


**ANAT3231 - Cell Biology
Lecture 5**
School of Medical Sciences
The University of New South Wales



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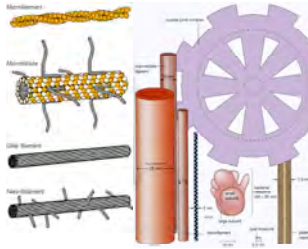
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Lecture 5 Overview

- Cytoskeleton
 - Functions
 - Structure
 - 3 Filament systems
- Intermediate Filaments
 - Function
 - Structure
 - Adhesive junctions
 - Regulation
 - Disorders
- Lecture 5 on the Web
 - <http://cellbiology.med.unsw.edu.au/units/science/lecture0505.htm>



Images: The Cell ch8f2.gif & The World of the Cell

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Cytoskeleton Function

- Cell Shape
- Cell motility
- Cell mechanical strength

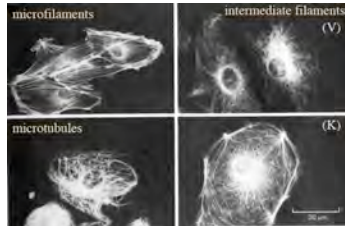
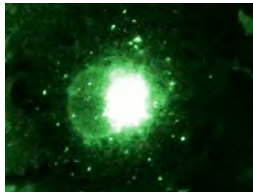


Image: MBoC

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Motility- Intracellular

- organelle movement
- vesicle transport
- mitosis & meiosis
- chromosome segregation
- gene expression
- transcription factor binding
- mRNA transport
- translation
- protein export
- transmitter release



MBoC13_3.mov

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Cellular Motility

- Mitosis & Meiosis
- cleavage, cytokinesis, migration
- Development
- sperm-egg fusion
- formation of primitive layers
- migration of cells
- formation of neural connections

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Cellular Motility

- Mature (somatic)
- wound healing
- blood cells, fibroblasts
- phagocytosis
- macrophages
- killer T cells
- neural regeneration
- process regrowth
- glial migration



Mouse macrophage

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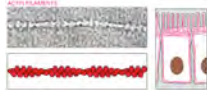
The Cytoskeleton

- Cytoskeleton composed of 3 structural elements
 - Microfilaments (actin)
 - Intermediate filaments (various)
 - Microtubules (tubulin)
- Small number of conserved proteins form filaments
- Several 1000 cytoskeleton-associated proteins
 - regulate cytoskeletal activity
 - Human genome 30,000 genes

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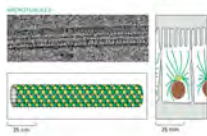
3 Filament Systems

ACTIN FILAMENTS




Actin filaments (also known as microfilaments) are thin filamentous structures of the protein actin. They appear in flexible structures, with a diameter of 5-9 nm, that are important sites of force generation. Microtubules are hollow, with their protofilament ends. Intermediate filaments are intermediate in size with they are most highly conserved in the cytosol, and through the plasma membrane.

MICROTUBULES



Microtubules are long, hollow tubular structures of the protein tubulin. With an outer diameter of 25 nm, they are made mostly of two main structural subunits, α -tubulin and β -tubulin, which are arranged in a protofilament structure. Microtubules are hollow, with their protofilament ends. Intermediate filaments are intermediate in size with they are most highly conserved in the cytosol, and through the plasma membrane.

INTERMEDIATE FILAMENTS

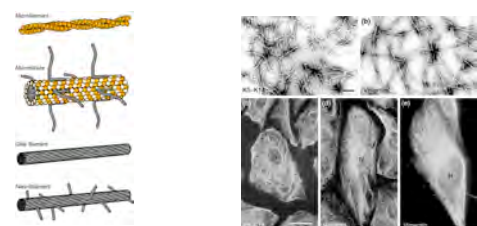


Intermediate filaments are rope-like structures with a diameter of around 10 nm. They are made of intermediate filament proteins, which assemble in a rope and fibrous structure. The rope of intermediate filament forms a filamentous lattice that is attached to the nucleus and beneath the cell surface membrane. Other types provide stress and contractility, and support the cell.

