Salt- and histone H1-induced structural changes of reconstituted minichromosomes

M.Böttger, S.Scherneck, C.-U.von Mickwitz, H.Fenske and R.Lindigkeit

Zentralinstitut für Molekularbiologie der Akademie der Wissenschaften der DDR, DDR-1115 Berlin-Buch, GDR

Received 14 February 1979

ABSTRACT

Structural changes of reconstituted SV 40 minichromosomes have been studied in relation to the salt concentration and addition of histone H1 by sedimentation and electron microscopy. Sedimentation data are represented as functions of the NaCl concentration and the Debye-Hückel electrostatic screening radius $1/\kappa$. The latter representation which proved to provide more information revealed three structural states of the SV 40 reconstitutes which can be additionally characterized by electron microscopy as follows: Expanded or relaxed conformation including free DNA spacers between the nucleosomes at low salt concentration (approx. 0.001 M-0.05 M NaCl), increasing condensation at moderate salt concentration (approx. 0.05 M-0.3 M NaCl) and expansion of this condensed state above approx. 0.3 M NaCl. The condensation of the reconstitutes at moderate salt concentration does not require the presence of histone H1. H1 seems to stabilize the condensed state against electrostatic expansion. The condensation might be promoted by salt-dependent conformational changes of naked superhelical DNA as revealed by sedimentation measurements.

INTRODUCTION

Three properties may be discussed to be involved in the higher order organization of chromatin, the arrangement of chromatin DNA in superhelical loops after deproteinization¹⁻⁵, the involvement of histone H1 in the chromatin condensation in vitro^{6,7} and in vivo^{8,9}, and the ability of nucleosomes to interact¹⁰⁻¹⁴. An attractive experimental object to study these properties in relation to chromatin structure by physicochemical methods is the SV 40 minichromosome¹⁵ because it retains the behaviour of a circular molecule with its property to restrict the rotary freedom

of the chromatin fibre. This property is lost in isolated chromatin. After extraction from nuclei of lytically infected cells the minichromosome contains besides the four histones H2A, H2B, H3, and H4 also histone H1¹⁶⁻²¹. The structure of this "native" minichromosome is very compact, a diameter of only 300 Å has been observed under "physiological" salt conditions^{16,19,21}. After removal of H1 or decreasing the salt concentration it shows the relaxed, beaded circular form in the electron microscope which consists of the DNA ring molecule and 20 - 24 nucleosomes joined by short DNA spacers¹⁵⁻²¹. Furthermore, it has been shown that the reconstitution in vitro of DNA and the four nucleosomal histones H2A, H2B, H3, and H4 leads to chromatin-like structures²². Such reconstitutes from SV 40 DNA and the nucleosomal histones retain morphologically the beaded circular appearance of the relaxed minichromosome²³⁻²⁵.

We want to report here sedimentation and electron microscopic results obtained with reconstituted minichromosomes in relation to salt concentration and addition of histone H1. We show that these reconstitutes are subject to similar salt-dependent structural changes as native minichromosomes. The addition of histone H1 seems to induce a small additional condensation and to stabilize this condensed form.

MATERIAL AND METHODS

SV 40 DNA and reconstituted SV 40 minichromosomes

SV 40 was extracted from infected CV-1 cells (M.O.I. of 10^{-4} PFU/cell) using the Hirt procedure²⁶ and purified as described previously²⁷.

The reconstitution of histone/DNA complexes has been carried out using a modification of the procedure of Oudet et al.²². The DNA used for reconstitution contained about 90 % of the covalently closed ring Component I. SV 40 DNA in 2 M NaCl, $2 \cdot 10^{-3}$ M Na citrate, $2 \cdot 10^{-4}$ M EDTA, pH 7.0 was mixed with various amounts of the four calf thymus histones H2A, H2B, H3, and H4 dissolved in 10^{-3} M HCl, 10^{-3} M DDT. The mixture was dialysed for a few hours against the above

DNA buffer. This was followed by dialysis against decreasing concentrations of NaCl at constant concentrations of the other buffer salts according to the following scheme: 1.5 M (12 hrs.), 1.1 M (12 hrs.), 0.75 M (12 hrs.), 0.5 M (12 hrs.), 0.4 M (24 hrs.), 10^{-3} M (12 hrs.).

