Intermediate Filaments

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Intermediate filaments form a class of insoluble cytoplasmic filaments of dimensions intermediate in size between those of microtubules (about 25 nm in diameter) and microfilaments (about 7 nm in diameter). Thus, they are generally known as intermediate-sized filaments (IF)[1] or 10-nm filaments. They have been identified in virtually all differentiated types of cells and it seems possible that IF-like proteins also are present in even the simplest eukaryote cell types. With immunological, biochemical, and molecular biological techniques, it has been possible to distinguish at least five distinct classes of proteins that constitute IF in different cells or tissues (30, 31, 46, 67): desmin or skeletin in myogenic cells; neurofilaments in neuronal cells; glial filaments in astroglia and related cells; vimentin or decamin in mesenchymally derived cells; and a more complex group of keratins or cytokeratins in epithelial tissues. Some embryonic cells seem not to contain IF, but further studies are required to confirm this (36). Although this simple cell-type classification system is of considerable value in understanding IF and has provided information on cellular origins during development, there is a growing list of addenda and exceptions. For example, it was recognized some time ago that some cells express vimentin in addition to their cell-type class of IF subunit (32, 67). Some fibroblastic cell lines have been shown to possess keratin-like IF proteins associated with their nuclear surfaces (69). We expect that more exceptions will be identified in the future, possibly requiring a revised system of IF subunit nomenclature.

Each IF system is currently the subject of intense research and the understanding of IF is increasing rapidly. Here, we will briefly review some of the salient features of the different types of IF and present recent data from our laboratories on structural-functional aspects of IF.

Keratin IF

Keratin IF are present in large amounts in various epithelial tissues throughout higher organisms. They are insoluble in neutral aqueous solutions but can be extracted in the form of their constituents subunits in denaturing solvents such as sodium dodecyl sulfate or 8 M urea. Subsequent one- or two-dimensional gel electrophoresis of tissue extracts has identified about 19 different subunits in human epithelial tissues, including specialized epithelia such as the epidermis, but when the keratin IF subunits of various epithelial derivatives (hair, nail, etc.) are included, the total number may approach 30 (35). Their molecular weights vary from 40,000 to 70,000. On two-dimensional gels, the subunits fall into distinct acidic and basic groups. Analyses using both gel electrophoresis (35) and monoclonal antibodies (61, 64) of any one epithelium usually show only a few subunits and always a coordinated doublet of at least one acidic and one basic subunit. Specific recombinant cDNA probes to an acidic and a basic human epidermal keratin subunit have been used to demonstrate the presence of these two types of keratins throughout vertebrate epithelia and perhaps also in simpler eukaryotes (13). Simple epithelia of ductal linings express keratins of small size, an acidic 46-kdalton and a basic 56-kdalton (35, 61, 64); more complex stratified squamous epithelia such a basal epidermal cells express acidic keratins of 46, 50, and 52 kdalton and basic keratins of 56 and 58 kdalton (59 and 60 kdalton in mouse) (40, 61, 64); terminally differentiating epidermal cells express the largest keratins, an acidic 56.5 kdalton (59 kdalton in mouse) and a basic 67 kdalton keratin (40). Some epithelially derived tumors express more subunits than in their cell type of origin, including the smallest (40 kdalton). Thus, keratins consist of a highly complex multigene family of proteins, subsets of which are differentially expressed in various epithelia, depending on the type, degree of complexity, and stage of differentiation.

Despite these variations, keratins all have similar properties. For example, immunologically, polyclonal and monoclonal antibodies elicited to certain subsets of keratins have been used to visualize the distribution of keratin filaments in many types of epithelia (Fig. 1) (9, 64). Such widely cross-reacting antisera have been useful in demonstrating the epithelial origin of certain types of cells (8, 9, 60) and promise to be useful in the diagnosis of tumors of epithelial origin (36, 58). The wide cross-reactivity of all of the existing keratin antisera described so far suggests that they have been elicited against structural features common to all keratin subunits. However, it has now been possible to produce keratin subunit-specific antibodies to carboxyl-terminal peptides of individual keratins.[2]

[1] *Abbreviations used in this paper:* IF, intermediate filaments; IFAP, intermediate filament-associated proteins; MF, microfilaments; MT, microtubules; STEM, scanning transmission electron microscopy.

