INTERMEDIATE FILAMENTS

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INTERMEDIATE FILAMENTS

The second of the three major cytoskeletal elements **intermediate filaments (IFs)** are seen in electron microscope as solid, unbranched filaments with a diameter of 10–12 nm.

To date, intermediate filaments have only been identified in animal cells.

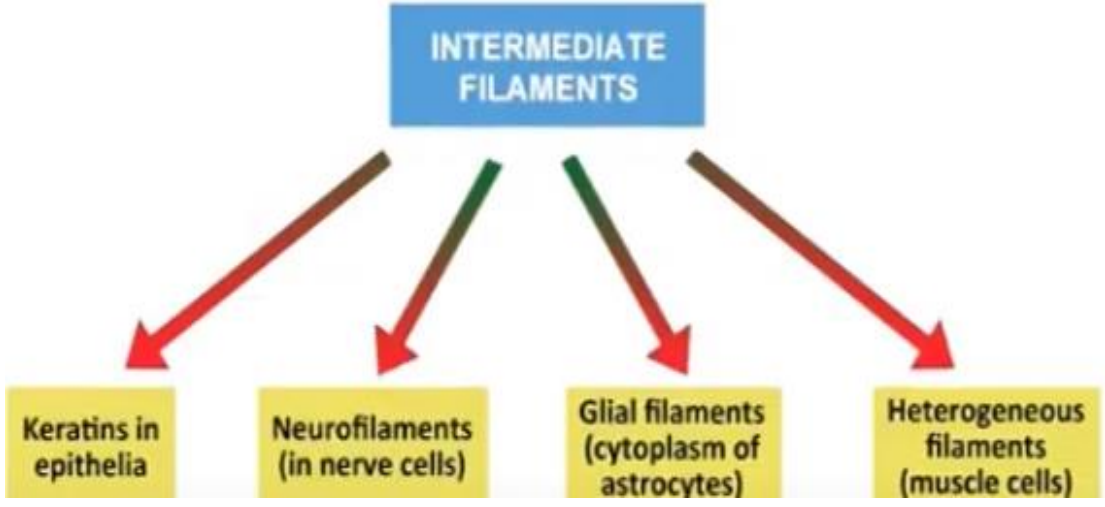
Intermediate filaments are strong, flexible ropelike fibers that provide mechanical strength to cells that are subjected to physical stress, including neurons, muscle cells, and the epithelial cells that line the body's cavities.

Unlike microfilaments and microtubules, IFs are a chemically heterogeneous group of structures that, in humans, are encoded by approximately 70 different genes.

The polypeptide subunits of IFs can be divided into 5 major classes based on the type of cell in which they are found as well as biochemical, genetic, and immunologic criteria.

Classes I-IV, which are found in the construction of cytoplasmic filaments, and consider type V IFs (the lamins), which are present as part of the inner lining of the nucleus.

Unlike the actin and tubulin isoforms, the various classes of IF proteins are widely divergent in sequence and vary greatly in molecular weight.

