Influence of histones and calcium and magnesium ions on the ultrastructure of chromatin in isolated nuclei of *Pinus silvestris* L. root meristem

HALINA MICHNIEWICZ

Institute of Physiology and Cytology, University of Łódź

(Received: July 21, 1975)

Abstract

The width of chromatin fibrils in nuclei fixed in situ is about 10 nm. In nuclei isolated in the presence of Ca^{+2} and Mg^{+2} ions the fibrils coalesce, and thus their width secondarily increases, whereas in nuclei isolated without the presence of the cations the diameter of fibrils increases somewhat as compared with that in nuclei in situ, probably owing to absorption of nonchromatin nuclear proteins.

Lysine histone extraction caused dispersion of condensed chromatin, and reintroduction of these proteins — its reconstruction. On the other hand, extraction and reintroduction of the arginine histone did not cause chromatin dispersion, but rather coalescence of the chromatin mass.

Lysine histone extraction from material isolated in the presence of Ca^{+2} and Mg^{+2} ions caused the appearance of a large number of 10-nm fibrils, only sporadically seen in the control material, and disappearance of the 30-nm forms. Reintroduction of the lysine histone reduced the number of single fibrils and enhanced the appearance of coalescent form with 30 nm diameter. Removal of arginine histones did not produce disappearance of single fibrils, but reduced their diameter. Reintroduction of this fraction caused coalescence of chromatin threads, owing to which 90 per cent of the population consisted of fibrils with diameter around 30 nm.

INTRODUCTION

The controversy concerning the width of the chromatin fibrils has lasted several years. When the method of surface spreading is applied, their diameter is 20—30 nm. On the other hand, the commonly obtained values in the case of chemical fixation and analysis of ultrathin sections, vary between 8 and 18 nm. Chromatin fibrils may be measured on the basis of

X-ray diffraction only in isolated material. The use of isolated chromatin and of different methods of measurement raise the question whether the values obtained may be compared with those from the electron microscope (Huberman, 1973). Pardon and Wilkins (1972) believe, on the basis of the results of the X-ray diffraction method that the deoxynucleo-histone spiral diameter is 10 nm and the pitch 12 nm. Huberman (1973) suggests, on the basis of comparison of the data obtained by way X-ray diffraction and observation in the electron microscope, that an irregular supercoil with pitch 4.5 nm and diameter 6 nm arises.

It is known that the methods of preparation themselves produce wide changes in the appearance and dimensions of the fibrils. These changes are differently interpreted by various authors. Zirkin (1972) considers that such wide differences in the fibril diameter cannot be the result of use of different methods and suggests that one diameter size cannot be assumed for characterization of chromatin fibrils of eukaryotic nuclei. Numerous authors (Zirkin, 1970; Ris, 1970; Olszewska, 1973) affirm that the fibrils with diameter 10 nm are the true forms, and the filaments of 20—30 nm described are artefacts. Other authors, however, claim that it is these 20—30-nm fibrils that accurately represent the native forms.

The chromatin fibril structure is stabilized by histone proteins. There exists, however much controversy as to the contribution of the particular histone fractions to the process of maintenance of the spatial conformation of the DNA threads. In the model proposed by Fredericq (1971) the DNA molecule is bound through the phosphate rests with arginine-rich histones and forms a thread 3 nm in diameter, which as the result of secondary spiralization gives forms 10 nm in diameter. The 10-nm fibrils are spatially stabilized by bonds between the lysine histone molecules. These bonds may form between the pitches of the same spiral or between neighbouring DNA molecules.

The present study was undertaken to compare the ultrastructure of nuclei fixed in situ with those isolated in the presence of Ca^{+2} and Mg^{+2} ions or in the absence of these bivalent cations. The changes were also investigated occurring after extraction and reintroduction of histone proteins.

MATERIAL AND METHODS

The experimental material consisted of roots of 17-day *Pinus silvestris* seedlings cultured on moist sawdust under 16-h daylight of about 4000 lux intensity at 23° C. The investigations were performed on 3-nm segments of root meristem.

