Proc. Natl. Acad. Sci. USA
Vol. 74, No. 4, pp. 1595–1599, April 1977
Cell Biology

Higher-order structure of human mitotic chromosomes

(nucleosomes/solenoids/unit fiber/helices/packing ratio)

ARNE LETH BAK*, JESPER ZEUTHEN†, AND F. H. C. CRICK‡

* Institute of Medical Microbiology and † Institute of Human Genetics, University of Aarhus, DK-8000 Aarhus C, Denmark; and ‡ The Salk Institute for Biological Studies, P.O. Box 1809, San Diego, California 92112

Contributed by F. H. C. Crick, January 14, 1977

ABSTRACT **From observations on the partial disintegration of isolated human metaphase chromosomes we propose that human metaphase chromatids have a rather simple organization based on the folding and coiling of a long, regular, hollow cylindrical structure with a diameter of about 4000 Å. This cylindrical structure, the unit fiber, is postulated to be a super-solenoid formed by the coiling of a 300 Å solenoid, itself composed by coiling the basic string of nucleosomes. The structure of a human chromatid would thus be a hierarchy of helices, the contraction ratio of each coil, in ascending order of size, being approximately 7, 6, 40, and 5. This model appears to explain the estimated mass/unit length and accounts for many of the known features of human mitotic chromatids.**

It has been known for a long time that chromosomes consist of both DNA and protein, but the general nature of the association of the DNA with histones resulting in the formation of the basic string of nucleosomes has only recently been elucidated (see, for example, refs. 1 and 2). This packing of the DNA in the primary chromatin fiber results in a condensation in length of the original DNA duplex by a factor of about 7. A further packing of the DNA in interphase chromatin has very recently been proposed. It consists of a further level of coiling with the formation of a solenoidal structure formed from the basic string of nucleosomes and by which the total condensation is increased to a factor of about 40 (3, 4). To account for the condensation of chromatin in the mitotic chromosome, for which the total factor is of the order of 10,000, a further contraction is necessary. Electron microscopic investigations using both thin-sectioning and surface-spreading techniques represent chromosome structure as a tangled mass of fibers of about 300 Å diameter (5), which suggests that the solenoidal folding of the string of nucleosomes is indeed present in mitotic chromosomes. However, it seems more likely that a higher order of rather simple organization is necessary to explain the highly defined regular structure characteristic of individual chromosomes, especially taking into account the regularity of chromosome structure shown by the modern chromosome banding techniques.

MATERIALS AND METHODS

Cell Cultures. Human fetal fibroblasts of normal karyotype were grown in Eagle's minimal essential medium supplemented with 20% calf serum and antibiotics (penicillin at 200 IU/ml, streptomycin at 200 μg/ml, and mycostatin at 25 IU/ml).

Chromosome Isolation. Cultures were treated with Colcemid (Ciba) at a concentration of 1 μg/ml of medium for 16 hr prior to the isolation of mitotic cells by shaking. This procedure regularly resulted in the recovery of more than 90% mitotic cells, most of which were in late metaphase as judged from conventional chromosome preparations.

Recovered mitotic cells were washed twice in Hanks' balanced salt solution and suspended in ice-cold chromosome isolation buffer at pH 6.5 [1.0 M hexylene glycol (2-methyl-2,4-pentanediol) Eastman Organic; 20 mM $CaCl_2$; and 0.1 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) monosodium hydrate (Pipes) (Calbiochem)]. The concentration of $CaCl_2$ in the buffer above (20 mM) was chosen to cause the greatest contraction of chromatin (6). The cells were allowed to lyse in this solution for 1–20 min, after which the majority of nonmitotic cells and intact nuclei were removed by repeated differential centrifugations (500 rpm, 5 min) and cytoplasmic debris was removed similarly by repeated differential centrifugations (2000 rpm, 10 min) in the Sorvall RC2B centrifuge using the SS34 rotor at 4°. This procedure is slightly modified from that previously given (7)[§], and is based on the procedure of Wray and Stubblefield (8).

