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Review

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

I. 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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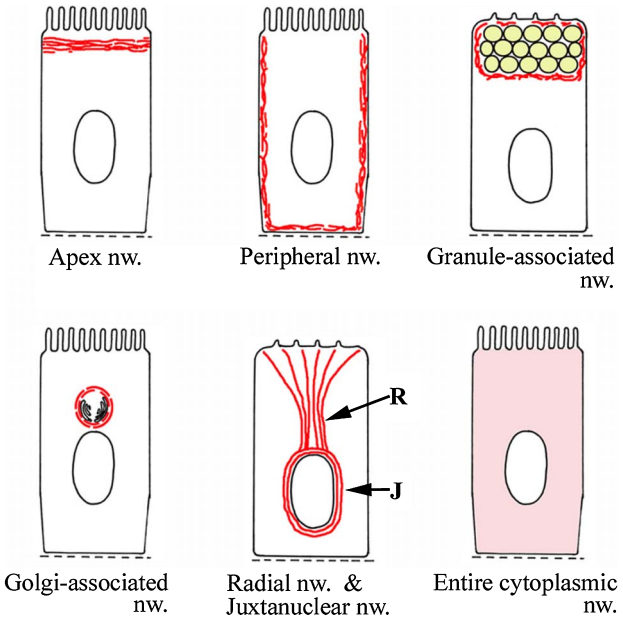

Fig. 1. Schematic illustration showing the localization of the seven kinds of IF networks in the cytoplasm of a polarized cell. Red lines indicate IF networks. An apex network (nw.) exists under the apical cell membrane. A peripheral network is distributed just beneath the basolateral cell membrane. A granule-associated network surrounds a mass of secretory granules. A Golgi-associated network surrounds the Golgi apparatus. A radial network (R) is located from the perinuclear region to the specific area of the cell membrane. A juxtanuclear network (J) surrounds the nucleus. An entire cytoplasmic network is distributed throughout the entire cytoplasm.

Table 1. *Mammalian intermediate filament proteins*

Type I: acidic keratins
K9-K28 (cytokeratin), K31-40 (hair keratin)
Type II: basic keratin
K1-K8 (cytokeratin), K71-K80 (cytokeratin), K81-K86 (hair keratin)
Type III
Desmin, Vimentin, Peripherin, Glial fibrillary acidic protein (GFAP), Syncoilin
Type IV
Light neurofilament protein (NF-L), Medium NF (NF-M), Heavy NF (NF-H), α-internexin, Synemin α, Synemin β, Nestin
Type V: Nuclear lamins
Lamin A, Lamin AΔ10, Lamin C1, Lamin C2, Lamin B1, Lamin B2, Lamin B3
Type VI
CP49 (Phakinin), Filensin (CP115)

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 synemins are 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].

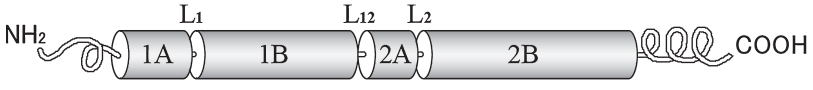

Fig. 2. Structural model of IF protein. The central α-helical rod domain is subdivided into the coil segments 1A, 1B, 2A and 2B by the short non-helical linker regions L_1, L_{12} and L_2. The rod domain is flanked by the non-helical N-terminal head domain and the C-terminal tail domain.

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

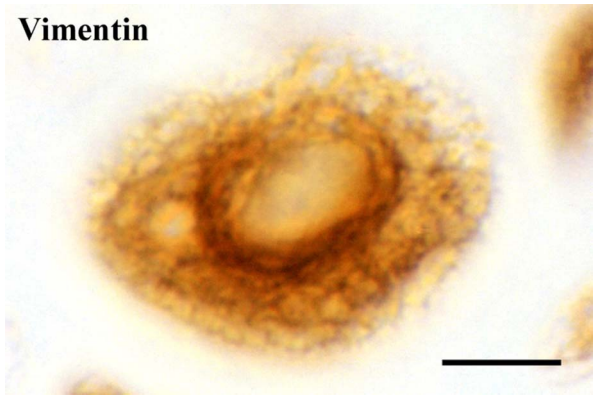

Fig. 3. Immunostaining of vimentin in a chondrocyte of rabbit tracheal cartilage. Vimentin IFs are concentrated in a perinuclear region from which they appear to radiate to the cell periphery. Bar=5 μm.

Table 2. *Composition of the seven kinds of intermediate filament networks in the cytoplasm of rabbit polarized cells*

Network (nw.)	IF proteins	Cells	References
Apex nw.	Keratin 5/18	Absorptive cells of duodenum	[63]
Peripheral nw.	Keratin 20	Goblet cells of intestinal epithelium	[62, 65]
		Serous and mucous cells of salivary and duodenal glands	[62, 65]
		Mucous cells of pyloric gland	[62]
		Epithelial cells of gastric surface	[62]
		Neck mucous cells and chief cells of gastric gland	[62]
Granule-associated nw.	Keratin 20	Goblet cells of intestinal epithelium	[62, 65]
		Epithelial cells of gastric surface	[62]
		Neck mucous cells of gastric gland	[62]
		Mucous cells of pyloric and duodenal glands	[65]
Golgi-associated nw.	Keratin 8/14	Duct epithelial cells of salivary glands	[64, 106]
		Bile duct cells of liver	[64, 106]
		Chief cells of gastric gland	[64]
	Keratin 8/14+Keratin 7/17	Serous cells of salivary and duodenal glands	[64]
		Surface cells and neck mucous cells of stomach	[64]
		Absorptive cells of intestinal epithelium	[63, 64]
	Keratin 7/14	Acinar cells of pancreas	[64]
	Keratin 7/14+Keratin 8/17	Ciliated cells of trachea	[64]
	Keratin 8/14+Nestin+ NF-L+GFAP	Immature neurons of spinal ganglion	[66]
Radial nw. and Juxtanuclear nw.	Keratin 20	Gastrointestinal endocrine cells of digestive tract	[62, 65]
		Endocrine cells of the pancreatic islets	[62]
	Vimentin	M cells of ileal Peyer's patch and of villus epithelium of small intestine	[61]
		M cells of appendix and of palatine tonsil	[40, 41]
Entire cytoplasmic nw.	Vimentin	Neural crest	[66]
	Nestin	Embryonic neural stem cells of spinal ganglion	[66]
	Keratin 8/14+Nestin+ NF-L+GFAP	Postnatal neural stem cells of spinal ganglion	[66]
	Keratin 7/17+Keratin 5/18+ Keratin 20	Absorptive cells of duodenum	[63]

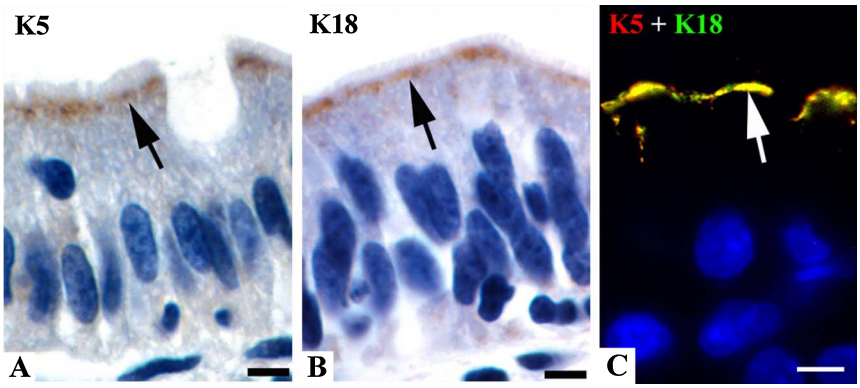

Fig. 4. Immunostaining of keratin 5 and 18 in the absorptive cells of the rabbit duodenum. Bar=5 μm. **A:** Keratin 5 (K5) is localized as a thin layer (arrow) in apical areas of the cell. **B:** The localization of keratin 18 (K18) resembles that of keratin 5. **C:** Double immunofluorescence staining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

