Behavior of Crocidolite Asbestos during Mitosis in Living Vertebrate Lung Epithelial Cells

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ABSTRACT

Asbestos has been described as a physical carcinogen in that long thin fibers are generally more carcinogenic than shorter thicker ones. It has been hypothesized that long thin fibers disrupt chromosome behavior during mitosis, causing chromosome abnormalities which lead to cell transformation and neoplastic progression. Using high-resolution time lapse video-enhanced light microscopy and the uniquely suited lung epithelial cells of the newt Taricha granulosa, we have characterized for the first time the behavior of crocidolite asbestos fibers, and their interactions with chromosomes, during mitosis in living cells. We found that the keratin cage surrounding the mitotic spindle inhibited fiber migration, resulting in spindles with few fibers. As in interphase, fibers displayed microtubule-mediated saltatory movements. Fiber position was only slightly affected by the ejection forces of the spindle asters. Physical interactions between crocidolite fibers and chromosomes occurred randomly within the spindle and along its edge. Crocidolite fibers showed no affinity toward chromatin and most encounters ended with the fiber passively yielding to the chromosome. In a few encounters along the spindle edge the chromosome yielded to the fiber, which remained stationary as if anchored to the keratin cage. We suggest that fibers thin enough to be caught in the keratin cage and long enough to protrude into the spindle are those fibers with the ability to snag or block moving chromosomes.

INTRODUCTION

Epidemiological studies and experiments on laboratory animals have long linked asbestos exposure to various diseases. Crocidolite fibers in particular have been associated with the development of pulmonary interstitial fibrosis (asbestosis), lung cancer, and malignant mesotheliomas of the pleura, pericardium, and peritoneum (reviewed in Refs. 1–3). In the case of lung cancer, but not mesotheliomas, asbestos exposure and cigarette smoke have demonstrated a multiplicative effect (4). The mechanisms by which asbestos fibers cause disease remain largely unknown (5–7). There is strong evidence that asbestos is a complete carcinogen, both initiating and promoting tumorigenesis (reviewed in Ref. 8). However, unlike most carcinogens, asbestos by itself does not appear to be mutagenic as is evident in a variety of gene mutation assays (9–12). Alternatively, it does induce both structural and numerical chromosomal abnormalities in a wide range of mammalian cells, including mesothelial cells (11, 13–17). The ability of asbestos to cause a variety of different diseases, affect different target cells, induce cell transformation, and promote tumor growth indicates that asbestos must have multiple mechanisms of action. The dimensions, durability, and the physicochemical nature of the surface of a fiber are all believed to contribute to disease development (8).

Asbestos-induced mesotheliomas and bronchogenic carcinomas are characterized by specific types of nonrandom chromosomal abnormalities, which include deletions, translocations, and aneuploidy of specific chromosomes (11, 13–17). These abnormalities are believed to be the mechanism by which cell transformation and tumor progression occur in asbestos-induced cancers, possibly by activating oncogenes and eliminating tumor suppressor genes within the genome (14, 18). It has been hypothesized that asbestos may cause chromosomal abnormalities by interfering with chromosome segregation during mitosis (19, 20). The incidence of chromosome laggards, chromosome bridges, and sticky chromosomes during anaphase increases with asbestos treatment (21–23). Observations on fixed material have shown asbestos fibers near and, in some cases, penetrating chromosomes within the spindle (17, 21, 24).

Since the ability of a fiber to transform a cell is related primarily to its size and shape, and not its chemistry, asbestos is considered to be a physical carcinogen (25). Long (>4 μm), thin (<0.25 μm) fibers are generally more tumorigenic than shorter, thicker ones (Ref. 26; reviewed in Refs. 1, 27). Reducing the length of a fiber by milling reduces its carcinogenicity (28, 29). The importance of fiber dimensions to carcinogenicity is not simply related to fiber aerodynamics and the selective expulsion of certain-sized fibers from the lung or to the ability of certain-sized fibers to penetrate the lung lining, since the same relationship is also seen when various-sized fibers are added to cultured cells or implanted into the pleurae of rats (11, 19, 29). At the cellular level, compared with shorter fibers, longer fibers are more easily phagocytized (19), but less easily transported along microtubules once inside the cell (30, 31). However, both long and short fibers accumulate in the perinuclear region, placing them at the time of mitosis in a position to interact with chromosomes on the spindle (5, 19). It has been hypothesized that long thin fibers may affect chromosome segregation sterically, either by directly blocking chromosome movement to a pole or by becoming entangled in the spindle apparatus (21, 22, 24, 32). Long fibers found within the cleavage furrow after the completion of karyokinesis indeed have been shown to affect chromosome distribution sterically by inhibiting the completion of cytokinesis, resulting in tetraploid or binucleated cells (17, 22, 31, 33).

