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Centrosome and Microtubule Instability in Aging *Drosophila* Cells

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Abstract Several cytoskeletal changes are associated with aging which includes alterations in muscle structure leading to muscular atrophy, and weakening of the microtubule network which affects cellular secretion and maintenance of cell shape. Weakening of the microtubule network during meiosis in aging oocytes can result in aneuploidy or trisomic zygotes with increasing maternal age. Imbalances of cytoskeletal organization can lead to disease such as Alzheimer's, muscular disorders, and cancer. Because many cytoskeletal diseases are related to age we investigated the effects of aging on microtubule organization in cell cultures of the *Drosophila* cell model system (Schneider S-1 and Kc23 cell lines). This cell model is increasingly being used as an alternative system to mammalian cell cultures. *Drosophila* cells are amenable to genetic manipulations and can be used to identify and manipulate genes which are involved in the aging processes. Immunofluorescence, scanning, and transmission electron microscopy were employed for the analysis of microtubule organizing centers (centrosomes) and microtubules at various times after subculturing cells in fresh medium. Our results reveal that centrosomes and the microtubule network becomes significantly affected in aging cells after 5 days of subculture. At 5–14 days of subculture, 1% abnormal out of 3% mitoses were noted which were clearly distinguishable from freshly subcultured control cells in which 3% of cells undergo normal mitosis with bipolar configurations. Microtubules are also affected in the midbody during cell division. The midbody in aging cells becomes up to 10 times longer when compared with midbodies in freshly subcultured cells. During interphase, microtubules are often disrupted and disorganized, which may indicate improper function related to transport of cell organelles along microtubules. These results are likely to help explain some cytoskeletal disorders and diseases related to aging. J. Cell. Biochem. 74:229–241, 1999.

Key words: cytoskeleton; mitosis; cell division; cell culture; abnormal polarity

The cytoskeleton plays crucial roles in maintaining a variety of cellular functions from fertilization throughout development. During aging, cytoskeletal functions decrease [Taylor et al., 1992; Schultz et al., 1997] which can lead to disease such as Alzheimer's [Schwarz et al., 1996] and heart dysfunctions [DeMeester et al., 1998], and can ultimately result in cell death [DeMeester et al., 1998; Schwarz et al., 1996; Lewis et al., 1998]. Several cytoskeletal alterations are associated with aging which includes structural changes in microfilament organization leading to muscular atrophy. The effects of aging on the microtubule system includes changes in cellular secretion [Patrone et al., 1992], in wound healing, and in transport of cell organelles [Skoufias and Scholey, 1993; Schatten and Chakrabarti, 1998]. Microtubule dysfunction during aging has also been linked to the rise in aneuploid zygotes with increasing maternal age [Eichenlaub-Ritter et al., 1988a,b]. Aneuploidy, i.e., abnormal numbers of chromosomes, can result in Down's syndrome. Microtubules are crucial for maintaining cell shape [Fulton, 1984], for protecting the cell against shear forces [Lazarides, 1987], for transport of cell organelles and intracellular particles [Koonce and McIntosh, 1990], for guiding RNA and proteins to appropriate locations [Fyrberg and Goldstein, 1990], and for the formation of the mitotic apparatus during cell division [Mazia, 1961]. The organization of microtubules during cell division is regulated by centrosomes, central cytoskeletal cell organelles which are composed of a variety of different proteins, some of which are intrinsic and permanently

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associated with centrosome structure [Joswig and Petzelt, 1990; Joswig et al., 1991], while others are reorganized throughout the cell cycle and serve cell-cycle specific functions [Petzelt et al., 1997a,b]. Structural changes are not only observed throughout the cell cycle but they can be experimentally induced under altered ionic conditions [Schatten et al., 1992], with agents such as dithiothreitol [Schatten, 1994], chloral hydrate and diazepam [Schatten and Chakrabarti, 1998], or with heat shock [Debec and Marcaillou, 1997]. This argues that centrosomes and microtubule organization are susceptible to chemical and environmental alterations. It also suggests that they are susceptible to altered metabolism and ionic conditions during aging.

Centrosomes are structural cell components which are closely associated with the nucleus during interphase. They duplicate during S-phase [Balczon, 1997] and become reorganized and distributed to the two mitotic poles during cell division. Centrosomes organize the bipolar mitotic apparatus and are therefore crucial for chromosome separation. Centrosomes split during telophase and become equally distributed to the daughter cells. This program holds true during normal cell division but becomes altered under adverse conditions [Schatten and Chakrabarti, 1996, 1998; Debec and Marcaillou, 1997]. Abnormal centrosomes have been implicated in cases of infertility [Schatten, 1994] and in diseases such as cancer [Boveri, 1914; Pihan et al., 1997; Lingle et al., 1998; Schatten et al., 1998a,b]. Centrosome abnormalities will lead to improper microtubule organization and unequal chromosome separation resulting in aneuploidy. We report in this paper that abnormal centrosomes are found in cells during aging, which may indicate structural instabilities and centrosome disorders which can lead to abnormal chromosome distribution that is also implicated in age-related cancers.

MATERIALS AND METHODS

Cell Lines and Culture

The Schneider S-1 and Kc23 cell lines were cultured as monolayers in corning polystyrene 25 cm^2 canted neck sterile tissue flasks with 0.2 µm vented caps. The *Drosophila* Kc23 cell line was cultured in D22 insect medium (Sigma, St. Louis, MO) supplemented with 5% fetal bovine serum (Hyclone, Logan, UT). The Schneider cell line was cultured in Schneider's medium (Sigma) supplemented with 15% fetal bovine serum (Hyclone). The cultures were kept in 23°C controlled environment incubator and subcultured in a sterile vertical air flow hood after either 3 or more days, depending on the nature of the experiments, with a final dilution of 1×10^6 cells/ml.