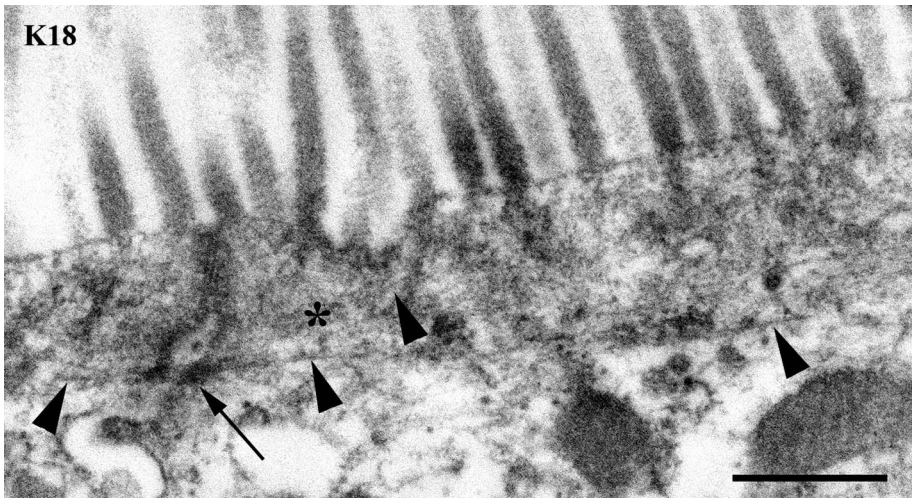

Fig. 5. Immunoelectron microscopic staining of keratin 18 (K18) in absorptive cells of the rabbit duodenum. Keratin 18-positive filaments (arrowheads) are localized just under the terminal web (asterisk) and anchored to a desmosome (arrow). Some of them are observed in the terminal web and in the upper cytoplasm of the terminal web. Bar=0.5 μm.

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

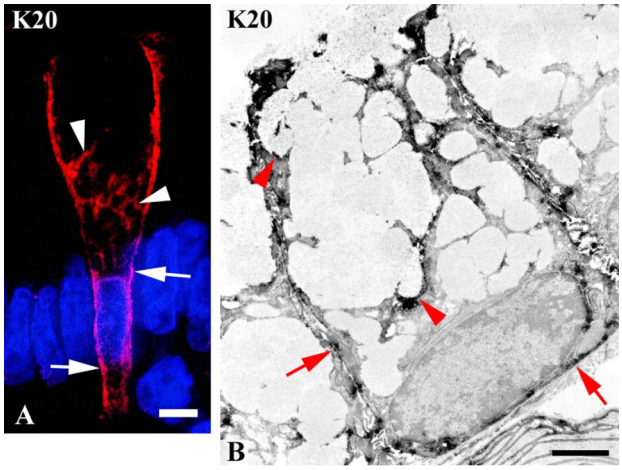

Fig. 6. Peripheral network and granule-associated network. **A:** Immunofluorescence staining of keratin 20 (K20) in a goblet cell of the rabbit duodenum. Keratin 20 (red) is distributed just beneath the basolateral cell membrane (arrows) and around a mass of mucigen granules (arrowheads). Bar=5 μm. **B:** Immunoelectron microscopical staining of keratin 20 in mucous cells of the rabbit duodenal gland. Keratin 20-containing filaments exist just under the cell membrane (arrows) and around the mucigen granules (arrowheads). Bar=2 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

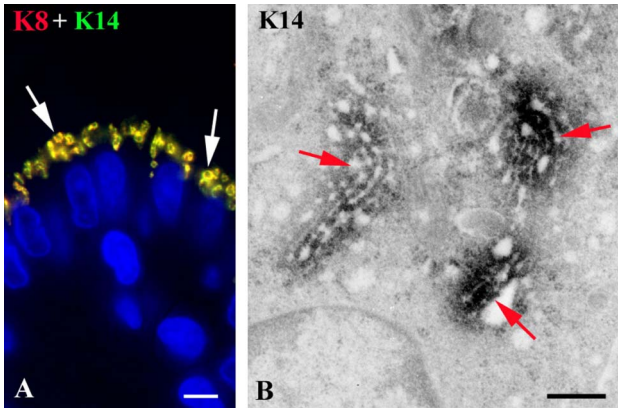

Fig. 7. Golgi-associated network in absorptive cells of the rabbit duodenum. **A:** Double immunofluorescence staining of keratin 8 (K8: red) and 14 (K14: green). Both keratins are co-localized as specific ring structures (arrows) in the supranuclear region of the cell. Bar=5 μm. **B:** Immunoelectron microscopical staining of keratin 14. Keratin 14 is localized around the Golgi apparatus (arrows). Bar=0.5 μm.

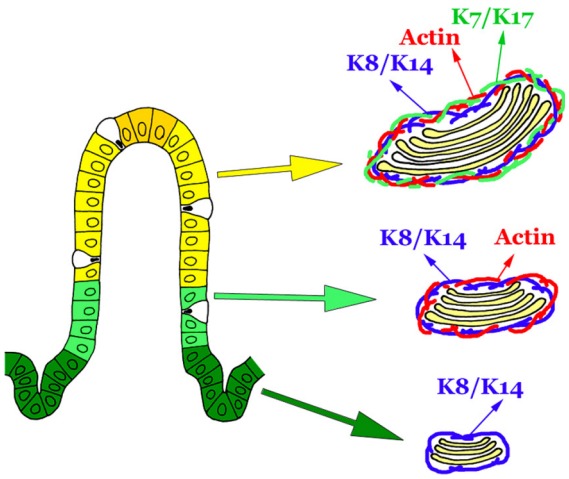

Fig. 8. Schematic representation of changes in the composition of the Golgi-associated network during the migration of absorptive cells along the crypt-villus axis. The Golgi-associated network consists of keratin 8/14 filaments (K8/K14) alone in the immature absorptive cells at the upper crypt. This 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.

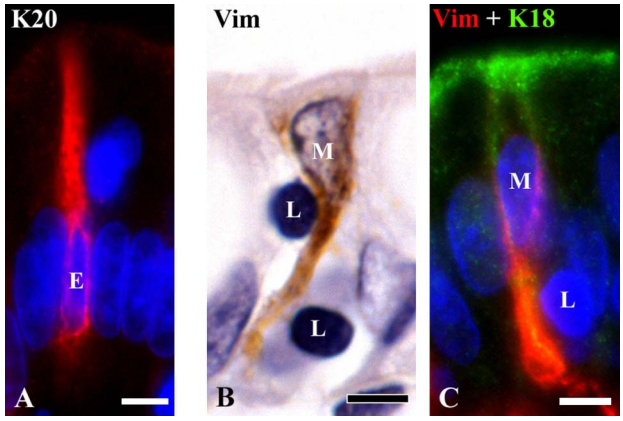

Fig. 9. Radial network and juxtanuclear network. Bar=5 μm. **A:** Strong staining of keratin 20 (K20: red) is observed in a few particular columnar cells (E) scattered throughout the villous and cryptic epithelia of the rabbit duodenum. These keratin 20-positive columnar cells were confirmed to be enteroendocrine 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 immunofluorescence 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.