Preparation and Examination of Chromosomes. Isolated chromosomes were fixed by addition of methanol/acetic acid 3:1, sedimented (5000 rpm, 10 min), and spread on glass slides for light microscopy and on Formvar-plus-carbon-coated grids for electron microscopy. Slides for light microscopy were stained using conventional Giemsa staining (Gurr's improved R66 Giemsa stain, Searle Diagnostic). Material spread on grids for electron microscopy was stained with ammonium molybdate (2%) or uranyl acetate (2%) for 15 min. Thin sections for electron microscopy were prepared by dehydration of the fixed material in acetone, embedded in Vestopal, and sectioned in an LKB 8800 Ultrotome III microtome to produce sections of a thickness of 600–800 Å. Thin sections were stained in uranyl acetate (7.5%) for 15 min. Preparations were inspected and photographed in the light microscope using a Leitz Orthoplan photomicroscope and photographic negatives were analyzed by projection and on photographic enlargements (×7000). Samples of isolated chromosomes were also examined as wet mounts in chromosome isolation buffer in a Leitz Orthoplan photomicroscope equipped with phase contrast or Nomarski interference contrast optics. Specimens for electron microscopy were examined in a JEOL JEM-100 S or JEM-100 B instrument operated at 80 or 50 kV, and magnifications of ×2000 to ×5000 were used for the examination of spreads and magnifications of ×25,000 to ×50,000 were used for the examination of chromosome material in thin sections.

RESULTS AND DISCUSSION

Diameter of the Unit Fiber. The isolated chromosomes, after treatment with chromosome isolation buffer, were observed to disintegrate and generate fibers which we assume must be present also in the intact mitotic chromosome (7). The apparent diameter of these unit fibers measured using the light microscope is about 0.4 μm. A single fiber, when in a fairly extended configuration, appears at this resolution to be rela-

[§] The magnifications of the figures printed in ref. 7 are incorrect, the stated values being roughly five times the correct ones, which range from ×1500 to ×3000.

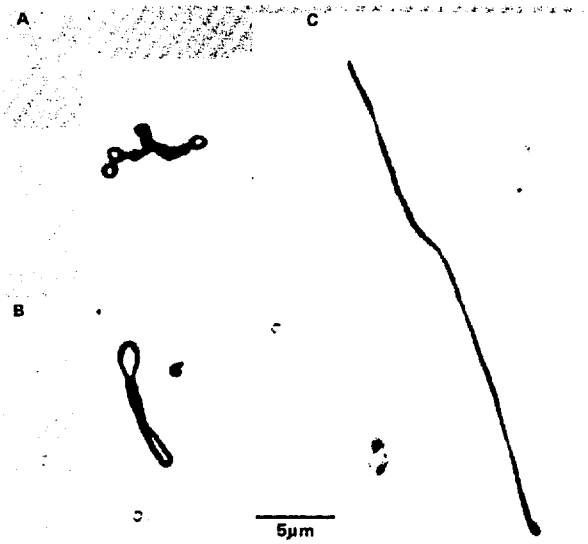

FIG. 1. Examples of partially disintegrated isolated human metaphase chromosomes at different stages of disintegration, from an almost intact chromosome to an extended fiber. Characteristic of all of these structures is that they appear to be composed of a well-defined fiber of about 0.4 μm diameter, the unit fiber. (Light microscopy after Giemsa staining.)

