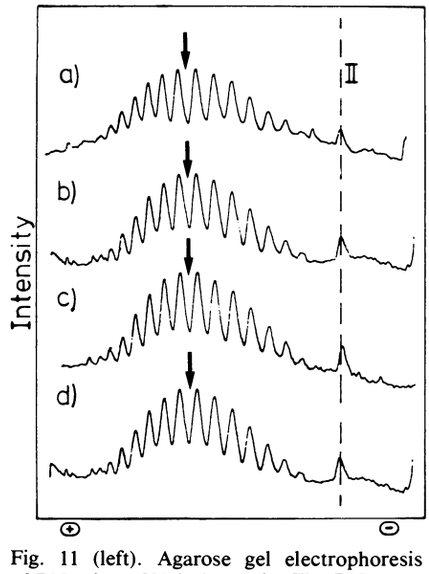


Fig. 10. Model of SV40 chromatin showing a possible mechanism for the condensation of the extended beaded string conformation into the compact globular form.

Supercoils in DNA from SV40 Chromatin

The DNA isolated from purified SV40 virions is superhelical and consists of a Gaussian set of topological isomers; the members within this distribution differ by integral values of their topological winding number (*33*) or linking number (*35, 36*). The linking number corresponds to the number of times one strand of the DNA double helix is wound around the other strand. Superhelical turns occur in closed-circular DNA whenever there is a difference in the number of helical turns and the linking number. The average number of superhelical turns in DNA from SV40 virions was determined by agarose gel electrophoresis to be −24 to −26 (*41, 46*). The reference state for these measurements is 0.2*M* sodium chloride and 37°C; the minus sign indicates left-handed supercoiling. Superhelical turns in free SV40 DNA are the consequence of the formation of nucleosomes (*30*). Because the average number of nucleosomes in SV40 chromatin and the number of superhelical turns in protein-free DNA is the same, formation of one nucleosome operationally causes one superhelical turn in free DNA. To test whether the DNA extracted from extended and compact intranuclear SV40 chromatin and that from SV40 virions would differ in superhelicity, DNA isolated from compact SV40 complexes and from H1-depleted open complexes was analyzed by agarose gel electrophoresis (*41*) and compared to a reference DNA extracted from purified SV40 virions. The H1-depleted complexes were dialyzed to lower the ionic strength and were incubated with an excess of DNA-relaxing enzyme (*38*) prior to the extraction and the analysis of their DNA (Fig. 11). Relaxing enzyme provides transient swivels for the DNA, allowing it to adjust its linking number when necessary. Under the conditions chosen, the topological isomers of SV40 DNA migrated as a Gaussian set of separate DNA bands, each differing from its neighbors by one superhelical turn (*41, 47*) (Fig. 11). The center of the Gaussian distributions of DNA bands is located at the same position in all channels of the gel,



