Mem. Fac. Educ., Shimane Univ. (Nat. Sci.), Vol. 6, pp. 99–104, December 1972.

THE SPIRAL STRUCTURE OF AMPHIBIAN SOMATIC CHROMOSOMES AS OBSERVED WITH A LIGHT MICROSCOPE

By

Takeshi SETO*

Introduction

The structure of metaphase chromosomes continues to be the subject of considerable controversy. Different investigators led a variety of evidences from a variety of sources on chromosomal structures with light and electron microscopy. Consequentially a variety of models of the chromatid structure ranging from a single long stranded-type to multistranded-types have been proposed (reviewed by Wolf 1969, Prescott 1970).

Although the ultrastructural research of the cell has been rapidly developed, the preparative technique for viewing chromosomes in the electron microscope have so far been inadequate either to reveal or preserve the fiber organization of an isolated chromosome. Therefore, using a light microscope for demonstrating the chromosome structure is still evaluated.

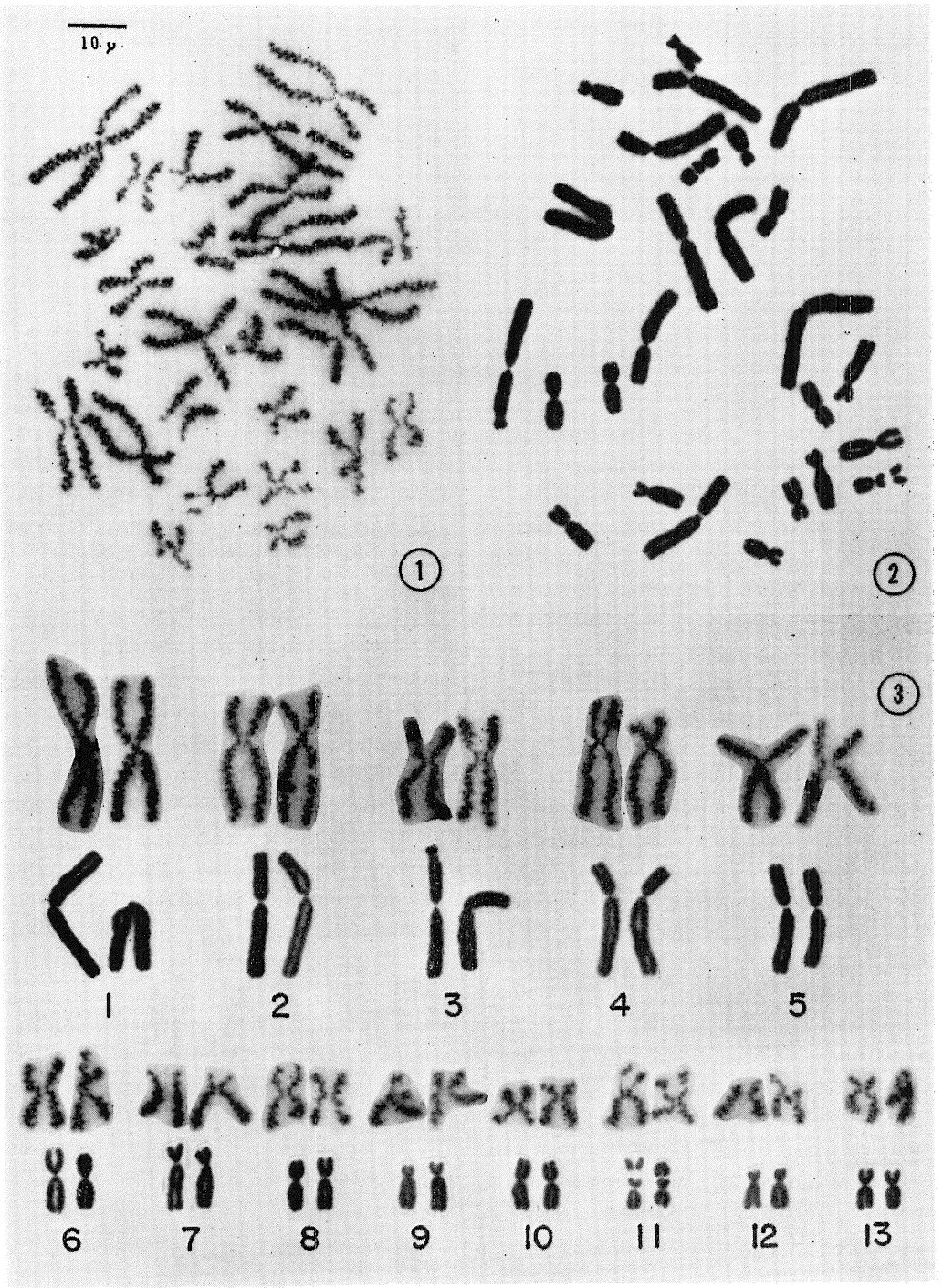
Recent progress of tissue culture methods in combination with a new technique of chromosome spreading has facilitated the study of the inner structure of mammalian chromosomes with a light microscope (Brooke *et al.* 1962, Ohnuki 1965, 1968, Goh 1967, Khan 1969). Ohnuki (1965) has developed a simple technique which was capable of giving satisfactory results for the demonstration of spirals of human chromosomes in cultured leukocytes without serious distortion of chromosome configuration. According to his study, the chromosomes were partially dissociated and clearly demonstrated somatic coils when the cells were pretreated with a certain hypotonic solution composing of equimolar solution of KCl, $NaNO_3$, CH_3COONa.