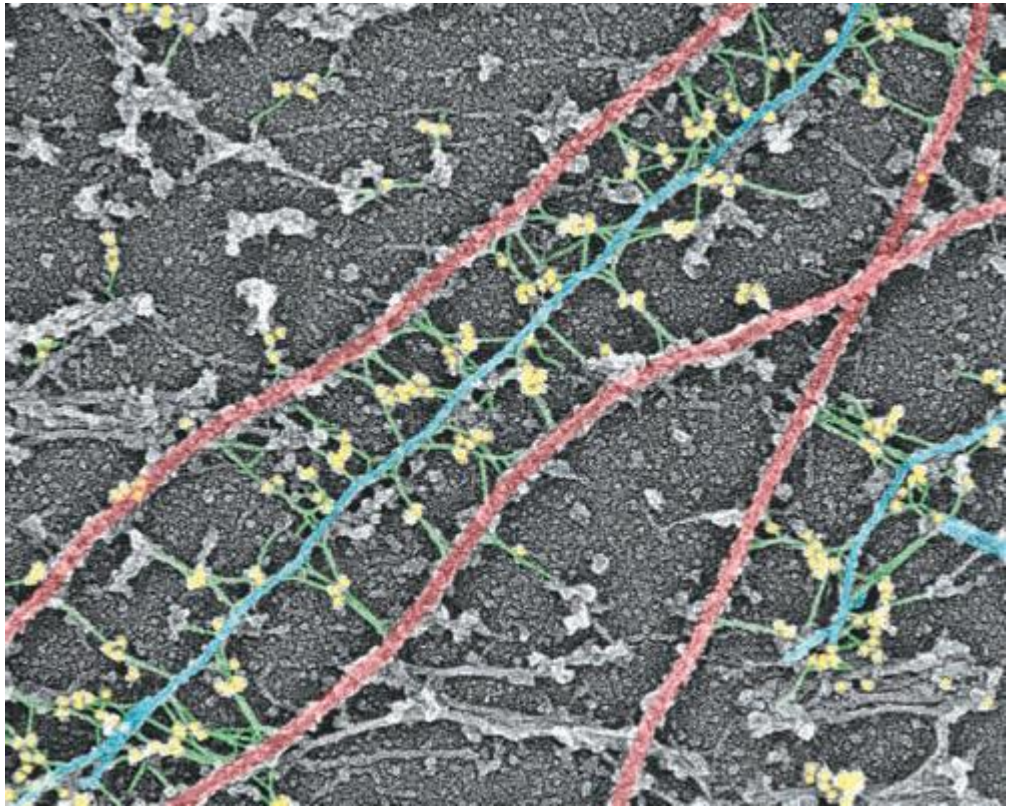


Properties and distribution of major mammalian Intermediate Filament proteins

Different kinds of epithelia use different keratins to build up their Intermediate filaments .

Over 20 different kinds of keratins have been found.

IF protein	Sequence type	Primary tissue distribution
Keratin (acidic) (28 different polypeptides)	I	Epithelia
Keratin (basic) (26 different polypeptides)	II	Epithelia
Vimentin	III	Mesenchymal cells
Desmin	III	Muscle
Glial fibrillary acidic protein (GFAP)	III	Astrocytes
Peripherin	III	Peripheral neurons
Neurofilament proteins		Neurons of central and peripheral nerves
NF-L	IV	
NF-M	IV	
NF-H	IV	
Nestin	IV	Neuroepithelial
Lamin proteins		All cell types (Nuclear envelopes)
Lamin A	V	
Lamin B	V	
Lamin C	V	



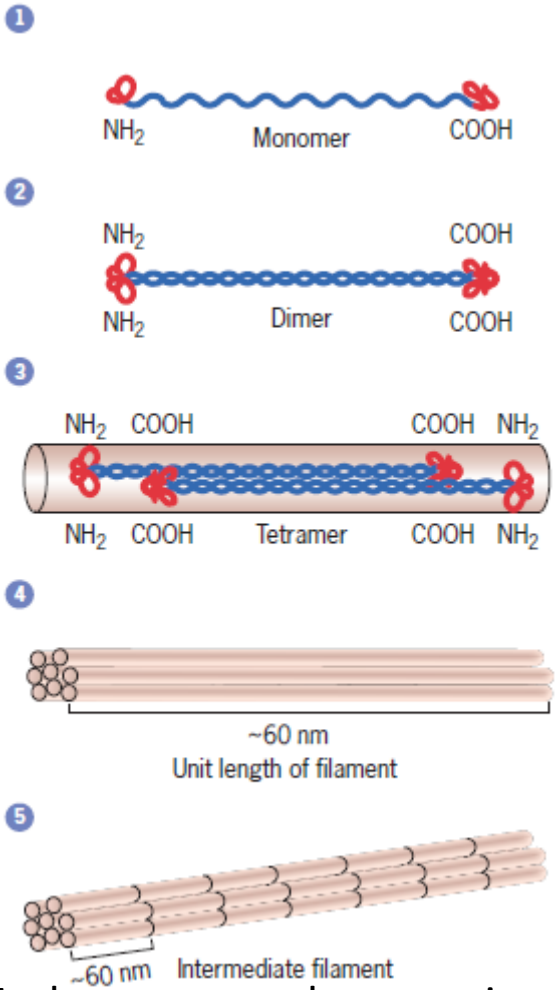
IFs radiate through the cytoplasm of a wide variety of animal cells and are often interconnected to other cytoskeletal filaments by thin, wispy cross-bridges.

In many cells, these cross-bridges consist of an elongated dimeric protein called ***plectin*** *that can exist in numerous isoforms. Each* plectin molecule has a binding site for an intermediate filament at one end and, depending on the isoform, a binding site for another intermediate filament, microfilament, or microtubule at the other end.

•Although IF polypeptides have diverse amino acid sequences, all have **similar structural organization** and form similar-filaments.