Fluorescence and Immunofluorescence Microscopy

Cells detached from the flasks with a cell scraper were prepared for fluorescence and immunofluorescence microscopy by first washing them in phosphate-buffered saline (PBS) and then attaching them to coverglass coated with poly-L-lysine (1 mg/ml; Sigma). After 3–5 min, cells attached to the coverglass were processed for the detection of cytoskeletal structures as follows.

Microtubule detection followed the protocols described before [Schatten et al., 1988; Schatten, 1994]. Briefly, cells were fixed for 6 min in cold methanol at −20°C, rehydrated in PBS, and stained with a 1:5 dilution of the mouse monoclonal antibody E7 (Developmental Studies, Hybridoma Bank, IA) which recognizes β-tubulin [Schatten, 1994]. Cells were washed three times for 20 min each in PBS containing 0.1% Triton X-100 (PBST) followed by second antibody staining with 1:40 diluted FITC-conjugated goat anti mouse antibody (Zymed, San Francisco, CA). After three washes in PBST, DAPI (Sigma) was added at a concentration of 4 µg/ml in the final wash and cells were mounted in Moviol (Calbiochem, San Diego, CA) containing 2.5% DABCO (Aldrich, Milwaukee, WI).

Centrosome detection followed the protocols described in Schatten et al. [1986, 1987, 1988] with modifications as follows. Cells were fixed in 3.7% formaldehyde for 10 min, followed by three washes in PBS, fixation in methanol at −20°C for 3–5 min and three washes in PBS. Distinct centrosome staining was detected with either the human autoimmune serum 5051 [Calarco et al., 1983] diluted 1:100 or with the mouse monoclonal MPM2 antibody (Upstate Biotechnology Inc., Lake Placid, NY) diluted 1:100 which recognizes phosphorylated epitopes of centrosomes in mitotic tissue culture cells [Vandre et al., 1984] and stains centrosomes as previously described [Schatten and Chakrabarti, 1994]. Cells were either treated with a secondary human antibody diluted 1:40 for detection of 5051 or with the mouse IgG

antibody diluted 1:40 for the detection of MPM2. DNA was stained with DAPI (Sigma) at a concentration of 4 μg/ml. Cells were analyzed with a Zeiss Axiophot microscope equipped with appropriate epifluorescence filters. Parallel observations of living cells were performed on an Olympus light microscope to verify the accuracy of results obtained on fixed samples.

Scanning Electron Microscopy

Schneider S-1 and Kc23 cells were fixed in 1% glutaraldehyde in 0.08 M sodium cacodylate buffer (pH 7.4) for 1 h at room temperature and washed in the same buffer. The samples were post-fixed with 1% osmium tetroxide in distilled water for 1 h, dehydrated in a graded series of ethanol, critical point dried, sputter coated with gold, and viewed in a JEOL JSM-35 scanning electron microscope.

Transmission Electron Microscopy

Cells were fixed with 2.5% glutaraldehyde and 0.05% formaldehyde mixture in 0.08 M sodium cacodylate buffer (pH 7.4) for 1 h at room temperature and washed in the same buffer. The samples were post-fixed with 1% osmium tetroxide in distilled water for 1 h, dehydrated in ethanol, and embedded in Epon or soft Spurr [Spurr, 1969]. Ultrathin sections were contrasted with 3% aqueous uranyl acetate and lead citrate [Reynolds, 1963] before analysis with a Philips 410 or a JEOL 1200EX transmission electron microscope.

