ELECTRON MICROSCOPIC STUDY ON CONSTITUENT CHROMOFILAMENTS OF METABOLIC CHROMOSOMES

by

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INTRODUCTION

Improved techniques for cutting sections thin enough for electron microscopy have enabled us to make a full study of the microstructure of chromosomes. The chromosomes in the metabolic stage (usually called resting stage) are made of coiled chromofilaments closely appressed, so that the detailed microstructure of constituent chromofilaments of metabolic chromosomes is hopelessly concealed even in ultrathin sections[1]. The smear preparation method has successfully revealed the longitudinally running filaments of salivary gland chromosomes[2,3,4,5], which filaments have not been discussed in any of the previous literature on ultrathin sections[6,7]. Recently, YASUZUMI and co-workers[8,9,10,11] have demonstrated that the metabolic chromosomes in the blood cell nuclei of various animals are composed of at least eight chromofilaments, and that each chromofilament consists of nodules of various sizes and fine connecting filaments. RIS[12] has also shown that the lampbrush-chromosomes of amphibia are made of submicroscopic fibrils, each forming a small-gyred helix. In the present study, with the help of ultrasonic vibration the author has succeeded in making much clearer the microstructure of constituent chromofilaments of metabolic chromosomes than in previous reports[8,9,10].

A part of this work was presented at the 11th Electron Microscope Congress of America held in Pocono Manor, Pennsylvania, in November 1953[13].

METHODS AND RESULTS

In the present experiment the red blood cells of triton *Triturus pyrrhogaster* were used. Much care was taken to carry out the procedure aseptically. The blood was collected immediately from the heart of the triton in a 2% sodium oxalate solution, and centrifuged for 10 minutes at 5,000 r.p.m., and then the leucocytes, thrombocytes and plasma were removed. When hypotonic solution was added to the sediment thus obtained, the red cells were completely destroyed, and their nuclei were precipitated on the bottom through centrifugation. The nuclei were well washed with the physiological saline. In the first series of experiments, the nuclear solution was placed in the apparatus of ultrasonic vibration with a frequency of 450,000, 2,000 V, and 210 mA for 30 seconds.

Many nuclear components have appeared in various distintegrated conditions By means of shadow casting we can easily demonstrate the helical structure of metabolic

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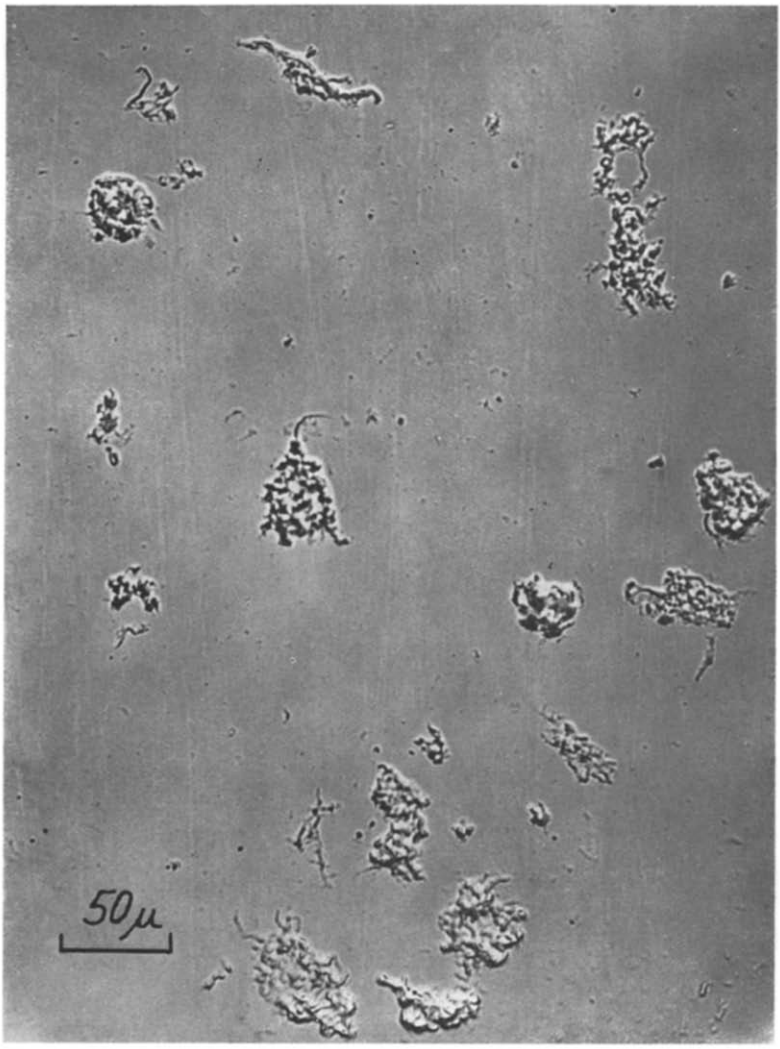

