Studies on the Elementary Fibril of Chromatin  
in Pollen Mother Cells

Aiko Sakai-Wada

Department of Biology, Faculty of Science, Nara Women's University, Nara, 630 Japan

Received January 11, 1979

Structural studies on chromosomes have been carried out by many investigators, but the detailed structure is not yet established. However, it is certain that chromosomes consist of fine threads of chromatin 200–300 Å in width, as reported by many investigators (DuPraw 1965, Gall 1966, Abuelo and Moor 1969, Bram and Ris 1971, Sakai 1975, Schwarzacher 1976). Moreover, it is also known that the 200 to 300 Å chromatin threads are formed by folding or coiling of thinner threads of chromatin of about 100 Å width (100 Å chromatin threads), as described by Ris (1969), DuPraw (1970), Heumann (1974), Baldwin et al. (1975), Bram et al. (1975) and Sakai (1975). Now the 100 Å chromatin threads are generally accepted as the "elementary fibrils of chromatin", and the substructure of the elementary fibrils has become the object of studies in this field.

In electron microscopic studies Olins and Olins (1974), Olins et al. (1975),Senior et al. (1975) and Howze et al. (1976) observed particles of 100 Å diameter aligned in tandem along the elementary chromatin fibrils. These particles have been called "v-bodies" (or "nucleosomes" by Oudet et al. 1975). From neutron-scatter studies Baldwin et al. (1975) proposed a globular model of 100 Å diameter in which the apolar segments of histones form the core surrounded by DNA complexed with the basic parts of the histone. Bram et al. (1975) also proposed a "coiled-coil model" of the chromatin subunit structure, formed from a series of globules of the type proposed by Baldwin et al. (1975).

Most of these investigations were carried out on functional nuclei of animal cells, amphibian and chiken erythrocytes and mammalian thymus, kidney and liver, and a few studies were made on dividing nuclei (Howze et al. 1976). The present work was carried out to clarify the substructure of elementary fibrils of chromatin and to determine the locations of various components, such as DNA, protein and RNA in the elementary fibrils. Studies were made by electron microscopy on dividing nuclei of plant cells, that is, chromosomes of pollen mother cells in the first meiotic metaphase.

Material and methods

Pollen mother cells in the first meiotic metaphase from Tradescantia reflexa were used. Specimens were examined as whole mounts and ultrathin sections.

Whole mounts. Anthers with pollen mother cells in the first meiotic metaphase were crushed with a razor in 0.3 M sucrose solution containing 1 mg/ml cel-
lulase, adjusted to pH 5.59 with phosphate buffer. The pollen mother cells flowed out into the sucrose solution and after 15 minutes at room temperature, they were collected by centrifugation and washed three times with 0.3 M sucrose solution adjusted to pH 5.59 with phosphate buffer. On transfer to distilled water, the pollen mother cells become swollen, the cell wall broke and the chromosomes were released in the distilled water. Unbroken cells and cell wall were removed by low speed centrifugation, and then 2.5% glutaraldehyde was added to the supernatant, and the chromosomes released were collected by centrifugation. They were washed three times with distilled water and a drop of the suspension was put on a polyvinyl formal membrane supported by a platinum loop and dried in a refrigerator. Then dry preparations were treated by the following procedures.

A. Untreatment preparations. Preparations were stained with aqueous 1% uranyl acetate, dehydrated in a graded series of increasing concentrations of acetone, immersed in 100% isopentane and dried in air at room temperature.

B. DNAase treatment. Preparations were treated with 1 mg/ml of DNAase solution (pH 6.3) containing 2.5 mM MgSO₄, for 1 hour at 37°C.

C. RNAase treatment. Preparations were treated with 1 mg/ml of RNAase for 1 hour at 37°C.

D. Pronase treatment. All proteins in chromatin were removed by treatment with a saturated solution of pronase adjusted to pH 7.5 with phosphate buffer for 1 hour at 37°C.