[2] Roop, D. R., C. K. Cheng, L. Titterington, C. A. Meyers, J. R.

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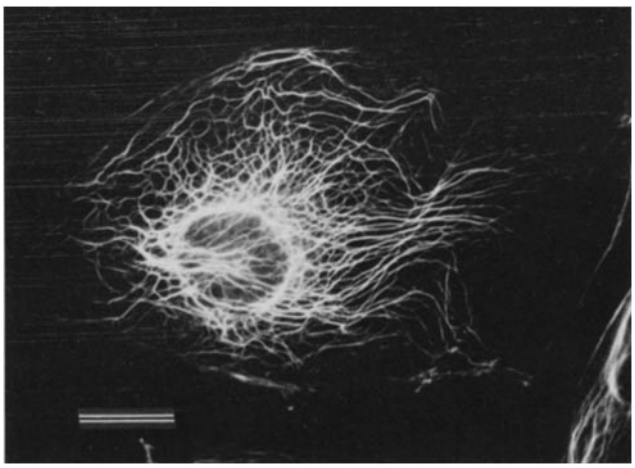

FIGURE 1 Indirect immunofluorescence microscopy of a PtK_1 cell grown in culture using a polyclonal antikeratin antibody elicited against a mouse epidermal keratin preparation (27). Bar, 10 μm.

Biochemically, keratin IF subunits differ from other IF subunits in having higher contents of glycine and serine, which we now know is due to unusual sequences rich in these residues located on their terminal domains (see below). Many purified keratin subunits have been induced to self-assemble in vitro into native-type IF (47, 52, 59), but at least two different subunits, one acidic and one basic, seem to be required for this process. Interestingly, copolymer IF can also be formed from mixtures of an acidic keratin subunit and either of the acidic proteins desmin or vimentin (52). The structural basis or rationale of assembly-compatible combinations of subunits required for keratin IF assembly in vitro and in vivo remains to be determined. The availability of subunit-specific keratin antisera and specific recombinant DNA probes to individual keratin subunits should prove to be valuable in resolving fundamental questions relating to keratin gene expression during epithelial cell differentiation as well as keratin IF assembly in vivo and functions in cells.

Neuronal and Glial IF

Neurofilaments are major structural components of axons and dendrites and often appear to be aligned and associated with microtubules (MT) along the axes of these processes. Their likely structural role is evident in such tissues as the giant axons of *Myxicola* in which the neurofilaments constitute the bulk of the protein of the axoplasm in the form of a structured gel. Isolated vertebrate neurofilaments consist of a triplet of proteins of about 200, 150, and 68 kdaltons (6, 25), which may all participate in assembly in vitro, but only the latter protein has been shown to assemble by itself (15, 34, 68). Interestingly, native and in vitro–assembled neurofilaments, unlike all other IF types, display side-arm appendages. Recent biochemical data suggest that the two larger subunits are integral components of the neurofilaments in vivo, with large portions projecting from the filament periphery (16). It has been speculated that such projections mediate interactions between neighboring neurofilaments and/or other structures, such as MT (42, 63). Invertebrate neurofilaments are distinctly different, but also consist of groups of subunits of about 150 and 60 kdaltons, although the relative amounts of the two size classes vary between species. For example, squid brain neurofilaments contain both size classes (66), but *Myxicola* giant axons contain only the larger subunit (18). Nevertheless, each of these participates in cycles of filament assembly/disassembly in vitro (66). Neurofilaments in general are exquisitely sensitive to Ca^{2+}-activated proteases, which may somehow regulate their function in axons (18). It has been suggested that the apparent spiral arrays of the larger subunits on the neurofilament periphery provide a mechanism for axonal transport (63).

Glial IF are clearly distinct from neurofilaments and are unique products of glial cells and cells of glial origin. They consist of a single protein, glial fibrillary acidic protein of 51 kdaltons (19, 43), that can form native-type IF in vitro with characteristic immunological and solubility properties (41).

Muscle IF

The IF protein of adult smooth muscle was first identified as an insoluble residue retained after extraction of the contractile proteins and is variously known as skeletin (44) or, more commonly, desmin (32). It is now known to be present in all types of muscle, although often in small amounts. Desmin is an acidic protein of 53 kdaltons, consists of two isoelectric variants differing only in their degree of phosphorylation, and can be reassembled in vitro (26, 49).

