Vimentin Intermediate Filaments: Function and Implication in Cell Differentiation

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ABSTRACT. We have demonstrated that vimentin intermediate filament protein is one of the most sensitive and useful markers for early retinal and neuroepithelial cell differentiations. This review focuses on recent studies concerning the role of vimentin intermediate filaments involved in the determination of cell structure, in cell division and in intracellular signal transduction, and discusses the mechanism of vimentin expression sensitively reflecting cell differentiation in the early development of the eye and spinal cord.

Key words: vimentin intermediate filaments — cytoskeletal proteins — cell differentiation — retina — neuroepithelium

The cellular cytoskeletal network is composed of three fibrillar systems; i.e., actin microfilaments, intermediate filaments (IFs) and microtubules. This network is a highly dynamic structure that is continually reorganized as cells change shape, move, divide and maintain the speciffic arrangements of organelles.[1-10] Despite advances in understanding of the function of microfilaments[5,7,8,10] and microtubules,[4,6,7,9] the biological roles of IFs remain largely unknown.

We have previously indicated that vimentin expression is acutely reflected in the cell differentiation of neuroectodermal cell lineage.[11-13] In this review, recent studies of vimentin IFs are examined, and the mechanisms of vimentin expression sensitively reflecting cell differentiation are discussed.

1. Characteristics of the intermediate filament proteins

Presently, nearly 50 different IF proteins have been identified, and they are expressed either tissue specifically or developmental stage-specifically. As shown in Table 1, IF proteins have been classified into six groups on the basis of their amino acid and cDNA sequence similarities.[2,3,14,15] In contrast to other five groups of IFs which form a characteristic network in cytoplasm, lamins, type V IF proteins, are exclusively found in the nucleus and assemble to form a fibrous meshwork, the nuclear lamina, underlying the nuclear membrane. This structure provides a framework for the nucleus and facilitates chromatin organization.[16-18]

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, including

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TABLE 1. Intermediate filament proteins and main sites of their expression

Type	Proteins	Sites of expression
I	Acidic keratins	
	Keratin (Cytokeratin) 9-20	Epithelial cells
	Acidic hair keratin 1-4	Hair follicles
II	Neutral or Basic keratins	
	Keratin (Cytokeratin) 1-8	Epithelial cells
	Basic hair keratin 1-4	Hair follicles
III	Vimentin	Mesenchymal cells and neuroectodermal cells
	Desmin	Muscle cells
	Glial fibrillary acidic protein	Neuroglial cells
	Peripherin	Peripheral neurons
IV	Neurofilament proteins (NFs)	
	NF-L, NF-M, NF-H	Neurons of central and peripheral nerves
	Internexin	Embryonic neurons
V	Lamins	
	lamin A, lamin B, lamin C	Nucleoplasm of most cells
VI	Nestin	Precursors of neurogenic or myogenic cells
	Filensin	Lens fiber cells

other cytoskeletal components, are lost.[19] Therefore, the IF networks are suited to provide structural support to the cells, such as cell-shape formation and maintenance, the positioning and distribution of organelles including the nucleus, and the maintenance of the integrity of cell-cell and cell-matrix contacts.[20-23]

As shown in Fig 1, all of the IF proteins have a common tripartite structure consisting of a central α-helical rod domain and non-helical amino-terminal head and carboxy-terminal tail domains. The size and sequence of the rod domain of different IF proteins are similar, but those of 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.[2,3,14,24,25] In addition, the head domain plays a crucial role in the IF assembly, and the organization of IFs is controlled by phosphorylation and dephosphorylation of serine residues in head domain.[2,3]

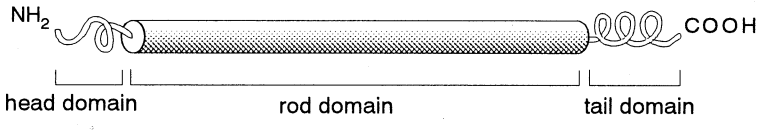

Fig 1. Structure of IF proteins. Most IF proteins share a similar rod domain that is approximately 310 amino acids long (350 amino acids in lamins) and forms an extended α-helix. The amino-terminal head and carboxy-terminal tail domains are non-helical and vary greatly in size and sequence in different IFs.

2. Vimentin intermediate filaments

Vimentin, a type III IF protein, is expressed not only in mesenchymal cells but also in the following cells originating from ectoderm; astrocytes[26-28] and ependymal cells[26] in the rat brain, pituicytes in the human neurohypophysis,[29] Müller cells,[30-32] pigment epithelial cells[33] and ciliary epithelial cells[34,35] in the eye of many mammals including humans, and supporting cells of both the crista ampullaris[36] and Corti's organ[37] in the guinea pig and rat inner ear. Among endodermal cells, strong expression of vimentin is observed in rabbit M cells ("membranous cells" or "microfold cells") of the follicle-associated epithelium of gut-associated lymphoid tissues, including intestinal Payer's patches,[38,39] appendices[38,39] and palatine tonsils.[40]

a. Intracellular distribution of vimentin IFs

The distribution of vimentin IFs in the cytoplasm is involved in cellular polarity. In unpolarized cells including fibroblasts[41] and chondrocytes (Fig 2), vimentin IFs are concentrated in the perinuclear region and form a juxtanuclear cap from which they radiate to the cell membrane. In the case of developing and mature epithelial cells exhibiting a polarized structure with specialized apical and basal faces, vimentin IFs are generally located from the perinuclear region to the basal plasma membrane.[11-13,30,36,42] Retinal pigment epithelial cells have circumferential bundles of vimentin IFs at the zonula adherens region.[43] Isolated pigment epithelial cells, however, lose their polarized epithelial structure at an early stage in culture, and the distribution of vimentin IFs shows an unpolarized cell-type pattern. After several weeks in culture, the epithelial cells regain a polarized cuboidal shape and reform circumferential bundles of vimentin IFs.[33] As shown in Fig 3, the M cells of rabbit Payer's patch possess dense bundles of vimentin IFs extending from the nuclear periphery to the plasma membrane surrounding interepithelial lymphocytes. In

