Intermediate filaments at a glance

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Journal of Cell Science 114, 4345-4347 (2001) © The Company of Biologists Ltd

Eukaryotic cells feature two ubiquitous fibrous cytoskeletal polymers in their cytoplasm: F-actin and microtubules. A third fibrous polymer, intermediate filaments (IFs), appeared more recently in evolution (Fuchs and Cleveland, 1998; Erber et al., 1998). Two common traits define members of the family of IF proteins. First, these proteins exhibit a characteristic tripartite domain organization, a generic version of which is shown in the poster. A centrally located domain of fixed length (either ~310 or ~352 residues) is dominated by α-helical segments featuring long-range heptad repeats of hydrophobic/apolar residues (subdomains 1A, 1B, 2A and 2B). This rod domain features highly conserved signature motifs at its N- and C-terminal extremities (shown as magenta boxes). It is flanked by non-helical head and tail domains, which vary considerably in length and primary structure and contribute significantly to the impressive heterogeneity encountered within this family. Second, IF proteins can self-assemble into cytoskeletal filaments, which usually appear as homogeneous, apolar fibers that have a 10-12 nm diameter upon visualization by negative staining and electron microscopy. Examples of keratin and vimentin filament assemblies reconstituted from purified recombinant proteins are shown in the poster at high and low magnification, respectively. The central rod domain represents a major driving force during the self-assembly of all IF proteins. In addition to contributing to assembly (albeit in an IF-protein-specific fashion), the end domains are important sites of regulation and interaction with other cellular elements. A minimum of 67 functional genes encode IF proteins in the human genome (see accompanying table) (Hesse et al., 2001) and, generally speaking, orthologous genes exhibit highly conserved sequences and regulation in mammals and other higher vertebrates.

IF genes can be classified according to various criteria (Hermann and Aebi, 2000), and this is exemplified in the accompanying table. Genomic structure

Journal of Cell Science

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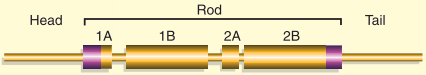
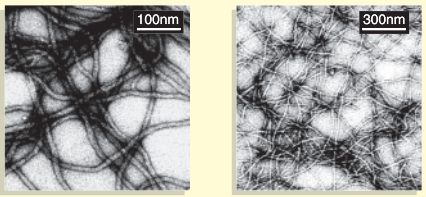
1. Classification of IF genes and proteins

Name	*No. of gene(s)*	*Sequence type*	*Assembly group*	*Pred. prot size (kDA)*	*Distribution (prototypical)*
Keratin (acidic)	>25	I	A	40-64	Epithelia
Keratin (basic)	>25	II	A	52-68	Epithelia
Vimentin	1	III	B	55	Heterogeneous
Desmin	1	III	B	53	Muscle
GFAP	1	III	B	50-52	Astrocytes/glia
Peripherin	1	III	B	54	PNS neurons
Syncoilin	1	III/IV (?)	B	54	Muscle
NF-L	1	IV	B	62	CNS neurons
NF-M	1	IV	B	102	CNS neurons
NF-H	1	IV	B	110	CNS neurons
α-Internexin	1	IV	B	66	CNS neurons
Lamin A/C	1	V	C	72/62	Nucleus
Lamin β1	1	V	C	65	Nucleus
Lamin β2	1	V	C	78	Nucleus
Nestin	1	VI (?)	B	240	Heterogeneous
Synemin	1	VI (?)	B	182	Muscle
Desmuslin	1	VI (?)	B	140	Muscle
Phakinin/CP49	1	Orphan	D	46	Lens
Filensin	1	Orphan	D	83	Lens

2. Genetic secondary structure of IF proteins

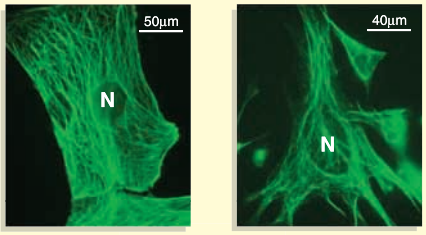


3. Ultrastructure of IFs reconstituted in vitro



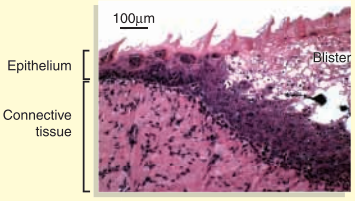
Keratin 5-14 co-polymer — Vimentin homopolymer