A number of other possible routes for asbestos-induced aneuploidy have also been proposed (reviewed in Ref. 20). For example, certain types of asbestos fibers may have an affinity, through charge-charge interactions, for specific components of the chromosome or spindle apparatus (11, 21, 33). In this case, fibers would disrupt chromosome segregation by sticking to chromosomes or to the spindle apparatus, or by causing defective chromosomes or spindles by absorbing the components before they assemble. Alternatively, long thin asbestos fibers may be more likely to disrupt the phagolysosomal membrane that surrounds them, releasing hydrolytic enzymes into the cytoplasm (34) and possibly poisoning the cell. Chromosomes of "sick" cells indeed tend to stick together and form bridges during anaphase.3

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3 Unpublished observations.
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To determine how various-sized asbestos fibers interact with the chromosomes and the spindle during mitosis it is necessary to follow their behavior within living cells amenable to high-resolution optical analyses. In this context we have shown previously that the lung epithelial cells of the newt, Taricha granulosa, are ideally suited for studying the in vivo behavior of asbestos fibers during interphase (30). The large size, flatness, and optical clarity of these cells allow one to visually track individual asbestos fibers and to observe how they interact with various cell components. Importantly, newt lung epithelial cells have the same basic fundamental structural organization, biochemical pathways, and mechanisms supporting cellular processes as their counterparts in humans. In this report we detail for the first time how crocidolite asbestos fibers behave within living cells during mitosis.

MATERIALS AND METHODS

Cell Cultures. Primary cultures of T. granulosa lung epithelial cells were derived from small lung fragments as described previously (35). Briefly, lung fragments were cultured at 23°C on 25-mm² glass coverslips in Rose multipurpose chambers filled with half-strength L-15 medium supplemented with 10% fetal calf serum and antibiotics (36). Within 7–10 days, monolayer sheets of epithelial cells migrate up to 1–2 mm from the tissue fragments. At this time, the medium was replaced with fresh medium containing 0.5 mg/ml of International Union Against Cancer crocidolite asbestos (a kind gift from Dr. T. M. Fasy, Mount Sinai Medical Center, New York, NY). As reported previously (30) numerous crocidolite fibers become incorporated by phagocytosis into T. granulosa epithelial cells within the first 24 h of exposure. For high-resolution microscopy, coverslips with mitotic cells containing asbestos fibers were removed from the Rose chambers and mounted on microperfusion chambers filled with fresh medium (see Ref. 30).

Video-enhanced Light Microscopy. The video-enhanced light microscopic system used consisted primarily of a Nikon Microphot-FX differential interference contrast light microscope equipped with ×60 (numerical aperture = 1.4) and ×40 (numerical aperture = 0.85) objectives. This microscope was coupled to a Hamamatsu C2400 Chalnicon camera, the output of which was routed through an Image-i image processor (Universal Imaging Corp., West Chester, PA). Image processing was used to eliminate background optical noise within the system and to enhance the signal/noise ratio by averaging the image over 16 frames. After processing, the image was ported to and stored on Panasonic TQ-FH224 optical memory disks using a Panasonic TQ-2028 optical memory disk recorder (ADCO Aerospace, Fort Lauderdale, FL). Cells were viewed at 23°C using heat-filtered 546 nm light. During time lapse microscopy, images of cells were captured and recorded every 1–4 s, and the illumination was shuttered between each exposure. For analyses of fiber lengths and rates of motion, sequential images from these recordings were played back, image by image, through a time-base corrector (For.A Corp., Natick, MA) into Image-1, which contains the appropriate analytical programs. For producing figures, selected video images were photographed onto Plus-X film (Eastman Kodak Co., Rochester, NY) using a freeze-frame video recorder (Polaroid Corp., Cambridge, England). This film was then developed in Rodinal (AGFA Corp., Ridgefield Park, NJ).

Immunofluorescence Light Microscopy. The procedures for the indirect immunofluorescent staining of microtubules (36, 37) and keratin (38) shown in Fig. 1 have been described previously. The monoclonal antibody against β-tubulin (TU-27B) and the polyclonal antibody against keratin (K5) used were kind gifts from Drs. L. Binder (University of Alabama, Birmingham, AL) and B. S. Eckert (State University of New York, Buffalo, NY), respectively.