MBoC Figure 16-2

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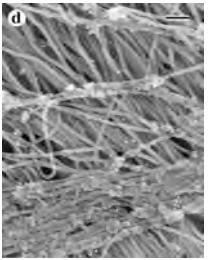
Cell Mechanical Strength



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About Intermediate Filaments

- Medline
 - 7,266 Refs
- Textbook
 - Essential Cell Biology Ch 16 p514-518
- Broad Range sizes
 - IF class proteins have Mr from 40 - 210 kDa

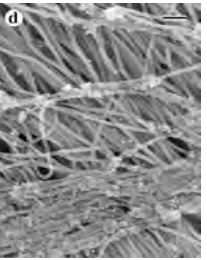


SEM from <http://www.stir.ac.uk/Departments/NaturalSciences/DBMS/courses/30CB/plfig4d.jpg>

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Physical Characteristics

- 10 nm diameter
- Named by relative size
- Provide rope-like resistance to mechanical stress
 - in muscle- link Z discs of adjacent myofibrils



SEM from <http://www.stir.ac.uk/Departments/NaturalSciences/DBMS/courses/30CB/plfig4d.jpg>

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Movie: GFP Vimentin



Movie 1: The IF array is dynamic. Shown is the behavior of the IF array in an NIH3T3 cell expressing GFP-vimentin. The movements of the IF array in this movie are typical of other arrays we have recorded. Bar, 15 μ m.

Intermediate Filament © Dr M.A. Hill, 2005 Slide 12 Mol Biol Cell, 1999 May; 10(5): 1289-1295

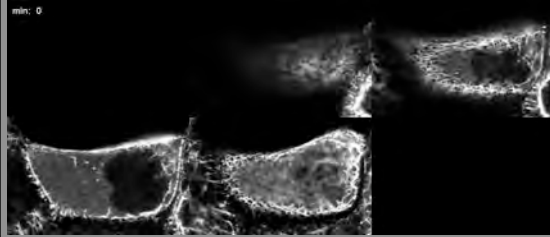
Movie: GFP Vimentin



Movie 3: Vimentin IFs extend, retract, and translocate. IFs at the edge of the cell undergo continuous changes in position. The filaments extend, retract, and shift. Shown is the first frame of the movie, which demonstrates the movements of IFs at the edge of the cell. Bar, 10 μ m.

Intermediate Filament © Dr M.A. Hill, 2005 Slide 14 Mol Biol Cell, 1999 May; 10(5): 1289-1295

Movie: GFP Cytokeratin



Movie 4: breakdown of cytokeratin filament network at the onset of mitosis. Movie shows five focal planes from bottom to top. At the beginning of the recording (early prophase) partial filament breakdown has already taken place in central cytoplasmic regions and increased diffuse cytoplasmic staining can be seen. At that time point the nucleus is still intact. Filament disassembly continues from the centre of the cell towards the periphery, where the originally fine network concentrates in more solid structures and disintegrates into granules. Note, that at the end of the sequence CKFs are completely absent. Instead, significant diffuse cytoplasmic staining and granules, most of which are located in the cell periphery, are detected in the rounded cells. At some intermediate time points rodlets arise in the cytoplasm (e.g., level 2, 7.5 min) but disappear later.

Intermediate Filament © Dr M.A. Hill, 2005 Slide 15 http://www.uni-mainz.de/FB/Medizin/Anatomie/Leube/pubs/11/movies_11.html

Types of Proteins

- IF subdivided into 6 classes based on homology
 - 3 new IF-like proteins identified may expand the number to 9
- Many different types of filament proteins
- Similar IF in many cell types
- Also different specific IF for different cell types
- Heterogeneous subunits
 - Can also form homopolymeric

