The energy components of stacked chromatin layers explain the morphology, dimensions and mechanical properties of metaphase chromosomes

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The measurement of the dimensions of metaphase chromosomes in different animal and plant karyotypes prepared in different laboratories indicates that chromatids have a great variety of sizes which are dependent on the amount of DNA that they contain. However, all chromatids are elongated cylinders that have relatively similar shape proportions (length to diameter ratio approx. 13). To explain this geometry, it is considered that chromosomes are self-organizing structures formed by stacked layers of planar chromatin and that the energy of nucleosome–nucleosome interactions between chromatin layers inside the chromatid is approximately $3.6 \times 10^{-20}$ J per nucleosome, which is the value reported by other authors for internucleosome interactions in chromatin fibres. Nucleosomes in the periphery of the chromatid are in contact with the medium; they cannot fully interact with bulk chromatin within layers and this generates a surface potential that destabilizes the structure. Chromatids are smooth cylinders because this morphology has a lower surface energy than structures having irregular surfaces. The elongated shape of chromatids can be explained if the destabilizing surface potential is higher in the telomeres (approx. 0.16 mJ m$^{-2}$) than in the lateral surface (approx. 0.012 mJ m$^{-2}$). The results obtained by other authors in experimental studies of chromosome mechanics have been used to test the proposed supramolecular structure. It is demonstrated quantitatively that internucleosome interactions between chromatin layers can justify the work required for elastic chromosome stretching (approx. 0.1 pJ for large chromosomes). The high amount of work (up to approx. 10 pJ) required for large chromosome extensions is probably absorbed by chromatin layers through a mechanism involving nucleosome unwrapping.

1. Introduction

In eukaryotic cells, the extremely long genomic DNA molecules, which are only 2 nm in diameter but up to more than 1 m in length in some species, are associated with histone proteins to form a huge number of nucleosomes. The nucleosome core is a short cylinder (11 nm diameter and 5.7 nm height) formed by 147 bp of DNA wrapped in 1.7 superhelical turns around an octamer of core histones [1]. Nucleosome cores connected with linker DNA and complemented with histone H1 constitute long chromatin filaments that are folded within the nucleus [2–6]. During mitosis, in order to avoid entanglements of different filaments and to preserve DNA integrity, each chromatid contains only one chromatin filament that is densely packaged [7] before being transferred to the daughter cells. The spatial organization of chromatin within metaphase chromosomes is a fundamental question of biology and several structural models have been suggested on the basis of results obtained using different experimental approaches. These models can be grouped into three categories that are schematized in figure 1a. The loop–scaffold models (scheme a) were based on early electron microscopy images showing large DNA loops emanating from histone-depleted metaphase
chromatins [8–10]. Micromechanical studies [11], and immunofluorescence [12] and cryo-electron microscopy [13] results suggested models in which chromatin is irregularly folded within condensed chromatids (scheme b). The thin-plate model (scheme c) was based on electron microscopy and AFM studies of the structure and mechanical properties of the multilayer plates that emanate from partially denatured chromosomes under metaphase ionic conditions [14,15]. Further studies using polarizing microscopy, cryo-electron microscopy and electron tomography [16] and AFM-based nanotribology [17] showed that in each layer the chromatin filament forms a flexible two-dimensional network (5–6 nm thick) in which nucleosomes are irregularly oriented, allowing the interdigitation of the successive layers that are stacked in the metaphase chromatids.

Figure 1. Chromosome dimensions and structural models for metaphase chromatin folding. (a) Schematic illustration of different models for chromatin structure in metaphase chromosomes: (a) chromatin fibres (grey lines) form loops that are bound to a central protein scaffold (brown) [8–10], (b) chromatin fibres are irregularly folded [11–13], and (c) chromatin has a laminar structure with many staked layers oriented perpendicular to the chromatid axis [2,14–18]; parallel lines represent the side view of the stacked layers. (b) Examples showing the differences in metaphase chromosome sizes that exist among animal species: crested newt (T. cristatus) (a), human (b), and D. melanogaster (c); the three chromosome sets are represented at the same magnification; figure reproduced from [19] with permission from the Royal Society.