•The polypeptides of IFs all contain a ***central, rod-shaped, α-helical domain*** of similar length and homologous amino acid sequence. This long fibrous domain makes the subunits of intermediate filaments very different from the globular tubulin and actin subunits of microtubules and microfilaments. The central fibrous domain is flanked on each side by globular domains of variable size and sequence (step 1).

•Two such polypeptides spontaneously interact as their α-helical rods wrap around each other to form a ***ropelike dimer*** approximately 45 nm in length (step 2). Because the two polypeptides are aligned *parallel* to one another in the same orientation, the dimer has polarity, with one end defined by the C-termini of polypeptides and opposite end by their N-termini.



A model of intermediate filament assembly and architecture. Each monomer has a pair of globular terminal domains (red) separated by a long α-helical region (step 1). Pairs of monomers associate in parallel orientation with their ends aligned to form dimers (step 2). Depending on the type of IF, the dimers may be composed of identical monomers (homodimers) / nonidentical monomers (heterodimers). Dimers in turn associate in antiparallel, staggered fashion to form tetramers (step 3), thought to be the basic subunit in the assembly of IF. 8 tetramers associate laterally to form a unit length of the IF (step 4). Highly elongated IF are then formed from end-to-end association of these unit lengths (step 5).

Intermediate Filament Assembly and Disassembly

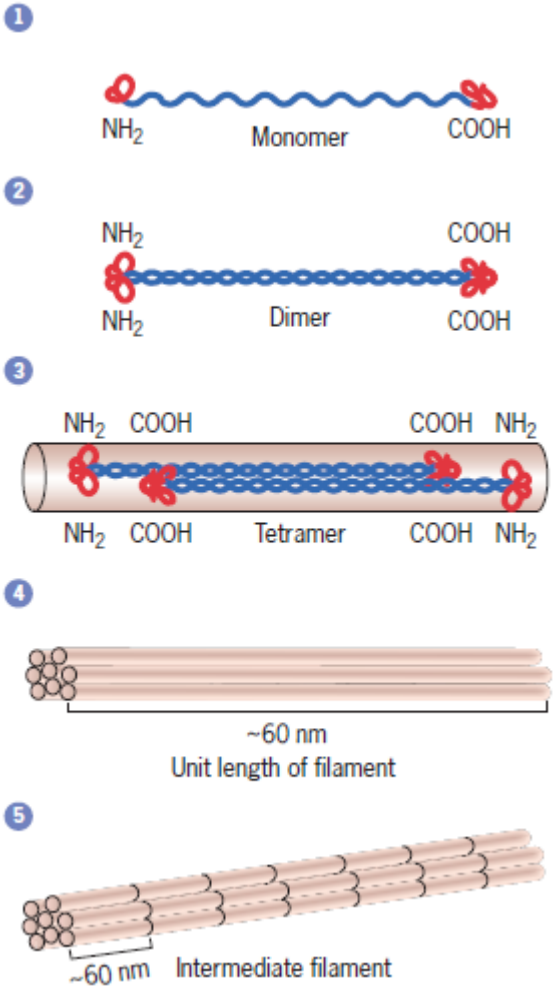
The basic building block of IF assembly is thought to be a *rodlike tetramer formed by 2 dimers* that become aligned side by side in a staggered fashion with their N- and C-termini pointing in opposite (antiparallel) directions (step 3).

Because the dimers point in opposite directions, the tetramer itself lacks polarity. *8 tetramers associate with one another in a side-by-side (lateral) arrangement* to form a filament that is one unit in length (about 60 nm) (step 4).

Subsequent growth of the polymer is accomplished as these unit lengths of filaments associate with one another in an end-to-end fashion to form the highly elongated intermediate filament (step 5).

None of these assembly steps is thought to require the direct involvement of either ATP or GTP.

