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The structure of SV 40 chromatin

By H. Zentgraf,† W. Keller‡ and Ulrike Müller‡
† *Institute of Virus Research, German Cancer Research Centre, Heidelberg, Federal Republic of Germany*
‡ *Department of Microbiology, University of Heidelberg, Heidelberg, Federal Republic of Germany*

[Plate 1]

Simian virus 40 (SV40) nucleoprotein complexes were studied with the electron microscope. Depending on the isolation procedure, SV40 chromatin has two different conformations: complexes isolated in the presence of 0.15 M NaCl appeared as very compact globular structures, while those isolated in the presence of 0.6 M NaCl had the typical 'beads-on-a-string' appearance of the primary nucleofilament. Concomitant with this structural change was a variation in the histone pattern and sedimentation behaviour of the complexes: with NaCl at 0.15 mol l^{-1} the isolated complexes contained both the nucleosomal histones and histone H1, and sedimented in sucrose gradients at 70*S*. Increasing the ionic strength to 0.6 M NaCl resulted in the removal of histone H1 from the complexes and in a decrease of the sedimentation coefficient to 40*S*. DNA relaxing enzyme is associated with the SV40 nucleoprotein complexes. The numbers of superhelical turns in DNA from compact and open types of complexes were found to be the same. Therefore the transition from the condensed to the open structure of viral chromatin does not require a change in the topological winding number of its DNA.

Recently several reports have shown by electron microscopy that the SV40 nucleoprotein complex is organized into nucleosomes (Griffith 1975; Germond *et al.* 1975; Cremisi, Pignatti, Croissant & Yaniv 1976; Varshavsky, Bakayev, Chumackov & Georgiev 1976). The main protein components of the viral nucleoprotein complex are histones (Lake, Barban & Salzman 1973; Meinke, Hall & Goldstein 1975) which originate from the host cells (Frearson & Crawford 1972; Fey & Hirt 1974). In addition to the four nucleosomal histones (H2A, H2B, H3, H4) histone H1 also has been found recently in SV40 chromatin. Thus SV40 nucleoprotein can be regarded as a model of cellular chromatin. In this report we present a brief account of our results on ultrastructural and biochemical aspects of SV40 chromatin. A full description will be given in a forthcoming publication.

SV40 nucleoprotein complexes were isolated by a modification of standard procedures from nuclei of infected cells (see legend to figure 1) and were centrifuged through sucrose gradients, containing different concentrations of NaCl. The sedimentation behaviour varied depending on the salt concentration (figure 1). At 0.15 M NaCl the SV40 chromatin sedimented with approximately 70*S*. Increasing the ionic strength to 0.6 M NaCl resulted in a decrease in the sedimentation coefficient to approximately 40*S*. Complexes isolated in the presence of 0.15 M NaCl will be referred to as 'A-complexes' and those isolated from gradients with 0.6 M NaCl will be referred to as 'B-complexes'.

The protein composition of the different SV40 chromatin forms was analysed by SDS gel electrophoresis (results not shown). A-complexes contained the usual complement of

intranucleosomal histones as well as a clearly distinguishable double-band which co-migrated with histone H1. In contrast, histone H1 was absent from B-complexes.

SV40 chromatin obtained from sucrose gradients run with different salt concentrations were examined by electron microscopy. They revealed striking differences in appearance from each other (figure 2, plate 1). A-complexes always showed a highly condensed globular structure when fixed with glutaraldehyde immediately after dilution of the sample (final salt concentration 0.02 M NaCl). In some of these compact 29 nm (average diameter) structures,