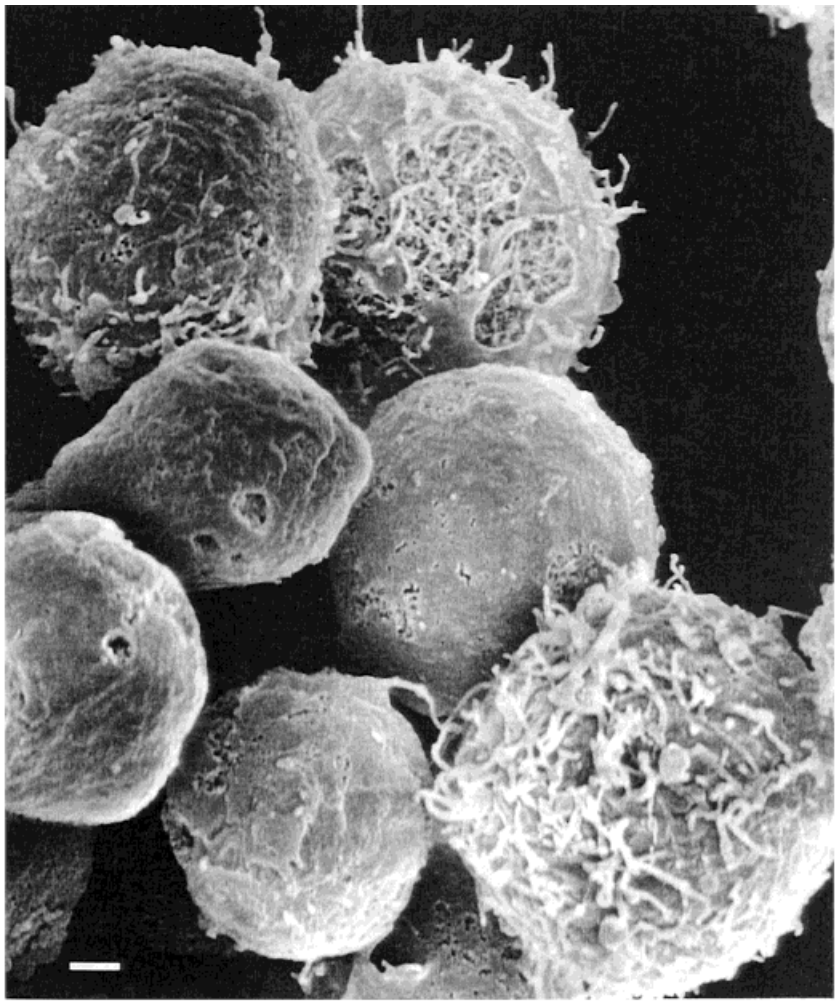
RESULTS

Figure 1 presents the *Drosophila* Schneider S-1 cell system in a scanning electron micrograph at 1 day after subculture. Smooth surfaces are depicted in most cells with a few elongated microvilli. The transmission electron micrograph in Figure 2 shows a cell at 1 day after subculture during mitosis. Regular breakdown of the nuclear envelope, the alignment of chromosomes at the equatorial plate, and regular organization of centrosomes at the two spindle poles (arrow at centrosome of one spindle pole) are indications of normal mitosis.

Immunofluorescence microscopy detects staining with antitubulin and anticentrosome antibodies during regular (Fig. 3A,B), and abnormal (Fig. 3C–F) mitosis. Bipolar spindle orientation is seen for microtubules in Figure 3A, left. Staining with centrosome antibodies reveals two bright foci at two mitotic poles (Fig. 3B, right) from where microtubules are organized. The effects of aging on centrosomes and the microtubule-based mitotic apparatus are shown for Schneider S-1 and *Drosophila* Kc23 cells in Figs. 3C–F. At 5 days of subculture aging effects can be detected which results in tripolar microtubule (Fig. 3C at 7 days) and centrosome (Fig. 3D at 7 days) organizations. This effect is observed in 1% of all cells (100 counted) in a culture that contains 3% of cells in mitosis. When left for longer periods of time centrosomal material becomes dispersed with multiple foci staining for centrosomes (Fig. 3F) and dispersed microtubule staining (Fig. 3E). Tripolar mitoses will cause imbalance of chromosome distribution while multipolar mitoses will result in cell death. The irregular centrosome and microtubule organizations are indications for centrosome instability within the centrosome structure. The figures shown here utilized a monoclonal antibody to β-tubulin (E7) to detect microtubules, and a monoclonal antibody to phosphoproteins (MPM2) to detect centrosomes. The images in Figure 3 depict mitotic figures at 1 (Fig. 3A,B), and at 7–9 days after subculture with tripolar (Fig. 3C,D) and multipolar (Fig. 3E,F) mitotic organizations. If left for prolonged periods of time, i.e., past 7–14 days, the index for mitotic aberrations increases and cell debris in the cultures increases which is interpreted to be the result of cell death.

Microtubules are also affected in the midbody of cells at 5 to 7 days of subculture. In freshly subcultured cells midbodies are short and connect the two dividing cells (Fig. 4A) before separation into daughter cells. In aging cells, midbodies become elongated and can be up to 10 times longer than in freshly subcultured cells (Fig. 4B). These findings are supported with scanning electron microscopy (data not shown).

Other effects of aging are seen during interphase (Fig. 5), on the microtubule system and cell organelles. Randomly distributed and short microtubules (Fig. 5A,B; arrows) are seen in aging cells that are not found in cells at 1–4 days of subculture. Mitochondria appear increased in number (Fig. 5C,D; black arrows) and vary in shape. The images in Figure 5C–F also show other cell organelles that are not seen in cells at 1–4 days of subculture. These include ringshaped membranous formations (Fig. 5C; white arrows), myelin figures (Fig. 5D; arrow



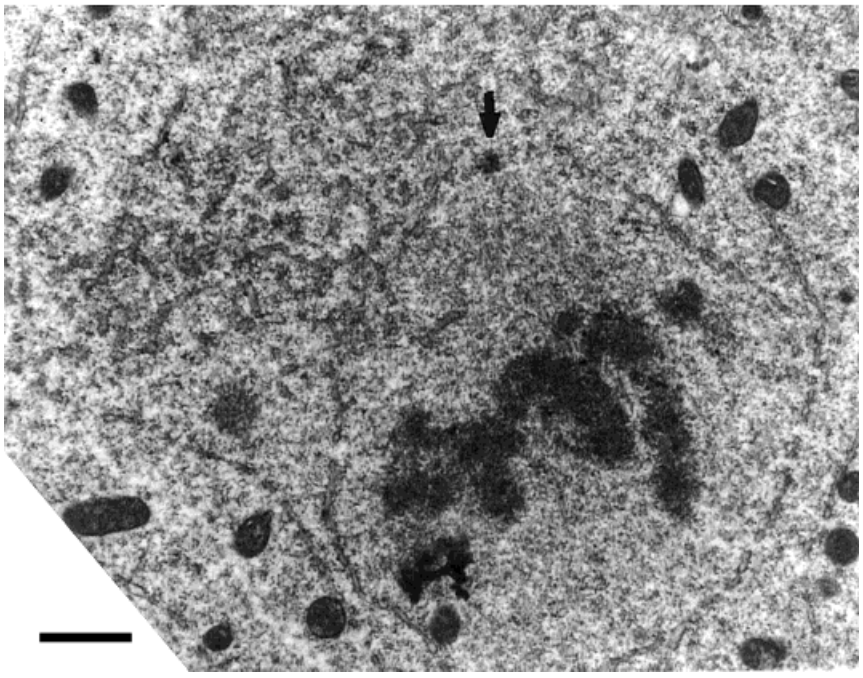


Fig. 1. Scanning electron micrograph of Schneider S-1 cells after 1 day of subculture. Cell surfaces are either smooth or covered with microvilli. Scale bar = 1 μm.