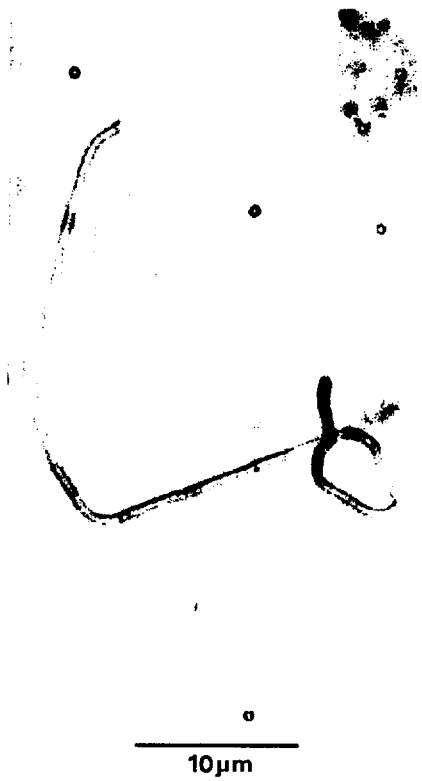

FIG. 2. An example of the image of the unit fiber photographed slightly above the focal plane showing the characteristic appearance of doubleness due to a phase effect which suggests a tubular structure for the unit fiber. (Light microscopy after Giemsa staining.)

tively uniform (Fig. 1*C*). In some instances the diameter appears to vary as much as ±20% between the different regions on the fiber. The diameter of most of the unit fibers was more constant than this. The diameter of different fibers does not vary over a wide range. The measured average diameter of 853 individual fibers is 0.39 μm with a standard deviation of ±0.04 μm. Some of this variation may be due to errors of observation and to small differences in the method of preparation.

Length of the Unit Fiber. Our evaluation of the length distribution of the unit fibers is based on the length measurements of 933 individual fibers. The shortest fibers have a well-defined length of about 11 μm. Our best estimate of the length of the longest fibers is about 60 μm. Since our data for the length distribution of the unit fibers still contain relatively few values for lengths of the longest fibers, this value could be in error by some 10%. The lengths of different human metaphase chromatids varies from 2 to 10 μm. (Our isolated chromosomes are more contracted, with lengths of 1–5 μm.) We interpret the unit fibers we observe as coming either from separated whole chromatids or from the broken fragments of the larger chromatids, the larger ones being more likely to be broken than the shorter ones. This would imply that the unit fiber is folded to about one-fifth of its length when coiled to constitute a metaphase chromatid.

"Doubleness" of the Unit Fiber. Under the best optical conditions all the fibers appear double when viewed slightly above the focal plane (Fig. 2). It was originally suggested that this might mean that the structure itself was double (7), but the fact that doubleness is seen in nearly all fibers makes this unlikely. This apparent doubleness of the unit fiber is most probably due to a phase effect caused by an inherent inhomogeneity of the unit fiber. A very plausible explanation is that the fiber is a thin-walled tube. This interpretation is reinforced by the electron microscopical observations and by the theoretical arguments given below.

Mass/Unit Length. We have not yet measured directly the mass/unit length of the unit fiber but we can make a plausible estimate of it. To do this we need to make one important assumption: This is that the fibers we see are *not* made by the joining together, during the extraction process, of fibers from different chromosomes. We shall also assume that the largest fibers we see represent the entire length of the unit from one of the larger human chromatids, and that the shortest fibers are derived, unbroken, from the shortest chromatids. From these assumptions we can calculate the contraction ratio for the DNA, as follows.

The total amount of DNA in the haploid human genome is 1.8×10^{12} daltons. This corresponds to 3×10^9 base pairs or a total length of about 10^6 μm. From this figure we estimate that, using the *relative* DNA contents of the human chromosomes (9), the largest chromatids contain DNA having a total length of about 8×10^4 μm. The longest unit fiber we observe is about 60 μm in length, so the contraction ratio for the unit fiber calculates, on these assumptions, to about 1300. Similar contraction ratios can be found using the DNA content of the smallest human chromosomes and the length of the smallest unit fibers, which are about 11 μm.