E. Treatment to remove histones. Preparations were treated for 3 hours at 4°C with 0.25N HCl containing 2 M NaCl.

F. DNAase+RNAase treatment. Preparations were treated with DNAase as described in B, and then with RNAase as described in C.

G. DNAase+pronase treatment. Preparations were treated as described in B and D.

After these treatments the same procedure was used as for untreated preparations, as described in A. Then the preparations on a polyvinyl formal membrane supported in a platinum loop were placed on a copper grid and examined under an HUM 12A electron microscope.

Ultrathin sections. Material were fixed for 1 hour in a mixture of 5% glutaraldehyde and 4% paraformaldehyde adjusted to pH 7.0 with phosphate buffer. Post-fixation was carried out in 1% OsO₄ (pH 7.0) for 3 hours. After dehydration in a graded series of increasing concentrations of acetone, materials were embedded in epoxy resin. Sections were made and stained with lead citrate. These preparations were examined under an electron microscope.

Fig. 1 is an ultrathin section and Figs. 2-9 are whole mount. Figs. 1-3. 1, part of chromosome in the first meiotic metaphase. Aggregates of fine fibrils of about 250 Å width are seen. ×42,000. 2, part of an untreated chromosome. 300 Å chromatin threads are seen through the figure. By the twist of a 300 Å chromatin thread, high-ordered chromatin thread is contributed (in the square) and higher-ordered chromatin threads consisting of twisted 300 Å chromatin threads are seen between opposite thick arrows. The thin arrows indicate a 300 Å chromatin thread composed by a coiled 100 Å chromatin thread. ×141,000. 3, part of an untreated chromosome that is more expanded than that in Fig. 2. Arrows indicate parts of granular chromatin threads. ×141,000.
Observations

In ultrathin sections aggregates of fine threads of about 250 Å in width were observed in chromosomes in the first meiotic metaphase (Fig. 1). Owing to the very complicated arrangement of these threads and the fact that the sections were very thin, the three dimensional arrangement of the fine threads in the chromosome could not be decided. Therefore, to examine the three dimensional structure of the chromosome and the locations of components such DNA, protein and RNA in the chromatin thread, we examined whole mount of metaphase chromosomes.

Figs. 2 and 3 show parts of untreated chromosomes. In the slightly extended chromosome, chromatin threads with about 300 Å in width (300 Å chromatin threads) are observed as basic chromatin threads (Fig. 2). These threads may be the same as the fine threads seen in chromosomes of ultrathin sections. It can also be seen that thicker chromatin threads are formed by twisting of the 300 Å chromatin threads (Fig. 2 inside the rectangle), and that larger chromatin threads or chromonemata are formed by aggregation and coiling of two or more thicker chromatin threads (Fig. 2, between thick arrows). It can also be seen that the 300 Å chromatin threads consist of coils of thinner threads of about 100 Å width (100 Å chromatin threads) (thin arrows in Fig. 2). In more extended chromosomes than that shown in Fig. 2, fine fibrils of about 50–70 Å in width are seen among the 100 Å and 300 Å chromatin threads (Fig. 3). These fine fibrils are not slender, but granular due to a tandem arrangement of small granules of about 70 Å diameter along the fine fibrils (Fig. 3, arrows). The granules are the same sizes as the v-bodies reported by Olins and Olins (1974), Woodcock et al. (1976) and Howze et al. (1976), but the tiny filaments connecting v-bodies as reported by Olins and Olins (1974) and Howze et al. (1976) could not be observed.