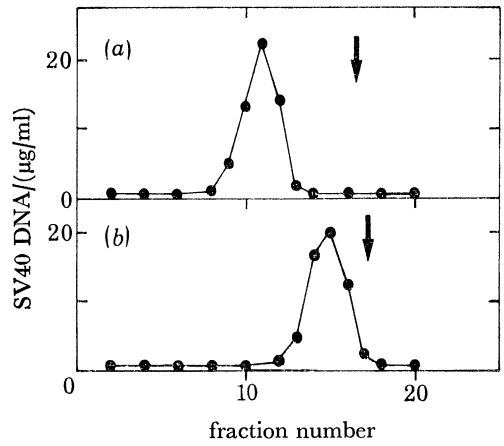

FIGURE 1. Sucrose gradient centrifugation of SV40 chromatin. Nuclei of SV40-infected CV-1 cells were extracted by a modification of the procedure described by Green *et al.* (1971) in the presence of 0.15 M NaCl. Samples (1 ml) of nuclear extract were centrifuged for 2 h at 39000 rev/min at 4 °C in a Spinco SW40 rotor through 5–20 % sucrose gradients containing 0.02 M Tris-HCl, pH 7.8; 0.2 mM EDTA; 1 mM dithiothreitol, and either 0.15 M NaCl (*a*), or 0.6 M NaCl (*b*). Fractions were collected from the bottom of the gradients and aliquots of 25 μl were analysed for their content of SV40 DNA by agarose gel electrophoresis and subsequent densitometry (Keller 1975*a*). The arrows indicate the peak positions of [^{3}H]SV40 DNA markers (21*S*) which were run in separate tubes. The peak position of the SV40 chromatin corresponds to sedimentation cofficients of approximately 70*S* for (*a*) (A-complexes) and 40*S* for (*b*) (B-complexes).

a few closely packed spheres of an average diameter of 17 nm were visible (figure 2). When A-complexes were diluted and kept at room temperature for a few minutes before fixation, a partial unfolding of the condensed structures was observed. Globular forms with loosely packed 17 nm spheres were interspersed with beaded nucleofilaments. When the diluted samples were kept for longer times at room temperature before fixation, all compact forms had been converted to the typical beaded string morphology (average diameter of the nucleosomes 11.7 nm) of the primary nucleofilament. The morphology of the compact A-complexes described here is clearly different from the '110 Å fibre'† described by Griffith (1975) for the native structure of SV40 chromatin (for details see figure 2). In contrast, B-complexes never underwent a structural transition depending on ionic strength. They always remained in the open nucleosomal configuration. These findings confirm earlier observations (Green, Miller & Hendler 1971; Goldstein, Hall & Meinke 1973) which suggested that the salt dependent reduction in sedimentation rate reflects a conformational change of the viral nucleoprotein complex.

From the data obtained by sedimentation analysis, SDS gel electrophoresis, and electron microscopy we derive the following conclusions. Raising the ionic strength leads to a reduction

† Å = 10^{-10} m = 0.1 nm.

