CROSS-BRIDGES ON THE MICROTUBULES OF COOLED INTERPHASE HeLa CELLS

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INTRODUCTION

Electron micrographs of spindle microtubules of plant (5, 6, 11) and animal cells (2, 6) in division show electron-opaque projections at right angles to the tubule axes. These have been interpreted as cross-bridges, and it has been proposed that they may participate in the movement of chromosomes (7). Cross-bridges have also been observed in a variety of specialized structures that consist of closely ordered arrays of parallel microtubules, e.g. the axonemes of *Actinosphaerium* (9, 10), the axostyles of flagellates (4), and K_m fibers of *Stentor* (1). The cytoplasmic microtubules of interphase cells, on the other hand, are free of cross-bridges; they are surrounded by an electron-transparent matrix or exclusion zone, into which ribosomes and other cytoplasmic particulates do not penetrate (8).

In experiments in which interphase HeLa cell cultures were cooled to 4°C to depolymerize the microtubules, we have regularly observed the transitory appearance of electron-opaque projections during the cooling and subsequent warming of cells. In this note, we describe these structures and comment on their interpretation.

MATERIAL AND METHODS

Monolayers of HeLa cells were cultured at 37°C in Falcon plastic Petri dishes (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.), in Eagle's minimum essential medium supplemented with 15% fetal calf serum. The cultures were cooled by transfer to a refrigerator at 4°C; the rate of cooling was determined by inserting into the culture medium of one dish a hypodermic thermistor probe connected to a telethermometer (Yellow Springs Instrument Company, Yellow Springs, Ohio, model 43T).

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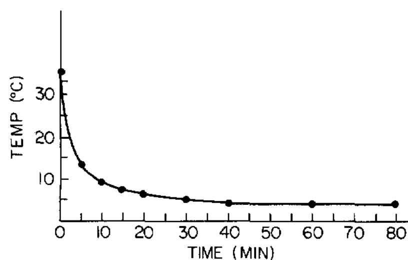

FIGURE 1 Changes in temperature in the culture medium after transfer to a refrigerator at time zero. Samples for electron microscopy were fixed at 10 min (ca. 9°C), 20 min (ca. 6°C), 40 min (ca. 4°C), and 60 min (ca. 4°C).

The cooling rate is shown in Fig. 1. Samples for electron microscopy were fixed at 10, 20, 40, and 60 min after the transfer. Other cultures remained in the refrigerator for 90 min and were then returned to the incubator at 37°C and fixed 30 min later. Each culture was rinsed in Hanks's balanced salt solution at a corresponding temperature and then fixed by addition of 3.5% glutaraldehyde (at 25°C) in phosphate buffer at pH 7.0. The cultures were postfixed in osmium tetroxide, embedded in Epon *in situ*, and sectioned either parallel to, or perpendicular to, the original growth surface as described previously (3). Measurements of cross-bridges were made from prints at a final magnification of 270,000.

RESULTS AND DISCUSSION

When HeLa cells are incubated and fixed at 37°C, microtubules are found to radiate from the cytocenter to the periphery, so that groups of closely associated tubules are infrequent (3). A search of such material for grouped microtubules was made, and in a few instances apparent cross-bridges were found (Fig. 9).

When HeLa cells are cooled to 4°C and fixed at this temperature, the number of recognizable microtubules is decreased and there is an increase in the prevalence of 90–100-A filaments. The latter tend to occur in bundles lying parallel to the cell membrane. In the samples fixed at 60 min in the experiments reported here, and in other samples maintained at 4°C for longer periods, microtubules are absent and the filaments persist.

HeLa cells fixed during the cooling process (10 and 20 min) had persisting microtubules. These tended to occur in parallel array, so that profiles of as many as 10 microtubules could be found in a single section cut parallel to the surface of cell attachment (see Figs. 2–8 for examples). Such microtubules from cooling cells differed from the control material in showing the presence of electron-opaque projections extending from the tubules, often linking adjacent tubules in the fashion of cross-bridges.

