

Structural organization of spacer chromatin between transcribed ribosomal RNA genes in amphibian oocytes

Ulrich Scheer¹⁾

Division of Membrane Biology and Biochemistry, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg/
Federal Republic of Germany

Received July 21, 1980

Accepted September 18, 1980

Chromatin structure, rDNA, amphibian oocytes

Transcribed nucleolar chromatin, including the spacer regions interspersed between the rRNA genes, is different from the bulk of non-transcribed chromatin in that the DNA of these regions appears to be in an extended (B) conformation when examined by electron microscopy. The possibility that this may reflect artificial unfolding of nucleosomes during incubation in very low salt buffers as routinely used in such spread preparations has been examined by studying the influence of various ion concentrations on nucleolar chromatin structure. Amplified nucleolar chromatin of amphibian oocytes (*Xenopus laevis*, *Pleurodeles waltlii*, *Triturus cristatus*) was spread in various concentrations of NaCl (range 0 to 20 mM). Below 1 mM salt spacer chromatin frequently revealed a variable number of irregularly shaped beads, whereas above this concentration the chromatin axis appeared uniformly smooth. At all salt concentrations studied, however, the length distribution of spacer and gene regions was identical. Preparations fixed with glutaraldehyde instead of formaldehyde, or unfixed preparations, were indistinguishable in this respect. The observations indicate that (i) rDNA spacer regions are not compacted into nucleosomal particles and into supranucleosomal structures when visualized at chromatin stabilizing salt concentrations (e.g., 20 mM NaCl), and (ii) spacer DNA is covered by a uniform layer of proteins of unknown nature which, at very low salt concentrations (below 1 mM NaCl), can artificially give rise to the appearance of small granular particles of approximately nucleosome-like sizes. These particles, however, are different from nucleosomes in that they do not foreshorten the associated spacer DNA. The data support the concept of an altered nucleohistone conformation not only in transcribed chromatin but also in the vicinity of transcriptional events.

Introduction

It has been amply documented both by biochemical and ultrastructural studies that transcribed regions of nucleolar chromatin are organized differently from the bulk of non-

transcribed chromatin (e.g. [2, 5-7, 9-11, 13, 18-21, 33, 37, 42, 45, 46, 50, 54]). While it is widely agreed that transcribed rDNA portions occur in an extended, non-nucleosomal state upon dispersal in solutions of very low ionic strength and spreading for electron microscopy (e.g. [9-11, 26, 46]), the specific conformation of the so-called non-transcribed spacer regions interspersed between the tandemly arranged rRNA genes has been subject to some controversial debate. For instance, while we [9-11, 42, 46, 50] and others [14, 36, 38] have presented evidence, in several plant and animal species, for a non-foreshortened (B-conformation) state of the rDNA spacer under such preparative conditions, other authors have reported that this DNA is compacted into nucleosomes to an extent comparable to that of inactive chromatin [5, 23, 24, 54]. The latter conclusion, however, was based solely on the observation within spacer regions of certain granular particles of sizes similar to those of nucleosomes which, of course, does not necessarily indicate nucleosomal organization, i.e. a defined DNA compaction. For instance, it has been shown recently that the spacer regions of *Xenopus laevis* reveal a beaded appearance suggestive of a nucleosomal organization [24, 36] although it is clear from length comparisons with isolated rDNA molecules or defined restriction fragments therefrom that this spacer rDNA is only slightly, if at all, compacted when examined in electron microscopic spread preparations [36, 46]. Therefore, the nucleosomal nature of these particles is questionable.

Since it is possible that nucleosomes, especially when depleted of histone H1, may unfold upon incubation in solutions of ionic strengths below 1 mM equivalents of monovalent cations (e.g. [4, 12, 15, 25, 32, 49, 51]), it is conceivable that nucleosomes associated with the spacer rDNA *in vivo* could be selectively unfolded during the low salt treatment of nucleolar chromatin. The particles observed in spacer regions would then represent, for example, various aspects of largely linearized nucleosome-sized histone complexes still bound to the DNA (for detailed models of nucleosome unfolding see, e.g., [39, 52]). In order to distinguish between a stable, non-nucleosomal conformation and artificial unfolding induced by low salt treatment, I have studied the morphology and length distribution of nucleolar spacer chromatin in spread preparations made in the presence of elevated

¹⁾ Dr. habil. Ulrich Scheer, Abteilung für Membranbiologie und Biochemie, Institut für Zell- und Tumorbologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg/
Federal Republic of Germany.