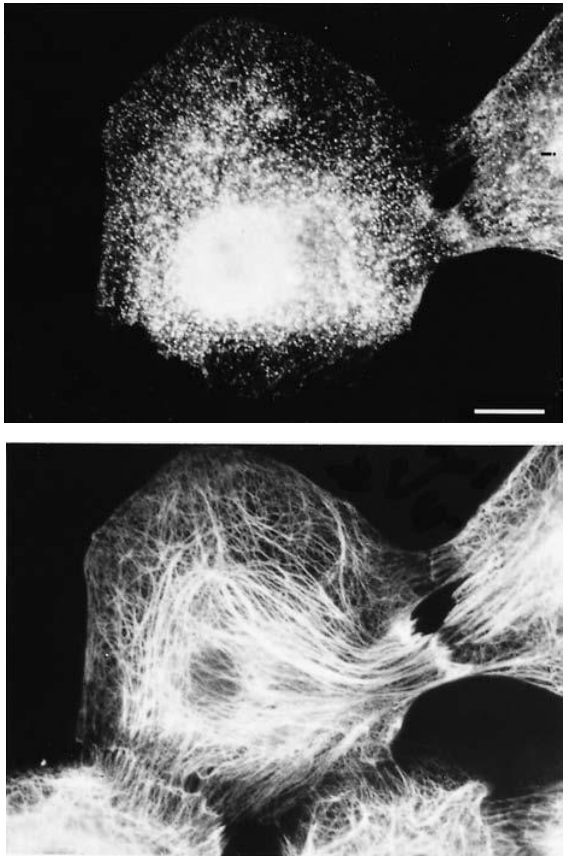
Because the tetrameric building blocks lack polarity, so too does the assembled filament, which is another feature that distinguishes IFs from other cytoskeletal elements.



Intermediate filaments tend to be less sensitive to chemical agents than other types of cytoskeletal elements and more difficult to solubilize. Because of their insolubility, IFs were initially thought to be permanent, unchanging structures, so it came as a surprise to find that they behave dynamically in vivo.

When labeled keratin subunits are injected into cultured skin cells, they are rapidly incorporated into existing IFs. Surprisingly, the subunits are not incorporated at the ends of the filament, as might have been expected by analogy with microtubule and microfilament assembly, but rather into the filament's interior. The results depicted here might reflect the exchange of unit lengths of filament (as shown in step 4) directly into an existing IF network.

Unlike the other two major cytoskeletal elements, assembly and disassembly of IFs are controlled primarily by phosphorylation and dephosphorylation of the subunits. For example, phosphorylation of vimentin filaments by protein kinase A leads to their disassembly.



Types and Functions of Intermediate Filaments

•Keratin filaments constitute the primary structural proteins of epithelial cells (including epidermal cells, liver hepatocytes, and pancreatic acinar cells). Figure (last page) *shows a schematic* view of the spatial arrangement of the keratin filaments of generalized epithelial cell.

•Keratin-containing IFs radiate through the cytoplasm, tethered to the nuclear envelope in the center of the cell and anchored at the outer edge of the cell by connections to the cytoplasmic plaques of desmosomes and hemidesmosomes. T*he interconnections* between IFs and the cell's microtubules and microfilaments transform these otherwise separate elements into an integrated cytoskeleton. Because of these various physical connections, the ***IF network is able to serve as a scaffold for organizing and maintaining cellular architecture and for absorbing mechanical stresses applied by the extracellular environment***.

•The cytoplasm of neurons contains loosely packed bundles of IFs whose long axes are oriented parallel to that of the nerve cell axon*. These* IFs, or neurofilaments, are composed of 3 distinct proteins: NF-L, NF-H, and NF-M, all of the type IV group. Unlike the polypeptides of other IFs, NF-H and NF-M have sidearms that project outward from the neurofilament. These sidearms are thought to maintain the proper spacing between the parallel neurofilaments of the axon (see Figure 9.13*b). In the early stages of differentiation* when the axon is growing toward a target cell, it contains very few neurofilaments but large numbers of supporting microtubules. Once the nerve cell has become fully extended, it becomes filled with neurofilaments that provide support as the axon increases dramatically in diameter.