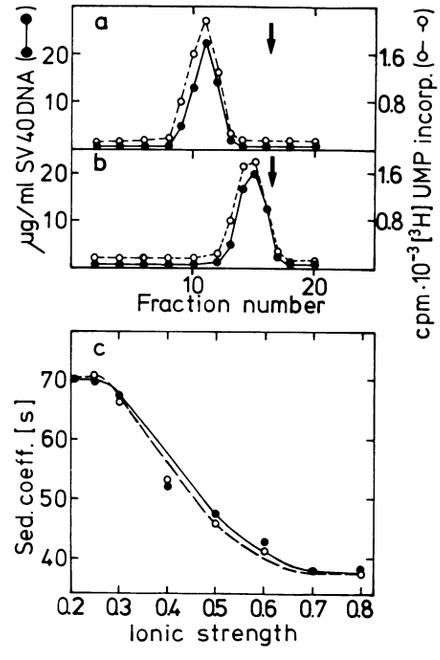


Fig. 11 (left). Agarose gel electrophoresis of DNA from SV40 chromatin. The DNA was extracted from the peak fractions of sucrose gradients in 0.1*M* NaCl and of high salt gradients (Fig. 2) and analyzed on a 25-cm-long slab gel of 1.4 percent agarose (*41*). The gel and the tank buffer contained ethidium bromide (0.03 μg/ml). Electrophoresis was performed at 2 V/cm for 38 hours. (a) The DNA from native complexes; (b) DNA from H1-depleted complexes; (c) DNA from H1-depleted complexes that were dialyzed against 0.2*M* NaCl, 10 m*M* tris-HCl (*p*H 7.9), 0.2 m*M* EDTA, and incubated with 50 units of DNA-relaxing enzyme (*37*) at 37°C for 10 minutes prior to extraction; (d) DNA from purified SV40 virions. Migration was from right to left. The position of nicked-circular SV40 (form II) is so marked. The arrows indicate the average intensity locations of the Gaussian DNA band sets. Fig. 12 (right). RNA synthesis in gradient fractions containing SV40 chromatin. Fractions of sucrose gradients sedimented in the presence of increasing concentrations of sodium chloride were assayed for RNA-synthesizing activity in reaction mixtures containing in a total volume of 0.1 ml: 0.03*M* tris-HCl, *p*H 7.9; 0.25 m*M* adenosine, cytidine, and guanosine triphosphate; 6×10^{-7}*M* [^{3}H]uridine triphosphate (41 Ci/mmole); 0.075*M* $(NH_4)_2SO_4$; 5 m*M* $MgCl_2$; 2 m*M* $MnCl_2$; 0.75 m*M* dithiothreitol; 0.5 m*M* EDTA; and 10 to 50 μl of gradient material. The mixtures were incubated at 37°C for 30 minutes; RNA was precipitated by adding 2 ml of ice-cold 5 percent trichloroacetic acid containing 10 m*M* $Na_4P_2O_7$. The precipitates were collected on Whatman GF/C filters, washed with 2 percent trichloroacetic acid or 10 m*M* $Na_4P_2O_7$ and ethanol, and dried in air; radioactivity was counted in a toluene-based scintillation fluid. (a) 0.05*M* and (b) 0.5*M* sodium chloride; arrows show the position of 21*S* SV40 marker; (c) the peak positions of SV40 DNA and RNA synthesis activity of a series of gradient centrifugations with increasing concentrations of sodium chloride are plotted as a function of ionic strength.

showing that the number of superhelical turns in the DNA from all samples is the same. Thus, it appears that the number of superhelical turns in SV40 DNA is set by the number of nucleosomes formed on it and that the higher-order folding of the primary nucleofilament into the condensed state is not accompanied by a change in the linking number.

Nucleosome formation results in a change of the linking number. The difference in the linking number between closed-circular relaxed DNA and closed-circular DNA isolated from SV40 chromatin causes a corresponding difference in the number of superhelical turns resulting in a difference in electrophoretic mobility. Thus, the electrophoretic method measures the difference in linking number between otherwise identical DNA molecules (*3, 36*).

The finding of one superhelical turn per nucleosome may at first sight be interpreted to indicate that the DNA helix is wound around each nucleosome in a single loop. However, it is difficult to reconcile this interpretation with current models of DNA folding in chromatin. There are good arguments favoring the existence of more than one supercoil per nucleosome (*3, 48*). As pointed out by F. H. C. Crick [personal communiction; also discussed in (*3*) and (*49*)] the possibility must be considered that the helical twist (number of base pairs per helix turn) of DNA free in solution is slightly different from that of DNA that is complexed with histones in chromatin. It is conceivable that, on removal of the histones, the primary DNA helix becomes slightly unwound. This would result in a decrease of the number of helical turns and would be accompanied by a corresponding reduction of the number of supercoils because of the interdependence of these two properties in closed circular DNA. Therefore, the number of superhelical turns measured with free SV40 DNA need not be the same as the number of supercoils present in the DNA while organized in the nucleoprotein complex. To clarify this relationship, an experimental measurement of the number of base pairs per helix turn would be required. In chromatin, this number most likely is very close to ten, because deoxyribonuclease I cuts DNA on the nucleosome at intervals of ten base pairs (*8*). A corresponding value for free DNA in solution is not known with equal precision.