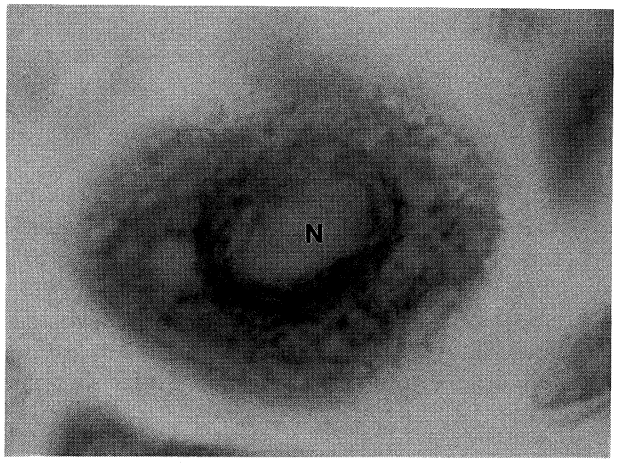

Fig 2. Immunohistochemical detection of vimentin in rabbit chondrocyte using the monoclonal antibody Vim3B4 and the avidin-biotin-peroxidase complex (ABC) technique. Vimentin IFs are concentrated around the edges of the nucleus (N) and appear to radiate from this region to the cell surface. ×2500.

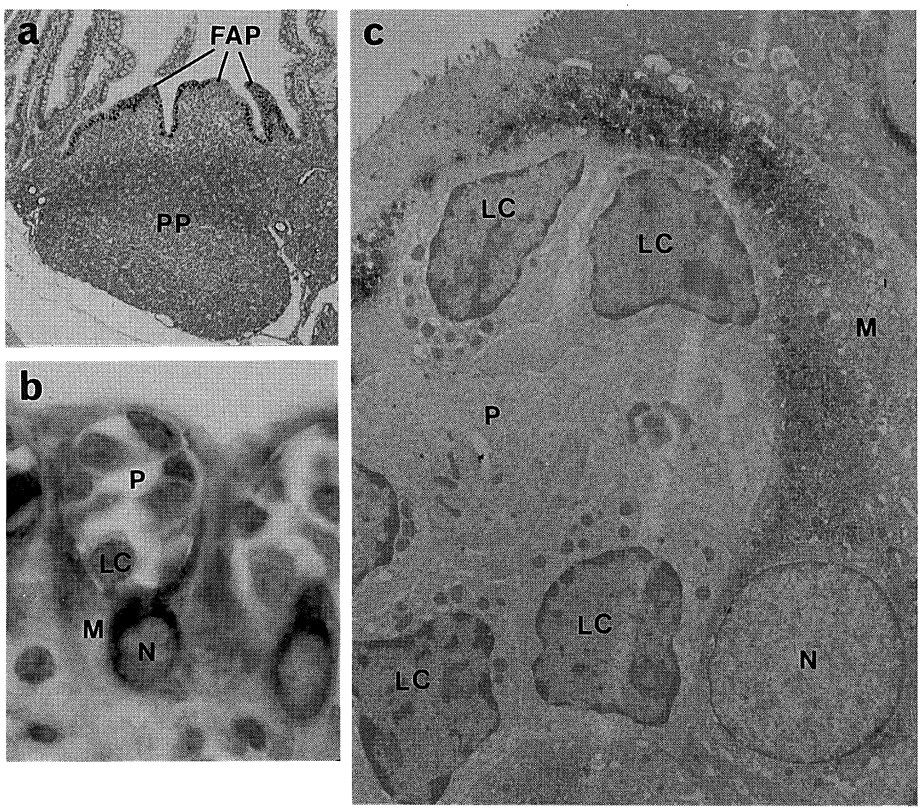

Fig 3. Vimentin IF expression in the M cells of a rabbit ileal Payer's patch.
a,b. Immunohistochemical staining of vimentin by the ABC technique in paraffin sections. Counterstained with hematoxylin. a. In the follicle-associated epithelium (FAP) of a Payer's patch (PP), M cells are selectively stained. ×40. b. High magnification of the follicle-associated epithelium. M cells (M) possess dense bundles of vimentin extending from the edges of the nucleus (N) to the plasma mambrane surrounding interepithelial lymphocytes (LC). P; basolateral pocket of M cells. ×1000. c. Similar distribution of vimentin IFs in M cells is confirmed by ultrahistochemical staining using the ABC-Os technique. ×4000.

the secretory dendritic cells in the bursa of Fabricius of neonate chickens, the accumulation of vimentin IFs on one side of the nucleus endows the cells with a polarized appearance, and the major cell process containing vimentin IFs extends from this "cap-like" vimentin positive area of the cytoplasm.[44] Thus, there exists a definite correlation between distribution of vimentin IFs and cell polarity. This correlation is highly suggestive of the involvement of vimentin IFs in functional cell differentiation or in intracellular signal transduction as will be mentioned later.

b. Maintenance of cell structure and vimentin

Vimentin IFs form a characteristic network which connects them, and furthermore, they connect directly or indirectly with actin microfilaments,[45,46] microtubules,[47] other IFs[48,49] and the cell membrane.[41,46] Thus, vimentin IFs could play a major role in the formation of a complicated cytoplasmic

cytoskeletal system serving the maintenance of cell structure. In addition, vimentin is preferentially phosphorylated among cytoplasmic proteins including cytoskeletal proteins.[50] Phosphorylation of serine residues in head domains of vimentin molecules results in disassembly of the vimentin network, and dephosphorylation of the residues results in reorganization of the network.[51] Therefore, phosphorylation of vimentin causes a specific disassembly of the cytoskeletal architecture and then leads to rounding of the cell.[46,52] Similar phosphorylation of vimentin has been recognized in the degranulation of mast cells.[53] Cytoskeletal networks containing vimentin IFs act as a barrier between the granule membrane and the plasma membrane in quiescent mast cells. When these cells are stimulated with Ca^{++}, protein kinase C phosphorylates vimentin. As a result, disruption of the cytoskeletal barrier occurs, and the granular membrane can fuse with the plasma membrane.