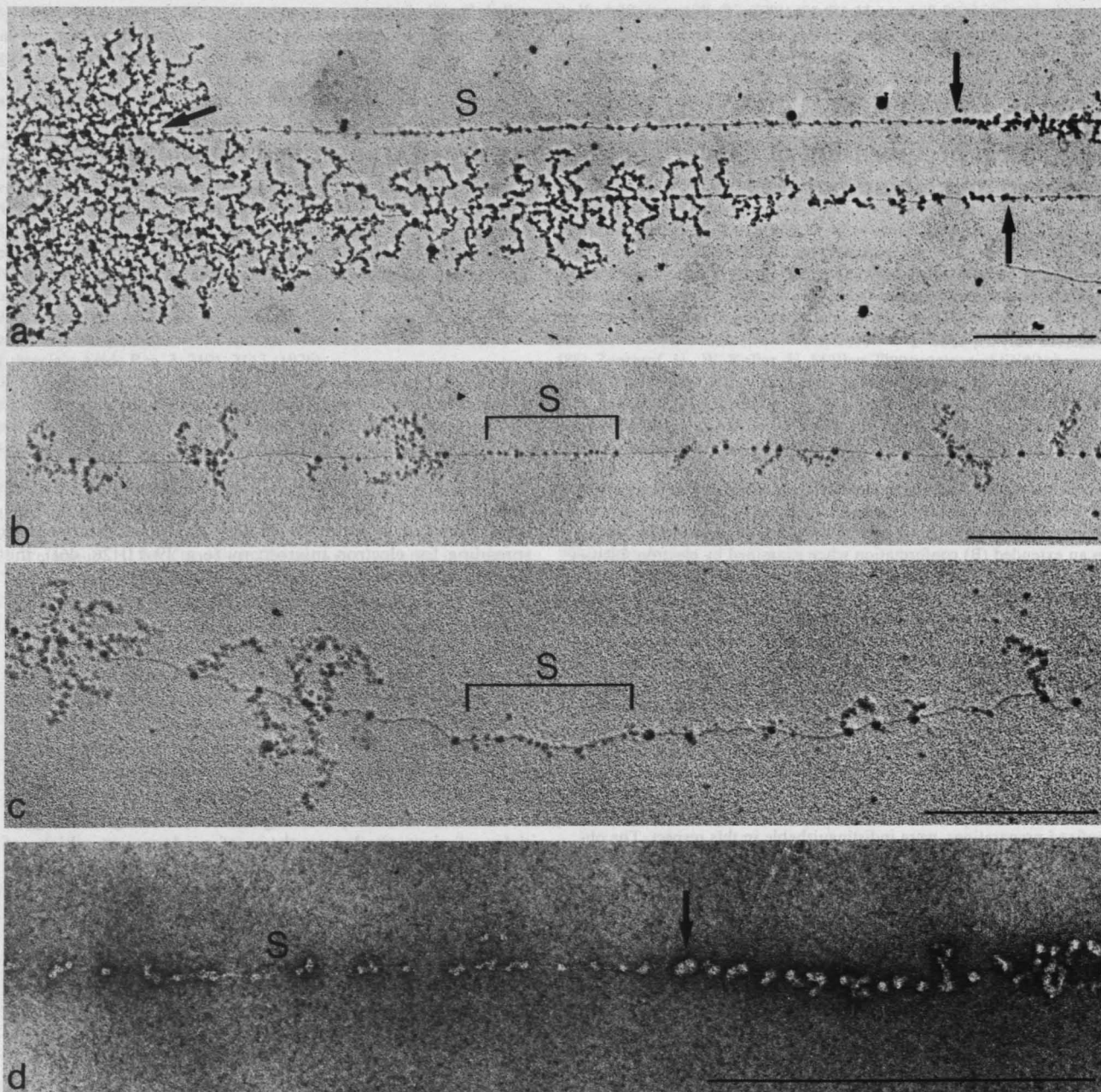


Fig. 1. Beaded aspects of rDNA spacer chromatin (S) as revealed after dispersal and spreading of nucleoli from oocytes of *T. cristatus* (a, d) and *P. waltlii* (b, c) in very low salt buffers (0.1 mM borate) followed by positive (a-c) or negative staining (d). Two rDNA containing strands are shown in (a); the arrows indicate the transcriptional initiation and transcript release sites. Note the differences in size and shape between spacer particles and RNA polymerases (particularly evident at higher magnification in the negatively stained preparation, d; the arrow denotes the beginning, i.e. the first RNA polymerase, of a transcriptional unit). Figures b and c show rRNA genes at markedly reduced transcriptional activity as typically present in large, full grown oocytes. In contrast to the beaded morphology of the spacer regions (S in b and c), the axes of transcriptional units that are only sparsely covered by transcripts appear smooth and non-beaded (b, c). Note that the spacer of *Pleurodeles* is much shorter than that of *Triturus* (for comparative data see [11]).—Bar 0.5 μm .—a. 38 000 \times .—b. 40 500 \times .—c. 54 500 \times .—d. 121 000 \times .

sodium chloride concentrations, including conditions known to stabilize nucleosome integrity (e.g. [4, 25, 49]). In addition, several conditions of fixation and preparation of nucleolar chromatin have been compared.

Materials and methods

Nuclei were manually isolated from medium-sized or nearly full-grown oocytes of the amphibia *Xenopus laevis*, *Pleurodeles waltlii* and *Triturus cristatus carnifex* in "3:1-saline" (75 mM KCl, 25 mM NaCl, 10 mM Tris-HCl, pH 7.2). In order to minimize the inclusion (co-transfer) of salts, each nucleus was washed briefly in a large volume of the specific dispersion medium prior to its final transfer into a droplet of dispersion medium, placed on a siliconized glass slide at about 10°C. Then the nuclear envelope was punctured with a fine

needle to release the nuclear content. Chromatin dispersal was induced in either 0.1 mM borate buffer (pH 8.0 to 9.0) or in 1 mM borate buffer (same pH range) containing various amounts (1 to 100 mM) of NaCl. After 15 min of incubation the droplet with the dispersed nuclear content was centrifuged through a solution of 1% paraformaldehyde, 0.1 M sucrose, containing the same buffer and NaCl concentration as in the dispersion medium, on to a freshly glow-discharged carbon coated grid as described by Miller and Bakken [29]. In order to monitor the influence of formaldehyde on the structure of nucleolar chromatin, some preparations were centrifuged through (i) 1% glutaraldehyde, 0.1 M sucrose, and (ii) 0.1 M sucrose without fixative added, containing various borate buffer and NaCl concentrations. After centrifugation, the electron microscopic grids were dried of 0.4% aqueous Photoflo (Kodak) solution in order to minimize surface tension forces [29], stained in ethanolic 1% phosphotungstic acid, dehydrated in 100% ethanol, air dried and finally rotary shadowed with Pt/Pd (80:20) at an angle of ca. 8°. The preparations were examined in Philips 301 or Zeiss EM 10 electron microscopes operated at 60 kV. DNA molecules were visualized under identical conditions. DNA plasmids (pBR 322, pMB 9) were linearized by a limited DNase I digestion, dissolved in the specific dispersion medium, centrifuged and stained as described above for the chromatin preparations. For negative staining, the electron microscopic grids were briefly washed in distilled water after the centrifugation step (without drying of the Photoflo-solution), a drop of 1% uranyl acetate was added, excess fluid removed, and the preparation air-dried.

Results

When spread in buffers containing 0.1 mM sodium borate or even lower salt concentrations, the amplified rRNA genes of the three amphibian species studied showed the characteristic tandem arrangement of transcriptional units separated by apparently nontranscribed spacer regions [11, 28]. Typically, the rRNA genes of growing oocytes were densely covered with transcript fibrils of increasing lengths, thus allowing precise identification of sites of transcriptional initiation and transcript release (Fig. 1a). Most spacer regions were free of transcripts (for occasional occurrence of "spacer transcripts" see [8, 43, 46]) but often contained a variable number of irregularly shaped particles (Figs. 1a, d; 3f; cf. also [8, 9]). The number of such particles per micrometer spacer chromatin varied from 14 to 46 (for similar data see also [8, 9, 54]). Their diameters were highly variable, 5 to 11 nm, but usually appeared smaller than the diameter of the basal particles of the RNA transcripts, i.e. the RNA polymerases, both in positively (Figs. 1a-c) and negatively (Fig. 1d) stained preparations. At higher magnification the spacer-attached particles could be distinguished from typical nucleosomes by three criteria (compare, e.g., Figs. 3e, f): (i) Their sizes were much more heterogeneous and generally slightly smaller than those of nucleosomes; (ii) their shapes were highly irregular, often forming angular, prolate or bipartite structures (e.g., Figs. 1a, d); and (iii) their pattern of spacing was irregular and frequently dense clusters of particles were separated by particle-free regions (compare, e.g., Figs. 1a, d and 3f with Fig. 3e).

