Structure of Intermediate Filaments

Chang-Hun Lee

Department of Biological Chemistry, Johns Hopkins University, School of Medicine
To whom correspondence should be addressed. E-mail clee75@jhmi.edu

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1. Introduction

As their names indicate, cytoskeletons have important roles of mechanical supports and scaffolds to maintain structure and function of cells, tissues and organisms. In metazoan animal cells, intermediate filaments form complex networks like other cytoskeletal elements: microtubules and microfilaments. The name ‘intermediate filament’ is derived from the fact that the diameter of intermediate filament (8–12 nm) is just intermediate between the size of microtubule (~25 nm) and that of microfilament (5–8 nm). The main function of intermediate filaments is believed to provide cells and tissues with viscoelastic resistance to mechanical stress caused by external forces or internal processes, e.g., cell migration. Mouse genetic models and patients with intermediate filament-related diseases such as skin blistering disorders, muscle dystrophies and laminopathies have showed that defective intermediate filaments cannot maintain the structural integrity of cells and tissues upon mechanical trauma.

Intermediate filament proteins are composed of a huge multigene family. Each family has unique functions and is expressed in specific regions (Table 1). For example, keratins (type I and type II intermediate filament proteins) are expressed in epithelial cells whereas vimentin (type III) is expressed in mesenchymal cells. Desmin, another type III protein, is expressed only in muscle cytoplasm while lamins are found in nuclear membranes of all cell types. Accordingly, mutations in keratins can cause skin blistering diseases, and abnormal desmin can promote muscle dystrophy. With unknown mechanism, lamin mutations are suspected of causative factors for accelerated aging disease, Hutchison–Gilford progeria syndrome, where abnormal shapes of nuclei in patients have been reported. Differential expression pattern can also be seen even within a same tissue, skin, for instance. At the basal layer of skin epithelia, mitotically active keratinocytes produce K5/K14 keratin pairs. As the cells differentiate, K1/K10 pairs replace pre-existing keratin networks of K5 and K14. When the skin is wounded, however, K6, K16 and K17 proteins are expressed until the wound is healed. The context-dependent expression of intermediate filament proteins suggests that each intermediate filament protein has a specific function on its demand. Coulombe and other pioneers of the field found that intermediate filament proteins are required for many cell life cycle steps other than structural...
support function; including cell growth and cell death\textsuperscript{6-12}. Newly uncovered functions of intermediate filament expand our knowledge on the physiological roles of intermediate filament proteins beyond mechanical function. Despite the growing interests on them, structural information of intermediate filament proteins is sparse. Some good reviews cover the topics of assembly mechanisms and related molecular structures of intermediate filaments\textsuperscript{13,14}. This paper focuses on technical aspects of structural studies and related achievements on the intermediate filaments.

### Table 1. Expression locations and related diseases of intermediate filaments

<table>
<thead>
<tr>
<th>Type</th>
<th>Protein names</th>
<th>Number of genes</th>
<th>Distribution</th>
<th>Related diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>I II</td>
<td>Keratins (acidic)</td>
<td>&gt;50</td>
<td>Epithelia</td>
<td>Epidermolysis bullosa simplex, Epidermolytic hyperkeratosis, Ichthyosis bullosa of Siemens, Inflammatory bowel disease, Loose–anagen syndrome, Meesman corneal dystrophy, Monilethrix, Oral white–sponge nevus, Pachyonychia congenita, Palmoplantar keratoderma, Pseudofolliculitis barbae, Steatocystoma multiplex, Chronic pancreatitis, Cirrhosis and hepatitis</td>
</tr>
<tr>
<td>III</td>
<td>Vimentin</td>
<td>1</td>
<td>Fibroblast, endothelia, leukocytes</td>
<td>Dilated cardiomyopathy, type II, Desmin–related myopathy</td>
</tr>
<tr>
<td></td>
<td>Desmin</td>
<td>1</td>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GFAP</td>
<td>1</td>
<td>Astrocytes/Glia</td>
<td></td>
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<tr>
<td></td>
<td>Peripherin</td>
<td>1</td>
<td>PNS neurons</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Syncoilin</td>
<td>1</td>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>NF–L</td>
<td>1</td>
<td>CNS neurons</td>
<td>Charcot–Marie–Tooth disease,</td>
</tr>
<tr>
<td>NF-M</td>
<td>1</td>
<td>CNS neurons</td>
<td></td>
<td>types 2E and 1F</td>
</tr>
<tr>
<td>NF-H</td>
<td>1</td>
<td>CNS neurons</td>
<td></td>
<td>Amyotrophic lateral sclerosis</td>
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<tr>
<td>- internex</td>
<td>1</td>
<td>CNS neurons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nestin</td>
<td>1</td>
<td>Heterogeneous</td>
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<td></td>
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<tr>
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<td>1</td>
<td>Muscle</td>
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<td></td>
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<tr>
<td>Desmuslin</td>
<td>1</td>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
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<td>V</td>
<td>Lamin A/C</td>
<td>1</td>
<td>Nucleus (differentiated tissues)</td>
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<td></td>
<td>Lamin B1</td>
<td>1</td>
<td>Nucleus (ubiquitous)</td>
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<tr>
<td></td>
<td>Lamin B2</td>
<td>1</td>
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<tr>
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<td>Phalkinin/C</td>
<td>1</td>
<td>Lens</td>
<td></td>
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<td>Lens</td>
<td></td>
</tr>
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This table was rebuilt from the original tables of two papers [3,12].
2. Microscopic views of intermediate filament networks