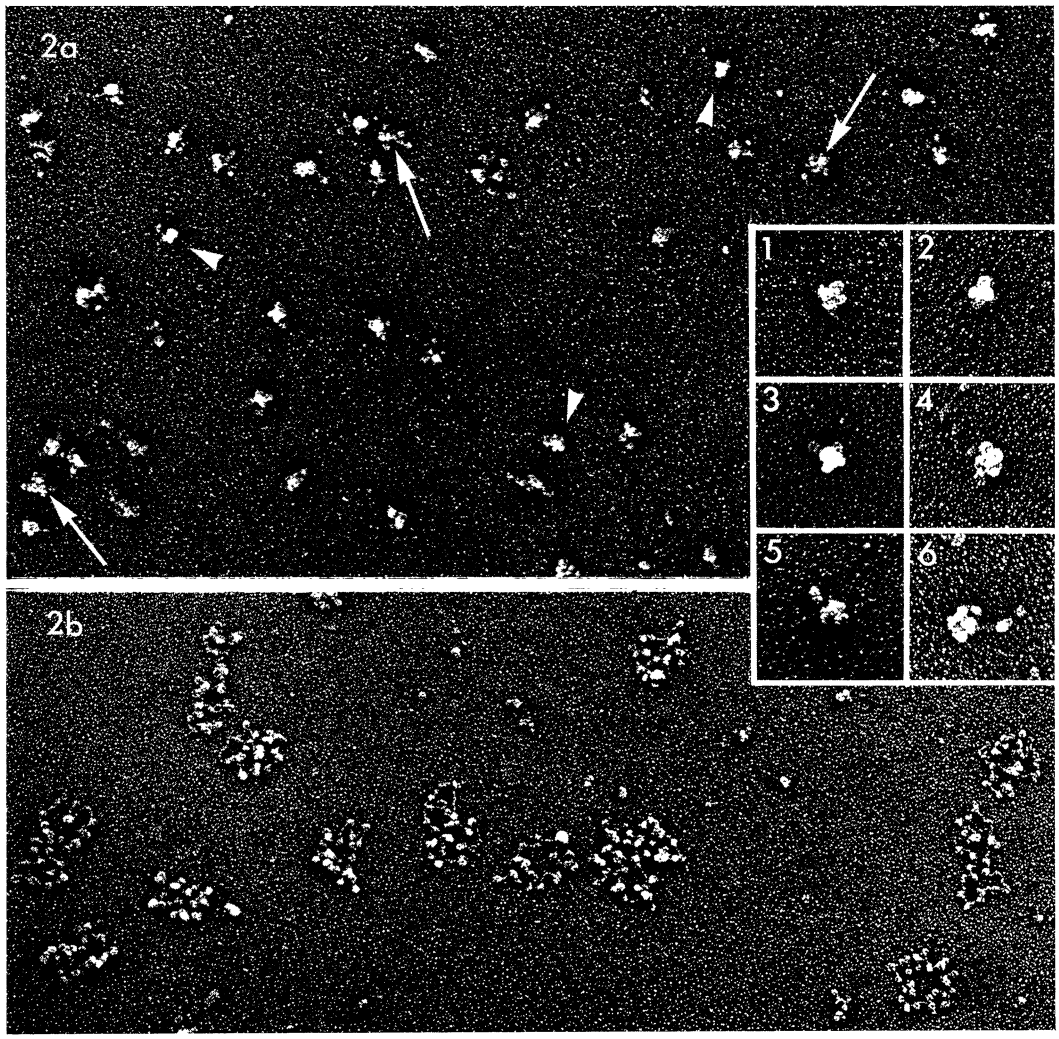

FIGURE 2. Electron microscopy of SV40 nucleoprotein complexes. A-complexes (*a*), isolated under 0.15 M salt conditions, have a compact, nearly globular form, while B-complexes, which are isolated in the presence of 0.6 M salt, reveal a beads-on-a-string morphology (*b*). Besides globular structures (arrow-heads in *a*) more or less loosely packed forms (arrows in *a*) are visible in spread preparations of A-complexes. At higher magnification A-complexes frequently seem to consist of a few spheres, which are larger than nucleosomes (inset; for details see text). In B-complexes the length of the internucleosomal bridge shows a great variability which might be a consequence of the loss of nucleosomes from the complexes when isolated under high-salt conditions.

The spreading conditions were similar to the method described by Griffith (1975). A- or B-complexes were diluted tenfold with double-distilled water and kept at room temperature at various times (for details see text) before fixing with glutaraldehyde (final concentration 0.2%). Fixation was for 15 min at 4 °C. Small drops of the samples were pipetted onto glow-discharged carbon-coated copper grids. After 1 min the grids were put face down on drops of double-distilled water placed on Parafilm. This washing step was repeated once. Finally the grids were stained in uranyl acetate (Davis, Simon & Davidson 1971), rinsed in ethanol and air-dried. The samples were rotary-shadowed with platinum-palladium (80:20) at an angle of 8°. Electron micrographs were taken with a Zeiss electron microscope EM 10A at 40 kV and at magnifications of 20000–35000 times. The magnification indicator was controlled by comparison with a grating replica. (Magns: figure 2*a*, ×58900; figure 2*b*, ×73100; insets 1, 2, 5, ×112500; inset 3, 4, 6, ×94500.)

(*Facing p.* 300)

of the sedimentation rate of SV40 chromatin, which is accompanied by the removal of histone H1. Concomitant with these effects is the change from a condensed gobular form to a beaded string morphology. Histone H1 therefore may be responsible for the maintenance of the compact form of the A-complexes which represent a higher-order level of organization in SV40 chromatin. This might be analogous to the higher order coiling of the primary nucleofilament in eucaryotic chromatin (Finch & Klug 1976; Franke *et al.* 1976; Kiryanov *et al.* 1976; Tsanev & Petrov 1976).