Histones

The four histones H2A, H2B, H3, and H4 were prepared by extraction with 0.25 N HCl of calf thymus chromatin pre-extracted successively with 0.35 M NaCl to remove most of the nonhistone proteins and with 5 % HClO_4 to remove histone H1. The histones were further purified on QAE-Sephadex²⁸. The composition of the mixture of the four histones was analysed by polyacrylamide gel electrophoresis²⁹.

Histone H1 has been isolated as described previously²⁷.

Sedimentation velocity analysis

Moving boundary sedimentation was performed in an analytical ultracentrifuge Spinco E equipped with absorption optics, monochromator, and photoelectric registration at rotor speeds from 15,000 - 36,000 rpm and temperatures close to room temperature (23 °C). To avoid convection disturbances, the temperature regulating system was not used. After accelerating the rotor the temperature remained constant within about ± 0.1 °C during a run. Sedimentation coefficients $s_{20,w}^{\text{app}}$ were obtained from the 50 % points of the sedimenting boundary in the usual way. They were corrected for solvent viscosity and temperature according to

$$s_{20,w}^{\text{app}} = s_t \cdot \eta_{t,\text{solv}} / \eta_{20,\text{H}_2\text{O}}$$

Corrections in the buoyancy factor were not made because of the uncertainties in the partial specific volume $\bar{v}$. In some cases s_{20} values (corrected only for temperature) are given.

The sedimentation experiments were performed in the dialysis buffer as above containing besides varying amounts of NaCl, $2 \cdot 10^{-3}$ M Na citrate, and $2 \cdot 10^{-4}$ M EDTA, pH 7.0. The DNA concentration of reconstitutes was in most cases about 30 $\mu\text{g/ml}$.

The salt titrations were carried out by successive addition of small volumes of highly concentrated NaCl solu-

tions in the centrifuge cell by means of a Beckman micro-pipette. The cell content was thoroughly mixed before each run. In some cases the dialysis was also stopped at higher NaCl concentrations or the salt concentration was adjusted in a test tube. The results of these different procedures were identical.

The histone/DNA weight input ratios were calculated from the histone content known by weighing and the DNA concentration by its absorbance ($E_{260}^{1\text{cm}, 1\%} = 200$). The H1 titration and other calculations were performed in the same way (cf. ref. 27).

The Debye-Hückel electrostatic screening radius $1/\kappa$ for aqueous solutions was calculated from the equation

$$1/\kappa = 3.1 \text{ \AA} / \sqrt{\mu}$$

μ being the ionic strength.

Electron microscopy

Reconstituted minichromosomes prepared as above in a concentration of about 1 μg DNA/ml and in NaCl concentrations of 0.015 M, 0.15 M, and 0.3 M besides $2 \cdot 10^{-3}$ M Na citrate, $2 \cdot 10^{-4}$ M EDTA, pH 7.0, were placed for 1 min onto positively charged Formvar/carbon grids as previously described^{30,31}, washed off with 1 % wet uranyl acetate, air dried and rotary shadowed with platinum at about 9°.

RESULTS

Sedimentation analysis of salt-induced conformational changes of reconstituted minichromosomes

First we checked the effect of increasing histone/DNA weight ratios on the sedimentation behaviour of reconstitutes at different salt concentrations. Increasing sedimentation coefficients $s_{20,w}^{\text{app}}$ should indicate the formation of complexes. As shown in Fig. 1, $s_{20,w}^{\text{app}}$ continuously increases with increasing histone/DNA weight input ratios at the three salt concentrations considered (0.001 M, 0.15 M, and 0.4 M NaCl). The complex at a histone/DNA ratio of 1:1, which should roughly correspond in the histone composition to H1-depleted "native" minichromosomes, shows only a slight increase in

Fig.1: Sedimentation coefficients $s_{20,w}^{app}$ of reconstituted minichromosomes with the histones H2A, H2B, H3, and H4 as a function of the histone/DNA weight input ratio at varying NaCl concentrations. Constant DNA concentration of approximately 30 $\mu\text{g/ml}$. The solvent contained besides NaCl as given, 2 mM Na citrate, 0.2 mM EDTA, pH 7.0.

