Optical Properties of Plant Chromosomes during Mitosis

Maria Luiza S. Mello and B. de Campos Vidal

Setor de Citologia e Citopatologia, Instituto de Biologia, Universidade Estadual de Campinas, 13100 Campinas, SP, Brasil

Received November 21, 1972

The systematic exploitation of the anisotropic phenomena induced by complexing the DNA with chromophores has been lately used for the purpose of raising representative data about the macromolecular orientation of the DNA and nucleoproteins under special states of chromosome and chromatin condensations.

The polarizing microscopy has been employed by Kuwada and Nakamura (1934) for the investigation of the plant chromosome framework. The anisotropical properties of animal chromosomes stained with toluidine blue have been used by Vidal (1972a, b) to detect some inhomogeneities (banding) with characteristic dispersions of the birefringence in the chromosome filaments.

In the present work the patterns of chromosome molecular ordering and the eventual modifications on the availability of the DNA phosphate groups which are not complexed with proteins are investigated in plant chromosomes during mitotic process.

Material and methods

Root tips of the onion (Allium cepa) were fixed in Carnoy's fixative for 1 hour, then subjected to a 1 N HCl: ethanol (1: 1) solution for 1 hour, squashed in 45% acetic acid, the coverslip being removed with liquid CO₂. The material was then stained with a toluidine blue solution according to reported procedures (Vidal 1972a, b), dehydrated and mounted in Eukitte.

Observations were made with a Zeiss Pol-photomicroscope equipped with a MPM microscope photometer and an EMI 6256 photomultiplier.

The dispersion of the birefringence was investigated by determining the optical path differences with a λ/20 rotatory compensator according to Bräce-Köhler's, at wavelengths from 480 to 640 nm provided by a monochromator ruler. The long axis of the chromosomes during mitosis and of chromatin filaments (interkaryo-kinesis nuclei) were oriented parallel to the NE-SW or NW-SE directions (45° from the polarizing azimuths of the analyser and the polarizer).

Absorption spectral curves were determined with the unpolarized light extinctions measured with the cytophotometer using a Neofluar 100/1.25 objective, optovar 2.0, photometric diaphragm 0.16 mm and field diaphragm 0.3 mm; the

1 This investigation was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo.
area of the specimen measured was 1.29 μ².

Results

The chromosomes of *Allium cepa* stained with toluidine blue display anisotropic phenomena during all the mitotic phases. In every case under observation

**FIG. 1**

![Graph](image-url-here)  

Fig. 1. Spectral curves of the birefringence and of the unpolarized light absorption on chromatin and chromosomes. I=interkaryokinesis; M=metaphase; A=anaphase; E=extinction; \( \Gamma' \) = optical path differences.
the curves of the dispersion of the birefringence determined for the chromosomes and for the chromatin (interkaryokinesis) showed one inflection point at about 520 nm (anomalous dispersion of the birefringence), the values being negative for light of wavelengths under 520 nm and positive over this wavelength (Fig. 1) (Each point in the curves is the mean of at least 3 measurements). The smallest values of optical path differences (o.p.d.) in the transition zone were displayed by interkaryokinesis chromatin, whereas the metaphase chromosomes yielded the largest ones. The absorption spectral curves on interkaryokinesis and anaphase showed maxima placed at 570 and 570–580 nm respectively (Fig. 1).

One prometaphase cell was specially observed as showing certain chromosomal zones condensed and densely stained (Fig. 2) and which corresponded to highly anisotropic patterns (Fig. 3). Intense birefringence was also observed on the other hand, in zones which correspond to apparently "unbanded" regions. The chromosome parts named A showed a yellow anomalous colour when oriented in NE-SW direction, the same as happened to the zone B, which is placed at 90° from A. The zone C which is parallel to A displayed a bluish-violet colour. The anomalous dispersion of the birefringence of the zone A appeared to be different from those of regions B and C, mainly in terms of the birefringence sign (Fig. 4). Both regions B and C presented one extinction maximum at 580 nm when the absorption spectral curves are taken into account; the absorption curve for the region A, however, apparently has a nearly plateau-like region or two peaks (one at 560 nm and another at 590 nm).
Fig. 4. Spectral curves of the birefringence and of the unpolarized light absorption on different zones (A, B, C) of prometaphase chromosomes. E = extinction; \( \Gamma \) = optical path difference.
Discussion