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, which touches the intraepithelial lymphocytes (Fig. 9B and C). The existence of these networks was confirmed immunoelectron microscopically (Fig. 10) and ultrastructurally [65]. Similar distribution of vimentin filaments has also been observed in the M cells of the appendices [40, 68] and of palatine tonsils [41].

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

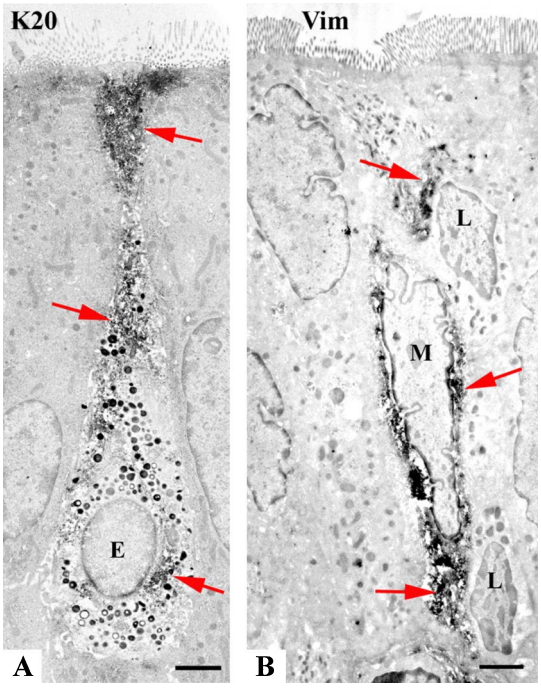

Fig. 10. Immunoelectron micrographs of radial and juxtanuclear networks. Bar=2 μm. **A:** A keratin 20 (K20)-positive columnar cell (E) was confirmed to be an enteroendocrine cell, because all keratin 20-positive columnar cells accumulate many secretory granules in their basal cytoplasm. In these cells, keratin 20-containing filaments (arrows) are detected in the perinuclear region and between this region and the apical cell membrane. **B:** In the M cell (M), vimentin (Vim: arrows) is localized around the nucleus and from the edge of the nucleus to the cell membrane, which touches intraepithelial lymphocytes (L).

lamina is involved in the organization of nuclear functions [21, 24, 112]. Second, the radial network of enteroendocrine cells localizes from the nuclear periphery to the apical cell membrane, which contains many receptors for binding specific extracellular signals [26, 67, 127, 128]. In contrast, the radial network of M cells localizes from the nuclear periphery to the cell membrane, which is in contact with the intraepithelial lymphocytes required for efficient M cell formation [23, 76, 77, 120]. Third, vimentin is preferentially phosphorylated among cytoplasmic proteins including cytoskeletal proteins [85]. Similarly, keratin 20 has an especially dynamic character as described previously (see section III-2). Therefore, vimentin filaments and keratin 20-containing filaments can be easily modified by the phosphorylation-dephosphorylation system. The possibility of this hypothesis is supported by the following studies. Berfield *et al.* [8] observed changes in the nuclear structure and chromatin aggregation subsequent to phosphorylation of vimentin IFs, when renal mesangial cells were stimulated with insulin. Inada *et al.* [57] demonstrated a direct interaction between keratin and tumor necrosis factor 1-associated death domain protein (TRADD). Gilbert *et al.* [42] reported that keratin filaments contribute to the antiapoptotic signaling in mouse hepatocytes and mammary cells. In addition, Hyder *et al.* [56] 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

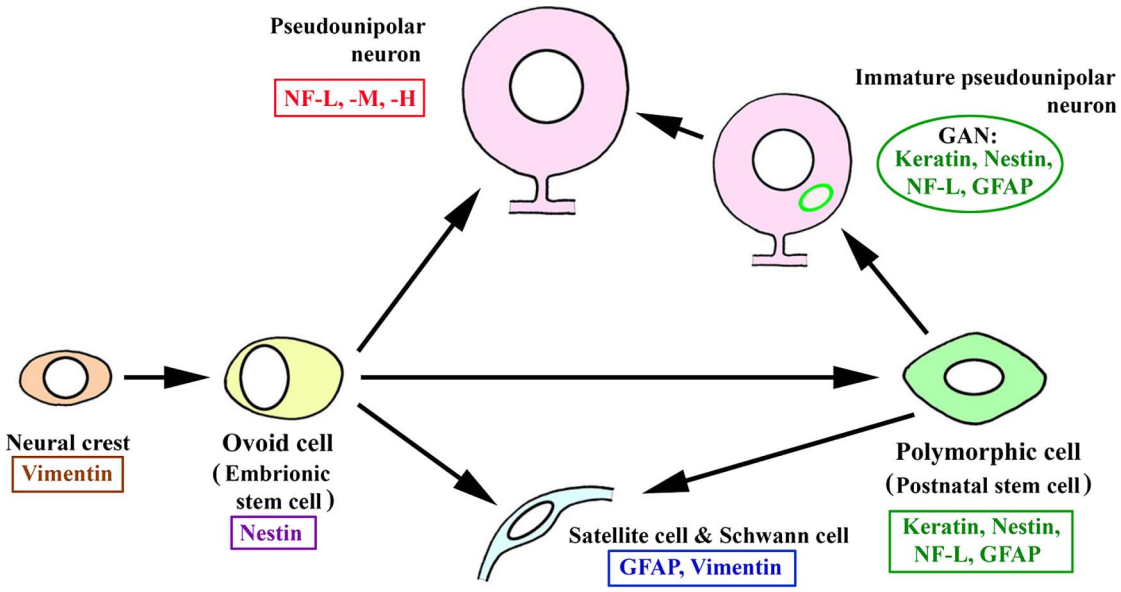

Fig. 11. Schematic representation of the embryonic and postnatal neurogenesis in the developing and adult rabbit spinal ganglia. Ovoid cells, which originate from the vimentin-positive neural crest, differentiate into NF-positive pseudounipolar neurons, GFAP/vimentin-positive glial cells (satellite cells and Schwann cells), and keratin-positive polymorphic cells during prenatal life. The polymorphic cells, which express keratin 8, keratin 14, nestin, NF-L and GFAP, differentiate into pseudounipolar neurons and glial cells after birth. When the polymorphic cells differentiate into neurons, the immature neurons transiently express these five kinds of IF proteins as a Golgi-associated filament network (GAN).

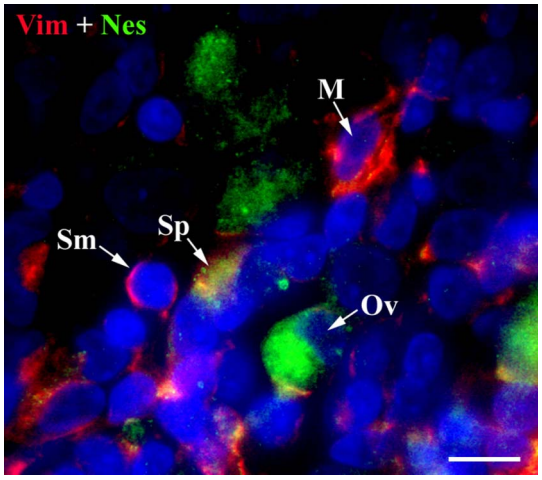

Fig. 12. Double immunofluorescence staining of vimentin (Vim: red) and nestin (Nes: green) in the rudiments of the rabbit spinal ganglion at 15 days of gestation. Small cells (Sm) express vimentin alone and ovoid cells (Ov) express nestin alone, but spindle-shaped cells (Sp) co-express both proteins. Mesenchymal cells (M) also express vimentin. Bar=10 μm.