Fig. 2. Transmission electron micrograph of Schneider S-1 cell in mitosis at 1 day after subculture. Clearly aligned chromosomes at the metaphase plate and bipolar spindle pole orientation indicate normal mitosis. Arrow points to one centrosome at one pole in this TEM section. Scale bar = 0.5 μm.

heads) similar to those reported by Debec and Marcaillou [1997] in heat shocked Kc23 cells, and unusual cell structure formations (Fig. 5E,F; arrows).

The pattern for abnormal mitosis might be set up during interphase when centrosomal material becomes reorganized to establish the mitotic poles. The irregularly shaped nucleus in Figure 6A might indicate abnormal centrosome separation along the pre-mitotic nucleus. This will lead to mitoses with irregular and imbalanced chromosome alignment as seen in Figure 6B. Irregular shaped nuclei are not seen in freshly subcultured cells. The abnormal organelle organizations in aging cells seen in Figures 5 and 6A,C might be indications of abnormal cellular metabolism.

DISCUSSION

This article reports on centrosome and microtubule alterations in *Drosophila* cells which are seen during long-term culture. Because many of the effects are also observed during aging in whole organisms [Schwarze et al., 1998; Labuhn and Brack, 1997; Benguria et al., 1996; Cottam and Milner, 1997; Lin et al., 1998; Tower, 1996; Cristofalo et al., 1998; Kassem et al., 1997; Rubin, 1997; Lee and McCulloch, 1997; Vickers et al., 1992; Munoz et al., 1998; Dimri et al., 1995] and in age-related disease [Wu et al., 1998; Bennett et al., 1997; Fujisawa et al., 1998; Schatten et al., 1998a,b] we relate our findings to aging. The gradual loss of homeostatic maintenance and physiological fitness in the whole organism is caused by subcellular changes which are directly or indirectly related to cytoskeletal functions. Drosophila has been used as a genetic model to study the effects of aging [Benguria et al., 1996; Rogina et al., 1998; Minois and LeBourg, 1997; Schwarze et al., 1998; Labuhn and Brack, 1997; Lin et al., 1998; Shikama and Brack, 1996; Tower, 1996]. More recently, *Drosophila* cell lines have become popular because they are amenable to genetic manipulations. This will facilitate research on telomeres which have been implicated in the aging process [Fossel, 1998]. Cytoskeletal mutants of the *Drosophila* Kc23 cell line have been used to study the function of cytoskeletal components including centrioles [Szöllösi et al., 1986]. More recently, *Drosophila* cell lines have been proposed to study the effects of aging on the growth and response of hormones [Cottam and Milner, 1997]. Many studies on aging have been conducted on donor cells of different ages, but these studies are not conclusive and have recently been reevaluated [Cristofalo et al., 1998]. Cellular aging may well occur during long-term culture of cells in vitro and may reflect processes of aging which occur in vivo [Rothen-Rutishauser et al., 1998]. Studies of the effect of aging on the morphology and physiology of *Drosophila* cell lines is only at the beginning, although problems of genotypic or phenotypic changes in cell lines with age are recognized in other areas of animal cell culture. Here we propose that the effects of aging can be studied in *Drosophila* during long-term culture. Intracellular debris was observed in our studies which has been associated with cellular aging in other cell systems [Kassem et al., 1997]. The cytoskeletal dysfunctions shown here in aging *Drosophila* cells are similar to those causing age-related disease. This includes microtubule dysfunction in Alzheimer disease [Cook et al., 1979], in liver pathological manifestations [Taylor et al., 1992], and in aneuploidy which causes Down's syndrome [Cook et al., 1979]. Aneuploidy is defined as karyotypic abnormality in which specific chromosomes are present in too many or too few copies. Polyploidization associated with in vitro cellular aging [Fujisawa et al., 1998] may contribute to cellular immortalization. Abnormal microtubule and centrosome organization play a role in cases of genetic instability during cancer [Lingle et al., 1998; Schatten et al., 1998a,b] and can be causes for age-related disease.

The effects of aging on cells during reproduction are well known and can result in birth defects [Eichenlaub-Ritter, 1988a,b] or in reduction in fertilizability [Kim et al., 1996]. Both effects are associated with weakening of the cytoskeletal system or with cytoskeletal dysfunctions. Aneuploidy is the result of abnormal separation of chromosomes which is directly linked to abnormal microtubule organization associated with aging [Eichenlaub-Ritter, 1988a,b]. Centrosomes are part of the cytoskeletal network and play a critical role in the organization of microtubules. Our current knowledge of centrosomes allows the conclusion that the abnormal microtubule organization in aging oocytes can be related to abnormal centrosome organization. The causes for centrosome abnormalities are not known. This field is still young and the biochemical and biophysical

