Claims that Intermediate Filaments Contain F-Actin Are Unwarranted

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ABSTRACT   Examination of sectioned, embedded material showed that almost all intermediate filaments normally present in cultured chick embryo fibroblasts, IMR-33 cells, and BHK-21 cells were eliminated by trypsinization. At the same time atypical filaments of intermediate size were formed by close apposition of thin filaments. Previous observations (Buckley et al., 1978, *J. Cell Biol.* 78:644–652) on negatively stained and on critical-point-dried whole-cell mounts confused these different filament types and, on the basis of heavy meromyosin binding to the atypical intermediate-sized filaments, we suggested that many normal intermediate filaments contain F-actin. The present results show that this suggestion was unwarranted and we therefore retract it.

The idea that many nonmuscle cell movements might be based on actin-myosin interactions is now generally accepted (9). Because the intermediate-sized filaments of the subcortical cytoplasm (where organelles reside and move) are numerically dominant (6) and closely associated with organelles (see, e.g., reference 10), we wondered if some of these filaments might contain F-actin. As described elsewhere (4, 5), heavy mero-myosin (HMM) labeling studies of negatively stained and of critical-point-dried whole cells that were extracted and mildly trypsin-treated seemed to indicate that this was so: trypsiniza-tion appeared neither to change the intermediate filament morphology nor to alter significantly the relative proportions of thin and intermediate-sized filaments. As many intermedi-ate-sized filaments survived trypsinization and as virtually all surviving filaments bound HMM, we suggested that many normal intermediate filaments contained a core of F-actin, possibly in a multistranded form.

We have investigated this phenomenon further, using a wider range of cultured cells and supplementing the whole-cell studies with observations on sectioned material. Trypsinization eliminated virtually all intermediate filaments normally present and caused some thin filaments to aggregate to form filaments of intermediate size that could bind HMM. Accordingly, the interpretation we originally placed on the data from whole-cell mounts was unwarranted and we retract our suggestion that many normal intermediate filaments contain F-actin.

MATERIALS AND METHODS

Cells

IMR-33 (gerbil fibroma) and BHK-21 cell lines (originally obtained from the American Type Culture Collection, Rockville, Md.) and primary cultures of 10-d chick embryo connective tissue cells were cultured in Ham's F12 culture medium (Grand Island Biological Co. [GIBCO], Grand Island, N. Y.) supple-mented with 10% fetal calf serum (GIBCO) maintained at 38°C in an atmosphere of 95% air/5% CO_2. As required, cells were transferred to Rose chambers and grown on either carbon-coated cover glasses (when required for resin embedment and sectioning [1]) or on Formvar/carbon-coated cover slips (when required for critical-point drying [3]). Glycerol-pyrophosphate extraction, trypsinization, and HMM labeling were carried out as described previously (4, 5).

Fixation, Embedment, Sectioning, and Electron Microscopy

Glycerol was progressively diluted out until the cells were finally washed in 0.1 M KCl, 5 mM $MgCl_2$, 20 mM imidazole-HCl, pH 7. Cells were fixed in 0.8% glutaraldehyde in 0.264 M sucrose, 0.02% tannic acid (Mallinckrodt Inc., St. Louis, Mo.), 10 mM sodium cacodylate, pH 7.0 at room temperature for 2–4 h and postfixed at room temperature for 10 min in 1% osmium tetroxide, then for 10 min in 1% uranyl acetate. For sectioning, cells were handled essentially as described elsewhere (1) except that Epon was used for flat embedding, and an excised segment was placed directly into a flat embedding chuck. Thin sections were examined at 80 kV with a Philips EM201 microscope fitted with a 0.07-mm objective aperture and photographed using Kodak Kodalith, LR 2572 70-mm film. Stereoscopic micrographs were taken at ±6° from the horizontal.

RESULTS

The results obtained were essentially independent of the particular cell type investigated. As the findings from critical-point-dried cells were altogether similar to those already reported (5), only the results obtained on sectioned embedded material will be described here.