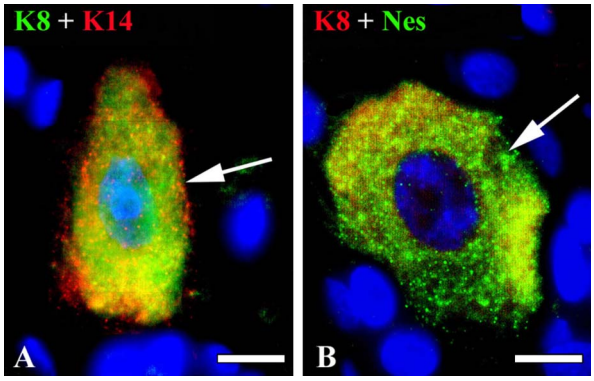

Fig. 13. Double immunofluorescence staining of two kinds of intermediate filament proteins in the adult rabbit spinal ganglia. Bar=10 μm. **A:** Keratin 8 (green) and keratin 14 (red) are detected throughout the entire cytoplasm of a few polymorphic cells (arrow). **B:** Keratin 8 (red) and nestin (green) are also detected throughout the entire cytoplasm of a few polymorphic cells (arrow).

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.

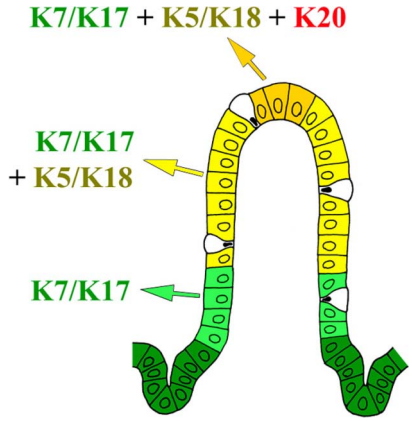

Fig. 14. Schematic representation of changes in the composition of the entire cytoplasmic network during the migration of absorptive cells along the villus axis. This network consists of keratin 7/17 filaments (K7/K17) alone in the immature absorptive cells at the villus base. This network is reinforced 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.

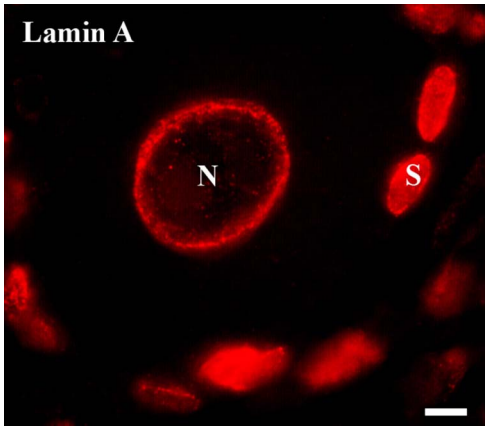

Fig. 15. Immunofluorescence staining of lamin A (red) in the rabbit spinal ganglion. Strong staining is observed at the nuclear rim. N, nucleus of the pseudounipolar neuron. S, nucleus of the satellite cell. Bar=5 μm.

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. Just before cell exfoliation, keratin 20 enters this network. Since the keratin 20-containing filaments are dynamic structures, they seem to be added to this network as the most reasonable IFs for the cells changing in shape for exfoliation.

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]. Lamin 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

1. Akita, Y. (2008) Protein kinase Cepsilon: multiple roles in the function of, and signaling mediated by, the cytoskeleton. *FEBS J.* 275; 3995–4004.
2. Albers, K. and Fuchs, E. (1992) The molecular biology of intermediate filament proteins. *Int. Rev. Cytol.* 134; 243–279.
3. Almahbobi, G., Korn, M. and Hall, P. F. (1994) Calcium/calmodulin induces phosphorylation of vimentin and myosin light chain and cell rounding in cultured adrenal cells. *Eur. J. Cell Biol.* 63; 307–315.
4. Ameen, N. A., Figueroa, Y. and Salas, P. J. I. (2001) Anomalous apical plasma membrane phenotype in CK8-deficient mice indicates a novel role for intermediate filaments in the polarization of simple epithelia. *J. Cell Sci.* 114; 563–575.
5. Arin, M. J. (2009) The molecular basis of human keratin disorders. *Hum Genet.* 125; 355–373.
6. Atencia, R., Asumendi, A. and García-Sanz, M. (2000) Role of cytoskeleton in apoptosis. *Vitam. Horm.* 58; 267–297.
7. Bauer, C. and Traub, P. (1995) Interaction of intermediate filaments with ribosomes in vitro. *Eur. J. Cell Biol.* 68; 288–296.
8. Berfield, A. K., Raugi, G. J. and Abrass, C. K. (1996) Insulin induces rapid and specific rearrangement of the cytoskeleton of rat mesangial cells in vitro. *J. Histochem. Cytochem.* 44; 91–101.
9. Boisseau, S. and Simonneau, M. (1989) Mammalian neuronal differentiation: early expression of a neuronal phenotype from mouse neural crest cells in a chemically defined culture medium. *Development* 106; 665–674.
10. Bousquet, O., Ma, L., Yamada, S., Gu, C., Idei, T., Takahashi, K., Wirtz, D. and Coulombe, P. A. (2001) The nonhelical tail domain of keratin 14 promotes filament bundling and enhances the mechanical properties of keratin intermediate filaments in vitro. *J. Cell Biol.* 155; 747–753.
11. Bouwens, L. and De Blay, E. (1996) Islet morphogenesis and stem cell markers in rat pancreas. *J. Histochem. Cytochem.* 44; 947–951.
12. Bouwens, L. (1998) Cytokeratins and cell differentiation in the pancreas. *J. Pathol.* 184; 234–239.
13. Bragulla, H. H. and Homberger, D. G. (2009) Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia. *J. Anat.* 214; 516–559.
14. Cabibi, D., Licata, A., Barresi, E., Craxi, A. and Aragona, F. (2003) Expression of cytokeratin 7 and 20 in pathological conditions of the bile tract. *Pathol. Res. Pract.* 199; 65–70.
15. Capetanaki, Y., Smith, S. and Heath, J. P. (1989) Overexpression of the vimentin gene in transgenic mice inhibits normal lens cell differentiation. *J. Cell Biol.* 109; 1653–1664.
16. Carmo-Fonseca, M. and David-Ferreira, J. F. (1990) Interactions of intermediate filaments with cell structures. *Electron Microsc. Rev.* 3; 115–141.
17. Carraway, K. L. and Carraway, C. A. (1989) Membrane-cytoskeleton interactions in animal cells. *Biochim. Biophys. Acta* 988; 147–171.
18. Charrier, C., Coronas, V., Fombonne, J., Roger, M., Jean, A., Krantic, S. and Moyse, E. (2006) Characterization of neural stem cells in the dorsal vagal complex of adult rat by in vivo proliferation labeling and in vitro neurosphere assay. *Neuroscience* 138; 5–16.
19. Chetty, R., Pillay, P. and Jaichand, V. (1998) Cytokeratin expression in adrenal phaeochromocytomas and extra-adrenal paragangliomas. *J. Clin. Pathol.* 51; 477–478.
20. Chou, C. F., Riopel, C. L. and Omary, M. B. (1994) Identification of a keratin-associated protein that localizes to a membrane compartment. *Biochem. J.* 298; 457–463.
21. Cohen, T. V., Hernandez, L. and Stewart, C. L. (2008) Functions of the nuclear envelope and lamina in development and disease. *Biochem. Soc. Trans.* 36; 1329–1334.
22. Coleman, T. R. and Lazarides, E. (1992) Continuous growth of vimentin filaments in mouse fibroblasts. *J. Cell Sci.* 103; 689–698.
23. Debard, N., Sierro, F. and Kraehenbuhl, J.-P. (1999) Development of Peyer's patches, follicle-associated epithelium and M cell: lessons from immunodeficient and knockout mice. *Semin. Immunol.* 11; 183–191.
24. Dechat, T., Pfleghaar, K., Sengupta, K., Shimi, T., Shumaker, D. K., Solimando, L. and Goldman, R. D. (2008) Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. *Genes Dev.* 22; 832–853.
25. Dellagi, K., Vainchenker, W., Vinci, G., Paulin, D. and Brouet, J. C. (1983) Alteration of vimentin intermediate filament expression during differentiation of human hemopoietic cells. *EMBO J.* 2; 1509–1514.
26. Dockray, G. J. (2003) Luminal sensing in the gut: an overview. *J. Physiol. Pharmacol.* 54; 9–17.
27. dos Remedios, C. G., Chhabra, D., Kekic, M., Dedova, I. V., Tsubakihara, M., Berry, D. A. and Nosworthy, N. J. (2003) Actin binding proteins: regulation of cytoskeletal microfilaments. *Physiol. Rev.* 83; 433–473.
28. Dráberová, E. and Dráber, P. (1993) A microtubule-interacting protein involved in coalignment of vimentin intermediate filaments with microtubules. *J. Cell Sci.* 106; 1263–1273.
29. Eggert, M., Radomski, N., Linder, D., Tripier, D., Traub, P. and Jost, E. (1993) Identification of novel phosphorylation sites in murine A-type lamins. *Eur. J. Biochem.* 213; 659–671.
30. Eng, L. F. and Smith, M. E. (1985) Recent studies of the glial