RESULTS

Intermediate Filaments Prohibit Most Fibers from Interacting with the Forming Spindle and Chromosomes. After incorporation into an interphase cell, the majority of asbestos fibers are transported to and accumulate around the nucleus (Fig. 1A; Refs. 5, 19, 30), where they remain throughout the prophase stage of mitosis. Despite this fact, we found that the forming spindle remained relatively free of fibers (Figs. 1D and 2–5). The spindle in vertebrate cells forms in that region previously occupied by the nucleus. In epithelial cells, this region is often substantially larger than the spindle, and it is optically clear in that it is virtually devoid of mitochondria and other large organelles (Fig. 2; Ref. 38). It has been shown previously that this clear area is created by keratin intermediate filaments, which form a “cage” around the interphase and prophase nucleus (Fig. 1B). In many cell types this cage persists for various periods of time after nuclear envelope breakdown (Fig. 1E; Ref. 38) and prohibits the migration of large organelles and cell inclusions, including asbestos fibers, into the region of the forming spindle. As mitosis progresses, the keratin cage usually slowly collapses around the spindle (38) so that by the time spindle formation is complete (i.e., metaphase), mitochondria and other excluded bodies may come to lie very close to the spindle

Fig. 1. Differential interference-contrast (A, D), antikeratin immunofluorescent (B, E), and antitu- bulin immunofluorescent (C, F) micrographs of newt lung epithelial cells. The majority of incorpo- rated crocidolite fibers (arrows) accumulate near the nucleus during interphase (A) but remain out- side the spindle region during mitosis (D). During the transition from interphase to mitosis, a cage of keratin intermediate filaments surrounding the interphase nucleus continues around the prophase nucleus (B) and around the mitotic spindle (E). In contrast, the long cytoplasmic microtubules of interphase (C) disassemble and are replaced by the shorter astral microtubules of the mitotic spindle (F). Bar, 30 μm.
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Fig. 2. Selected micrographs, from a time lapse video-enhanced differential interference contrast recording of a forming mitotic spindle. The spindle is located in a clear area (small arrowheads) produced by a keratin cage (0 min). As mitosis progresses, the keratin cage collapses around the spindle, allowing mitochondria and crocidolite fibers (large arrow) to enter the clear area and to move closer to the chromosomes on the spindle (47–78 min). A crocidolite fiber (large arrowhead), 12.9 \( \mu \text{m} \) long and 0.4 \( \mu \text{m} \) wide, displaying saltatory motions exhibited unique behavior in that it penetrated through a chromosome (small arrow) and dragged it poleward. The time lapse sequence shows the transported fiber (large arrowhead) entering the spindle region (0 min), moving out of the spindle (34 min), moving toward the spindle pole with the snagged chromosome (37, 47, and 51 min), turning away from the pole (56 min), the freed chromosome (small arrow) expelled from the spindle by the astral ejection force (63 min), and the fiber without the attached chromosome moving close to the pole (78 min). Penetration of the fiber through the chromosome is particularly evident at 47 and 56 min. Time (in min) in upper right corner. Bar, 15 \( \mu \text{m} \).

periphery. During this time, asbestos fibers within the keratin cage also moved with the cage to the edge of the spindle and closer to the chromosomes (Fig. 2). However, since the majority of these fibers never completely pass through and escape the keratin cage, relatively few fibers were actually seen associated with the spindle or its polar regions.

Crocidolite Fibers Exhibit Microtubule-mediated Transport during Mitosis. In vertebrates, the mitotic spindle is assembled after nuclear envelope breakdown from an interaction between the chromosomes and spindle poles, the latter of which consist of two radial microtubule arrays known as asters (Fig. 1F). For this study we have defined the spindle to be composed of its associated asters and the microtubule-rich region between the asters containing the chromosomes and spindle proper. Once within the spindle, crocidolite fibers sometimes displayed saltatory movements similar to those previously reported to occur along microtubules in interphase cells (30, 31). It was apparent that the microtubule-mediated transport of membrane-bound fibers observed in interphase cells (30) also occurred during mitosis. In mitotic cells these movements were directed along astral microtubules radiating from the centers of the two spindle poles. The average rate of transport [0.46 \( \mu \text{m/s} \); 24 particles undergoing a total of 35 \( \pm \) 0.22 (SD) translocations] was not significantly different from that seen during interphase [0.48 \( \mu \text{m/s} \); 25 particles undergoing a total of 32 \( \pm \) 0.25 (SD) translocations] data from (Ref. 30) \( (P = 0.86; t \) distribution, df = 65). The maximum velocity observed was 0.98 \( \mu \text{m/s} \) compared with 1.18 \( \mu \text{m/s} \) during interphase (30). The average length of the transporting fibers studied was 4.5 \( \mu \text{m} \) with a range of 1.3–12.9 \( \mu \text{m} \).