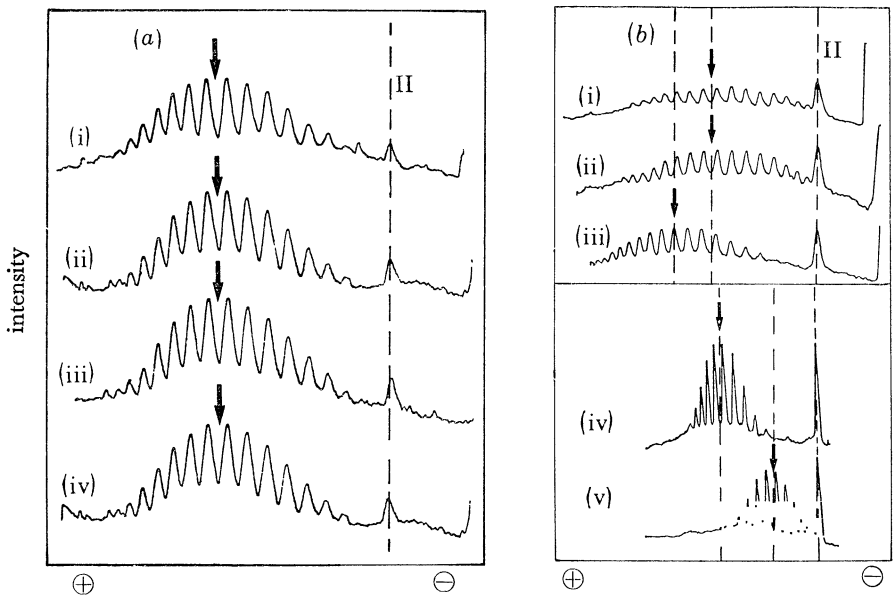

FIGURE 3. (*a*) Agarose gel electrophoresis of DNA from SV40 chromatin. DNA was extracted from the peak fractions of 0.15 M NaCl–sucrose gradients (A-complexes) and of 0.6 M NaCl–sucrose gradients (B-complexes; figure 1) using phenol and sodium dodecyl sulphate (SDS) (Keller 1975*b*). Samples of 0.3 μg were analysed on a 25 cm long slab gel of 1.4% agarose as described (Keller 1975*b*). Ethidium bromide (0.03 μg/ml) was included both in the gel and in the tank buffer. Electrophoresis was carried out at 2 V/cm for 38 h. Channel (i), DNA from A-complexes; channel (ii), DNA from B-complexes; channel (iii), DNA from B-complexes which were dialysed against 0.2 M NaCl, 10 mM Tris–HCl, pH 7.9, 0.2 mM EDTA, and incubated with 50 units of purified DNA-relaxing enzyme (Keller 1975*a*) at 37 °C for 10 min prior to extraction; channel iv, DNA from purified SV40 virions. Migration was from right to left. Gels were stained in a solution of 0.5 μg/ml of ethidium bromide and photographed under ultraviolet illumination (Keller 1975*a*). The photographs were scanned with a densitometer. The position of nicked-circular SV40 DNA (form II) is shown by 'II'. The arrows indicate the average intensity locations of the Gaussian DNA band sets. See text for further explanations.

(*b*) The effect of temperature on the average helix rotation angle of free DNA and on DNA in SV40 chromatin; detection of endogenous DNA-relaxing enzyme activity. Panels (i)–(iii): Aliquots (0.2 ml) of A-complexes (figure 1*a*) were incubated at either 0 °C (panel (i)), or at 37 °C (panel (ii)), or in the presence of 10^{-7} M ethidium bromide at 37 °C (panel (iii)) for 30 min. DNA was extracted and analysed as described in the legend to figure 3*a*. Panels (iv) and (v): Purified SV40 DNA I (0.25 μg) was incubated in reaction mixtures (25 μl) containing 0.2 M NaCl, 10 mM Tris–HCl, pH 7.8, 0.2 mM EDTA, and 10 units of DNA-relaxing enzyme (Keller 1975*a*) for 30 min at either 0 °C (panel (iv)), or at 37 °C (panel (v)), extracted, and analysed on a 12 cm long slab gel of 1.4% agarose. The arrows indicate the average intensity location of the Gaussian DNA band sets. See text for further discussion.