$s_{20,w}^{app}$ at these salt concentrations. H1-depleted "native" minichromosomes should sediment, however, with higher $s_{20,w}$ values. Therefore, in our conditions, not all histones are bound to DNA, or a part of them is bound in a way not characteristic for nucleosomes. On the other hand, with increasing histone/DNA ratio, the $s_{20,w}^{app}$ values increase further exceeding the values expected for H1-depleted minichromosomes (55 S in 0.01 M NaCl, 70 S in 0.15 M NaCl, cf. ref. 16). This means that the amount of histones which can be bound to DNA is higher compared with the content of histones of "native" minichromosomes. Saturation binding of histones as described by Voordouw et al.³² is not reached up to a histone/DNA ratio of 1.75:1.

In Fig. 2 $s_{20,w}^{app}$ values of the reconstitutes at different histone/DNA ratios are given as a function of the NaCl concentration. $s_{20,w}^{app}$ increases with increasing salt concentration, reaches a maximum between 0.15 M and 0.3 M NaCl

Fig.2: Sedimentation coefficients $s_{20,w}^{app}$ of SV 40 reconstitutes at several histone/DNA weight input ratios and SV 40 DNA as a function of the NaCl concentration. The data were obtained by increasing the NaCl concentration. DNA concentration in all cases approximately 30 μ g/ml, solvent conditions as in Fig.1. The arrow indicates precipitation.

and decreases with further increasing salt concentration. The reconstitutes of histone/DNA of 1.75:1 precipitate between 0.15 and 0.35 M NaCl (indicated by an arrow). This precipitation results in a decrease of the concentration of the sedimenting particles by pelleting the precipitated part and finally in the complete disappearance of the boundary in the centrifuge cell. The part of particles remaining in solution at beginning precipitation shows symmetrical boundaries without indications of association or aggregation. Above 0.35 M NaCl the reconstitutes are soluble. At a histone/DNA ratio of 1.5:1 a considerable part of reconstitutes remains in solution around 0.2 M NaCl. Therefore, the high $s_{20,w}^{app}$ values in the range of "physio-

logical" salt concentration might reflect a conformational change rather than aggregation. A condensation of the reconstitutes should be considered. As further shown in Fig. 2 $s_{20,w}^{app}$ of superhelical SV 40 DNA does not change above approximately 0.02 M NaCl.

In Fig. 3 we have plotted the $s_{20,w}^{app}$ values given in Fig. 2 against $1/\kappa$, the Debye-Hückel screening radius. This parameter describes the electrostatic screening of charged groups as the radius of the domain of counterions and is proportional to the reciprocal square root of ionic strength. From such a plot, some additional informations on the salt-dependent expansion of the reconstitutes might perhaps be obtained. It is obvious that there exist three quasi-linear curve segments which might represent three different structural states of the reconstitutes and their salt-dependent changes. Therefore, this representation of

Fig.3: Sedimentation coefficients $s_{20,w}^{app}$ of SV 40 reconstitutes at two histone/DNA weight input ratios as a function of the Debye-Hückel electrostatic screening radius $1/\kappa$, $s_{20,w}^{app}$ values taken from Fig. 2. The dashed line indicates $s_{20,w}^{app}$ of reconstitutes at a histone/DNA ratio of 1.5:1 after addition of histone H1 in 0.15 M NaCl. The Mol H1/Mol DNA ratio was 11, the NaCl concentration was decreased by dialysis.

$s_{20,w}^{app}$ as a function of $1/\alpha$ seems indeed to provide more structural information than Fig. 2. The transition from one structural state to another does not seem to be a continuous one. It is characterized by a sharp kink in this plot. The region in which precipitation of the reconstitutes of 1.75:1 histone/DNA has been observed is approached by straight lines. This seems to be justified by comparison with the analogous curve at 1.5:1 histone/DNA. It should be further mentioned that with increasing histone/DNA ratio in Figs. 2 and 3 the maxima of the curves seem to be shifted to higher salt concentrations. This could indicate a higher stability of the reconstitutes with increasing histone/DNA ratio.