Sectioned Cells

NON-TRYPSIN-TREATED: Outside a wide empty zone surrounding the nucleus of glycerinated myosin-extracted cells, remnants of mitochondria, endoplasmic reticulum, and other membranous organelles were unevenly disposed through the subcortical space. Similarly, the distribution of subcortical filaments was extremely uneven and markedly different from that found in sections of unextracted cells. In accord with the findings of Ishikawa et al. (6), most of these subcortical filaments were intermediate in size. In most areas, relatively few thin filaments could be identified; occasional short "wispy" ones occurred free or attached to the surfaces of organelles, to surviving microtubules, or to intermediate filaments. However, as noted elsewhere (2), curious focal concentrations of tangled thin filaments were found in scattered small areas. The intermediate filaments were swollen (mean diameter, 14.5 ± 0.3 nm SEM; $n = 115$), remarkably uniform in width, and unbranched (Fig. 1a). Although single micrographs occasionally showed apparent branching, in every case both the "branches" and the filament of origin shared the same diameter and stereoscopy showed that these apparent branches were attributable to superposition of individual filaments. The characteristic intermediate filaments were always readily distinguishable from other types of cytoplasmic filament, even when these were of intermediate size. Variable numbers of filaments of intermediate size, which appeared to be formed by close apposition of converging thin filaments (arrowheads in Fig. 1a), were found irregularly distributed throughout both cortical and subcortical cytoplasm, especially towards the cell margins. Because these intermediate-sized filaments sometimes branched (giving off thin filaments), they probably corresponded to the branching intermediate filaments seen in similarly extracted cells that had been negatively stained (4) or critical-point dried (5). We stress again that in sectioned material the atypical intermediate-sized filaments involved in these network formations were morphologically distinct from the unbranching, uniform-caliber intermediate filaments described above.

TRYPSIN-TREATED: There was a moderate increase in the disorder of the cytoplasm of trypsin-treated extracted cells. Moreover, in many cells the nuclear envelope was breached, allowing nuclear material to spill into the cytoplasm. In most trypsin-treated cells, virtually all of the typical intermediate filaments had disappeared (Fig. 1b). Where remnants remained, they appeared as evenly swollen segments measuring up to 16 nm in diameter. Thin filaments were never seen to extend from the ends of these segments. In a small number of trypsin-treated cells, significant numbers of apparently normal (unbranching) intermediate filaments survived.

Most of the cells' thin filaments survived the trypsin treatment. As before, many intermediate-sized filaments, which appeared to be formed by close apposition of convergent thin filaments, were scattered through the cytoplasm. Thus, contrary to the impression gained by examining critical-point-dried (5) and negatively stained (4) whole-mount preparations, the overall effect of the trypsin treatment was largely to eradicate the
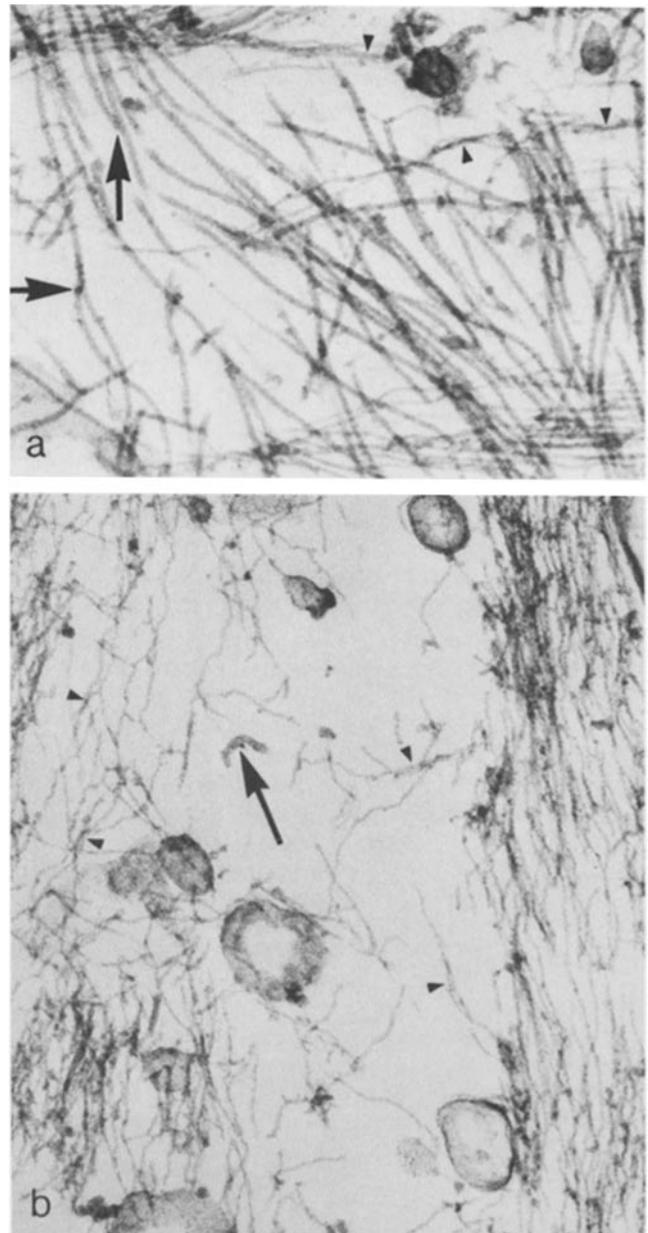
FIGURE 1 Sections through the cytoplasm of glycerol/myosin-extracted cultured chick embryo fibroblasts. (a) Before trypsin treatment. Most filaments are of intermediate size but uniformly swollen; some of them appear to branch (arrows); however, note that the filament of origin and the branches have the same diameter. There are also several thin-filament aggregates (arrowheads) that are intermediate in diameter. (b) After trypsin treatment. Here the loss of the typical intermediate filaments is almost complete, with only a single swollen segment remaining (arrow). Numbers of cortical and subcortical thin filaments have survived. However, in several places these have converged to form reticular arrays within which thicker (intermediate-sized) filaments (arrowheads) can be found. × 70,000.

