Bi 336 Lecture Notes from Nov. 20-Dec. 2. These notes are based on Dr. Boone's outline given on his website. However, they have been modified by Dr. Carter.

FINAL EXAM DEC. 9, 12:30 TO 2:20
The exam will focus on the material covered in Dr. Carter's presentations. Also, carefully review the figures and figure descriptions given in the book for those figures shown in Dr. Carter's presentations. Also, make sure you can answer the questions given on the study sheets. Also, read the material in the text book that covers the material given in lecture. The exam will consist of multiple choice or true and false. There will be 70 or more questions, so bring a 100 answer scantron Form No. 882-E (like you used on the last test). The scores will be made equivalent to a possible value of 50 pts.

Lecture Notes:

"Cell Ecology"
Interactions between cells and their environment.

- Extracellular space
- Interactions between cells
- Sealing the extracellular space
- Gap junctions and plasmodesmata
- Cell walls

Tissue Organization
in Animals

- Connective
- Epithelial
- Nervous
- Muscle

(All are associated with ECM, but connective has high ratio of ECM to cells)

Extracellular Matrix (ECM)

- Defined: the organized material beyond the plasma membrane.
- Organization by physical, enzymatic factors:
  - self assembly--free energy changes
  - binding specificity
  - pH, redox
  - enzyme activity
  - hormones
  - force fields (mechanical, electrical)

(The important role of
carbohydrates in cell-cell

interactions)

"The information-carrying potential of oligosaccharides is far greater than that of proteins and nucleic acids of equivalent molecular weight, and their presence on cell surfaces and on many proteins suggests an importance which was previously unrecognized. Oligosaccharides are now thought to mediate cell-cell recognition, including the infection of cells by bacteria and viruses, moderate the behaviour of enzymes and other proteins, and play a variety of rôles in the immune response."

(Fig. of amphiphilic hexose not in text)

Some components of the ECM of animals: collagen, fibronection, proteoglycan. (Fig 7.5)

Fibronectin
Fig. 7.5.
(Also, Fig. Of fibronectin gene not in text)

- Evolutionarily conserved protein
- Has functional domains; interacts differentially
- Has arginine-glycine-aspartate (RGD) sites
- Tissue variable forms from differential RNA splicing

Collagen
Fig 7.6

- Fibrous protein, abundant.
- Gene family; allows variations per tissue
- Secreted as procollagen, enzyme cleaves, then self-assembly into triple helix
- Fibroblasts may organize
- Can be tethered by fibronectin, integrin, actin
- Examples: tendons (fibrillar), cornea (crossed), basement membrane (random)

Proteogylcans
Fig. 7.9

- Protein core with glycosamine (GAG) side chains.
- GAGs neg. charged
- GAGs bind cations
- Cations bind water
- $_{H}O_{H}$ = gel
- If water can't flow it resists compression.
- Therefore function of proteoglycans is…..? [cushions, protects tissue]

Cell-Cell Interactions

Fig 7.19

- Depends on distinguishing self, nonself
- Mediated by recognition factors
- selectins, Ig, integrins, cadherins (all glycoproteins)

Integrins, Fig. 7.5

- Integral gycoprotein- spans membrane. (cytoplasmic and extracellular head)
- Subunits: ", $-- not covalently bound to each other
- Gene family for each ", $ unit--specificity, 22 known
- Inside/outside communication (also outside/inside)
- Requires Ca
- Binds to arginine/glycine/ aspartate residues (RGD)
- Conserved among animals.

Selectins, Fig. 7.20

- Transmembrane molecule
- Glycoproteins
- Lectins (binds to specific carbohydrate)
- Requires Ca ion
- Mediates transient interactions with blood

Immunoglobulin Superfamily (IgSF)
Fig. 7.21

- Glycoproteins
- Members of SF share similar domains (aa sequence, conformation)
- Most are antibodies (immune response)
- Others have generalized cell adhesion or neural development functions
- Can link with integrins

The immunoglobulin superfamily (IgSF) is the largest known group of related proteins. In a single species, the IgSF includes hundreds of members believed to have arisen from a single primordial Ig-like domain during evolution of multicellular metazoans (Williams and Barklay, 1988; Doolittle, 1995). (Fig. 7.21)