Intermediate Filament © Dr M.A. Hill, 2005 Slide 16

Intermediate Filament Types

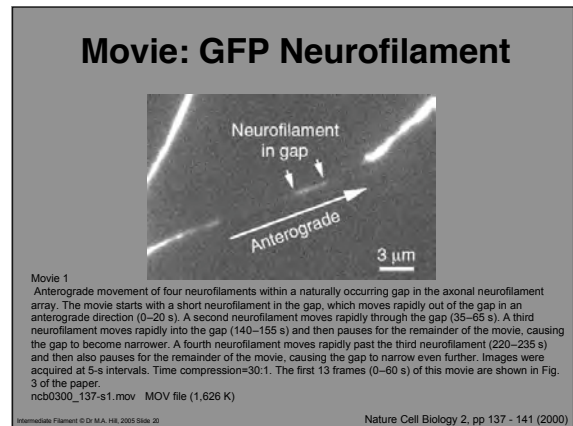
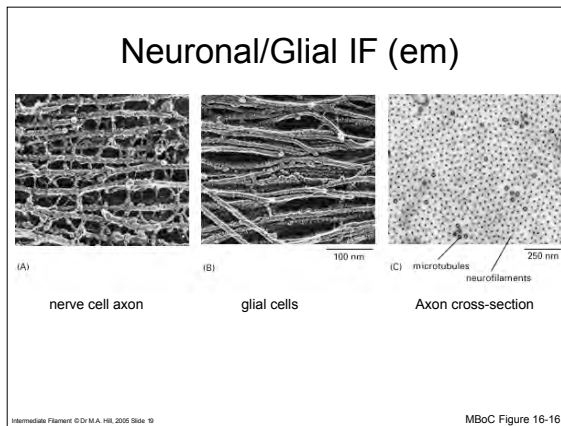
Class	Protein Identity	M.W. (kDa)	Cell type
Type I	keratin	40-54	epithelial
Type II	desmin	52-58	epithelial
	filipin	53	muscle
	CPAP	51	astroglia
	peripherin	54	neuronal
Type III	vimentin	55	mesenchymal, neural, neuronal cells
Type IV	α -Internexin	66	connective (neuronal)
Type V	β -Internexin	70	neuronal
	SP-L	68	neuronal
	SP-M	110	neuronal
	SP-H	130	neuronal
	nestin	140	CFE, neuro-emb
Type VI	Lamins A	70	nuclei
	Lamins B	64	nuclei
	Lamins C	56	nuclei
	Septin A,B,C	30-40	cytoskeletal
	Myosin Class III	20-40	muscle
	Tubulin	50	microtubule
	Phalloidin	variable	microtubule
	Shikonin D/A		microtubule

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IF Types and Cell

IF	Subunit	Cell Type
Keratin	Type I	epithelial
	Type II (40-65 kDa)	
Neurofilament	NF _L (70 kDa)	neurons
	NF _M (140 kDa)	
	NF _H (210 kDa)	
Vimentin	Vimentin (55 kDa)	fibroblasts
	Vimentin + glial fibrillary acidic protein (50 kDa)	
	Vimentin + desmin (51 kDa)	Muscle cells
Nuclear lamina	Lamins A, B, C (65-75 kDa)	All nucleated cells

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IF Gene Conservation

- Vimentin, desmin, and glial fibrillary acidic protein genes
 - each contain 8 introns at identical positions
 - 6 introns located in regions encoding alpha-helical seq
 - Most of introns in less closely related keratin genes occurred at similar or identical positions

DNA → mRNA

exon intron exon
Coding mRNA

Secondary structure: alpha helix

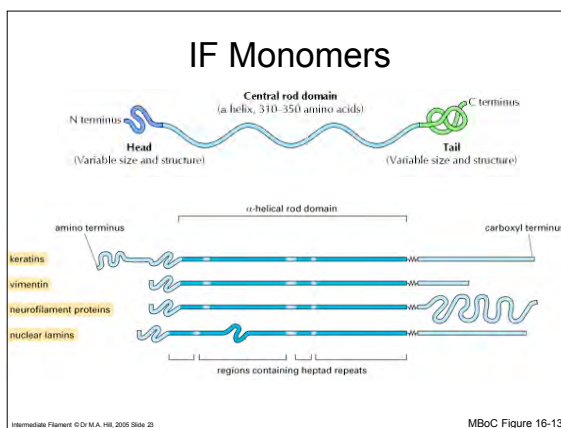
Intermediate Filament © Dr M.A. Hill, 2005 Slide 20 Image MBoC ch3f30.gif