Both morphological and immunological studies have demonstrated that desmin is located at the region of the Z line in muscle (8, 30, 33). Thus, desmin is thought to mechanically integrate the contractile actions of the muscle fiber by linking adjacent myofibrils laterally through the Z discs and to the plasma membrane (30). Desmin may therefore play an important role during myogenesis in organizing the lateral order of adjacent Z discs, and a considerable body of evidence has been adduced in support of this model. Early myotubes in cell culture express both vimentin and desmin IF arranged throughout the cytoplasm, but, as differentiation proceeds and the Z discs assemble, the desmin IF begin to associate with the Z discs, with a concomitant reduction in the cytoplasmic arrays of vimentin and desmin (3, 14, 20). Elucidation of the details of this process remains an exciting area of research.

Mesenchymal IF

Many cells of mesenchymal origin as well as cells derived from them grown in culture contain abundant IF whose properties distinguish them from those of other specialized cells. This protein has been isolated from many types of cell and usually is known as vimentin (10) or decamin (65). A characteristic feature of vimentin-containing cells is their response to treatment with MT inhibitors such as colchicine; the IF withdraw from their cytoplasmic arrays to form a perinuclear birefringent "cap" and redeploy throughout the cytoplasm on removal of the drug from the culture medium (45) (Fig. 2). In addition, the distribution of vimentin IF can undergo major configurational changes during the cell cycle without apparent disaggregation (45). The IF of BHK-21 cells, for example, transiently "cap" after cell division and spreading of the daughter cells or following replating after trypsinization (45). Various perturbations of cultured cells, such as microinjection of antivimentin antibodies, infections with viruses, or additions to the culture medium of toxins and other chemicals

Stanley, P. M. Steinert, and S. H. Yuspa. Carboxyl-terminal amino acid sequences of keratin subunits elicit highly specific antibodies. *J. Biol. Chem.* In press.

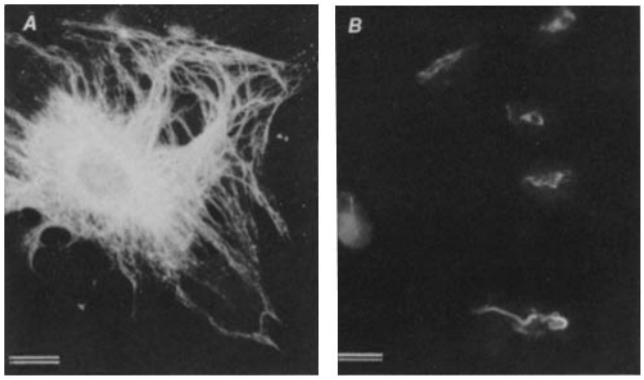

FIGURE 2 Indirect immunofluorescence microscopy of mouse 3T3 cells grown in culture in the absence (*A*) and presence (*B*) of colchicine. Bar, 10 μm. Courtesy of Dr. Michael Gottesman.

such as vanadate, can also reversibly disrupt the normal cytoplasmic array of vimentin IF, usually resulting in collapse toward the nucleus.

The capacity of cultured cells to concentrate their vimentin IF by capping has facilitated their isolation and characterization (45), although it has subsequently been learned that native IF can also be isolated in high yield and in an enriched form simply by taking advantage of their insolubility in aqueous solvents of high ionic strength (53). Purified vimentin can self-assemble in vitro into native-type homopolymer IF (Fig. 3) under conditions of physiological ionic strength and pH (4, 52, 53). Vimentin IF, like all other types of IF assembled in vitro, can be reversibly disassembled into smaller "protofilamentous" particles by simple manipulation of the solvent.