4. Organization of cytoplasmic IFs in cells



Epidermal keratinocyte — Skin fibroblast

5. Function of keratin IF networks in epithelia



Dorsal tongue epithelium from a K6-null mouse

(See poster insert)

and nucleotide sequence homology throughout the rod domain define six major types (I-VI). At the protein level, polymerization properties define four assembly groups (A-D). Type I and type II IF genes encode the 'acidic' and 'basic' keratins, respectively, which co-polymerize in an exclusive and obligatory fashion (assembly group A) to give rise to the IFs present in the cytoplasm of all epithelial cells. Type I and type II genes are regulated in a pairwise, tissue- and differentiation-specific fashion in epithelial tissues. Assembly group B includes proteins encoded by type III, type IV and type VI IF genes, which encode cytoplasmic proteins. The prototypic type III gene, vimentin, is expressed in a plethora of non-epithelial cell types, and its product can homopolymerize to form 10-nm filaments in vitro as well as in vivo. Few other proteins in assembly group B are capable of this feat; most require the involvement of another 'group B' sequence, often vimentin, for proper assembly. Many of the type III and type VI genes are expressed in muscle cells. Notable exceptions are GFAP (glial cells), peripherin (peripheral neurons) and nestin (many types of progenitor cells and mesenchyme derived cells). Type IV sequences, which consist of the neurofilament triplet proteins (NF-Light, NF-Medium and HF-Heavy) and α-internexin, are expressed in neurons. In common with keratins, neurofilaments are strict obligatory heteropolymers, the L, M and H subunits interacting with a 5:3:1 molar ratio (Fuchs and Cleveland, 1998). As is the case for NF-M and particularly NF-H, type VI sequences have the distinction of containing unusually long non-helical tail domains (Steinert et al., 1999; Coulombe et al., 2000).

Type V IF genes encode the lamins, which form the meshwork of filaments located in the nuclear lamina. Lamin proteins have additional sequence motifs, including six extra heptad repeats within subdomain 1B, and a nuclear localization sequence (NLS), a chromatin-binding site and a CAAX isoprenylation signal sequence in the tail domain (Wilson et al., 2001). Although synthesized in the cytoplasm, lamin proteins cannot co-polymerize with other IF proteins, and thus they form assembly group C. Convincing evidence indicates that the ancestral IF gene was lamin-like in its features (Erber et al., 1998). Indeed, all the cytoplasmic IF sequences discovered so far in invertebrate species have six extra heptad repeats within subdomain 1B, and many show homology to the lamin tail domain. Moreover, the *Drosophila melanogaster* genome contains a lamin gene but no cytoplasmic IF gene (Wilson et al., 2001). Finally, phakinin/CP49 and filensin are the building blocks of the cytoplasmic beaded filaments characteristic of the eye lens - they defy classification in many respects and form their own assembly group (Herrmann and Aebi, 2001).

The cell-type-specific regulation of cytoplasmic IF genes is often accompanied by a specific organization of filaments. For instance, many epithelial cells feature a pan-cytoplasmic network in which filaments are attached at the surface of the nucleus (or run close to it; see 'N' in the micrograph depicting an epidermal keratinocyte) and radiate towards the periphery, where they typically are anchored at desmosome and hemidesmosome adhesion sites. In fibroblasts in primary culture, a vimentin IF network typically extends towards the cytoplasmic periphery as well but does not fully populate the cytoplasm and is not attached at cell-cell adhesion sites (see micrograph). There are many instances, however, in which IF proteins localize to a discrete compartment of a given differentiated cell type - desmin, for example, along with synemin and nestin is enriched at the Z-line in differentiated muscle cells. As in the case of F-actin and microtubules, the IF organization in situ is a highly regulated and dynamic phenomenon that is controlled by accessory proteins (Coulombe et al., 2000) and post-translational modifications (Omary et al., 1998). Organization is undoubtedly a major determinant of the function of IF polymers in vivo.

All major types of IF polymer have been implicated as mechanical scaffolds in differentiated cell types in situ. Defects in IF polymer structure or organization engender fragility states that translate into loss of cellular integrity following exposure to shearing forces (Fuchs and Cleveland, 1998). As illustrated in the poster, this occurs in the dorsal tongue epithelium of K6-null neonatal mice once they start suckling from the mother (Wong et al., 2000). Inherited mutations affecting the primary structure of IF proteins are responsible for a vast number of typically rare, usually dominantly inherited, disease conditions that arise from cell-fragility states (Fuchs and Cleveland, 1998; Irvine and McLean, 1999; Wilson et al., 2001). The majority of these genetic lesions are missense mutations affecting highly conserved residues at either the N- or the C-terminus of the central rod domain (Irvine and McLean, 1999). Recent evidence indicates that a hot-spot mutation affecting type I keratin sequences alters the micromechanical properties of keratin filament assemblies (Ma et al., 2001).

There is also emerging evidence that IF proteins play additional roles, which are not only cell type specific but also context dependent. Examples include the contribution of neurofilaments to the radial growth of neurons (Fuchs and Cleveland, 1998), the role of keratins in affording protection against chemical stress (Ku et al., 2001) and promoting a specific epithelial cytoarchitecture (Herrmann and Aebi, 2000; Coulombe et al., 2000), and the role of lamins during nuclear envelope assembly (Wilson et al., 2001; Lopez-Soler et al., 2001). Continuing studies involving gene manipulation in mice (Magin, 1998) and other model organisms, such as *Ceanorhabditis elegans* (Karabinos et al., 2001), should provide insights into the function of IF polymers and the significance of IF sequence diversity.

We apologize to our colleagues in the field for only referring to recent articles. We are grateful to the National Institutes of Arthritis, Musculoskeletal and Skin Diseases (NIAMS, NIH, Bethesda, MD, USA) for grant support.

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