Isolation of nuclei. Nuclei were isolated by the method described by Gabara and Michniewicz (1974). Homogenization of the

meristematic root segments in the presence of Ca^{+2} and Mg^{+2} ions was run in a 50 per cent glycerin solution of 20 mM CaCl_2 and 20 ml MgCl_2 . For preliminary purification the homogenate was filtered through 4 layers of cheesecloth and then through a Shott G4 funnel. Further purification was achieved by centrifugation in an ascending gradient of gum arabic concentrations, pH 7.0 — 20 mM CaCl_2 and 20 mM MgCl_2 . Parallely nuclei were isolated in the absence of calcium and magnesium ions.

Histone extraction. Histone were extracted from the isolated nuclei of *Pinus silvestris* root meristem according to Spelsberg and Hnilica (1971). The method of histone extraction and reintroduction into the nuclei is described in an earlier paper (Michniewicz 1976).

The nuclear fraction obtained and the root meristem segments from *Pinus silvestris* were fixed in 2 per cent OsO_4 in 0.1 M cacodylate buffer, pH 7.0, containing 0.5 mg/ml CaCl_2 . The material was stained in a saturated uranyl acetate solution in 50 per cent ethanol, and after dehydration in an ascending alcohol gradient embedded in Epon. Ultrathin sections were photographed in a TESLA 512 A electron microscope. Chromatin fibrils diameter was measured on microphotographs at a magnification of 175 000 (primary magnification $\times 35\,000$). The measurements were performed with a magnifying glass giving a $\times 1.5$ magnification with the use of an ocular micrometer with smallest scale grading of 0.1 mm, this corresponding to 0.57 nm.

RESULTS

1. Nuclei in situ

Chromatin in the nuclei examined in situ is of reticular form, dispersed, with numerous condensed chromatin agglomerations. Condensed chromatin shows a distinct fibrillar structure (Plate I, fig. 1).

Chromatin fibrils of two kinds were noted. Single spiralized forms with diameter of 9.06 ± 0.03 (Plate V, fig. 1) which constituted 36.8 per cent of the population and forms consisting of two fibrils coiled usually around a common axis (Plate V, fig. 2). In both kinds of fibrils spiralization is well visible.

2. Isolated nuclei

2. 1. Nuclei isolated without the presence of bivalent ions

The chromatin of isolated nuclei shows wide differences as compared with that of nuclei in situ (Gabara and Michniewicz, 1974). In the interior of the nucleus dispersed chromatin prevails.

Agglomerations of condensed chromatin were not numerous, but they showed a distinct fibrillar structure (Plate I, fig. 2). The chromatin fibril

Table 1
Kind of fibrils under various experimental conditions (nm)

Kind of fibrils	In situ	Nuclei isolated without Mg^{++} and Ca^{++}	Nuclei isolated in the presence of Mg^{++} and Ca^{++}						
			Control	$(NH_4)_2SO_4$	0.5 M NaCl	Ethanol HCl	$(NH_4)_2SO_4$ + lysine-rich histone	0.5 M NaCl + lysine-rich histone	Ethanol: HCl + arginine rich histone
Single	9.06 ± 0.03 36.8%	10.05 ± 0.06 32.7%	11.00	10.51 ± 0.11 46.3%	9.36 ± 0.09 47.8%	8.45 ± 0.15 41.7%	9.99 ± 0.42 20.8%	11.42 ± 0.13 2.9%	11.42 ± 0.16 4.7%
	13.68 ± 0.16 63.2%	17.71 ± 0.50 67.3%	21.69 ± 0.63 49.2%	17.59 ± 0.26 53.7%	16.39 ± 0.23 57.2%	16.02 ± 0.15 50.0%	19.41 ± 0.47 79.2%	19.53 ± 0.05 74.3%	18.27 ± 0.64 4.8%
Coalesced			31.59 ± 0.43 50.8%			28.55 ± 0.56 8.3%		30.57 ± 0.1 22.9%	34.26 ± 1.00 90.5%

Plate I

Fig. 1. Picture of nuclear chromatin in situ. $\times 35\,000$; Fig. 2. Picture of chromatin from nuclei isolated without the presence of Ca^{+2} and Mg^{+2} ions. $\times 35\,000$; Fig. 3. Picture of chromatin from nuclei isolated in the presence of Ca^{+2} and Mg^{+2} ions. $\times 35\,000$