Transcription of SV40 Chromatin

Viral chromatin extracted from cells infected by polyoma virus or SV40 has been shown to be transcriptionally active in vitro; when incubated under appropriate conditions with ribonucleoside triphosphates, virus-specific RNA is synthesized as a result of the presence of RNA polymerase molecules that had initiated RNA synthesis prior to the isolation of the viral nucleoprotein complexes (*26*). The SV40 chromatin extracted from nuclei as described in this article also contains transcriptionally active nucleoprotein complexes. Presumably, however, the transcriptionally active forms constitute only a small fraction of the total number of nucleoprotein complexes (*26*). We have investigated the sedimentation behavior of such transcription complexes in sucrose gradients that contained different concentrations of sodium chloride. We found a peak of RNA synthesis at all salt concentrations tested (Fig. 12). Moreover, the position of the peaks of RNA synthesis in all cases coincided with the position of the viral chromatin in the gradients. The RNA synthesized in vitro on SV40 chromatin is virus-specific, and most of it is being transcribed from the L strand (*50*) of SV40 DNA (*51*). The rate and the extent of RNA synthesis in vitro did not differ appreciably between viral nucleoprotein complexes in the compact and the open state (Fig. 12, and unpublished results). We found it surprising that the sedimentation rate of the transcriptionally active nucleoprotein complexes underwent the same gradual transition from 70*S* at moderate ionic strength to 40*S* at higher salt concentrations (Fig. 12c). We had expected that transcriptionally active complexes would behave as a separate class upon sedimentation. As was shown above, the shift in sedimentation rate at higher ionic strength signifies a structural transition from a compact to an extended conformation. Thus, at physiological ionic strength ($\mu = 0.2$), transcriptionally active complexes appear to be in the same type of condensed superstructure as inactive nucleoprotein complexes. This was unexpected because it is generally believed that transcriptionally active chromatin is in a more open conformation (*52*).

We do not know the mechanism by which RNA polymerase manages to move along a chromatin fiber. Possibly, the higher order organization and also the nucleosome structure is transiently and locally disrupted during the act of transcription; if histone H1 is holding together adjacent nucleosomes, these cross-links have to be disconnected when the RNA polymerase moves along a given stretch of DNA. If there are only one or a few nascent RNA chains present per SV40 nucleoprotein complex, the overall condensed structure of the viral chromatin may be maintained. Possibly, transcription on cellular chromatin could take place on chromatin fibers that are in a higher state of condensation than is the primary nucleofilament.

Conclusion

If we can extrapolate from the findings made on viral nucleoprotein complexes to cellular chromatin it appears that the higher-order organization of chromatin does not interfere with the processes of DNA replication and transcription. The detailed structure of chromatin at the supranucleosomal level and the maintenance of this conformation by histone H1 should be challenging subjects for future studies.

References and Notes

1. A. L. Olins and D. E. Olins, *Science* **183**, 330 (1974).
2. P. Oudet, M. Gross-Bellard, P. Chambon, *Cell* **4**, 281 (1975).
3. J. T. Finch, L. C. Lutter, D. Rhodes, R. S. Brown, B. Rushton, M. Levitt, A. Klug, *Nature (London)* **269**, 29 (1977).
4. S. C. R. Elgin and H. Weintraub, *Annu. Rev. Biochem.* **44**, 725 (1975).
5. R. D. Kornberg, *Science* **184**, 868 (1974); *Annu. Rev. Biochem.* **46**, 931 (1977); K. E. van Holde, C. G. Sahasrabuddhe, B. R. Shaw, *Nucleic Acids Res.* **1**, 1579 (1974).
6. A. J. Varshavsky, V. V. Bakayev, G. P. Georgiev, *Nucleic Acids Res.* **3**, 477 (1976); J. P. Whitlock and R. T. Simpson, *Biochemistry* **15**, 3307 (1976).
7. M. Noll and R. D. Kornberg, *J. Mol. Biol.* **109**, 393 (1977).
8. M. Noll, *Nucleic Acids Res.* **1**, 1573 (1974).
9. C. L. F. Woodcock, J. P. Safer, J. E. Stanchfield, *Exp. Cell Res.* **97**, 101 (1976).
10. J. T. Finch, M. Noll, R. D. Kornberg, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3320 (1975).
11. J. T. Finch and A. Klug, *ibid.* **73**, 1897 (1976).
12. M. Renz, P. Nehls, J. Hozier, *ibid.* **74**, 1879 (1977); J. Hozier, M. Renz, P. Nehls, *Chromosoma* **62**, 301 (1977).
13. E. J. Dupraw, in *DNA and Chromosomes* (Holt, Rinehart & Winston, New York, 1970), p. 180.
14. A. L. Bak, J. Zeuthen, F. H. C. Crick, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1595 (1977).
15. L. Sperling and A. Klug, *J. Mol. Biol.* **112**, 253 (1977).
16. B. G. Carpenter, J. P. Baldwin, E. M. Bradbury, K. Ibel, *Nucleic Acids Res.* **3**, 1739 (1976).
17. H. Ris and B. L. Chandler, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 1 (1963); H. Ris and D. F. Kubai, *Annu. Rev. Genet.* **4**, 263 (1970).
18. A. S. Pooley, J. F. Pardon, B. M. Richards, *J. Mol. Biol.* **85**, 533 (1974); G. I. Kiryanov, T. A. Manamshjan, V. Y. Polyakov, D. Fais, J. S. Chentsov, *FEBS Lett.* **67**, 323 (1976); R. Tsanev and P. Petrov, *J. Microsc. Biol. Cell.* **27**, 11 (1976).
19. W. W. Franke, U. Scheer, M. F. Trendelenburg, H. Spring, H. Zentgraf, *Cytobiologie* **13**, 401 (1976).
20. A. L. Olins, *Cold Spring Harbor Symp. Quant. Biol.*, in press.
21. V. C. Littau, C. J. Burdick, V. G. Allfrey, A. E. Mirsky, *Proc. Natl. Acad. Sci. U.S.A.* **54**, 1204 (19 → A. E. Mirsky, C. J. Burdick, E. H. Davidson, V. C. Littau, *ibid.* **61**, 592 (1968).
22. E. M. Bradbury, B. G. Carpenter, H. W. E. Rattle, *Nature (London)* **241**, 123 (1973); M. A. Billett and J. M. Barry, *Eur. J. Biochem.* **49**, 477 (1974); E. M. Bradbury, S. E. Danby, H. E. Rattle, V. Giancotti, *ibid.* **57**, 97 (1975); K. Brasch, *Exp. Cell Res.* **101**, 396 (1976).
23. J. Tooze, Ed., *The Molecular Biology of Tumor Viruses* (Cold Spring Harbor Laboratory Press, New York, 1973); G. C. Fareed and D. Davoli, *Annu. Rev. Biochem.* **46**, 471 (1977); T. J. Kelly and D. Nathans, *Adv. Virus Res.* **21**, 85 (1977).
24. M. H. Green, H. I. Miller, S. Hendler, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1032 (1971); M. White and R. Eason, *J. Virol.* **8**, 363 (1971).
25. M. H. Green, *J. Virol.* **10**, 32 (1972); D. A. Gold-