Closed circular DNA (form I) from SV40 virions is supercoiled and consists of a Gaussian set of topological isomers; the members within this distribution differ by integral values of their topological winding number α (Bauer & Vinograd 1974) or linking number L (Fuller 1971; Crick 1976). The difference in linking results in a corresponding difference in the

number of superhelical turns. Under appropriate conditions the topological isomers can be separated by agarose gel electrophoresis (Keller 1975*b*). Compared with a reference DNA relaxed at 37 °C in 0.2 M NaCl, SV40 DNA I contains an average of 24–26 negative superhelical turns (Keller 1975*b*; Shure & Vinograd 1976). Supercoiling is a consequence of the association of histones to DNA to form nucleosomes (Germond *et al.* 1975). In order to test whether the higher-order folding of this beaded string leads to a change in the degree of superhelicity of its DNA, agarose gel electrophoresis was carried out with DNA samples extracted from compact A-complexes and from unfolded B-complexes, and compared with DNA obtained from SV40 virions. Before the extraction of the DNA the chromatin samples were incubated under conditions where nicking–closing events mediated by DNA-relaxing enzyme could proceed (see below). The result is presented in figure 3*a*. The centre of the Gaussian distributions of DNA bands was located at the same position in all channels of the gel showing that the number of superhelical turns in the DNA from all samples was identical. From this we conclude that the folding of the beaded string form of SV40 chromatin into a higher order conformation occurs with a packing mode such that a concomitant change in the linking number of its DNA is not required.

DNA-relaxing enzyme is present within the compact form of SV40 chromatin (W. Keller, unpublished results). This provides for swivels in the DNA where the topological constraint is temporarily absent. For this reason, a change in the helix rotation angle (θ) between adjacent base pairs with temperature in DNA associated with histones can be measured as a difference in superhelicity, and can be compared with the known temperature dependent variation of θ in free DNA. The result of such an experiment is shown in figure 3*b*. When protein-free SV40 DNA samples were incubated with DNA-relaxing enzyme at 0 °C or at 37 °C and subsequently analysed by agarose gel electrophoresis, the centre of the respective Gaussian distributions of topological isomers was displaced by approximately five bands (figure 3*b*, panels iv and v). Thus, at the conditions of electrophoresis, the DNA which had been relaxed at 0 °C contained five superhelical turns more than the DNA relaxed at 37 °C. The change from 0 to 37 °C effected an unwinding of the helix by 5 times $(-360°) = -1800°$. Since SV40 DNA has about 5200 base pairs, the reduction in θ was roughly $-0.01°\ K^{-1}$, in agreement with previous measurements (Depew & Wang 1975; Pulleyblank *et al.* 1975).

When the same experiment was done with SV40 *chromatin* (A-complexes) and its DNA subsequently analysed by gel electrophoresis, the constituent DNA band sets did not show any difference in mobility (figure 3*b*, panels i and ii). Thus, in contrast to the behaviour of free DNA, the DNA complexed with histones was unable to alter measurably its average helix rotation angle in response to a temperature shift. This result suggests that DNA in chromatin is rigidly held in place by its histone contacts. However, in a gel analysis of DNA from A-complexes which had been incubated in the presence of a low concentration of ethidium bromide before extraction, a shift of the centre of the band distribution by about two and a half turns was readily detected (figure 3*b*, panel iii). Ethidium bromide had forced a certain extent of unwinding in the DNA by intercalation. This led to a constraint which was removed via nicking–closing reactions mediated by the DNA-relaxing enzyme associated with SV40 chromatin. As a result of this the DNA contained two and a half more superhelical turns than control DNA after removal of its bound proteins as well as the intercalated ethidium. This experiment serves to demonstrate that the endogenous DNA-relaxing enzyme is capable of carrying out nicking–closing reactions on DNA even in the compact form of chromatin.