Fig. 1. Picture of chromatin from nuclei in situ. $\times 35\,000$; Fig. 2. Picture of chromatin from nuclei isolated in the presence of Ca^{+2} and Mg^{+2} ions and subjected to extraction with 0.5 M NaCl, pH 6.0. $\times 35\,000$; Fig. 3. Picture of chromatin from nuclei isolated in the presence of Ca^{+2} and Mg^{+2} ions, subjected to extraction with 0.5 M pH 6.0, and reintroduction of the lysine histon fraction. $\times 35\,000$

Fig. 1. Picture of chromatin from nuclei in situ. $\times 35\,000$; Fig. 2. Picture of chromatin from isolated nuclei in the presence of Ca^{+2} and Mg^{+2} ions and subjected to extraction with $2.0\text{ M } (\text{NH}_4)_2\text{SO}_4$. $\times 35\,000$; Fig. 3. Picture of chromatin from nuclei isolated in the presence of Ca^{+2} and Mg^{+2} ions, subjected to extraction with $2.0\text{ M } (\text{NH}_4)_2\text{SO}_4$ and reintroduction of lysine histones. $\times 35\,000$

Fig. 1. Picture of chromatin from nuclei in situ. $\times 35\,000$; Fig. 2. Picture of chromatin from nuclei isolated in the presence of Ca^{+3} and Mg^{+2} ions and subjected to extraction with a mixture of ethanol and HCl . $\times 35\,000$; Fig. 3. Picture of chromatin from nuclei isolated in the presence of Ca^{+2} and Mg^{+2} ions, subjected to extraction with an ethanol— HCl mixture and reintroduction of arginine histones. $\times 35\,000$

Kinds of chromatin fibrils

Fig. 1. Single fibril. $\times 525\,000$; Fig. 2. Double fibril coiled around a common axis. $\times 525\,000$; Fig. 3. Double fibril — two single fibrils coiled around their own axes. $\times 525\,000$; Fig. 4. Double fibrils, spiralization obliterated. $\times 525\,000$; Fig. 5. Single fibril stretched. Interior not filled. $\times 525\,000$; Fig. 6. Double fibril, dense spiralization, contours obliterated by presence of coat. $\times 525\,000$; Fig. 7. Forms consisting of 2 double fibrils coiled around one another $\times 525\,000$; Fig. 8. Forms consisting of several coalesced double fibrils $\times 525\,000$

diameter was changed. Single fibrils forming spirals with a 10.1 ± 0.1 nm diameter were observed, constituting 32.7 per cent as well as coarser forms with a diameter of 17.7 ± 0.5 nm forming the rest of the population (Table 1). On the electron microphotographs it is seen that the coarser forms are composed of two single fibrils which may be coiled around a common axis (Plate V, fig. 2). Forms were also observed consisting of two fibrils each of which was spiralized around its own axis, this greatly increasing the diameter (Plate V, fig. 3).

2. 2. Nuclei isolated in the presence of Ca^{+2} and Mg^{+2} ions

Chromatin in nuclei isolated in the presence of Ca^{+2} and Mg^{+2} ions preserves its fibrillar structure (Plate I, fig. 3). A greater number of condensed chromatin aggregations was observed as compared with the nuclei isolated without the participation of bivalent cations. Single fibrils 11.0 nm in diameter were sporadically observed. On the other hand very numerous (49.2%) were double fibrils. The fibril diameter increased to 21.7 ± 0.6 nm (Table 1).

The double forms consisted of two elementary fibrils each of which was spiralized around its own axis, this increasing the diameter (Plate V, fig. 3). Besides, in the nuclei isolated in the presence of bivalent ions much larger forms were found with diameter 31.6 ± 0.43 nm constituting 50.8 per cent. They formed as the result of coalescence of two double fibrils as may be seen in the electronmicroscopic pictures (Plate V, fig. 8).

2. 2. 1. Nuclei isolated (in the presence of Ca^{+2} and Mg^{+2} ions) after extraction with 0.5 M NaCl, pH 6.0 and reintroduction of the lysine histon fraction.

Nuclei subjected to extraction with 0.5 M NaCl which selectively removes the lysine-rich fractions F1, showed complete disappearance of condensed chromatin (Michniewicz, 1976). Neither were thicker, coalesced fibrillar structures observed (Plate 2, fig. 2). Single fibrils with diameter 9.4 ± 0.1 nm, on the contrary, appeared in large quantities (47.8%), while they only sporadically were observed in the control nuclei of this experimental series. The double fibrillar structures with diameter 16.4 ± 0.2 nm composed the remaining part of the population (Table 1).