Coexistence of Different IF Subunits in a Single Cell

The preceding discussion has summarized the distinctive properties of the various types of IF, but a number of studies have shown that some specialized cell types can also coexpress vimentin IF under certain circumstances, including normal development, cellular transformation, and growth in culture. Some established epithelial cell lines, such as PtK_2 cells and HeLa cells, contain prominent arrays of both keratin and vimentin IF, of which only the latter collapse on exposure to colchicine (2, 11). This indicates that the two IF systems form separate networks in the cells. The expression of vimentin is not a universal characteristic of cultured or transformed epithelial cells, suggesting that the expression of the vimentin and keratin IF subunits is tightly regulated during normal differentiation and is variably and abnormally altered during transformation or accommodation to growth in cell culture. Early differentiating myotubes and cultured BHK-21 cells contain both desmin and vimentin in what appear to be overlapping or coincident cytoplasmic arrays; both systems are susceptible to colchicine (20, 45). In vitro experiments suggest that desmin and vimentin in fact copolymerize into the same IF (49). In this case, it seems that that the two IF systems cooperate in their cellular functions.

Structural Features of IF

One of the more perplexing questions that arose in the study of IF was that of how subunits of widely differing masses and properties could all form morphologically similar structures. We now know that IF are a structurally homologous family of cytoskeletal proteins. All IF give an α-type x-ray diffraction pattern (Fig. 3*c*), which indicates they contain regions of coiled-coil α-helix aligned approximately parallel to the filament axis. Complete or partial amino acid sequences are now known for several IF subunits (17, 23, 24, 39, 54, 55, and footnote 3), and several interesting features have become clear (Fig. 4). All subunits contain a large central α-helical domain of similar size that can form four extended tracts of coiled-coil α-helix interspersed with short non-α-helical inclusions. Analyses of the exact size of these α-helical tracts and of sequence homologies between different IF subunits have shown that there are at least three distinct types of α-helical domain: type I for acidic keratins; type II for basic keratins; and type III for vimentin and desmin (and possibly glial fibrillary acidic protein) (5, 23, 24, 55, 56, and footnote 3). In contrast, the amino- and carboxyl-terminal domains of IF subunits vary widely in both size (from <50 residues in small

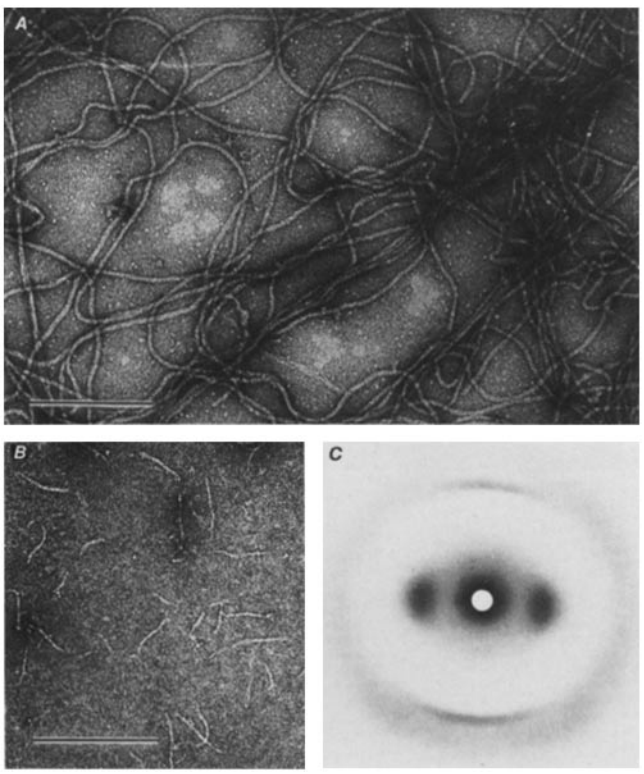

FIGURE 3 Homopolymer vimentin IF reassembled in vitro (4). (*A*) The IF are uniformly 8–10 nm wide, have "smooth" edges, and are many micrometers long. Bar, 0.2 μm. (*B*) Disassembled IF ("protofilaments") formed by reducing the ionic strength of the solution are 2–3.5 nm wide and 50–100 nm long. Intact IF can be reformed by simply adding salt. Note that whereas the assembly-disassembly conditions for vimentin, desmin, and glial fibrillary acidic protein are generally similar, those for keratin IF and invertebrate and vertebrate neurofilaments are quite different (52, 53). In no case is there a requirement for accessory proteins, metal ions, high-energy phosphates, or other cofactors. Bar, 0.2 μm. (C), Fiber prepared from a pellet of IF was used for wide-angle x-ray diffraction (47). This is a typical α-type pattern as determined by the meridional arcs at 0.517 nm and equatorial spots at 0.98 nm.