Electron microscopy of reconstituted minichromosomes

The question of whether these reconstitutes possess the basic structure of relaxed minichromosomes, i. e. of a beaded circle consisting of nucleosomes arrayed along the DNA, cannot be answered by sedimentation measurements. Electron microscopic evidence for the presence of nucleosome-like structures on SV 40 DNA and the appearance of the reconstitutes as beaded circles in solutions of low salt concentration (0.015 M NaCl, $2 \cdot 10^{-3}$ M Na citrate, $2 \cdot 10^{-4}$ M EDTA) is given in Fig. 4a. Up to 24 nucleosomes with diameters of about 100 Å in uranyl acetate stained specimens connected by short DNA filaments can be counted at a histone/DNA weight input ratio of 1.5:1. Reconstitutes at this input ratio are not distinguishable from H1-depleted "native" minichromosomes described by several authors¹⁶⁻¹⁹. At lower input ratios the number of nucleosomes per DNA molecule decreases (data not shown here).

In solutions with higher salt concentration (0.15 M or 0.3 M NaCl, $2 \cdot 10^{-3}$ M Na citrate, $2 \cdot 10^{-4}$ M EDTA) these reconstituted minichromosomes are more or less condensed (Fig. 4b). Free DNA spacers can hardly be detected in the condensed particles; the nucleosomes seem to be here in close spatial contact. Almost the whole material is equally distributed and the formation of aggregates of several particles is very seldom. The shape of these particles is

Fig.4: Electron micrographs of reconstituted SV 40 mini-chromosomes with the histones H2A, H2B, H3, and H4, histone/DNA weight input ratio 1.5:1. Spreading according to Dubochet et al.³⁰ without fixation. The bars indicate 100 nm.
A) Low salt concentration, 0.015 M NaCl, 2 mM Na citrate, 0.2 mM EDTA, pH 7.0.
B) Moderate salt concentration, 0.15 M NaCl, 2 mM Na citrate, 0.2 mM EDTA, pH 7.0.

somewhat irregular and also very similar to "native" mini-chromosomes depleted of histone H1 in "physiological" salt concentration¹⁷.

Interaction of reconstituted minichromosomes with histone H1

As we have seen in Figs. 2 and 3 and by electron microscopy (Fig. 4b), a condensation of reconstituates around about 0.2 M NaCl can be observed. On the other hand, several authors have shown that native minichromosomes containing histone H1 are able to adopt a still compacter conformation at approximately the same salt concentration^{16,17,21}. In order to check this possibility, we have added histone H1 to reconstituates at three different salt concentrations, 0.5 M, 0.25 M, and 0.001 M NaCl (Fig. 5a). The histone/DNA input ratio used was 1.5:1 because of the risk of aggregation at higher histone content. Whereas at 0.001 M and 0.5 M NaCl no changes in s_{20} were observed, there was a slight increase at 0.25 NaCl. This increase could

Fig.5: Uncorrected sedimentation coefficients s_{20} of SV 40 reconstituates at several NaCl concentrations a) and solubility in 0.25 M NaCl b) as functions of the H1 content expressed as molar ratio H1/DNA. DNA concentration in all cases approximately 30 μ g/ml, solvent as in Fig. 1.

possibly be explained by a small further condensation of reconstitutes if we presume that at 0.001 M NaCl the complete binding of H1 does not effect a noticeable change in s_{20} . At 0.5 M NaCl H1 should be bound only weakly. Fig. 5b shows that the solubility of the reconstitutes in 0.25 M NaCl after addition of histone H1 is strongly reduced. At an input ratio of about 20 Mol H1/Mol DNA only 20 % of the reconstitutes are soluble. The unsoluble part is pelleted, effecting a decrease in the concentration of reconstitutes which is measured in the ultracentrifuge. At 0.001 M and 0.5 M NaCl the reconstitutes remain in solution; there was no noticeable loss of sedimenting material.

In order to check whether the H1-complexed reconstitutes are better stabilized against the electrostatic expansion some samples with a histone/DNA ratio of 1.5:1 in 0.15 M NaCl and an input ratio Mol H1/Mol DNA of 11 were brought to lower salt concentration by dialysis or dilution with bidistilled water. At a higher H1/DNA ratio the loss of material by aggregation would be too strong, thus not allowing meaningful measurements (cf. Fig.5b). One sample, e.g., had in 0.15 M NaCl without H1 a $s_{20,w}^{app}$ of 80 S, in the presence of H1 a $s_{20,w}^{app}$ of 85 S, and in 0.06 M NaCl of 86 S. Below 0.06 M NaCl $s_{20,w}^{app}$ dropped rapidly to 40 S in 0.03 M NaCl. The control without H1 sedimented with only 62 S in 0.06 M NaCl (Fig. 3). These findings indicate a certain stabilization of reconstitutes in the presence of histone H1. It should be expected that at higher H1 content this stabilization would be more pronounced.

