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Intermediate filaments in health and disease

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Abbreviation: IF, intermediate filaments

Abstract

Intermediate filaments constitute one of the major classes of cytoskeletal proteins of mammalian cells. The 40 or more known intermediate filament proteins have been classified into five types which show very specific rules of expression in specialized cell types, and include: type I and type II keratins of epithelial cells; type III vimentin (mesenchymal cells), desmin (muscle), glial fibrillary acidic protein (astroglia), and peripherin (peripheral neurones); type IV neurofilaments of neuronal cell types; and type V nuclear lamins. Intermediate filaments differ from ubiquitous microfilaments (actin) and microtubules (tubulin) in several important ways, including their structures, intracellular dynamics, and functions. Notably, intermediate filament chains possess a characteristic central α-helical domain that forms coiled-coil rods which in turn assemble into 10-15 nm diameter filaments. The end domains which flank the rod domain are highly variable in sequence and confer specific functions to the filaments in different cell types. Recently, a number of diseases have been identified in human that involve mutations in genes encoding intermediate filament chains. These include seven different keratin diseases, usually caused by mutations in rod domain sequences which often result in serious skin (epidermal) abnormalities; and many cases of sporadic amyotrophic lateral sclerosis caused by mutations in the end domain sequences of neurofilament chains.

Introduction

The cytoskeleton of mammalian cells consists of an elaborate cytoskeleton composed in large part of three major classes of structural proteins. These are: ~25 nm diameter microtubules, composed of the globular tubulin proteins; ~7 nm diameter microfilaments composed of the ovoid-shaped actin molecule; and a class of filaments of diameter roughly intermediate in size, usually 10-15 nm, which are now termed intermediate filaments (IF). In contrast to the other two, the IF are composed of α-helical fibrous proteins. We now know that IF are common constituents of virtually all differentiated cell types and are present in both the nucleus (as the lamin karyoskeleton) and the cytoplasm (Steinert and Roop, 1988; Goldman and Steinert, 1990; Fuchs and Weber, 1994; Parry and Steinert, 1995). They were first recognized more than 100 years ago as "tonofibrils" in epithelial cells, or "neurofibrils" in neuronal cells, but only as recently as 1978 was it realized that these and other proteins are actually members of a common family (Steinert *et al.*, 1978). The common features that characterize an IF protein chain include: the ability to self-assemble *in vitro* into a typical 10-15 nm IF without the aid of energy or cofactors; and a common chain motif consisting of a central highly α-helical rod domain flanked by variable end domain sequences.

More than 40 different IF chains are now known in humans, all expressed from separate genes. The precise sequences of these chains have allowed their classification into five types (Table 1). Their expression is strikingly tissue-specific, which suggests that the IF type present in a cell is closely related to its function. The most numerous IF chains are the keratins, which are always co-expressed in type I/type II pairs, and indeed, the exact pair of chains expressed is often characteristic of a particular epithelium (Eichner *et al.*, 1984). For example, simple single-celled epithelia commonly co-express keratins 8 and 18; the more complex stratified squamous epithelial cell types co-express keratins 5 and 14; and terminally differentiating epithelia express keratins 1 and 10 in the epidermis, or sets of trichocyte keratins in hairs and nails.

Intermediate filament organization in cells

Light and electron micrographs of partially extracted epithelial cells have shown that keratin IF in particular adopt a very complex array in cells. They form an elaborate cage-like network around the nucleus and in fact connect to the nuclear surface at nuclear pores where they may contact directly the lamin karyoskeletal network (Jones *et al.*, 1982). In addition, cables of