Fig. 1. Light micrograph of metabolic chromosomes isolated from erythrocyte nuclei of *Triturus pyrrhogaster* with ultrasonic vibration. Chrom shadow casting.

chromosomes without staining with the ordinary light microscope, as seen in Fig. 1. However, it is difficult to differentiate the gyres of helical threads and the chromatin granules, and also to calculate the number of metabolic chromosomes in the figure.

The Shimazu electron microscope of the magnetic type SM-1A was used in this experiment. In the electron microscopic figures the difference between the helical threads and the chromatin granules is easily understood, as seen in Fig. 2 and 3. The helical structure of the metabolic chromosome is destroyed by vibration and untangled, so that it is difficult to obtain a complete metabolic chromosome. In Fig. 2, the totally opaque bodies are easily understood to be chromatin granules, and the helical threads (marked by *M*), being attached to the chromatin granules, are nothing but the metabolic chromo-

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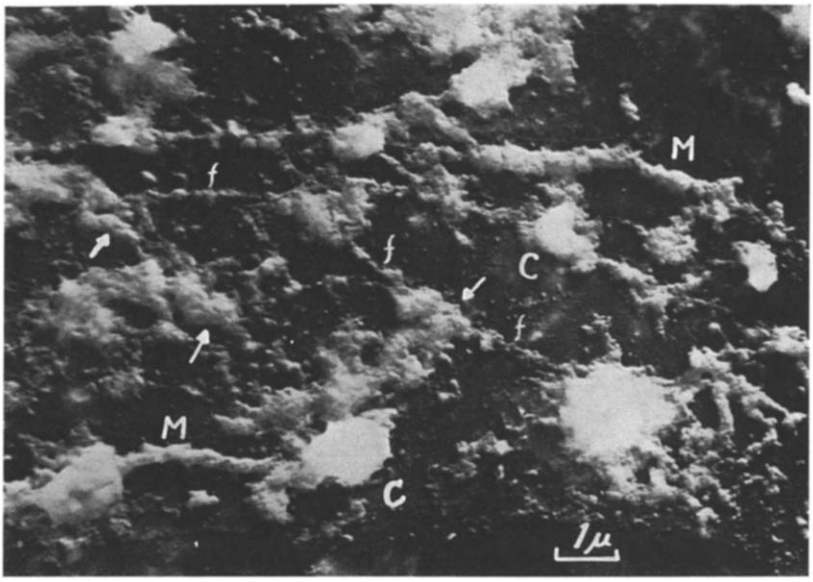

Fig. 2. Electron micrograph of metabolic chromosomes in various conditions. The dense bodies marked by *C* are chromatin granules. The helical threads are visible at the points marked by *M*. The metabolic chromosomes are destroyed at the points marked by the arrows. The constituent chromofilaments are visible at the points marked by *f*.