The application of this technique to the amphibian chromosomes was considered to be valuable for observing more apparent spiralization since the animal used here has the lower chromosome number and as a consequence the chromosomes are larger than human. This paper describes, as a preliminary report, the general view of frog chromosome structure using above method, especially on the visualization of morphological features of major coils of the chromonemata, the centromere region, and the secondary constrictions.

Material and Methods

The bone marrow cells used as a material for this study were obtained from an adult

*Department of Biology, Faculty of Education, Shimane University, Matsue, Japan.


10 μ
1
2
3
1
2
3
4
5
6
7
8
9
10
11
12
13

bullfrog (*Rana catesbeiana* SHAW). A routine chromosome preparation from bone marrow cells in femur and tibia was made through a method described before (Seto 1965a, 1965b). The method for demonstrating the spiral structure of chromosomes followed the Ohnuki's original procedure (1965). After collecting the colchicine treated cells in a centrifuge tube, the cells were treated with a 4 : 2 : 0.8 hypotonic mixture of equimolar solution (0.05 M) of KCl, $NaNO_3$, and CH_3COONa for more than 100 minutes at room temperature (24−26°C). Then the cells were fixed with chilled acetic alcohol consisting of 3 parts absolute ethanol and 1 part glacial acetic acid. Fixed chromosomes were spreaded on a slide glass by the air drying method and stained with the carbol fuchsin (Carr 1961). Well-spreaded metaphases showing clear coiling structure were photomicrographed with a 35mm PM-6 camera (Olympus) and Neopan-F film (Fuji).

Observations and Remarks

Features of Spiralized Chromosomes

The somatic chromosome complement of bullfrog, 26 in diploid number, consists of five pairs of large metacentric or submetacentric chromosomes and eight pairs of small elements. A karyological study of this species has been reported previously (Seto 1965a).

The appearance of metaphase chromosomes of treated and non-treated with the special hypotonic solution was comparatively shown in Figures 1 and 2. The intact metaphase chromosome when viewed with the optical microscope appeared as a tightly homogeneously staining structure and no real internal structure could be discerned. In contrast with the general appearance of non-treated chromosomes, well defined spiral chromonemata became clearly visualized in the treated cells. Appearance of spiralized chromosomes numbered approximately 10 per cent of whole metaphase cells after use of a hypotonic solution of the special salt composition.

Karyograms illustrated a good contrast between two complements of the metaphase cells under different conditions of the pretreatment (Fig. 3). In general, the gyres of coiled chromonema were recognizable in metaphase cells, though inner structure of the chromosomes were only defined to the major coils. As it was very difficult to trace accurately all spirals from end to end of complete chromosome arms in the karyotype, analysis of the direction of spiralized coils were tried in a few representative chromosomes of larger sized (Fig. 4). It was only noted in the observation that either right- or left-handed spiralization occurred randomly on either side of the centromere or in both sister chromatids, and there was no consistent direction detected in any of coils of the spiralized chromosomes.

(*Explanations of Figures*) Photomicrographs of metaphase chromosomes obtained from the bullfrog bone marrow. The preparation was made by air-drying method and carbol fuchsin stain. All figures were taken with an ordinary light microscope and a 35mm camera (Olympus) with ×100 oil immersion objectives. Figure 1, illustrating the structural conformation of metaphase chromosomes ; spiral structures of major coils are seen in the each chromosomes of the cell taken the special hypotonic treatment. Figure 2, a metaphase cell made by the ordinary chromosome preparation. Figure 3, karyograms of *Rana catesbeiana* constructed from cells shown in Figures 1 & 2. The autosomes are arranged into two groups, a gross secondary constriction is seen in No. 11 chromosomes.