Cadherins
Fig. 7.22

- Family of glycoproteins
- Mediate Ca++ dependent cell adhesion
- Transmit signals from ECM to cytoplasm
- Self recognition (same type of cell)

Cadherins, con't
Fig 7.27a

- In humans, 40 different kinds known
- Most variety found in brain
- Holds epithelial-derived tissues together (e.g., kidney, skin)
- Ca++ integrated between repeats--holds rigid
- Zips together with like cadherins from another cell
- Bound to cytoskeleton

Sealing Extracellular Space

- In animals "tight junctions" isolate regions of extracellular space
- In plants, impregnation of matrix with solids isolates regions.
- Reason: to control the pathway of diffusion of solutes

Tight Junctions, Fig. 7.30a

- Membrane proteins shared or conjoined.
- Forms ring around cells
- Solutes are restricted in ECM.
- Diffusion is restricted within plane of membrane.
- Common in epithelial tissues

Gap Junctions, Fig. 7.32

- Allow intercellular communication
- Found in verts and inverts, but different proteins
- "Connexons" are found in verts.
- Allow direct channel between cells
- Ion exchanges, etc.

The ECM of Plant Cells--plant cell walls
Fig. 7.35

- Cellulose microfibrils - give strength, shape
- Hemicellulose - cross links
- Pectins: neg. charged - gel, glue
- Glycoproteins - allow changes, recognition, and ?
- Other polymers: lignin, suberin, wax

Calcium Bridges (Fig. not in text)

Function of Cell Walls

- Support

- Protection
- Translocation of water, ions, nutritive substances, hormones
- Specializations can direct transport --lignin, suberin, wax

Wall Specialization: Casparian Strip
(Fig. not in text)

- An impervious strip (suberin) within cell walls of cells surrounding the root vascular tissue. Directs solutes from apoplast to symplast.

Plasmodesmata, (Fig. 7.34)

- Symplastic channel across cell walls between cells
- ER tube goes through
- Cytoplasmic continuum
- amino acids, other charged molecules can pass through; some viruses regulate by "movement protein"

Cellulose Synthesis, Fig. 7.37b

- Cellulose synthase rosettes extrude cellulose polymer made from glucose subunits.
- Cellulose polymer joins with others to form cellulose microfibril.
- Orientation is related to microtubules

Concept Summary

- Carbos give specificity by conformation
- Glycoproteins carry carbos, bind, allow communication
- Cations (especially Ca++) regulate, bridge, form gels,
- ECM organized by physical factors (e.g. collagen)
- ECM supports, protects, allows cell recognition, interaction
- Fibronectin binds to many types of molecules- (RGD) specificity by RNA splicing
- Cell-cell interactions mediated by glycoproteins, e.g. integrins, selectins, IgSF, cadherins-- specificity by gene families, gycomoieties
- Tight junctions, Casparian strips-- partition solutes
- Plasmodesmata/ Gap junctions allow cytoplasm continuum
- Integral proteins (cellulose synthase, connexons, integrins) allow inside/out communications & connections

Some Approaches to the Study of Sub-cellular Biology
Autoradiography
Fig. 8.3

- Early studies
- Incubation with radioactive amino acids
- Pulse-chase, various times
- Fix, expose film
- Metabolic fate of protein visualized on autoradiograph.

Endomembrane Concept
Fig. 8.3

- Studies show membrane structures as interrelated in a dynamic system
- Leader sequences: Amino acid 'zip codes' provide 'addresses'
- Materials are transported via transport vesicles
- Biosynthetic pathway modifies proteins
- Secretion: constitutive, regulated, membrane recycling.

GFP-green fluorescent protein

- gene isolated from luminescent jellyfish
- gene product absorbs blue light/emits green light
- GFP gene inserted into expression vector will transform cells, fluoresce.
- can be tagged onto another gene
- can follow fate of gene product thru cell cycle by periodic exposure to blue light.--non destructive
- new types of GFP emit at different wave lengths--what is advantage of this?