The mass/unit length could be calculated from this number if we knew the ratio of protein to DNA in chromosomes. If we take this ratio as 1:2 (8), then we can estimate, from the known density of DNA and protein, the approximate value for the hydration, assuming that in the wet state the unit fiber has a diameter of 0.4 μm. This calculation suggests that the extent of hydration is in the region of 90%. Even if rather more protein were assumed to be present the hydration would still be high. Thus *the unit fiber is a highly hydrated structure.* This is very

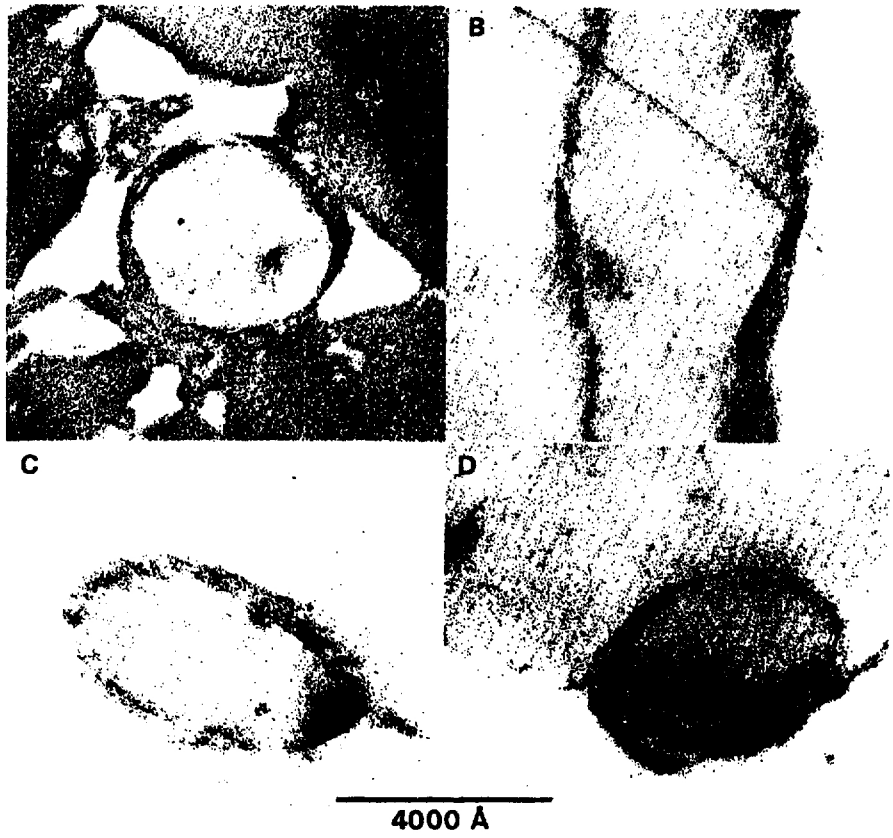

FIG. 3. Electron micrographs of cross sections of 600 to 800 Å thickness of the unit fiber which is suggested to be a supersolenoid. Samples were embedded in Vestopal and stained with uranyl acetate. The diameter of the spiralized thin fiber is about 300 Å, which corresponds to the dimensions of the solenoid (3, 4). The diameter of the larger thin-walled supersolenoid structure is about 4000 Å (= 0.4 μm).

compatible with our interpretation of its "doubleness" as due to its being a thin-walled hydrated tube. Note that if the longest fibers we see were fragments of a single chromatid the calculated degree of hydration would be even higher. One can only arrive at a low value of hydration by assuming that the largest fibers consist of the fibers from two or more chromatids joined together. There is no evidence for this and we consider it unlikely.

Unit Fiber as a Supersolenoid. The above arguments are independent of the exact arrangement of DNA and protein in the unit fiber. We now assume that the DNA in one chromatid consists of one long DNA molecule as suggested by recent work (see, for example, ref. 10), that the nucleofilament—the string of closely apposed nucleosomes—is indeed folded into a solenoid of about 300 Å diameter (3, 4), and that the solenoid is folded once more into a further helix, or supersolenoid, of a diameter about 4000 Å (= 0.4 μm) to give the unit fiber. Because the contraction ratio for the solenoid itself is believed to be about 40 the calculated contraction ratio for the unit fiber comes to some figure in the region of 1300–1500, depending on the exact parameters assumed, in very good agreement with the estimate given earlier.