Even with macroscopic vision, we can easily see common and representative structures of intermediate filaments: hair, nail and scale on skin. In wool industries and hair cosmetics, strength and beauty of intermediate filaments have been focused on for many decades. Now people know that a simple biochemical treatment, generally called perm, changes disulfide bonds in hair so that it can modify structural alignment of hair filament making curly wave patterns. With a variety of optical microscopes, we can see intermediate filament networks in micrometer scale or nanometer scale. Figure 1 shows an example of intermediate filament network in cells. Immunostaining of its component protein, using a cytokeratin antibody in this case, clearly demonstrated meshwork structure of keratin filaments in a mouse skin keratinocyte. One should note that intermediate filament networks extend from perinuclear region to outer cell periphery area. As Intermediate filaments in cytoplasm are connected to desmosomes and hemidesmosomes at plasma membrane, each cell can build up multi-directional linkages inside and multiple joints with other cells or extracellular membrane components outside simultaneously (Figure 2)\textsuperscript{15-17}. Recent finding of direct binding of Nesprin in outer nuclear membrane with keratins suggests that a cell has continuous internal connection between its nucleus and the extracellular matrix through intermediate filaments\textsuperscript{18}. Along with lamins in nuclear envelope, internal boundaries of cytosol can be defined with the intermediate filament network of the cell.

Electron microscope has been a conventional tool to observe each 8 – 10 nm filament from cells or from a reconstituted protein sample in vitro. Filament assembly process and anomalies caused by mutations were studied by exploiting high resolution power of electron microscopy. Indeed, electron micrographs have provided us interesting details of intermediate filament structures that are not seen with light microscopes. A group of researchers found “ring-core” structure from the transmission electron micrograms of thin sections of fully keratinized fiber (hard keratin like hair)\textsuperscript{19}. X-ray diffraction studies also support the idea that intermediate filaments exhibit tubular shape with hollowed center or low density axis along their longitudinal direction\textsuperscript{20,21}. Recently, Lars Norlen and Ashraf Al-Amoudi used cryo-electron microscopy to observe the native structure of skin keratin filament in situ, and postulated...
cubic rod packing and membrane templating model\textsuperscript{22}. Briefly, filament architecture of cytokeratin network in stratum corneum keratinocytes of skin seems to have cubic–like symmetry, and the symmetric matrices of keratin in cytoplasm can be formed by assistance with cell membranes, not by self-assembly only. If the observed symmetry really reflected molecular architecture of keratin networks in vivo, there might be a restricted rule of defining an angle/distance of the junction point of two filaments. It will be interesting to investigate the biochemical mechanism of inter-filament interaction. The suggested role of cell membrane as a guideline of intermediate filaments symmetry is also an interesting topic in this field. The idea of intervening lipids (cell membrane) into intermediate filament networks has been suggested with different viewpoints\textsuperscript{23,24}. It seems that not only proteins but also lipid molecules are involved in the formation of intermediate filament networks with unknown mechanism. Therefore, studies on the role of lipids in intermediate filaments and their interaction mechanism may be good questions.