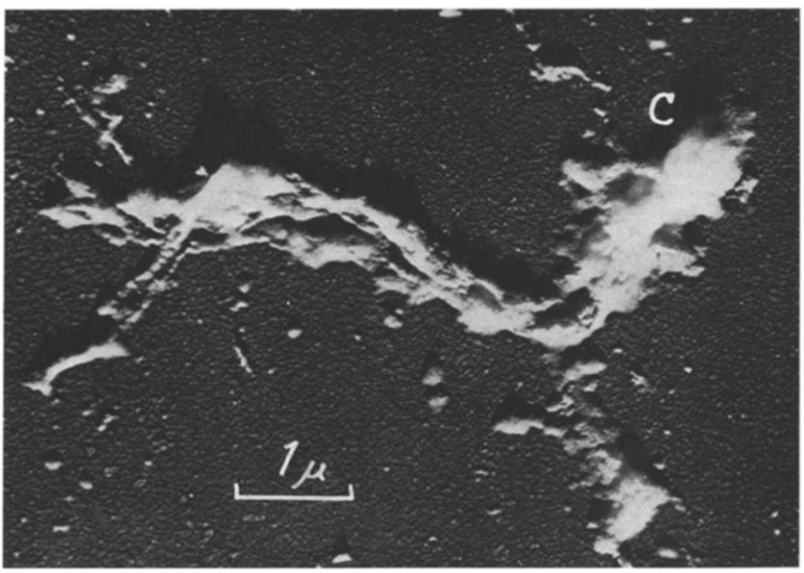

Fig. 3. Electron micrograph of a isolated metabolic chromosome, which is composed of a chromatin granule (heterochromatin) marked by *C*, and helical threads. The helical threads are partially decomposed.

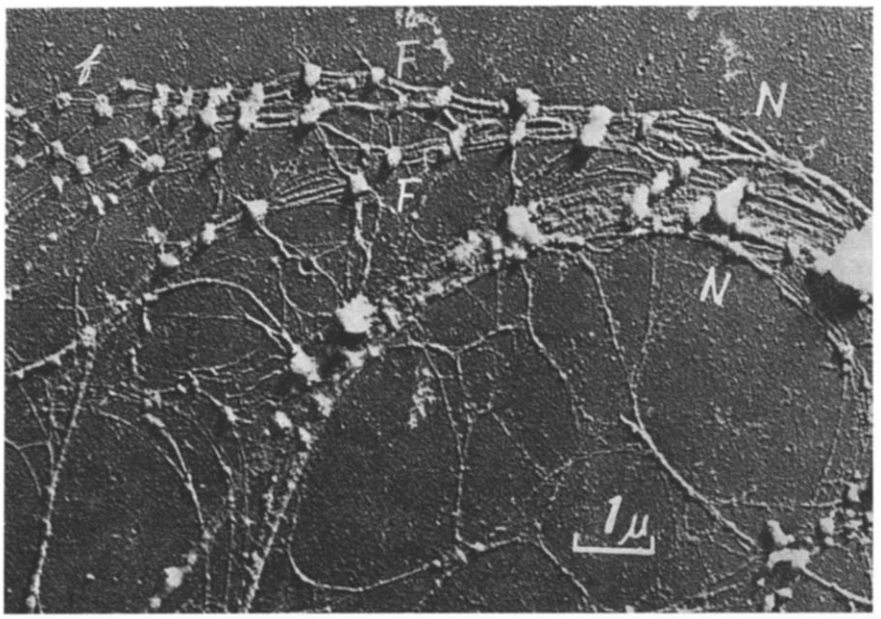

Fig. 4. Electron micrograph of chromofilaments, which consist of opaque particles and intercalary filaments. *N*: Chromonema; *F*: Chromofibril; *f*: Chromofilament.