fibrillary acidic protein. *Ann. N. Y. Acad. Sci.* 455; 525–537.
31. Erman, A., Veranic, P., Psenicnik, M. and Jezernik, K. (2006) Superficial cell differentiation during embryonic and postnatal development of mouse urothelium. *Tissue Cell.* 38; 293–301.
32. Etienne-Manneville, S. (2004) Actin and microtubules in cell motility: which one is in control? *Traffic* 5; 470–477.
33. Franke, W. W., Appelhans, B., Schmid, E. and Freudenstein, C. (1979) The organization of cytokeratin filaments in the intestinal epithelium. *Eur. J. Cell Biol.* 19; 255–268.
34. Fuchs, E. (1996) The cytoskeleton and disease: genetic disorders of intermediate filaments. *Annu. Rev. Genet.* 30; 197–231.
35. Furukawa, K. and Hotta, Y. (1993) cDNA cloning of a germ cell specific lamin B3 from mouse spermatocytes and analysis of its function by ectopic expression in somatic cells. *EMBO J.* 12; 97–106.
36. Furukawa, K., Inagaki, H. and Hotta, Y. (1994) Identification and cloning of an mRNA coding for a germ cell-specific A-type lamin in mice. *Exp. Cell Res.* 212; 426–430.
37. Gant, T. M. and Wilson, K. L. (1997) Nuclear assembly. *Ann. Rev. Cell Dev. Biol.* 13; 669–695.
38. Gao, Y. and Sztul, E. (2001) A novel interaction of the Golgi complex with the vimentin intermediate filament cytoskeleton. *J. Cell Biol.* 152; 877–894.
39. Gardner, M. K., Hunt, A. J., Goodson, H. V. and Odde, D. J. (2008) Microtubule assembly dynamics: new insights at the nanoscale. *Curr. Opin. Cell Biol.* 20; 64–70.
40. Gebert, A., Hach, G. and Bartels, H. (1992) Co-localization of vimentin and cytokeratins in M-cells of rabbit gut-associated lymphoid tissue (GALT). *Cell Tissue Res.* 269; 331–340.
41. Gebert, A. (1995) Identification of M-cells in the rabbit tonsil by vimentin immunohistochemistry and in vivo protein transport. *Histochem. Cell Biol.* 104; 211–220.
42. Gilbert, S., Loranger, A. and Marceau, N. (2004) Keratins modulate c-Flip/extracellular signal-regulated kinase 1 and 2 anti-apoptotic signaling in simple epithelial cells. *Mol. Cell. Biol.* 24; 7072–7081.
43. Gilyarov, A. V. (2008) Nestin in central nervous system cells. *Neurosci. Behav. Physiol.* 38; 165–169.
44. Goldberg, M. W., Fiserova, J., Huttenlauch, I. and Stick, R. (2008) A new model for nuclear lamina organization. *Biochem. Soc. Trans.* 36; 1339–1343.
45. Goldman, R., Goldman, A., Green, K., Jones, J., Lieska, N. and Yang, H.-Y. (1985) Intermediate filaments; possible functions as cytoskeletal connecting links between the nucleus and the cell surface. *Ann. N. Y. Acad. Sci.* 455; 1–17.
46. Goldman, R. D., Grin, B., Mendez, M. G. and Kuczmarski, E. R. (2008) Intermediate filaments: versatile building blocks of cell structure. *Curr. Opin. Cell Biol.* 20; 28–34.
47. Graw, J. (1996) Genetic aspects of embryonic eye development in vertebrates. *Dev. Genet.* 18; 181–197.
48. Green, K. J., Böhringer, M., Gocken, T. and Jones, J. C. R. (2005) Intermediate filament associated proteins. *Adv. Protein Chem.* 70; 143–202.
49. Grimm-Günter, E. M., Revenu, C., Ramos, S., Hurbain, I., Smyth, N., Ferrary, E., Louvard, D., Robine, S. and Rivero, F. (2009) Plastin 1 binds to keratin and is required for terminal web assembly in the intestinal epithelium. *Mol. Biol. Cell* 20; 2549–2562.
50. Guarino, M. (1993) Plexiform schwannoma. Immunohistochemistry of Schwann cell markers, intermediate filaments and extracellular matrix components. *Pathol. Res. Pract.* 189; 913–920.
51. Heatley, M. K. (2008) Immunohistochemical biomarkers of value in distinguishing primary ovarian carcinoma from gastric carcinoma: a systematic review with statistical meta-analysis. *Histopathology* 52; 267–276.
52. Hennekes, H., Peter, M., Weber, K. and Nigg, E. A. (1993) Phosphorylation on protein kinase C sites inhibits nuclear import of lamin B2. *J. Cell Biol.* 120; 1293–1304.
53. Herrmann, H. and Aebi, U. (2000) Intermediate filaments and their associates; multi-talented structural elements specifying cytoarchitecture and cytodynamics. *Curr. Opin. Cell Biol.* 12; 79–90.
54. Herrmann, H., Hesse, M., Reichenzeller, M., Aebi, U. and Magin, T. M. (2003) Functional complexity of intermediate filament cytoskeletons; from structure to assembly to gene ablation. *Int. Rev. Cytol.* 223; 83–175.
55. Herrmann, H., Bär, H., Kreplak, L., Strelkov, S. V. and Aebi, U. (2007) Intermediate filaments; from cell architecture to nanomechanics. *Nat. Rev. Mol. Cell Biol.* 8; 562–573.
56. Hyder, C. L., Pallari, H. M., Kochin, V. and Eriksson, J. E. (2008) Providing cellular signposts: post-translational modifications of intermediate filaments. *FEBS Lett.* 582; 2140–2148.
57. Inada, H., Izawa, I., Nishizawa, M., Fujita, E., Kiyono, T., Takahashi, T., Momoi, T. and Inagaki, M. (2001) Keratin attenuates tumor necrosis factor-induced cytotoxicity through association with TRADD. *J. Cell Biol.* 155; 415–425.
58. Iwatsuki, H., Sasaki, K., Suda, M. and Itano, C. (1996) Cytoskeletal proteins and epithelium differentiation of the optic vesicle of the chick embryo; An immunohistochemical analysis. *Acta Histochem. Cytochem.* 29; S832–S833.
59. Iwatsuki, H. (1999) Vimentin intermediate filaments; Function and implication in cell differentiation. *Kawasaki Med. J.* 25; 39–58.
60. Iwatsuki, H., Sasaki, K., Suda, M. and Itano, C. (1999) Vimentin intermediate filament protein as differentiation marker of optic vesicle epithelium in the chick embryo. *Acta Histochem.* 101; 369–382.
61. Iwatsuki, H., Ogawa, C. and Suda, M. (2002) Vimentin-positive cells in the villus epithelium of the rabbit small intestine. *Histochem. Cell Biol.* 117; 363–370.
62. Iwatsuki, H., Ogawa, C. and Suda, M. (2002) Keratin 20 expressed in secretory cells of the rabbit digestive system. *Acta Histochem. Cytochem.* 35; 55.
63. Iwatsuki, H. and Suda, M. (2005) Maturation of three kinds of keratin networks in the absorptive cells of rabbit duodenum. *Acta Histochem. Cytochem.* 38; 237–245.
64. Iwatsuki, H. and Suda, M. (2005) Keratin composition of the Golgi-associated networks in various epithelial cells. *Acta Histochem. Cytochem.* 38; 159.
65. Iwatsuki, H. and Suda, M. (2007) Keratin 20 expressed in the endocrine and exocrine cells of the rabbit duodenum. *Acta Histochem. Cytochem.* 40; 123–130.
66. Iwatsuki, H. and Suda, M. (2010) Transient expression of keratin during neuronal development in the adult rabbit spinal ganglion. *Anat. Sci. Int.* 85; 46–55.
67. Jeon, T. I., Zhu, B., Larson, J. L. and Osborne, T. F. (2008) SREBP-2 regulates gut peptide secretion through intestinal bitter taste receptor signaling in mice. *J. Clin. Invest.* 118; 3693–3700.
68. Jepson, M. A., Simmons, N. L., Hirst, G. L. and Hirst, B. H. (1993) Identification of M cells and their distribution in rabbit intestinal Peyer's patches and appendix. *Cell Tissue Res.* 273; 127–136.
69. Jessen, K. R., Morgan, L., Stewart, H. J. S. and Mirsky, R. (1990) Three markers of adult non-myelin-forming Schwann cells, 217c(Ran-1), A5E3 and GFAP: development and regulation by neuron-Schwann cell interactions. *Development* 109; 91–103.
70. Jing, R., Wilhelmsson, U., Goodwill, W., Li, L., Pan, Y., Pekny, M. and Skalli, O. (2007) Synemin is expressed in reactive astrocytes in neurotrauma and interacts differentially with vimentin and GFAP intermediate filament networks. *J. Cell Sci.* 120; 1267–1277.
71. Jones, J. C. R., Goldman, A. E., Steinert, P. M., Yuspa, S. and Goldman, R. D. (1982) Dynamic aspects of the supramolecular organization of intermediate filament networks in cultured epidermal cells. *Cell Motil.* 2; 197–213.
72. Karam, S. M. (1999) Lineage commitment and maturation of epithelial cells in the gut. *Front. Biosci.* 4; D286–D298.