Table 1. Human intermediate filament proteins

Type	Name	Number	Proteins	Tissues of chains
Type I	Keratins (acidic)	>10	Keratins 9-20	Various epithelia
		~5	Trichocyte keratins Ha1-Ha4, Hax	Hair/nails
Type II	Keratins (neutral-basic)	≥8	Keratins 1-8	Various epithelia
		~5	Trichocyte keratins Hb1-Hb4, Hbx	Hair/nails
Type III		1	Vimentin	"Mesenchymal" cells
		1	Desmin	Muscle
		1	Glial fibrillary acidic protein	Astroglia
		1	Peripherin	Peripheral nerves
Type IV	Neurofilaments	1	Nestin	Neuroectodermal stem cells?
		1	α-Internexin	Differentiating neurones
		3	NF-L, NF-M, NF-H	Mature neurones/axons
Type V	Lamins	4	Lamins A, B1, B2, C	Nuclear lamina

keratin IF, corresponding to the tonofibrils seen in early studies, extend from the nuclear region and course throughout the cytoplasm in complex sinusoidal-like arrays. They impact the cell periphery at specialized junctions termed desmosomes. In this way, the keratin IF play an essential mechanico-structural role in the cell (Goldman and Steinert, 1990). Because of their apparent continuity into neighboring cells in three dimensions by way of the desmosome junctions, keratin IF play an essential role in the mechanical integrity in an entire epithelium.

Other types of IF may have similar structural functions. For example, neurofilaments are abundant components of axons, where together with microtubules, they serve as major structural components of axons. They are also involved in the movement of macromolecules along the axon, a process termed axonal transport (Liem, 1993). Nestin functions as a structural precursor of neurofilaments in neuroectodermal stem cells (Lendahl *et al.*, 1990). Proteins such as vimentin and desmin are thought to be involved in stabilization or maintenance of cell phenotype during differentiation (Fuchs and Weber, 1994). Nuclear lamins form a mesh-like scaffold along the nuclear membrane, are connected to nuclear pores and perhaps the cytoplasmic IF, appear to attach chromatin, and thus may play a role in gene expression (Gerace, 1986).

Intermediate filament associated proteins

In addition, other quantitatively minor proteins are involved in maintenance of IF supramolecular organization in cells. These are collectively termed IF associated proteins, and have been classified in terms of size and function (Steinert and Roop, 1988; Goldman and Steinert, 1990; Parry and Steinert, 1995). Some are involved in the tight bundling of IF such as filaggrin in the epidermis or certain high sulfur proteins of hair. Others are involved in the association of IF at the cell peripheries, such as desmoplakin and plectin of desmosomes in many cell types, or loricrin in the epidermis.

Intermediate filaments are highly dynamic structures

However, these concepts that IF serve only to provide support the nucleus and provide tensile strength to the cell are incomplete. A variety of recent studies has shown that IF are highly dynamic as well. Nuclear lamins are reversibly disassembled during the onset of mitosis and then reassembled at metaphase, by processes which are tightly controlled by cycles of phosphorylation and then dephosphorylation, respectively (Chou *et al.*, 1990; Peter *et al.*, 1990; Goldman *et al.*, 1992). Many cells disassemble/reassemble their vimentin IF networks coincident with mitosis in the same way. Phosphorylation at specific sites on the amino-terminal end domain causes IF disassembly, and spontaneous reassembly on dephosphorylation. However, most epithelial cells do not disassemble their keratin IF, and in this case, the IF are retained largely intact until cytokinesis, at which time they are split into two (Jones *et al.*, 1982). In postmitotic terminally differentiating epithelia, the IF networks undergo an extraordinary degree of protein