Single-molecule techniques (optical and magnetic tweezers) and computer modelling have provided a wealth of information about the mecanochemical properties of chromatin [5,20]. It was found that stretching forces of 4–6 pN cause the unfolding of individual chromatin fibres [21,22] and that higher forces (20–40 pN) produce nucleosome unwrapping and eventually dissociation of core histones [21,23,24]. The force–extension curves corresponding to the unfolding and refolding of the fibres indicated that this process is reversible and allowed the calculation of the energy corresponding to the disruption of the attractive interactions between nucleosomes in folded fibres. The energy required for breaking a single nucleosome–nucleosome interaction is between 3.4 and 14 $k_B T$ [21,22] ($\approx 3.6 \times 10^{-20}$ J considering that $k_B T = 4.1 \times 10^{-21}$ J. $k_B$ is the Boltzmann constant and $T$ corresponds to the room temperature). These experimental results are compatible with the internucleosome interaction energy obtained in modelling studies of chromatin fibres having different degrees of nucleosome stacking [25–27]. Furthermore, the stretching experiments indicated that the first stage of nucleosome unwrapping is also reversible; the energy required for the unwrapping of the outer DNA turn of a single nucleosome is $9–20 k_B T$ [24,28]. Assuming that chromatin fibres form a zigzag structure without internucleosome interactions, it was possible to model fibre unfolding considering that the work involved in this transformation corresponds to the partial unwrapping of nucleosomal DNA [29]. The unwrapping of the inner DNA turn is an irreversible process that requires more energy [26,28]; the activation barrier energy for this transformation is $36–38 k_B T$ per nucleosome [24].

Other laboratories have studied the micromechanical properties of mitotic chromosomes using microneedles and micropipette manipulation [30–34]. Individual chromosomes showed a linear elastic response for stretching forces up to approximately 5 nN, corresponding to a fivelfold extension. Higher forces were needed to obtain longer extensions, which were accompanied by an irreversible plastic deformation of chromosomes. In the stretching experiments, chromosomes showed a viscoelastic behaviour corresponding to an internal viscosity that is approximately $10^5$ times the viscosity of water [33]. The Young elastic modulus obtained from force–extension curves in the elastic region for chromosomes of different species is 0.2–1 kPa. Equivalent values were obtained for the Young modulus calculated from the bending rigidity measured experimentally for different metaphase chromosomes [35]. These results indicated that chromosomes are equivalent to isotropic elastic rods and that their internal structure, which is responsible for the observed stretching and bending elastic properties, is homogeneous across the entire chromatid cross section. Taking into account the low elastic stiffness observed for chromosomes, they have to be considered as very soft structures. Nevertheless, chromosomes have outstanding mechanical properties [31,32]. Chromosomes recover their initial shape and dimensions after repeated extension–relaxation cycles in the reversible elastic region of the stretching experiments, and they can be extended up to 80 times their native length without suffering breakage.

Metaphase chromosomes of different species have different sizes (see examples in figure 1b), but apparently they have structural similarities. These similarities have never been explained quantitatively. After many years of advances in many fields of structural biology, the physical elements that determine the global shape and basic properties of condensed chromosomes remain unknown. This represents a major gap in our knowledge of cell structure [36], which is particularly frustrating because the metaphase chromosome is one of the most fascinating structures of living matter. Moreover, probably the tremendous success obtained by X-ray crystallography in the resolution of relatively large protein–nucleic acids complexes [1,37] has reduced the current interest in huge structures, for example metaphase chromosomes, that cannot be crystallized.
Fortunately, however, cutting-edge nanotechnology research has demonstrated that self-assembly of different structures of biological origin produces extremely complex micrometre-scale materials that can be modelled using basic concepts of supramolecular chemistry [38–41]. In this context, recent research has revealed that multilayer plates (identical to those found in metaphase chromosomes) can be self-assembled from chromatin fragments obtained by micrococcal nuclease digestion of metaphase chromosomes [18]. This finding has reinforced the thin-plate model for metaphase chromosome structure (figure 1a,c) and suggests that the whole chromosome could be a self-organizing structure. The immense capabilities of soft matter for the building of complex structures, and the possibility to understand them without considering all interactions on the atomic scale [42], has stimulated the research presented in this study. If the metaphase chromosome is considered as a typical supramolecular structure, which has a maximum stability when the total energy of the whole structure is minimum, it is possible to explain the geometry and mechanical properties of the condensed chromatids. The energy values obtained in the fibre extension experiments and in modelling studies considered above have been used in this work, and the size of chromosomes of diverse species has been measured from microscopy images obtained in different laboratories. It is demonstrated that the symmetry breaking produced by the different internucleosome interaction energies in different regions of the condensed chromatids allows a consistent physical explanation of the observed dimensions of metaphase chromatids. The results obtained in experimental studies of chromosome stretching performed in other laboratories (see above) have been used to test the proposed supramolecular structure, and it is shown that the dynamic properties of this structure can explain the mechanical behaviour of condensed chromosomes.