73. Kasper, M. (1992) Cytokeratins in intracranial and intraspinal tissues. *Adv. Anat. Embryol. Cell Biol.* 126; 1–82.
74. Katagata, Y., Aoki, T., Kawa, Y., Mizoguchi, M. and Kondo, S. (1999) Keratin subunit expression in human cultured melanocytes and mouse neural crest cells without formation of filamentous structures. *J. Investig. Dermatol. Symp. Proc.* 4; 110–115.
75. Kende, A. I., Carr, N. J. and Sobin, L. H. (2003) Expression of cytokeratins 7 and 20 in carcinomas of the gastrointestinal tract. *Histopathology* 42; 137–140.
76. Kernéis, S., Bogdanova, A., Kraehenbuhl, J.-P. and Pringault, E. (1997) Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science* 277; 949–952.
77. Kernéis, S. and Pringault, E. (1999) Plasticity of the gastrointestinal epithelium: the M cell paradigm and opportunism of pathogenic microorganisms. *Semin. Immunol.* 11; 205–215.
78. Kim, S. Y., Lee, S. H., Kim, B. M., Kim, E. H., Min, B. H., Bendayan, M. and Park, I. S. (2004) Activation of nestin-positive duct stem (NPDS) cells in pancreas upon neogenic motivation and possible cytodifferentiation into insulin-secreting cells from NPDS cells. *Dev. Dyn.* 30; 1–11.
79. Kirfel, J., Magin, T. M. and Reichelt, J. (2003) Keratins: a structural scaffold with emerging functions. *Cell. Mol. Life Sci.* 60; 56–71.
80. Kivelä, T. and Uusitalo, M. (1998) Structure, development and function of cytoskeletal elements in non-neuronal cells of the human eye. *Prog. Retin. Eye Res.* 17; 385–428.
81. Kobayashi, S., Chiu, F. C., Katayama, M., Sacchi, R. S., Suzuki, K. and Suzuki, K. (1986) Expression of glial fibrillary acidic protein in the CNS and PNS of murine globoid cell leukodystrophy, the twitcher. *Am. J. Pathol.* 125; 227–243.
82. Kreft, M. E., Sterle, M., Veranic, P. and Jezernik, K. (2005) Urothelial injuries and the early wound healing response: tight junctions and urothelial cytodifferentiation. *Histochem. Cell Biol.* 123; 529–539.
83. Ku, N.-O., Zhou, X., Toivola, D. M. and Omary, M. B. (1999) The cytoskeleton of digestive epithelia in health and disease. *Am. J. Physiol.* 277; G1108–G1137.
84. Kumano, I., Iwatsuki, H. and Sasaki, K. (2002) Ganglion cell differentiation and intermediate filaments in the cervical dorsal root ganglion of the chick embryo. *Kawasaki Med. J.* 28; 57–69.
85. Lai, Y.-K., Lee, W.-C. and Chen, K.-D. (1993) Vimentin serves as a phosphate sink during the apparent activation of protein kinases by okadaic acid in mammalian cells. *J. Cell Biochem.* 53; 161–168.
86. Le Clainche, C. and Carlier, M. F. (2008) Regulation of actin assembly associated with protrusion and adhesion in cell migration. *Physiol. Rev.* 88; 489–513.
87. Lee, M. J., Lee, H. S., Kim, W. H., Choi, Y. and Yang, M. (2003) Expression of mucins and cytokeratins in primary carcinomas of the digestive system. *Mod. Pathol.* 16; 403–410.
88. Loewinger, L. and McKeon, F. (1988) Mutations in the nuclear lamin proteins resulting in their aberrant assembly in the cytoplasm. *EMBO J.* 17; 2301–2309.
89. Maddox-Hyttel, P., Alexopoulos, N. I., Vajta, G., Lewis, I., Rogers, P., Cann, L., Callesen, H., Tveden-Nyborg, P. and Trounson, A. (2003) Immunohistochemical and ultrastructural characterization of the initial post-hatching development of bovine embryos. *Reproduction* 125; 607–623.
90. Maison, C., Horstmann, H. and Georgatos, S. D. (1993) Regulated docking of nuclear membrane vesicles to vimentin filaments during mitosis. *J. Cell Biol.* 123; 1491–1505.
91. Maison, C., Pyrpasopoulou, A. and Georgatos, S. D. (1995) Vimentin-associated mitotic vesicles interact with chromosomes in a lamin B- and phosphorylation-dependent manner. *EMBO J.* 14; 3311–3324.
92. Marceau, N., Loranger, A., Gilbert, S., Daigle, N. and Champetier, S. (2001) Keratin-mediated resistance to stress and apoptosis in simple epithelial cells in relation to health and disease. *Biochem. Cell Biol.* 79; 543–555.
