Vol. 79

INTERNATIONAL

Review of Cytology

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VOLUME 79

1982

ACADEMIC PRESS *A Subsidiary of Harcourt Brace Jovanovich, Publishers*
New York London
Paris San Diego San Francisco São Paulo Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 52–5203

ISBN 0–12–364479–8

PRINTED IN THE UNITED STATES OF AMERICA

82 83 84 85 9 8 7 6 5 4 3 2 1

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INTERNATIONAL REVIEW OF CYTOLOGY, VOL. 79

The Formation, Structure, and Composition of the Mammalian Kinetochore and Kinetochore Fiber

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I. Introduction

The initiation of chromosome movement during cell division can be correlated with the formation of a fiber, composed primarily of microtubules (MTs) and associated proteins which connects each chromosome to the polar area of the spindle (e.g., Begg and Ellis, 1979a,b). The region on the chromosome where the MTs attach is referred to as the kinetochore and the MTs themselves are known as kinetochore MTs (K-MTs).

In the past ultrastructural studies have provided us with a wealth of information with regards to the structure of kinetochores from a variety of organisms (see reviews of Luykx, 1970; Bajer and Mole-Bajer, 1972; Kubai, 1975; Fuge, 1977; Heath, 1979). However, until recently little was known about the formation and chemistry of this organelle and the mechanism by which it functions. The greatest single factor in slowing progress in this area has been the lack of success in obtaining bulk isolates of kinetochores for *in vitro* analysis. Recently, alternate approaches, including the use of enzymes, immunoelectron, and electron microscopic cytochemical staining procedures, have been developed and have proven useful for dissecting the macromolecular organization of this organelle. Similar-

ISBN 0-12-364479-8

ly, the perfection and routine use of correlative light and electron microscopic methods, of lysed cell systems augmented with polymerization competent MT protein (tubulin), and of potent but reversible inhibitors of MT assembly have also advanced our understanding concerning the composition and formation of the kinetochore fiber (K-Fiber).

The purpose of this article is to summarize recent advances in understanding the formation, structure, and chemistry of the mammalian kinetochore and its associated fiber. Its narrrow emphasis reflects an almost complete lack of knowledge concerning the formation and chemistry of kinetochores in other types of organisms.

II. Kinetochore versus Centromere

The terms "centromere" and "kinetochore" were coined by early light microscopists as synonyms to denote that region on the chromosome which becomes attached to the spindle during mitosis and meiosis (see reviews of Schrader, 1953; Ris and Witt, 1981). In most organisms this region corresponds to a narrow constriction on the chromosome known as the primary constriction (Fig. 1). The primary constriction frequently contains a chromatin component, situated peripheral to the kinetochore, which fails to decondense after cell division. This "constitutive heterochromatin" (Brown, 1966) is similarly more resistant to treatments which disperse the remainder of the chromosome (e.g., Rattner *et al.*, 1975, 1978; Roos, 1977; Brinkley *et al.*, 1980; Ris and Witt, 1981). It can be detected with the light microscope (LM) after various staining procedures (see reviews of Commings *et al.*, 1973; Commings, 1978). The DNA within this "pericentromeric" heterochromatin is generally replicated later in S phase than the bulk of the remaining chromosomal DNA and contains, in some organisms (e.g., calf, mouse, humans, flies), tandem arrays of highly repeated nucleotide sequences (i.e., satellite DNA–see Rae, 1972; John and Miklos, 1979).

The heterochromatin of the primary constriction is appropriately referred to as pericentromeric or procentromeric due to its location peripheral to the kinetochore (centromere). It should be stressed that its exact relationship with the kinetochore as well as its function remain unknown. Some have suggested that it is involved in (1) chromatid adhesiveness until the onset of anaphase (Stubblefield, 1973; Vig, 1981), (2) synapsis and recombination during meiosis (Miklos and John, 1979), (3) karyotype evolution via translocations of the Robertsonian type (references in Yunis and Yasmineh, 1972), or (4) protecting the kinetochore from evolutionary changes (Yunis and Yasmineh, 1972). The proximity of the pericentromeric heterochromatin to the kinetochore has even led some investigators to speculate that the (mammalian) kinetochore is formed, in