The appearance of the rRNA genes and the spacer chromatin was not influenced by the specific fixative included in the sucrose solution used as "cushion" in the centrifugation. Preparations exposed to 1% glutaraldehyde, 1% formaldehyde, or to no fixative were practically indistinguishable. Likewise, omission of the detergent treatment (Photoflo) and

the subsequent drying step did not result in any noticeable alteration (e.g. Fig. 1d).

When rRNA genes were isolated from nuclei of maximally grown, nearly mature oocytes, the density of transcriptional complexes was more or less reduced, reflecting decreased pre-rRNA synthesis (cf. [37, 45]). This allowed better analysis of the structure of the axial chromatin. While the chromatin of the gene regions invariably appeared as thin and smooth fibers, spacer regions were often characterized by their "beaded" appearance (Figs. 1b, c). Even in those cases in which the start and the end of a gene region were not covered by RNP transcripts, the structural transition between beaded spacer and smooth, non-beaded gene chromatin was still recognized (Fig. 1c).

When the nucleolar chromatin was spread in the presence of increasing salt concentrations, up to 20 mM NaCl (adjusted to pH 8.0 to 9.0 with 1 mM borate buffer), spacer chromatin particles were much less frequent and, in many cases, were totally absent. Instead the spacer chromatin appeared smoothly contoured and uniform in thickness, 6 to 8 nm in positively stained and metal shadowed preparations (Figs. 2a, b; 3a-c, h) and 3 to 5 nm in negatively stained preparations (Fig. 3d). Salt concentrations above 20 mM made it almost impossible to trace the spacer chromatin due to aggregation of the masses of RNP fibrils associated with the gene regions. The length distributions of the spacer regions and the transcribed portions appeared identical, irrespective of the inclusion of various ionic strengths of NaCl in the chromatin dispersal medium. Therefore, the change in the morphology of spacer chromatin from beaded to smooth appearance was not accompanied by a change of length. Deproteinized DNA mounted under identical conditions appeared slightly thinner and showed less contrast after positive staining and rotary shadowing which suggests the absence of proteins (cf. Figs. 3g, h). Only occasionally, a few isolated (Fig. 2a) or clustered (Fig. 2b) particles were still seen in spacer chromatin, preferentially in regions adjacent to termini of transcriptional units (e.g. Fig. 2b).

When rRNA genes with reduced density of RNP transcripts such as present in fully grown oocytes (see above) or after treatment with inhibitors of transcription (cf. [27, 44]), were spread in the presence of different concentrations of NaCl, chromatin of gene and spacer regions were both smooth and practically indistinguishable (Figs. 3c, d).

It should be emphasized that under the spreading conditions resulting in the non-beaded appearance of rDNA spacer chromatin, transcriptionally inactive chromatin present in the same preparation showed either the typical beads-on-a-string aspect of closely spaced nucleosomes (Figs. 2b, 3e) or globular forms of supranucleosomal packing (cf. [47, 48]).

Discussion

Nucleosomal structures ("beads-on-a-string") were first visualized by electron microscopy of spread preparations of chromatin dispersed in solutions of very low salt concentrations [30, 31, 53] similar to the conditions used in the Miller technique [28, 29]. Thus, the nucleosomal structure is obviously preserved under these conditions. It is unlikely that the extended state of spacer rDNA demonstrated in this study is