93. Marmiroli, S., Bertacchini, J., Beretti, F., Cenni, V., Guida, M., De Pol, A., Maraldi, N. M. and Lattanzi, G. (2009) A-type lamins and signaling: the PI 3-kinase/Akt pathway moves forward. *Cell Physiol.* 220; 553–561.
94. McCallum, S. J., Erickson, J. W. and Cerione, R. A. (1998) Characterization of the association of the actin-binding protein, IQGAP, and activated Cdc42 with Golgi membranes. *J. Biol. Chem.* 273; 22537–22544.
95. Melissourgos, N. D., Kastrinakis, N. G., Skolarikos, A., Pappa, M., Vassilakis, G., Gorgoulis, V. G. and Salla, C. (2005) Cytokeratin-20 immunocytology in voided urine exhibits greater sensitivity and reliability than standard cytology in the diagnosis of transitional cell carcinoma of the bladder. *Urology* 66; 536–541.
96. Merdes, A., Gounari, F. and Georgatos, S. D. (1993) The 47-kD lens-specific protein phakinin is a tailless intermediate filament protein and an assembly partner of filensin. *J. Cell Biol.* 123; 1507–1516.
97. Merrill, T. G., Sprinz, H. and Tousimis, A. J. (1967) Changes of intestinal absorptive cells during maturation; an electron microscopic study of prenatal, postnatal, and adult guinea pig ileum. *J. Ultrastruct. Res.* 19; 304–326.
98. Millard, T. H., Sharp, S. J. and Machesky, L. M. (2004) Signalling to actin assembly via the WASP (Wiskott-Aldrich syndrome protein)-family proteins and the Arp2/3 complex. *Biochem. J.* 380; 1–17.
99. Minin, A. A. and Moldaver, M. V. (2008) Intermediate vimentin filaments and their role in intracellular organelle distribution. *Biochemistry (Mosc.)* 73; 1453–1466.
100. Moll, R., Schiller, D. L. and Franke, W. W. (1990) Identification of protein IT of the intestinal cytoskeleton as a novel type I cytokeratin with unusual properties and expression patterns. *J. Cell Biol.* 111; 567–580.
101. Moll, R., Divo, M. and Langbein, L. (2008) The human keratins: biology and pathology. *Histochem. Cell Biol.* 129; 705–733.
102. Montes, J. F., Estrada, G., López-Tejero, M. D. and García-Valero, J. (1996) Changes in the enterocyte cytoskeleton in newborn rats exposed to ethanol in utero. *Gut* 38; 846–852.
103. Moran, C. A., Rush, W. and Mena, H. (1997) Primary spinal paragangliomas; a clinicopathological and immunohistochemical study of 30 cases. *Histopathology* 31; 167–173.
104. Nagle, R. B. (1994) A review of intermediate filament biology and their use in pathologic diagnosis. *Mol. Biol. Rep.* 19; 3–21.
105. Ogawa, C., Iwatsuki, H., Sasaki, K. and Kumano, I. (2001) Keratin filaments in epithelial cells of the excretory ducts of rabbit submandibular glands—An immunohistochemical and ultra-immunohistochemical study. *Acta Anat. Nippon* 76; 389–398.
106. Ogawa, C., Iwatsuki, H., Suda, M. and Sasaki, K. (2002) Golgi-associated filament networks in duct epithelial cells of rabbit submandibular glands; immunohistochemical light and electron microscopic studies. *Histochem. Cell Biol.* 118; 35–40.
107. Omary, M. B., Ku, N.-O., Liao, J. and Price, D. (1998) Keratin modifications and solubility properties in epithelial cells and in vitro. *Subcell. Biochem.* 31; 105–140.
108. Oriolo, A. S., Wald, F. A., Ramsauer, V. P. and Salas, P. J. (2007) Intermediate filaments: a role in epithelial polarity. *Exp. Cell Res.* 313; 2255–2264.
109. Oshima, R. G. (2007) Intermediate filaments; a historical perspective. *Exp. Cell Res.* 313; 1981–1994.
110. Pang, Y. Y. S., Schermer, A., Yu, J. and Sun, T. T. (1993) Suprabasal change and subsequent formation of disulfide-stabilized homo- and hetero-dimers of keratins during esophageal epithelial differentiation. *J. Cell Sci.* 104; 727–740.
111. Pannese, E. (1974) The histogenesis of the spinal ganglia. *Adv. Anat. Embryol. Cell Biol.* 47; 7–97.
112. Parnaik, V. K. (2008) Role of nuclear lamins in nuclear organization, cellular signaling, and inherited diseases. *Int. Rev. Cell Mol. Biol.* 266; 157–206.
113. Resto, V. A., Krane, J. F., Faquin, W. C. and Lin, D. T. (2006)