[3] Steinert, P. M., W. W. Idler, L. D. Johnson, D. A. D. Parry, and D. R. Roop. Partial amino acid sequences of mouse and human epidermal keratin subunits of molecular weight 67,000. Submitted for publication.

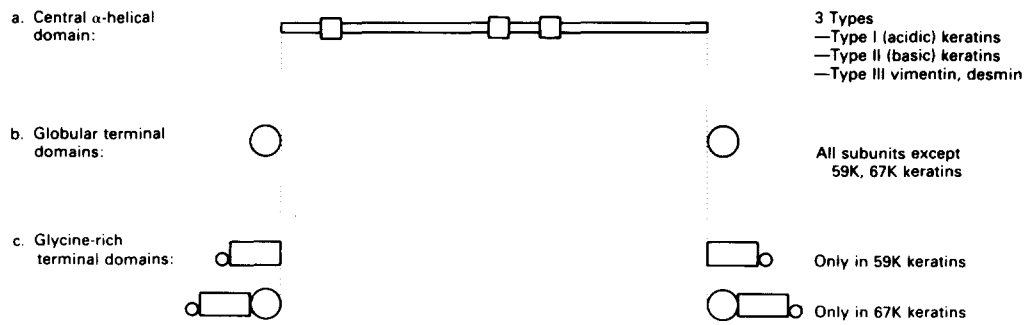

FIGURE 4 Schematic line diagram of IF subunit secondary structure. All subunits contain a common rodlike central α-helical domain and have globular amino- and carboxyl-terminal domains that vary widely in size, sequence, and conformation.

subunits to >200 in large subunits) and amino acid sequence, especially at the very ends of the subunits. These domains are basic in charge and generally adopt a random coil or globular conformation, except possibly for certain large keratins that contain unusual stretches rich in glycine and serine residues of unknown conformation (Fig. 4). All IF are constructed of subfilamentous or protofilamentous particles (Fig. 3*b*). It had been suggested that the protofilament consists of three aligned subunits to form a three-chain coiled-coil unit (48), but recent subunit cross-linking experiments (22, 37) and analyses of the available sequence data (5) indicate that the protofilament consists of a pair of two-chain coiled coils. The details of how these protofilaments are arranged in intact IF remain to be settled. Some electron microscope negative-staining studies have demonstrated that IF can be unravelled into 4 "protofibrils," each of which contains two or three protofilaments (1, 28); other model-building studies suggest that IF are polymorphic and can consist of either seven or 10 protofilaments (5). Recent scanning transmission electron microscopy (STEM) mass measurements of unstained IF have shown that there are two mass forms: "light-weight" IF contain 22–23 subunits/50 nm and "mature" IF contain about 33–34 subunits/50 nm (56, 57). Nevertheless, within each mass class, all IF, irrespective of their subunit content, contain the same number of subunits per unit length of IF. Presumably, any structural model for IF must conform to these mass data. All models so far agree that the conserved α-helical regions of the IF subunits form the basis of IF structural homology. However, few data are available on the arrangement and location within the IF of the non-α-helical terminal domains of the subunits. The STEM data have suggested that IF are as much as 15–16 nm wide due to the existence of low-density peripheral mass that is not normally visualized by negative or positive staining (57). Some proteolytic digestion studies suggest that large portions of the non-α-helical domains of the subunits are located at the IF periphery (16, 48, 54). These preliminary observations suggest that the variable properties and functions of IF are mediated through variations in the size, sequence, and conformation of the protruding terminal sequences of their constituent subunits (48, 54). This concept has already been successfully exploited in the production of highly subunit-specific keratin antisera using terminal peptides as immunogens.[2]

