EFFECT OF THE IONIC ENVIRONMENT ON THE INCORPORATION OF THE INTERMEDIATE-SIZED FILAMENT PROTEIN VIMENTIN INTO RESIDUAL CELL STRUCTURES UPON TREATMENT OF EHRlich ASCITES TUMOUR CELLS WITH TRITON X-100

II. ULTRASTRUCTURAL ANALYSIS

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SUMMARY

Ehrlich ascites tumour cells were extracted in buffers containing Triton X-100 and mono-, di- and polyvalent cations and then analysed by phase-contrast and electron microscopy. The results of this ultrastructural analysis confirm those of the biochemical analysis in the accompanying paper that the stability of intermediate-sized filaments is dependent on the ionic environment. Furthermore, the organization of filaments in long parallel arrays is dependent on the presence of divalent cations and can be inhibited, to some extent, by the presence of monovalent cations. The stability of other detergent-resistant structures, the boundary lamina, microfilaments, microtubules, centrioles, polyribosomes and the nuclear cortex, is also affected by the ionic environment but to a lesser extent.

INTRODUCTION

The analysis of detergent-resistant residual cell structures (also termed 'detergent-resistant cytoskeletons'; Brown, Levinson & Spudich, 1976; Osborn & Weber, 1977) has been an important starting point for the study of the inter-relationship of the various cytoplasmic filament systems and their interaction with other cytoplasmic structures. Most of these investigations have been done by either indirect immunofluorescence microscopy (e.g. see Osborn & Weber, 1977; Lehto, Virtanen & Kurki, 1978; Hynes & Destree, 1978; Franke, Schmid, Weber & Osborn, 1979) or electron microscopy techniques (e.g. see Bell, Miller, Carraway & Revel, 1978; Small & Celis, 1978; Heuser & Kirschner, 1980). The results of these morphological studies have demonstrated that the detergent-resistant residual cell structures consist of intermediate-sized filaments and an actin-containing lattice-like network (Small & Celis, 1978; Wolosewick & Porter, 1979) and several other cytoplasmic structures (Ben-Ze'ev, Duerr, Solomon & Penman, 1979; Lenk, Ransom, Kaufmann & Penman, 1980).

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However, it is often difficult to compare the results of these studies because of the use of different buffers containing Triton X-100 for the extraction of cells: for instance, phosphate-buffered saline (Hynes & Destree, 1978; Franke et al. 1979; Cabral, Willingham & Gottesman, 1980; Campbell et al. 1979), 140 mM-NaCl and 5 mM-MgCl₂ (Osborn & Weber, 1977; Franke et al. 1979), 50 mM-NaCl and 2·5 mM-MgCl₂ (Lenk & Penman, 1979; Lenk, Storch & Maizel, 1980), 100 mM-PIPES and 0·5 mM-MgCl₂ (Heuser & Kirschner, 1980), 0·6 M-KCl (O'Connor, Gard & Lazarides, 1981) and 40 mM-Tris-HCl (Virtanen, Kurkinen & Lehto, 1979; Virtanen, Lehto, Lehtonen & Badley, 1980; Lehto et al. 1978).

In the preceding paper (Traub & Nelson, 1981) it was shown that the stability of vimentin-containing intermediate-sized filaments, as indicated by their incorporation into Triton X-100-resistant residual cell structures, is dependent on the ionic environment. In particular, when cells were extracted with low ionic strength buffers in the presence of 4 mM-Mg-acetate or 1·2 mM-spermidine all vimentin was retained in the Triton X-100-resistant residual cell structures; whereas in the presence of 1 mM-Mg-acetate very little vimentin was bound. At physiological KCl concentration about 50% of the vimentin was incorporated into the residual cell structures. A synergistic effect of mono- and di- or polyvalent cations on the association of vimentin with the nucleus was also detected. Furthermore, it was shown that the disassembly of vimentin-containing intermediate-sized filaments induced by swelling cells in low ionic strength buffers could be reversed, to a considerable extent, by restoration of isotonicity. These results were based on the quantitative extraction of vimentin and analysis by polyacrylamide gel electrophoresis.

In order to confirm and extend this biochemical analysis at the ultrastructural level, residual cell structures were analysed by phase-contrast and electron microscopy following treatment of Ehrlich ascites tumour cells with various selected buffers. The buffers were selected from the results of the preceding paper to demonstrate the differences in the morphology of the residual cell structures depending on the ionic environment, particularly with respect to the distribution of intermediate-sized filaments.