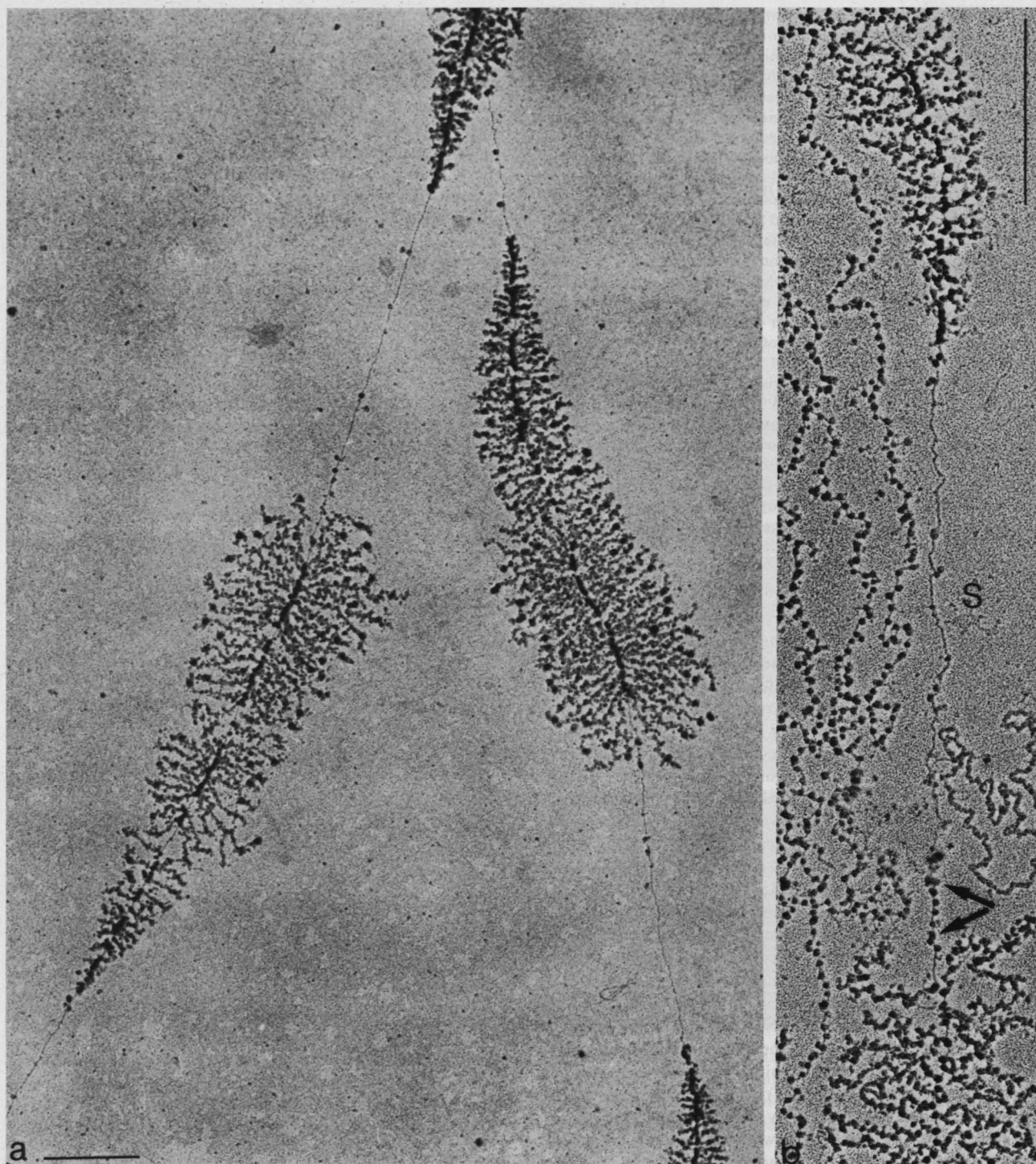


Fig. 2. Smooth and non-beaded appearance of rDNA spacer chromatin (S) from *Triturus* oocytes after spreading in 10 mM (a) and 1 mM (b) NaCl. Occasionally, small groups of particles are still associated with apparent spacer regions, preferentially in regions close to the transcript release site (arrows in b); these, however, may include RNA polymerase particles. Transcriptionally inactive chromatin spread under the same conditions is arranged into regularly sized and relatively closely spaced nucleosomes (left part of b). — Bar 0.5 μ m. — a. 31 500 \times . — b. 60 500 \times .

artificially induced by the low salt concentrations present in the spreading solution. Partial unfolding of nucleosomes in electron microscopic preparations has been reported at very low ionic strengths (below 1 mM monovalent cations) and was shown to be readily reversible upon raising the NaCl concentration to 20 mM [32]. Other authors have described similar changes of nucleosome structure in solutions containing less than 1 mM salt, however only after depleting the

chromatin of histone H1 [49]. In addition, several physicochemical studies using chromatin or nucleosome core particles have indicated structural transitions from a compact to a more loosened and/or extended nucleosomal configuration at low ionic strength of 1 mM or less [4, 12, 15, 25]. Thus, the present demonstration of the absence of nucleosomal particles in both gene and spacer regions of transcriptionally active nucleolar chromatin, after preparation in buffers containing 5 to 20 mM NaCl, provides support for the existence of non-nucleosomal, i.e. non-foreshortened, chromatin of rDNA. This is in agreement with earlier conclusions from this [7, 9–11, 42, 46, 50] and other [36, 38] laboratories. (The only reported case of DNA foreshortening in a region between two adjacent transcribed rRNA genes has been the central portion of the palindromic rDNA molecules of *Physarum polycephalum* [16]: this region obviously is not equivalent to the spacer of the tandemly arranged rRNA genes.) Whether this structural alteration in the spacer regions reflects changes induced by the adjacent transcriptional units or is a consequence of the occurrence of some relatively rare transcriptional events in spacer regions (cf. [8, 41]; see also [1]) remains to be seen.

In contrast to a statement by Pruitt and Grainger [34] who claimed the occurrence of higher order folding of DNA in transcriptionally active nucleolar chromatin of *Xenopus* oocytes as a function of ionic strength (reported contraction ratios of the spacer rDNA greater than 20:1), none of the numerous chromatin spreads made under various ionic and fixation conditions showed any indication of supranucleosomal or other higher order packing structures, neither in spacer regions nor in transcriptional units. Moreover, the absence of higher order packing of chromatin after treatment with glutaraldehyde or without any fixative, clearly speaks against the possibility that formaldehyde, which is routinely included in spread preparations as fixation agent in most laboratories, selectively induces unfolding of higher order formations as suggested by Ratner and Hamkalo [35]. In addition, length measurements of repeating units in nucleolar chromatin dispersed and spread at varying ionic strengths between 0.1 and 20 mM sodium salt did not reveal statistically significant differences and, in the case of *Xenopus laevis*, always agreed with the length distribution of the corresponding rDNA (cf. [36, 46]).

The molecular mechanism responsible for the extension of the transcribed nucleolar chromatin, including the spacer regions, is unknown. In particular, it is not known whether (modified?) histones are associated with the rDNA or whether they are partly or completely removed and/or replaced by other proteins. Although the presence of some histones in fractions of isolated nucleoli from *X. laevis* oocytes has been reported [17, 36] their distribution with respect to various rDNA sequences (gene vs. spacer regions) or in different transcriptional states (active vs. inactive) is unknown. Biochemical data obtained from other cell systems indicate that rDNA containing chromatin can be different from bulk chromatin by a significant lower histone/DNA ratio (*Tetrahymena pyriformis*, [22]) and that histones can be disposed differently in gene and spacer regions (*Physarum polycephalum*, [21]). The observation that antibodies against histone H2B injected into amphibian oocyte nuclei do not interfere

with transcription of rRNA genes but severely inhibit transcription of lampbrush loops [47] is also suggestive of a different histone composition or arrangement in active nucleolar chromatin. Therefore, general comparisons of nucleolar chromatin with other kinds of chromatin do not seem to be justified at present.