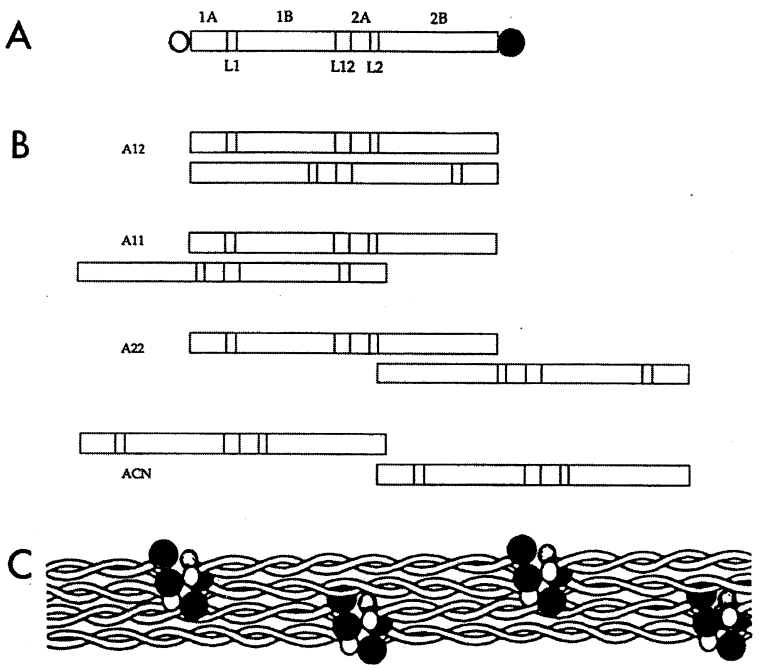

Figure 1. Models of the hierarchical structure of IF. **A.** Generic model of an IF chain showing the four α-helical rod domain segments interspersed by non-α-helical linkers. The end domains are shown as open and larger closed circles to denote polarity, but their structures are not known. **B.** Four generic alignments of pairs of molecules within IF. The exact dimensions of overlap differ slightly in different types of IF. The three antiparallel alignments indicated the existence of a fourth alignment (ACN) for parallel molecules in the same row. Note however that in this case there is a slight overlap of their ends. Almost all mutations known to cause keratin diseases occur in or very near to these overlapping sequences. **C.** Using these rules it is possible to construct a two-dimensional map of an IF. This drawing shows two strands of molecules which are thought to form a protofibril. We think that intact IF are polymorphic because they can contain from three to six such protofibrils. However, the details of how this two-dimensional map folds in three dimensions is not yet known. Again, current views suggest that the rod domains pack to form a central core of the IF of about 8-10 nm in diameter, from which most of the end domains protrude onto the surface, to give a total IF diameter of 10-15 nm. This model allows for the dynamic exchange of molecules known to occur in living cells, simply by insertions or deletions of molecules into the rows.

exchange between intact IF and soluble pools of keratins. This exchange occurs at innumerable sites along the entire length of the IF (Miller *et al.*, 1991; Vikstrom *et al.*, 1992). Therefore this process is fundamentally different from microtubule and microfilament assembly/disassembly which is highly polar instead. Similar protein exchange also occurs in vimentin IF (Vikstrom *et al.*, 1989) in fibroblasts and other cell types, and neuronal IF (Angelides *et al.*, 1989; Okabe *et al.*, 1993). Again, these processes are controlled by phosphorylation since phosphatase inhibitors such as okadaic acid which poison the reverse reaction promote massive IF network disassembly into soluble pools of hyperphosphorylated protein (Steinert, 1988; Lee *et al.*, 1992; Kartasova *et al.*, 1993). Altogether, present data show that most IF are highly dynamic structures (Steinert and Liem, 1990) exchanging protein along their length in response to different phases of the cell cycle, cell movement, differentiation, etc.

Hierarchical structure of intermediate filaments

Organization of IF protein chains

As mentioned, all IF chains contain a central rod domain of conserved structure. This consists of four defined α-helical segments (1A, 1B, 2A, 2B) interspersed by non-α-helical linkers (L1, L12, L2) (Figure 1A). These possess a repeating heptad sequence motif $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$ characteristic of proteins that can form a coiled-coil α-helix. The 1B segment of all lamin and many invertebrate IF chains is exactly 42 residues (6 heptads) longer than that of vertebrate cytoplasmic IF chains, which supports the idea that vertebrate IFs arose during evolution from a lamin precursor. The exact sequences of these segments define the five sequence types: members of a given type show ≥80% sequence homology, while those of different types are usually ≤50% homologous. All IF chains also possess end domains which are extraordinarily variable in size and sequence, but can be subdivided into several subdomains.