MATERIALS AND METHODS

Cell culture

Ehrlich ascites tumour (EAT) cells were grown in suspension culture using minimum essential medium supplemented with 5% foetal calf serum as described previously (Egberts, Hackett & Traub, 1976). The harvesting of cells was performed as described in the preceding paper.

Extraction of cells

Unless stated otherwise, all operations were carried out at 2 °C. The extraction of cells has been described in detail in the preceding paper. Briefly, cells were resuspended in basal buffer consisting of 10 mM-Na-cacodylate (pH 7·6), 1 mM-EGTA (cacodylate buffer was substituted for the Tris buffer used in the preceding paper to improve fixation and to exclude artifacts in the sections) containing 0·5% (w/v) Triton X-100 and additional salts as described in the text.
Fig. 1. Micrographs of residual cell structures following extraction of EAT cells in basal buffer containing 0.5% (w/v) Triton X-100. A. Phase-contrast micrograph; \( \times 260 \). B. Electron micrograph of residual cell structure; \( \times 9700 \). C. High magnification electron micrograph of the edge of the nucleus (\( n \)), which is surrounded by the nuclear cortex (nc); \( \times 46900 \).

and figures. The cells were extracted 3 times, applying 10 up-and-down strokes per extraction in a tightly fitting all-glass Dounce homogenizer. Between individual extractions, the Triton X-100-resistant residual cell structures were sedimented by centrifugation at 1500 \( g \) for 5 min. Prior to fixation for electron microscopy (see below), the residual cell structures were washed briefly in extraction buffer without Triton X-100.

In a second set of experiments, cells were resuspended in basal buffer supplemented with either 1 mm or 4 mm-Mg-acetate. The cells were allowed to swell for 30 min at 2 °C and then
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...processed for electron microscopy. Finally, cells that had been swollen in basal buffer containing 1 mM-Mg-acetate were restored to isotonicity by the addition of KCl to a final concentration of 150 mM. After a further 30 min at 2 °C, the cells were processed for electron microscopy.

Microscopy

Residual cell structures and swollen cells were analysed by phase-contrast microscopy before and after fixation in 2 % glutaraldehyde (see below). Cells were viewed in a Zeiss inverted IM 35 microscope.

For electron microscopy, cells were fixed in 2 % glutaraldehyde for 30 min in the same buffer used for the extraction procedure without Triton X-100. Following extensive washing, cells were post-fixed in 2.5 % osmium tetroxide in 100 mM-Na-cacodylate (pH 7.2) for 30 min. Cells were again washed extensively and then incubated in 2.5 % tannic acid in 100 mM-Na-cacodylate (pH 7.2) for 30 min; all operations were carried out at 2 °C. The cells were dehydrated in an ethanol/propyleneoxide series and embedded in Epon 812. Silver sections were cut with a DuPont diamond knife and counterstained in 2 % uranyl acetate and 2 % lead citrate. Sections were viewed in a Zeiss EM9 electron microscope.

RESULTS

Residual cell structures and swollen cells were analysed by phase-contrast microscopy before and after fixation in 2 % glutaraldehyde. The fixation procedure had no observable effect on the morphology of the residual cell structures.

The extraction of cells in basal buffer containing 0.5 % Triton X-100 solubilizes all intermediate-sized filaments and other cytoplasmic structures leaving naked nuclei (Fig. 1 A, B). Most of the nuclei remain intact, although very swollen (Fig. 1 A, B), with a diffuse chromatin structure. Surrounding the chromatin is an electron-dense boundary, which probably represents the nuclear cortex (Fig. 1 C). Fuzzy material occasionally observed in close proximity to the outside of the nuclear cortex is probably chromatin from the small percentage of nuclei that had been lysed during extraction (Fig. 1 C).

The addition of 1 mM-Mg-acetate to the basal buffer apparently has the effect of stabilizing several cytoplasmic structures thus rendering them resistant to solubilization during extraction with Triton X-100 (Fig. 2). The nuclei still appear swollen, although the chromatin has a more granular form (Fig. 2 D). In all cases the nuclear envelope is completely solubilized (Fig. 2 D). Surrounding the nucleus is a boundary lamina, 8–10 nm thick but without the typical trilaminar pattern of the plasma membrane (Fig. 2 B, D). In a narrow band between the nucleus and the boundary lamina there is a diffuse network of intermediate-sized filaments with some polyribosomes.