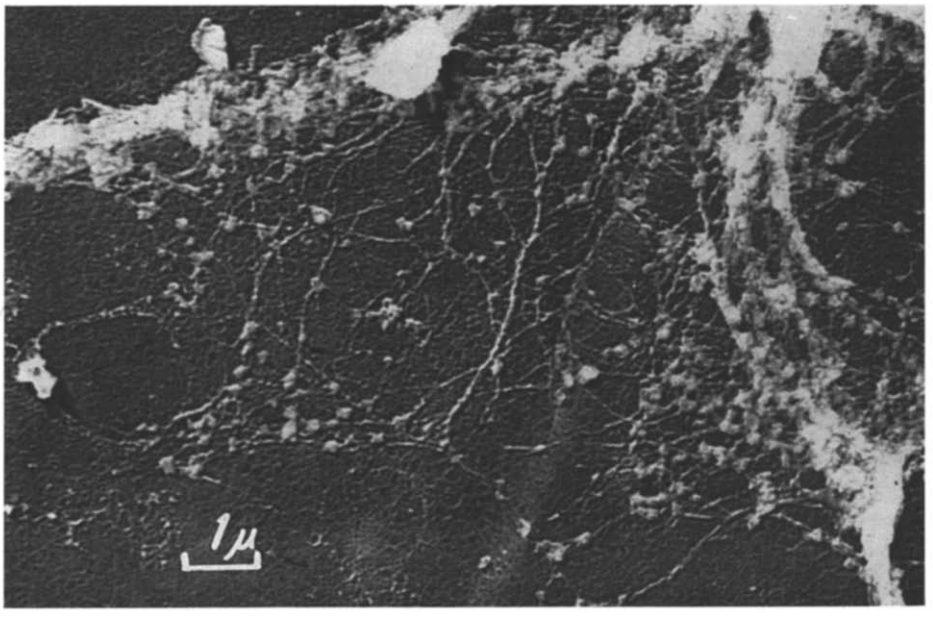

Fig. 5. Electron micrograph of chromofilaments which appeared in a reticular condition. The opaque particles and fibrous elements are clearly visible.