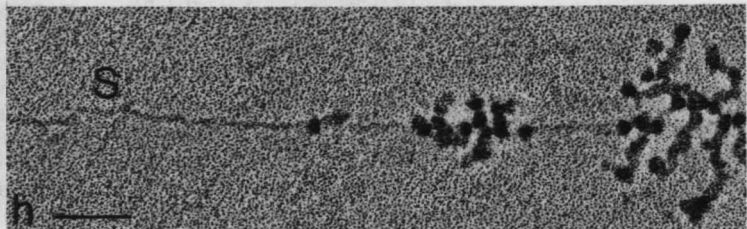
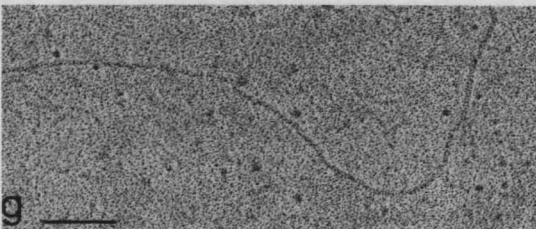
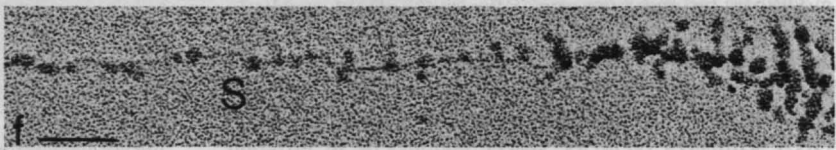
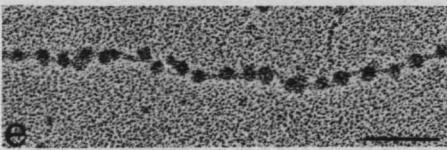
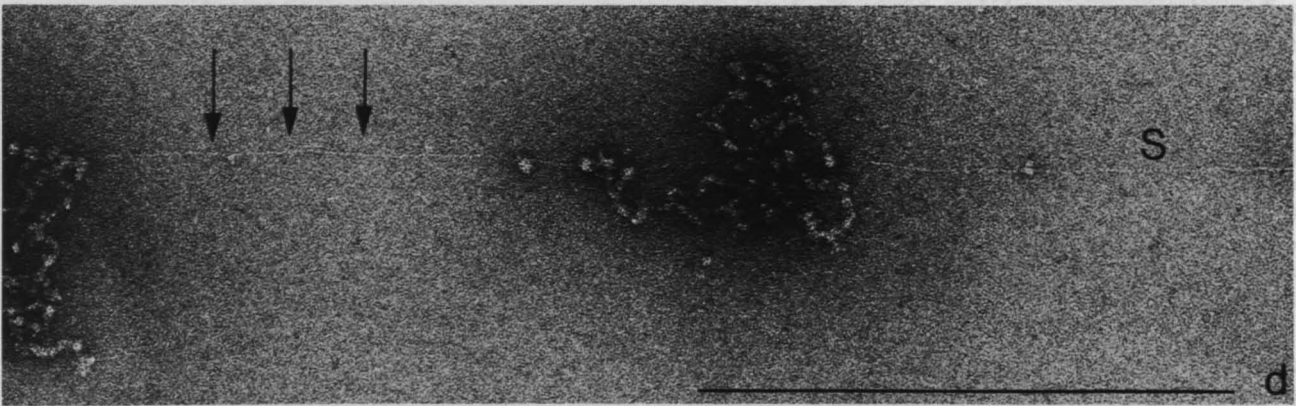
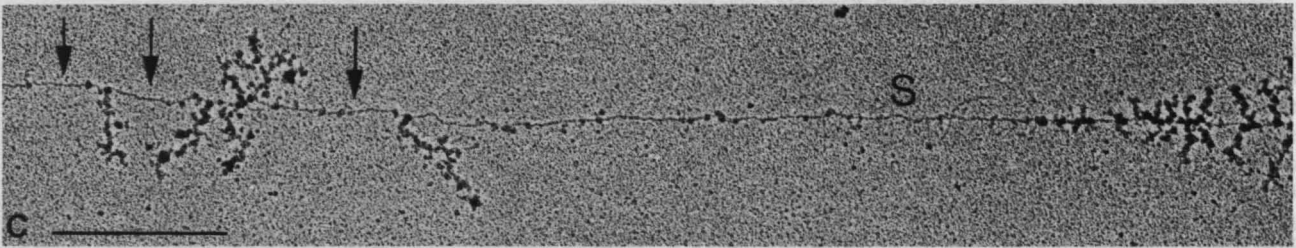
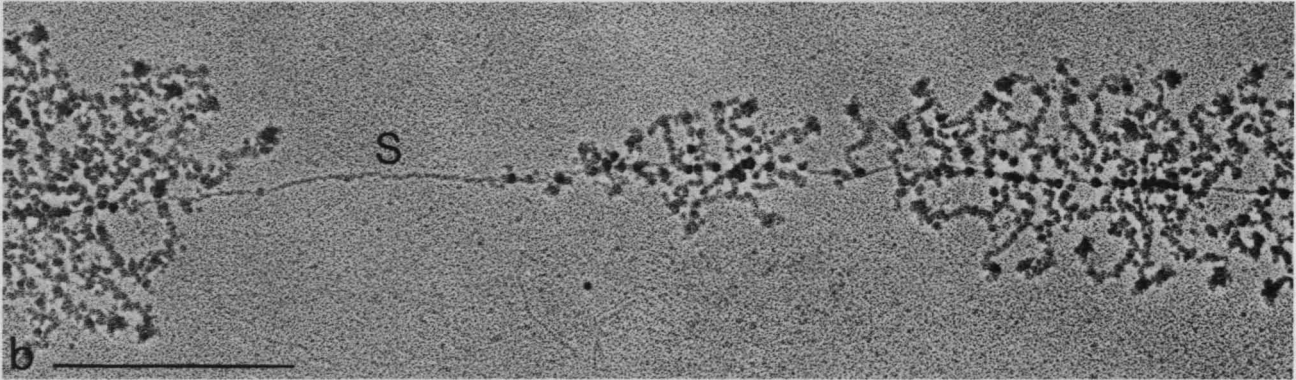
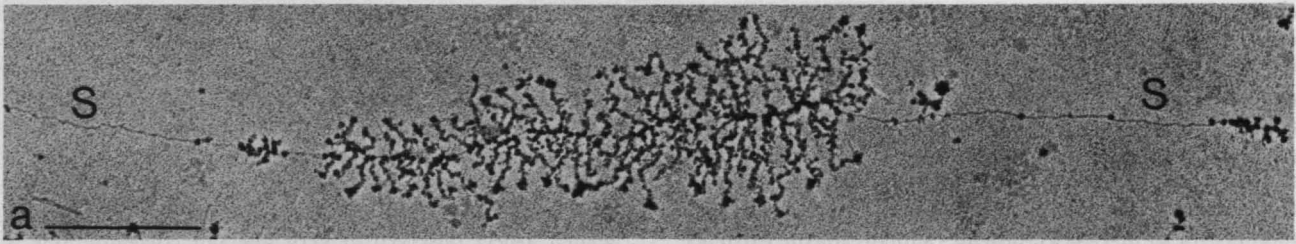
The specific nucleoprotein arrangement in spacer chromatin as described remains unclear. Since the non-nucleosomal beaded aspect observed at very low salt concentrations is confined to the spacer and is absent from the gene region, both in states of high and low transcriptional activity, differences in protein composition and/or arrangement of the chromatin axis must exist between these two regions. Low salt treatment, which is known to decrease hydrophobic interactions, might cause local disturbances of protein-protein interaction, thus leading to artificial rearrangements into particulate structures, which are formed in the spacer intercepts but not in actively transcribed regions which are associated with, and possibly protected by, transcriptional complexes. However, the alternative explanation that the low salt treatment results in such irregularly spaced and shaped associations of nuclear sap proteins, perhaps by ionic interaction, cannot be excluded.