IF Genes- Keratin

- Keratin
 - acidic keratins encoded by genes KRT9 to KRT19
 - genes located on mouse chromosome 11
 - human chromosome 17
 - except for KRT18 which may be located on human chromosome 12
 - basic keratins encoded by genes KRT1 to KRT8
 - located on mouse chromosome 15
 - human chromosome 12

See chromosomes http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search

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IF Structure

- common structural core
 - 300-330 amino-acids
 - flanked by extra amino- and carboxy-terminal domains
 - composed of a heptad repeating unit
 - containing a greater than average allowance of hydrophobic residues and sequences
 - form alpha-helical conformations
- repeating hydrophobic residues create a coiled-coil dimer

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IF Structure

- Dimers
 - alpha-helical coiled-coils
- Tetramers
 - two alpha-helical coiled-coiled dimers
 - associate head-to-head (sometimes with a staggered)
- Protofilament
 - Two protofilaments associate side-by-side to form a protofibril
- Four Protofibrils (eight protofilaments)
 - form a super-helically twisted 10 nm filament

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IF Structure

The diagram illustrates the assembly of an intermediate filament in several stages:

- Monomer:** A single polypeptide chain with an N-terminus, a central rod domain, and a C-terminus.
- Staggered dimer:** Two monomers associate side-by-side in a staggered fashion.
- Parallel dimer:** Two monomers associate side-by-side in a parallel fashion.
- Antiparallel tetramer:** Two dimers associate in an antiparallel manner.
- Protofibril:** Two tetramers associate side-by-side.
- Protofilament:** Two protofibrils associate side-by-side.
- Intermediate filament:** Four protofilaments associate to form a super-helically twisted filament.

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IF Polymerisation

- Nearly all monomer incorporated into polymer
- No energy required for IF polymerisation
 - Compare to microfilament and microtubule
 - Use energy to polymerise

The diagram shows a monomer with the following regions:

- N terminus:** A blue squiggly line.
- Head:** A blue squiggly line, labeled as having a variable size and structure.
- Central rod domain:** A blue wavy line, labeled as containing alpha helices (310-350 amino acids).
- Tail:** A blue squiggly line, labeled as having a variable size and structure.
- C terminus:** A green squiggly line.

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Dimer/Tetramer Structure

The diagram shows:

- DIMER:** Two monomers associated side-by-side, with N and C termini labeled.
- TETRAMER:** Two dimers associated in an antiparallel manner, with N and C termini labeled.

Intermediate Filament © Dr M.A. Hill, 2005 Slide 28 Image: Paul McLaughlin (see refs)

IF Polymer

The diagram shows the assembly of an intermediate filament polymer:

- monomer:** A single polypeptide chain.
- dimer:** Two monomers associated side-by-side.
- Anti-parallel tetramer:** Two dimers associated in an antiparallel manner.
- Protofibril:** Two tetramers associated side-by-side.
- Protofilament:** Two protofibrils associated side-by-side.
- Intermediate filament:** Four protofilaments associated to form a super-helically twisted filament.

Intermediate Filament © Dr M.A. Hill, 2005 Slide 29 MBoC Figure 16-14

Protofilament/Filament

The diagram shows:

- PROTOFILAMENT:** A long, thin, cylindrical structure composed of two protofibrils.
- INTERMEDIATE FILAMENT:** A thicker, super-helically twisted structure composed of four protofilaments.