2. Results

2.1. Metaphase chromosomes of different species show a great variety of sizes but have relatively similar shape proportions

As can be seen in the examples presented in figure 1b and in the electronic supplementary material, figure S1, chromosomes of different animal and plant species exhibit dramatic differences in size. The chromosome dimensions of selected species covering a broad range of genome sizes are presented in table 1. It can be observed that the diameter and length of the chromatids increases with the amount of DNA that they contain. In agreement with this observation, individual species, such as Drosophila melanogaster [43] and chicken [44], having chromatids that contain very different amounts of DNA, have karyotypes with a great heterogeneity of chromosome sizes ([19,45,46]; figure 1b,c). The measurements shown in table 1 cover from the small chromatids (approx. 35 Mb) of rice ([47,48]; see the electronic supplementary material, figure S1a) up to the very large chromatids (approx. 7450 Mb) of P. japonica (see the electronic supplementary material, figure S1b,b), a species having the largest genome so far reported [49,50]. There are species having chromosomes smaller than those of rice; for instance, chromosome 4 of Drosophila (4 Mb [43]) and the microchromosomes of chicken (approx. 5 Mb [44]). These small chromosomes (sometimes called dot chromosomes because they appear as circular objects in the karyotypes) have not been included in table 1 because their diameters (less than 0.3 μm) are artefactually enlarged in the micrographs owing to resolution problems of optical microscopy (see Methods in the electronic supplementary material).

Despite the large differences in size, metaphase chromatids of different animal and plant species are elongated cylinders that are approximately similar from a geometrical point of view. For the species presented in table 1, the ratio \( \Phi \) between chromatid length \( L \) and diameter \( D \) is higher for large chromatids (more than 1000 Mb; average \( \Phi \approx 19 \)) than for smaller chromatids (less than 1000 Mb; average \( \Phi \approx 9 \)). Chromatids of many more species should be measured to confirm whether these differences are generally valid, but for the approximate calculations presented in this work (see below), it is reasonable to use the average value of \( \Phi \) (≈13) obtained from all chromatids considered in table 1. Taking into account that metaphase chromatose contain 0.17 pg of DNA μm\(^{-3} \), which is equivalent to a density \( \rho \approx 166 \) Mb μm\(^{-3} \), a chromatid containing a specific amount of Mb of DNA \( (N_{MB}) \) has a volume \( V = N_{MB}/\rho \). For a cylindrical chromatid \( V = \pi D^2 L/2 \), but considering that \( L = \Phi D \approx 13D \), the chromatid volume can be expressed exclusively as a function of \( D \),

\[
V = \pi \left( \frac{D^2}{2} \right) \Phi D = \frac{\pi D^3 13}{4}.
\]

Therefore, \( N_{MB}/\rho \approx \pi D^3 13/4 \) and this leads to a formula allowing the calculation of the approximate dimensions \( (D \text{ and } L \text{ in } \mu m) \) of any chromatid containing \( N_{MB} \) of DNA

\[
D \approx \sqrt[3]{\frac{4N_{MB}}{13\pi \rho}} \approx 0.084(N_{MB})^{1/3} \quad L \approx 13D.
\]
The dimensions of chromatids containing different amounts of DNA calculated using this formula as compared with the corresponding observed values are represented in figure 2. There is some deviation of the calculated from the observed lengths for some large chromosomes (Triturus cristatus and P. japonica) but, as can be seen in panels a and b, respectively, most of the values of $D$ and $L$ calculated using this chromosome-dimensions formula fits reasonably well with the experimental results. This indicates that a length to diameter ratio of about 13 represents adequately the approximate shape proportions of metaphase chromosomes.

2.2. Surface energy differences of stacked chromatin layers can explain the morphology and dimensions of metaphase chromosomes

If the short centromere constriction is not taken into account to simplify modelling, condensed chromatids can be considered as smooth cylinders of constant diameter that are limited by two circular plane surfaces, the telomeres. Cylinders and planes have a constant mean curvature that minimizes surface area under a volume constraint [52]. This suggests that chromatin layers have a constant mean curvature that minimizes surface area for a constant volume can be expressed as a function of $R$ and $V$,

$$S = 2\pi R^2 + \frac{2V}{R} \quad (2.3)$$

and the minimum surface area for a constant volume can be obtained by equating to zero the partial derivative of $S$ with respect to $R$

$$\left(\frac{\partial S}{\partial R}\right)_V = 4\pi R - \frac{2V}{R^2} = 0. \quad (2.4)$$

This gives $2\pi R = V/R^2$ and, substituting $V$ by $\pi R^2 L$, it results that $2R = L$. Therefore, in principle, the minimum area corresponds to cylinders having $D = L$. However, as indicated above, native chromatids are much more elongated. In this section, it is shown that the study of the factors that reduce $D$ and the relative surface of the telomeres leads to a simple physical explanation of chromosome morphology and dimensions.