IF-associated Proteins

Just as the functions of MT and microfilaments (MF) in cells are mediated through associated regulatory proteins, it is now becoming clear that IF are likewise attended by associated proteins (IFAP). Several proteins have been identified as possible static or regulatory mediators of the interactions of IF with themselves or other cytoplasmic systems. Some of these IFAP seem to be tissue specific or of limited distribution, such as synemin and paranemin in muscle and some mesenchymally derived cells (30, 31, 38), whereas others have a more widespread distribution (21, 62). Several IFAP are very large proteins and they may thus form cross-linked networks within the cytoskeleton (62). Neurofilaments may have "built-in" regulatory or cross-bridging systems composed of the projecting non-α-helical terminal domains of their large subunits that may interact with microtubule-associated proteins (16, 42, 63). Perhaps the IFAP with the most clearly defined roles to date are those of terminally differentiating keratinizing tissues. Hair and related "hard" keratinocytes contain classes of very cysteine-rich ("high-sulphur") matrix proteins or IFAP that form extensive disulfide bond cross-links with the relatively cysteine-rich non-α-helical domains of the keratin IF subunits, contributing to a rigid, stable, and insoluble keratinized tissue (12, 46). Similarly, cornified epidermal cells contain an IFAP, filaggrin, which contains glycine-serine-rich domains[4] that may form noncovalent β-linkages with the glycine-serine-rich sequences on the terminal domains of epidermal keratin IF subunits to form a stable, insoluble, but more flexible structure (50). It seems clear from these two examples that to effect the specialized functions of the keratin IF in these tissues, evolution has adapted the sequences of the terminal non-α-helical domains of the IF subunits and the IFAP to permit highly specific interactions with one another. In view of the diversity of IF distribution and possible function, we expect that more IFAP will be identified in the future.

What Is the Function of IF in Cells?

IF are more insoluble than most other constituents of the cytoskeleton, which suggests that their functions are somewhat different from those of the other major components, MT and MF. In general, IF, unlike MT and MF, have half-lives well in excess of cell-cycle times, and they do not undergo reversible assembly-disassembly processes during the cell cycle and thus probably do not "tread-mill." One theme common to many studies is that IF have a more mechanical and a less dynamic role in cells. Mechanical functions have already been postulated for the IF of muscle and neuronal cells (18, 30, 31). This role is especially evident in epidermal cells grown in culture. In the light microscope, keratin IF bundles or cables appear to emanate from a perinuclear location and extend to

[4] Hinter, H., T. J. Lawley, and P. M. Steinert. A monoclonal antibody to high molecular weight precursors of human epidermal filaggrin. Submitted for publication.

