1 # ANAT3231 - Cell Biology
Lecture 5

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3 ## 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

4 ## Cytoskeleton Function

- Cell Shape
- Cell motility
- Cell mechanical strength

5 ## Motility- Intracellular

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

6 ## Cellular Motility

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

7 ## Cellular Motility

- Mature (somatic)
- wound healing

- blood cells, fibroblasts
- phagocytosis
- macrophages
- killer T cells
- neural regeneration
- process regrowth
- glial migration

8 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 proteins
  - regulate cytoskeletal activity
    - Human genome 30,000 genes

9 3 Filament Systems

10 Cell Mechanical Strength

11 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

12 Physical Characteristics

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

13 **Movie: GFP Vimentin**

14 **Movie: GFP Vimentin**

15 **Movie: GFP Cytokeratin**

16 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

17 Intermediate Filament Types

18 IF Types and Cell

19 Neuronal/Glial IF (em)

20 **Movie: GFP Neurofilament**

21 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

22 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

23 IF Monomers

24 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

25 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 protofilamenents)
  - form a super-helically twisted 10 nm filament

26 IF Structure

27 IF Polymerisation

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

28 Dimer/Tetramer Structure

29 IF Polymer

30 Protofilament/Filament

31 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

32 IF and Cell Junctions

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

33 Desmosomes

34 (Hemi)Desmosomes

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

35 Keratins

36 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

37 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

38 Nuclear Lamina

39 Nuclear Lamins

40 **Movie: GFP Lamin A**

41 **Movie: GFP Lamin B**

42 **Movie: GFP Lamin C**

43 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 etal., J Struct Biol 2000, 129:324

44 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

45 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 chromatides
  - nuclear membrane targeting
  - nuclear lamina assembly
  - formation of replication-competent nucleus

46 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

47 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

48 Epidermis

49 IF Diseases- Keratin

50 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

51 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

52 References

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- 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/coursenotes/30CB/INTFILNO.html
- http://www.ultranet.com/~jkimball/BiologyPages/J/Junctions.html

53 References

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    - 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

54 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.