The length of the projections, as measured in the plane of section, ranged from 100 to 250 A; the width appeared to be more regular, with a mean value of 51 A (SD 18 A). The apparent electron opacity is less than, or equal to, the density of the microtubule wall as observed in side view. Occasional bridges appear to terminate halfway between adjacent tubules, and pairs of such half-bridges are observed: each protrudes from a tubule slightly out of register so that they do not meet (Fig. 8).

Sections perpendicular to the cell attachment surface were made to examine the appearance of the tubule groups in cross-section. The cross-bridges seen from this aspect are shown in Figs. 9–14. Half-bridges are occasionally observed to project from the isolated border of a tubule (Figs. 11, 12, 14).

The distance along the tubule axis between

FIGURES 2–8 Representative groups of parallel microtubules from interphase cells cooled for 10 min, sectioned parallel to the growth surface. Arrows indicate points along the length of the tubules where projections are found. All Figs., × 162,000.

FIGURE 2 Obliquely sectioned group that contains at least eight microtubules. Arrows indicate the levels of two representative electron-opaque projections.

FIGURE 3 A group of six microtubules, with some of the projections at the levels indicated by arrows.

FIGURE 4 A group of two or three tubules showing fairly regularly spaced projections, particularly near the region indicated by arrows.

FIGURES 5 and 6 Projections that show a regularly spaced series of densities.

FIGURE 7 Projections as seen in an extremely thin section.

FIGURE 8 Pair of microtubules with half-bridges, one pair not in contact shown at the level of the upper arrow.

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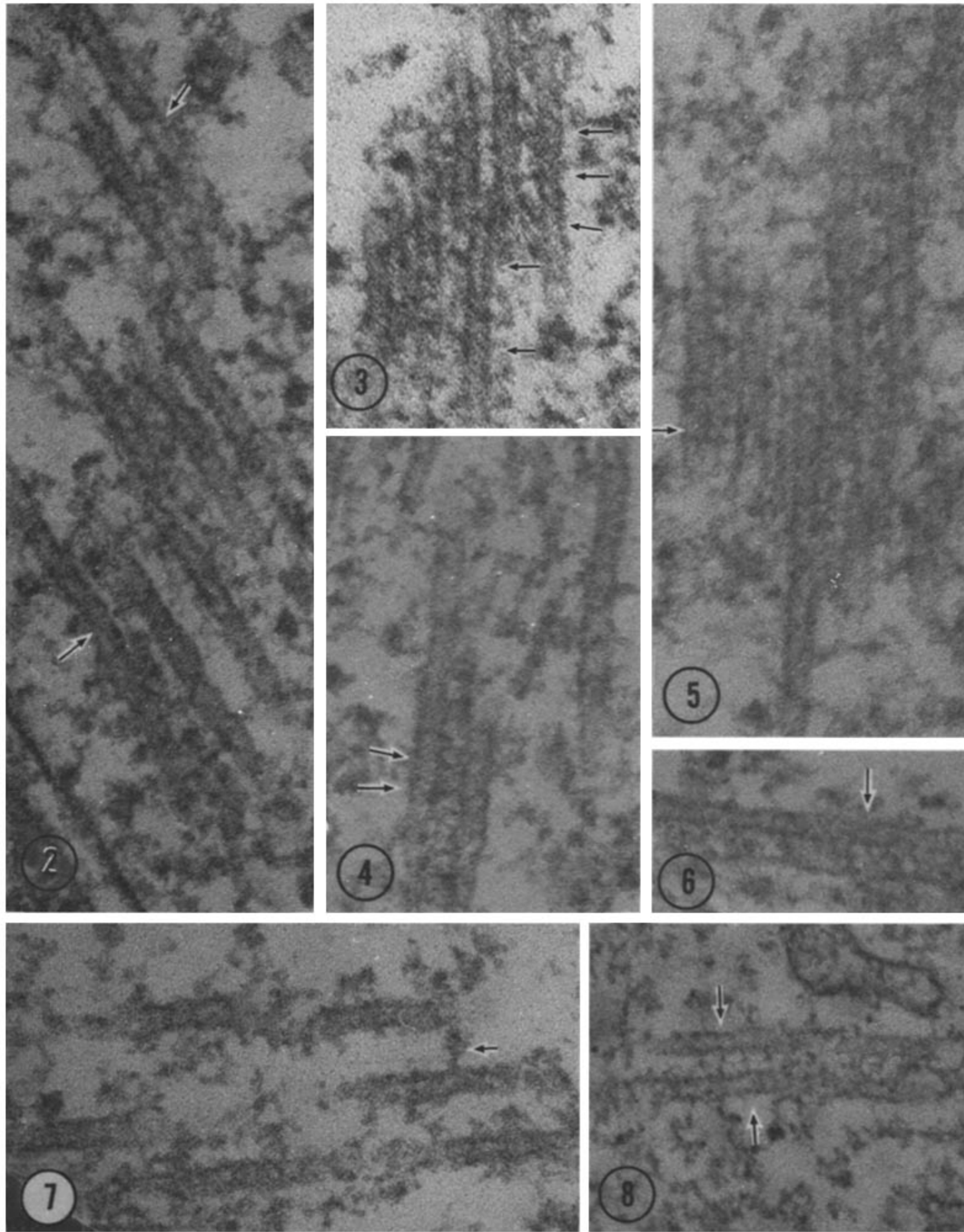