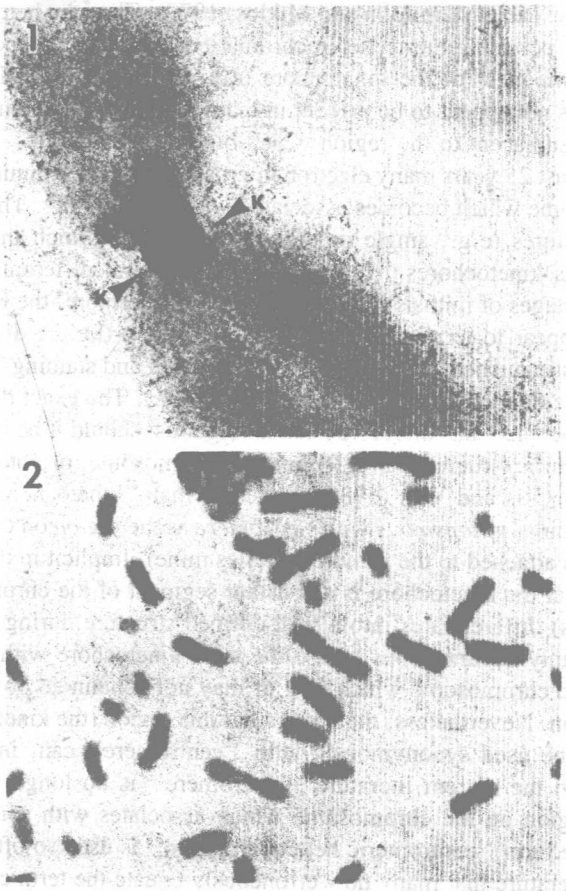

FIG. 1. Mouse L929 chromosomes isolated from a colcemid-arrested mitotic cell by detergent lysis. The kinetochores (K) appear as electron opaque plaques on the surface of the pericentromeric heterochromatin (H). See text for details. ×12,800. (Courtesy of J. B. Rattner.)

FIG. 2. Human metaphase chromosomes stained by a Giemsa technique (C_d staining) which reveals two identical dots, one on each side of the centromere. See text for details. (Courtesy of H. Eiberg by permission of *Nature* (*London*), 1974.)

part, from DNA continuous with the heterochromatin (e.g., Brinkley and Stubblefield, 1970; Yunis and Yasmineh, 1972; Hennig, 1973; Pepper and Brinkley, 1980). This is not an unreasonable assertion in light of the recent evidence suggesting the presence of DNA in the kinetochore (see Section IV). Yet heterochromatin (and satellite DNA) has not been detected in and may be absent from the primary constriction of a number of plants and animals including some

mammals (see Table VI in John and Miklos, 1979). Thus the heterochromatized DNA of the pericentromeric heterochromatin should not be considered as a structural component of the kinetochore (e.g., Nicklas, 1971; Brenner *et al.*, 1980): it does not appear to be present in many organisms and when it is present it is located subjacent to the region which binds spindle MTs.

Over the past 25 years many electron microscopists have examined the area on the chromosome which becomes associated with spindle MTs. They often found distinct structures (e.g., single or multilayered disks) which they specifically referred to as kinetochores. However, such structural differentiations are not found in all stages of mitosis (e.g., prophase). Furthermore, the K-MTs in some organisms appear to terminate directly on chromatin (i.e., a discrete structure cannot be distinguished, after conventional fixation and staining, from the chromosome—see Section III; also Ris and Witt, 1981). The exact definition of the term "kinetochore" has therefore been ambiguous: should it be used with reference to a specific structure, a region on the chromosome, or both? To eliminate this confusion Ris and Witt (1981) suggested that "kinetochore be used in its original meaning, *synonymous* with *centromere* as the *region* on the chromosome that becomes attached to the spindle" (italics mine). Implicit in this definition is the notion that the kinetochore is a constant segment of the chromosome which (in mammals) differentiates into a well-defined structure during mitosis.