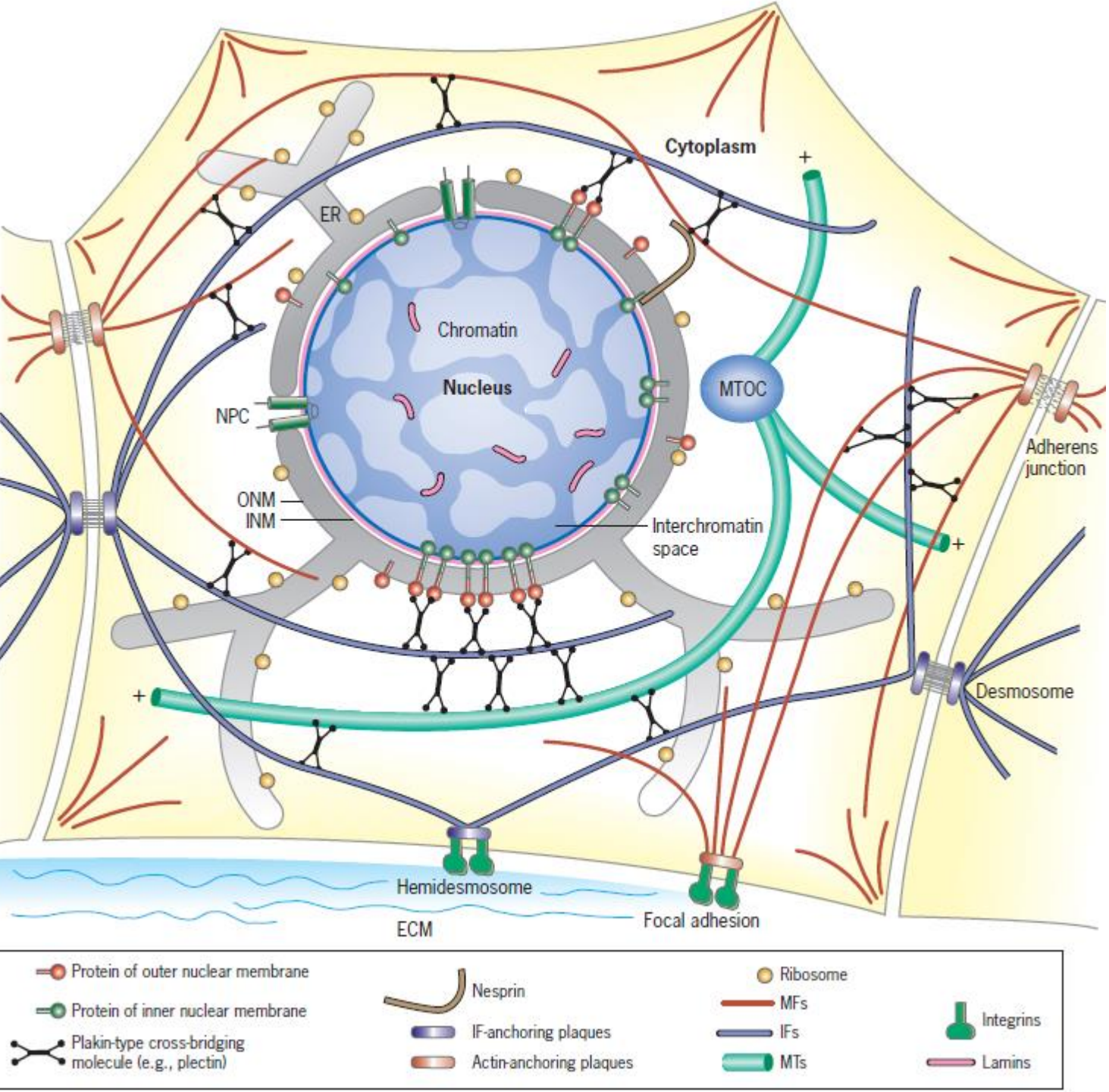
•Aggregation of NFs is seen in several human neurodegenerative disorders, including ALS and Parkinson's disease. These NF aggregates may block axonal transport, leading to the death of affected neurons.

•IFs play major role in imparting mechanical strength to cells situated in epithelial layers.

•Desmin plays a key structural role in maintaining the alignment of the myofibrils of a •muscle cell, and the absence of these IFs makes the cells extremely fragile. An inherited human disease, named *desminrelated myopathy, is caused by mutations in the gene that* encodes desmin. Persons with this disorder suffer from skeletal muscle weakness, cardiac arrhythmias, and eventual congestive heart failure.

•Not all IF polypeptides have such essential functions. For example, mice that lack the vimentin gene, which is expressed in fibroblasts, macrophages, and white blood cells, show relatively minor abnormalities, even though the affected cells lack cytoplasmic IFs.

•It is evident from these studies that IFs have tissue-specific functions, which are more important in some cells than in others.



The organization of IF in an epithelial cell. IFs radiate throughout cell, being anchored at both outer surface of nucleus and inner surface of PM. Connections to the nucleus are made via proteins that span both membranes of the nuclear envelope and to PM via specialized sites of adhesion such as desmosomes and hemidesmosomes. IFs are also interconnected to both other types of cytoskeletal Fibers (MT and MF) by members of plakin family of proteins, such as the dimeric plectin molecule