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In situ observation of multiple levels of intermediate chromatin fibers on metaphase chromosomes of *Triticum aestivum* L.

Jianyue Wang and Feixiong Zhang*

College of Life Sciences, Capital Normal University, Beijing 100048, China

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Abstract

Metaphase chromosomes of root-tip meristematic cells of wheat (*Triticum aestivum* L.) were carefully examined under the transmission electron microscope. It was obvious that only in one section of a metaphase cell, besides 10 and 25-30 nm fibers, a serial of intermediate chromatin fibers between 30 nm and metaphase chromosome such as 50-60, 100-120 and 200-300 nm, could be identified on three metaphase chromosomes in the cell. The higher degrees of chromatin fibers were helically coiled from the lower levels of chromatin fibers and at last, 750~800 nm metaphase chromosomes were formed from 200-300 nm fibers. It was concluded that the 30 nm chromatin fibers exist truly in the metaphase chromosomes which are hierarchically organized from the 10 nm ones and there are at least 4 levels of helical coils from 30 nm to metaphase chromosomes.

Key words: Metaphase chromosome, intermediate chromatin fiber, transmission electron microscopy, *Triticum aestivum* L.

Introduction

Chromosomes is a complex cellbody composed of DNA, histone, non-histone proteins and RNA, etc. (Khorasanizadeh 2004). It has long been focused on how the four major components constitute the higher order structure of chromosomes. Since nucleosome was discovered in the early 1970s (Olins and Olins 1974), it is now well established that nucleosome is the basic repeating structural unit of eukaryotic chromatin comprising ~200 bp of DNA associated with a histone octamer that consists of two copies each of H2A, H2B, H3, and H4 (Luger et al. 2012). The arrays of nucleosomes containing linker DNA and histone H1 tend to form irregular fibers ~30 nm in diameter called solenoid or 30 nm chromatin fiber (Luger et al. 2012; Thoma et al. 1979, Finch and Klug 1976). This is the second structural level of DNA organization (Robinson et al. 2006). However, it is still controversial that if the 30-nm chromatin fibers do exist in chromosomes (Eltsov et al. 2008; Maeshima et al. 2010) then how the 30 nm chromatin fibers organize into metaphase chromosomes. There are several models, namely, loop-scaffold, radial-loop and helical coils, hierarchical folding-axial glue (condensin), chromatin network, polymer melt and thin-plate model have been proposed to explain the mechanisms involved in the formation of chromosomes from chromatin fibers, but each one has its limitation (Daban 2011). Therefore, the aim of the present study was to investigate the ultrastructure features such as chromatin fibres of chromosomes in wheat (*Triticum aestivum* L.).

Materials and methods

Seeds of wheat (*Triticum aestivum* L.) were pre-imbibed for 2 h and then placed in 10 cm Petri dish on moistened filter papers for germination at 25^{o}C in darkness. When the length of the roots was approximately 0.5-1.0 cm, the root-tips were carefully excised and fixed immediately in 3% glutaraldehyde (Sigma, Shanghai, China) in 0.1 M phosphate buffer (pH 7.2) for 4 h at room temperature. After rinsing with the phosphate buffer, the specimens were postfixed in 2% osmium tetroxide (Sigma, Shanghai, China) in the same buffer for 2 h. After a thorough wash with distilled water, the specimens were dehydrated in an ethanol-acetone series and embedded in Epon 812 (Zhongjingkeyi Technology Co., Ltd., Beijing, China). Sections were

*Corresponding author's e-mail: fxzhang@hotmail.com
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cut on a Leica UC6 ultratome (Leica Microsystems, Beijing, China) at a thickness of 60-70 nm. After staining with uranyl acetate and lead citrate the sections were observed under an H-7500 transmission electron microscope (Hitachi Instruments (Shanghai) Co., Ltd., Shanghai, China).

Results and discussion

While examining the ultrastructural features of chromosomes in root-tip meristematic cells of *Triticum aestivum*, we observed that different levels of chromatin fibers as 10, 25-30, 50-60, 100-120 and 200-300 nm existed in metaphase chromosomes. The present results also indicated that the higher degrees of chromatin fibers were helically coiled from the lower ones, and at last, 750~800 nm metaphase chromosomes were formed from 200-300 nm fibers. To the best of our knowledge, it is the first time to identify those chromatin fibers on metaphase chromosomes in only a cell. So, the fidelity of chromosome structures should be more reliable. Our results support the helical coiling model put forward in the late 1970s (Bak et al. 1977, 1979). Figure 1-A indicated a metaphase cell with 5 chromosomes, numbered 1, 2, 3, 4 and 5, respectively, in root-tip meristematic cells of *Triticum aestivum*. Arrows indicate the centromere regions. By checking the whole magnification of metaphase chromosome 1 shown in Fig. 1B, it was demonstrated that the diameter of the chromosome was around 750 nm (Fig. 1B, brace), and obviously, this chromosome was helically coiled from seven levels of chromatin fibers with thickness of 200-300 nm (Fig. 1B, brackets).