characteristic intermediate filaments. The absence of residual F-actin was unlikely to be attributable to osmium-induced destruction (8) after exposure to trypsin, because using tannic acid in the primary glutaraldehyde fixative and reversing the order of treatment with osmium tetroxide and uranyl acetate (procedures known to protect F-actin against the destructive effects of osmium) made no difference to the end result, the eradication of the typical intermediate filaments.

TRYPSIN-TREATED, HMM-LABELED: Virtually all residual filaments showed clearcut arrowhead labeling. Well-defined bundles of HMM-labeled cortical filaments could be seen at the cell margins, just within the plasma membrane, whereas variable concentrations of HMM-decorated filaments were scattered unevenly between organelles in the subcortical cytoplasm. However, there were too few labeled filaments to account for the combined numbers of thin and intermediate filaments found in non-trypsin-treated cells, a finding consistent with the loss of the intermediate filaments normally predominant in the subcortical cytoplasm. Because HMM-labeled actin is protected against the destructive effects of osmium tetroxide (7, 8), there was no question here that subcortical actin-containing filaments had been lost by this mechanism.

DISCUSSION

From the experiments on sectioned material, it was clear that the trypsin treatment eradicated virtually the entire normal population of intermediate filaments in cultured IMR-33, BHK-21, and chick embryo connective tissue cells. This conclusion was based on (a) the low concentration of labeled subcortical filaments in the trypsin-treated HMM-labeled cells (cf. the combined concentrations of intermediate and thin filaments in non-trypsin-treated control cells) and, more significantly, on (b) the widespread loss of intermediate filaments from the subcortical cytoplasm of trypsin-treated controls. Here it was striking that most intermediate filaments disappeared leaving no visible filamentous material behind. Where scattered intermediate filament remnants were found, these appeared as swollen, abruptly terminating segments that exhibited no continuity with thin filament material.

It would therefore seem clear that we were incorrect, in our earlier study (4), in assuming that the predominant intermediate filaments had survived trypsin treatment. Although there were many intermediate-sized filaments in our negatively stained and critical-point-dried whole-cell mounts, the results obtained here on sectioned embedded cells indicated that these filaments were different from those present before trypsination. Therefore, our previous data should not be taken to indicate the presence of F-actin in the predominant intermediate filaments normally present and, accordingly, we retract this suggestion. Moreover, since the trypsin treatment allowed most of the cells' cortical and other thin filaments to survive, the apparent lack of residual intermediate filaments makes it seem likely that the intermediate filaments of these cells were devoid of F-actin.

In addition to retracting our earlier suggestion, we wish to draw attention to the type of artifact that gave rise to it. As described above and elsewhere (2), cell permeabilization and extraction procedures that are used to allow HMM labeling considerably disrupt the cells' filament systems, especially those of the subcortical cytoplasm. One pronounced effect is to enable many of the thin filaments to redistribute, many of them often concentrating together in discrete foci (see Fig. 7 in reference 2). Some thin filaments also become abnormally relocated close to other cytoplasmic structures, including intermediate filaments and other thin filaments. Although the cell permeabilization, extraction, and trypsin treatments were com-
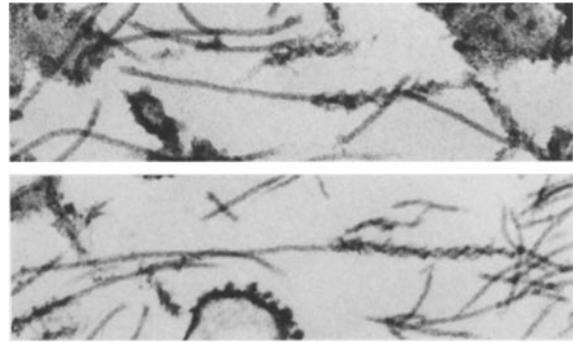

FIGURE 2 Superposition artifact often seen in HMM-labeled cells (these examples are from IMR-33 cells). On first sight, it appears that part of the intermediate filament has been labeled with HMM, but in all cases stereo pairs showed that this resulted from the superposition of intermediate filaments and labeled microfilaments. × 70,000.

mon to both the earlier and more recent sets of experiments, it seems very likely that the use of whole-cell mount preparations in the earlier experiments led to systematic errors of interpretation associated with fortuitous superposition of microfilaments (Fig. 2). It appears that both negative staining and critical-point drying failed to maintain the very fine separation that can exist between adjacent filaments, thus making them appear to be a single structure.

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