Furthermore, the decrease in $s_{20,w}^{app}$ of 80 S to 62 S of the control sample without H1 with decreasing NaCl concentration to 0.06 M indicates the reversibility of the salt-dependent condensation.

Salt-dependent conformational changes of covalent closed ring DNA

As mentioned in the introduction, the salt-dependent structural changes of the reconstitutes might be influenced by conformational changes of the ring DNA. In order to check this hypothesis, we performed sedimentation measure-

Fig.6: Sedimentation coefficients $s_{20,w}^{I,app}$ of PM2 DNA and SV 40 DNA as functions of the Debye-Hückel electrostatic screening radius $1/\kappa$. PM 2 DNA was solved in NaCl as given, pH 7.0; SV 40 DNA contained additionally 2 mM Na citrate, 0.2 mM EDTA, pH 7.0. DNA concentrations approximately 30 $\mu\text{g/ml}$.

ments of SV 40 Component I DNA in a broad range of salt concentrations. These $s_{20,w}^{app}$ values, together with those of earlier measurements of PM 2 Component I DNA³³, are given in Fig. 6 as functions of the Debye-Hückel radius $1/\kappa$. $s_{20,w}^{app}$ values of PM 2 DNA were plotted formerly only as a function of the salt concentration³³. The Component I DNA of both sources shows quasi-linear curve segments in this plot, while the curves differ.

DISCUSSION

The dependence of the sedimentation coefficients $s_{20,w}^{app}$ of the reconstituted minichromosomes on the salt concentration has been studied in detail. Qualitatively, our results resemble the sedimentation data of Griffith and Christiansen^{16,17} describing the salt-dependent con-

densation-relaxation behaviour of native H1-depleted minichromosomes. They are further in close accordance with results of Voordouw et al.³², obtained on reconstituted minichromosomes. The presentation of the data as a function of the Debye-Hückel radius $1/\kappa$ has not been used so far. Obviously, some additional interesting conclusions may be drawn from this plot. As shown in Fig. 3, there exist three quasi-linear curve segments which might represent three different structural states of the reconstitutes. Neglecting changes of the partial specific volume $\bar{v}$, we discuss these structural states and their conformational changes in the following way: (i) $1/\kappa > 15 \text{ \AA}$: Extended state with free DNA spacers between nucleosomes as visualized by electron microscopy (Fig. 4a); increasing electrostatically driven expansion with increasing $1/\kappa$. (ii) $6-8 \text{ \AA} < 1/\kappa < 15 \text{ \AA}$: With decreasing $1/\kappa$, obviously, formation of a more condensed state perhaps by nucleosome-nucleosome interaction; evidence for condensed reconstitutes without free DNA spacers from electron microscopy (Fig. 4b). Aggregation can be excluded. (iii) $1/\kappa < 6-8 \text{ \AA}$: With decreasing $1/\kappa$ (increasing salt concentration) beginning dissociation of the histone-DNA interaction which again leads to expansion.

It seems interesting furthermore that no continuous transitions occur between these structural states. The curve segments in Fig. 3 are separated by sharp, kink-like transitions. Inside these states, however, continuous alterations are present. We argue that these sharp transitions have a cooperative character. An explanation for the linearity of the curve segments in Fig. 3 cannot yet be given. This would require a concrete structural model of the minichromosomes which includes the polyelectrolyte behaviour of the histone/DNA complexes. Unwrapping of DNA spacers between the nucleosomes as shown by electron microscopy might be involved.