Recent Experiments with GFP. Re: Biochem. J. (2001)357, 529-536

- No GFP tag no PM label
- positive fluorescence on PM with GFP beta tag
- positive fluorescence on PM with GFP alpha tag
- antibody stain is positive
- GFP advantage: non-destructive, dynamic

(understand terms in title and results given here)

Preparing a microsomal fraction
Fig 8.5

- Disrupt tissue
- Spin 20,000 g/ 20 min
- Spin supernatant 50,000 g/ 2 hr.
- Retrieve pellet
- Resuspend in buffer
- Gradient separation
- Analysis: PAGE, EM, immunocytochemistry

Cell fractionation by differential and density gradient centrifugation: Enrichment for a phloem-specific protein.

Methods:

- disrupt tissue
- differential centrifugation
- PAGE fractions for enrichment
- load enriched fraction on density gradient
- dialyze fractions
- PAGE
- electron microscopy of enriched fractions

Sub-cellular localization of a phloem-specific lectin. A: lanes 2-5 pine phloem; lanes 6-9 pine xylem. Top, SDS-PAGE; bottom, western blot. B: immunogold localization on phloem protein crystals. C & D: purified protein (by glycosamine affinity column).* (Re: Planta (1989) 179: 506-515)

- *understand all italicized terms

- Requires protein purification or leader sequence
- Antibody/antigen interaction
- Sensitivity enhanced with fluorescent tags.

Figure: immunofluorescence of pre-mRNA splicing factors in nuclear subcompartments during interphase (figure not in book)

Mutants
Fig. 8.7

- Yeasts are major subjects for eukaryotic cell bio studies
- Single cell; easy to mutate, screen.
- Can grow as haploids (no masking mutation)
- Mutants deficient in secretion used (SEC genes)
- Info obtained on membrane dynamics

The Endomembrane System
Endomembrane Concept
Fig. 8.3--overview

- Studies show membrane structures as interrelated in a dynamic system
- Materials are transported between components via transport vesicles
- Biosynthetic processes within system modifies proteins
- Membranes arise from pre-existing membranes--not *de novo*

Endoplasmic Reticulum, Figs. 8.9a, 8.10

- Most versatile organelle of cells
- Composed of interconnected membranous cisternae (tubes or flattened bags)
- RER-has ribosomes for protein synthesis via translation; I^oglycosylation
- SER-metabolic processing, membrane prep., secretions.

Plasmodesmata, (Fig. 7.34)

- Symplastic channel across cell walls between cells
- ER tube goes through
- Cytoplasmic continuum

RER

- Can separate by density-gradient centrifugation
- Has ribosomes bound to cytosolic side of membrane

- Can be continuous with nuclear membrane, but not with plasma membrane
- Cisternae interconnected and flattened.

SER
- No ribosomes
- Interconnected and tubular

ER is a dynamic system that can continually change shape in living cells.

Details, SER

- SER extensive in skeletal muscle cells, kidney tubule cells, glands, oil producing cells of plants
- Some functions: synthesize hormones; detox by oxygen (-OH) transfer to solubilize for excretion; G-6-Phosphatase (dephosphorylation of glucose stores necessary for mobilization out of tissue); sequestration of Ca^{++}.

Protein Synthesis: membrane bound or free

RER
- Secreted proteins
- Integral membrane proteins.
- Soluble proteins

Cytosolic
- Protein remaining in cytosol
- Peripheral membrane proteins
- For inside of nucleus, mitochondria, plastids, peroxisomes

Gunter Blobel, nobel prize for Medicine 1999 “for the discovery that proteins have intrinsic signals that govern their transport and localization in the cell"

- Known for work on the signal hypothesis:
- A signal sequence on N-terminous directs proteins to final cell site.

Cotranslational Protein synthesis on RER, a model. Fig 8.12

1. mRNA/ribosome begins translation in cytoplasm, thru signal seq.
2. SRP binds to sig seq, docks on ER at translocon & SRP receptor
3. GTP>GDP on G proteins provides energy for opening translocon, re-binding
4. Translation proceeds inserting protein thru pore
5. New protein associates with chaperone, finalizing conformation

Integral membrane protein synthesis--a model, Fig 8.12

- Same as for secretory protein only mid-way thru there is a recognition sequence that stops transfer thru pore.
- Ribosome tilts to provide release of cytosolic portion

- Protein is released from translocon and the hydrophobic region is inserted into bilayer.