In both kinds of structures spiralization was compact and the interior of the spiral was electron-dense.

Reintroduction of the lysine histone into the nuclei deprived of these proteins caused the reappearance of condensed chromatin aggregations (Plate II, fig. 3). The major part of the fibril population consisted of double structures (Plate V, fig. 4). Their diameter reached 19.5 ± 0.1 nm (Table

1). Spiralization was not so distinct as in nuclei extracted with 0.5 M NaCl. This is probably due to the formation of a coat over the fibrils.

Introduction of the lysine histone fraction caused the formation of larger forms with diameter 30.6 ± 0.1 nm (Plate V, fig. 8). The appearance of these structures was probably connected with the joining of double fibrils by way of interaction between lysine histones forming the coat around them.

2.2.2. Nuclei after extraction with 2.0 M $(\text{NH}_4)_2\text{SO}_4$, pH 6.0, and reintroduction of lysine histones

Nuclei subjected to extraction with ammonium sulphate which removes together the lysine-rich and slightly lysine-rich fractions, are shown in Plate III, fig. 2. Chromatin dispersion in this case was greater than after extraction with 0.5 M NaCl. Condensed chromatin disappeared completely, and the interior of the nucleus was filled with a loose reticular fibrillar structure (Michniewicz, 1976).

Two kinds of chromatin fibrils were observed: single ones 10.5 ± 0.1 nm in diameter and double ones with a diameter of 17.6 ± 0.3 nm. Each kind constitutes about one half of the population (Table I). Spiralization of the observed forms was distinct (Plate V, fig. 5). The spirals were somewhat stretched, owing to which the pitch of the spiral increased as compared with that in double fibrils occurring in control nuclei. The spiral interior does not seem to be filled, a clear space is visible between the pitches (Plate V, fig. 5).

Reintroduction of the lysine histone causes reconstruction of the condensed chromatin masses (Michniewicz, 1976). The amount of condensed chromatin formed was larger than in the control nuclei. Condensed chromatin preserves its fibrillar structure (Plate III, fig. 3). Single fibril diameter was 10.0 ± 0.4 nm. The contribution of these structures decreased by about one half amounting to 20.8 per cent. On the other hand, the number of double fibers increased, and their diameter was slightly larger as compared with that in material with lysine histones removed (Table 1). Both kinds of fibrils showed compact spiralization, their interior was frequently filled (Plate V, fig. 6), and the contours were obliterated by the relatively thick coat surrounding these structures.

Reintroduction of the lysine histone into the nuclei after extraction with ammonium sulphate did not result in coalescence of fibrils. This was probably associated with the fact that the lysine histone introduced rebuilds the cross-link between the neighbouring pitches of the spiral, this producing a stronger spiralization, and hence a reduction in the diameter of single fibrils.

2.2.3. Nuclei after extraction with ethanol-HCl and reintroduction of the arginine histone

The picture of chromatin from nuclei subjected to arginine histone extraction is shown in Plate III, fig. 2. In these nuclei chromatin was not dispersed as in the case of removal of the lysine fraction. Extraction of the arginine fraction leads to chromatin coagulation, and in these sites the fibrillar structure is not visible. In the parts of the nucleus where the fibrillar structure was preserved single fibrils could be seen 8.5 ± 0.2 nm in diameter as well as double fibrils with an almost two times larger diameter. There were also coalesced fibrils with diameter 28.5 ± 0.6 nm (Table 1) but only in small numbers.

A very strong spiralization of the fibrillar structures examined was noted, giving in consequence a reduction of their diameter. This decrease in size is particularly pronounced in the case of single fibers.

Reintroduction of the arginine histone does not change the general appearance of the nucleus. Chromatin remains in most of the area in a dense compact form (Plate III, fig. 3).

The main part of the analysed fibril population (90.5 per cent) consisted of forms 34.3 ± 0.1 nm wide. These structure were formed of two or more double fibrils wound around themselves. (Plate V, fig. 7) or coalesced (Plate V, fig. 8).

The 10-nm fibrils observed in all the experimental series are spiralized threads. In all cases the diameter of these threads was 2.3 nm, and only slightly larger after reintroduction of the arginine histone.

