Seven Kinds of Intermediate Filament Networks in the Cytoplasm of Polarized Cells: Structure and Function

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Intermediate filaments (IFs) are involved in many important physiological functions, such as the distribution of organelles, signal transduction, cell polarity and gene regulation. However, little information exists on the structure of the IF networks performing these functions. We have clarified the existence of seven kinds of IF networks in the cytoplasm of diverse polarized cells: an apex network just under the terminal web, a peripheral network lying just beneath the cell membrane, a granule-associated network surrounding a mass of secretory granules, a Golgi-associated network surrounding the Golgi apparatus, a radial network locating from the perinuclear region to the specific area of the cell membrane, a juxtanuclear network surrounding the nucleus, and an entire cytoplasmic network. In this review, we describe these seven kinds of IF networks and discuss their biological roles.

Key words: intermediate filament network, organelle distribution, cell polarity, signal transduction, neural stem cell

1. Introduction

The cellular cytoskeletal network is composed of three fibrillar systems, namely, actin microfilaments, intermediate filaments (IFs) and microtubules. This network is a highly dynamic structure that is continually reorganized in diverse cellular processes including cell division, cell migration, cell adhesion, intracellular transport, and specific arrangements of organelles [1, 6, 32, 83, 131]. Despite advances in the understanding of the structure and function of the microfilament network [27, 86, 98] and microtubule network [39, 138], the structure of the IF-network and its biological role remain elusive.

IFs are the most stable components in the cells under physiological conditions. When cells are treated with concentrated salt solution and nonionic detergents, the IF networks are retained in their normal arrangement, whereas the vast majority of cytoplasmic and nuclear constituents are lost [71]. Moreover, IFs have a long half-life, roughly equivalent to the cell generation time, whereas the half-life of IF protein mRNA is very short. For instance, the half-life of vimentin mRNA in mouse fibroblasts is about 6 hr [22]. Therefore, for a long time it was thought that the IF network had a fixed architecture that protects cells against various forms of mechanical stress. However, since IFs are highly dynamic and reorganize by phosphorylation, glycosylation, and transglutamination [56, 92, 107], recent studies suggest that the IF network is involved in many important physiological functions, such as the distribution of organelles [16, 46, 99], signal transduction [56], cell polarity [108], and gene regulation [21, 24, 112]. On the other hand, little information exists concerning the structure of the IF networks performing these functions. We have examined the relation between cell differentiation and expression of IF protein in the polarized cells of the digestive, respiratory, nervous, and endocrine systems, as well as the eye, in a series of research studies [58–66, 105]. In these studies, we have clarified the existence of the following seven kinds of IF networks in the cytoplasm of polarized cells: an apex network, a peripheral network, a granule-associated network, a Golgi-associated network, a radial network, a juxtanuclear network, and an entire cytoplasmic network (Fig. 1). This article examines recent studies of IFs and discusses the functions of these seven kinds of IF networks.

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II. IF Proteins

Presently, at least 70 members of the IF protein family have been identified [132], and their expression is sensitively reflected in the cell differentiation occurring in histogenesis [58, 63, 78, 121, 137, 139] and disease [5, 34, 70, 80, 87, 104]. Therefore, they have been utilized as valuable histochemical markers of cell differentiation [59–61, 65, 66, 84]. As shown in Table 1, IF proteins are classified into six groups on the basis of their amino acid and cDNA sequence similarities [109]. The largest group of IF proteins consists of the type I and type II keratins. In humans, 54 functional keratin genes exist [101]. These keratins include cytokeratin expressed in epithelial cells and hair keratin expressed in hair and nails. Keratin filaments are composed of a specific combination of type I keratin and type II keratin [2]. This keratin pair formation is regulated tissue- and cell-specifically in complex patterns [13, 63, 139]. The type III IF proteins consist of the homopolymeric proteins; vimentin, desmin, glial fibrillary acidic protein (GFAP), peripherin and syncoilin. However, these proteins can also assemble as heteropolymers in vivo [53]. In the type IV IF proteins, three neurofilament proteins (NF), α-internexin, and nestin are expressed in neurons, whereas synemin is expressed in muscle [134]. In contrast to other groups of IF proteins which form a characteristic network in the cytoplasm, the type V IF proteins, lamins, form an intranuclear IF network, the nuclear lamina, underlying the inner nuclear membrane. The type VI IF proteins are the lens-specific IF proteins [96].