Ejection Properties of the Asters Only Minimally Affect Fiber Position. An astral ejection force exists in mitotic vertebrate epithelial cells that expels chromosome fragments and other large bodies away from the centers of the two polar asters (39). It is dependent on the dynamically unstable behavior of astral microtubules and is thought to be caused by the continuous growth of microtubule plus ends away from the aster (36). We found that the position of crocidolite fibers was only slightly affected by the ejection properties of the asters. Over time some of the fibers that were not actively tethered to and moving along astral microtubules tended to migrate away from the astral centers (Fig. 3; 50–88 min). This expulsion of fibers was not as prominent or as efficient as that of chromosome fragments, which are ejected in a slow (~0.04 \( \mu \text{m/s} \)) but relatively continuous motion (40). When it occurred, the ejection of crocidolite fibers took longer and fiber motion was discontinuous. Fibers which pointed directly toward the astral center, i.e., those that were arrayed parallel to astral microtubules, were less likely to be influenced by the astral ejection force (Fig. 4).

Physical Encounters between Crocidolite Fibers and Chromosomes Usually Result in Fiber Displacement. Crocidolite fibers within the spindle and along its edge were those fibers in position to physically interact with chromosomes on the spindle. Although relatively rare, chromosome fiber encounters did occur (Figs. 2–5). Such encounters were by chance and occurred either when a moving chromosome bumped into a stationary fiber or when a moving fiber contacted a stationary chromosome arm. During these encounters crocidolite fibers never stuck to, nor showed any affinity for, the chromosome (Fig. 3). Most encounters ended with the fiber yielding to the chromosome (Table 1); i.e., either the moving chromosome pushed the fiber aside (Figs. 3 and 4) or the moving fiber bounced off the chromosome. In the 10 cases in which a stationary fiber did not yield to the chromosome, the fiber was positioned along the edge of the spindle presumably anchored in the keratin cage. In 9 of these cases, the chromosome squeezed around the fiber; in the remaining case, the chromosome was momentarily snagged on the fiber and its movement was temporarily delayed.

We observed a chromosome yielding to a moving fiber only twice (Table 1). In one of these cases, a chromosome arm was displaced...
slightly by the fiber. In the other case, the moving fiber penetrated through the chromosome and dragged the whole chromosome, which had not yet attached to the spindle, toward the center of the aster (Fig. 2). As the fiber and chromosome moved poleward, they met resistance from the astral ejection force (Fig. 2; 51 mm). The fiber eventually turned away from the pole (Fig. 2; 56 mm), and the chromosome slipped off the fiber. The chromosome was then expelled by the astral ejection force to the periphery of the spindle (Fig. 2; 63 mm). The fiber, now lacking the attached chromosome, moved closer to the center of the aster (Fig. 2; 78 mm). The chromosome ultimately attached to the spindle and moved poleward on its own without the aid of the fiber.

Crocidolite Fiber-Chromosome Contacts May Induce Chromosome Breaks. In one cell a crocidolite fiber appeared to break a chromosome (Fig. 5). In this cell, rotation of the spindle dragged chromosome arms along the edge of the keratin cage and into a region containing numerous fibers (Fig. 5; 37 mm). Unfortunately, extensive birefringence from the many fibers prevented the identification of individual fibers within this region. However, as one chromosome arm was dragged through this region, a break in both chromatids suddenly appeared (Fig. 5; 37 mm). During anaphase, the resultant acentric chromosome fragment, consisting of both chromatids, was left behind at the equatorial plate of the spindle (Fig. 5; 58 mm). The fragment ended up in the cytoplasm of one of the daughter cells (Fig. 5; 105 mm).