DISCUSSION

According to the previously presented data (Michniewicz, 1976), histones exert a considerable influence on the degree of chromatin condensation as well as on the kind and of dimensions of chromatin fibrils. Lysine histone extraction caused dispersion of condensed chromatin, and at the site of compact masses a reticular fibrillar structure appeared. The rebuilding of compact masses after reintroduction of the lysine histone indicates that this change is actually associated with the removal of the histone fraction.

The differences in the influence of the particular histone fractions on the condensation process are probably caused by the different arrangement of the histone molecules in the DNH chain: it is supposed that the arginine histone molecules form transverse bonds between the DNA chains or between the pitches of the same chain (Fredericq, 1971). Chromatin decondensation due to lysine histone extraction is supposedly the result of breaking of these cross-link bonds joining neighbouring chromatin threads (Littau, 1965). On the other hand, removal of the arginine his-

tone may produce an additional interaction between the lysine histone molecules, this leading to the observed coalescence of chromatin masses.

The present observations confirm the reports of other authors that the lysine histones are responsible for the condensation process (Miller et al., 1972; Berlowitz et al., 1969). Littau et al. (1965) demonstrated that removal of lysine-rich histones, and solely of these histones, loosens the condensed chromatin structure, whereas reintroduction of these proteins into the nucleus induces reconstruction of the chromatin masses. The results of Brusch and Setterfield (1972, 1974) are, however, different. These authors observed that after lysine-rich histone extraction chromatin coagulates homogeneously in the form of gel. A certain dispersion of chromatin masses occurred only after extraction of the lysine-rich and arginine histones.

The present observations confirmed earlier data, namely, that in the interphase nuclei the diameter of the basic fibrillar structure lies within the range 8.0—12.0 nm (Ris, 1970; Zirkin, 1972; Olszewska, 1973a). Investigations of X-ray diffraction (Pardon and Wilkins, 1972) also showed that the diameter of nucleo-histone fibrils is about 10.0 nm.

There is much controversy in the literature concerning the true dimensions of chromatin fibrils. The difficulty in interpretation of the results lies in the impossibility of their confrontation with dimensions of chromatin fibrils in living cells. The range of measurement results reported by various authors varies between 3.0 and 50.0 nm, and it is probably due to the different methods of preparation.

The present investigations demonstrated that the process of isolation itself caused considerable differences in the size of the observed fibrils. It is known that bivalent cations are indispensable for maintenance of a supercoiled structure (Garret, 1971). It proved however, that the usually applied Ca^{+2} and Mg^{+2} ions produce, during the process of isolation of the nuclear fraction, significant changes in the dimensions and kind of chromatin fibrils observed. It results from the data obtained that these ions cause coalescence of the chromatin threads, owing to which single fibrils disappear and change to forms with a much larger diameter. The structures thus formed are similar to those described by a number of authors as basic native forms (Brusch and Adams, 1974).

Less pronounced differences as compared with the nuclei examined *in situ* were noted in nuclei isolated without the presence of bivalent cations. In this case also, however, an increase of the diameter was observed, particularly of the double fibrillar structures. This increase in size of the fibrils may be due to the binding of extrachromosome proteins with the fibrils during the process of isolation. Evidence of this phenomenon has been clearly presented by Wolfe and Grim (1967) who demonstrated that the fibril diameter increases when the cell is damaged during the isolation process. These authors suggest that the increase in

diameter occurs as the result of interaction of the 10-nm fibrils with extrachromosomal proteins. Therefore the 20—30-nm fibrils may be artefacts, and the true diameter of the fibrils is 10 nm. These suppositions were confirmed by Bornkam (1973) who demonstrated that hypotonic damage to the cell causes an increase in fibril diameter. Also Zirkin and Wolfe (1970) comparing by cytochemical methods the histone and nonhistone protein contents in interphase nuclei and metaphase chromosomes of intact cells and in isolated preparations demonstrated that extrachromosomal proteins bind with the chromosomes during the isolation procedure. These authors suggest that the same process occurs in isolated interphase nuclei. Solarì (1971) stresses the possibility of appearance of chromatin fibrils of various dimensions when different methods of preparation are applied. He demonstrated that in the presence of bivalent cations the use of alcohols for drying and the presence of haemoglobin which binds with DNA may increase the diameter of fibrils from chick erythrocytes from 3.7 to 31.3 nm.