Fig. 2. Micrographs of residual cell structures following extraction of EAT cells with basal buffer containing 1 mM-Mg-acetate and 0.5 % (w/v) Triton X-100. A. Phase-contrast micrograph; x 260. B. Low-power electron micrograph of residual cell structures; x 10 200. C. High magnification of part of the residual cytoplasm containing intermediate-sized filaments (if), ribosomes (r) and endoplasmic reticulum-derived membranes (cm); x 35 500. D. Electron micrograph of the edge of the residual cell structure. The nucleus (n) is surrounded by intermediate-sized filaments (if), a few polyribosomes (r) and the boundary lamina (bl); x 30 200.
The intermediate-sized filaments appear to be randomly orientated and do not form parallel arrays (Fig. 2c; compare with Fig. 3c). The filaments also appear to be localized close to the nuclear cortex. Occasionally, membrane systems are observed that are probably derived from elements of the endoplasmic reticulum (Fig. 2c).

The effect of increasing the Mg-acetate concentration in the basal buffer to 4 mM is dramatic. Even in the light microscope the cells are observed to be surrounded by cytoplasmic material (Fig. 3A). The nuclei have a more normal appearance with condensed chromatin, some of which is localized at the periphery of the nucleus (Fig. 3B). The residual cell structure is surrounded by a boundary lamina. The volume of the residual 'cytoplasm' appears to be much greater than in the residual cell structures obtained by extraction of cells in 1 mM-Mg-acetate (Fig. 2; for comparison see also Figs. 4–6). The residual 'cytoplasm' contains many ribosomes and endoplasmic reticulum-derived membranes, often in association with one another (Fig. 3D). There are large numbers of intermediate-sized filaments, which are more electron-dense than those observed after extraction in 1 mM-Mg-acetate (Fig. 2c) and form long parallel arrays of filaments giving the appearance of bundles (Fig. 3C, E). The filaments appear to be organized in these arrays since distinct regions of either only longitudinally sectioned or only transversally sectioned filaments are seen (Fig. 3C). There is also some evidence that some areas of the residual cell, particularly in the perinuclear region, contain intermediate-sized filaments exclusively; in general, areas of polyribosomes have fewer filaments (Fig. 3D).

When 150 mM-KCl is substituted for the Mg-acetate in the basal buffer the volume of the residual 'cytoplasm' between the boundary lamina and the nucleus appears to be reduced (Fig. 4A, B; for comparison see Fig. 3A, B). The nuclei have less condensed chromatin with clearly defined nucleolar regions and a delineating nuclear cortex (Fig. 4B, D). The residual cell structure is surrounded by the boundary lamina. There is almost a complete absence of polyribosomes and only a few of the endoplasmic reticulum-derived membranes remain (Fig. 4D). There are fewer intermediate-sized filaments present and they appear to be less organized than they were following extraction of cells with 4 mM-Mg-acetate (compare Figs. 4C and 3C); the filaments are not arranged in parallel arrays and filaments in transverse section are almost equally dispersed amongst those in longitudinal section (Fig. 4C, D). Furthermore,
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there appears to be a more general distribution of filaments in the residual 'cytoplasm' and less perinuclear localization (Fig. 4D).

The effect of polyvalent cations on the stability of intermediate-sized filaments was analysed ultrastructurally, following extraction of EAT cells with basal buffer containing 1-2 mM-spermidine (Fig. 5). The residual cell structures have a very dense appearance (Fig. 5A, B). The boundary lamina remains intact, with an electron-dense band just underneath; this is probably produced by the aggregation of submembraneous microfilament bundles (Fig. 5C). The space between the boundary lamina and the nucleus contains very electron-dense unstructured material, which is probably the result of aggregation of intermediate-sized filaments and ribosomes (Fig. 5C, D).

The synergistic effect of mono- and polyvalent cations was analysed with 0-4 mM-spermidine and 50 mM-KCl in the basal buffer (Fig. 6). The morphology of the residual cell structures is similar, but not identical, to that shown in Fig. 4. In general, the space between the boundary lamina and the nucleus is greater (for comparison see Fig. 4B) and consists of large numbers of intermediate-sized filaments, polyribosomes and endoplasmic reticulum-derived membranes (Fig. 6D). The intermediate-sized filaments appear to be more organized than following extraction with 150 mM-KCl (compare Figs. 6C and 4C), however, they do not form such extensive parallel arrays of filaments as seen after extraction with 4 mM-Mg-acetate (compare Figs. 6C and 3C).