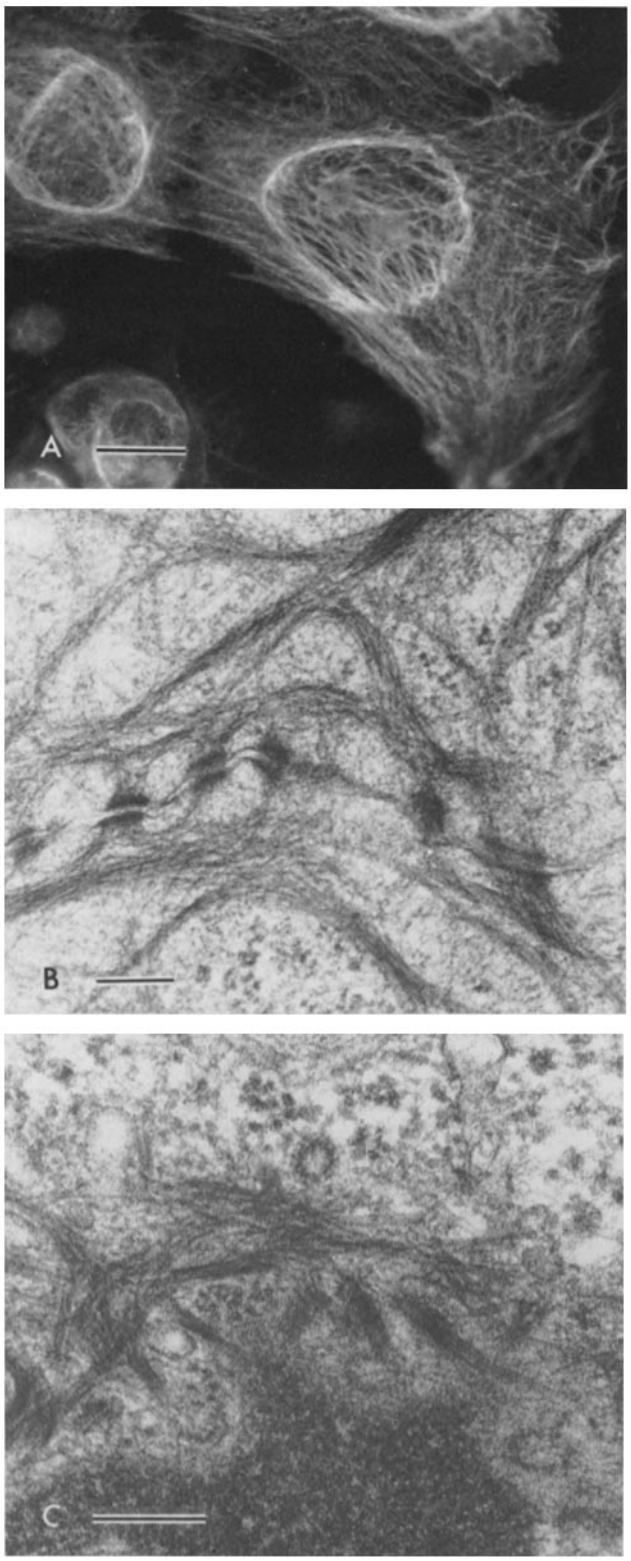

FIGURE 5 Ultrastructure of keratin IF in cultured mouse epidermal cells. (*A*) Indirect immunofluorescence microscopy showing bundles of cables of IF that appear to form a basket-weave or cagelike network around the nucleus and extend to the cell periphery where they appear to continue into neighboring cells. Bar, 10 μm. (*B*) In fact, the IF bundles of each cell terminate abruptly at intercellular desmosomal junctions. Bar, 0.2 μm. (*C*) Keratin IF bundles also extend toward the nuclear surface, where they appear in close contact with nuclear pore complexes. Bar, 0.2 μm.

the periphery of the cell (Fig. 5*a*; 27) where they abruptly terminate at intercellular desmosomal junctions between neighboring cells (Fig. 5*b*). Such images suggest the keratin IF form a mechanically continuous network throughout the tissue (27). This may explain the characteristic flexible and elastic-recovery properties of epithelial tissues. At higher resolution, the perinuclear basket-weave networks of keratin IF often appear to be closely associated with the nuclear envelope at the site of nuclear pore complexes (Fig. 5*c*). Thus, the keratin IF networks appear to be intimately involved in epithelial cell shape, nuclear centration, and cell-cell contact throughout the entire tissue (27). Likewise, the vimentin IF of mesenchymal cells are thought to be involved in nuclear centration, maintenance of cell shape, and movement of organelles (67).

Nevertheless, the existence of different types of IF in different cells suggests that they are a functionally heterogeneous class of proteins. Moreover, as mentioned above, cells can vary the function of their IF during such events as differentiation by changes in the expression of different subunits. Many factors must be involved in the maintenance of the cytoplasmic networks of the IF in cells. The collapse of vimentin IF into the nuclear region on treatment of mesenchymal cells with colchicine suggests close association vith MT. In epithelial cells, Ca^{2+} ions, injection of antikeratin antibodies, and combinations of MT and MF inhibitors (7, 27, 29) can cause the reversible collapse of the IF networks. The common phenomenen of IF collapse into the perinuclear region caused by various factors has suggested the existence of IF-organizing centers that direct or regulate the cytoplasmic distribution and even synthesis of IF (7). The molecular bases for all of these interactions remain unknown at this stage, but it is likely that IFAP are intimately involved.

Since IF are deployed throughout most cells, from the plasma membrane to or into the nuclear membrane, we wonder whether IF also are involved in information transport by mechanical or electrochemical means.

Most IF subunits are phosphoproteins containing from one to several moles of serine-phosphate per mole, but the role of the phosphate in IF structure and function remains unknown (51). IF subunits can be phosphorylated by cyclic nucleotide–dependent kinases, epidermal growth factor, and in response to a variety of stimuli, including cellular transformation (30, 31). Interestingly, in these cases, the phosphorylation is usually substoichiometric, indicating that site-specific phosphorylation of IF can have profound effects on the cells.

While a great deal is now known about the diversity and biochemistry of IF, much remains to be learned about their distribution and functions in cells, their synthesis and assembly, their gene structure and expression during development, and so on. The study of IF therefore remains an exciting and challenging area of cell and molecular biology.

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