The effect of magnesium ions on chromosome structure as observed by scanning electron microscopy (SEM) and scanning transmission electron microscope (STEM) tomography

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Abstract

The structural details of chromosomes have been of interest for many years; however, the enigma of how the metaphase chromosome is constructed has remained unsolved. Divalent cations, especially Mg$^{2+}$, are known to be required for chromatin condensation. However, details about the effect of Mg$^{2+}$ at the nanoscale are still limited. In this study, the effect of Mg$^{2+}$ on chromosome structure was investigated by means of scanning electron microscopy (SEM), because of its high magnification and resolution, as well as by scanning transmission electron microscope (STEM) tomography, because of its advantages in three-dimensionally imaging chromosomes without sectioning. We herewith report the reversibility between 11 and 30 nm chromosome structures according to the concentration of Mg$^{2+}$, as observed by SEM, and how three-dimensional chromosome structure is affected by Mg$^{2+}$ concentration by STEM tomography. Treatment with a buffer lacking Mg$^{2+}$ yields a less compact chromosome structure, with smaller fiber diameters, than for chromosomes treated with a buffer containing 5 mM Mg$^{2+}$. The changes of chromatin diameter are reversible after re-addition of Mg$^{2+}$. These findings signify the importance of an adequate concentration of Mg$^{2+}$ to chromosome structure. The advantages of SEM and STEM for chromosomal research were highlighted in the current study.

Keywords: Chromosome structure, Magnesium ion (Mg$^{2+}$), Scanning electron microscopy (SEM), Scanning transmission electron microscope (STEM) tomography

Introduction

Various models of chromosome higher-order structure have been proposed. Beyond the existence of 11 nm fibers, attempts to elucidate chromosome higher-order structure have remained elusive (Fukui and Uchiyama, 2007; Fukui, 2009; Bin & Belmont, 2012). The integrity of condensed chromatin in the nucleus has been reported to depend on the binding of cations (Engelhardt, 2004). At high ionic concentrations, chromosomes become granulated with the most frequently observed granules having a diameter of about 35 nm (Caravaca et al, 2005); the existence of such ca. 30 nm chromatin structure in native chromosomes is still controversial, however (Eltsov et al, 2008; Grigoryev and Woodcock, 2012; Nishino et al, 2012).

Magnesium is one of the most abundant cations in eukaryotic cells (Strick et al, 2001). However, the detailed role of Mg$^{2+}$ in chromosome organization has not yet been elucidated. In the current experiments, the effects of Mg$^{2+}$ concentration on chromosome structure were investigated by using optical microscopy, scanning electron microscopy (SEM), and scanning transmission electron microscope (STEM). The structural changes in chromatin after re-addition of Mg$^{2+}$ could be used to confirm the reversibility of chromatin dynamics depending on Mg$^{2+}$ concentration by using SEM. SEM was used to investigate the structure of chromatin under different Mg$^{2+}$ concentration because of its ability to image such nanoscale ultrastructure. The advantages of STEM tomography in obtaining three-dimensional (3D) information from chromosome images by tilting the sample stage were also assessed to gain more detailed information about chromosome structure at different concentrations of Mg$^{2+}$.

Materials and methods

Sample preparation

In this study, chromosomes from human cervical adenocarcinoma HeLa S3 cells were used. Chromosomes were isolated and suspended by the polyamine method (PA isolated chromosomes, Uchiyama et al, 2005, 2008; Hayashihara et al, 2008). The PA chromosomes were placed on a cover glass for optical microscopic observation, silicon substrate for SEM observation, or on a 75 mesh TEM grid for STEM tomography observation. Samples were incubated on ice for 10 min, and then subjected to two different buffers for 30 min: XBE5 (10 mM HEPES, pH 7.7, 5 mM MgCl$_2$, 100 mM KCl, 5 mM EGTA), and
XBE (10 mM HEPES, pH 7.7, 100 mM KCl, 5 mM EGTA). After XBE5/XBE treatment, the chromosomes were either stained with 4,6-diamidino-2-phenylindole (DAPI) for optical microscopy (Axioplan 2, Zeiss) observation or subjected to preparation for SEM/STEM.

**Sample preparation for SEM/STEM**

Chromosomes on grids were fixed with 2.5% glutaraldehyde diluted in XBE5 or XBE buffer and 0.2% tannic acid/XBE5 or XBE; post-fixed with 2% OsO4/Milli-Q; and then washed by using Milli-Q water three times, 5 min each. They were subsequently dehydrated with 70, 100, and 100% ethanol and dried by a critical point dryer (JCPD-5, JEOL, Tokyo, Japan). Samples for SEM were coated by an OsO4 coater (HPC-1S; Vacuum Device Inc., Japan), while samples for STEM were coated with carbon, and then observed by SEM/STEM.