Intermediate Filament © Dr M.A. Hill, 2005 Slide 30 Image: Paul McLaughlin (see refs)

IF Associated Proteins

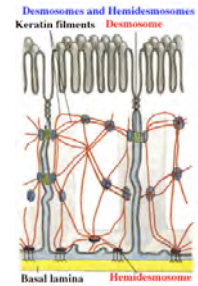
- cross-link IFs with one another
- Form
 - a bundle (also called a *tonofilament*)
 - a network
 - and link with other cell structures (plasma membrane)
- Only a few IFAPs have been identified
 - none of the known IFAPs sever or cap intermediate filaments, sequester IF proteins in a soluble pool, or act as a motor protein
 - IF organization and supportive function in various cells types depends on linkage to other cell structures via IFAPs

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Text modified from MCB

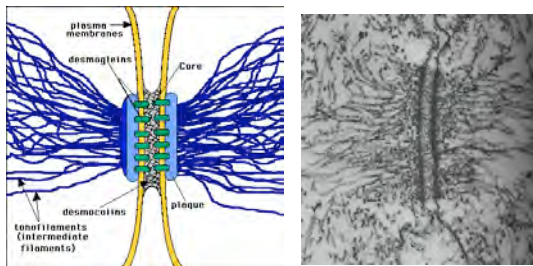
IF and Cell Junctions

- **Desmosome**
 - macula adherens
 - Cell-cell IF (keratin) junction
 - Through transmembrane linker glycoprotein
 - Desmocollins, desmogleins
- **Hemidesmosome**
 - Cell-matrix junction
 - Through transmembrane linker glycoprotein
 - laminin receptor



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Desmosomes

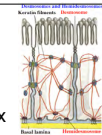


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Images from <http://ntri.tamuk.edu/class/ryan/celljunctions.html>

(Hemi)Desmosomes

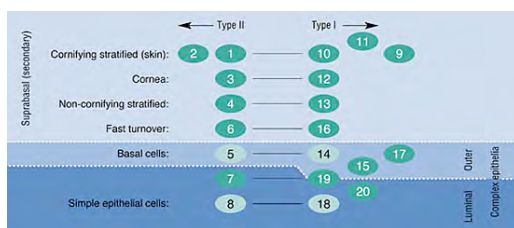
- Focal points of intercellular contact
- bind neighbouring cells together or to cell matrix
- Transmembrane linker glycoproteins
 - Homophilic binding
 - Calcium dependent



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Image: The Cell ch11f34b.gif

Keratins



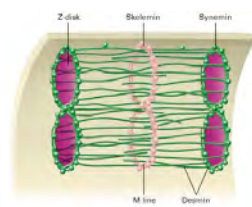
The diagram illustrates cytokeratin expression in different epithelial types. The numbers refer to the numerical classification of human cytokeratins.

Image from Novocastra <http://www.novocastra.co.uk/ifcdgs.htm>

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Desmin in Muscle

- Stabilize Sarcomeres in Muscle
- lattice of a band of desmin filaments surrounds sarcomere
- desmin filaments encircle the Z disk and are cross-linked to the plasma membrane by several IFAPs
 - paranemin and ankyrin.
- Longitudinal desmin filaments
 - cross to neighboring Z disks within myofibril
 - connections between desmin filaments around Z disks in adjacent myofibrils serve to cross-link myofibrils into bundles

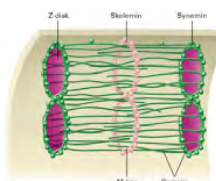


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MCB ch19f57.gif

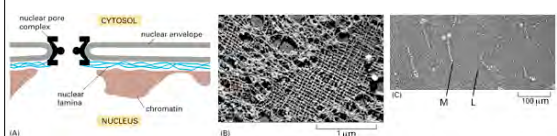
Desmin in Muscle

- lattice is also attached to sarcomere
 - through interactions with myosin thick filaments
 - possibly by skelemin in the H zone
- desmin filaments lie outside sarcomere
 - not active in contractile forces
 - structural role
 - maintain muscle integrity
- transgenic mice lacking desmin
 - supporting architecture disrupted
 - muscles are misaligned



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Nuclear Lamina



nuclear pore complex CYTOSOL nuclear envelope chromatin NUCLEUS

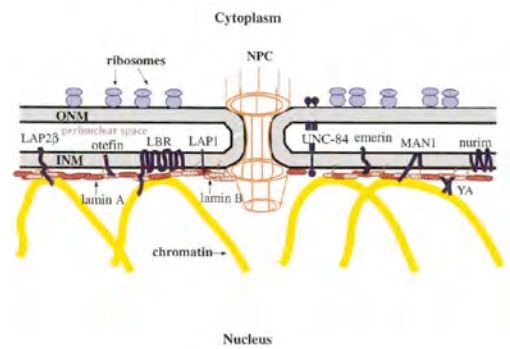
(A) (B) (C)

nuclear pore square lattice of intermediate filaments isolated lamin dimers (marked L)