It is certainly advantageous to use the term kinetochore with reference to a *region* on the chromosome which may or may not contain an obvious structural differentiation. Nevertheless, the notion that this region (the kinetochore) should continue to be used synonymously with "centromere" can, in itself, lead to confusion. In the current literature "centromere" is no longer equated solely with that region on the chromosome which associates with spindle MTs. For example, the term "centromeric heterochromatin" is used so often in the ultrastructural literature that many now erroneously equate the term centromere with the heterochromatin of the primary constriction (e.g., Fuge, 1977; Roos, 1977; Brinkley *et al.*, 1980) even though this heterochromatin cannot be considered to be a part of the kinetochore (see above). This association leads to such statements as "the kinetochore is a specialized chromosomal structure situated on the surface of the centromere" (Alov and Lyubskii, 1977) or, "this observation indicates that the kinetochores are physically distinct from the centromere" (Brenner *et al.*, 1980). Obviously, in these instances the terms kinetochore and centromere are not used as synonyms.

A similar situation is encountered in the current LM literature where centromere (and even kinetochore) is used to describe stained or unstained "dots" in the region of the primary constriction (Fig. 2). It is clear that the centromere (kinetochore) can sometimes be seen with the LM under suitable circumstances (e.g., Lima-de-Faria, 1958; Hard and Allen, 1977; Clapham and Ostergren, 1978). On the other hand, in many cases one can argue, as Roos (1975) has, that

these preferentially stained regions may in reality be areas of more densely packed heterochromatin, protein components unique to regions of this heterochromatin (e.g., Matsukuma and Utakoji, 1977), or even clear zones similar to those sometimes seen adjacent to the kinetochore in the electron microscope (e.g., Roos, 1975; Heneen, 1975a; Fig. 12 of Rieder and Borisy, 1981).

For example, Marks (1975; see also Denton *et al.*, 1977; Brat *et al.*, 1979) considered his differentially stained dots in the primary constriction of *Nigella* chromosomes to be kinetochores (centromeres) even though these dots bridged "the space across each chromatid." Brown and Loughman (1980) noted that C-banding is often used to locate the centromere/kinetochore (whereas in reality it locates the pericentromeric heterochromatin) and they then developed a silver stain which "unequivocably stains the centromeres" of Indian muntjac (minature deer) chromosomes. Similarly, Alves and Jonasson (1978) developed a direct Giemsa technique which they used "for the detailed cytological study of the mouse kinetochore." However, they stress that their observations indicate that they are staining mouse satellite DNA which they then equate with centromeric heterochromatin, which they consider equivalent to the *kinetochore (centromere)*. Finally, Moroi *et al.* (1981) attempted to localize, at the electron microscope (EM) level, antigen(s) which they had previously (Moroi *et al.*, 1980) shown with the LM to specifically bind to the centromere of mammalian chromosomes. At the ultrastructural level, though, these "centromere" antigen(s) were found to be distributed over a large area corresponding the the whole of the primary constriction (see however Brenner *et al.*, 1981), an observation which was interpreted by these authors to indicate the specificity of the antibody(s) to the centromere.

It is clear from the few examples noted above, and there are *many* more, that the (mis)use of the term "centromere" has progressed to the point where many even define it as a synonym for the *primary constriction* (e.g., DuPraw, 1970; Stack, 1974; Fuge, 1977; Clapham and Ostergren, 1978; Bostock and Sumner, 1978; Holmquist and Dancis, 1979; McIntosh, 1979). This being the case one can no longer argue that the terms kinetochore and centromere be used as synonyms, regardless of their original definition. It is no surprise that electron microscopists favor the term kinetochore since they are not referring to an area on the chromosome as broadly defined as the "centromere" currently is.

To eliminate this confusion I suggest that the term kinetochore be used as defined by Ris and Witt (1981) to note, at the ultrastructural level, *the precise region on the chromosome that becomes attached to spindle MTs*. In mammalian cells this region differentiates into a trilaminar disk structure during mitosis and appears to contain unique components (see Section IV) not found in the adjacent heterochromatin or on the remainder of the chromosome. However, unlike Ris and Witt (1981) I suggest that the term centromere be used, as it is *now* often used by cytologists and geneticists, in a less precise manner to note *the region on*

the chromosome (e.g., the primary constriction, pericentromeric heterochromatin, etc.) with which the kinetochore is associated.