The general anisotropical properties of the onion chromosomes are in accordance with most of the birefringence observations in toluidine blue-stained L-cells during mitosis (Vidal 1972b), i.e. the chromosomes display birefringence characteristics and the metaphase figures show the largest optical path differences due to a special condensed state of the chromonema. In the anaphase, on account of the chromosome despiralization, the o.p.d. values are recorded as similar to those of the phases which precede the metaphase. The inflection point was always found as being placed at about 520 nm, however the sign of the curves is the inverse of that found in L-cells (Vidal 1972b) and that can be explained in terms of differences in the direction of the DNA filaments spiralization (coilings).

The fact that condensed ("banded") regions of the prometaphase chromosomes visually display high anisotropic values is also in accordance with previous observations in animal somatic cells like mouse L-cells (Vidal 1972b) and in insect meiotic chromosomes (Mello and Vidal 1972b, Mello 1972-manuscript in preparation). (We consider these condensed anisotropic regions as banded in the sense of zones with a more or less heterogeneous distribution of matter as compared with the other regions of the chromosome framework). On the other hand, the intense birefringence phenomenon found in two apparently unbanded zones (as for instance zone A) is probably due to mechanical efforts introduced by the squashing, in a similar way to the findings in stretched polytene chromosome filaments (Vidal 1972-unpublished data) and hemipteran chromatin fibers (Mello 1972-manuscript in preparation).

According to Kuwada and Nakamura (1934) if the chromonema is coiled into a spiral to form the chromosome, each turn of the spiral or the length of the chromonema is disposed perpendicular to the length of the chromosome structure and, therefore, the double refraction which the chromosome presents would be positive with respect to its length; if the chromonema spiral is in the same way thrown again into coiling to form a double-coiled spiral the double refraction of this chromosome would be negative with respect to its length. If we consider that the inversion of the sign of the anomalous dispersion of the birefringence in the zone A was due to a mechanical stretching of the chromosome, this finding would suggest a change in the direction of the DNA fibers with respect to the long axis of the chromosome, i.e. there would be a modification (reduction) in the number and orientation of the coilings in this region as compared with other chromosome regions, such fact being in accordance with Kuwada and Nakamura's hypothesis.

When the affinity of the chromosomal material to the toluidine blue is considered in terms of the wavelengths of the absorption peaks, one observed that it does not practically change during mitosis (570–580 nm). Considering the significance of the metachromasia (Lison 1960) and the relationship between this phenomenon and the proximity of the DNA PO₃⁻ groups where the toluidine blue molecules are attached (Davison and Butler 1956) it can be assumed that the occurrence of chromosome proteins and their relationships with the nucleic acid phosphate groups in fact do not change during this time of the cell life. However in the case
of stretched portions of the chromosomes displaying modified anisotropical patterns, the absorption spectral curves show a plateau-like region where two maxima are nearly suggested (560 and 590 nm). Therefore we can state that mechanical efforts promoted molecular displacements at the level of the chromosome ultrastructure producing longitudinal dislocation of some of the phosphate groups and the separation of toluidine blue dimers as giving rise to absorption peaks at about 600 nm (bathochromic effect) (Davison and Butler 1956, Mello and Vidal 1972).

Summary

Interkaryokinesis chromatin and mitotic chromosomes of the onion root tips stained with toluidine blue display anomalous dispersion of the birefringence with one inflection point at about 520 nm. The largest values of the optical path differences are shown by the metaphase chromosomes which are presumed to be more condensed. Banding associated with intense birefringence was also found in prometaphase chromosomes.

Chromosome proteins and their relationships with the DNA do not change during mitosis as measured by constancy in the wavelength of the light absorption peaks.

The patterns of the anomalous dispersion of the birefringence and of the absorption spectral curves on chromosome regions stretched during the squashing show how the cytophysical findings can tell about changes in the molecular ordering of the chromosome structure.

Acknowledgements

The equipment used in this work was given by Fundação de Amparo a Pesquisa do Estado de São Paulo (68/749), Conselho Nacional de Pesquisas (12607/69) and Alexander von Humboldt Foundation (III–XI/184 vH).

References

 — and — 1972b. Changes in anisotropical properties and nuclear stainability during spermiogenesis in the grasshopper. Submitted to publication.