1 μm 100 μm

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Nuclear Lamins



Cytoplasm ribosomes NPC ONM LAP25 otefin LBR LAP1 UNC-84 emerin MAN1 nurin INM lamin A lamin B chromatin Nucleus

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Movie: GFP Lamin A



© University Maastricht Dept. Path. Cell Biology

View the sequence of events occurring after metaphase of a CHO cell transfected with lamin A-GFP
Department of Molecular Cell Biology & Genetics of the Medical Faculty, Maastricht University, The Netherlands
<http://137.120.28.214/mcb/cytoskeleton.html#GFP>

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Movie: GFP Lamin B



Lamin B1-GFP during mitosis

Intermediate Filament © Dr M.A. Hill, 2005 Slide 41 <http://137.120.28.214/mcb/cytoskeleton.html#GFP>

Movie: GFP Lamin C



Lamin C-GFP during mitosis

Intermediate Filament © Dr M.A. Hill, 2005 Slide 42 <http://137.120.28.214/mcb/cytoskeleton.html#GFP>

Nuclear Lamins

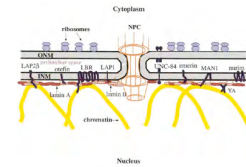
- Lamins found
 - at nuclear periphery
 - interior of nucleus
 - as distinct nucleoplasmic foci and possibly as a network throughout the nucleus
- Nuclear processes such as DNA replication may organize around these structures
 - See review article Moir et al., J Struct Biol 2000, 129:324

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Moir et al., J Struct Biol 2000, 129:324

Nuclear Lamins

- polymerize to form nuclear lamina
 - fibrous structure on inner face of nuclear membrane
- also form structures within the nucleoplasm
 - establish and maintain shape and strength of interphase nucleus
 - role in nuclear processes such as DNA replication
- Mutations in human lamin A/C gene linked to several diseases
 - including Emery-Dreifuss muscular dystrophy



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Moir and Spann, Cell Mol Life Sci., 2001 58:1748-57

Nuclear Lamins

- cell-cycle-dependent dynamics of the nucleus in higher eukaryotes
- lamins and lamin-binding proteins
- functions during steps of post-mitotic nuclear reassembly
 - cross-linking of chromatids
 - nuclear membrane targeting
 - nuclear lamina assembly
 - formation of replication-competent nucleus

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Nuclear lamins: building blocks of nuclear architecture, Goldman et al GENES & DEVELOPMENT, 2002 16:533

IF Antibodies- Cancer detection

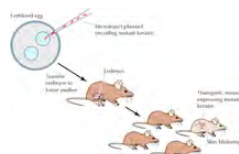
- common malignant tumours of breast and gastrointestinal tract contain keratins and lack vimentin
 - derived from epithelial cells
 - which contain keratins but not vimentin
 - underlying stromal mesenchymal cells
 - which contain vimentin but not keratins
- epithelial cancers and mesenchymal cancers are sensitive to different treatments
 - IF protein identity in tumour cell allows selection of most effective treatment for destroying the tumour

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Text modified from MCB

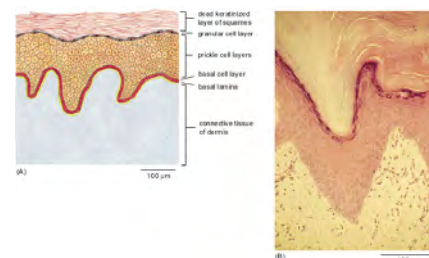
IF Diseases

- OMIM 62 entries for IF
- Epidermolysis bullosa
 - Mutation in keratin gene
- Emery-Dreifuss Muscular Dystrophy
 - Mutations in emerin and Lamin A/C gene
- Nuclear Lamins and lamin associated proteins
 - involved in autoimmune diseases
- Alexander Disease
 - Mutations in GFAP