Coiled-coil α-helical molecules

The first step in IF assembly is the formation of a two-chained molecule in which the two chains align in parallel and axial register so that the adjacent rod domain sequences form a coiled-coil (Parry *et al.*, 1985). In the case of keratins, this is always a type I/type II heterodimer (Hatzfield and Weber, 1990; Steinert, 1990), usually consisting of specific co-expressed pairs of chains characteristic of a given epithelial cell type. In the case of all other IF chains, the molecule is usually a homodimer, so that the entire IF is formed from the single chain, although members within the type III-V families form facultative copolymers, such

as vimentin-desmin hybrids in developing myotubes, and NF-L/NF-M/NF-H copolymers in mature axons. Also type III-type IV hybrid IF can be formed *in vitro* and *in vivo* during development (Liem, 1993).

Higher-order organization of molecules in IF

The second step is the alignment of a pair of molecules in antiparallel in three very specific ways termed A12, A11 and A22 (Figure 1B). These alignments were determined by chemical crosslinking studies (Geisler *et al.*, 1992; Steinert and Parry, 1993; Steinert *et al.*, 1993a,b,c). In addition, the exact relative displacements of the pairs of molecules differ for types I/II, type III and type IV, and type V molecules. Because these alignments are slightly offset with respect to each other, the keratin and type III chains can not co-assemble into IF, but type III and type IV chains can co-assemble both *in vitro* and *in vivo* because their molecular alignments are identical. Arising from the crosslinking studies, a fourth molecular parallel alignment was discovered, termed ACN, in which the end of the 2B segment of one molecule overlaps by about 1 nm, corresponding to approximately 10 amino acid residues, with the beginning of the 1A segment of the next molecule in the same axial row (Steinert *et al.*, 1993a).

These four modes of assembly direct higher orders of assembly of molecules in IF, but the details of how assembly stops at 10-15 nm wide IF are not yet known. However, some principles are clear. First, from quantitative scanning transmission electron microscope studies, it seems the IF can contain up to 16 molecules in cross-section, although this number is highly variable (Steven *et al.*, 1982, 1983; Engel *et al.*, 1985). That is, both IF assembled *in vitro*, and native IF isolated from cells *in vivo*, are polymorphic. Some data obtained from partial disassembly studies, in which IFs were seen to unravel, suggest a hierarchical assembly (Aebi *et al.*, 1983) (Figure 1C). A linear strand of pairs of molecules is thought to form a protofilament. A pair of these forms a protofibril. Several protofibrils, ranging from three to six, then assemble into the complete IF. Current views suggest that the IF consists of a central core of about 10 nm diameter composed largely of the packed rod domains of the molecules. Much of the end domain sequences are thought to protrude from this core to form a brush-like structure. Thus the conserved rod domains pack together in a common way so as to serve as a scaffold to expose the highly variable end domains so they can perform their specific functions in cells. A number of studies have indicated that some portions of the end domains are required for the higher order packing of molecules, since when they are experimentally removed, the rod domains form curious paracrystalline structures instead of IF (Stewart *et al.*, 1989). Also, as mentioned, phosphorylation of the end domains of types III and V IF chains causes disassembly of the IF. Specific sequences located immediately on either side of the rod domains are essential for keratin IF assembly (Steinert and Parry, 1993).

Functions of end domains

To date, largely only anecdotal information is available, based on the particular sequence characteristics of various chains. For example, the sequences of the keratin chains expressed in complex stratified squamous epithelia are rich in glycine residues, often configured as tandem peptide repeats. There has been speculation that these highly flexible sequences are used in these tissues in order to interact with other epithelial cell peripheral structural proteins such as loricrin to coordinate cellular structure (Steinert *et al.*, 1991). The carboxyl-terminal tails of neurofilament chains are characterized by very long lysine/serine rich peptide repeats that are highly phosphorylated in axons by specific phosphatases, and are thought to be important in spatial organization within the axon, and essential for axonal transport (Liem, 1993). Various studies have suggested that the end domains of vimentin and desmin are involved in specific interactions with nuclear lamins, spectrin/ankyrin of the subplasma membrane of many cells, associate with polysomes in the cytoplasm, etc.