As shown in Figure 2, all of the IF proteins have a common tripartite structure consisting of a central α-helical rod domain and non-helical N-terminal head and C-terminal tail domains. The size and sequence of the rod domain of the different IF proteins are similar, except for lamins. The lamin rod domain is slightly longer. In contrast, the head and tail domains are highly variable. The rod domains interact with each other to form the core of the filament, whereas the head and tail domains interact with various cytoplasmic elements including other cytoskeletal components [10, 55, 99]. In addition, the head and tail domains play a crucial role in IF assembly, and the organization of IFs is controlled by phosphorylation and dephosphorylation of serine residues in the head and tail domains [53, 92]. The C-terminal tail domain of lamins contains a nuclear localization signal. Therefore, only lamins can form the IF network in the nucleus [52, 88].
III. IF Networks in the Cytoplasm of Polarized Cells

The distribution of IFs in the cytoplasm is involved in cellular polarity. In unpolarized cells, as reported by Goldman et al. [45], IFs form two kinds of networks in the cytoplasm, namely, a juxtanuclear network surrounding the nucleus and a radial network located from the juxtanuclear network to the cell periphery (Fig. 3). In the polarized cells with apical and basal faces, we clarified the existence of the following seven kinds of IF networks in their cytoplasm: an apex network, a peripheral network, a granule-associated network, a Golgi-associated network, a radial network, a juxtanuclear network, and an entire cytoplasmic network (Fig. 1). The composition of these networks is characteristic of a particular cell differentiation program (Table 2), and many cells have two or more networks in the cytoplasm according to their functions.

1. Apex network

Franke et al. [33] were the first to note that keratin is concentrated in the terminal web and in a special zone subjacent to the terminal web of the intestinal absorptive cells. As shown in Figure 4, this apex network of the rabbit absorptive cells is composed of keratin 5/18 filaments [63]. This network develops very little in the crypt cells, and then develops steadily until the cells move upward onto the villus base. It was confirmed by an immunoelectron microscopical

<table>
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<th>Network (nw.)</th>
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<tbody>
<tr>
<td>Apex nw.</td>
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<td>Peripheral nw.</td>
<td>Keratin 20</td>
<td>Goblet cells of intestinal epithelium</td>
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<td></td>
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<td>Serous and mucous cells of salivary and duodenal glands</td>
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<td>Mucous cells of pyloric gland</td>
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<td>Epithelial cells of gastric surface</td>
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<td>Neck mucous cells and chief cells of gastric gland</td>
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<td>Epithelial cells of gastric surface</td>
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<td></td>
<td></td>
<td>Mucous cells of pyloric and duodenal glands</td>
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<td>[64, 106]</td>
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<td>Chief cells of gastric gland</td>
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<tr>
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<td>Serous cells of salivary and duodenal glands</td>
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<td>[63]</td>
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study that this network is tightly anchored to the desmosomes and extends into the terminal web (Fig. 5). Keratin IFs can anchor to desmosomes by adaptor proteins of desmoplakin [141] and connect to actin filaments in the terminal web by plastin 1 [49]. Therefore, this network may serve to maintain cell-cell contact and may be involved in reinforcement of the terminal web. It has been reported that epithelial cells exhibiting a polarized structure require the keratin filament-organization at the apical domain, and that a deficiency in this organization leads to the disruption of cell polarity [4, 115, 130]. Therefore, the apex network may also participate in the generation of cell polarity.

2. Peripheral network

Keratin 20 is selectively expressed in all exocrine and endocrine cells of the rabbit duodenum, but immature and mature absorptive cells do not express keratin 20 [65]. Therefore, it seems that keratin 20 is closely related to the secretory function in the rabbit duodenum. In the exocrine cells, keratin 20 is distributed just beneath the basolateral cell membrane and forms a thin peripheral network (Fig. 6A). The existence of this peripheral network was confirmed by an immunoelectron microscopical study (Fig. 6B) and an ultrastructural study [65]. The existence of this network was also confirmed in other exocrine cells of the rabbit digestive system, such as the serous and mucous cells of the salivary glands and pyloric glands, the epithelial cells of the gastric surface, the neck mucous cells and chief cells of the gastric glands, and the pancreatic acinar cells [62].