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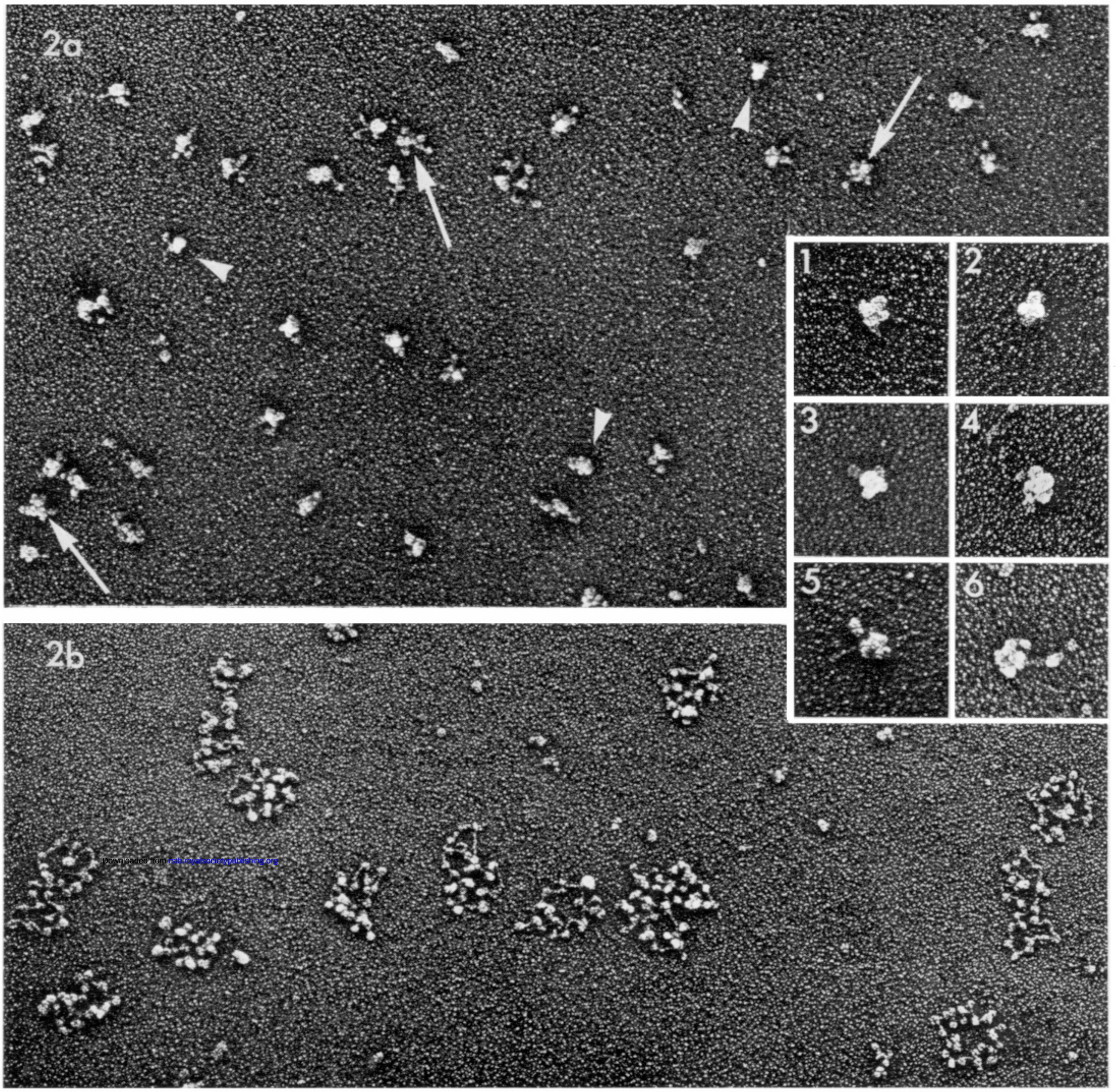

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