Intermediate filaments in diseases

One of the most exciting developments in the study of IF has been the recent discovery that mutations in genes encoding several IF proteins cause serious pathology. Basically, two types of experimental approaches have been used. In one, classic genetic linkage analyses using large multi-generational families have provided strong statistical relationships between disease phenotype and specific chromosomal regions known to encode various IF genes. Subsequent sequencing of patient DNA has then verified the direct causal relationship. In a second approach, DNA constructs bearing severely mutated forms of some IF chains have been inserted into the germ lines of transgenic mice, which produced negative phenotypes reminiscent of human diseases. Again, subsequent sequencing of DNA from human patients has confirmed the causal relationship between the disease and the specific IF gene.

Keratin diseases

Starting from late 1991, a total of seven different diseases are now known to be caused by mutations in several of the keratin genes (reviewed in Fuchs and Weber, 1994; McLean and Lane, 1995; Parry and Steinert, 1995; Richard *et al.*, 1995) (Table 2). Most of

Table 2. Human keratin diseases

Disease Name	Keratin Chain(s)	Tissues Affected
Epidermolysis bullosa simplex	keratins 5, 14	Basal epidermis of all body locations; certain internal stratified squamous epithelial tissues several variants of severity
Epidermolytic hyperkeratosis	keratins 1, 10	Suprabasal epidermis of all body locations several variants of severity
Ichthyosis bullosa of Siemens	keratin 2e	Suprabasal epidermis of all body locations
Pachyonychia congenita I, II and Steatocystoma multiplex	keratins 6a, 16, 17	Many internal epithelia, hair and nail abnormalities
Epidermolytic palmar-plantar keratoderma	keratin 9	Suprabasal epidermis of palms and soles
Non Epidermolytic palmar-plantar keratoderma	keratin 1	Suprabasal epidermis of palms and soles
White Sponge Nevus [Monilethrix	keratins 4, 13 hair keratins?	Buccal mucosa, tongue, softpalate abnormalities Hair abnormalities]

these are autosomal dominant mutations, which means that the abnormal protein product of only one of the two alleles can cause disease. The first keratin IF chain disease discovered was epidermolysis bullosa simplex in which the basal epidermal cells detach from the underlying connective tissue, resulting in variably severe blistered skin with serious if not lethal consequences for the patient, especially in newborn children. Ultrastructurally, the cells become filled with insoluble clumps of keratin proteins instead of the beautifully organized three-dimensional array of keratin IF bundles. It is thought that these disorganized clumps destroy the mechanical integrity of the cell and entire epithelium, resulting in cytolysis, which manifests macroscopically as blisters. Histologically similar findings are apparent in most of the other keratin diseases listed in Table 2. In the majority of cases, the disorder is due to a simple point mutation in a specific keratin gene, resulting in an inappropriate amino acid substitution in the keratin. This change renders the protein non-functional so that it accumulates in the cytoplasm. In addition, the differentiation program of the epithelial cells are disrupted, leading to hyperkeratosis and hyperproliferation. This results in ichthyosis (epidermis), pachyonychia (nails), hypohydrosis (sweat glands), alopecia (hair), etc.

Interestingly, two rare cases of epidermolysis bullosa simplex have been reported in which both alleles of the keratin 14 gene have been ablated; that is, the individual does not express any keratin 14 protein (Rugg *et al.*, 1994; Chan *et al.*, 1994). In both cases, the disease was less severe than in others caused by point mutations of only one allele. Thus the absence of protein produces a milder phenotype than the presence of abnormal protein. However, the possibility that some other keratin may be expressed instead of keratin 14 has not been ruled out.