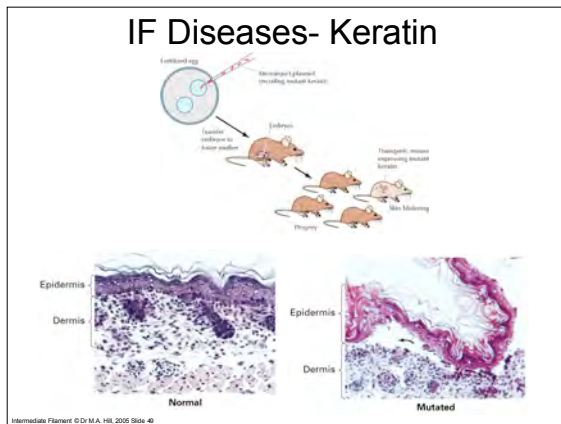
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Epidermis



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MBOC Figure 22-19



GFAP

- class-III intermediate filament
- cell-specific marker
- during CNS development
 - distinguishes astrocytes from other glial cells
- GFAP defects cause Alexander disease
 - rare disorder of central nervous system

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IF Disease- Alexander Disease

- rare disorder of central nervous system
 - progressive leukoencephalopathy
 - widespread accumulation of Rosenthal fibers
 - cytoplasmic inclusions in astrocytes
- Most common form infants and young children
 - progressive failure of central myelination leading to death usually within the first decade
- Infants
 - develop a leukoencephalopathy with macrocephaly, seizures, and psychomotor retardation
- juvenile or adult form
 - ataxia, bulbar signs and spasticity, more slowly progressive course

Intermediate Filament © Dr M.A. Hill, 2005 Slide 51 Source: Expaty

References

Web

- <http://www.cytoskeleton.com/aif.htm>
- <http://www.cytochemistry.net/Cell-biology/filam.htm>
- <http://nessie.bch.ed.ac.uk/PAUL/TEACHING/IF/if.htm>
- <http://www.stir.ac.uk/Departments/NaturalSciences/DBMS/coursenot/es/30CB/INTFILNO.html>
- <http://www.ultranet.com/~kimball/BiologyPages/J/Junctions.html>

Text

- Intermediate filaments: structure, dynamics, function and disease, Fuchs, E, and Weber, K. Annu. Rev. Biochem. 1994, 63, 345-82
- The cytoskeleton and disease: Genetic disorders of intermediate filaments. Fuchs, E Annu. Rev. Genet 1996. 197-231

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References

- ANAT3231 Lecture
 - <http://cellbiology.med.unsw.edu.au/units/science/lecture0505.htm>
- Essential Cell Biology Ch16
- Molecular Biology of the Cell Ch16
- The Cell Ch8
- Molecular Cell Biology Ch19

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MBoC IF Summary

- Intermediate filaments are strong, rope-like polymers of fibrous polypeptides that resist stretch and play a structural or tension-bearing role in the cell.
- A variety of tissue-specific forms are known that differ in the type of polypeptide they contain:
 - keratin filaments of epithelial cells, the neurofilaments of nerve cells, the glial filaments of astrocytes and Schwann cells, the desmin filaments of muscle cells, and the vimentin filaments of fibroblasts and many other cell types.
 - Nuclear lamins, which form the fibrous lamina that underlies the nuclear envelope, are a separate family of intermediate filament proteins.
- Monomers of different types of intermediate filaments differ in amino acid sequence and have very different molecular weights.
 - all contain a homologous central rod domain that forms an extended coiled-coil structure when the protein dimerizes. Two coiled-coil dimers associate with each other to form a symmetrical tetramer, which in turn assembles in large overlapping arrays to form the nonpolarized intermediate filament. The rod domains of the subunits form the structural core of the intermediate filament, whereas the domains at either end can project outward.
- One function of the variable terminal domains may be to allow each type of filament to associate with specific other components in the cell, so as to position the filaments appropriately for a particular cell type.

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