The described morphological change in nucleolar chromatin at low salt concentrations around 1 mM monovalent cations might also provide an explanation why different authors, sometimes with the same biological material, have described spacer chromatin either as "beaded" or as "smooth" (for refs. see Introduction). Ion concentrations present in both the nuclei and the media used for nuclear isolation are about 100 mM monovalent alkali salt (for endogenous ion concentrations in amphibian oocyte nuclei see [3, 40]). Therefore, it is not unlikely that different amounts of ions present in the nuclei or the media have been co-transferred with the chromatin material and thus have influenced the morphology of nucleolar chromatin. Extreme dilutions in low salt buffers, therefore, should result in structural rearrangements such as the appearance of "non-nucleosomal beads" in spacer regions of nucleolar chromatin.

Acknowledgements. I am indebted to Drs. W. W. Franke (this institute), A. Olins and D. E. Olins (University of Tennessee, Oak Ridge, Tennessee, USA) and M. Williams (Department of Biological Sciences, Purdue University, West Lafayette, Indiana, USA) for stimulating discussions and for reading and correcting the manuscript. Mr. K. Mähler is acknowledged for his excellent photographic assistance. This work was supported by the Deutsche Forschungsgemeinschaft (grant Sche, 157/3).

References

- [1] Boseley, P., T. Moss, M. Mächler, R. Portmann, M. Birnstiel: Sequence organization of the spacer DNA in a ribosomal gene unit of *Xenopus laevis*. *Cell* **17**, 19–31 (1979).
- [2] Cech, T. R., K. M. Karrer: Chromatin structure of the ribosomal RNA genes of *Tetrahymena thermophila* as analyzed by trimethylpsoralen crosslinking in vivo. *J. Mol. Biol.* **136**, 395–416 (1980).
- [3] Century, T. J., J. R. Fenichel, S. B. Horowitz: The concentrations of water, sodium and potassium in the nucleus and cytoplasm of amphibian oocytes. *J. Cell Sci.* **7**, 5–13 (1970).
- [4] Dieterich, A. E., R. Axel, C. R. Cantor: Salt-induced structural changes of nucleosome core particles. *J. Mol. Biol.* **129**, 587–602.



[5] Foe, V. E.: Modulation of ribosomal RNA synthesis in *Oncopeltus fasciatus*: An electron microscopic study of the relationship between changes in chromatin structure and transcriptional activity. *Cold Spring Harbor Symp. Quant. Biol.* **42**, 723–740 (1978).

[6] Foe, V. E., L. E. Wilkinson, C. D. Laird: Comparative organization of active transcription units in *Oncopeltus fasciatus*. *Cell* **9**, 131–146 (1976).

[7] Franke, W. W., U. Scheer: Morphology of transcriptional units at different states of activity. *Phil. Trans. Roy. Soc. London B* **283**, 333–342 (1978).

[8] Franke, W. W., U. Scheer, H. Spring, M. F. Trendelenburg, G. Krohne: Morphology of transcriptional units of rDNA. *Exp. Cell Res.* **100**, 233–244 (1976).

[9] Franke, W. W., U. Scheer, M. F. Trendelenburg, H. Spring, H. Zentgraf: Absence of nucleosomes in transcriptionally active chromatin. *Cytobiol.* **13**, 401–434 (1976).

[10] Franke, W. W., U. Scheer, M. F. Trendelenburg, H. Zentgraf, H. Spring: Morphology of transcriptionally active chromatin. *Cold Spring Harbor Symp. Quant. Biol.* **42**, 755–772 (1978).

[11] Franke, W. W., U. Scheer, H. Spring, M. F. Trendelenburg, H. Zentgraf: Organization of nucleolar chromatin. In: H. Busch (ed.): *The Cell Nucleus*. Vol. 7, pp. 49–95. Academic Press, New York 1979.

[12] Fulmer, A. W., G. D. Fasman: Ionic strength-dependent conformational transitions of chromatin. Circular dichroism and thermal denaturation studies. *Biopolymers* **18**, 2875–2891 (1979).

[13] Giri, C. P., M. A. Gorovsky: DNase I sensitivity of ribosomal genes in isolated nucleosome core particles. *Nucleic Acid Res.* **8**, 197–214 (1980).

[14] Glätzer, K. H.: Lengths of transcribed rDNA repeating units in spermatocytes of *Drosophila hydei*: Only genes without an intervening sequence are expressed. *Chromosoma* **75**, 161–175 (1979).

[15] Gordon, V. C., C. M. Knobler, D. E. Olins, V. N. Schumaker: Conformational changes of the chromatin subunit. *Proc. Nat. Acad. Sci. USA* **75**, 660–663 (1978).

[16] Grainger, R. M., R. C. Ogle: Chromatin structure of the ribosomal RNA genes in *Physarum polycephalum*. *Chromosoma* **65**, 115–126 (1978).

[17] Higashinakagawa, T., H. L. Wahn, R. H. Reeder: Isolation of ribosomal gene chromatin. *Develop. Biol.* **55**, 375–386 (1977).

[18] Jamrich, M., E. Clark, O. L. Miller: Absence of nucleosomes in the actively transcribed rDNA of *Calliphora erythrocephala* larval tissue and of quail myoblasts. ICN-UCLA Symposium, in press (1980).

[19] Johnson, E. M., V. G. Allfrey, E. M. Bradbury, H. R. Matthews: Altered nucleosome structure containing DNA sequences complementary to 19S and 26S ribosomal RNA in *Physarum polycephalum*. *Proc. Nat. Acad. Sci. USA* **75**, 1116–1120 (1978).