In chromatids formed by stacked layers of planar chromat, the energy $e_{0}$ per nucleosome (figure 3) has two components: the energy $e_{wa}$ due to all interactions within each layer and the energy $e_{wa}$ due to the interactions with the interdigitated nucleosomes of the two adjacent layers.
and the energy $E_{\text{nn}}$ corresponding to the interactions with nucleosomes of two adjacent layers. For a chromatid containing $N_n$ nucleosomes, the energy stabilizing the whole structure is

$$E_0 = N_n e_0 = N_n e_{\text{wl}} + N_n e_{\text{nn}} = E_{\text{wl}} + E_{\text{nn}}.$$  \hfill (2.5)

The observed small thickness of layers and other microscopy results suggested that chromatin plates are interdigitated favouring face-to-face interactions between nucleosomes of adjacent layers ([15,16]; figure 3c). An equivalent interdigitation between the successive helical turns of 30 nm chromatin fibres was proposed previously [2,53,54], and electron microscopy [55,56], X-ray crystallography [1,4,57,58] and modelling studies [5,25–27,59] indicated that nucleosomes have a high tendency to interact through their faces. Furthermore, the intense scattering peak corresponding to a spacing of approximately 6 nm found in a recent synchrotron X-ray scattering study of human mitotic chromosomes [60] also suggests a lateral association between nucleosomes in condensed chromatids. The DNA density in metaphase chromosomes is high [7,51] and edge-to-edge contacts between nucleosome cores must also be considered. However, the attractive energy of edge-to-edge interactions is only approximately 10 times lower than that corresponding to face-to-face interactions [5,25]. Thus, it is very likely that the energy $e_{\text{nn}}$ that stabilizes the stacking of the successive layers in chromosomes is essentially due to face-to-face interactions between nucleosomes. The energy per nucleosome in internucleosome interactions has been obtained experimentally [21,22] and in modelling studies [25–27] in several laboratories (see Introduction); taking into account all the values obtained in these studies, it is reasonable to consider that $e_{\text{nn}} \approx 3.4$–14 $k_B T$. On the other hand, the easy sliding between layers in chromatin plates observed in electron microscopy experiments [15,16], suggested that the forces holding the chromatin filament within a layer are higher that the interactions between adjacent layers. This is consistent with the relatively large the Young modulus [15] and the high mechanical resistance [17] found for chromatin plates in AFM experiments in aqueous media, and indicates that the energy $e_{\text{wl}}$ stabilizing the structure of layers is higher than $e_{\text{nn}}$.

Nucleosomes in the surface of the chromatid are in contact with the medium. These nucleosomes cannot fully interact with bulk chromatin and have an extra energy (figure 3). If $N_T$ and $N_L$ are, respectively, the number of nucleosomes in the surface of the telomeres and in the lateral surface of the chromatid, the total energy is

$$E = E_{\text{wl}} + E_{\text{nn}} + E_T + E_L = N_n e_{\text{wl}} + N_T e_T + N_L e_L,$$  \hfill (2.6)

where $e_T$ and $e_L$ are the energies required for the transfer of one nucleosome from the bulk metaphase chromatin to the surface of a telomere and to the lateral surface of the chromatid, respectively. $E_T = N_T e_T$ is a positive energy because nucleosomes in the telomere surface can only interact with the nucleosomes of one layer; $E_L = N_L e_L$ is also positive because nucleosomes in the lateral surface of the chromatid are exposed to the medium (they are not completely surrounded by nucleosomes) and are less stabilized by interactions with bulk chromatin. These considerations are consistent with the observed smooth surface and constant diameter of the chromatids. As schematized in figure 3, irregular stacking of chromatin layers destabilizes the chromatid, because it increases the lateral surface area and more nucleosomes become exposed to the medium. The destabilizing energy $E_L$ corresponding to a regular stacking (scheme a) is lower than $E_L$ of an irregular stacking (scheme b).

$E_T$ and $E_L$ can be related, respectively, to the areas of the two telomere surfaces ($S_T = 2\pi R^2$) and of the lateral surface ($S_L = 2\pi RL$) of the chromatid. The total surface energy is

$$E_{T+L} = E_T + E_L = S_T \mu_T + S_L \mu_L = 2\pi R^2 \mu_T + 2\pi RL \mu_L.$$  \hfill (2.7)

where $\mu_T$ and $\mu_L$ are surface potentials and correspond, respectively, to the amount of energy required to increase $S_T$ and $S_L$ by unit area. Considering that $V = \pi R^2 L$, the total surface energy can be expressed as a function of $R$ and $V$ and the two surface potentials

$$E_{T+L} = 2\pi R^2 \mu_T + \frac{2V}{R} \mu_L.$$  \hfill (2.8)

To have a maximum stability, native chromatids must have a minimum surface energy. For a constant volume, the minimum surface energy corresponds to

$$\left(\frac{\partial E_{T+L}}{\partial R}\right)_V = 4\pi R \mu_T + \frac{2V}{R^2} \mu_L = 0.$$  \hfill (2.9)