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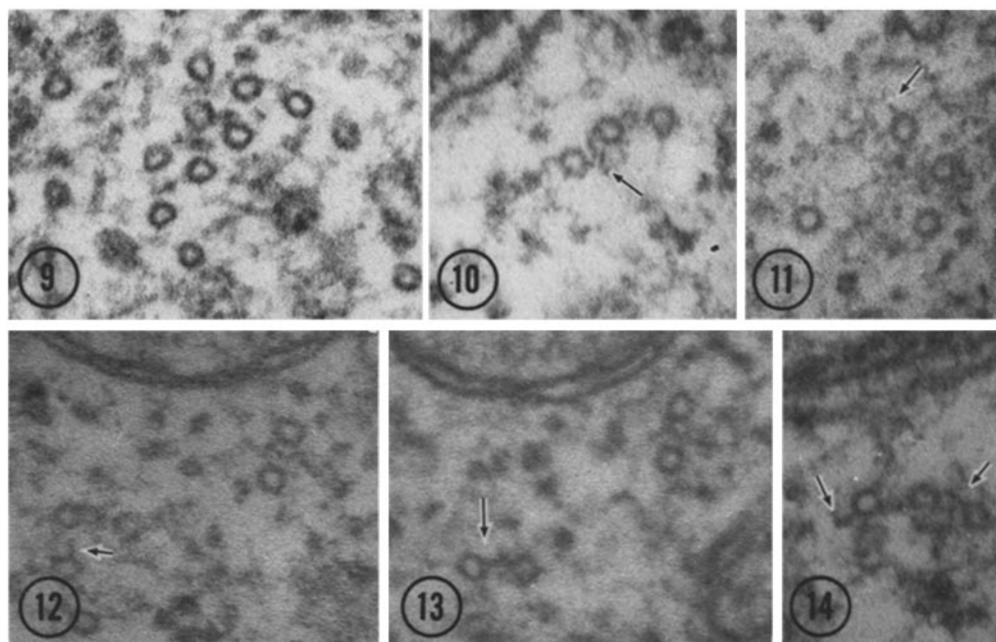

FIGURES 9–14 Cross-sections of microtubules in cells sectioned perpendicular to the growth surface. All Figs., × 162,000.

FIGURE 9 Group of microtubules in a control cell, incubated and fixed at 37°C.

FIGURES 10–14 Groups of microtubules from cells cooled for 10 min, showing electron-opaque projections from the tubule walls. Structures having the appearance of bridges or half-bridges are shown by arrows. Figs. 12 and 13 are through the same cell at different levels.

successive bridges ranged from 150 to 750 A. Although some micrographs suggested a regularly periodic arrangement (Figs. 5, 15, 16), optical diffraction of selected micrographs did not give unequivocal evidence of a regular periodicity along the length of the tubules.