Almost the same results were depicted from the enlargement of the boxed area in metaphase chromosome 2 (Fig. 1C). Here, the diameter of the chromosome was around 800 nm (Fig. 1C, brace). Four levels of chromatin fibers in the helical state with thickness of 220-300 nm could be seen in this chromosome (Fig. 1C, brackets).

Further examining of Fig. 1C revealed that there were two parallel chromatin fibers with the diameter of 100-120 nm spread out from one of the 220-300 nm fibers (Fig. 1C, hollow curves). In the boxed area of Fig. 1C, the 100-120 nm fiber (indicated with a hollow curve) was consisted of two thinner fibers of 50-60 nm in diameter (Fig. 1C, hollow arrows). Fig. 1E is the magnification of the boxed area of Fig. 1C. There were three parallel chromatin fibers with the diameter of 25-30 nm (Fig. 1E, triangle arrows) which appeared in the 50-60 nm fiber (Fig. 1E, boxed area).

Fig. 1D is the whole magnification of metaphase chromosome 4 in Fig. 1A. Two parallel chromatin fibers with the diameter of 25-30 nm could be easily seen (Fig. 1D, boxed area, arrows). In the magnification of the boxed area of Fig. 1D, a 10 nm thin fiber helically cross the long axis of one of the 25-30 nm fibers (Fig. 1F, boxed area, and arrow). A lot of evidences have

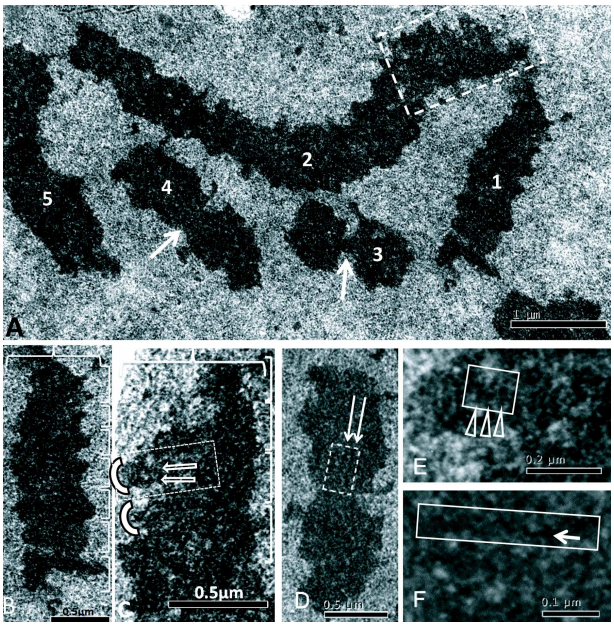

Fig. 1. Electron micrograph of a metaphase cell in root-tip meristematic cells of *Triticum aestivum* prepared by conventional procedures.
(A) A metaphase cell containing 5 chromosomes numbered 1-5 from right to left, respectively. Arrows indicate the centromere regions. (B) The whole magnification of chromosome 1 in Fig. 1A. The brace indicates the chromosome with the diameter of around 750 nm. The brackets indicate the chromatin fibers with thickness of 200-300 nm. (C) The enlargement of the boxed area in chromosome 2 in Fig. 1A. The brace indicates the chromosome with the diameter of around 800 nm. The brackets, hollow curves and hollow arrows indicate the chromatin fibers with thickness of 220-300, 100~120 and 50~60 nm, respectively. (D) The whole magnification of chromosome 4 in Fig. 1A. The arrows indicate the fibers with thickness of 25-30 nm. (E) The enlargement of the boxed area in Fig. 2C. The triangle arrows indicate three parallel chromatin fibers with the diameter of 25-30 nm in the boxed area. (F) The enlargement of the boxed area in Fig. 2D. The arrow indicates a 10 nm thin fiber helically across the long axis in the boxed area. Bar in A: 1 μm. Bars in B to D: 0.5 μm. Bar in E: 0.2 μm; Bar in F: 0.1 μm

been accumulated in support of the opinion that the nucleosome chain forms the 30 nm chromatin fibers (Finch and Klug 1976; Woodcock et al. 1984; Luger et al. 2012). But Eltsov et al. (2008, 2010) disagree with the hypothesis when using cryo-electron microscopy images to investigate the mitotic chromosomes of HeLa S3 cells. Therefore, further investigations are needed to elucidate evidence that how the 30 nm chromatin fibers organize into metaphase chromosomes.