Disassembly of the cytoskeletal system also arises from a decrease in the amount of vimentin. Administration of heparin in hypertensive rat models lowers their blood pressure.[54] This lowering effect is endothelium-dependent. The heparin treatment causes a significant decrease in the amount of vimentin in endothelial cells resulting in disassembly of the cytoskeletal system. Subsequently, relaxation of endothelium causes capillary relaxation.

In addition to cytokeratin IFs, vimentin IFs also play a role in both cell-cell and cell-matrix contacts. Vimentin IFs are tightly anchored to two specialized structures of the membrane, desmosomes and hemidesmosomes, which serve to maintain cell-cell and cell-matrix contacts, respectively. For connection of vimentin IFs to the dense plaques of these junctional complexes, the following IF-associated proteins act as linker proteins; desmoplakin I and II,[55] IF-associated protein 300,[56] bullous pemphigoid 230 and 180[57] and plectin.[58]

The N-terminal head domain of vimentin carries a high net positive charge due to a wealth of arginine residues, whereas its C-terminal tail domain is usually negative-charged.[2] Therefore, the vimentin head domain can attract polyanionic compounds including phospholipid in membrane and nucleic acids in ribosomes. Actually, vimentin IFs interact with the nuclear envelope,[21,41] mitochondria,[59,60] Golgi apparatus,[61] ribosomes[62] and centrosomes,[63] and are involved in the storage and distribution of these organelles in the cytoplasm. In addition, vimentin IFs play an essential role in enucleation, since they act as an anchor for the nucleus. Vimentin expression ceases when the nuclei of the lens fibers[64,65] and mammalian erythroblasts[66] are extruded, whereas vimentin persists in nucleated avian erythrocytes.[21,67] The experimental analysis of the correlation between vimentin IFs and enucleation has been taken a step further by using transgenic mice.[68] Overexpression of vimentin in the lens fibers of transgenic mice interferes with denucleation, and the animals develop cataracts at 6-12 weeks of age.

Vimentin IFs are also involved in the storage of cytoplasmic inclusions. During adipogenesis, the expression of vimentin IFs in adipocytes is increased in correlation with the onset of lipid droplet formation, and vimentin IFs form a complex cage surrounding the lipid droplets. This cage consists of a monolayer of regularly arranged vimentin IFs and borders between cytoplasm and lipid droplets.[69,70]

c. Cell division and vimentin

Besides microtubules and actin microfilaments, vimentin IFs also play an important role in cell division in collaboration with nuclear lamin IFs. Three types of lamin IF proteins have been recognized ; lamin A, lamin B and lamin C. Lamin A and C make up the nuclear lamina, an IF meshwork underlying the nuclear membrane. Lamin B connects this meshwork with the inner nuclear membrane.[3,17,18] When the cell enters mitosis, cyclin-dependent cdc2 kinase phosphorylates serine residues in the head domains of both the molecules of vimentin and the lamins.[51,57] Takai *et al*[72] reported that protein kinase C also acts in the phosphorylation of vimentin IFs in mitosis. The phosphorylation of vimentin causes a disassembly of cytoskeletal architecture and cell rounding, as described previously in section "2-b". Increase in the phosphorylation of lamins leads to depolymerization of nuclear lamina, as a result of which the nuclear membrane is fragmented into small vesicles.[3,17,18] In contrast to lamin A and C which are released as free dimers, lamin B remains bound to these small vesicles, and the vesicles are docked on vimentin IFs by mediation of lamin-B.[73,74] At the end of mitosis, inactivation of cdc2 leads to the dephosphorylation of vimentin and lamins. Thus, lamin B-binding vesicles dissociate from vimentin filaments and assemble around chromatin. The vesicles then fuse with each other to form a complete nuclear envelope. The cytoskeletal architecture in cytoplasm is finally reorganized at the end of mitosis.[51,73,74]

d. Intracellular signal transduction system

Each cell is bombarded by chemical signals from other cells, and these signals bind to cell-surface receptors that activate several intracellular signal transduction systems. Peter[75] first suggested the possibility of the participation of the cytoskeletal network in intracellular signal transduction, since very small amounts of hormone bound to the cell surface receptors and altered diverse biochemical reactions within all regions of the cell. However, this area is still conceptually fuzzy. Recent evidence has suggested that actin microfilaments are associated with intracellular signal transduction because of their dynamic structure.[76,77] The possibility that vimentin IFs also are a part of that system should be considered for the following three reasons. First, vimentin IFs have a high binding affinity to both the plasma membrane[41,46] and DNA sequence elements in the nucleus.[78] Second, vimentin IFs are very stable under physiological conditions. Vimentin has a long half-life, roughly equivalent to the cell generation time, whereas the half-life of vimentin mRNA is very short, about 6 hr, in mouse fibroblasts.[79] Third, vimentin networks are dynamic structures and can be easily modified by the phosphorylation-dephosphorylation system, as described previously in section "2-b".

Recently, the possibility that vimentin IFs are a part of the intracellular signal transduction system has been supported by the results of several studies. Yano *et al*[27] elucidated a close relationship between the cell surface receptors and vimentin IFs in cultured rat astrocytes. Binding of glutamate to its receptor on the astrocyte surface stimulated Ca^{++}/calmodulin-dependent protein kinase II, and then vimentin IFs were phosphorylated by the enzyme. A similar phenomenon was observed in mast cells.[53] When mast cells were stimulated with Ca^{++}, protein kinase C phosphorylated vimentin IFs. In

addition, Berfield *et al*[80] observed the changes in the nuclear structure and chromatin aggregation subsequent to phosphorylation of vimentin IFs, when renal mesangial cells were stimulated with insulin. The cells subsequently produce large amounts of collagens I and III.

It is important to note that vimentin IFs in developing epithelial cells generally distribute from the nucleus to basal plasma membrane, which contains various receptors for extracellular signals. For example, during eye development, the latero-central epithelium of the optic vesicle differentiates into the neural retina as a result of the action of fibroblast growth factor (FGF) from epidermal ectoderm.[81,82] As shown in Fig 4, at the first step of retinal differentiation, the latero-central epithelial cells of the optic vesicle possess dense bundles of vimentin IFs from the basal plasma membrane containing FGF receptors to the nuclear periphery. A similar phenomenon is observed in the developing neural plate, as described later. Therefore, it seems that vimentin IFs are part of the intracellular signal transduction system connecting cell surface receptors with chromosomes and leading to changes in gene expression in response to extracellular signals.