Keratin 20 is expressed predominantly in undifferentiated epithelial cells at the early stage of organogenesis [11, 12] and in some tumor cells [14, 51, 75, 95, 113]. These cells exhibit successive changes in cell shape for proliferation, movement, or invasion. The superficial cells of the uroepithelium, which are subject to great changes owing to the emptying and distension of the urinary bladder, also

Fig. 4. Immunostaining of keratin 5 and 18 in the absorptive cells of the rabbit duodenum. Bar=5 μm. A: Keratin 5 (K5) is localized as a thin layer (arrow) in apical areas of the cell. B: The localization of keratin 18 (K18) resembles that of keratin 5. C: Double immunofluorescence staining of keratin 5 (red) and keratin 18 (green) confirms tight co-localization of both keratin proteins as an apex network (arrow).

Fig. 5. Immunoelectron microscopic staining of keratin 18 (K18) in absorptive cells of the rabbit duodenum. Keratin 18-positive filaments (arrowheads) are localized just under the terminal web (asterisk) and anchored to a desmosome (arrow). Some of them are observed in the terminal web and in the upper cytoplasm of the terminal web. Bar=0.5 μm.
express a large amount of keratin 20 [31, 82, 100, 114]. Therefore, a network consisting of keratin 20-containing filaments is considered to have an especially dynamic character, and to be easily modified by the phosphorylation-dephosphorylation system. This assumption is supported by a biochemical study. Zhou et al. [151] reported that goblet cells undergo dramatic phosphorylation of keratin 20 when they secrete mucigen granules. The shape of these exocrine cells changed remarkably during the secretory cycle. Thus, these cells may select keratin 20-containing filaments as advantageous IFs in their peripheral network. In addition, this network is anchored to desmosomes and hemidesmosomes. Therefore, this network may also serve to maintain cell-cell and cell-matrix contacts. Keratin IFs consist of a specific combination of type I keratins and type II keratins, but a partner for keratin 20 in this network could not be identified, since no keratin is co-localized with keratin 20 in the secretory cells of the rabbit digestive system. However, the possibility that the filaments are composed of keratin 20/20 homodimers could not be ruled out, since Pang et al. [110] described the presence of a keratin 13/13 homodimer in the rabbit esophageal epithelium.

3. Granule-associated network

As shown in Figure 6A, keratin 20 is also distributed around the mass of mucigen granules and forms a granule-associated network in the mucus secreting cells of the rabbit digestive system. The existence of this network was confirmed immunoelectron microscopically (Fig. 6B) and ultrastructurally [65]. However, this network could not be plainly observed in the serous secreting cells. The shape of the masses of mucigen granules also changed remarkably during the secretory cycle. Therefore, mucus secreting cells may select keratin 20-containing filaments as advantageous IFs to form this network, since these filaments seem to have an especially dynamic character as mentioned above.

4. Golgi-associated network

As shown in Figure 7A, the Golgi-associated network is observed as a specific ring structure in the supranuclear region. This specific ring structure was confirmed to be a Golgi-associated filament network surrounding the Golgi apparatus by an immunoelectron microscopical study (Fig. 7B). The existence of this network was also confirmed ultrastructurally [66]. Dense bundles of IFs are observed around the Golgi apparatus. The existence of this network is recognized in the various kinds of cells of the rabbit large salivary glands, stomach, small and large intestines, pancreas, trachea, and spinal ganglion [63, 64, 66, 106].

Absorptive cells of the small intestinal villi are derived from the stem cells in the crypts [72]. Immature absorptive cells move upward onto the villi, and reach functional maturity during cell migration to the mid-portion of the villi [97, 143]. As shown in Figure 8, the Golgi-associated network in the immature absorptive cells at first consists of keratin 8/14 filaments alone. When the cells migrate out of the crypt to the villus base, actin filaments enter this network. In addition, keratin 7/17 filaments enter this network in mature cells at the mid-villus. Additional changes in their components could not be recognized during the cells migration from the mid-villus to the villus tip [63]. The ultrastructure of the Golgi apparatus in absorptive cells changes as the cells migrate along the crypt-villus axis and their maturation is completed at the mid-villus [72, 97]. Therefore, it seems that the Golgi-associated network of the absorptive cells is reinforced by the addition of actin filaments.
filaments and keratin 7/17 filaments to keratin 8/14 filaments following maturation of the Golgi apparatus.