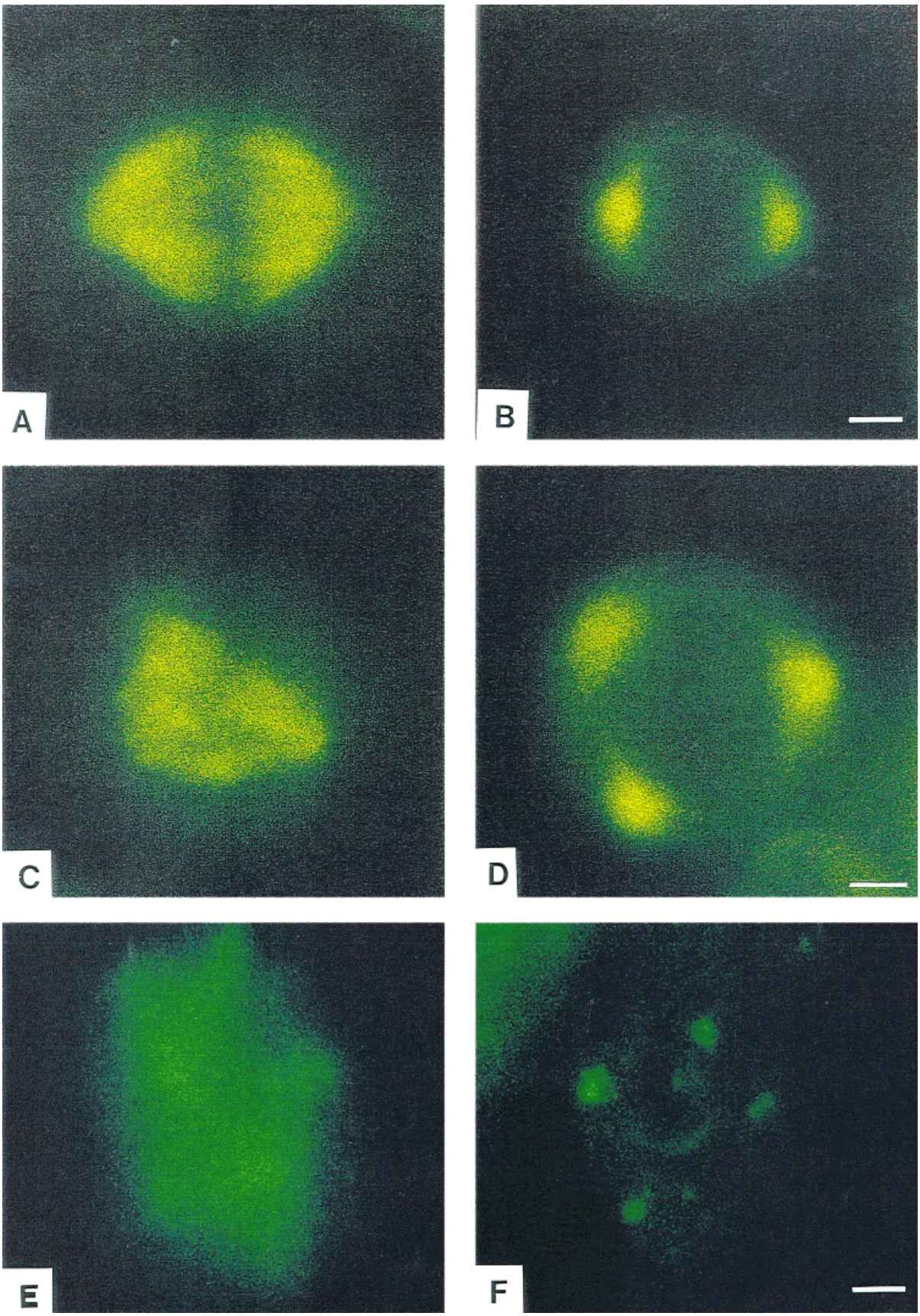


Figure 3

nature of centrosome regulation is largely unexplored.

Much of our knowledge on centrosome behavior comes from studies of reproduction in invertebrate and mammalian systems. During fertilization in most mammalian and invertebrate systems, sperm contributes centrosomal material that forms the microtubule-based sperm aster which moves maternal and paternal genomes in close apposition. Centrosomal material reorganizes after nuclear envelope breakdown and becomes located at the mitotic poles in order to organize the mitotic apparatus during mitosis and cell division.

It has been shown that defects in centrosomal material can account for some cases of infertility as a result of failure of sperm aster formation. In mammalian tissue culture cells, centrosomes are associated with the nuclear envelope during interphase through mechanisms which are only partly understood. Upon nuclear envelope breakdown the interphase centrosomes undergo substantial structural and molecular changes [Petzelt et al., 1997a,b; Debec and Montmory, 1992] which capacitates centrosomal material to function as the main microtubule organizing center during mitosis and cell division. During normal cell cycles, centrosome changes are precisely determined but can become irregular in disease such as cancer and in cells during aging. Because many cancers are related to age, including cancers of the breast, prostate, and colon, centrosomal instability during aging may contribute to the onset of cancer [Boveri, 1914]. The unequal separation of chromosomes may introduce genomic imbalances which will result in abnormal mitoses as characteristic for cancer [Boveri, 1914; Fukasawa et al., 1996; Pihan et al., 1997; Lingle et al., 1998; Schatten et al., 1998a,b].

Fig. 3. Immunofluorescence micrographs. **A:** Normal bipolar mitotic apparatus in Schneider S-1 cell subcultured for one day and stained with the monoclonal anti-tubulin antibody E7 (1:5) depicting regular microtubule organization. **B:** Schneider S-1 cell stained with the monoclonal phosphoprotein antibody MPM2 (1:100) depicting regular bipolar organization of centrosomes after 1 day of subculture. C–F: Schneider S-1 cells subcultured for 7 to 9 days depicting abnormal tripolar organization of microtubules stained with the monoclonal anti-tubulin (1:5) antibody **(C)** and of centrosomes stained with the monoclonal MPM2 phosphoprotein antibody **(D)**. Abnormal multipolar mitoses are seen in E and F stained for microtubules with the monoclonal E7 antibody **(E)** and stained for centrosomes with the monoclonal MPM2 antibody **(F)**. Schneider S-1 cells. Scale bars = 0.75 μm.