**SEM/STEM observation**

Chromosomes were observed by SEM (S-5200, Hitachi, Tokyo, Japan) with an accelerating voltage of 10 kV in secondary electron (SE) mode. SEM data were analyzed by using ImageJ software (Abramoff et al., 2004). The diameters of chromatin structure (n = 50 positions on each chromosome) were measured at random positions. To obtain tomography data, chromosomes were observed by STEM (Tecnai G2, FEI). The sample stage was tilted at 2° intervals from -70° to +70° and image series were taken at each position. The data were then aligned and analyzed by using IMOD software (Kremer et al., 1996).

**Results and Discussion**

The effect of Mg2+ on chromosome structure

In this study, the role of Mg2+ in chromosome higher-order structure was evaluated, particularly the reversibility between dispersed 11 nm- and compact 30 nm-fiber containing structures. Chromosome structure and the diameter of chromatin varied with the different treatments (Fig. 1). Chromosomes treated with buffer lacking Mg2+ showed a more dispersed structure as observed by optical microscopy (Fig. 1C) as well as by SEM (Fig. 1D) compared to those treated with 5 mM Mg2+ buffers (Figs. 1A, B), indicating that Mg2+ is essential for maintenance of chromosome structure. These differences are consistent with previous reports (Adolph et al., 1986; Dwiranti et al., 2014). Mg2+ is one of the most abundant cations in the eukaryotic cells and has important functional roles in multiple cellular processes. Strick et al. (2001) showed that Mg2+ is effective at the integral part of mitotic chromatid and it was equally distributed over the entire chromatids. The Mg2+ concentration of an interphase

![Figure 1](Image)
Indian Muntjac nuclei/chromatin was revealed as 2-4 mM, and the concentration of Mg$^{2+}$ in a mitotic cell was 5-17 mM measured by using SIMS (Secondary ion mass spectrometry) imaging. The concentration of the entire cell showed the range of 1-3 mM both in interphase and mitotic cells. It indicated the cation redistribution between cytosol and chromosomes during the cell cycle. In interphase cells, Mg$^{2+}$ distributed throughout the cytosol with specific high accumulations probably at the endoplasmic reticulum and Golgi complex, whereas the nucleus was reduced in the concentration of Mg$^{2+}$. In contrast to interphase, mitotic cells showed high concentrations of Mg$^{2+}$ on chromatin. SIMS analysis demonstrated Mg$^{2+}$ enrichment on mitotic chromatin. These results indicate the importance of Mg$^{2+}$ for chromatin condensation.

Chromosomes maintained their compact structure in the presence of 5 mM Mg$^{2+}$ and transformed into a more dispersed structure when treated with a buffer without Mg$^{2+}$. The change in chromosome structure affected by Mg$^{2+}$ concentration was reversible as shown in Figure 2. Fiber diameters were smaller in chromosomes treated with a buffer without Mg$^{2+}$ (Figs. 2B, B') compared to those treated with a buffer containing 5 mM Mg$^{2+}$ (Figs. 2A, A'). Re-addition of 5 mM Mg$^{2+}$ resulted in recovery of mean chromatin fiber diameter observed by SEM (Figs. 2C, C'). Mean chromatin diameters are depicted in Figure 2D. Figure 2 clearly shows the reversibility of chromatin structural changes upon restoration of 5 mM Mg$^{2+}$. The phenomenon of conformational freedom of chromatin allowing dynamic unfolding and refolding has been described by Poirier et al. in 2002 by using a combination of chemical-micromechanical techniques and adjustment of cation concentration. The results obtained in this study give an important insight into nanoscale ultrastructural changes in high magnification and resolution by using SEM. These phenomena strongly suggest that Mg$^{2+}$ is responsible in the compaction of 11 nm chromatin fibers into a 30 nm structure and that the chromosome structure might be highly elastic depending on the ionic strength.

**The effect of Mg$^{2+}$ on chromosome structure observed by STEM tomography**

To gain deeper understanding regarding chromosome structure under different Mg$^{2+}$ concentrations, STEM tomography was employed. The dispersed and fibrous nature of chromatin when it was treated with buffer lacking Mg$^{2+}$ was also demonstrated by STEM tomography. This system enabled us to visualize the entire chromosomal structure in three dimensions without sectioning, which is difficult to do by conventional transmission electron microscopy. The advantages of the STEM tomography have been described by Aoyama et al., (2008).