Immunohistochemical distinction of intestinal-type sinonasal adenocarcinoma from metastatic adenocarcinoma of intestinal origin. *Ann. Otol. Rhinol. Laryngol.* 115; 59–64.
114. Romih, R., Jezernik, K. and Masera, A. (1998) Uroplakins and cytokeratins in the regenerating rat urothelium after sodium saccharin treatment. *Histochem. Cell Biol.* 109; 263–269.
115. Salas, P. J., Rodriguez, M. L., Viciana, A. L., Vega-Salas, D. E. and Hauri, H. P. (1997) The apical submembrane cytoskeleton participates in the organization of the apical pole in epithelial cells. *J. Cell Biol.* 137; 359–375.
116. Sax, C. M., Farrell, F. X., Zehner, Z. E. and Piatlgorsky, J. (1990) Regulation of vimentin gene expression in the ocular lens. *Dev. Biol.* 139; 56–64.
117. Scheffler, B., Walton, N. M., Lin, D. D., Goetz, A. K., Enikolopov, G., Roper, S. N. and Steindler, D. A. (2005) Phenotypic and functional characterization of adult brain neuropoiesis. *Proc. Natl. Acad. Sci. U S A* 102; 9353–9358.
118. Schmidt, A. and Hall, M. N. (1998) Signaling to the actin cytoskeleton. *Ann. Rev. Cell Dev. Biol.* 14; 305–338.
119. Sechi, A. S. and Wehland, J. (2004) Interplay between TCR signalling and actin cytoskeleton dynamics. *Trends Immunol.* 25; 257–265.
120. Sharma, R., Schumacher, U. and Adam, E. (1998) Lectin histochemistry reveals the appearance of M-cells in Peyer's patches of SCID mice after syngeneic normal bone marrow transplantation. *J. Histochem. Cytochem.* 46; 143–148.
121. Shaw, G. and Weber, K. (1982) Differential expression of neurofilament triplet proteins in brain development. *Nature* 298; 277–279.
122. Sonnenberg, A. and Liem, R. K. (2007) Plakins in development and disease. *Exp. Cell Res.* 313; 2189–2203.
123. Starr, D. A. (2007) Communication between the cytoskeleton and the nuclear envelope to position the nucleus. *Mol. Biosyst.* 3; 583–589.
124. Starr, D. A. (2009) A nuclear-envelope bridge positions nuclei and moves chromosomes. *J. Cell Sci.* 122; 577–586.
125. Stephens, D. J. and Banting, G. (1999) Direct interaction of the trans-Golgi network membrane protein, TGN38, with the F-actin binding protein, neurabin. *J. Biol. Chem.* 274; 30080–30086.
126. Stephenson, J. L. and Byers, M. R. (1995) GFAP immunoreactivity in trigeminal ganglion satellite cells after tooth injury in rats. *Exp. Neurol.* 131; 11–22.
127. Sternini, C., Anselmi, L. and Rozengurt, E. (2008) Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing. *Curr. Opin. Endocrinol. Diabetes Obes.* 15; 73–78.
128. Strader, A. D. and Woods, S. C. (2005) Gastrointestinal hormones and food intake. *Gastroenterology* 128; 175–191.
129. Stuurman, N., Heins, S. and Aebi, U. (1998) Nuclear lamins; their structure, assembly, and interactions. *J. Struct. Biol.* 122; 42–66.
130. Sugimoto, M., Inoko, A., Shiromizu, T., Nakayama, M., Zou, P., Yonemura, S., Hayashi, Y., Izawa, I., Sasoh, M., Uji, Y., Kaibuchi, K., Kiyono, T. and Inagaki, M. (2008) The keratin-binding protein Albatross regulates polarization of epithelial cells. *J. Cell Biol.* 183; 19–28.
131. Suresh, S. (2007) Biomechanics and biophysics of cancer cells. *Acta Biomater.* 3; 413–438.
132. Szeverenyi, I., Cassidy, A. J., Chung, C. W., Lee, B. T., Common, J. E., Ogg, S. C., Chen, H., Sim, S. Y., Goh, W. L., Ng, K. W., Simpson, J. A., Chee, L. L., Eng, G. H., Li, B., Lunny, D. P., Chuon, D., Venkatesh, A., Khoo, K. H., McLean, W. H., Lim, Y. P. and Lane, E. B. (2008) The Human Intermediate Filament Database: comprehensive information on a gene family involved in many human diseases. *Hum. Mutat.* 29; 351–360.
133. Takeuchi, H., Kubota, T., Sato, K., Llena, J. F. and Hirano, A. (2002) Epithelial differentiation and proliferative potential in spinal ependymomas. *J. Neurooncol.* 58; 13–19.
134. Titeux, M., Brocheriou, V., Xue, Z., Gao, J., Pellissier, J. F., Guicheney, P., Paulin, D. and Li, Z. (2001) Human synemin gene generates splice variants encoding two distinct intermediate filament proteins. *Eur. J. Biochem.* 268; 6435–6449.
135. Trevor, K. T., McGuire, J. G. and Leonova, E. V. (1995) Association of vimentin intermediate filaments with the centrosome. *J. Cell Sci.* 108; 343–356.
136. Tsujimura, K., Ogawara, M., Takeuchi, Y., Imajoh-Ohmi, S., Ha, M. H. and Inagaki, M. (1994) Visualization and function of vimentin phosphorylation by cdc2 kinase during mitosis. *J. Biol. Chem.* 269; 31097–31106.
137. Uusitalo, M. and Kivelä, T. (1995) Development of cytoskeleton in neuroectodermally derived epithelial and muscle cells of the human eye. *Invest. Ophthalmol. Vis. Sci.* 36; 2584–2591.
138. Valiron, O., Caudron, N. and Job, D. (2001) Microtubule dynamics. *Cell Mol. Life Sci.* 58; 2069–2084.
139. Van Leenders, G., Dijkman, H., Hulsbergen-Van De Kaa, C., Ruiter, D. and Schalken, J. (2000) Demonstration of intermediate cells during human prostate epithelial differentiation in situ and in vitro using triple-staining confocal scanning microscopy. *Lab. Invest.* 80; 1251–1258.
140. Verstraeten, V. L., Broers, J. L., Ramaekers, F. C. and van Steensel, M. A. (2007) The nuclear envelope, a key structure in cellular integrity and gene expression. *Curr. Med. Chem.* 14; 1231–1248.
141. Waschke, J. (2008) The desmosome and pemphigus. *Histochem. Cell Biol.* 130; 21–54.
142. Weiner, O. H., Murphy, J., Griffiths, G., Schleicher, M. and Noegel, A. A. (1993) The actin-binding protein comitin (p24) is a component of the Golgi apparatus. *J. Cell Biol.* 123; 23–34.
143. Weiser, M. M., Walters, J. R. F. and Wilson, J. R. (1986) Intestinal cell membranes. *Int. Rev. Cytol.* 101; 1–57.
144. Wiche, G. (1998) Role of plectin in cytoskeleton organization and dynamics. *J. Cell Sci.* 111; 2477–2486.
145. Woodcock, C. L. F. (1980) Nucleus-associated intermediate filaments from chicken erythrocytes. *J. Cell Biol.* 85; 881–889.
146. Woodring, P. J., Hunter, T. and Wang, J. Y. (2003) Regulation of F-actin-dependent processes by the Abl family of tyrosine kinases. *J. Cell Sci.* 116; 2613–2626.
147. Xu, Y., Tamamaki, N., Noda, T., Kimura, K., Itokazu, Y., Matsumoto, N., Dezawa, M. and Ide, C. (2005) Neurogenesis in the ependymal layer of the adult rat 3rd ventricle. *Exp. Neurol.* 192; 251–264.
148. Yamada, M., Nakagawa, M., Yamamoto, M., Furuoka, H., Matsui, T. and Taniyama, H. (1998) Histopathological and immunohistochemical studies of intracranial nervous-system tumours in four cattle. *J. Comp. Pathol.* 119; 75–82.
149. Zhang, B., Chen, Y., Han, Z., Ris, H. and Zhai, Z. (1998) The role of keratin filaments during nuclear envelope reassembly in *Xenopus* egg extract. *FEBS Lett.* 428; 52–56.
150. Zhao, M., Momma, S., Delfani, K., Carlen, M., Cassidy, R. M., Johansson, C. B., Brismar, H., Shupliakov, O., Frisen, J. and Janson, A. M. (2003) Evidence for neurogenesis in the adult mammalian substantia nigra. *Proc. Natl. Acad. Sci. U S A* 100; 7925–7930.
151. Zhou, Q., Cadrin, M., Herrmann, H., Chen, C. H., Chalkley, R. J., Burlingame, A. L. and Omary, M. B. (2006) Keratin 20 serine 13 phosphorylation is a stress and intestinal goblet cell marker. *J. Biol. Chem.* 281; 16453–16461.