The secondary constriction in relation to the chromonemata structure in No. 11 chromosomes was especially noticed. The secondary constriction in the homogeneously stained chromosome that was non-spiralized usually appeares as an nonstaining and amorphous gap in a certain region of the chromosome arm. As demonstrated in the Figure 4c the chromonemata appeared consistently as uncoiling entity in the region of a secondary constriction.

Several observations have been reported concerning the existence of half-chromatids in plant and animal chromosomes (cf, Wolff 1969) ; anaphase chromosomes have repeatedly been observed to be split into two chromatids indicating that metaphase chromatids consisting of 2 or more subunits of strands. Therefore the presence of half-chromatids seems to be extended enough as to justify their real participation in the chromosome structure. For instance, with lateral root tips of *Vicia faba*, Trosko and Wolff (1965) demonstrated the chromosome feature of which each chromatid contained four strands at the optical level. The present observation failed to demonstrate such half-chromatid structure in any spiralized element in the complement. Possibly more highly dissociated preparations should be required for observing the half-chromatid structure.

Centromere Region

The centromere in somatic chromosomes of many organisms appears simply as a nonstaining constriction with no morphological evidence of structure in the ordinary chromosome preparations. (Figs. 4A and 4B). On the contrary, the centromere region, termed by Comings and Okada (1971), in spiralized chromosomes proved to be euchromatic indicating that two heteropycnotic spots were existed on each chromonemata and

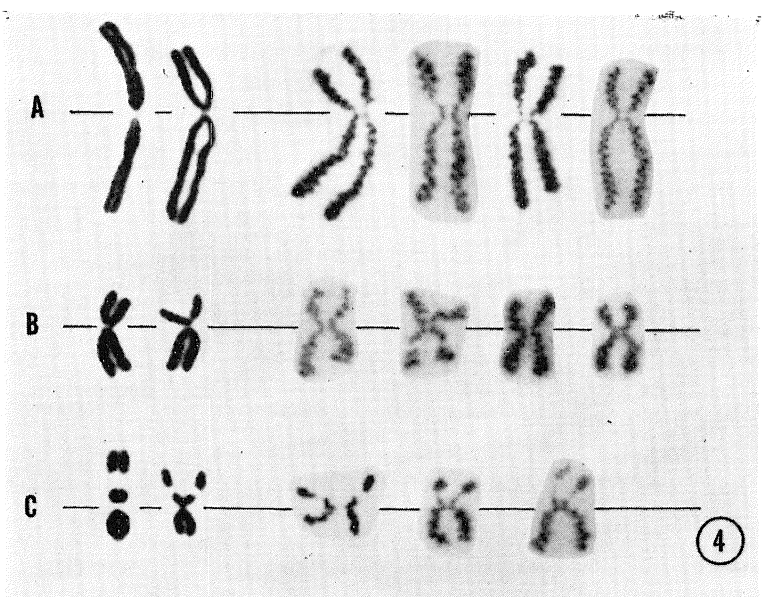


Figure 4. Representative chromosomes of spiralized and non-spiralized selected from different cells ; A and B illustrating the structural conformation of chromonema at centromere region ; C, secondary constrictions in No. 11 chromosomes showing uncoiling chromonema.

a simple uncoiled strand was connected in between these spots. In human somatic chromosomes, Khan (1969) has observed the centromere region characterized by a quadruple structure which was formed by fibrous connections present between the four centromeric chromomeres.

The centromeric chromomeres which appeared as a well demarcated spot were rather conspicuous than chromonema strand in the region. Fibrous connection did not always indicate a square form when sister chromatids were located very closely each other.

At the optical level most studies of centromere structure were conducted in plant meiotic chromosome. In vertebrates, also meiotic chromosomes were unusually stained with basic dyes such as iron hematoxylin, Sudan black B, or aceto-orcein and showed centromeres as dark staining bodies (Chen and Falek 1969, Kezer and Macgregor 1971, Comings 1971, Polani 1971). Chen and Falek (1969) described that if the heterochromatic bodies observed in the preparations of human meiotic chromosomes stained with aceto-orcein they were recognized indeed centromere. With amphibian spermatocytes, Kezer and Macgregor (1971) have studied the centromeric heterochromatin and clearly demonstrated four centromere granules in each diplotene bivalent. In the present observation with somatic chromosomes the centromere region was appeared as a simple stranded entity containing two dark spots in each chromonemata.