We have looked to see whether we could observe the cross section of such a structure by examining thin sections of the fibers in the electron microscope. Several cross sections of the predicted dimensions have been found, some of which are illustrated in Fig. 3. These pictures show a fiber, often somewhat irregular, of a diameter of 300 Å coiled to form a hollow structure of about 4000 Å diameter. In some cases, as in Fig. 3*D*, the helical character of the supersolenoid was apparent in the 600 to 800 Å sections. This finding agrees with the hollowness of the structure suggested by the "doubleness" of its image in the light microscope and its calculated high degree of hydration.

Is the Unit Fiber Crosslinked? It is, at the moment, unclear what holds the unit fiber in its rather regular configuration. This regularity could be due to the exact way the nucleosomes pack, perhaps with the help of certain "structural" nonhistone proteins, but the large size of the structures, compared to atomic dimensions, makes it unlikely that this is the sole method of stabilizing it. We therefore wonder whether successive turns of the supersolenoid are in some way joined together either by protein or RNA or both, to help maintain the regular packing of the structure. If this were indeed true it would have important implications for both chromosome structure and function.

Nonmitotic Chromosomes. It would be surprising if the very regular structure of the unit fiber were maintained unaltered in interphase, in all phases of meiosis, in the lampbrush chromosomes, or in giant polytene chromosomes. However, if cross ties exist some of them might persist under these very different conditions and thus account, for example, for the bands and interbands of the giant polytene chromosomes. There are two cases, however, in which the structure of the chromosomes

FIG. 4. Electron micrograph of a unit fiber spread on a grid and stained with uranyl acetate. The fiber is seen to be folded and could be a circular structure.

might approximate more closely that of the unit fiber. These are the extended structures found in the pachytene stage of meiosis and in prematurely condensed chromosomes produced by fusing cells in G_1 with mitotic cells (11). In both of these cases the chromosomes have diameters closer to the diameter of the unit fiber but appear rather more lumpy when stained. This may be because not all the cross ties needed to produce a regular superstructure are in place in these conditions.

Final Level of Folding. We envisage that the unit fiber is folded one further time to produce the mitotic chromosome. We estimate the observed diameter of wet, unfixed human chromatids in chromsome isolation buffer to be in the region of 1.0 μm, based on phase contrast or Nomarski interference contrast microscopy. There is thus enough space to allow for the postulated further contraction by a factor of about 5. By observation using Nomarski interference contrast microscopy, chromatids were observed to be composed of fibers with the expected diameter of the unit fiber of 0.4 μm, that appeared to be coiled in some helical fashion. The question arises whether this folding could be in the form of a further single or double helix. It has previously been suggested on the basis of the observation of disintegrated structures of the same type as illustrated earlier (Fig. 1*A* and *B*) that this folding could be double helical, and that the mitotic chromatid could be generated from the unit fiber by folding it back onto itself to result in the characteristic figure-eight structures which are abundant in our preparations (see Fig. 1*B* and Fig. 4), and by a final supercoiling of the formed loops to generate the fully condensed chromatid (7). Also we now have photographs which could suggest that the unit fibers at least in some cases may be circular (Fig. 4), which would make the folding and subsequent supercoiling easier to understand. However, we are well aware that this interpretation poses serious problems in relation to a number of known genetic phenomena such as crossing-over, sister chromatid exchange, and translocation. For instance, to explain the relatively well-established conservation of the banding pattern and genetic map as well as their colinearity after translocation of a chromosomal segment would require that the chromosome breaks in some way arise symmetrically on the two parallel segments of the folded, or possibly circular, unit fiber. For these reasons, a single helical model for the condensed chromatid would be easier to accept. Evidently much further work will be necessary in order to clarify the final level of folding of the unit fiber within the chromatid.