As shown here by sedimentation analysis, the salt-induced condensation of reconstituted minichromosomes does not require the presence of histone H1. Whereas similar results were obtained by Christiansen and Griffith^{16,17}

with H1-depleted minichromosomes, other workers have found conflicting results^{21,24}. It should be mentioned here that, according to Bradbury et al.^{6,7}, the chromatin condensation by salt requires the presence of H1. A possible explanation for the different behaviour of H1-free reconstitutes and minichromosomes on the one hand and chromatin on the other is presented later. According to our results, H1 might induce at most a small additional condensation perhaps by organizing a more ordered structure as indicated by slowly re-increasing s_{20} values in Fig. 5a. The observation that reconstitutes containing H1 remain condensed upon dilution into salt concentrations of about 0.06 M NaCl in which the reconstitutes without H1 are already expanded (Fig. 3) argues that one additional role of H1 is to stabilize a condensed chromatin conformation. Clearly, the full degree of condensation and the full stabilization against relaxation as seen with native minichromosomes^{16,17} cannot be reached in this system involving possibly some erroneous histone-DNA interactions. The increasing aggregation of reconstitutes with the addition of H1 (Fig. 5b) can be expected for this reason and if we consider an analogous behaviour of complexes of H1 and SV 40 DNA²⁷.

It should be mentioned that published sedimentation data of several workers measured as a function of the salt concentration of native minichromosomes containing histone H1¹⁹, reconstituted minichromosomes without H1³², and unsheared chromatin^{34,35} provide similar linear curves as in Fig. 3 if plotted against the Debye-Hückel radius $1/\kappa$. Especially the sedimentation data of "native" minichromosomes of Müller et al.¹⁹ plotted in this form support the conclusion of a stabilization of the condensed state as outlined above. These data almost coincide with our data of Fig. 3 for reconstitutes of 1.5:1 histone/DNA after addition of histone H1.

Finally, we want to discuss a possible influence of the superhelicity of DNA on the structural changes of the reconstitutes. There are several interesting findings which might indicate such an influence. Chromatin DNA of eukaryotes

is organized in independent superhelical loops¹⁻⁵ possibly maintained by matrix fixation³⁶. These loops which are called domains in chromatin⁵ are topologically restricted with regard to their freedom of rotation. By this implication, as shown by Worcel and Benyajati³⁷ the close array without spacers of nucleosomes on the DNA is effected. Histone H1 is not necessary for this close array. Only after induction of single strand breaks in the DNA are free spacers produced. It seems to be, therefore, a reasonable assumption that conformational changes of the SV 40 DNA might additionally be responsible for the observed structural changes of the reconstitutes. The salt-dependent conformational changes of both superhelical DNA's studied here have a different character (Fig. 6). This could be a consequence of differences in the superhelix density of PM 2 and SV 40 DNA. As shown by Campbell et al.³⁸ PM 2 DNA exists below 0.05 M NaCl as a toroidal superhelix, above in the interwound form. This behaviour is reflected in Fig. 6. SV 40 DNA is known to exist in several interwound forms, with and without branches, depending on the superhelix density and the ionic strength respectively³⁹. It seems interesting that the points of transition between these different conformational states (15 Å) coincide with that of the reconstitutes at the transition between extended and condensed form (Fig. 3). By a change of the DNA conformation which enables a closer neighbourhood of different distant DNA segments a stronger interaction of the nucleosomes themselves might be possible. This might lead to the condensed form of the reconstitutes also in the absence of histone H1. Clearly, the presence of nucleosomes reduces the content of superhelix turns²³. However, in the range of salt concentrations considered the induction of some new superhelix turns should be expected^{33,39}. On the other hand, it seems clear that also the full neutralization of the remaining free DNA phosphates will enhance the ability of the nucleosomes to interact with each other and to generate the condensed form.

ACKNOWLEDGEMENT

We thank Drs. K. E. Reinert, H. Triebel (Jena), and L. Karawajew for a critical reading of the manuscript and valuable discussions.