3. Cell differentiation and vimentin

We have demonstrated that vimentin could be one of the most sensitive and useful markers for early neural cell differentiation in chick embryos.

a. Differentiation of retinal cell linage

Eye primordia develop from the forebrain as lateral evaginations identified as optic vesicles. Subsequently, the lateral wall of each vesicle becomes invaginated so that each vesicle forms a two-layered optic cup. By detection of various kinds of molecules as markers for retinal cell differentiation, recent histochemical and immunohistochemical studies have concluded that the differentiation of retinal epithelial cells occurs at a late stage of the optic cup period. These marker molecules include carbonic anhydrase,[83] a cell surface antigen (RET-RE2),[84] a neural cell adhesion molecule (N-CAM),[84] a channel forming protein (connexin 43),[85] acidic and basic fibroblast growth factors,[86] a membrane protein (REMP)[87] and nuclear limb deformity protein.[88] However, our immunohistochemical study of chick embryos using anti-vimentin antibody clearly demonstrated that the differentiation of retinal epithelium into neural retina, pigment epithelium and pars caeca retinae occurs at a late stage of the optic vesicle period, prior to optic cup formation.[11,13] At this stage of eye development, the optic vesicle epithelium could be classified into three different portions on the basis of the amount of vimentin IFs; latero-central epithelium under the lens placode, medio-central epithelium facing the latero-central epithelium, and peripheral epithelium connecting the latero-central epithelium and the medio-central epithelium (Fig 4). The strongest expression of vimentin was observed in the latero-central epithelium, while the weakest expression was seen in the medio-central epithelium. Moderate expression of vimentin was observed in the peripheral epithelium. These differences in vimentin expression could be observed during optic cup formation (Fig 5). Therefore, the epithelium of the two-layered optic cup could also be separated into three portions; inner-central epithelium derived from the previous latero-central epithelium of the vesicle, outer-central epithelium derived from the previous

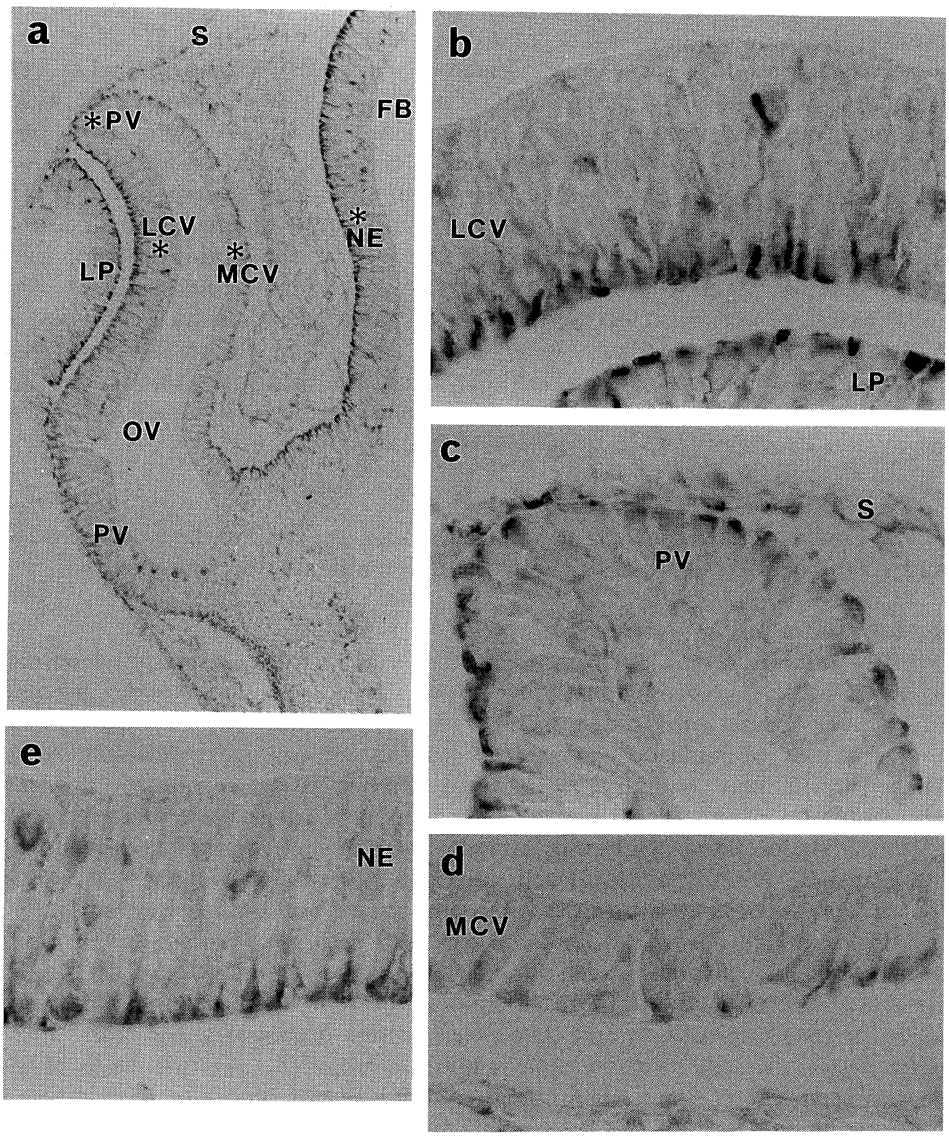

Fig 4. Immunohistochemical staining of vimentin in the optic vesicle epithelium of a chick embryo at a late stage of the optic vesicle period (Hamburger and Hamilton's stage 14). a. Low magnification of the optic vesicle (OV) and forebrain (FB). The lens placode (LP) begins to invaginate. The optic vesicle epithelium can be classified into three different portions on the basis of vimentin staining intensity; latero-central epithelium (LCV), medio-central epithelium (MCV) and peripheral epithelium (PV). Asterisks indicate the sites corresponding to 'b' (LCV), 'c' (PV), 'd' (MCV), and 'e' (NE: neuroepithelium). S: surface ectoderm. ×190. b. Among three epithelial portions, the strongest staining is observed in the latero-central epithelium (LCV), and the staining patterns are similar to those of the neuroepithelium in 'e'. ×1000. c. Moderate staining is observed in the peripheral epithelium (PV). ×1000. d. The weakest staining is observed in the medio-central epithelium (MCV). ×1000. e. Strong staining is also observed in the neuroepithelium (NE) of forebrain. ×1000.