These results clearly show that the ionic environment has a significant effect on the stability of not only intermediate-sized filaments but also several other structures in the Triton X-100-resistant cell residues. In order to observe the effect of the ionic environment on cells with widely intact membranes, cells were swollen in 1 mM and 4 mM-Mg-acetate, respectively, and then, in the former case, the isotonicity was restored by the addition of KCl to the buffer. Fig. 7 shows the result of swelling cells in basal buffer containing 1 mM-Mg-acetate. The cells are very swollen (Fig. 7A, B), with dense material localized on one side of the nucleus and with the rest of the cytoplasm relatively clear (Fig. 7A). These clear areas are apparently devoid of membranes, ribosomes and filaments (Fig. 7C). The cellular membranes (endoplasmic reticulum, mitochondria and Golgi apparatus) are very distorted by the swelling process; however, in all cases the plasma membrane remains intact. Amongst the membranes a few intermediate-sized filaments can be seen (Fig. 7C).

When cells are swollen in basal buffer containing 4 mM-Mg-acetate, 2 distinct populations of cells are observed (Fig. 8A-C). One population (Fig. 8B) consists of very swollen cells, similar to those incubated in 1 mM-Mg-acetate (for comparison

Fig. 4. Micrographs of residual cell structures following extraction of EAT cells with basal buffer containing 150 mM-KCl and 0-5 % (w/v) Triton X-100. A. Phase-contrast micrograph of residual cell structures surrounded by 'cytoplasm' (arrows); × 260. B. Low-power electron micrograph of residual cell structures; × 3600. C. High magnification of a typical area of the cell containing intermediate-sized filaments (if); × 35 100. D. Edge of a residual cell structure. The nucleus (n) is surrounded by randomly orientated intermediate-sized filaments (if) and the boundary lamina (bl). A few 'cytoplasmic' membranes are also present (cm); × 30 400.
Fig. 5. Micrographs of residual cell structures following extraction of EAT cells with 1.2 mM-spermidine and 0.5% (w/v) Triton X-100. A. Phase-contrast micrograph; x 260. B. Low-power electron micrograph of a residual cell; x 8600. C. High magnification of part of the boundary lamina showing aggregated submembraneous microfilament bundles (mf) and other electron-dense aggregates in the 'cytoplasm' (arrows); x 35,100. D. Another part of a residual cell structure showing the nucleus (n) surrounded by electron-dense aggregates (arrows) and the boundary lamina (bl); x 28,300.
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see Fig. 7 A, B); the second population (Fig. 8 C) consists of less swollen cells with condensed chromatin; the ratio of the number of these cells is about 1:2, respectively (Fig. 8 A). The less swollen cells have large numbers of intermediate-sized filaments, particularly in the perinuclear region, which form long parallel arrays of filaments (Fig. 8 E). In contrast, the very swollen cells (Fig. 8 B) have very few intermediate-sized filaments (Fig. 8 D).

Fig. 9 shows the results of restoring isotonicity to cells that had been swollen in 1 mM-Mg-acetate (see Fig. 7). The cells are less swollen (Fig. 9 A), although the cytoplasmic membranes are still distorted. However, there are large numbers of intermediate-sized filaments in the cells (Fig. 9 C), particularly close to the nucleus (Fig. 9 B).

DISCUSSION

EAT cells have a characteristic arrangement of intermediate-sized filaments in the form of organized arrays or bundles of filaments encircling the nucleus (our unpublished observations; see Kim & Okada, 1981). In the accompanying paper, it was shown by biochemical analysis that the amount of vimentin-containing intermediate-sized filaments that remain associated with the nucleus in Triton X-100-resistant residual cell structures is dependent on the ionic environment. The results of the ultrastructural analysis of these residual cell structures confirm the biochemical findings; although it is not possible to quantify amounts of intermediate-sized filaments retained in the residual cell structures, it is possible to observe the organization of the filaments and their relationship with other structures.