On the other hand Ris and Kubai (1970) observed 25-nm fibrils which, as they suggest, result from the coalescence (side-by-side) of two 10-nm fibrils. This supposition is confirmed by the demonstration that on the spreading surface 10-nm fibrils are obtained in the presence of chelating buffer and that fibrils fixed 10—20 sec after exposure to chelating agents seem to be a pair of 10-nm fibrils coiled around one another. Zirkin and Kim (1972), however, observed 20 nm fibrils in sections fixed in the presence of chelating buffers (phosphate and cacodylate).

The present observations also demonstrated the occurrence of forms with diameter of 13—22 nm, in spite of the use of the chelating cacodylate buffer. In most cases this chelating action was abolished by the addition of Ca^{+2} and Mg^{+2} ions in the course of isolation of nuclei. In the material isolated without the presence of these ions forms with 17.7 nm diameter prevailed. In all experimental series it could be observed that the thicker form consisted of at least two 10-nm fibrils, in agreement with the earlier suggestions of Ris (1970).

Zirkin and Kim (1972) suppose that spiralization of the fibrils is the mechanism causing an increase in their diameter. The present observations, however, demonstrated that differences in spiralization do not cause such wide divergence in the fibril dimensions, but that this phenomenon is mainly due to aggregation of the thinner forms.

The presently performed investigations demonstrated that extraction and reintroduction of the histone fraction exerts a marked effect on the kind of fibrillar structures, the degree of their spiralization and spacial configuration. Removal of lysine histones abolished the interaction between neighbouring DNH molecules, as indicated by the large number of 10-nm elementary fibrils which in controls fibers were observed only sporadi-

cally. Reintroduction of lysine histones led to a reconstruction of the bonds between the histone molecules and produced double forms.

The present observations confirmed earlier data (Borkamm, 1973) indicating that removal of histones by increasing NaCl concentrations (0.6—2.0 M) reduces the diameter of chromatin fibrils from 15—18 to 8—10 nm. Brasch et al. (1972, 1974) demonstrated that, after extraction of the lysine histone F1, the 20-nm fibrils disappear, while forms with diameter of 2—10 nm become visible. Littau (1965) also observed after the extraction of lysine histones the appearance of fibers 5 nm thick.

Numerous data indicate that the interactions between histones are actually important for maintaining the chromatin fibrils structure. It was demonstrated that digestion with proteases causes a diminution of the diameter of elementary fibrils as the consequence of loosening of the spiral of the DNH thread (Olszewska 1973b). Other evidence of the influence of interaction between histone molecules in chromatin is presented in the results of investigations on the action of urea (Varshavsky et al. 1971). Urea destroys the protein-protein interaction indispensable for preserving the superhelix configuration and causes elongation and narrowing of the chromatin fibrils to a diameter of 3.5—4.0 nm, this occurring without loss of proteins from DNH.

A reversible diminution of the diameter of chromatin fibrils from 20 nm to 5—10 nm was observed after exposure of nuclei to a gradient of decreasing ion concentration (Brasch and Setterfield, 1974). These changes in fibril diameter result probably from the action of changes within nucleohistone complexes, which cause loosening of the spiral organization of 20-nm fibrils.

Biochemical studies and X-ray diffraction demonstrated that histones have a tendency to association in pairs in the solution and to the formation of tetrameres. There is much evidence suggesting that the tetrameres occurring in the solution are also present in chromatin (Kornberg, 1974).

In the literature there is much controversy concerning the role played by the particular histone fractions in the maintenance of the chromatin organization. Brasch et al. suggest that loosely bound histones (the lysine-rich fraction I and the arginine fraction V) are mainly responsible for preserving the configuration of 20-nm units. Removal of the lysine-rich histone (I) and the arginine one (V) caused disappearance of the 20-nm fibrils, whereas the 10-nm ones remained intact (Brasch, 1972, 1974). On the other hand, Bonner et al. (1968) report that histone I is not responsible for the structural properties of DNH and ascribe this kind of influence to the moderate lysine fraction II. The contribution of the arginine fraction IV also seems probable here.

Literature data only exceptionally mention that the main role in ordering the superhelix is played by arginine-rich histones (Auer, 1970).

Since histone F1 is most easily removed by low salt concentrations, Smart and Bonner (1971) advanced a suggestion as to the distribution of the particular histone fractions in the DNH molecule. According to their hypothesis, histone F1 forms a sheath around the spirally coiled DNA thread, whereas moderately lysine-rich and arginine-rich histones are present inside this sheath, thus they are not so much exposed to the action of the external environment.