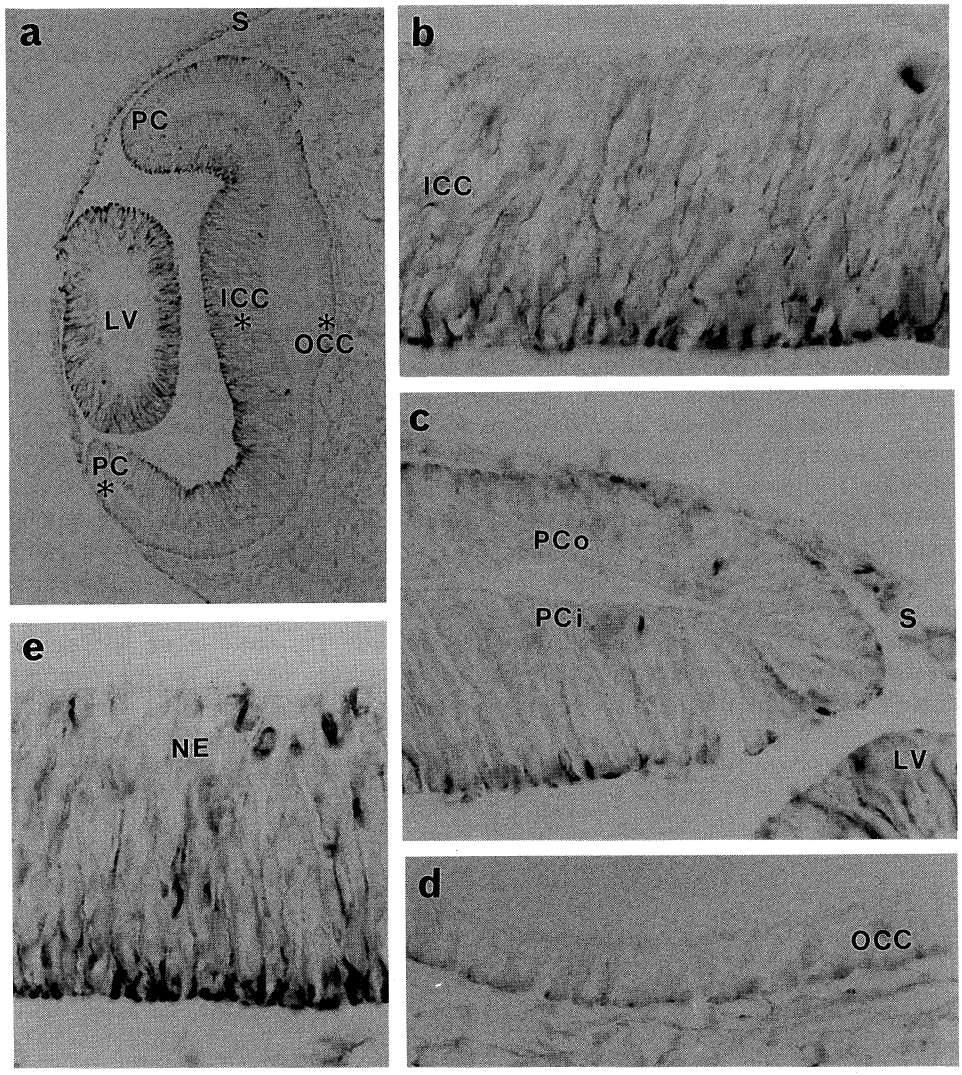

Fig 5. Immunohistochemical staining of vimentin in the optic cup epithelium of a chick embryo at stage 17. a. Overall view of the optic cup. On the basis of vimentin staining, the epithelium of the optic cup can also be separated into three different portions; inner-central epithelium (ICC), outer-central epithelium (OCC), and inner and outer peripheral epithelia (PC). Asterisks indicate the sites corresponding to 'b' (ICC), 'c' (PC) and 'd' (OCC). LV: lens vesicle. S: surface ectoderm. ×170. b. Among the three epithelial portions, the strongest staining is observed in the inner-central epithelium (ICC), and the staining patterns are similar to those of the neuroepithelium in 'e'. ×750. c. The inner and outer peripheral epithelia (PCi and PCo) exhibit moderate staining. ×750. d. The weakest staining is observed in the outer-central epithelium (OCC). ×750. e. The neuroepithelium (NE) of the forebrain shows strong staining. ×750.

medio-central epithelium, and inner and outer peripheral epithelia derived from the previous peripheral epithelium. The strongest expression of vimentin IFs was detected in the inner-central epithelium; i.e., the future neural retina. The inner and outer peripheral epithlia; i.e., the future pars caeca retinae, exhibited

a moderate expression of vimentin IFs. The weakest expression of vimentin IFs was in the outer-central epithelium; i.e., the future retinal pigment epithelium. The vimentin expression abruptly decreased in every portion of the optic cup when the formation of two-layered optic cup was completed (Fig 6). The relation between the vimentin expression and the differentiation process of retinal epithelial cells is schematically summarized in Fig 7.