III. Types of Kinetochores

The chromosomes in some organisms (particularly among the monocotyledons and arthropods—see Schrader, 1953; Maeki, 1980) lack a primary constriction and the chromosomal spindle fibers appear at the LM level to terminate along the entire length of each chromatid. These features, combined with the fact that the separating sister chromatids remain uniformly parallel to one another during anaphase (i.e., they exhibit holokinetic movement—Bauer, 1952), prompted Schrader (1935) to suggest that these chromosomes possessed a "diffuse" or "nonlocalized" kinetochore. This view gained considerable experimental support by the finding that chromosome fragments, generated in these organisms by UV irradiation, continue to function in a normal manner throughout mitosis (see references in Schrader, 1953; Hughes-Schrader and Schrader, 1961).

Data concerning the ultrastructure of diffuse kinetochores are sparse and are based, for any one organism (except *Luzula*), on random nonserial sections. Nevertheless, it suggests the existence of at least two morphologically distinct types of diffuse kinetochores. In the mitosis of *Rhodnius* (Buck, 1967) and *Oncopeltus* (Commings and Okada, 1972) the kinetic activity occurs along most or all of the chromosome length, and the kinetochore material is evenly distributed along its poleward edge in a loosely defined "plate" which characteristically stains lighter than the chromatin. This type of chromosome is generally referred to as holocentric (e.g., Braselton, 1971, 1981; Commings and Okada, 1972). On the other hand, the MTs associated with the meiotic kinetochores in *Philaenus* (Ris and Kubai, 1970), Lepidoptera (Maeki, 1981), and *Bombyx mori* (Friedlander and Wharman, 1970) appear to terminate at multiple sites along the chromosome directly on the chromatin. This type of chromosome is often referred to as polycentric. A reconstruction of mitotic chromosomes in *Luzula* and *Cyperus* (Braselton, 1971) suggests that the kinetic activity in these organisms is also restricted to numerous discrete units along the chromosome since the kinetochore material appears to be distributed as multiple, light staining irregularly shaped packets, which are often recessed within the chromosome. However, Braselton's (1971, 1981) conclusion that *Luzula* chromosomes are polycentric has been questioned by Bokari and Godward (1980) who also reconstructed the kinetochores of metaphase *Luzula* chromosomes from serial sections and concluded that a single kinetochore extends continuously along most of the length of each chromatid (as in *Rhodnius* and *Oncopeltus*). These authors note that fixation and embedding induces a lateral side-by-side adherence of adjacent chromosomes in *Luzula* and that photomicrographs of sections through these

fused chromosomes can easily be erroneously interpreted as photographs of single chromosomes with multiple kinetochores. Similarly, the polycentric condition reported to exist in the alga *Spirogyra* (Mughal and Godward, 1973) appears to have also been based on images of localized kinetochores on adjacent overlapping chromosomes.

An LM analysis of fixed specimens suggests that the kinetic activity in those Hemiptera which possess diffuse kinetochores becomes restricted to the terminal region of the chromosome during anaphase of meiosis (Hughes-Schrader and Schrader, 1961). This modified behavior does not appear to involve an irreversible change in the distribution of the kinetochore material since fragments of meiotic chromosomes continue to divide normally (Hughes-Schrader and Schrader, 1961). Indeed, the available ultrastructural data on Hemiptera (*Rhodnius* and *Oncopeltus*) indicate that the meiotic chromosomes lack the plate-like kinetochore structure characteristically associated with mitotic chromosomes; the K-MTs appear to terminate instead along the body of the metaphase chromosome within the chromatin (i.e., these chromosomes are now polycentric). However, there is currently no ultrastructural evidence to indicate that these K-MTs become redistributed and/or are restricted, during early anaphase, to the terminal ends of the chromosomes (e.g., Buck, 1967). Rather, Commings and Okada (1972) have suggested that the change in kinetochore structure between mitosis allows for the terminalization of chiasmata. In this respect, the terminalization of chiasmata may lead to a restriction of anaphase kinetochore activity which is more apparent than real.