Hierarchy of Folding. It is not a new idea that the mitotic chromosome is based on a hierarchy of helices (see, for example ref. 5). What is new is that we can suggest, at least for human chromosomes, the various levels of hierarchy. These can be summarized by listing the contraction ratio of each state, starting from the lowest, in the form

$$7 \times 6 \times 40 \times 5$$

it being understood that the numbers are at the present time only approximate. They certainly do not need to be integers, for example.

The remarkable and unexpected feature is the ×40 stage and the high degree of hydration associated with it. Moreover, this folding appears surprisingly regular, though further work will be needed to specify this regularity more precisely and to demonstrate the details of the underlying structure.

Other Species. It is a well-known generalization that the mitotic chromatids of all mammalian species, though varying greatly in length, have approximately the same diameter. It would therefore not be surprising if they all follow the $7 \times 6 \times 40 \times 5$ hierarchy of folding. Whether other eukaryotes will show this pattern or a similar one remains to be seen. One would especially like to know the folding in such organisms as *Drosophila*, various amphibia, and the fungi, to mention only a few. For human chromatids the startling regularity of the unit fiber that we deduce suggests to us that it is unlikely to be an artefact and that perhaps the broad outline of the folding of mammalian mitotic chromatids has now been revealed after many years of research and speculation.

We thank Mrs. Hanne Puibaraud for excellent technical assistance. We also thank Dr. Muriel Nesbitt and Dr. A. G. Searle for access to unpublished data and Dr. D. L. Lindsley, Dr. H. Stern and Dr. Muriel Nesbitt for helpful discussions. F.H.C.C. holds a Ferkauf Foundation Research Professorship. This work was supported in part by Grant 521/18 from the Danish Natural Science Research Council.

1. Kornberg, R. D. (1974) "Chromatin structure: A repeating unit of histones and DNA," *Science* **184**, 868–871.
2. Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) "Electron microscopic and biochemical evidence that chromatin structure is a repeating unit," *Cell* **4**, 281–300.
3. Finch, J. T. & Klug, A. (1976) "Solenoidal model for superstructure in chromatin," *Proc. Natl. Acad. Sci. USA* **73**, 1897–1901.
4. Carpenter, B. G., Baldwin, J. P., Bradbury, M. & Ibel, K. (1976) "Organisation of subunits in chromatin," *Nucleic Acid Res.* **3**, 1739–1746.
5. DuPraw, E. J. (1970) *DNA and Chromosomes* (Holt, Rinehart and Winston, New York).
6. Bradbury, E. M., Danby, S. E., Rattle, H. W. E. & Giancotti, V.

(1975) "Studies on the role and mode of operation of the very-lysine-rich histone H1 (F1) in eukaryote chromatin," *Eur. J. Biochem.* **57**, 97–105.

7. Bak, A. Leth & Zeuthen, J. (1976) "Evidence for a folded structure of human chromosomes," *Hereditas* **82**, 1–6.§
8. Wray, W. & Stubblefield, E. (1970) "A new method for the isolation of chromosomes, mitotic apparatus, or nuclei from mammalian fibroblasts at near neutral pH," *Exp. Cell Res.* **59**, 469–478.
9. Mendelsohn, M. L., Mayall, B. H., Bogart, E., Moore, D. H. & Perry, B. H. (1973) "DNA content and DNA-based centromeric index of the 24 human chromosomes," *Science* **179**, 1126–1129.
10. Kavenoff, R. & Zimm, B. H. (1973) "Chromosome-sized DNA molecules from *Drosophila*," *Chromosoma* **41**, 1–27.
11. Johnson, R. T. & Rao, P. N. (1970) "Mammalian cell fusion: Induction of premature chromosome condensation in interphase nuclei," *Nature* **226**, 717–722.