During optic cup formation, the neural retinal cell lineage, namely the latero-central epithelium of the optic vesicle and the inner-central epithelium of the optic cup, showed strong expression of vimentin similar to that of the neuroepithelium of the forebrain. The strong expression of vimentin IFs in these epithelial cells can be explained by the following findings. First, the neural retinal cell lineage and forebrain neuroepithelial cells show high proliferative activity, active migration of the nuclei, and successive changes in cell shape at this developmental stage.[89,90] As mentioned previously in section "2-b", vimentin IFs act in cell shape formation through interconnection to other cytoskeletal components.[45–49] Among intermediate filament proteins, vimentin is the most sensitive to phosphorylation and dephosphorylation,[50] and phosphorylation of vimentin causes a specific disassembly of the cytoskeletal architecture of the cell.[46,51,52] Since the cytoskeletal architecture containing vimentin IFs can easily be reconstructed by the phosphorylation-dephosphorylation system, vimentin is considered to be one of the IFs most responsible for successive changes in cell shape. Second, vimentin IFs serve as an anchor for the nucleus during interphase[21,41,91] and as a docking site for laminin-binding nuclear membrane during mitosis,[72–74] as mentioned previously in section "2-c".

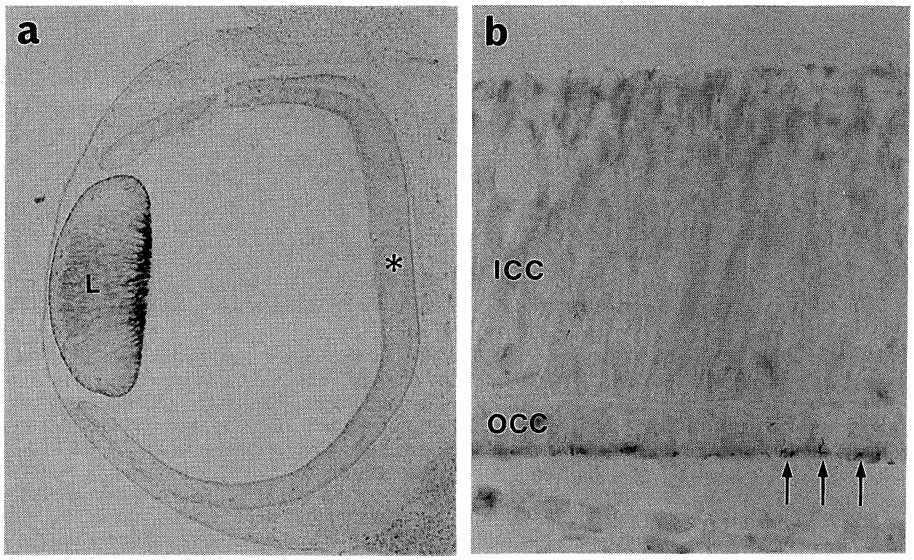

Fig 6. Immunohistochemical staining of vimentin in the optic cup epithelium of a chick embryo when the formation of the two-layered optic cup is completed (stage 21).
a. Overall view of the optic cup. The formation of the two-layered optic cup is completed. Anti-vimentin staining abruptly decreased in every epithelial portion. Asterisk indicates the site corresponding to 'b'. L: lens. ×75. b. Only the basal cytoplasm of both the inner- and outer-central epithelia (ICC and OCC, respectively) is faintly stained. The OCC possesses melanin granules (arrows). ×730.

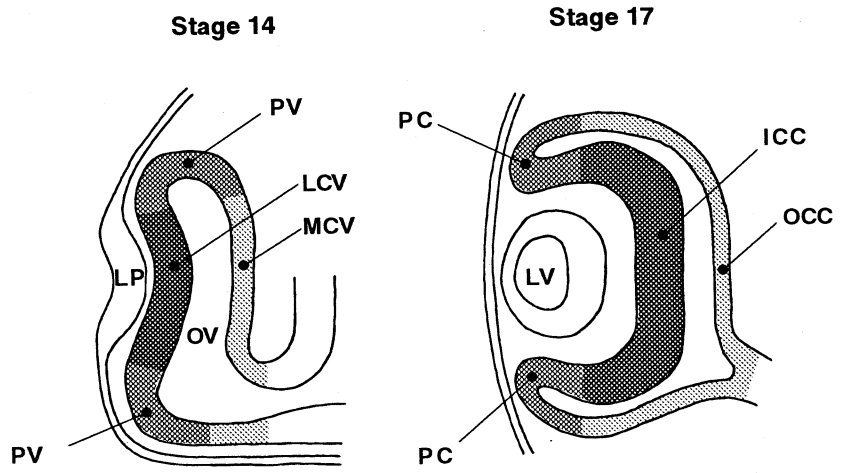

Fig 7. Schematic representation of the regional differences in vimentin expression in the epithelium of the optic vesicle at stage 14 and of the optic cup at stage 17. The density of dots in the epithelium shows the relative staining intensity for vimentin. ICC : inner-central epithelium. LCV : latero-central epithelium. LP : lens placode. LV : lens vesicle. MCV : medio-central epithelium. OCC : outer-central epithelium. OV : optic vesicle. PC : inner and outer peripheral epithelia of the optic cup. PV : peripheral epithelium of the optic vesicle.

Therefore, vimentin IFs appears to be the most useful IFs for cells exhibiting high active proliferation and continuous nuclear movement. In this regard, similar strong expression of vimentin IFs has been detected in many tumor tissues with high active proliferation and changes in cell shape.[92–97]

An abrupt decrease in vimentin expression in optic cup epithelial cells took place when the formation of the two-layered optic cup was completed. A variety of cell types transiently hold vimentin IFs during early development, and vimentin IFs are progressively replaced by tissue-specific IFs.[15,26,28,65,98] Therefore, it is reasonable to assume that the abrupt decrease in vimentin IFs reflects a change from vimentin IFs to other tissue-specific IFs.

b. Differentiation of neuroepithelial cells of spinal cord

Primary neurulation involves formation of the neural plate, the shaping of this plate into the neural groove, and the closure of the groove converting it into the neural tube. Thereafter, the ventral and dorsal neuroepithelial cells of the neural tube give rise to motor neuroblasts and sensory coordinative neuroblasts, respectively. By application of vimentin immunostaining in chick embryos, we clearly demonstrated that differentiation of the neuroepithelium of the spinal cord into motor, sensory, floor and roof areas occurred at an early stage of neural groove development, prior to neural tube formation.[12] At this stage of development, the level of vimentin expression allowed us to identify the following four areas in the neural groove wall ; a median hinge area in close vicinity to the notochord, a ventral area occupying about two thirds of the lateral wall of the groove, a dorsal area occupying about a third of the lateral wall, and a dorsolateral edge area bordering epidermal ectoderm. (Fig 8) Vimentin was expressed moderately in the ventral area, but only weakly in the dorsal area. In contrast, the expression of vimentin was largely suppressed in