Keratin filaments interact directly or indirectly with the intracellular membrane system [20, 149]. Actin filaments also bind to the membrane of the Golgi apparatus through various actin-associated proteins [94, 125, 142]. Some IF-associated proteins which mediate the interaction between keratin and actin have also been reported [48, 122, 144]. Furthermore, Montes et al. [102] observed a close association of keratin and actin filaments with the Golgi apparatus in the intestinal absorptive cells of newborn rats exposed to ethanol in utero. Actin and keratin are abnormally located in the trans Golgi and trans Golgi network in these absorptive cells. Therefore, the Golgi-associated network, which is composed of keratin filaments and actin filaments, seems to maintain the complex structure of the Golgi apparatus.

On the other hand, this network in pancreatic acinar cells and tracheal ciliated cells consists of keratin 7/14 filaments and actin filaments [64]. This discrepancy may be due to the cell type specificity of expression.

5. Radial network and juxtanuclear network

The radial network is distributed from the perinuclear region to the specific area of the cell membrane and the juxtanuclear network is localized around the nucleus. These two networks are observed in the enteroendocrine cells of the rabbit digestive system, endocrine cells of the rabbit pancreatic islets, and M cells of both the ileal Peyer’s patches and the villus epithelium of the small intestine [61, 62, 65]. In the endocrine cells, these networks consist of keratin 20-containing filaments, and the radial network extends from the edge of the nucleus to the apical cell membrane (Fig. 9A). On the other hand, in both types of M cells, vimentin filaments form these networks, and the radial network extends from the edge of the nucleus to the cell membrane surrounding intraepithelial lymphocytes (L). C: Double immuno-fluorescence staining of vimentin (Vim: red) and keratin 18 (K18: green) in the M cells of rabbit ileum. In the M cell, vimentin forms the radial and juxtanuclear networks, and keratin 18 form the apex and peripheral networks.

The possibility that actin microfilaments are associated with intracellular signal transduction has been considered because of their dynamic structure [118, 119, 146]. However, recent evidence has suggested that IFs are also associated with the intracellular signal transduction system [42, 46, 56, 79]. The possibility that the radial network and juxtanuclear network are a part of that system should be considered for the following three reasons. First, the cytoplasmic IFs have a high binding affinity to both the plasma membrane [3, 17, 20, 45] and nuclear envelope [45, 92, 149]. In addition, the cytoplasmic IFs are connected to the nuclear IF network, the nuclear lamina, through the KASH protein in the outer nuclear membrane and the SUN protein in the inner nuclear membrane [123, 124]. The nuclear

Fig. 8. Schematic representation of changes in the composition of the Golgi-associated network during the migration of absorptive cells along the crypt-villus axis. The Golgi-associated network consists of keratin 8/14 filaments (K8/K14) alone in the immature absorptive cells at the upper crypt. This network is reinforced by the addition of actin filaments at the villus base and keratin 7/17 filaments (K7/K17) at the mid-villus to keratin 8/14 filaments following maturation of the Golgi apparatus.

Fig. 9. Radial network and juxtanuclear network. Bar=5 μm. A: Strong staining of keratin 20 (K20: red) is observed in a few particular columnar cells (E) scattered throughout the villous and crypt epithelia of the rabbit duodenum. These keratin 20-positive columnar cells were confirmed to be enteroendocrine cells as shown in Figure 10A. Keratin 20 is localized around the nucleus and from the edge of the nucleus to the apical cell membrane. B: Vimentin (Vim)-positive columnar cells (M) are scattered throughout the villus epithelium of the rabbit small intestine. These cells were confirmed to be M cells by their ultrastructural and histochemical features [61]. In these cells, vimentin is localized around the nucleus and from the edge of the nucleus to the cell membrane surrounding intraepithelial lymphocytes (L). C: Double immuno-fluorescence staining of vimentin (Vim: red) and keratin 18 (K18: green) in the M cells of rabbit ileum. In the M cell, vimentin forms the radial and juxtanuclear networks, and keratin 18 form the apex and peripheral networks.
keratin and tumor necrosis factor-associated death domain filaments contribute to the antiapoptotic signaling in mouse renal mesangial cells when stimulated with insulin. Aggregation subsequent to phosphorylation of vimentin IFs, observed changes in the nuclear structure and chromatin is supported by the following studies. Berfield et al. demonstrated a direct interaction between keratin and tumor necrosis factor-1-associated death domain protein (TRADD). Gilbert et al. reported that keratin filaments contribute to the antiapoptotic signaling in mouse hepatocytes and mammary cells. In addition, Hyder et al. have suggested that multiple post-translational modifications of IFs play a significant role in signal transduction.