Although little is known about centrosome structure [Moritz et al., 1995; Thompson-Coffe et al., 1996], centrosome abnormalities in cells during aging are indicative of structural instability of a fibrous network that is compacted and decompacted in order to nucleate the dynamically changing microtubule organizations during interphase and mitosis. Typically, compacted centrosomal material will nucleate compact microtubule structures while decompacted material will give rise to wider microtubule organizations. It is not inconceivable that the elasticity of centrosome material may change during aging which will result in abnormal states of centrosome folding. Alternatively, ionic controls may be different in cells during aging which may change the conditions for centrosome compaction and decompaction and will result in abnormal centrosome protein folding [Mazia, 1984]. Several factors are necessary for normal progression of the centrosome cycle within the cell cycle which includes disulfide and sulfhydryl bond formation and reformation [Schatten, 1994; Schatten et al., 1993], and changes in pH and calcium [Schatten et al., 1992]. Structural errors in folding of centrosome protein may account for the tripolar and multipolar configurations which are found in cells during aging.

Nutrients may play a critical role in maintaining physiological conditions for centrosome and cytoskeletal functions. The cytoskeleton responds to environmental stimuli and transduces signals through the cytoskeletal network by mechanisms that are only partially understood [Patrone et al., 1992]. Several examples exist where the cytoskeleton is changed in response to altered environments [Schatten et al., 1998a]. Nutrition may play a role which in cell cultures is provided by serum [Wheaton et al., 1996] and by ions such as calcium, magnesium, and trace elements. Exhaustion of factors from the medium may have caused the effects on the cytoskeleton reported in this paper. Calcium and magnesium in particular are ions known to affect cytoskeletal functions [Murchison and Griffith, 1998]. The uncharacteristic organelle structures seen in Figures 5 and 6 and also observed after heat shock [Debec and Marcaillou, 1997] may be indications of altered physiological functions caused by adverse conditions.

The regulatory mechanisms for cytoskeletal functions play significant roles for maintaining

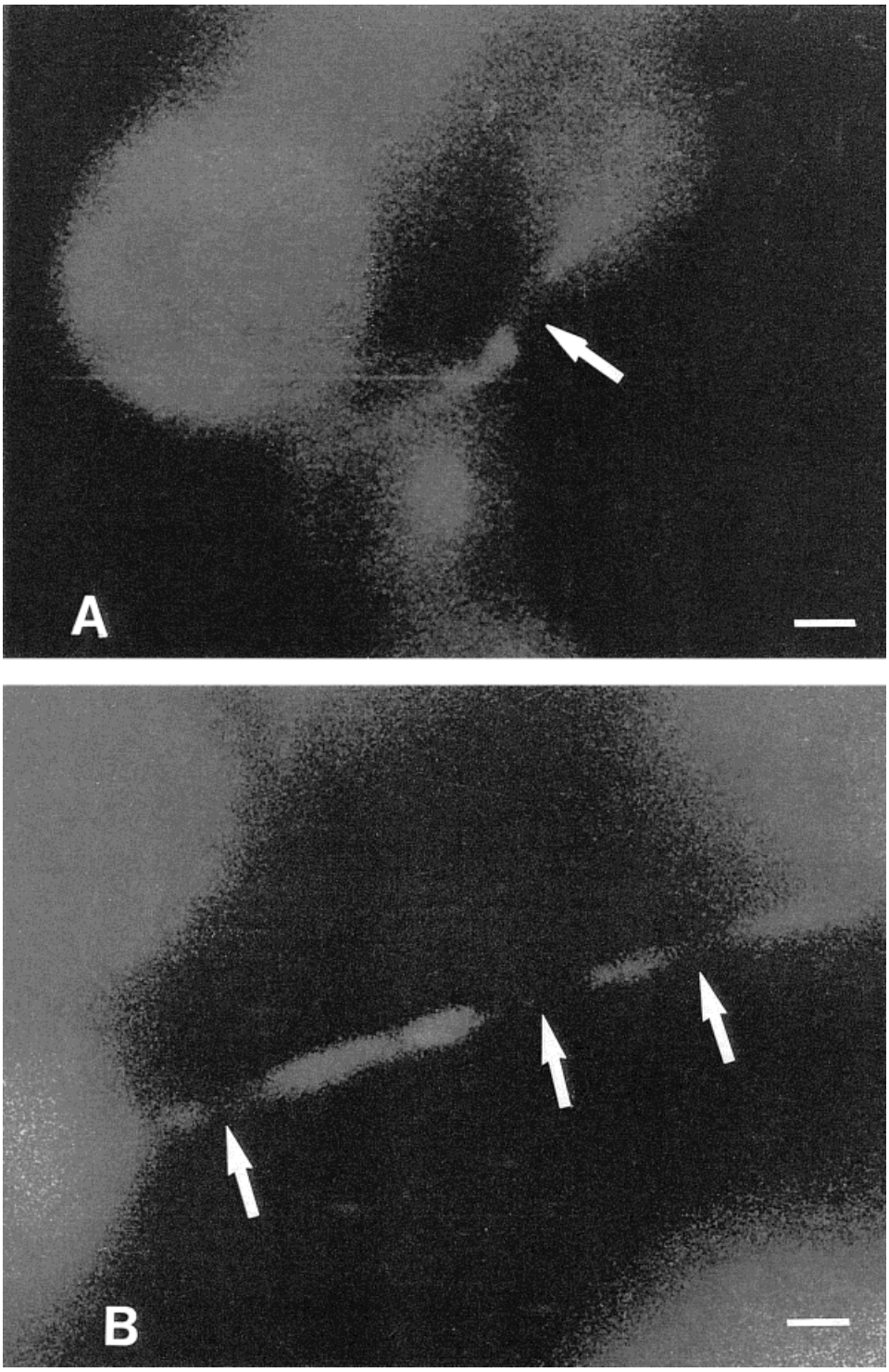


Fig. 4. Immunofluorescence images of cells in cell division depicting normal **(A)** and abnormal **(B)** midbodies when stained with the monoclonal E7 anti-tubulin (1:5) antibody. While midbodies in freshly subcultured control cells are short and staining is characteristically excluded from the microtubule interdigitating mid-zone (A; arrow), midbodies in cells after 5–9 days of subculture are five to 10 times longer and have irregular organization with several areas in which staining is excluded (B; arrows). Schneider S-1 cells. Scale bars = 1 μm.