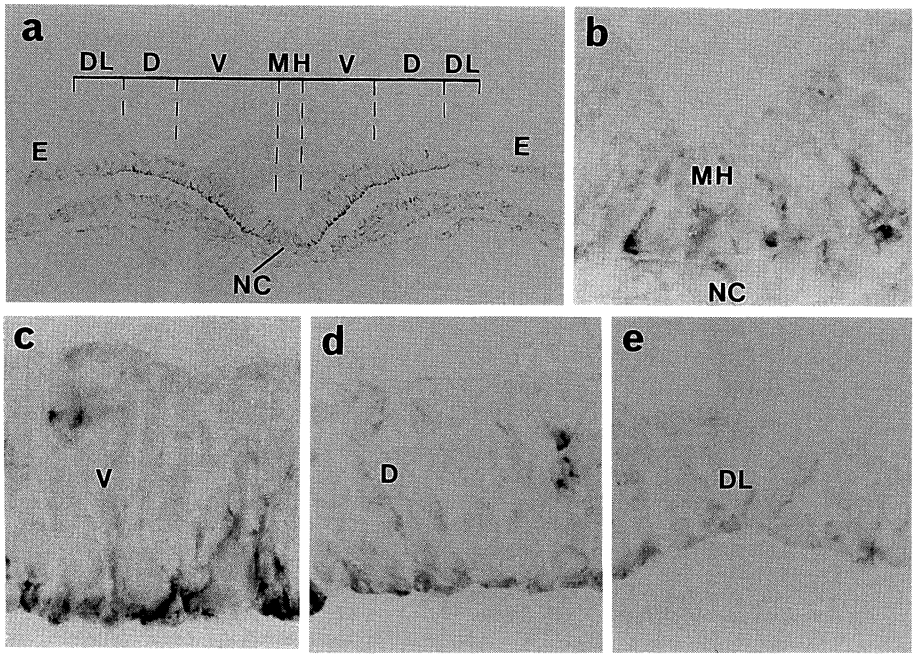

Fig 8. Immunohistochemical staining of vimentin in the neuroepithelium of a chick embryo at an early stage of the neural groove (stage 8). a. Low magnification of the neural groove at the future cervical spinal cord level. The neuroepithelium can be classified into four different areas on the basis of vimentin staining intensity; a median hinge area (MH), a ventral area (V), a dorsal area (D), and a dorsolateral edge area (DL). E: epidermal ectoderm, NC: notochord. ×100. b,e. The expression of vimentin in both the median hinge area (MH in 'b') and dorsolateral edge area (DL in 'e') is largely suppressed. ×1100. c. Moderate staining is observed in the ventral area (V). ×1100. d. Weak staining is observed in the dorsal area (D). ×1100.

both the median hinge and dorsolateral edge areas. These differences in vimentin expression could still be observed after neural tube formation was completed. Therefore, the neuroepithelium of the neural tube could also be classified into the following four areas; the basal plate derived from the previous ventral area of the neural groove, the alar plate derived from the previous dorsal area, the floor plate derived from the previous median hinge area, and the roof plate derived from the previous dorsolateral edge area (Fig 9). Moderate expression of vimentin IFs was detected in the basal plate representing the generation area of motor neurons. Weak expression of vimentin IFs was observed in the alar plate representing the generation zone of sensory coordinative neurons. In contrast, the expression of vimentin in both the floor and roof plates was suppressed. The relation between the vimentin expression and the differentiation process of neuroepithelial cells is schematically summarized in Fig 10.

At the early stage of neurulation, two factors, namely sonic hedgehog from the notochord and bone morphogenic protein from the surface ectoderm, regulate the differentiation of neuroepithelial cells. The former induces the differentiation of neuroepithelium into both the basal and floor plates.[99–104] The latter induces it into both the alar and roof plates.[105] In addition, cell