By employing different tilting angles, with increments of 2° from -70° to +70°, the varying structure of chromosomes with different concentrations of Mg$^{2+}$ was clearly demonstrated. Not only could the dispersion of chromatin clearly be visualized by STEM tomography, the expanded chromosome structure could also be seen when the chromosome was treated with the buffer lacking Mg$^{2+}$. Chromosome images taken at different angles are shown in Figure 3. The results show that the chromosomes were compact when treated with 5 mM Mg$^{2+}$ (Figs. 3A-E) but became fibrous when treated with buffer lacking Mg$^{2+}$ (Figs. 3F-J). These data are in good accordance with the SEM results and highlight the importance of Mg$^{2+}$ for chromosome maintenance. By using STEM tomography, the structure of compact chromosome treated with 5 mM Mg$^{2+}$ and the dispersed, thin, and long structure of chromosome treated with 0 mM Mg$^{2+}$ signified the effect of Mg$^{2+}$ on chromosomes three dimensionally, they were

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**Figure 2.** The reversibility of chromatin diameter change with Mg$^{2+}$. Chromosome was more compact (A, A') when treated with buffer containing 5 mM Mg$^{2+}$, with mean chromatin diameter of ca. 30 nm (D), and became more fibrous (B, B') when treated with buffer without Mg$^{2+}$, with mean chromatin diameter of ca. 11 nm (D). Chromosome structure with similar mean chromatin diameter to the control (ca. 30 nm) was achieved after restoring the Mg$^{2+}$ concentration to 5 mM (C, C') and Mg$^{2+}$ re-addition. Bars: 500 nm (A, B, C), 100 nm (A', B', C'). n = 150 positions on chromosome, p < 0.005 (D).
Effects of Mg$^{2+}$ on chromosomes as observed by SEM and STEM.

observed not only on the surface of the chromosomes, but also in the inside of them from the different tilting angles. It is also the advantage of STEM tomography to analyze the entire chromosome like as Focused Ion Beam (FIB)/SEM (Hamano et al., 2014; Kaneyoshi et al., 2014) and could avoid the possibility of sample disruption during sectioning.

The effect of Mg$^{2+}$ on the structural maintenance of the chromosome, especially the transformation between 11 and 30 nm fiber structures, was visualized by SEM. In addition, the striking changes to chromosome structure due to variable concentrations of Mg$^{2+}$ were shown to happen in a reversible manner. Furthermore, for the first time, chromosome structure changes were revealed in 3D by STEM tomography. Chromosomes were observed with a compact granular structure when treated with buffer containing 5 mM Mg$^{2+}$. However, when a buffer lacking Mg$^{2+}$ was used, the chromosomes showed a less condensed structure, with a smaller chromatin fiber diameter.

While the role of cations in chromosome structure might be explained by the modulation of the electrostatic portion of nucleosome-nucleosome interactions (Poirier et al., 2002), cations may also stabilize histone-DNA interactions within the nucleosome core (Yang and Hayes, 2011). The crystallization studies of B-DNA decamers or dodecamers in the presence of Mg$^{2+}$ confirmed a direct cation interaction with the major and minor grooves of DNA and leading its stabilization and conformation (Minasov et al., 1999; Chiu and Dickerson, 2000). Previous report (Gueroult et al., 2012) also showed that Mg$^{2+}$ induces strong changes on the electrostatic potential of the electronegative atoms. DNA structure and dynamics are sensitive to divalent cations in the major groove. Cation binding is sequence-dependent and modulates the intrinsic sequence-dependent properties of DNA. Further, in the presence of 5 mM Mg$^{2+}$, scaffold proteins undergo a lateral aggregation (Earnshaw and Laemmli, 1983), emphasizing the importance of Mg$^{2+}$ concentrations to scaffold proteins that are essential for chromosome organization.

Some conclusions can be drawn from the SEM and STEM visualization employed in this study. These results provide convincing evidence that Mg$^{2+}$ ions play an important role in the maintenance of chromosome structure. Therefore the conditions of Mg$^{2+}$ concentration is essential in the course of sample preparation for the study on chromosome higher order structure, especially by the high resolution techniques such as, SEM, TEM, STEM, Focused ion beam (FIB)/SEM, Serial block face (SBF) imaging, and so on. Because, the chromatin structure seems not to be rigid, but rather elastic, allowing for different dynamics with different cation concentrations. This study also highlights the benefits of SEM and STEM tomography for chromosome research. The revelation of the detailed factors affecting chromosome structure will further open the new insights into chromosome higher-order structure.

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**Figure 3.** STEM images of chromosomes at different Mg$^{2+}$ concentrations. Chromosomes were compact when they were treated with buffer containing 5 mM Mg$^{2+}$ (A–E, K) and were dispersed when in buffer without Mg$^{2+}$ (F–J, L). Images were taken at -62.6° (A), -37.3° (B), 0° (C), 35.7° (D), 61.6° (E), -66° (F), -42° (G), -2° (H), 36° (I), and 68° (J) tilting angles. Arrows indicated chromosomes. Bars: 1 µm (J), 500 nm (K, L).
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