The present observations seem to agree with such a model of the DNH thread. Extraction of the F1 fraction produced the disappearance of the coat surrounding the fibrils, and the stronger spiralization observed is probably the result of additional interaction between the remaining lysine histones. On the other hand, reintroduction of lysine histones causes a rebuilding of the coat covering the fibrils. The appearance of structures formed by coalescence of two or more threads may be due to the formation of bonds between the histones forming the coat around the neighbouring fibrils. Removal of the moderate — lysine rich histones together with lysine-rich histones caused, beside disappearance of the coat, a loosening of spiralization, and after reintroduction of the lysine histones, in numerous cases a core filling the spiral was observed. It is supposed that this core is formed by the moderately lysine-rich histone.

To sum up, the results of the present investigations suggest that the presence of Mg^{+2} and Ca^{+2} ions results in longitudinal coalescence of the 10-nm elementary chromatin fibrils, giving forms with a diameter of about 30 nm. Histones exert a significant influence not only on the degree of chromatin condensation, but also on the kind and dimensions of the fibrillar structures. The observations here described do not, however, give grounds to ascribe to any one histone fraction a particular role in the above discussed processes.

The author is deeply indebted to Professor Maria Olszewska for his valuable advice, criticism and discussion in the course of this work.

REFERENCES

- Auer G., Zettenberg A., Killander D., 1970, Changes in binding between DNA and arginine residues in histone induced by cell crowding. *Exp. Cell. Res.* 62: 32—38.
- Bahr S. F., 1970, Human chromosome fibers. Consideration of DNA-protein packing and of looping patterns. *Exp. Cell. Res.* 62: 39—45.
- Berlowitz L., Pallota D., Sibley C. H., 1969, Chromatin and histones. Binding of tritiated actinomycin D to heterochromatin in mealy bugs. *Science* 164: 1527—1529.
- Bonner J., Dahus M. E., Fambrough D., Huang R. S., Marushige K., Tuan D. Y. H., 1968, The biology of isolated chromatin. *Science* 159: 47—55.
- Borkamm G. W., Sonnenbichler J., 1973, The electron microscopic structure

- of nucleoprotein fibrils from metaphase chromosomes treated with salt. *Chromosoma* 43: 261—268.
- Brasch K., Seligy V. L., Setterfield G., 1971, Effect of low salt concentration on structural organization and template activity of chromatin in chicken erythrocyte nuclei. *Exp. Cell. Res.* 65: 61—72.
- Brasch K., Setterfield G., Neelin J. M., 1972, Effect of sequential extraction of histone protein on structural organization of avian erythrocyte and liver nuclei. *Exp. Cell. Res.* 74: 27—41.
- Brasch K., Adams G. H., Neelin J. M., 1974, Evidence for erythrocyte specific histone modification and structural changes in chromatin during goose maturation. *J. Cell. Sci.* 15: 659—677.
- Brasch K., Setterfield G., 1974, Structural organization of chromosomes in interphase nuclei. *Exp. Cell. Res.* 83: 175—185.
- Fredericq E., 1971, The chemical and physical properties of nucleo-histones. [In:] *Histones and nucleohistones*, ed. D. M. P. Philips, Plenum Press, London and New York.
- Gabara G., Michniewicz-Porazińska H., 1974, A comparison of the ultrastructure of nuclei in situ with that of nuclei isolated from the root meristem of *Pinus silvestris*. *Folia Histochem. Cytochem.* 12: 183—192.
- Garret R. A., 1971, Low angle X-ray diffraction from dilute nucleohistone gels. *B. B. A.* 246: 553—560.
- Hippel H. P., McGhee, 1972, DNA-protein interactions. *Ann. Rev. Bioch.* 41: 231—232.
- Kornberg R. D., 1974, Chromatin structure: A replicating unit of histones and DNA. *Science* 184: 868—871.
- Littau V., Burdick C. J., Allfrey V. G., Mirsky A. E., 1965, The role of histones in the maintenance of chromatin structure. *Proc. Nat. Acad. Sci. USA* 54: 1204—1212.
- Michniewicz H., 1976, The effect of histone extraction on ³H-actinomycin D binding to isolated nuclei of *Pinus silvestris* roots. *Folia Histochem. Cytochem.* 14: 39—48.
- Miller G. J., Berlowitz L., Regehon W., 1972, Chromatin and histones in hen erythrocyte nuclei. *Exp. Cell. Res.* 71: 409—421.
- Olszewska M. J., 1973a, Ultrastructure of the elementary fibril in extended and condensed chromatin and in metaphase chromosomes. *Acta Soc. Bot. Pol.* 42 (2): 265—279.
- Olszewska M. J., 1973b, Effect of some proteolytic enzymes on chromatin ultrastructure. *Folia Histochem. Cytochem.* 11: 345—346.
- Pardon J. F., Wilkins M. H. F., Richards B. M., 1967, Molecular structure. Super helical model for nucleohistone. *Nature* 215: 508—509.
- Pardon J. F., Wilkins M. H. F., 1972, A super-coil model for nucleohistone. *J. Mol. Biol.* 68: 115—124.
- Ris H., Kubai D. F., 1970, Chromosome structure. *Ann. Rev. Gen.* 4: 263—294.
- Spelsberg T. C., Hnilica L. S., 1971, Protein in template restriction. RNA synthesis in vitro. *B. B. A.* 228, 1: 202—208.
- Smart J. E., Bonner J., 1971, Studies on the role of histones in relation to the template activity and precipitability of chromatin at physiological ionic strengths. *J. Mol. Biol.* 58: 675—684.
- Solari A. J., 1971, Experimental changes in the width of chromatin fibers from chicken erythrocytes. *Exp. Cell. Res.* 67: 161—170.
- Varshavsky A. Y., Ilyin I. V., Kadyckov V. I., Senchenkov E. P., 1971, Collapse of extend deoxyribonucleoprotein molecule upon increase of the ionic strengths of solution. *B. B. A.* 246: 583—588.