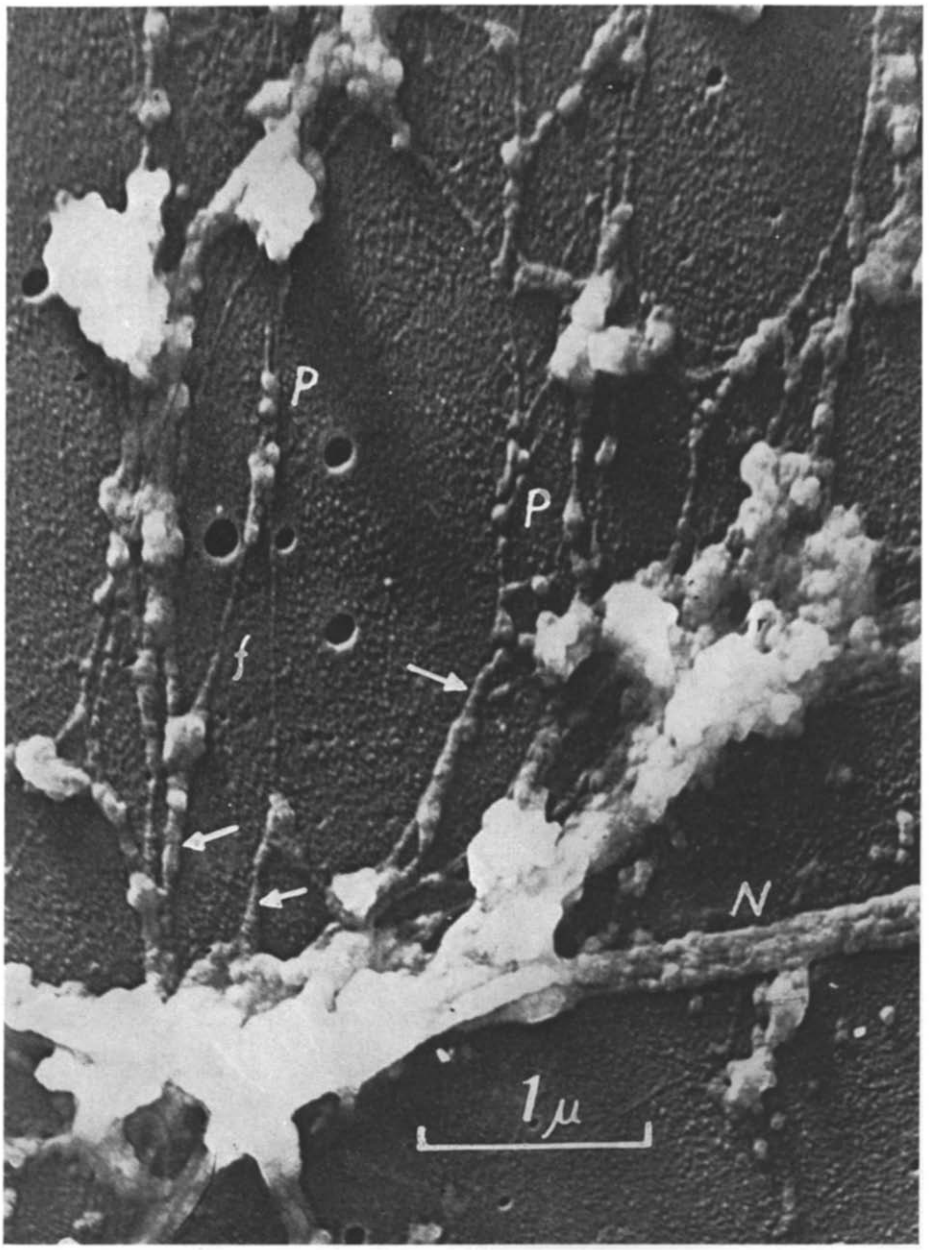

Fig. 6. Electron micrograph of chromofilaments isolated with the help of a blendor. The coiled structure of the chromofilaments are clearly visible at the points marked by the arrows. *P*: Opaque particle; *f*: Intercalary filament; *N*: Uncoiled chromonemata.

somes. The metabolic chromosomes are destroyed by vibration, as seen at the points marked by the arrows. The constituent chromofilaments of chromsomes are visible at the points marked by *f*, due to the destruction by vibration.

Fig. 3 shows a isolated metabolic chromosome, which is composed of a Feulgen-positive chromatin granule (heterochromatin) and a Feulgen-negative helical thread, which is separated into a pair of chromonemata. Each chromonema is disintegrated into the constituent filaments at the distal end.

After a minute's treatment, the chromosomes are totally disintegrated into their constituent elements which are composed of filamentous and globular elements. Fig. 4 shows that the metabolic chromosome is composed of chromofilaments which consist of opaque particles *ca.* 60–120 mμ in diameter and intercalary filaments *ca.* 10 mμ in width. This image reminds us of the polythene structure of the salivary gland chromosome. The part marked by *N* in Fig. 4 represents clearly a pair of chromonemata, which are divided into a pair of chromofibrils(*F*), each chromofibril being further bifurcated till at least 32 chromofilaments(*f*) are formed.

After treatment for 5 minutes, the bundle of chromofilaments is often destroyed, so that the chromofilaments appear in a reticular condition. The opaque particles *ca.* 80 mμ in diameter and fibrous elements *ca.* 10 mμ in width can be seen in Fig. 5.

In the second series of experiments the nuclear solution was placed in a blendor for 15 minutes at 3° C, and was centrifuged. The precipitation consisted of metabolic chromosomes and their derivatives from the erythrocyte nuclei, as was already reported[8,9,10]. This treatment was repeated until the chromofilaments were obtained. Micro-drops of the specimen were placed on the collodion film of a sample holder. The specimen was fixed with osmic vapour and was allowed to dry in the air. The specimen was then shadowed with chromium at a shadowing angle of 25°.

It has been show here that when the blood cell nuclei are placed in a blendor for 90 minutes at 3° C and pH 5.4, the resulting chromofilament gives evidence of its being longitudinally differentiated (Fig. 6). The chromofilaments are composed of two kinds with respect to the penetration of electron beams, the opaque spherical particles and the less opaque intercalary filaments. The apparent diameter of these particles is about 50–100 mμ, but the width of the filament is variable. In more detailed studies the chromofilaments show a coiled microstructure at the points marked by the arrows in Fig. 6. The intercalary filaments are of an elastic nature and elongated to the width of about 10 mμ. In fact, the appearance of chromofilaments suggests that the extensibility of the chromofilaments is due to self uncoiling.