Of great significance is the fact that most of the inappropriate amino acid substitutions occur in the regions where adjacent protein chains overlap in keratin IF: specifically, the first several amino acids of the 1A segment, the last few amino acids of the 2B segment of the rod domain, a few in the L12 segment, and the H1 subdomain of type II keratin chains (Figure 2). Indeed, numerically, the large majority of mutations identified so far occur in the beginning of the 1A segment. Further, in several diseases, there is a direct correlation between the severity of the disease and the types and exact locations of the substitutions. For example, proline insertions in these three regions always cause severe disease; proline insertions elsewhere in the keratin chains cause milder disease (Letai *et al.*, 1992). Apparently therefore, the overlap sequences are critical determinants of IF structure.

One curious observation which troubled workers for several years is that some cases with remarkably similar phenotypes are not caused by mutations in the keratin IF genes. More recently, an explanation for this has begun to emerge. At least some cases of epidermolysis bullosa simplex are not caused by mutations in keratins 5 or 14, but by mutations in the gene for the IF associated protein plectin (Gache *et al.*, 1996; Eady *et al.*, 1996). This very large protein is believed to be essential for the organization of IF at or

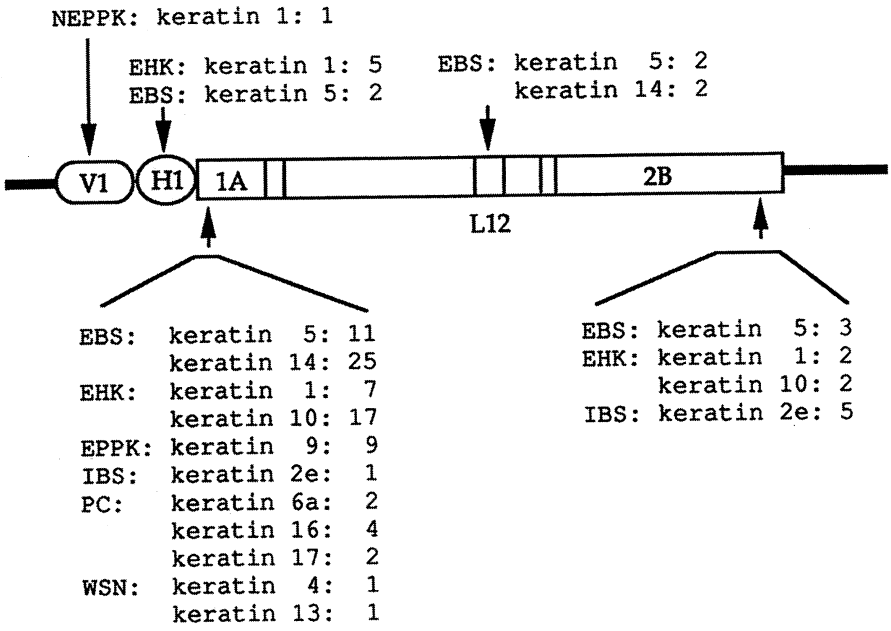

Figure 2. Diagrammatic representation of known mutations in keratin IF diseases. The arrows point to the four regions where mutations have been discovered in the genes encoding the listed keratin chains. Also shown is the number of cases of each mutation reported in the literature (Fuchs and Weber, 1994; Parry and Steinert, 1995; McLean and Lane, 1995; Richard *et al.*, 1995). Note that the type II keratin chains (1 and 5) possess an H1 subdomain immediately adjacent to the beginning of the rod domain. The abbreviations used for the diseases are: EBS, epidermolysis bullosa simplex; EHK, epidermolytic hyperkeratosis; EPPK, epidermolytic palmar-plantar keratoderma; NEPPK, non-epidermolytic palmar-plantar keratoderma; IBS, ichthyosis bullosa of Siemens; PC, pachyonychia congenita (and variants); WSN, white sponge nevus.

near the cell periphery of numerous cell types including muscle and epidermis (Foisner *et al.*, 1987). Future work may show that other cases of some of these diseases are caused by mutations in genes for the various IF associated proteins.