**DISCUSSION**

This study is the first to describe the behavior of phagocytized crocidolite asbestos fibers during mitosis in a living cell. Considering the long latency period between asbestos exposure and disease in humans, we were not surprised that most crocidolite-containing cells completed mitosis normally. During this study, cells which had moderate (~10–20) numbers of fibers showed no signs of cytotoxicity,
such as excessive vacuolization, collapsed spindles, or sticky chromosomes. This may be due in part to the phagolysosomal membrane, which effectively isolates each crocidolite fiber from the rest of the cell, and which probably prevents physicochemical reactions between the fiber surface and potential intracellular targets including chromosomes. Contrary to earlier reports (41, 42; reviewed in Ref. 43), we have recently found that most if not all fibers remain surrounded by a membrane weeks after their incorporation into vertebrate epithelial cells (31; see also Ref. 44). Indeed, many of the fibers we observed in mitotic cells by differential interference contrast microscopy displayed an obvious membrane bulge, which indicated that they were still enclosed within an intact phagolysosome (Figs. 3 and 4).

Phagocytized crocidolite fibers undergo saltatory motions that ultimately lead to their perinuclear accumulation (30, 31). This "saltatory" type of motion is characteristically exhibited by other non-fiber-containing phagosomes and phagolysosomes as they are transported along microtubules within the interphase cell (45-47). This motion is mediated by several different classes of membrane-bound mechanochemical (molecular motor) proteins which move organelles along microtubule surfaces in different directions, depending on the type of motor (48-50). As the cell enters mitosis the relatively stable, long, curvy cytoplasmic microtubules of interphase (Fig. 1C) disassemble and are replaced by more dynamic, shorter, straighter astral microtubules that ultimately form the spindle (Fig. 1F; see Ref. 51). In this study, we found that the saltatory motions exhibited by crocidolite fibers during interphase (30) continue during mitosis. We attribute this behavior to the abundance of microtubules within the mitotic cell and to the presence of membrane-based microtubule motors around the fiber. Indeed, the average rate that fibers moved along astral microtubules was similar to that along interphase cytoplasmic microtubules (0.46 μm/s during mitosis versus 0.48 μm/s during interphase).

However, the microtubule-mediated motion of fibers during interphase appears to be size dependent in that fibers >5 μm rarely exhibited saltatory motions (30). By contrast we found that longer fibers (up to 12.9 μm) were frequently transported during mitosis (Fig. 3). This difference in fiber behavior during interphase and mitosis can be explained by the following assumptions. Phagolysosomes containing long fibers are in contact with many more microtubules than phagolysosomes containing short fibers because of their greater surface area. As a result their molecular motors interact with many more microtubules at any given time. During interphase, microtubule density is high but neighboring microtubules are often oriented in different directions (Fig. 1C). The molecular motors on the surface of a long fiber can be expected to interact with and move along many microtubules with different orientations. Since the fiber is pulled in many directions simultaneously, it will exhibit little net motion. By contrast, each of the two half-spindles in a mitotic cell contain highly polarized arrays of near parallel microtubules (Fig. 1F). Under this condition the molecular motors on the surface of a long fiber can be expected to interact and move along many microtubules oriented in the same general direction, resulting in movement of the fiber in that direction. Under this scenario, saltatory movement of longer fibers could occur during interphase in cell types with less complex microtubule networks (31).

We found that the astral ejection force, which expels chromosome fragments and large bodies away from the two spindle poles, had only a minimal effect on crocidolite fibers. On the basis of what we know regarding how this ejection force works, this was to be expected. Current evidence suggests that the mechanism for producing this force is based on the constant outward growth of dynamically unstable microtubule plus ends, which in effect creates a steric "wall" of microtubule ends that are constantly moving away from the astral center (36). Other data suggest that the magnitude of the ejection force depends primarily on the cross-sectional surface area of the object that faces the polar microtubule array; i.e., the smaller the cross-sectional surface area the lesser is the magnitude of the ejection force (40). Because astral microtubules are radially arrayed, the further an object is from the astral center, the lower is the density of microtubules that it encounters. As a fiber at the periphery of the astral array interacts with and moves along a microtubule toward the astral center (via molecular motors), resistance and drag will align it parallel to the microtubule, at which point it presents its minimal cross-sectional surface area to the pole. Therefore, thin fibers would present a relatively small target for the outward growing microtubules of the aster. Of course, the expulsion of crocidolite fibers from the aster by

| Table 1 Chromosome-fiber encounters (146 encounters in 29 cells) |
|-------------------|-----------------|-----------------|-----------------|
|                   | Total           | Fiber yields    | Chromosome yields | Neither yields  |
| Chromosome moves into fiber | 115             | 95 (82.6)       | 10⁸ (8.7)        | 10 (8.7)        |
| Fiber moves into chromosome | 31             | 29 (93.5)       | 2⁶ (6.5)         | 0 (0)           |
| Total              | 146             | 124             | 12              | 10              |

- Numbers in parentheses, percentage.
- Chromosomes squeezed around fiber. In one case, the chromosome was snagged momentarily on the fiber.
- In one case, the chromosome was slightly displaced; in the other case, the chromosome was snagged and dragged by the fiber.