This leads to $\mu_T/\mu_L = V/2\pi R^2$ and, substituting $V$ by $\pi R^2 L$, it results that $\mu_T/\mu_L = L/2R = L/D$. Thus, taking into account that in native chromatids $L/D = \Phi \approx 13$ (see the preceding section), it can be concluded that $\mu_T/\mu_L \approx 13$. Furthermore, according to equations (2.6) and (2.7), $N_T e_T = S_T \mu_T$ and $N_L e_L = S_L \mu_L$, and considering that the areas $S_T$ and $S_L$ must be directly proportional to the number of nucleosomes $N_T$ and $N_L$, respectively, it can be concluded that $e_T/e_L = \mu_T/\mu_L \approx 13$. As nucleosomes in bulk chromatin can interact with nucleosomes of two adjacent layers, but nucleosomes in the telomere surface can interact only with nucleosomes of one adjacent layer, $e_T \approx e_{\text{nn}}/2$. Therefore, taking the value of $e_{\text{nn}}$ considered above, $e_T \approx 1.7$–7 $k_B T$ and $e_L \approx 0.13$–0.54 $k_B T$ per nucleosome. Finally, from the DNA density of chromatids, the number of nucleosomes per unit of surface area can be estimated (approx. $9 \times 10^{12}$ nucleosomes m$^{-2}$) and this allows the calculation of the approximate values of the small surface potentials in telomers and in lateral surface: $\mu_T \approx 0.063$–0.26 mJ m$^{-2}$ and $\mu_L \approx 0.0048$–0.02 mJ m$^{-2}$.

According to the surface energy minimization considered in the preceding paragraph, $\mu_T/\mu_L = L/D = \Phi$. Therefore, if more accurate values of $\Phi$ become available in the future (see preceding section), the $\mu_T/\mu_L$ ratio should be adjusted according to new values. However, to justify the elongated structure of native chromatids, $\mu_T$ must be significantly higher than $\mu_L$. From a structural point of view, the fact that $\Phi > 10$ indicates that nucleosomes in the telomere surfaces are much more in contact with the medium than those in the chromatid lateral surface. This is consistent with the thin-plate model schematized in figure 3, because whereas nucleosomes in telomeres can interact only with one adjacent layer, nucleosomes in the lateral surface are less stabilized by the attractive interactions within the layer but can interact with the nucleosomes of two adjacent layers.

The results presented in figure 4 demonstrate that the surface potentials ratio $\mu_T/\mu_L = 13$ can explain the dimensions of chromatids containing different amounts of DNA and having a DNA density equal to that found experimentally for
Figure 4. Surface energy of metaphase chromosomes as a function of their diameter and length. The plots correspond to chromatids containing different amounts of DNA: (a) 30, (b) 300 and (c) 3000 Mb. The three chromatids have the same DNA density $\rho = 166$ Mb $\mu$m$^{-3}$ [51], and the corresponding volume $V = N_n \rho / \rho$ is maintained constant in each plot (i.e. $R$ and $L$ satisfy the equation $\pi R^2 L = N_n \rho / \rho$). The relative energies of nucleosomes in the surface of the telomeres ($E_T = 2 \pi R^2 \mu T$) and in the lateral surface of the chromatid ($E_L = 2 \pi R \mu L$) were calculated considering that $\mu_T / \mu_L = U / D = 13$ (see text); according to the approximate value of the surface potentials $\mu_T$ and $\mu_L$ given in the text, 1 arbitrary unit $\approx 1.2 \times 10^{-15}$ J. The total surface energy ($E_{T+L} = E_T + E_L$) show minimum values (indicated by blue arrows) corresponding to diameters of approximately 0.3 (a), approximately 0.6 (b) and approximately 1.2 (c) $\mu$m. In each plot, there is a range of chromatid dimensions (indicated by blue rectangles) that have energies closely similar to the minimum value.

2.3. Consistency test for the proposed supramolecular structure: the interactions between adjacent stacked chromatin layers and within layers can explain quantitatively the experimentally observed mechanical properties of metaphase chromosomes

As indicated in the Introduction, metaphase chromosomes can be extended reversibly up to five times their native length. The Young moduli obtained in chromosome extension experiments in the reversible elastic region [30–32,34,35] has allowed the calculation of the stretching work $W_{st}$ (see Methods in the electronic supplementary material, equation (S1.1)) done by the pulling forces applied to chromosomes of different sizes (from approx. 178 (Xenopus laevis) to approx. 3382 (N. viridescens) Mb per chromatid). The results obtained are presented in table 2. The $W_{st}$ obtained from experimental results corresponding to larger (irreversible) extensions of $N. viridescens$ chromosomes (see Methods in the electronic supplementary material) is also presented in this table. In this section, it is shown that the comparison of $W_{st}$ to the energies that stabilize the structure of chromosomes formed by many stacked layers of chromatin leads to a simple physical explanation of the mechanical properties of condensed chromosomes.