The largest concentrations of intermediate-sized filaments, particularly those that were organized in long parallel arrays, were observed following extraction of EAT cells in basal buffer containing 4 mM-Mg-acetate (Fig. 3). This compares well with the results of the biochemical analysis, which showed that about 95% of the vimentin was retained in these residual cell structures (accompanying paper). A similar amount of vimentin was found associated with the nucleus of the residual cell structures following extraction of EAT cells with 1-2 mM-spermidine in the basal buffer. The ultrastructural analysis of these cell residues, however, showed large aggregates of very electron-dense material, which probably consisted of intermediate-sized filaments and ribosomes (Fig. 5). White (1972 a, b) has observed a similar effect of cationic polyelectrolytes on microtubules in platelets. It had been shown previously that microtubules are surrounded by a clear zone (Behnke, 1970), which is alcian blue-positive (see White & Gerrard, 1979). The effect of the cationic polyelectrolytes was to cause this clear zone around individual microtubules to become moderately electron-dense. White & Gerrard (1979) concluded from these results that microtubules are surrounded by a negatively charged zone, although they were uncertain as to the physiological significance of this. Since intermediate-sized filaments appear more electron-dense in 4 mM-Mg-acetate (Fig. 3) and 1-2 mM spermidine (Fig. 5), it is possible that they are also surrounded by a negatively charged zone similar to microtubules. Thus, di- and polyvalent cations may stabilize intermediate-sized
Fig. 6. For legend see p. 91.
Fig. 7. For legend see p. 91.
Fig. 8. For legend see opposite.
filaments and also play a role in their organization by a process of inter-connecting them into parallel arrays or bundles. This hypothesis is also supported by the results of Fukuyama, Murozuka, Caldwell & Epstein (1978) on the assembly and alignment of keratin filaments in vitro in the presence of divalent cations.

The effect of divalent cations on the retention and organization of intermediate-sized filaments is also indirectly demonstrated by the results of extracting EAT cells with monovalent cations. Following extraction in 150 mM-KCl, there are fewer intermediate-sized filaments present (compare Figs. 3 and 4) than after extraction with 4 mM-Mg-acetate. The filaments appear to be randomly orientated and do not form parallel arrays (see Fig. 4c). The biochemical analysis of these residual cell structures showed that approximately 50% of the vimentin had been retained. Thus, even in the absence of di- and polyvalent cations, charged macromolecules can interact when their net charges are compensated by monovalent counterions. However, this interaction is not so stable as, for instance, in the presence of Mg$^{2+}$ (accompanying paper). The combination of 50 mM-KCl and 0.4 mM-spermidine in the extraction buffer stabilized a larger number of filaments (Fig. 6), with 75% of the vimentin retained in the residual cell structures as shown by the biochemical analysis. Although the intermediate-sized filaments had a more organized appearance, they did not form parallel arrays characteristic of residual cell structures obtained in basal buffer containing 4 mM-Mg-acetate (compare Fig. 6c with Fig. 3c, e). The biochemical analysis of the synergistic effect of mono- and di- or polyvalent cations showed that even at optimal Mg$^{2+}$ or spermidine concentrations it was impossible to achieve quantitative

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Fig. 6. Micrographs of residual cell structures following extraction of EAT cells with basal buffer containing 0.4 mM-spermidine, 50 mM-KCl and 0.5% (w/v) Triton X-100. a. Phase-contrast micrograph. Note the presence of 'cytoplasm' surrounding the nucleus (arrows); × 260. b. Low-power electron micrograph of residual cell structures; × 4400. c. High magnification of typical region of the cell containing intermediate-sized filaments (i/f) and ribosomes (r); × 35000. d. Part of a residual cell structure showing the relative distributions of the nucleus (n), boundary lamina (bl), intermediate-sized filaments (i/f) and endoplasmic reticulum-derived membranes (em); × 30600.

Fig. 7. Micrographs of EAT cells swollen in basal buffer containing 1 mM-Mg-acetate. a. Phase-contrast micrograph. Note the localization of dense material on one part of the cell (arrowheads). The rest of the cytoplasm is relatively clear (arrows); × 260. b. Low-power electron micrograph of a swollen cell; × 7100. c. High magnification of a typical swollen cell. The cytoplasm membrane systems (endoplasmic reticulum (er), mitochondria (m)) are distorted by the swelling although the plasma membrane (pm) is intact. A few intermediate-sized filaments (i/f) can be seen. Parts of the cells (cyt) are devoid of membranes and filaments; × 23300.

Fig. 8. Micrographs of EAT cells swollen in basal buffer containing 4 mM-Mg-acetate. a. Phase-contrast micrograph showing 2 populations of cells (a, b); × 260. b. Electron micrograph of a typical cell of population b; × 6000. c. A typical cell of population a; × 6000. d. High magnification of part of the cell shown in Fig. 8b showing the presence of a few intermediate-sized filaments (i/f) amongst the endoplasmic reticulum (er). The cell is surrounded by the intact plasma membrane (pm); × 24700. e. High magnification of part of the cell shown in Fig. 8c. Large numbers of intermediate-sized filaments (i/f) can be seen surrounding the nucleus (n); × 24700.
incorporation of vimentin as long as monovalent cations were included in the extraction buffer. Thus the results of this ultrastructural analysis confirm those of the biochemical analysis, that monovalent cations probably inhibit the cross-linking of filaments into stable networks by competing with di- and polyvalent cations for critical binding sites.