Multiple levels of intermediate chromatin fibers in metaphase chromosomes

Since the early of 1970s, a number of new techniques and methods have been employed to study the detailed structural features of chromosomes from different levels and angles. Nevertheless, the conclusions are still controversial. Thus, nearly 10 models such as folded-fiber, multiple coiling, loop-scaffold, radial-loop and helical coils, hierarchical folding-axial glue (condensin), chromatin network, polymer melt and thin-plate model, have been proposed to explain the mechanisms involved in the formation of chromosomes from chromatin fibers, but each one has its limitation. However, there is more useful information aggregated to the helical coiling model. At first, Bak et al. (1977, 1979) reported that there existed 400 nm unit fibers between 30 nm chromatin fibers and metaphase chromosomes in humans. This conclusion was confirmed by Taniguchi and Takayama (1986) as well as Rattner and Lin (1985), although the diameters of the fibers illustrated by them were 200-300 nm. Later on, several evidences were put forward to demonstrate that, no matter in animal or plant chromosomes, there were not only one, but more degrees of intermediate chromatin fibers that existed between 30 nm and metaphase chromosomes. Belmont et al. (1987) observed chromatin fibers of 12, 24, 40-50, 80-100 and 130-300 nm in less perturbed metaphase chromosomes of *Drosophila melanogaster*. But the results were not obtained in one cell and the relationship among these fibers was also not fully illustrated. In CHO cells, they also indicated 60-80, 100-130 and 200-250 nm fibers in G1 (Belmont and Bruce 1994) and prophase chromatin (Kireeva et al. 2004). Hao et al. (1990) made a detailed examination on the decondensation of telophase chromosomes in root-tip meristematic cells of *Allium* and identified 50-70, 170-200 and 400-550 nm chromatin fibers. Both Kireeva et al. (2004) and Hao et al. (1990) presented evidences that the higher levels of chromatin fibers were formed by the coiling of the lower level ones, and at last the metaphase chromosomes were formed by the coiling of the highest levels of chromatin fibers (Ris 1981).

As stated above, there have been only a few reports so far indicating that there are multiple levels of intermediate chromatin fibers between 30 nm chromatin fibers and metaphase chromosomes and the relationship among them. Since some of the results were obtained from different phases and different cell types, it is hard to be convinced that they can reflect the natural statue of metaphase chromosomes; some others were from the treatment by physical or chemical methods to the cells or chromosomes, hence the reality of chromosomal structures revealed was broadly questioned.

Careful examination of chromosomal structures in root-tip meristematic cells of *Triticum aestivum* under a TEM, revealed that different levels of chromatin fibers of 10, 25-30, 50-60, 100-120 and 200-300 nm existed in metaphase chromosomes. Evidently, the higher levels of chromatin fibers were formed by the coiling of the lower levels ones, and at last, the 750~800 nm metaphase chromosomes were formed by the coiling of 200-300 nm chromatin fibers. Though our results are similar with Belmont et al. (1987) and Balmont and Bruce (1994) who indicated a similar observation in CHO cells and Hao et al. (1990) described in plant cells, it was obtained from three metaphase chromosomes (in only one cell) following all the experimental procedures under normal conditions. Thus the fidelity of chromosomal structures should be more reliable.

The reality of 30 nm chromatin fibers in metaphase chromosomes

The 30 nm chromatin fibers were first identified by Finch and Klug in 1976 on isolated chromatin from rat liver nuclei under TEM. Since then, this kind of structure has been verified in different laboratories with different techniques such as scan electron microscopy (SEM) (Adolph and Kreisman 1985), scanning transmission electron microscopy (STEM) (Woodcock et al. 1984; Gerchman and Ramakrishnan 1987), cryo-EM (Horowitz et al. 1994), electron spectroscopic imaging (ESI) (Bazett-Jones 1992), X-ray diffraction (Widom and Klug 1985), X-ray or neutron scattering (Williams and Longmore 1991), electric and photochemical dichroism (Mitra et al. 1984), nuclease digestion (Staynova 2000), X-ray crystallography (Richmond et al. 1984) and *in vitro* reconstitution system (Huyanh et al. 2005; Song et al. 2014), etc. Thus, two main structural models, the one-start helix (solenoid) models

and the two-start helix (zigzag) models, have been proposed during the past three decades (Maeshima et al. 2010). However, the reality of 30 nm chromatin fibers in interphase nuclei and chromosomes has long been controversial. The major concerns are as follows: a) the isolated chromatin fibers were buffer-extracted and/or nuclease-digested, the identified 30 nm fibers can not represent the native state, b) many *in situ* analysis did not support the existence of 30-nm chromatin fibers, c) if the *in vitro* reconstitution system can reflect the *in vivo* condition and d) can 30 nm fibers be detected in native chromosomes? Therefore, the best way to verify the reality of the fibers in native chromosomes is to find them in untreated chromosomes.

In present study, the 30 nm chromatin fibers were clearly shown in the native etaphase chromosomes. Thus, the fibers do exist in the metaphase chromosomes. From their relationship with the 10 nm and 50-60 nm chromatin fibers, it can't imaged that how the chromosomes to be formed normally if there are no 30nm chromatin fibers existed.

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