In chromosomes treated with DNAase, the 100 Å chromatin threads appear as tubes with an electron opaque periphery of about 25 Å thickness and an electron less opaque core of about 45 Å in width (Fig. 4, arrows). The less opaque core is probably apparent because some substances existing in the core of 100 Å chromatin thread have been removed by DNAase treatment. After removal of DNA and RNA, chromatin threads of about 90 Å in width (90 Å chromatin thread) are mainly seen instead of 100 Å chromatin thread (Fig. 5). The 100 Å chromatin threads may be reduced in size by these treatments. At some regions the 90 Å chromatin threads appear as tubes with an electron opaque periphery of about 25 Å thickness and an electron translucent core of about 35 Å in width (Fig. 5, Figs. 4–9. 4, DNAase-treated chromosome. 100 Å chromatin threads appear like a tube (arrows). ×274,000. 5, DNAase+RNAase-treated chromosome. Chromatin threads look like a rope. Thick arrow indicates part of an electron translucent core in 90 Å chromatin thread and thin arrows show translucent particles of about 25 Å diameter in the core. ×274,000. 6, chromosome treated with 0.25 N HCl containing 2 M NaCl. The arrows indicate a 100 Å chromatin thread that is somewhat less electron dense than that seen in Figs. 2 and 3. ×274,000. 7, pronase-treated chromosome. The peripheries and particles of 25 Å diameter of the core area of 100 Å chromatin threads (arrows) appear electron opaque. ×274,000. 8, RNAase-treated chromosome. ×274,000. 9, DNAase+pronase-treated chromosome. Only the periphery of threads is electron opaque (arrow). ×274,000.
thick arrow), but most 90 Å chromatin threads look like ropes, since the electron opaque peripheries cross the core area (Fig. 5, thin arrows), and as a result of this crossing, the translucent particles of about 25 Å diameter appear to have a tandem arrangement. This figure suggests that substances in the extreme periphery and core area are removed by DNAase and RNAase. The electron opaque periphery seen in this figure, may be formed with substances that are resistant to DNAase and RNAase. Fig. 6 shows part of a chromosome after removal of histones with 0.25 N HCl containing 2 M NaCl. The core area of the 100 Å chromatin thread is rather less electron dense (Fig. 6, arrows) than in untreated preparations (Figs. 2 and 3), but there are no distinct changes, such as those seen after treatment with DNAase or DNAase+RNAase.

Then the chromosomes were treated with pronase to remove all proteins. These preparations contained 100 Å chromatin threads with an electron opaque periphery of 10 Å thickness and electron opaque particles of 25 Å diameter arranged in a row in the core area (Fig. 7). Another area from the periphery and the particles in the 100 Å chromatin thread appeared electron translucent (Fig. 7, arrows). The electron translucent parts may result from digestion with pronase. The appearance of the chromosomes is like the negative picture obtained after treatment with DNAase+RNAase (compare Fig. 7 with Fig. 5). When the materials were treated with RNAase, chromatin threads of 90 Å width were observed, but these was no consistent change in the appearance of the threads (Fig. 8). After treatment with DNAase+pronase, only the extreme periphery of the 100 Å chromatin threads appeared electron opaque (Fig. 9, arrow). Namely, substances that were not removed with DNAase+pronase were localized in the periphery.

The following conclusion and conjectures are made from the above results. Findings after treatment with only DNAase (Fig. 4), with a combination of DNAase+RNAase (Fig. 5) and with pronase (Fig. 7) suggest that DNA is localized in the core area of the 100 Å chromatin threads. RNA, if present in the 100 Å chromatin threads, may be situated in the extreme periphery, because the 100 Å chromatin threads became thinner after treatment with RNAase (Figs. 5 and 8) and only the extreme periphery remained after DNAase+pronase treatment (Fig. 9). From findings after treatment with 0.25 N HCl containing 2 M NaCl (Fig. 6) and pronase (Fig. 7) it is concluded that proteins are located all along the 100 Å chromatin threads. The appearance of 100 Å chromatin threads after removal of histones with 0.25 M HCl containing 2 M NaCl (Fig. 6) was very different from that after treatment with pronase (Fig. 7). In the former the center of the 100 Å chromatin threads was somewhat less opaque than in untreated preparations (Figs. 2 and 3), but in the latter, great variations were observed as seen in Fig. 7. These findings suggest that histones and many non-histone proteins are located in the 100 Å chromatin threads.