stein, M. R. Hall, W. Meinke, *ibid.* **12**, 887 (1973); T. Seebeck and R. Weil, *ibid.* **13**, 567 (1974); R. T. Su and M. L. Depamphilis, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3466 (1976).
26. R. J. Shmookler, J. Buss, M. H. Green, *Virology* **57**, 122 (1974); M. H. Green and T. L. Brooks, *ibid.* **72**, 110 (1976).
27. J. McMillen and R. A. Consigli, *J. Virol.* **14**, 1326 (1974); W. Meinke, M. R. Hall, D. A. Goldstein, *ibid.* **15**, 439 (1975).
28. A. J. Varshavsky, V. V. Bakayev, P. M. Chumackov, G. P. Georgiev, *Nucleic Acids Res.* **3**, 2101 (1976).
29. J. D. Griffith, *Science* **187**, 1202 (1975).
30. J. E. Germond, B. Hirt, P. Oudet, M. Gross-Bellard, P. Chambon, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1843 (1975).
31. C. Cremisi, P. F. Pignatti, O. Croissant, M. Yaniv, *J. Virol.* **17**, 204 (1976).
32. H. Zentgraf, W. Keller, U. Müller, *Philos. Trans. R. Soc. London Ser. B* **283**, 299 (1978).
33. W. Bauer and J. Vinograd, in *Basic Principles in Nucleic Acid Chemistry*, P. O. P. T'so, Ed. (Academic Press, New York, 1974), vol. 2, pp. 262–305.
34. G. Christiansen and J. D. Griffith, *Nucleic Acids Res.* **4**, 1837 (1977); J. D. Griffith and G. Christiansen, *Cold Spring Harbor Symp. Quant. Biol.*, in press; A. J. Varshavsky, S. A. Nedospasov, V. V. Schmatchenko, V. V. Bakayev, P. M. Chumackov, G. P. Georgiev, *Nucleic Acids Res.* **4**, 3303 (1977).
35. F. B. Fuller, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 815 (1971).
36. F. H. C. Crick, *ibid.* **73**, 2639 (1976).
37. J. C. Wang, *J. Mol. Biol.* **55**, 523 (1971); J. J. Champoux and R. Dulbecco, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 143 (19 W. Keller, *ibid.* **72**, 2550 (1975); H.-P. Vosberg and J. Vinograd, *Biochem. Biophys. Res. Commun.* **68**, 456 (1976).
38. D. Nathans and K. J. Danna, *J. Mol. Biol.* **64**, 515 (1972).
39. R. Hancock, *ibid.* **86**, 649 (1974).
40. M. L. Depamphilis, P. Beard, P. Berg, *J. Biol. Chem.* **250**, 4340 (1975).
41. W. Keller, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4876 (1975).
42. H. H. Ohlenbusch, B. M. Olivera, D. Tuan, N. Davidson, *J. Mol. Biol.* **25**, 299 (1967).
43. In the electron microscope, the material from this region of the sucrose gradient (Fig. 4b, fraction 14) showed small capsidlike structures of irregular, often filamentous shapes. The absence of these structures from sucrose gradient fractions containing 0.6*M* sodium chloride (Fig. 4) indicate that they may represent salt-unstable capsomeric aggregates [C. F. T. Mattern, K. K. Takemoto, A. M. DeLava, *Virology* **32**, 378 (1967)].
44. M. Bellard, P. Oudet, J.-F. Germond, P. Chambon, *Eur. J. Biochem.* **70**, 543 (1976).
45. M. Renz and L. Day, *Biochemistry* **15**, 3220 (1976).
46. M. Shure and J. Vinograd, *Cell* **8**, 215 (1976).
47. R. E. Depew and J. C. Wang, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4275 (19 D. E. Pulleyblank, M. Shure, D. Tang, J. Vinograd, H.-P. Vosberg, *ibid.* **72**, 4280 (1975).
48. F. H. C. Crick and A. Klug, *Nature (London)* **255**, 530 (1975); H. M. Sobell, C.-C. Tsai, S. G. Gilbert, S. C. Jain, T. D. Sakore; *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3068 (1976); J. F. Pardon, D. L. Worcester, J. C. Wooley, R. I. Cotter, D. M. Lilley, B. M. Richards, *Nucleic Acids Res.* **4**, 3199 (1977).
49. M. Levitt, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 640 (1978).
50. J. Sambrook, P. A. Sharp, W. Keller, *J. Mol. Biol.* **70**, 57 (1972).
51. M. Botchan, E. Lacy, T. Maniatis, W. Keller, unpublished data.
52. H. Weintraub, A. Worcel, B. Alberts, *Cell* **9**, 409 (1976).
53. R. W. Davis, M. Simon, N. Davidson, *Meth. Enzymol.* **21**, 413 (1971).
54. J. O. Thomas and R. D. Kornberg, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2626 (1975).
55. W. Keller and I. Wendel, *Cold Spring Harbor Symp. Quant. Biol.* **39**, 199 (1974).
56. D. R. van der Westhuyzen, E. L. Böhm, C. van Holt, *Biochim. Biophys. Acta* **359**, 341 (1974). The histones were extracted from the nuclei of HeLa cells.
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Research Instrument Sharing