The juxtanuclear network seems to be involved in the storage and distribution of the nucleus, besides a role in the intracellular signal transduction system. In the lens fibers and erythroblasts, their juxtanuclear networks are composed of vimentin filaments, and the networks play an essential role in enucleation. Vimentin expression ceases when the nuclei of the lens fibers [47, 116] and mammalian erythroblasts [25] are extruded, whereas vimentin persists in nucleated avian erythrocytes [145]. The experimental analysis of the correlation between vimentin IFs and enucleation has been taken a step further by using transgenic mice. Overexpression of vimentin in the lens fibers of transgenic mice interferes with denucleation, and the animals develop cataracts at 6–12 weeks of age [15].

6. Entire cytoplasmic network

The entire cytoplasmic network extends throughout the cytoplasm. In general, the constitutive protein of this network is acutely reflected in the cell differentiation occurring in histogenesis and disease. Therefore, the analysis of this network has generated useful information about cell lineage and cell differentiation.

As shown in Figure 11, we recognized two kinds of cell lineages in the neurogenesis in the developing and adult rabbit spinal ganglion by studying the changes in the composition of this network [66]. Spinal ganglia arise from the neural crest [111], and migrating neural crest cells exclusively express vimentin [9]. As shown in Figure 12, these neural crest cells differentiate into nestin-positive ovoid cells with an eccentric nucleus through the spindle-shaped cells co-expressing vimentin and nestin in the rudiments of the spinal ganglia. Some ovoid cells co-express nestin with either NF-L or GFAP. Nestin has been utilized as a histochemical marker for identifying neural stem cells of the central nervous system [18, 43, 117, 147, 150]. NF-L is expressed in embryonic neurons [54, 121], and GFAP is expressed in satellite cells [81, 126] and Schwann cells [30, 69]. In addition, these nestin-positive ovoid cells cannot be observed in newborn and adult ganglia. Therefore, they seem to be an embryonic neural stem cell of the spinal ganglion. In the rudiments of the spinal ganglia, a few keratin-positive polymorphic cells also exist among the ovoid cells. These polymorphic cells co-express five kinds of IF proteins, namely, keratin 8, keratin 14, nestin, NF-L, and GFAP. Since cells co-expressing vimentin and keratin cannot be detected in the rudiments, it seems that the keratin-positive polymorphic cell is not derived directly from the neural crest, but from the nestin-positive ovoid cells. These keratin-positive polymorphic cells can also be detected in newborn and adult ganglia (Fig. 13). The possibility that the keratin-positive polymorphic cell is a postnatal neural stem cell of the spinal ganglion can be considered for the following three reasons. First, keratin has been detected not only in undifferentiated neuronal cells [74, 89], but also...
in dedifferentiated tumor cells of the nervous system [19, 50, 73, 103, 133, 148]. Therefore, keratin, in addition to nestin, can also be utilized as a valuable histochemical marker for neuronal stem cells. Second, the polymorphic cells have the ability to differentiate into both neurons and glial cells, since they contain NF-L and GFAP in addition to keratin and nestin. Third, a few neurons in the adult ganglion also express these five kinds of IF proteins as a Golgi-associated network. However, neurons expressing these five kinds of proteins cannot be detected in either the embryonic or newborn spinal ganglia. Therefore, it is conjectured that the polymorphic cells expressing the five kinds of IF proteins differentiate into neurons after birth, and that immature neurons transiently express the five kinds of IF proteins as a Golgi-associated network when polymorphic cells differentiate into the neurons.
Interestingly, vimentin appears temporarily in this network when a bipolar neuron changes into a pseudounipolar neuron, and it disappears when the change is completed [84]. Since vimentin IFs are dynamic structures, they seem to be added to this network as the most reasonable IFs for neurons changing in cell shape.