[20] Johnson, E. M., H. R. Matthews, V. C. Littau, L. Lohstein, E. M. Bradbury, V. G. Allfrey: The structure of chromatin containing

DNA complementary to 19S and 26S ribosomal RNA in active and inactive stages of *Physarum polycephalum*. *Arch. Biochem. Biophys.* **191**, 537–550 (1978).

[21] Johnson, E. M., G. R. Campbell, V. G. Allfrey: Different nucleosome structures on transcribing and nontranscribing ribosomal gene sequences. *Science* **206**, 1192–1194 (1979).

[22] Jones, R. W.: Histone composition of a chromatin fraction containing ribosomal deoxyribonucleic acid isolated from the macronucleus of *Tetrahymena pyriformis*. *Biochem. J.* **173**, 155–164 (1978).

[23] Laird, C. D., L. E. Wilkinson, V. E. Foe, W. Y. Chooi: Analysis of chromatin-associated fiber arrays. *Chromosoma* **58**, 169–192 (1976).

[24] Martin, K., Y. N. Osheim, A. L. Beyer, O. L. Miller: Visualization of transcriptional activity during *Xenopus laevis* oogenesis. In: W. Beermann, W. J. Gehring, J. Gurdon, F. C. Kafatos, J. Reiner (eds.): *Results and Problems in Cell Differentiation*. Springer-Verlag, Heidelberg 1980 (in press).

[25] Martinson, H. G., R. J. True, J. B. E. Burch: Specific histone-histone contacts are ruptured when nucleosomes unfold at low ionic strength. *Biochemistry* **18**, 1082–1089 (1979).

[26] McKnight, S. L., K. A. Martin, A. L. Beyer, O. L. Miller: Visualization of functionally active chromatin. In: H. Busch (ed.): *The Cell Nucleus*. Vol. 7, pp. 97–122. Academic Press, New York 1979.

[27] Meyer, G. F., W. Hennig: The nucleolus in primary spermatocytes of *Drosophila hydei*. *Chromosoma* **46**, 121–144 (1974).

[28] Miller, O. L., B. R. Beatty: Visualization of nucleolar genes. *Science* **164**, 955–957 (1969).

[29] Miller, O. L., A. H. Bakken: Morphological studies of transcription. *Acta endocrinol. Suppl.* **168**, 155–177 (1972).

[30] Olins, A. L., D. E. Olins: Spheroid chromatin units (ν bodies). *Science* **183**, 330–332 (1974).

[31] Oudet, P., M. Gross-Bellard, P. Chambon: Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. *Cell* **4**, 281–300 (1975).

[32] Oudet, P., C. Spadafora, P. Chambon: Nucleosome structure II: Structure of the SV40 minichromosome and electron microscopic evidence for reversible transitions of the nucleosome structure. *Cold Spring Harbor Symp. Quant. Biol.* **42**, 301–312 (1978).

[33] Puvion-Dutilleul, J.-P. Bachelier, J.-P. Zalta, W. Bernhard: Morphology of ribosomal transcription units in isolated subnuclear fractions of mammalian cells. *Biol. Cell.* **30**, 183–194 (1977).

[34] Pruitt, S. C., R. M. Grainger: Visualization of changes in chromatin conformation during ribosomal gene activation in *Xenopus* oocytes. *J. Cell Biol.* **83**, 164a (1979).

[35] Rattner, J. B., B. A. Hamkalo: Nucleosome packing in interphase chromatin. *J. Cell Biol.* **81**, 453–457 (1979).

[36] Reeder, H., H. L. Wahn, P. Botchan, R. Hipskind, B. Sollner-Webb: Ribosomal genes and their proteins from *Xenopus*. *Cold Spring Harbor Symp. Quant. Biol.* **42**, 1167–1177 (1978).

[37] Reeves, R.: Nucleosome structure of *Xenopus* oocyte amplified ribosomal genes. *Biochemistry* **17**, 4908–4916 (1978).

[38] Renkawitz, R., S. A. Gerbi: Ribosomal DNA of the fly *Sciara coprophila* has a very small and homogeneous repeat unit. *Molec. gen. Genet.* **173**, 1–13 (1979).

[39] Richards, B. M., J. F. Pardon, D. M. J. Lilley, R. I. Cotter, J. C. Wooley, D. L. Worcester: Nucleosome sub-structure during transcription and replication. *Phil. Trans. Roy. Soc. Lond. B* **283**, 287–289 (1978).

[40] Riemann, W., C. Muir, H. C. Macgregor: Sodium and potassium in oocytes of *Triturus cristatus*. *J. Cell Sci.* **4**, 299–304 (1969).

[41] Rungger, D., H. Achermann, M. Crippa: Transcription of spacer sequences in genes coding for ribosomal RNA in *Xenopus* cells. *Proc. Nat. Acad. Sci. USA* **76**, 3957–3961 (1979).

[42] Scheer, U.: Changes of nucleosome frequency in nucleolar and non-nucleolar chromatin as a function of transcription: An electron microscopic study. *Cell* **13**, 535–549 (1978).

[43] Scheer, U., M. F. Trendelenburg, W. W. Franke: Transcription

Fig. 3. Smooth appearance of spacer chromatin (S) from oocytes of *X. laevis* (a), *P. waltlii* (b) and *T. cristatus* (c, d) after spreading in 1 mM (b, c), 5 mM (a) or 10 mM (d) NaCl. The chromatin axis between distantly spaced transcript fibrils (e.g., at the arrows in c and d) is indistinguishable from that present in spacer intercepts (S), both after positive staining (c) and negative staining (d). For comparison, the ultrastructure of non-transcribed nucleosomal chromatin (e), spacer regions in transcribed nucleolar chromatin prepared in very low salt buffer (f) or in the presence of 5 mM NaCl (h), and of deproteinized DNA (g) is shown at the same magnification after identical staining and shadowing procedures. — Bar 0.5 μ m (a–d), 0.1 μ m (e–h). — a. 41000 \times . — b. 64000 \times . — c. 46000 \times . — d. 141000 \times . — e. to h. 100000 \times .

of ribosomal RNA cistrons. *Exp. Cell Res.* **80**, 175-190 (1973).