Continued availability of advanced instruments must involve sharing; how are instruments shared?

Charles L. Coulter

Summary. Continued progress in many areas of science depends on access to advanced modern instruments and the data they provide. Costly instruments have been shared in a number of disciplines for many years, and common patterns of shared usage have developed independently. The scientific and financial aspects of large instrument usage are discussed from the points of view of the instrument centers, the users, and the funding agencies. The instrument problem is not one problem but many, and coordinated solutions must be implemented with well-defined goals based on knowledge of the needs of the users and developers of instruments.

Access to advanced instrumentation facilities is essential for the conduct of most research projects in the fields of physics, chemistry, biology, and biophysics and has been a key factor in most recent advances in basic research in the physical and biological sciences. Awareness of this dependence on instruments and the increasing difficulties in obtaining funds to replace and upgrade instruments has led to growing concern about the problems of effective support, management, and utilization of research instruments. The National Academy of Sciences (NAS), the National Science Foundation (NSF), the National Institutes of Health (NIH), and the Energy Research and Development Administration [now part of the Department of Energy (DOE)] (*1–7*) have all recently sponsored or carried out studies, both formal and informal, of the need for and management of costly instrumentation resources. In addition, a broader study (*8*) of the state of scientific research in U.S. universities drew attention to the increasing concern within a wide spectrum of university departments about maintaining up-to-date instrument facilities for both teaching and research. While good cases can and are being made for alleviating these problems by asking Congress and the appropriate granting agencies to provide more funds in existing and new programs, these cases could be supported by better characterizing the usage patterns for current instruments.

One aspect of the overall problem that has received little attention is the current effectiveness of shared instrumentation facilities in various settings. Are presently available instruments being used in an equitable and cost-effective manner? Do various disciplines have discrete types of users, or are there broad models for shared usage that cut across disciplines? In this article some aspects of instrument sharing in natural science areas are reviewed in order to identify common features of use and in general to better characterize the user communities. Several fields of science have been selected as examples for discussion on the basis of the need of research scientists in these disciplines for access to costly instruments and on the basis of differing patterns of shared usage in these areas.

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