To date, only one disease variant has been attributable to an inappropriate amino acid substitution in the end domain sequence of a keratin chain (Kimonis *et al.*, 1994). This involves the loss of a lysine residue which has been shown to be required for transglutaminase crosslinking of keratin IF to loricrin, a protein component of the cell periphery of epidermal cells (Steinert and Marekov, 1995). In this disease, termed non-epidermolytic palmar-plantar keratoderma, there is no tissue disintegration or blistering, but there is massive hyperkeratosis and papillomatosis limited to the palms and soles, with only slight or mild involvement of other body locations.

In addition, recent genetic linkage work has implicated the type II keratin gene cluster in a hair disease, monilethrix. However, mutations in a hair keratin gene have not yet been described (Healy *et al.*, 1995; Stevens *et al.*, 1996).

Sporadic amyotrophic lateral sclerosis

The pathology of this disease involves progressive degeneration of neurons in the cortex, brain stem and spinal cord, due to the abnormal accumulation of neurofilaments in proximal axons. About 10% of the cases are familial, which are now known to be caused by mutations in the Cu, Zn superoxide dismutase gene (Deng *et al.*, 1993; Rosen *et al.*, 1993). However, about 90% of the cases are sporadic, and are now known to be due to mutations in the repeating sequence regions of the long carboxyl-terminal tail domains of the neurofilament chains. This discovery was made primarily through use of transgenic animal model systems (Côté *et al.*, 1993; Xu *et al.*, 1993; Collard *et al.*, 1995): the overexpression of the NF-L, NF-M and NF-H chains caused impairments in axonal diameter, and axonal transport, with symptoms reminiscent of the human diseases (Heins *et al.*, 1993; Wong *et al.*, 1995). Sequencing of human patient DNA confirmed these observations (Figliewicz *et al.*, 1994; Lee *et al.*, 1994).

Altogether, these IF disease studies suggest that mutations in different domains of the IF chains may cause quite different types of diseases. Mutations in rod domain sequences promote failure of IF *assembly* with resultant disruption of cell and tissue integrity, leading to blistering. Mutations in the end domain sequences apparently allow normal IF assembly, but may affect the subsequent *function* or *supramolecular organization* of IF in cells.

Other animal disease models

The use of transgenic technology has enabled construction of mice in which a particular gene has been ablated or "knocked out". Some IF genes have now been investigated this way. Surprisingly, the vimentin knockout mouse is relatively normal; that is, it seems to grow normally, breeds and has no obviously negative phenotype (Colucci-Guyon *et al.*, 1994). However, more careful analyses will be necessary to eliminate the likelihood of more subtle changes. It

should be pointed out that these experimental animals live in a highly artificial environment, very different from wild conditions. It could be argued that if vimentin confers only a modest survival advantage to the wild animal, evolution long ago would have selected in favor of its expression. On the other hand, the desmin knockout mouse has serious neuromuscular and cardiovascular deficiencies which limit its survival (Li *et al.*, 1994). These animals may serve as useful models for certain human cardiac and muscular diseases. The knockout of the keratin 8 gene leads to the death of the embryo (Baribault *et al.*, 1993), thus indicating the critical roles of this keratin, and presumably its keratin 18 partner, during development.

Future directions

Of course the major benefit of the large body of expression, structure and recent disease work on IF and related proteins will have enormous impact on studies of human diseases. For those IF diseases for which the causative gene(s) have already been identified, prenatal diagnosis is now a reality (Rothnagel *et al.*, 1994). Much work now will be focused on strategies for gene therapy, which raises a large number of technical, social and ethical questions for the future. Thus IF remain an interesting and important genre of molecular and cellular biology.

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