- [44] Scheer, U., M. F. Trendelenburg, W. W. Franke: Effects of actinomycin D on the association of newly formed ribonucleoproteins with the cistrons of ribosomal RNA in *Triturus oocytes*. *J. Cell Biol.* **65**, 163-179 (1975).
- [45] Scheer, U., M. F. Trendelenburg, W. W. Franke: Regulation of transcription of genes of ribosomal RNA during amphibian oogenesis. *J. Cell Biol.* **69**, 465-489 (1976).
- [46] Scheer, U., M. F. Trendelenburg, G. Krohne, W. W. Franke: Lengths and patterns of transcriptional units in the amplified nucleoli of *Xenopus laevis*. *Chromosoma* **60**, 147-167 (1977).
- [47] Scheer, U., J. Sommerville, M. Bustin: Injected histone antibodies interfere with transcription of lampbrush chromosome loops in oocytes of *Pleurodeles*. *J. Cell Sci.* **40**, 1-20 (1979).
- [48] Scheer, U., J. Sommerville, U. Müller: DNA is assembled into globular supranucleosomal chromatin structures by nuclear contents of amphibian oocytes. *Exp. Cell Res.*, **129**, 115-126 (1980).
- [49] Thoma, F., T. Koller, A. Klug: Involvement of histone H1 in the

organization of the nucleosome and of the salt-dependent superstructures of chromatin. *J. Cell Biol.* **83**, 403-427 (1979).

- [50] Trendelenburg, M. F., U. Scheer, H. Zentgraf, W. W. Franke: Heterogeneity of spacer lengths in circles of amplified ribosomal DNA of two insect species, *Dytiscus marginalis* and *Acheta domestica*. *J. Mol. Biol.* **108**, 453-470 (1976).
- [51] Tsanev, R.: The substructure of nucleosomes. In: H. Busch (ed.): *The Cell Nucleus*. Vol. 4, pp. 107-133. Academic Press, New York (1978).
- [52] Weintraub, H., A. Worcel, B. Alberts: A model for chromatin based upon two symmetrically paired half-nucleosomes. *Cell* **9**, 409-417 (1976).
- [53] Woodcock, C. L. F., J. P. Safer, J. E. Stanchfield: Structural repeating units in chromatin. I. Evidence for their general occurrence. *Exp. Cell Res.* **97**, 101-110 (1976).
- [54] Woodcock, C. L. F., L.-L. Y. Frado, C. L. Hatch, L. Ricciardiello: Fine structure of active ribosomal genes. *Chromosoma* **58**, 33-39 (1976).

Coexistence of nucleosomal and various non-nucleosomal chromatin configurations in cells infected with herpes simplex virus

Ulrike Müller¹⁾

Division of Membrane Biology and Biochemistry, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg/
Federal Republic of Germany

Claus H. Schröder, Hanswalter Zentgraf

Institute of Virus Research, German Cancer Research Center, Heidelberg/Federal Republic of Germany

Werner W. Franke

Division of Membrane Biology and Biochemistry, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg/
Federal Republic of Germany

Received July 30, 1980

Accepted September 19, 1980

Non-nucleosomal—viral chromatin

Coexistence of four different forms of chromatin was observed by electron microscopy in nuclear spread preparations of monkey kidney cells during late stages of infection with herpes simplex virus (HSV-1 ANG). Besides typical nucleosomal (i) chromatin, thin (3–5 nm) strands morphologically indistinguishable from protein-free DNA were frequent, without (ii) or with (iii) sparse 10–22 nm large granules different from nucleosomes. In addition, uniformly thick (mean 17 nm), heavily stained chromatin strands (iv) were seen. The non-nucleosomal character of types (iii) and (iv) chromatin was also demonstrated by their resistance to histone removal in Sarkosyl and heparin. All four forms were seen in capsid-associated HSV-DNA molecules, and various combinations of these forms occurred in adjacent regions of the same DNA molecule, including the vicinity of replication branch points. Especially frequent were regions of chromatin types (ii) or (iii) alternating with thickly coated intercepts of type (iv) chromatin, the latter often displaying “bubble”-like strand separations. The appearance of chromatin types (ii)–(iv) was dependent on viral replication. These chromatin arrays were compared with structures observed in purified HSV-DNA from these cells. Patterns of single-stranded regions were found in HSV-DNA that were similar to those observed in the thickly coated type (iv) chromatin. It is concluded that, in these nuclei, non-nucleosomal arrangements can be formed, at least on viral DNA, under conditions of continued DNA synthesis and inhibited protein synthesis, and that single-stranded DNA is packed into a characteristic thick strand of non-nucleosomal chromatin by association with a special, probably virus-coded protein.

Introduction

In most eukaryotic cells the bulk of the chromatin is organized in nucleosomes which are visualized by electron microscopy of spread chromatin in extended filaments of 10 to 12 nm large beads-on-a-chain [17, 23, 39]. A modified form of chromatin arrangement, with an essentially maintained nucleosome-like pattern of histone complexes, has been described in chromatin regions engaged in transcription [2, 26, 37], in particular in transcribed nucleolar chromatin in which the DNA appears to be in a largely extended conformation [8, 9, 19, 27, 35]. During our studies on the organization of chromatin in virus-infected cells we have observed, in cells infected with herpes simplex virus, the coexistence of nucleosomal and various novel forms of non-nucleosomal chromatin, even on the same viral DNA molecule.

Materials and methods

Cells

African green monkey kidney cells, RC-37 (Italodiagnostic Products, Rome, Italy), were used for infection and replication of herpes simplex virus (HSV), type 1, strain ANG, as described [32, 33]. The HSV-1 ANG standard virus stock (LP5) was plaquepurified and passaged five times at low multiplicity (0.01 PFU/cell; [34]). Upon infection of cells with high passage virus stock (HP4), the amount of newly synthesized viral DNA was not significantly different from that of standard virus infections as described for other HSV-1 ANG virus stocks containing I-particles [34]. For control, a temperature-sensitive HSV-1 ANG mutant (ts7) was used. The mutant was isolated as described by Schaffer et al [30] and failed to replicate its DNA in infected cells at the non-permissive temperature of 39.5 °C. Alternatively, infected cells were treated with 300 µg/ml disodium phosphonoacetate (PAA) added to the medium in order to prevent replication of viral DNA [24].

¹⁾ Dr. Ulrike Müller, Division of Membrane Biology and Biochemistry, Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, Federal Republic of Germany.