In a view with electron microscopy of isolated chromosomes from the Chinese hamster cells, Stubblefield and Wray (1971) observed the four small dense masses of the fibers and the interchromatid fibers were apparently seen connecting the dense masses at the centromere region. Such structure might correspond to the dense spot observed in this study. However optical microscope hardly permit of observing further detail structure of the centromere region.

Although optical views of chromosome structure do not always bear satisfactorily result, for viewing chromosomes by electron microscopy have also been inadequate so far either to reveal or preserve the fiber organization. Thus more intimate observation of chromosome structure with a light microscope has to be required.

Summary

1. Using the Ohnuki's hypotonic solution for the demonstration of spiral structure in human somatic chromosomes, a coiling structure of frog somatic chromosomes was studied in bone marrow cells.

2. Special hypotonic pretreatment and the routine air-drying method facilitated the exaggeration of coiling chromonemata embedded in the chromosome entities.

3. Though inner structure of the chromosomes was only defined to the major coils, the gyres of coiled chromonemata were recognizable in metaphase cells.

4. At the secondary constriction the chromonemata appeared usually as uncoiling entity.

5. The centromere region in the chromosome appeared to be characterized by a quadruple chromomere structure which was formed fibrous connections present between four dense spots.

Acknowledgments: The author wishes to express his appreciation to Professor Masaru Akiyama for his kind support and encouragement.

References

Brooke, J. H., Jenkins, D. P., Lawson, R. K., and Osgood, E. E.: Human chromosome uncoiling and dissociation. Ann. Human Genet., Lond. **26**, 139–143 (1962).

Carr, D. H, and Walker, J. E.: Carbol fuchsin as a stain for human chromosomes. Stain Techn. **36**, 233–236 (1961).

Chen, A. T. L., and Falek, A.: Centromeres in human meiotic chromosomes. Science **166**, 1008–1010 (1969).

Comings, D. E.: Heterochromatin of the Indian muntjac. —Replication, condensation, DNA ultracentrifugation, fluorescent and heterochromatin staining. Exp. Cell Res. **67**, 441–460 (1971).

Comings, D. E. and Okada, T. A.: Fine structure of kinetochore in Indian muntjac. Exp. Cell Res. **67**, 97–110 (1971).

Goh, K. O.: Inner chromosomal structures in the somatic metaphase of rat kangaroo (*Potorous tridactylis*): A light microscopic observation. Cytologia **32**, 416–419 (1967).

Kezer, J., and Macgregor, H. C.: A fresh look at meiosis and centromeric heterochromatin in the red-backed salamander, *Plethodon cinereus cinereus* (Green). Chromosoma (Berl.) **33**, 146–166 (1971).

Khan, M. H.: The structure of the centromere of the human somatic chromosomes. La Kromosomo **75**: 2443–2444 (1969).

Ohnuki, Y.: Demonstration of the spiral structure of human chromosomes. Nature **208**, 916–917 (1965).

Ohnuki, Y.: Structure of chromosomes I. Morphological studies of the spiral structure of human somatic chromosomes. Chromosoma (Berl.) **25**, 402–428 (1968).

Polani, P. E.: Centromere localization at meiosis and the position of chiasmata in the male and female mouse. Chromosoma (Berl.) **36**, 343–374 (1972).

Prescott, D. M.: The structure and replication of eukaryotic chromosomes. Advanc. Cell Biol. **1**, 57–117 (1970).

Seto, T.: Cytological studies in lower vertebrates II. Karyological studies of several species of frogs (Ranidae). Cytologia **30**, 437–446 (1965a).

Seto, T.: A simple method for chromosome preparations from amphibian bone marrow. Zool. Mag. (Japan) **74**, 243–244 (1965b).

Stubblefield, E. and Wray, W.: Architecture of the Chinese hamster metaphase chromosome. Chromosoma (Berl.) **32**, 262–294 (1971).

Trosko, J. E., and Wolff, S.: Strandedness of *Vicia faba* chromosomes as revealed by enzyme digestion studies. J. Cell Biol. **26**, 125–135 (1965).

Wolff, S.: Strandedness of chromosomes. Intern. Rev. Cytol. **25**, 279–296 (1969).