The diffuse kinetochores of some organisms (e.g., *Philaenus*, Lepidoptera) appear to consist primarily of DNA and protein (i.e., chromatin) since, after conventional fixation and staining, the associated MTs appear to terminate directly on the chromatin without evidence of an additional structural component. On the other hand, the kinetochores on all of the holocentric chromosomes (including *Luzula*) contain additional material which stains lighter than the chromatin. In one case (*Luzula;* Braselton, 1980) this material has been shown to contain an RNase-sensitive component which can be selectively stained by a method (Bernhard, 1969) which preferentially stains ribonucleoprotein (RNP). To my knowledge there is no additional information regarding the cytochemistry of diffuse kinetochores.

In contrast to the diffuse kinetochore, the kinetochore in most organisms is located on only a small segment of the chromosome (i.e., the primary constriction—see Section II). During anaphase this segment leads the way poleward, bending the chromosome into the familiar "V" or "J" shape. Early investigators found that these "localized" kinetochores could, in some cases, be preferentially stained (see reviews of Schrader, 1953; Lima-de-Faria, 1958; Mazia, 1961). However, their small sizes (in most cases about the limit of resolution of the LM) prohibited a detailed analysis of their composition and structure.

Ultrastructural investigations have since revealed that the localized kinetochore varies considerably in structure according to the stage of division and the organism. In general it appears during metaphase as either (1) a single or multilayered disk on the surface of the chromosome (e.g., mammals, some algae, insects, slime moulds, protozoa, ciliates—see references in Luykx, 1970; Fuge, 1974, 1977; Heath, 1979; Bostock and Sumner, 1978), (2) a ball of ill-defined material embedded in a more electron opaque chromatin cup (e.g., most plants including *Haemanthus, Lilum, Allium*—see references in Bajer and Mole-Bajer, 1972; Alov and Lyubskii, 1977), or featureless and difficult to differentiate from the chromatin (e.g., some insects, fungi, yeast, and protista—see references in Luykx, 1970; Kubai, 1975; Fuller, 1976; Heath, 1979). These latter types generally possess only one K-MT.

The remainder of this article will focus primarily on the formation, structure, and composition of the mammalian kinetochore (and its associated fiber). For a more detailed description of kinetochore morphology, in a variety of nonmammalian cells, the reader should consult the reviews cited above.

IV. Mammalian Kinetochore Structure and Chemistry

A. Interphase

Discrete patches of material shown to be precursors of mitotic kinetochores are visible within the interphase nuclei of some plants (Church and Moens, 1976; Moens and Moens, 1981) and protozoa (e.g., Kubai, 1973; Ris and Kubai, 1974). Similar structures are not apparent in the interphase nuclei of mammals after conventional fixation and staining. It has recently been shown however that sera from patients with the CREST variant of progressive systemic sclerosis contain high titers of an antibody which binds specifically to the centromere region of chromosomes from mammals (Moroi *et al.*, 1980; Tan *et al.*, 1980; Fritzler and Kinsella, 1980), flies (Will *et al.*, 1981), and probably to the same region in other types of organisms. This antibody has been demonstrated by indirect immunoelectron microscopy to be specific for antigen(s) associated with the mitotic kinetochore (Brenner *et al.*, 1981; Fig. 3). More important in the context of this section is the fact that this antibody binds to discrete spherical patches, approximately 0.22 μm in diameter, within the nuclei of *interphase* mammalian cells (Figs. 4 and 5). The number of these interphase staining foci corresponds with the number of chromosomes within the cell. Since this antibody binds to mitotic kinetochores, as well as localized foci within interphase nuclei, at least some component(s) of the mitotic kinetochore remain associated with the interphase chromatin throughout the cell cycle. This observation prompted Pepper *et al.* (1980) to suggest "that a kinetochore organizer exists in the genome which retains some structural integrity in the decondensed chromatin of interphase nuclei" (see also Section V).