As shown in Figure 14, in the duodenal absorptive cells, the composition of this network changes with cell maturation [63]. Keratin 7/17 filaments first enter this network at the villus base. As the cells mature, the network is reinforced by the addition of keratin 5/18 filaments (K5/K18) following maturation of the cell. Just before cell exfoliation, keratin 20 (K20) enters this network.

IFs connect directly or indirectly with the actin microfilaments [49, 122], microtubules [28, 53], cell membrane [3, 17, 20, 45], nuclear envelope [45, 92, 149], mitochondria [3], Golgi apparatus [38], ribosomes [7], endoplasmic reticulum [16], and centrosomes [135]. Many IF-associated proteins are involved in these connections [54, 99]. Therefore, the entire cytoplasmic network of IFs can play a major role in the formation of a completed cytoplasmic cytoskeletal system serving in the maintenance of the cell structure, the storage and distribution of cell organelles including nucleus, and the resistance to external mechanical force.

IV. IF Networks in the Nucleus

Since the C-terminal tail domain of type V IF proteins, the lamins, possesses a nuclear localization signal [52, 88], the lamins can form an intranuclear IF network, the nuclear lamina (Fig. 15). Lamins are divided into A-type (lamin A, lamin AΔ10, lamin C1, and lamin C2) and B-type (lamin B1, lamin B2, and lamin B3) based on sequence homologies [24, 140]. Lamins C2 and lamin B3 are exclusively expressed in germ cells [35, 36]. The nuclear lamina, composed of A-type and B-type lamins, exists just under the inner nuclear membrane [44]. Since both types of lamins interact with inner nuclear membrane proteins, the nuclear lamina gives shape and stability to the nuclear envelope [37, 129]. In addition, A-type lamins interact with chromosomes, and they are involved in chromatin organization, DNA replication, transcription, DNA repair, and RNA splicing [21, 24, 112]. Since A-type lamins interplay with signal molecules [93], they may play an important role in the signal transduction system.

When the cell enters mitosis, cyclin-dependent cdc2 kinase phosphorylates the cytoplasmic IF proteins forming the juxtanuclear network [136] and lamins forming the nuclear lamina [29]. This phosphorylation leads to depolymerization of the juxtanuclear network and nuclear lamina, as a result of which the nuclear membrane is fragmented into small vesicles [37]. In contrast to lamin A and C, which are released as free dimers, lamin B remains bound to these small vesicles. At the end of mitosis, inactivation of cdc2 kinase leads to the dephosphorylation of lamins. During this process, lamins reassociate to form the nuclear lamina, and the vesicles fuse with each other to form a complete nuclear envelope [90, 91].

V. Conclusions

Seven kinds of IF networks exist in the cytoplasm of polarized cells, namely, the apex network, peripheral network, granule-associated network, Golgi-associated network, radial network, juxtanuclear network, and entire cytoplasmic network. The apex network, located just under the terminal web, may serve to maintain cell-cell contact and participate in the generation of cell polarity. The peripheral network, lying just beneath the cell membrane, seems to
play some role in maintaining the shape of the cell, as well as cell-cell and cell-matrix contacts. The granule-associated network, surrounding a mass of mucigen granules, may play some role in maintaining the shape of the mass. The Golgi-associated network, surrounding the Golgi apparatus, seems to be involved in the maintenance of the complex structure of the organelle. The radial network, located from the nucleus to the specific area of the cell membrane, may be associated with the intracellular signal transduction system. The juxtanuclear network, surrounding the nucleus, seems to be involved in the storage and distribution of the nucleus, in addition to a role in the intracellular signal transduction system. The entire cytoplasmic network may play a major role in the maintenance of cell structure, as well as in the storage and distribution of cell organelles in the cytoplasm. In addition to these cytoplasmic IF networks, the nuclear IF network, the nuclear lamina, exists in the nucleus lining the inner nuclear membrane. This network gives shape and stability to the nuclear envelope, provides anchorage sites for chromosomes, and is involved in chromatin organization, DNA replication, transcription, DNA repair, RNA splicing, and signal transduction. Since the composition of the IF networks in the cytoplasm begins to change prior to changes in cell function during organogenesis, immunohistochemical examination of the IF composition may become a powerful tool for achieving earlier detection of the onset of various diseases.

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VII. References


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