KINETOCHORE ULTRASTRUCTURE IN VINCristINE-TREATED MAMMALIAN CELLS

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SUMMARY
The ultrastructure of chromosomes from tissue culture cells blocked in division with vin-
cristine sulphate or colchicine was examined. Kinetochores had a distinctive morphology
consisting of trilaminar dense plates closely applied to the chromosomes. In longitudinal
sections, kinetochores appeared as separate plates attached to opposite sides of the chromosomes.
In transverse sections of some prometaphase chromosomes, however, the kinetochore resembled
an incomplete ring-like structure encircling the chromatid pair. These observations indicate
that sister kinetochores may be continuous during early stages of their formation.

INTRODUCTION
The kinetochore (centromere) represents a specialized region on the chromosome
for attachment of spindle tubules of the mitotic apparatus. In electron-microscopic
studies of mammalian chromosomes the kinetochores have been described as osmio-
philic plate or disk-like structures, closely adherent to the chromosome, but with
little organized substructure (Robbins & Gonatas, 1964; Krishan & Buck, 1965;
Murray, Murray & Pizzo, 1965). More recently, this dense plate has been further
resolved into 3 layers, two electron-dense fibrillar zones separated by an intermediate

Previous ultrastructural studies have shown that vincristine sulphate (VCR) is a
potent mitotic inhibitor acting primarily on the spindle tubules (George, Journey &
Goldstein, 1965; Journey, Burdman & George, 1968). Mitotic inhibition with this
drug provided large numbers of chromosomes with intact kinetochores for electron-
microscopic examination. The fine structure of kinetochores in VCR-treated cells
has been described earlier but this report concerns the presence of circular or band-
like kinetochores on prometaphase chromosomes in mammalian cells.

MATERIALS AND METHODS
Chinese hamster fibroblasts, derived from embryonic spleen, were grown as monolayers
in Medium 199, supplemented with 10 % calf serum. HeLa cells maintained in serial culture for
several years were also used in this study. The cultures were treated with 0.2-1.0 µg/ml of
vincristine sulphate (Oncovin, Eli Lilly & Co.) for varying periods and then fixed and processed
for electron microscopy following procedures already published (George et al. 1965). In
several experiments, mitotic inhibition was achieved with 1.0 µg colchicine/ml and the cells
processed in a similar manner.
OBSERVATIONS

Exposure of cells to VCR for periods of 6 h or longer permitted accumulation of large numbers of cells blocked in division. Mitotic inhibition occurs in prometaphase for polar migration of centrioles was prevented and the chromosomes were not arranged along a well defined equatorial plate. A large number of chromosomes with a variety of kinetochore profiles were examined in these blocked cells. Kinetochore regions on chromosomes were apparent even at low magnifications since such regions had a lower electron density than condensed chromatin. In some longitudinal sections, the kinetochores were found at a metacentric location and appeared as a pair of parallel dense bands within a constriction of the chromosome (Fig. 1). In some profiles of chromosomes, only a single kinetochore plate was included in the section (Fig. 2). On other chromosomes, however, the sister kinetochores were localized along chromatids without indentations, and in fact, often arched out from the chromatid surface (Figs. 3, 4). These observations would indicate that, at some stage of formation, the kinetochores are discontinuous paired structures attached to opposite polar sides of the chromatid pair.

At low magnifications, the kinetochores appeared as dense plates embedded in a less electron-dense matrix that was contiguous with the chromatin. At higher magnification, this plate could be resolved into two parallel dense lamina separated by an intermediate light zone (Figs. 3 and 5–7). Each of these layers was about equal thickness, with a relatively uniform thickness (about 36 nm) for the triple unit on different chromosomes. This trilaminar unit was surrounded by a less-dense amorphous or fibrillar zone which was in intimate contact with the condensed chromatin. Measurement of kinetochore length revealed a marked variation among different chromosomes (120–700 nm), some of which may be attributed to random sectioning.

With regard to 3-dimensional organization, in transverse sections of prometaphase chromosomes, numerous examples were seen in which the kinetochore resembled a band almost encircling the chromosomes (Figs. 5–7). In one transverse section (Fig. 8), the separate trilaminar kinetochores applied to opposite poles of the chromosome were connected on one side by a single dense line. The basic architecture of these band-like kinetochores was similar to that seen in longitudinal sections, i.e. trilaminar. This band or girdle, however, was not seen to encompass the chromosome entirely; there were always breaks or gaps in it.