The stabilizing energy of chromatin layers within chromosomes containing $N_n$ nucleosomes has two main components (see equation (2.6)): the energy $E_{wl} = N_n \rho \rho_w$ corresponding to interactions within layers, and the energy $E_{inn} = N_n \rho \rho_{inn}$ corresponding to the interactions with nucleosomes of adjacent layers; since $N_T$ and $N_L$ are much lower than $N_n$, the extra energy of nucleosomes in the telomere surfaces ($E_T = N_T \rho_T$) and in the lateral surface of chromatids ($E_L = N_L \rho_L$) are not considered in this section because they cause a relatively small decrease in the total stabilizing energy of bulk chromatin. Furthermore, as indicated in the preceding section, and in accordance with what is expected for a laminar material, $E_{wl}$ is larger than $E_{inn}$. As can be seen in table 2, $W_{st}$ for a fivefold elongation is dependent on chromosome size. Note, however, that for all the chromosomes considered in this table, the values obtained for $E_{inn}$ are higher than $W_{st}$ corresponding to the elastic region. Therefore, these calculations indicate that for this level of chromosome stretching, it is not necessary to consider the strong stabilizing interactions within the layers; the breakage of part of the attractive nucleosome–nucleosome
interactions between layers can justify the work required for extensions up to five times the native length.

A fivefold extension produces a great deformation of the chromosome. However, this deformation does not preclude the recovery of the initial length [32]. From a structural point of view, it seems difficult to justify such a remarkable elasticity, but considering that the nucleosome–nucleosome interactions between layers can be regenerated, the retraction of the chromatin may be produced following a two-dimensional re-zipping mechanism that restores all the initial attractive interactions between nucleosomes in adjacent layers. This reversible transformation is represented with a simplified solid model in figure 5a,b. During elongation, the partial unzipping of the interactions between layers could also be responsible for the high internal viscosity and viscoelastic behaviour of chromosomes observed in micromechanical studies [33]. Furthermore, chromosome bending requires a lateral deformation of the chromosome (figure 5a), but again according to the multilayer chromosome model, presumably this local deformation gives rise to the dissociation of nucleosome–nucleosome interactions between layers. As this mechanism is equivalent to the mechanism of elongation, this can explain the experimental results obtained with *N. viridescens* and *X. laevis* chromosomes [35] showing that the Young modulus calculated from bending results is equivalent to that found in stretching experiments.

The values of *W*~el~ calculated for large extensions of *N. viridescens* (table 2) cannot be explained by considering *E*~mn~ alone. In these irreversible plastic deformations (figure 5c), the high values obtained for *W*~el~ are probably due to breakage of interactions within layers. The total energy *E*~el~ that stabilizes the structure of chromatin layers is not known. However, stretching experiments performed with single chromatin fibres demonstrated that forces larger than those involved in breaking internucleosomal interactions cause nucleosome unwrapping and dissociation ([21,23,24,26,28]; see Introduction). Furthermore, nanotribology results obtained with individual metaphase chromatin plates [17] indicated that during friction measurements, the kinetic energy of the AFM tip produce reversible deformations that probably include nucleosome dissociation, but do not cause DNA breakage even when the forces applied by the tip were higher than those required for the scission of the covalent DNA backbone. Therefore, the work applied to chromosomes for large extensions is probably absorbed by layers through a mechanism involving different levels of unwrapping and dissociation of nucleosomes.

The good mechanical strength observed for chromatid plates is due to the fact that they form a two-dimensional network [17]. Therefore, it is unlikely that the connection between consecutive layers can take place in specific points as represented schematically in figure 6a, because this would produce local heterogeneities in the connection points that could expose the chromatin filament to strong stretching forces without the protection given by the intralayer interactions of bulk chromatin. Considering early work indicating that metaphase chromatids have a global helical structure [9,61,62], it was suggested [16,18] that the chromatid layers could form a continuous helicoidal structure. This gives a feasible geometric solution (figure 6b) that allows chromatin to form a homogeneous two-dimensional network within the chromatids, which protects the integrity of DNA and avoids the random entanglement of DNA within the chromatid. Furthermore, from microscopy results and the experimental observation of mirror-symmetrical positioning of single-copy genes in sister chromatids, it was suggested [62,63] that sister chromatids in metaphase have an opposite helical handedness. Thus, the scheme in figure 6b represents a right-handed helicoid but, according to these observations, both right- and left-handed helicoids are required for the representation of chromatin folding in metaphase chromosomes.

### Table 2. Work required for chromosome stretching and estimated total energy of the internucleosome interactions between stacked layers. The work *W*~el~ (pJ) done by the stretching forces producing chromosome elongations up to 5, 10, 14 and 50 times their native length (*L*) was calculated as described in Methods (see the electronic supplementary material). Young moduli *Y* (kPa) corresponding to the elastic extension of the chromosomes considered in this table were obtained from [30–32,34,35]. The total energy due to nucleosome–nucleosome interactions *E*~mn~ (pJ) was calculated from the number of nucleosomes *N*~n~ in each chromosome (*N*~n~ ≈ number of bp per chromosome/200 bp per nucleosome) and considering previous experimental [21,22] and modelling [25–27] studies showing that the energy *E*~nn~ of a single internucleosome interaction is between 3.4 and 14 kT (*E*~nn~ = *N*~n~*E*~nn~; see equation (2.5)). All the values shown in this table correspond to chromosomes formed by two sister chromatids.