- Wolfe S. L., Grim J. N., 1967, The relationship of isolated chromosome fibers to the fibers of the embedded nucleus. *J. Ultrastruct. Res.* 19: 382—397.
- Zirkin B. R., Kim S. K., 1972, Fine structure of fibers in thin sections of condensed chromatin. *Exp. Cell. Res.* 75: 490—496.
- Zirkin B. R., Wolfe S. L., 1970, The chemical composition of nuclei and chromosomes isolated by the langmuir trough technique. *Chromosoma* 32: 162—170.

Author's address:

Halina Michniewicz

Institute of Physiology and Cytology,
University of Łódź,

Banacha Str. 12/16; 90-257 Łódź, Poland

Wpływ histonów oraz jonów wapnia i magnezu na ultrastrukturę chromatyny w izolowanych jądrach merystemu korzeniowego
Pinus silvestris L.

Streszczenie

Szerokość fibryli chromatynowych w jądrach utrwalanych in situ wynosi około 10 nm, w jądrach izolowanych w obecności jonów Ca^{+2} i Mg^{+2} następuje sklejanie fibryli, a tym samym wtórne zwiększenie ich szerokości, natomiast w jądrach izolowanych bez tych kationów następuje pewne zwiększenie średnicy w porównaniu z jądrami in situ, prawdopodobnie wskutek adsorbowania niechromatynowych białek jądrowych.

Ekstrakcja histonów lizynowych powodowała dyspersję chromatyny skondensowanej, a ponowne wprowadzenie tych białek — jej rekonstruowanie. Natomiast ekstrakcja i ponowne wprowadzenie histonu argininowego nie wywoływało rozpraszania chromatyny, a raczej sklejanie mas chromatynowych.

Ekstrakcja histonów lizynowych z materiału izolowanego w obecności jonów Ca^{+2} i Mg^{+2} powodowała pojawienie się znacznych ilości fibryli 10 nm, które tylko sporadycznie występowały w materiale kontrolnym, oraz zanik form 30 nm. Ponowne wprowadzenie frakcji lizynowych powodowało zmniejszenie ilości fibryli pojedynczych oraz powstanie form sklejonych o średnicy 30 nm. Usunięcie histonów argininowych nie powodowało zaniku fibryli pojedynczych, ale zmniejszenie ich średnicy. Ponowne wprowadzenie tej frakcji powoduje sklejanie się nici chromatynowych w wyniku czego 90% obserwowanej populacji stanowiły fibryle o średnicy ok. 30 nm.