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Title	Visualization of Chromosome Higher-Order Structure by New Methods
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Citation	大阪大学, 2014, 博士論文
Version Type	VoR
URL	https://doi.org/10.18910/50539
rights	
Note	

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Form 3

Abstract of Thesis

Name (Astari Dwiranti)	
Title	Visualization of Chromosome Higher-Order Structure by New Methods (新手法による染色体高次構造の可視化)

Abstract of Thesis

Chapter 1: General introduction

Chromosome higher-order structure has long been studied. One of the information known about chromosome structure has been that divalent cations especially Ca^{2+} and Mg^{2+} are required for the organization of chromosomes. However the detailed information about these cations on chromosome organization is still limited. The comprehensive study of chromosome higher-order structure and at the same time the development and introduction of new technologies are necessary to answer this mystery.

For chromosome study, scanning electron microscopy (SEM) has been useful because of its high magnification and resolution. However, the utilization of SEM is limited due to the time-consuming and multiple preparation steps. In conventional sample preparation, dehydration and drying could distort the biological structure. In addition, the necessity of metal coating could also conceal the real surface structure of the samples. The development of a new method enabling observation of chromosomes without dehydration, drying, and metal coating would be very effective for chromosome research. Thus, the objective of this study is to develop the new preparation method for chromosome visualization.

In this study, the ionic liquid (IL) method was developed which enabling sample preparation for chromosome observation by SEM without dehydration, drying, and metal coating. In addition, this new method was also combined with helium ion microscopy (HIM), focused ion beam/SEM (FIB/SEM), and transmission electron microscopy (STEM) tomography, to gain the information of chromosome surface, interior, and 3D structure in rapid and closer to the native condition.

Chapter 2: The effect of divalent cations on chromosome higher-order structure

Chapter 2 focuses on the effect of Ca^{2+} and Mg^{2+} on chromosome structure observed by optical microscopy and SEM, particularly the reversibility of 11-30 nm chromatin particles. Furthermore, the advantages of STEM tomography in providing 3D chromosome images by tilting the sample stage were also assessed to gain the more detailed results of chromosome structure in different concentration of Mg^{2+}.

Chapter 3: Uncoated chromosome visualization by helium ion microscopy (HIM)

Chapter 3 presents the advantages of HIM in enabling chromosome without metal coating in high resolution. The results showed that chromosome could clearly be observed by HIM for the first time even without metal coating. Different structure of chromosome in different Mg^{2+} concentration was also examined.

Chapter 4: Chromosome observation by scanning electron microscopy using ionic liquid

In this chapter, the advantages of IL method for chromosome observation by SEM are evaluated. The first chromosome images prepared by IL method without dehydration, drying, and metal coating are presented. Optimal conditions for chromosome observation by SEM using IL method including the kind, concentration, and the combination with Pt-blue staining have also been determined.

Chapter 5: Chromosome interior investigation by FIB/SEM with ionic liquid

In Chapter 5, the IL method was employed to the FIB/SEM which enables us to do slice-and-view the sample at the same time. This combination yields an insight with regard to different types of chromosome interior: with and without cavities, suggesting that chromosome interior may be flexible. In addition, it was found that the existence and sizes of cavities depend on the preparation procedures. The combination of IL method with FIB/SEM has been demonstrated to be beneficial for chromosome interior imaging in rapid and less laborious manner.

Chapter 6: General conclusions

In the present work, the effects of divalent cations on chromosome structure have been investigated. Furthermore, the limitations of sample preparation for chromosome observation by SEM have been overcome by developing the IL method. This new method has successfully been demonstrated to be applicable for other systems including HIM in providing high-resolution chromosome images, FIB/SEM in presenting chromosome interior information, and STEM tomography in imaging 3D chromosome. These methods enable us to observe chromosome surface and interior in high resolution, rapid, and closer to their native condition by avoiding dehydration, drying, and metal coating. The results achieved in this study not only would contribute to further chromosome research but also to the other biological samples visualization.

List of publications

1. Dwiranti A, lin L, Mochisuki E, Kuwabata S, Takaoka A, Uchiyama S, Fukui, K. 2012. Chromosome Observation by Scanning Electron Microscopy using Ionic Liquid. *Microscopy Research and Technique* 75: 1113-1118.
2. Dwiranti A, Hamano T, Takata H, Nagano S, Guo H, Ohnishi K, Wako T, Uchiyama S, Fukui, K. 2014. The Effect of Magnesium Ions on Chromosome Structure as Observed by Helium Ion Microscopy. *Microscopy and Microanalysis* 20: 184-188.
3. Hamano T, Dwiranti A, Kaneyoshi K, Fukuda S, Kometani R, Nakao M, Takata H, Uchiyama S, Ohmido N, Fukui K. Chromosome Interior Observation by Focused Ion Beam/Scanning Electron Microscopy (FIB/SEM) using Ionic Liquid Method. *Microscopy and Microanalysis* (in press).

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

中期染色体の高次構造は１９世紀に染色体が発見されて以降、２１世紀の今日に至るまで謎であり、その高次構造が不明である最後の細胞内の構造体である。しかしながら、染色体構造異常が発癌と深く係わっていることなどを考えると、染色体高次構造の解明は基礎生物学的に重要であるのみならず、応用面においても極めて重要な課題であることがわかる。このような位置づけにより、学位申請者は、多岐にわたる最新の可視化技術を用いて染色体の構造解析に取り組み、多くの新しい知見を得るのである。

まず、染色体の構造解析のために２価陽イオンである、マグネシウムイオンおよびカルシウムイオンが構造変化に及ぼす効果を見るのである。生じた構造変化はイオン濃度の調整により復元可能であることが分かり、かつその構造変化は進展著しいスキャニング・トランスミッション・エレクトロン顕微鏡（STEM）で観察される。

次に、従来に無い新しい顕微鏡であるヘリウム・イオン顕微鏡（HIM）による染色体高次構造の解明が、やはり２価陽イオン濃度を変化させる条件下で検討される。ヘリウム・イオン顕微鏡は従来の走査型電子顕微鏡（SEM）での結果とも比較され、導電性のコーティングを必要としない点、高い解像度などその優位性が明らかにされる。さらに、イオン液体を用いた新しい試料調整法が検討され、最適化が図られる。イオン液体は常温で液体の塩であり、その導電性、不揮発性、安定性から SEM や HIM 用の試料を調整する上で、従来にない新しい局面を開くのではないかと期待されたのである。実際にイオン液体を試料作製に用いた場合には試料調整時間が大幅に削減され、かつ良好な画像が得られるのである。

最後に、中期染色体の内部構造を直接可視化する方法として収束イオンビーム・走査型電子顕微鏡法（FIB/SEM）が試みられる。ここではイオン液体処理をした試料も含めてイオンビームを用いて染色体を直接削りこんで内部を可視化する方法を取る。これにより、従来は長時間を要した染色体の内部構造を容易に観察することができる。

以上のように、本論文は、新しい可視化技術や試料作成法を用いて２価陽イオンが染色体の及ぼす効果を種々の異なる条件下で解明を試みたものであり、その成果は染色体に限らず種々の生物試料の構造の解析に広く利用できるものである。よって本論文は博士論文として価値あるものと認める。