We have extended this investigation to include the effect of changes of the ionic environment on the stability of intermediate-sized filaments in EAT cells with widely intact membrane systems. Cells swollen in basal buffer containing 1 mM-Mg-acetate had very few intermediate-sized filaments (Fig. 7), in good agreement with the results of the biochemical analysis. However, in basal buffer containing 4 mM-Mg-acetate, 2 populations of cells were observed (Fig. 8). One population comprised cells with few intermediate-sized filaments and a similar appearance to cells swollen in basal buffer containing 1 mM-Mg-acetate. The other population of cells (60-70 % of the total cell population) had less distorted membranes and a nearly normal distribution of parallel arrays of intermediate-sized filaments in the perinuclear region. The biochemical analysis of these cells showed that 60-70 % of the vimentin was found in an insoluble (filamentous) form. It seems that the cells which, for an unknown reason, excluded Mg ions from their cytoplasm had completely disassembled filaments, whereas those cells that were permeable to the Mg ions had a stabilized filament system despite the hypotonic buffer conditions. Restoration of isotonicity to the cells swollen in basal buffer containing 1 mM-Mg-acetate resulted in the reassembly of large numbers of intermediate-sized filaments (Fig. 9). These results further substantiate the earlier results described above, that the stability of intermediate-sized filaments is dependent on the ionic environment.

Finally, the ionic environment appears to affect the integrity of several other cytoplasmic structures rendering them resistant to solubilization by Triton X-100. The nuclear cortex appears to be resistant to detergent extraction even in low ionic strength buffers without di- or polyvalent cations. The boundary lamina, which has been shown to be derived from the plasma membrane (Ben-Ze'ev et al. 1979; Nelson & Traub, 1981), and its associated submembranous array of microfilaments is apparently stabilized in basal buffer containing either mono-, di- or polyvalent cations. Microtubules seem to be stabilized in buffers containing monovalent and di- or polyvalent cations. The centrioles appear to be stable in all the buffers tested, except following extraction of cells in basal buffer alone (data not shown). Polyribosomes were present in all residual cell structures obtained with buffers that contained di- or polyvalent cations. Associated with, and sometimes in direct contact with, the ribosomes were short pieces of membraneous material. Maximum numbers of these membranes were present under conditions that stabilized the greatest number of polyribosomes (e.g. 4 mM-Mg-acetate). That ribosomes were sometimes seen in direct contact with these membranes suggests they were derived from the endoplasmic reticulum. In general, most of these structures were affected to a lesser extent than the intermediate-sized filament network by changes in the ionic environment.

It appears, therefore, that the ionic environment may play a greater role than has been hitherto realized in the organization of intermediate-sized filaments and their
interaction with other cytoplasmic structures in the cell. Indeed there is morphological evidence that microtubules and intermediate-sized filaments may be associated (Geiger & Singer, 1980; Borenfreund, Schmid, Bendich & Franke, 1980), although it has also been shown that it is possible to disrupt the microtubule system without affecting the distribution of intermediate-sized filaments (Virtanen et al. 1980). As described earlier, microtubules appear to be surrounded by a negatively charged zone and thus an association with intermediate-sized filaments could be affected by the ionic environment. There is also some evidence to suggest that intermediate-sized
filaments may interact with the plasma membrane (Schliwa, 1975; Weihing, 1979; Cooke, 1976), coated vesicles (accompanying paper), mitochondria (Toh, Lollaï, Mathy & Baum, 1980; Guillouzo, Guillouzo & Boisnard, 1978) and the nuclear periphery (Lehto et al. 1978; Woodcock, 1980). Furthermore, recent studies have shown that the cytoplasm of cultured cells comprises a 3-dimensional lattice (the microtrabecular lattice; Wolosewick & Porter, 1976, 1979) of interconnected filaments (see also Small & Celis, 1978; Heuser & Kirschner, 1980). However, there are no specializations at the points of intersection of the filaments (Heuser & Kirschner, 1980). The evidence presented in this paper indicates that, at least in the case of intermediate-sized filaments, the interconnection of filaments is stabilized in certain ionic environments. Thus conditions for the extraction of cells should be chosen not only to retain the maximum number of filaments but also to stabilize their spatial organization.

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