<table>
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<th>chromosome source</th>
<th><em>Y</em> (kPa)</th>
<th><em>W</em><del>el</del> (5<em>L</em>)</th>
<th><em>W</em><del>el</del> (10<em>L</em>)</th>
<th><em>W</em><del>el</del> (14<em>L</em>)</th>
<th><em>W</em><del>el</del> (30<em>L</em>)</th>
<th><em>E</em><del>mn</del> (pJ)</th>
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<td>0.012</td>
<td>0.032–0.13</td>
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<td>0.65</td>
<td>1.7</td>
<td>10</td>
<td>0.47–1.9</td>
</tr>
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</table>

3. Discussion

Typical chromatin plates can be self-assembled from chromatin fragments of metaphase chromosomes, indicating that chromatin structure can hold discontinuous DNA in the correct place within the plates [18]. However, when the relatively high lateral force of the AFM tip was applied to micrococcal nuclease-digested plates in nanotribology experiments, the structural integrity of chromatin layers was maintained only if the degree of digestion was very low [17]. Metaphase chromosomes subjected to stretching forces of 0.2–0.8 nN can tolerate a certain level of digestion with restriction enzymes, but after extensive digestion with micrococcal nuclease the mechanical strength of chromosomes disappears completely [11]; these results demonstrated that chromosomes do not have a mechanically
Reversible and irreversible transformations are indicated by parallel arrows and a single arrow, respectively.

2 left-handed helicoid, but in this case z thickness [15,16], electron microscopy, AFM and electron tomography measurements of layer (continuity of the single chromatin filament contained in a chromatid. (b) A helicoid could facilitate a continuous folding of the chromatin filament without any structural heterogeneity. Note that the side view of a chromatid with N stacked circular layers of radius R formed with thin sheets of planar chromatin in close contact (i.e., having a thickness approximately equal to the distance h between layers) is essentially equivalent to the side view of a helicoid of N turns constructed with a single sheet of chromatin having the same thickness than the planar layers. This right-handed helicoid of pitch h can be defined by the parametric equations: x = r cos θ, y = r sin θ, z = h q/2π, with 0 ≤ r ≤ R and 0 ≤ θ ≤ N 2π. Equivalent equations define a left-handed helicoid, but in this case z = -h q/2π. According to previous electron microscopy, AFM and electron tomography measurements of layer thickness [15,16], h ≈ 5–6 nm.

Figure 5. Simplified solid model for the discussion of some basic aspects of chromosome mechanics. The blue plastic discs represent part of a multilayered unstretched chromatid (a) and after extension up to 5 (b) and 10 (c) times its initial length. It is represented (using staples placed at random locations) that part of the interactions between adjacent layers is not broken even in large extensions. This facilitates the complete recovery (five times extension) or partial recovery (10 times extension) of the native length through a two-dimensional re-zipping mechanism based on the restoration of the initial interactions between layers. (d) It is shown that chromatid bending produces the breakage (concentrated in a lateral region) of the same interactions between layers considered in (b). Reversible and irreversible transformations are indicated by parallel arrows and a single arrow, respectively.

Figure 6. Structural possibilities for the maintenance of the covalent continuity of the single chromatin filament contained in a chromatid. (a) Three-dimensional scheme corresponding to part of a chromatid formed by stacked planar layers; the connections between layers introduce local structural heterogeneities that could be located in internal regions or in the periphery of the chromatid (some peripheral connections are schematically indicated by short vertical lines between layers). (b) A helicoid could facilitate a continuous folding of the chromatin filament within metaphase chromosomes. It will be very interesting to study the mechanism by which chromosomal proteins interact with planar chromatin. In these studies, it has to be taken into account that a significant part of the chromosome volume is occupied by water [67]. This and the relatively weak association between chromatin layers [15,16] suggest that chromosome structure may be dynamic. The rapid diffusion observed for topoisomerase II and other proteins in mitotic chromosomes in vivo [68–70] is consistent with the possibility that condensed chromatids are actually very dynamic structures.