Figure 1. One example of intermediate filament networks from skin keratinocytes in various dimensions. (a) immunostaining image of mouse keratinocyte with cytokeratin antibody\textsuperscript{52} (b) electron micrograph of negative staining sample of keratin filaments (c) DIC microscopy of keratin tonofilament \textit{in vitro}. All the figures are generated by the author and are unpublished.
Figure 2. Diagram of keratin filament networks in skin epithelial tissue. Red, pink and purple lines represent K5/K14, K1/K10, and crosslinked K1,K2,K10 networks, respectively.

On the other hand, atomic force microscopy (AFM) technologies have been applied to the intermediate filament field. The resolution of AFM image (several Angstrom) is approaching to atomic level, and information from AFM can reconstitute true three dimensional surface profile of one single molecule. The power of AFM images on several intermediate filament molecules successfully demonstrated super-helical coiled structure with a regular pitch on a given strip of intermediate filament\textsuperscript{25}. Advantages of AFM also include its ability of force measurement on one molecule. This paper will not discuss further, but many good papers describe biophysical force parameters of intermediate filaments, such as remarkable expansibility and flexible reaction upon exterior force\textsuperscript{26-29}.
3. Molecular level structures of intermediate filaments

Intermediate filaments are distinguishable from other cytoskeletal proteins even from the level of monomer. Shape of an intermediate filament monomer is filamentous whereas monomer units of other cytoskeletal proteins (actins or tubulins) are globular. The filamentous building blocks in intermediate filaments create distinctive mechanism of oligomerization and mechanical properties. Assembly or disassembly of filamentous monomer contains complicated dynamics of association and dissociation along with longitudinal motions and lateral movements, which cannot be explained by well-characterized models of microtubules and microfilaments. When external force is applied, microtubules and microfilaments generally undergo hardening process for adaptation. Intermediate filaments, on the other hand, get more elastic to make resilience. Apparently, longitudinal dispersion of applied force along filamentous units makes it possible to buffer the mechanical shock.

Molecular weights of intermediate filament monomers vary with the types from 40 to 80 kDa. Every monomer has tripartite domain structure composed of head, rod, and tail domain. Amino acid sequences of rod domains are highly conserved among intermediate filament family whereas those of head domains and tail domains are variable. Three loop-like structures called linkers (L1, L12 and L2) divide rod domain into four sub-domains called 1A, 1B, 2A, and 2B. Each sub-domain in rod region contains unique heptad repeats (abcdefg), which amino acid residues at ‘a’ position and ‘d’ position have usually hydrophobic residues or complementary charged groups with their paired partners. This ‘4+3’ composition of amphipathy reflects subtle variation of the number of amino acid residues per turn in α-helix (3.4 a.a / turn), therefore, hydrophobic amino acids at ‘a’ or ‘d’ position facing at the same side can drive hydrophobic interaction to form a tilted coiled-coil dimer structure with a defined pitch. The unique features of helices and coiled-coil in intermediate filaments have been got interested to many pioneers of structural biology like Pauling and Crick.

All the intermediate filament proteins have conserved sequence motifs at the tips of rod domain (N-terminal end of 1A and C-terminal end of 2B), and it is believed that those conserved motifs may initiate the coiling of monomer units into a coiled-coil dimer. In fact, mutations in these regions

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coincide with serious pathologies on some of intermediate filament disorders, implying that there may be a sort of correlation between the location of a mutation and severity of the related disease. It turned out that phenotypes of several mutations are not explained simply by the locations of those, possibly due to unknown filament assembly mechanism beyond dimer formation or disrupted interaction with other cellular compartments as mentioned above. However, the position effect of mutations in intermediate filament still gives us important clues of structural information of intermediate filaments.

Steinert, Strelkov, Parry and many researchers in this field have been studied for long time to understand the assembly steps beyond dimer and found that intermediate filaments are formed by dimerization, tetramer formation, and oligomerization with unknown steps. Apolarity of intermediate filament, another big difference with other cytoskeletal proteins, is conferred at the tetramer level from anti-parallel association of two dimers. It is well understood that tetramer is a major form of soluble pool in cytosolic intermediate filaments and that transition stage from tetramer to unknown higher order oligomer is rate determination step of intermediate filament formation. The diameter of intermediate filament suggests that there are 32 monomer units with a given cross-section of one filament unit. Therefore, identifying the stages between tetramer and 32-mer is an interesting topic to understand structure, assembly and mechanism of network formation of intermediate filaments. Also, unexplained mechanisms of disease-related mutations may be understood by studying the oligomerization steps beyond dimer formation.