Discussion

"Coiled-coil model" proposed by Bram et al. (1975) and "solenoid model" proposed by Finch and Klug (1976) have the following points in common: 1) chro-
matin threads are about 100 Å in diameter, 2) high-ordered chromatin threads are formed by coiling of 100 Å chromatin threads and 3) 100 Å chromatin threads consist of repeating granular structures of about 100 Å diameter. In the present work, it was also seen that the 100 Å-chromatin threads formed chromosomes. That is, although in ultrathin sections no substructures of 300 Å chromatin threads could be observed, in whole mounts the 300 Å chromatin threads were seen to be formed by the coiling of 100 Å chromatin threads (Fig. 2). Olins and Olins (1974) and Woodcock et al. (1976) concluded that the extended chromatin threads have a “beads-on-a-string” substructure, with particles called “v-bodies” which are about 70 Å in diameter and are connected by DNA fibers of about 15 Å diameter. Howze et al. (1976) observed fine fibrils of about 89±11 Å length between particles in the chromosomes of Chinese hamsters. In the present work, granules that seemed to be “v-bodies” or “nucleosomes” were clearly seen, as shown in Fig. 3, but not fine fibrils of DNA connecting the nucleosomes were detectable. The detection of these fibrils may depend on the degree of expansion of chromosomes when the preparations is made. It seems likely that the fibrils are also structural components of the present materials. Further studies are required on this problem.

Baldwin et al. (1975) considered that globular structure of about 100 Å diameter recognizing in chromatin fibril consists of a core which are made from apolar segments of four histones, H2A, H2B, H3 and H4, and are surrounded by DNA complexed with the basic segments of histone H1. In this work, granules considered to be nucleosome, were observed in 100 Å chromatin threads (Fig. 3), but the locations of DNA and histones in the 100 Å chromatin threads were not consistently as described by Baldwin et al. (1975), judging from the results of the treatments by DNAase (Fig. 4) and 0.25 N HCl containing 2 M NaCl (Fig. 6).

From light microscopic studies Mirsky and Ris (1951) concluded that metaphase chromosomes contain two kinds of proteins, histones and non-histones and later studies have supported this conclusion (Swift 1964). The non-histone protein remains after extraction of DNA and histone. In the present work, the existence of non-histone protein was suggested from findings after treatment with 0.25 N HCl-2 M NaCl and pronase.

It has been generally accepted that about 10% of the chromosome consists of RNA. Kaufman et al. (1948), Jacobson and Webb (1952), and LaCour (1963), Fan and Penman (1971) and Augenlicht (1977) demonstrated the existence of RNA in chromosomes cytochemically and autoradiographically. In the present work, the existence of RNA in chromatin threads was suggested from the results that only the peripheries remained after treatment with DNAase+pronase (Fig. 9), and that chromatin thread became tiny, about 90 Å ia diameter, after treatment of RNAase (Figs. 5 and 8).

Abstract

Electron microscopic studies on the substructure of chromatin were carried out using chromosomes in the first meiotic metaphase from pollen mother cells of Tradescantia reflexa. The chromosomes were composed of 300 Å chromatin
threads formed by coiling of 100 Å chromatin thread. The 100 Å chromatin threads contained globular structures of about 70 Å diameter. The location of DNA, protein and RNA were studied by treatment of the chromosomes with DNAase, 0.25 N HCl containing 2 M NaCl and RNAase.

Acknowledgements

This work was supported by a Research Grant from the Ministry of Education of Japan, No. 034077 (1975), No. 034077 (1976), No. 234044 (1977).

The author wishes to express her thanks to Emer. Prof. Dr. M. Shigenaga for valuable advice throughout these investigations.

References


Sakai, A. 1975. The fine structure of chromosomes in pollen mother cells of Tradescantia reflexa.
Cytologia 40: 743–749.