The structures described above were most common in the samples fixed 10 min after the start of cooling (sample temperature ca. 9°C), but were regularly found in 20- and even 40-min samples (see Fig. 15). At 60 min, the microtubules themselves were absent. However, when such cells were returned to the 37°C incubator and fixed 30 min later, cross-bridged microtubules were again observed (Figs. 16, 17).

These observations suggest that the formation of cross-bridge–like structures on microtubules is not a specific response to metaphase, nor a property of spindle microtubules that is absent in the cytoplasmic microtubules of interphase. The material that we see as cross-bridges must exist in interphase cells, since a transfer to suboptimal temperatures seems unlikely to induce new syntheses, e.g. of a "cross-bridge protein." It seems more likely that the appearance of cross-bridges reflects a change in physical state, perhaps a loosening of bonds between protein subunits of the microtubules that permits binding of materials to sites unavailable at 37°C.

It also seems likely that the close association of microtubules plays a role in the appearance of cross-bridges. The tubules in cooled cells generally come closer to each other than do those of cells at normal temperature, probably because of disruption of the normal radial tubule array, although closely associated groups with fairly regular spacing can be found in normal cells (Fig. 9). Such close associations may make cross-bridge binding more likely.

The matrix, or exclusion zone, may play an important part in determining whether or not cross-bridges appear. If the matrix is composed of structural units that bind but little electron-opaque stain, separation or shrinkage of these units on cooling might allow penetration of the stainable material that we see as cross-bridges into the interstices. If this view is correct, what we are interpreting as cross-bridges may be a "negative" image of the microtubule matrix itself.

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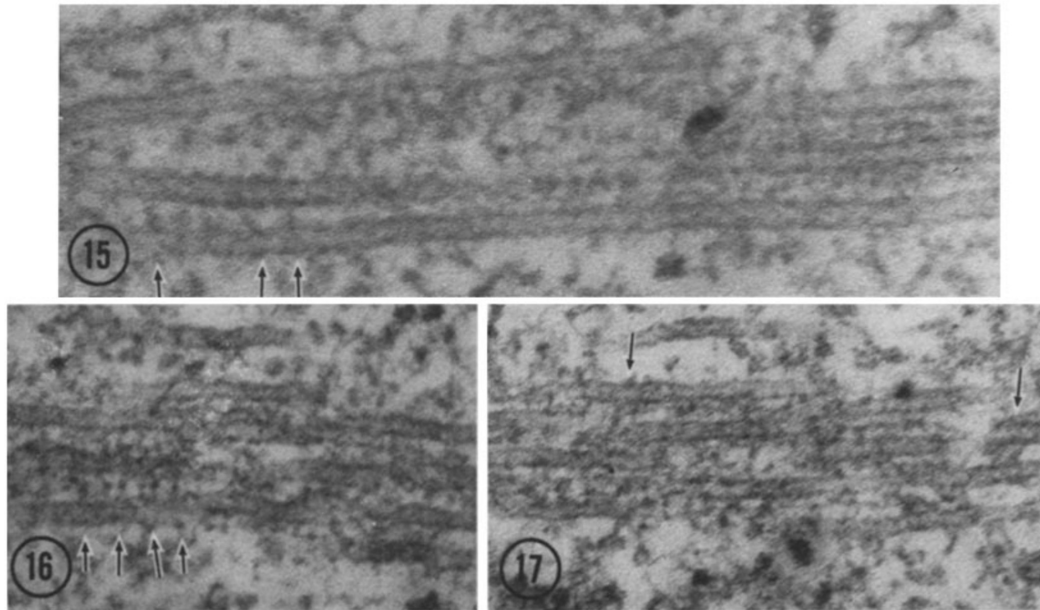

Figures 15–17 Electron-opaque projections from microtubules of treated cells, sectioned parallel to growth surface.

Figure 15 Group of five microtubules from a cell cooled for 40 min. Some of the projections are at the locations shown by arrows. × 180,000.

Figures 16 and 17 Serial sections through a cell fixed 30 min after transfer from 4°C to 37°C. Projections (examples at levels indicated by arrows) occur at many points. × 126,000.

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