4. Atomic level structures of intermediate filament proteins

Due to the filamentous structures and tendency to make high order oligomers, studying intermediate filament structures is practically hard with conventional methods of structural biology like X-ray crystallography and NMR spectroscopy. To bypass impediments on it, Steinter, Parry and their colleagues adapt Napoleonic “divide-and-conquer” strategy. From a set of separated domain polypeptides of vimentin, they were able to crystallize 1A domain and 2B domain (Figure 3a) that showed crystal clear coiled coil in high resolution. These structures made the first atomic view of intermediate
filament proteins, even though they had some pitfalls (monomeric 1A and partially truncated 2B domain). Crystal structures of 2B domain of human Lamin A and 2B domain of mouse desmin followed the achievement of first crystallization work.\textsuperscript{45} One of great ripple effects from structural information with atomic resolution is a possibility of homology modeling. By using crystal structure of vimentin 1A domain as a template, the corresponding region of dimer complex with keratin 5 and keratin 14 was modeled to understand mechanism of skin blistering disease called epidermolysis bullosa simplex\textsuperscript{46}. Even though some mutations still could not be explained, the model was able to dissect different potential and distinctive mechanisms of many mutations on keratins.

![Figure 3. Crystal structure of vimentin homo-dimer 2B domain (a) and 1B domain model of keratin 6/keratin 17 hetero-dimer (b). Red coloration on the surface of the molecular model depicts newly identified hydrophobic stripe that is able to stabilize tetramer \textit{in vitro} and to increase turnover rate \textit{in vivo}. The models here were generated from the published coordinate file (PDB: 1GK4) and the model data from author’s work. Displaying models and calculating surface were done using Swiss PDB viewer program or MolMol and POV-ray.](image)

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Even when a crystal structure with high sequence homology is not available, one can run a comparative modeling with a reliable template structure. Researchers in Coulombe lab successfully built models of 1B domain dimer of keratin 6 with keratin 16 or keratin 17 using crystal structure of Cortexillin I, an actin binding protein (PDB: 1D7M)\textsuperscript{47}. Many lines of evidences showed that 1B domain is involved with the tetramer stability of intermediate filaments in vitro and turn over rate in vivo, therefore, this model was intended to study the different potentials of tetramer stabilities of K6/K16 and K6/K17 pairs\textsuperscript{48,49}. Interestingly, unidentified hydrophobic stripe was found on their surfaces from the 1B domain models, and mutant studies proved that this hydrophobic stripe can drive physical interaction of 1B-1B domain during tetramer formation (Figure 3b)\textsuperscript{39}.

Model building was also able to depict the assembly steps beyond tetramer or molecular alignment within intermediate filaments. Recent trials of using modeled structures along with analytical ultra-centrifugation and/or X-ray scattering suggested possible molecular structures of octamer and 32mer\textsuperscript{50}. Those models can be under debate due to their low quality of structures, but it is clear that they were successful to make a new approach for understanding structures of intermediate filaments further.

5. Concluding remarks

As noticed, functions of microtubules and microfilaments can be well understood once their structural information is available. However, our knowledge on the structures of intermediate filaments is still very limited. Crystal structure of a full length intermediate filament protein is currently unavailable. Small portion of filament assembly is understood with many caveats. As new biophysical methods develop, nevertheless, our understanding on structure of intermediate filaments grows much easier and more reliable. For their ability to quantify the population of different size oligomers, light scattering measurement or calorimetric experiments are worth to be applied to study assembly mechanism of intermediate filaments. Electron tomography accompanied with experimentally determined crystal structures will be useful to reconstitute three dimensional images of intermediate filaments, which links between a sub-nanometer structure
(atomic level) and an obtained image with nanometer/micrometer range. Crystal structures of intermediate filament protein domains with identified binding partners will be very useful when non-mechanical function or regulation of intermediate filament proteins is of interest. Finally, understanding filament assembly with structures with fine resolution can provide us with good mechanistic models, and possibly therapeutic strategies, of intermediate filament-related diseases.

6. References