DISCUSSION

Our previous studies have demonstrated that VCR blocks cells in prometaphase by disruption of the mitotic spindle apparatus. The primary cytological effect of VCR was prevention of spindle tubule formation, for tubules were not seen in treated cells except at extremely low concentrations. There were no typical metaphase configurations in drug-treated cells, indicating that spindle tubules are essential for metakinesis, that is, the movement and precise alignment of chromosomes along the equatorial plate.
Other investigators have recognized the kinetochores of mammalian chromosomes as dense plate-like or disk structures (Robbins & Gonatas 1964; Murray et al. 1965) and more recently, they have been found to be composed of 3 distinct layers in colcemid-treated Chinese hamster cells (Brinkley & Stubblefield, 1966). This trilaminar appearance has been found in VCR-treated cells also and represents a distinctive and characteristic feature of kinetochore ultrastructure.

In a study of untreated rat mitotic cells, Jokelainen (1967) described disk-shaped kinetochores located on opposite sides of the metaphase chromosome and found no evidence of structural continuity between sister kinetochores. This report suggested that the kinetochore undergoes structural changes during prometaphase leading to a uniformity in size at metaphase, independent of the particular chromosome or cell type. We found that, although the thickness of the kinetochore plate was uniform, the length of kinetochore plates in sections of prometaphase chromosomes varied considerably. Further studies are necessary to determine whether the lack of uniformity is due to drug treatment, random sectioning, or transient changes in dimension during kinetochore differentiation.

As the chromatin condenses during mitosis, the kinetochore does not appear as a distinct structure until the nuclear envelope begins to break down. This would indicate that the kinetochore develops or takes form in early prophase, presumably at a specific site on any given chromosome. The location of the kinetochore region and length of chromosome arms serve to identify the different chromosomes in aceto-orcein squashes of cells blocked with colchicine. In these preparations, the chromatids remain firmly attached or contiguous in the kinetochore regions, although in many chromosome spreads, the arms of sister chromatids become separated. Of special interest in our study has been the demonstration of structural continuity of the kinetochore plates in some prometaphase chromosomes from VCR- or colchicine-treated cells. In several instances, the kinetochores resembled an incomplete band and there was some evidence of separation into 2 separate kinetochore plates. The transient existence of a band-like kinetochore region may be the structural basis for this point of strong attachment observed on prometaphase chromosomes in light-microscopic preparations.

During anaphase, kinetochore regions lead in poleward movement of chromosomes with their arms trailing behind. In order to obtain this separation of sister chromosomes to daughter cells, the kinetochores would have to be separate and discontinuous. The presence of a band-like structure, observed in our studies, may represent a stage in the early development or differentiation of the kinetochore. This band would then be transformed into 2 separate sister kinetochores at metaphase to allow separation and movement of the chromosomes to their respective poles.

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REFERENCES


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Fig. 1. An oblique longitudinal section through a chromosome from a Chinese hamster cell blocked with 0.2 μg VCR/ml for 2.5 h. The kinetochores (arrows) appear as paired dense plates within a constriction on the chromosome. × 49,000.

Fig. 2. Chromosome from a VCR-treated Chinese hamster cell; this longitudinal section includes only a single kinetochore. × 31,000.

Fig. 3. Two sister kinetochores with an arched configuration embedded in a less-dense matrix. × 51,000.

Fig. 4. Another view of 2 separate kinetochores as viewed in transverse section. × 42,000.
Fig. 5. Transverse section of a chromosome from a VCR-treated HeLa cell. The kinetochoore appears as a single, though incomplete, band around the chromosome. \( \times 44,000 \).

Fig. 6. Another example of the trilaminar and band-like kinetochore in HeLa cells treated with VCR. \( \times 57,000 \).

Fig. 7. Chromosomes from a HeLa cell treated with 1-0 \( \mu \)g colchicine/ml for 20 h. There is a gap in the kinetochore band on one side of the chromosome, but apparent continuity on the opposite side. \( \times 42,000 \).

Fig. 8. Two dense kinetochore plates are connected by a thin dense line on one side of the chromosome in this VCR-treated Chinese hamster cell. \( \times 35,000 \).