DISCUSSION

YASUZUMI and co-workers[8,9,10] have revealed that the metabolic chromosomes isolated from blood cell nuclei of various animals are composed of at least 8 chromofilaments. This conception has been supported in the smear preparation of erythrocytes of *Sebastodes matsubarae*[11,14]. In the present study the metabolic chromosomes are completely disintegrated with the help of ultrasonic vibration, resulting in uncoiling of coiled constituent elements which are composed of at least 32 chromofilaments. YASUZUMI AND KONDO[4] have already succeeded in identifing the difference of physico-chemical property between the sex chromosome and autosomes of *Drosophila virilis* and *Drosophila melanogaster* by ultrasonic vibration: when female larvae have been exposed

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to ultrasonic vibration for one minute, only the sex chromosome has been remarkably elongated. The result mentioned above and the present study clearly show that ultrasonic vibration is most useful for stretching the chromosome.

It has been generally believed that the protein of the chromosome is always submicroscopically folded at molecular or supramolecular levels, and can easily be stretched to several times its original length without rupture, probably by a mechanism of unfolding of polypeptide chains. In the present work it has been demonstrated that helices of chromofilaments become uncoiled under vibration or mechanical agitation. And the chromofilaments are composed of the fibrous and spherical protein-molecules. The Fig. 6 seems to support the models of molecular construction of the chromosome suggested by DARLINGTON AND MATHER[15].

The electron micrograph (Fig. 4) of the metabolic chromosome disintegrated by ultrasonic vibration looks like a premature figure of the salivary gland chromosome of Diptera. This is easily understood, if we examine the genesis of salivary gland chromosomes. At the beginning of their development salivary gland chromosomes are made of four chromatids. These chromatids are chromomeric in structure, that is, are made of chromomeres which are connected together by strands. After synapsis the compound chromosome grows larger in diameter and in length. It is generally known that reduplication with division accounts for a great increase in nuclear volume, often of the order of 512, 1024 or more times the initial volume.

The euchromatic bands of salivary gland chromosomes of Diptera have been shown directly or indirectly to contain the genes. The bands are made of chromomeres closely appressed, but a single chromomere must be regarded as compound in the sense that it is made of a number of homologous ultimate chromomeres. Assuming that the genes are found in the chromosome as morphological units, and that the opaque spherical particles of chromofilaments in metabolic chromosomes are identified with the ultimate chromomeres of salivary gland chromosomes, the opaque spherical particles 50–120 mμ in diameter correspond to the genetic unit.

The euchromatic bands, that is, the chromomeres in salivary gland chromosomes are positive in Feulgen-reaction, because the salivary gland chromosomes are in the division-stage. On the contrary, the particles of chromofilaments in metabolic chromosomes are Feulgen-negative, throwing off the coat of the nucleic acid. Evidently the nucleic acid remains aggregated resulting in the formation of the chromatin granule (heterochromatin)[16]. The nucleus is filled with gene strings and products of action of the gene neither of which are covered with the nucleic acid.

SUMMARY

The isolation of metabolic chromosomes from triton *Triturus pyrrhogaster* erythrocyte nuclei has successfully been performed by ultrasonic vibrations. The metabolic chromosome is composed of at least 32 constituent chromofilaments, which consist of opaque particles 50–120 mμ in diameter and intercalary filaments 10 mμ in width.

RÉSUMÉ

Des chromosomes métaboliques provenant de noyaux d'erythrocytes de triton *Triturus pyrrhogaster* ont été isolés à l'aide d'ultrasons. Ces chromosomes sont scindés par les ultrasons en au moins 32 filaments chromatiniques, composé chacun de particules opaques (50 à 120 mμ) reliées par des filaments connectifs (10 mμ).

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ZUSAMMENFASSUNG

Mit Hilfe von Ultraschall wurden die metabolischen Chromosomen aus den Erythrocyten-Kernen von Triton *Triturus pyrrhogaster* isoliert. Durch die Wirkung der Ultraschallwellen wurden die Chromosomen in wenigstens 32 Chromofilamente zerlegt. Diese bestehen aus undurchsichtigen Partikeln (50–120 mμ) und Verbindungsfilamenten (10 mμ).

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