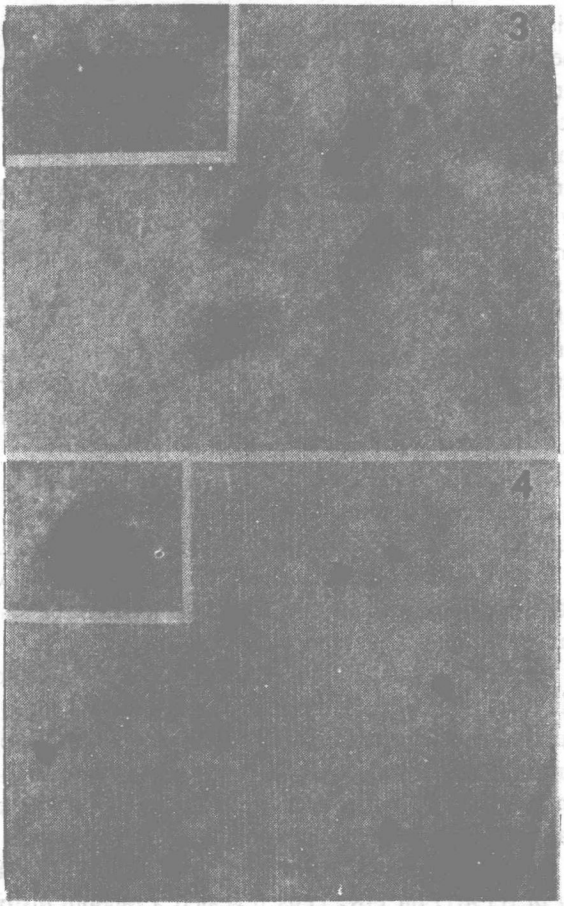

FIG. 3. Electron micrograph of colcemid-treated PtK_2 kinetochores stained with the anti-kinetochore serum by the immunoperoxidase method. Note the double-layered appearance of the kinetochore and the specificity of the staining reaction for this structure. ×16,000; inset, ×32,000. (From Brenner *et al.*, 1981.)

FIG. 4. Immunoperoxidase localization of the kinetochore antigen (Fig. 3) in an interphase PtK_2 cell. The antigen is restricted to localized foci or "presumptive kinetochores." See text for details. ×12,800; inset, ×42,300. (From Brenner *et al.*, 1981.)

Brenner *et al.* (1981) refer to these interphase staining foci as "presumptive kinetochores" because of their lack of structural similarity to similarly stained regions on metaphase chromosomes. These investigators note that the pre-kinetochores in PtK_2 (male rat kangaroo) cells become visibly duplicated during late G_2 of the cell cycle, after the completion of DNA synthesis (Fig. 5). At present it remains an open question as to whether this antigen becomes associated with the newly replicated chromatin prior to, during, or after the condensation of

the later replicating pericentromeric heterochromatin. (In this respect the question of whether prekinetochores are formed, in part, from the condensation of the pericentromeric heterochromatin can be approached by determining whether similar structures are present in mammals which appear to lack this heterochromatin.)

Information concerning the nature of this kinetochore antigen was obtained by Moroi *et al.* (1980) who first attempted to solubilize it with different reagents in hopes of later identifying the antigen–antibody complex. The lack of success of this method (see also Cox *et al.*, 1980) forced them to pretreat periodiate/lysine/paraformaldehyde-fixed RAMOS cells with a variety of enzymes and chemical reagents prior to reaction with the antiserum. Their results, assayed by an absence or significant decrease of immunofluorescent staining intensity, indicated that the antigen was probably a protein tightly bound to centromere DNA (see also Cox *et al.*, 1980) since it was destroyed by DNase and trypsin but not by RNase (Fig. 6). Brenner *et al.* (1981), using serum supplied by Moroi, further characterized this antigen by noting that the immunofluorescent staining intensity of the kinetochore was not diminished by initially preabsorbing the serum with tubulin, actin, or microtubule-associated proteins (MAPS). This was interpreted to indicate that the antigen was not a previously recognized or suspected component of the kinetochore (see Sections IV,C and D).