Chromosomes from dinoflagellates do not contain histones and are birefringent. It was suggested that these chromosomes are equivalent to cholesteric liquid crystals consistent of many stacked layers of DNA [71]. Liquid crystals formed in vitro by parallel columns of stacked nucleosome core particles are also birefringent [56]. By contrast, metaphase chromosomes are optically isotropic [16], indicating that they do not contain parallel columns of nucleosomes. In chromatin layers nucleosomes are irregularly oriented [16], but this high degree of disorder at short distances does not preclude the formation of a well-defined multilayered structure that can be considered a lyotropic lamellar liquid crystal, in which the high water
content provides fluidity to the whole system. Lipids and other molecules form spontaneously lyotropic liquid crystals (disordered at molecular distances but with long-range order), which exhibit different phases depending on the lipid concentration and on the solvent composition [52,72]. Self-assembled multilayer plates are formed when the chromatin concentration is high [18] and the buffer contains the relatively high concentrations of Mg$^{2+}$ found in metaphase chromosomes [73].

However, in contrast to current liquid crystals formed by self-assembly of small molecules, in the case of chromosomes a single molecule of DNA occupies the entire volume of each chromatid. This introduces covalent cross-links into the structure in addition to the electrostatic interactions responsible for histone–DNA and nucleosome–nucleosome associations [74–76]. Therefore, as in the case of other soft-matter structures [77], it is better to consider that chromosomes are hydrogels with a liquid crystal organization. Hydrogels containing covalent and ionic bonds are very interesting because they have better mechanical properties than typical hydrogels stabilized exclusively by covalent bonds [78]. As observed for stretched chromosomes in the elastic region, these hydrogels have the capacity of self-healing through a re-zipping mechanism that regenerates the broken ionic interactions. This self-healing capacity is required for the maintenance of chromosome integrity during anaphase [30], because the force exerted by the spindle (approx. 1 nN) is high enough to produce significant deformations of the chromatids. As expected for structures with components having large charges on their surfaces [74–76], divalent cations cause chromatin [2,22,27] and chromosome [67,73] condensation, but it has been observed [79] that crowding agents such as polyethylene glycol can also compact chromosomes even when the ionic concentrations are very low. This suggests that the crowded environment of the cell can generate entropic forces [80] which may also contribute to the recovery of the initial size of deformed chromatids.

Nucleosome unwrapping and dissociation may allow very large chromosome extensions without causing DNA damage. However, as the chromatin concentration in chromosomes is high, in contrast to the detachment of histones observed in unwrapped nucleosomes in individual fibres [21,24], it is very likely that histones of unwrapped nucleosomes in chromosomes remain bound to chromatin. Early studies performed with purified DNA and histones [81], and with chromatin containing histones in excess [82], showed that there are rapid spontaneous reactions for nucleosome re-association and folding. These reactions could facilitate the partial recovery of the initial length observed in chromatides stretched beyond the elastic limit [31,32].

It seems surprising that the difference between the small surface potentials $\mu_T$ and $\mu_L$ can determine the characteristic elongated dimensions of condensed chromatids. However, it has to be taken into account that in self-organizing systems, small energy differences are important even for the formation of very large structures [38,83]; for instance, in biological membranes, the tiny energy differences between lipids in the edges and within bilayers are responsible for the spontaneous closure of vesicles and cell membranes [52]. On the other hand, it is reasonable to consider that a continuous helicoid gives a homogeneous dynamic strength to chromatin within chromatids. Moreover, helicoids are minimal surfaces having zero mean curvature [52] and this may facilitate a maximum contact between the surfaces of the adjacent helicoidal turns, which is very convenient for the recovery of the native chromosome length observed in the reversible elastic region of stretching experiments. Finally, the possibility that planar chromatin may form both right- and left-handed helicoids is also somewhat surprising because both DNA and nucleosomes are chiral structures that have a fixed handedness. Note, however, that in previous in vitro studies of short chromatin fragments from chicken erythrocytes [53,84], the observed frequency of right- and left-handed helical fibres was roughly the same. The switching between right- and left-handed helical architectures has been observed in different chiral polymer systems [85]. Furthermore, it has been reported recently [40] that homochiral M13 virus particles can self-assemble producing micrometre-scale helical structures that can be both right- and left-handed, depending on small external perturbations.

4. Conclusion

The different energy terms considered above are responsible for specific structural and dynamic roles that can justify the global geometry and physical properties of metaphase chromosomes. It has been shown that the stacked chromatin layers generate surface energies that destabilize nucleosomes located in the telomeres and in the lateral surface of the chromatids. The minimization of the lateral surface explains the smooth cylindrical shape of the chromatids, and the symmetry breaking produced by the different values of the surface potentials in the telomeres and in the lateral surface explains the elongated structure of the chromatids. The results obtained in previous experimental studies of chromosome mechanics have been used as a test for the proposed supramolecular structure. It is demonstrated that the intermediate energy of nucleosome–nucleosome interactions between chromatin layers can account quantitatively for the work required for elastic stretching and further reversible recovery of the initial chromosome length. The intralayer energy, which includes the large term corresponding to nucleosome unwrapping, can justify the work required for large chromosome extensions. In summary, it can be concluded that the results obtained in this work provide a consistent physical explanation of the observed cylindrical shape, diameter and length, and mechanical properties of metaphase chromosomes.

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