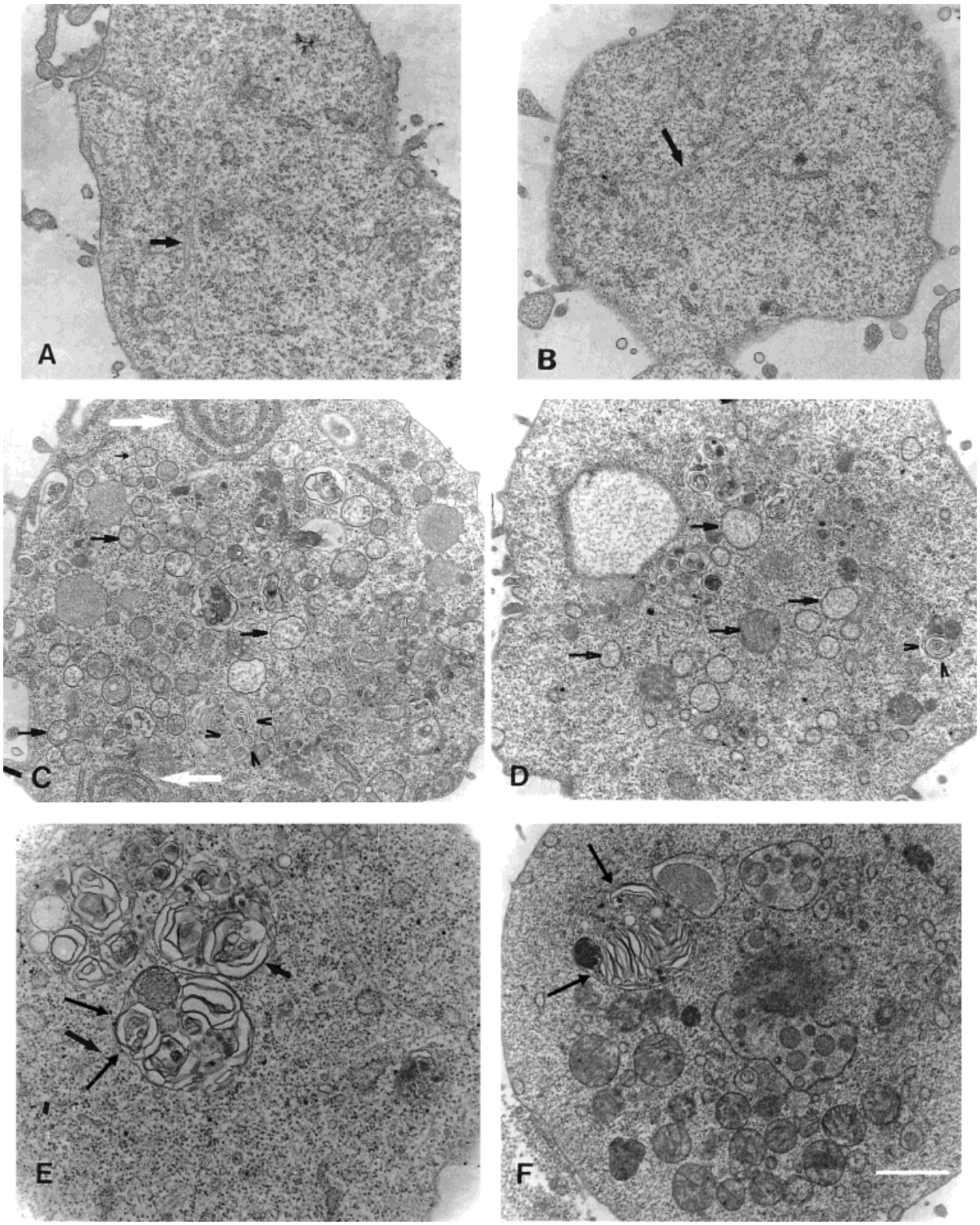


Fig. 5. A–F: Transmission electron micrographs of subcultured Kc23 **(A–D)** and Schneider S-1 **(E,F)** cells during interphase cultured for 5 (E,F) and 7 (A–D) days. Irregular stacks of microtubules (A; arrow) or longer microtubules in random orientation (B; arrow) are seen in aging cells. Mitochondria appear increased in number (C,D; black arrows) and vary in shape. The images in C–F show other cellular organelles that are not seen in freshly subcultured cells. These include ringshaped membranous formations (C; white arrows), myelin figures (C,D; arrowheads), and other unusual organelle formations (E,F; arrows). Scale bar = 1 μm.