REFERENCES

1. Ide, T. M., Nakane, M., Anzai, K., and Andoh, T. (1975) *Nature* 258, 445-447
2. Cook, P. R. and Brazell, I. A. (1975) *J. Cell Sci.* 19, 261-279
3. Hartwig, M. (1978) *Acta Biol. Med. Germ.* 37, 421-432
4. Benyajati, C. and Worcel, A. (1976) *Cell* 9, 393-407
5. Igo-Kemenes, T. and Zachau, H. G. (1977) *Cold Spring Harbor Symp. Quant. Biol.* 42, 109-118
6. Bradbury, E. M., Carpenter, B. G., and Rattle, H. W. E. (1973) *Nature* 241, 123-125
7. Bradbury, E. M., Danby, S. E., Rattle, H., and Giancotti, U. (1975) *Eur. J. Biochem.* 57, 97-105
8. Littau, V. C., Burdick, C. J., Allfrey, V. G., and Mirsky, A. E. (1965) *Proc. Nat. Acad. Sci. USA* 54, 1204-1212
9. Bradbury, E. M., Inglis, R. J., and Matthews, H. R. (1974) *Nature* 247, 257-261
10. Finch, J. T. and Klug, A. (1976) *Proc. Nat. Acad. Sci. USA* 73, 1897-1901
11. Campbell, A. S. and Cotter, R. I. (1977) *Nucleic Acids Res.* 4, 3877-3886
12. Dubochet, J. and Noll, M. (1978) *Proc. 12th FEBS-Meeting (Dresden)*, Pergamon Press, in press
13. Ramsay-Shaw, B. and Schmitz, K. S. (1976) *Biochem. Biophys. Res. Comm.* 73, 224-232
14. Carpenter, B. G., Baldwin, J. P., Bradbury, E. M., and Ibel, K. (1976) *Nucleic Acids Res.* 3, 1739-1746
15. Griffith, J. D. (1975) *Science* 187, 1202-1203
16. Christiansen, G. and Griffith, J. (1977) *Nucleic Acids Res.* 4, 1837-1851
17. Griffith, J. D. and Christiansen, G. (1977) *Cold Spring Harbor Symp. Quant. Biol.* 42, 215-226
18. Keller, W., Müller, U., Eicken, I., Wendel, I., and Zentgraf, H. (1977) *Cold Spring Harbor Symp. Quant. Biol.* 42, 227-244
19. Müller, U., Zentgraf, H., Eicken, I., and Keller, W. (1978) *Science* 201, 406-415
20. Varshavsky, A. J., Bakayev, V. V., Chumackov, P. M., and Georgiev, G. P. (1976) *Nucleic Acids Res.* 3, 2101-2113
21. Varshavsky, A. J., Nedospasov, S. A., Schmatchenko, V. V., Bakayev, V. V., Chumackov, P. M., and Georgiev, G. P. (1977) *Nucleic Acids Res.* 4, 3303-3325
22. Oudet, P. M., Gross-Bellard, M., and Chambon, P. (1975) *Cell* 4, 281-297
23. Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M., and Chambon, P. (1975) *Proc. Nat. Acad. Sci. USA* 72, 1843-1847

24. Bellard, M., Oudet, P., Germond, J. E., and Chambon, P. (1976) *Eur. J. Biochem.* 70, 543-553
25. Germond, J. E., Bellard, M., Oudet, P., and Chambon, P. (1976) *Nucleic Acids Res.* 3, 3173-3192
26. Hirt, B. (1967) *J. Mol. Biol.* 26, 365-369
27. Böttger, M., Scherneck, S., and Fenske, H. (1976) *Nucleic Acids Res.* 3, 419-429
28. Gilmour, R. and Paul, J. (1970) *FEBS Lett.* 9, 242-244
29. Panyim, S. and Chalkley, R. (1969) *Arch. Biochem. Biophys.* 130, 337-346
30. Dubochet, J., Ducommun, M., Zollinger, N., and Kellenberger, E. (1971) *J. Ultrastr. Res.* 35, 147-167
31. Weihe, A., von Mickwitz, C.-U., Grade, K., and Lindigkeit, R. (1978) *Biochim. Biophys. Acta* 518, 172-176
32. Voordouw, G., Kalif, D., and Eisenberg, H. (1977) *Nucleic Acids Res.* 4, 1207-1223
33. Böttger, M., and Kuhn, W. (1971) *Biochim. Biophys. Acta* 254, 407-411
34. Renz, M., Nehls, P., and Hozier, J. (1977) *Proc. Nat. Acad. Sci. USA* 74, 1879-1883
35. Lindigkeit, R., Böttger, M., von Mickwitz, C.-U., Fenske, H., Karawajew, L., and Hamann, H. (1978) *Proc. 12th FEBS-Meeting (Dresden)*, Pergamon Press Vol. 51, 209-218
36. Paulson, J. R. and Laemmli, U. K. (1977) *Cell* 12, 817-828
37. Worcel, A. and Benyajati, C. (1977) *Cell* 12, 83-100
38. Campbell, A. M. (1978) *Biochem. J.* 171, 281-283
39. Upholt, W. B., Gray, H. B., and Vinograd, J. (1971) *J. Mol. Biol.* 61, 21-38