The ability to specifically stain interphase prekinetochores allows one to approach the question of how these structures are arranged three dimensionally within the interphase nucleus. Moroi *et al.* (1981) found that the prekinetochores of human lymphoid and Chinese hamster cells were associated predominantly with either the surface of the nuclear envelope (NE) or with nucleoli. They concluded that the "centromere regions of the chromosomes in interphase are not randomly distributed within the nucleus." At the same time a similar study by Brenner *et al.* (1981) on PtK_2 cells revealed a random distribution of prekinetochores with no consistent association with the NE. They did note that a few prekinetochores had an affinity for the nucleoli, but they considered this to be a manifestation of the proximity between the kinetochore and the nucleolar organizer on the sex chromosomes of PtK_2. Although the results of these two studies differ, it is apparent from both that prekinetochores in mammalian cells are not clustered or polarized within the interphase nucleus as they are in *Allium* (Church and Moens, 1976). (This difference may be due, in part, to the stability of the chromocenter in those plant cells which have a long G_1 phase of the cell cycle.)

The immunological studies described above should be considered at present as preliminary since the possibility exists that the serum used by these investigators contained a variety of antibodies—some of which bound to antigens found only on mitotic kinetochores and others which bound to totally different antigens unique to the so-called "presumptive kinetochores" of interphase cells. Even though these results need confirmation with a monoclonal antibody system, they

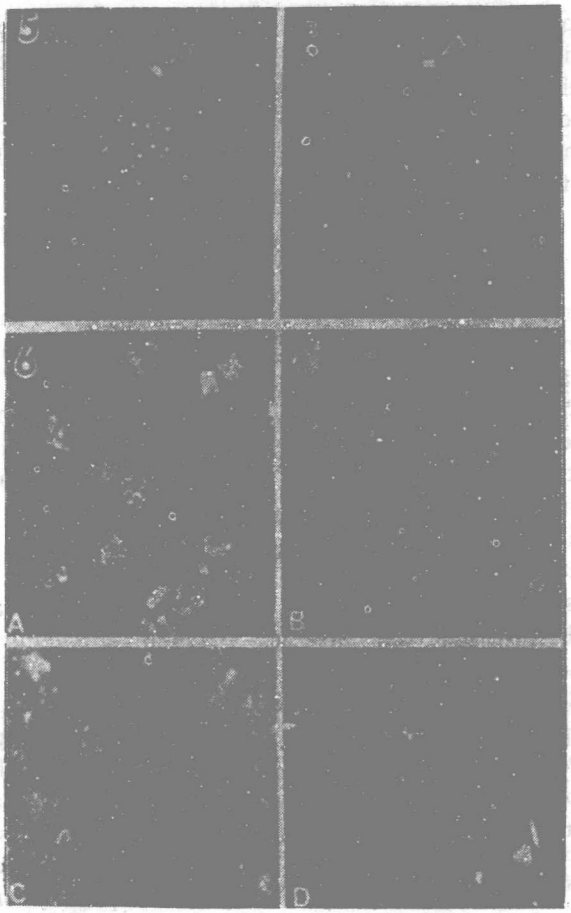

FIG. 5. (A and B) Interphase presumptive kinetochores as seen by indirect immunofluorescence. These cells have also been double-stained with anticentrosome antisera to reveal the centrosome (arrow). (A) Early interphase cell with single presumptive kinetochores. (B) Late interphase cell with double presumptive kinetochores. See text for details. ×832. (From Brenner *et al.*, 1981.)

FIG. 6. (A–D) The effects of various enzymatic treatments on the indirect immunofluorescent staining of interphase presumptive kinetochores with the kinetochore antibody in RAMOS cells (human B lymphocyte cell line). ×300. (From Moroi *et al.*, 1980.) (A) Control. (B) Image after digestion with DNase I. Kinetochores no longer stain after this treatment. (C) Image after digestion with RNase A. No significant changes in the kinetochore staining pattern are visible. (D) Image after digestion with a combination of trypsin and 0.01% $NaDOdSO_4$. Kinetochores no longer stain after this treatment (however, digestion with trypsin alone does not remove the kinetochore antigen—not shown).