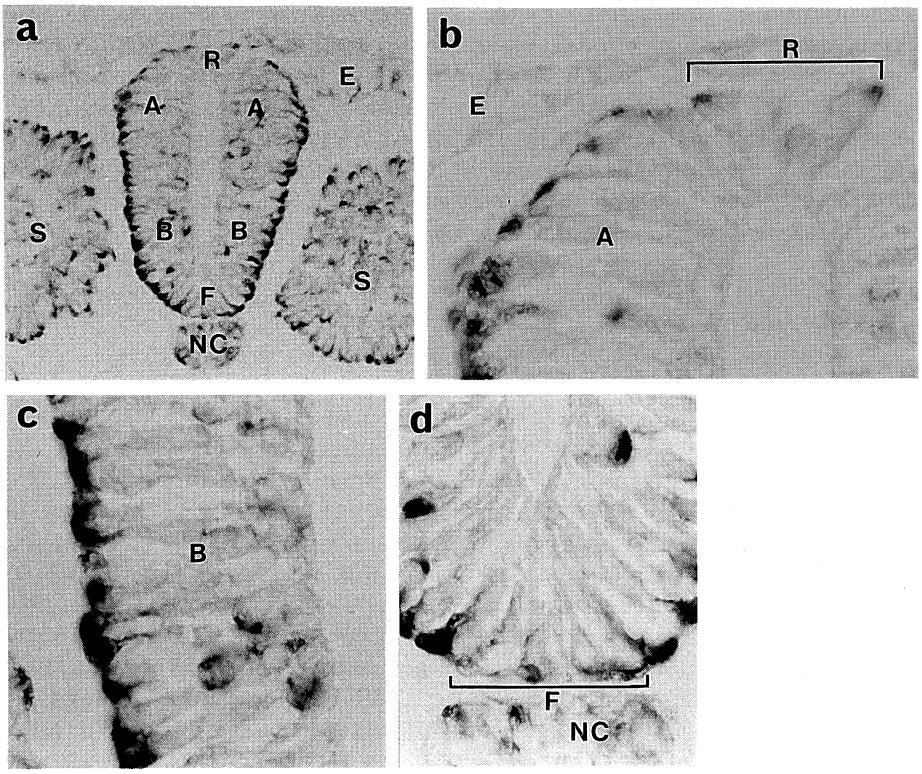

Fig 9. Immunohistochemical staining of vimentin in the neuroepithelium of a chick embryo at an early stage of the neural tube (stage 12). a. Low magnification of the neural tube at the future cervical spinal cord level. The epithelium of the neural tube can also be classified into four different areas on the basis of vimentin staining intensity; the floor plate (F), basal plate (B), alar plate (A), and roof plate (R). E: epidermal ectoderm, NC: notochord. S: somite. ×300. b. Weak staining is observed in the alar plate (A), but the expression of vimentin in roof plate (R) is suppressed. ×1100. c. Moderate staining is observed in the basal plate (B). ×1100. d. The expression of vimentin in flroor plate (F) is suppressed. ×1100.

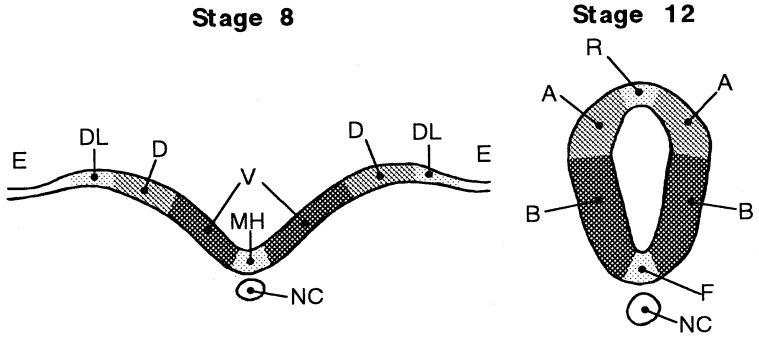

Fig 10. Schematic representation of the regional differences in vimentin expression in the neuroepithelium of the neural groove at stage 8 and of the neural tube at stage 12. The density of dots in the neuroepithelium shows the relative staining intensity for vimentin. A: alar plate. B: basal plate. D: dorsal area. DL: dorsolateral edge area. E: epidermal ectoderm. F: floor plate. MH: median hinge area. NC: notochord. R: roof plate. V: ventral area.

differentiation of the ventral motor area starts prior to that of the dorsal sensory area.[106] As mentioned previously in section "3-a", vimentin IFs are considered to be one of the most responsible IFs for the development of neuroepithelial cells showing high proliferation activity, active migration of nuclei and successive changes in cell shape. Therefore, it appears that the expression of vimentin IFs increases in the neuroepithelial cells of the ventral motor area before it does in the dorsal sensory area, and that the differences in vimentin expression between the two areas are maintained during neural tube formation.

4. Disease and vimentin

Numerous diseases are associated with abnormalities of IF organization.[3,14,107–111] Abnormal expression of vimentin IFs also occurs in various pathological conditions,[108] and some tumor cells especially exhibit an overexpression of vimentin.[92–97,111] Hendrix *et al*[96] reported that overexpression of vimentin IFs in the breast carcinoma cells led to augmentation of cell motility and invasiveness. Since tumor cells exhibit high active proliferation and successive changes in cell shape for movement or invasion, they may select vimentin IFs as the most advantageous IFs (the advantage of vimentin IFs for them was described in section "3-a"). Therefore, vimentin may become a good marker for determining the differentiation status of some tumor cells. In this regard, similar overexpression of vimentin IFs has been detected in stimulated fibroblasts. When quiescent fibroblasts were stimulated to proliferate by serum, a fourfold increase in vimentin mRNA levels was observed in their cytoplasm.[112] An interesting study has also been done concerning fostriecin, an antitumor drug, and its effect on vimentin. Although fostriecin has been used as an antitumor drug in phase I clinical trials, it inhibits protein phosphatase 1 and 2A, and induces vimentin hyperphosphorylation. Thus, this drug causes reorganization of the vimentin IF networks and the rounding of cells.[52] These effects of fostriecin are very similar to those of okadaic acid[113,114] and calyculin,[114–116] which are recognized as potent tumor promoters.[117,118] Therefore, fostriecin may have tumor-promoting activity.

Abnormal expression of vimentin IFs has also been implicated in some pathological conditions. High expression of vimentin in the lens fibers of the eye has been found to interfere with normal denucleation and elongation of the lens fibers, resulting in the development of pronounced cataracts in animals.[68,119] An increase in vimentin within Müller cells after retinal detachment is involved in glial scar formation.[30] In frog erythrocytes infected with frog erythrocytic virus, vimentin IFs are involved in the production or maintenance of the structural integrity of virus assembly sites.[91]

5. Conclusions

Vimentin IFs play a major role in the formation of a complicated cytoskeletal system through interconnection to other cytoskeletal components and are involved in the maintenance of cell structure and cell-cell contact. They are also involved in the storage and distribution of organelles, including the nucleus. In addition to microtubules and actin microfilaments, vimentin IFs also play an important role in cell division, in which they serve as docking

sites of lamin B-binding vesicles originating from the nuclear envelope. The possibility that vimentin IFs are part of the intracellular signal transduction system can also be considered. It seems that these functions of vimentin IFs depend on their polarized intracellular distribution and their dynamic structures that alternate between disassembly and reorganization by the phosphorylation-dephosphorylation system. The differentiation of both retinal and neuroepithelial cells is sensitively reflected by the expression of vimentin. Since vimentin IFs are dynamic structures, their expression may be most responsible for the development of retinal and neuroepithelial cells exhibiting high proliferative activity, active migration of the nucleus and successive changes in cell shape. Recent studies have shown that numerous pathologies are associated with modifications of IF organization. Some tumor cells especially exhibit an overexpression of vimentin IFs. Since changes in IF composition occur prior to changes in cell structure during organogenesis, immunohistochemical determination of abnormal IF composition may become a powerful tool for making earlier detection of the onset of various diseases.

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