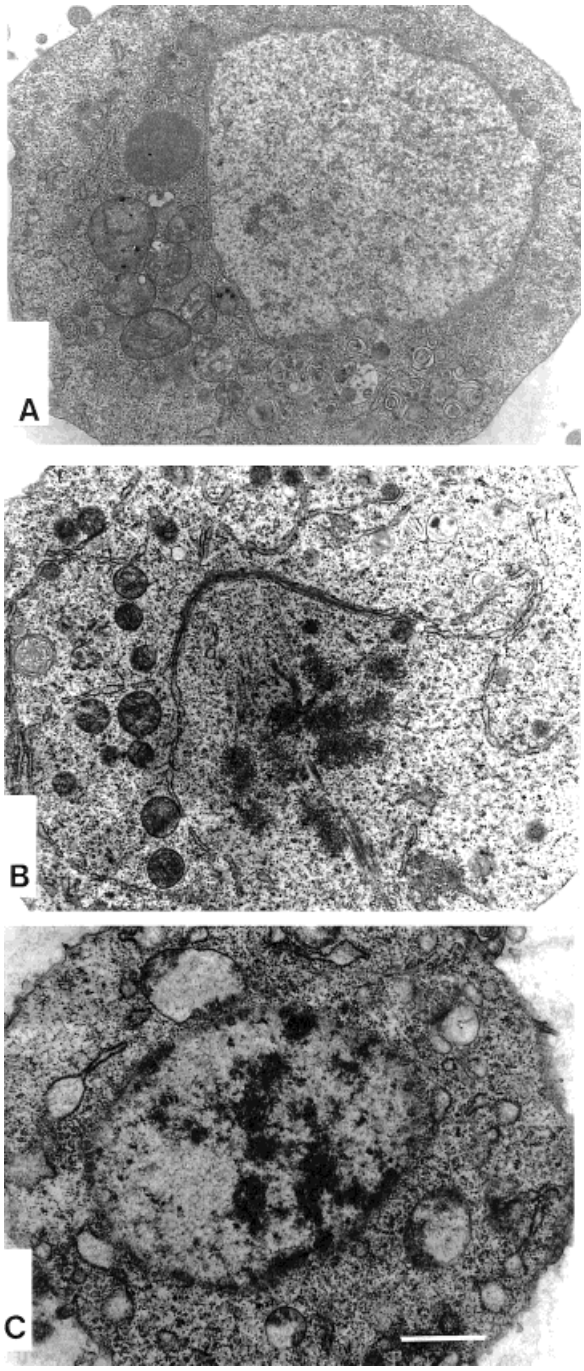


Fig. 6. Transmission electron micrographs of Kc23 **(A)** and Schneider S-1 **(B,C)** cells depicting irregular nucleus shape during interphase (A) and irregular alignment of chromosomes (B) at 7 days of subculture. Cell organelle structure is largely affected in cells at 14 days of subculture (C). Scale bar = 1 μm.

a viable cytoskeleton. In aging cells short and disrupted microtubules are frequently found which may indicate impaired microtubule functions. Microtubules serve a variety of different functions including transport of cell organelles [Koonce and McIntosh, 1990], and transport of centrosome subcomponents [Balczon, 1997]. The accumulation of cell organelles seen in aging cells which are not observed in freshly subcultured cells may be indications of insufficient organelle transport which will lead to impaired cellular metabolism. Insufficient transport of centrosome proteins along microtubules may result in abnormal centrosome protein composition. Alterations of cytoskeletal regulation can lead to disease such as muscular dystrophy, muscular atrophy, osteoporosis, loss of neuronal functions, reproductive disorders, or cancer.

Changes in microtubule organization have been observed during aging in other cell systems including eggs and oocytes during reproduction which can result in age-related birth defects [Eichenlaub-Ritter et al., 1988a,b]. These studies report on tripolar and multipolar mitoses which were stained with antitubulin antibody and indicate centrosome alterations. While microtubules are responsible for chromosome separation the organization of microtubules is correlated to centrosome organization. Centrosomes are the microtubule organizing centers which define the shape of the spindle. Abnormal centrosomes will organize abnormal spindles which will separate chromosomes unequally. Tripolar mitoses will result in three cells after cell division while multipolar cells will lead to cell death. Well functioning centrosomes are therefore crucial for the proper separation of chromosomes.

The causes leading to abnormal centrosomes during aging may lie in structural defects or changes in cell physiology during centrosome protein folding. While little is known about centrosome structure evidence for fibrous string-like material comes from studies with low voltage high resolution scanning electron microscopy (LVSEM) [Thompson-Coffe et al., 1996]. These studies are only the beginning of analyzing centrosomes on a structural level. Mazia [1984] predicted a flexible string model of centrosomes which will fold and unfold throughout the cell cycle and will result in the various microtubule formations. The exposure of gamma tubulin [Zheng et al., 1995; Mazia, 1984] ("microtubule initiating units") on the string would define the organization of microtubule formations. Folding and unfolding of centrosome structure is regulated by several factors. Studies with dithiothreitol [Schatten, 1994] and mercaptoethanol [Schatten et al., 1987] have shown

that disulfide and sulfhydryl bond formations are important for the folding and refolding process. Other factors that can change centrosome behavior include pH and calcium [Schatten et al., 1992]. These studies have been conducted in invertebrate systems, but studies on mammalian or insect cells in culture have not yet been performed. It might well be that pH changes and calcium are different in cells during aging.

It is feasible to study centrosome and cytoskeletal genetics in the insect cell system presented here. Mutants of the Kc23 cell line have been used for studies of the cytoskeleton [Szöllösi et al., 1986; Debec et al., 1995] and allowed comparison of cytoskeletal function with and without a centriolar mutation. Insect cell lines derived from *Drosophila melanogaster* are increasingly used as an alternative system to mammalian cell cultures [Sondergaard, 1994]. They are easy to maintain since they can grow at room temperature in suspension or in monolayer cultures and do not require CO_2. Because *Drosophila* cell lines are amenable to genetic manipulations they can serve for the investigation of gene regulation and the expression of proteins under adverse conditions. While other cell systems have been ideal models to investigate cytoskeletal functions [Schatten et al., 1986, 1987, 1992; Schatten, 1994; Schatten and Chakrabarti, 1998], the *Drosophila* system offers a new level to study such functions on a genetic basis. The insect cell system of *Drosophila* can also provide mutants in which to study disease.

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