![Fig. 5. Chromosome breakage in a mitotic cell containing crocidolite fibers. Crocidolite fibers (white areas) have accumulated along the edge of the spindle (0 min). As the spindle rotated, chromosome arms were dragged through a region with numerous fibers. A chromosome break (arrowhead) was observed in one of these arms shortly after this rotation was completed (37 min) and the resulting chromosome fragment (arrow) was left behind at anaphase (58 min). The fragment (arrow) was excluded from each of the daughter nuclei (105 min). Time (in min) in upper right corner. Bar, 15 μm.](image-url)
growing microtubules may also be inhibited by attachments (via molecular motors) of the fiber to neighboring microtubules.

It has been hypothesized that crocidolite asbestos may affect chromosome segregation and that this is a stochastic process based on steric considerations (21, 22, 24, 32). If true, the inability of our study to clearly demonstrate the disruption of chromosome segregation by crocidolite can be attributed to the fact that only very few of the many fibers within the cell are actually able to pass through the keratin intermediate filament cage to contact the spindle and that when they do they are not normally in a position to interfere substantially with chromosome behavior.

One of the more important observations of our study was that crocidolite fibers did not show any affinity for chromosomes; i.e., they did not stick to chromatid (Fig. 3). In rare occasions (2 of 146 encounters), an end of a fiber did penetrate and snag a chromosome (Fig. 2). However, in these rare situations, the chromatid did not adhere to the fiber and the chromosome eventually slipped off the fiber. We therefore conclude that crocidolite asbestos does not disrupt chromosome behavior by sticking to specific components of the chromosome or spindle apparatus.

Most chromosome-fiber encounters usually resulted in the fiber passively yielding to the chromosome (Table 1). Moving chromosomes, with an estimated force between 10^{-4} and 10^{-6} dynes (52, 53), easily displaced fibers found within the spindle. On the other hand, the generated force of a moving fiber was usually not enough to displace a chromosome. We observed only once a moving fiber significantly displacing a chromosome. In this particular case, a long (12.9 μm), thin (0.4 μm) fiber penetrated an unattached chromosome and dragged the entire chromosome poleward (Fig. 2). Perhaps the large surface area of this extra long fiber allowed more molecular motors to engage in force generation, and the majority of the force was directed in one direction. Whatever the situation, the combined net force of these molecular motors was great enough to move a large unanchored chromosome.

Perhaps more pertinent to the induction of chromosome abnormalities by asbestos may be the stationary fibers that do not yield to moving chromosomes. Such fibers were found primarily along the edge of the spindle and are believed to be anchored in the keratin cage. Unlike similar-sized fibers within the spindle, these fibers did not yield when hit by a moving chromosome. Instead, the chromosome usually became deformed as it squeezed around the stationary fiber, and in one case the chromosome was snagged and its movement was momentarily delayed by the fiber. Moreover, at least one chromosome break in our study may have been caused by an anchored fiber snagging a chromosome arm as the spindle was rotating (Fig. 5). By contrast, unanchored fibers, even large ones located between the chromosome and the pole, seem unable to affect chromosome segregation (Figs. 3 and 4). From our results we hypothesize that, for crocidolite fibers to affect chromosome movement, they must be strongly anchored to the cellular matrix. In this context long thin fibers may be more carcinogenic than shorter thicker ones because they are thin enough to penetrate the intermediate filament cage surrounding the forming spindle and long enough to extend well into the spindle while remaining anchored within the cage.

Mesothelial cells are 10 times more sensitive than bronchial epithelial cells to asbestos (14, 18). An important difference between mesothelial and epithelial cells is their intermediate filament network (54, 55). Mesothelial cells have different keratin types and produce more vimentin than do epithelial cells (54). Mesothelial cells normally have a high keratin/low vimentin content in vivo, but during cell division it appears that the keratin content drops and the vimentin content rises (55). Mesothelial cells round during cell division and, like certain immortalized epithelial cell lines, this rounding is probably due to the breakdown of the keratin filament network, including the keratin cage (56). Thus, it is possible that the remaining vimentin filament cage surrounding the spindle is more permeable to asbestos fibers, allowing more chances for a deleterious chromosome-fiber encounter.

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REFERENCES


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