Integral proteins have complex associations with membranes

- Integral proteins can have different interaction with membranes.
- How they insert depends on topogenic sequences, e.g., hydrophobic amino acids, charged a.a.

Topographic signals, Fig 4.18

- Hydrophobic alpha helix--within memb.
- Carbos added on lumenal side
- Often + charged on cytosolic face of membrane

Membrane Synthesis, Fig 8.14

- Orientation is determined during translation and maintained throughout endomembrane system
- Vesicles may travel to Golgi for processing
- From Golgi goes to PM or vacuole.
- Organelle, surface area increased by vesicles

Membrane synthesis, lipids, Fig 8.15

- Membrane lipid synthases have active sites on cytosol side
- can be swapped to cisternal side by flippases
- Distributed by preferential inclusion, vesicles, transferases

Glycosylation in RER:

- Primary glyc is a conserved process.
- Built up monomer by monomer on cytosol side on dolichol carrier.
- Donor monomer is nucleotide sugar, e.g. GDP-mannose
- Each nucleotide sugar addition has own enzyme, contiguous to carrier.

Modifications to core oligosaccharide

- After 2 N-acetylglucoseamines and 5 mannoses are added, dolichol flips and additional mannoses, glucoses added.
- Unit is transferred to co-translated polypep. at asparagine N
- Calnexin or other chaparone helps protein fold (re Fig 8.18)
- incorrectly folded proteins expelled to cytosol

ERGIC, Fig 8.23c

endoplasmic reticulum - Golgi intermediate compartment

- Transitional region of endomembrane system.
- In animals, ERGIC lies between nucleus and Golgi.
- Regions of RER become smooth and vesicate.

- They anastomose to larger vesicles & tubules.
- They move to Golgi complex, taking glycoprotein cargo.
- Movement may be facilitated by microtubule motor system.

Golgi Complex, Fig.8.20b, c

- Composed of stack of flattened cisternae with dialated, tubular margins with vesicles.
- Individual stacks may be interconnected (animals), free to move in plants.
- Function: processes proteins, synthesizes carbos.

Golgi Complex, Fig 8.25

- Functionally distinct compartments, cis to trans.
- Materials enter from ERGIC to cis Golgi.
- Compartments move through complex to trans region while processing occurs.
- Final products sorted into coated vesicles.
- Coated vesicles deliver cargo to final cell site.

Movement through the Golgi, Figs 8.23, 8.25

- Models have changed over the years.
- Current model has both larger cisternae and vesicles moving from cis to trans.
- Anterograde and retrograde (recycling) vesicle movement
- Not all material leaves out the trans side.

Vesicle Transport, Figs 8.25

- Vesicles become coated on cytoplasmic side as they pinch off from SER, Golgi, or plasma membrane.
- Coats are for mechanics and for destination signals
- Three types known: COP I, COP II, clathrin
- GTP of G proteins provide docking/fusion energy.

Coated Vesicles, Fig 8.24

Coat Protein II

- Added to SER vesicles
- Carry to ERGIC
- Carry enzymes for Golgi processing
- Carry I^o glycosylated translation products
- Coat is lost as fusion with ERGIC occurs

Coat Protein I

- less understood, but first described
- Move from Golgi back to ER (retrograde)
- May also move from cis to trans (anterograde)

Clathrin-Coated Vesicles, Fig 8.43

- Added to vesicles from trans Golgi network (TGN)
- Coats contain clathrin triskelions (a protein complex) over an inner adaptor protein coat.

- For lysosome or vacuole destinations.
- Where endocytosis occurs at plasma memb.

Posttranslational Protein Uptake (other destinations for translation products)

- Fully formed proteins are delivered to nuclei, peroxisomes, mitochondria, chloroplasts.
- Signal sequence within aa chain are recognized by cytoplasmic chaparones or directly by organelle receptors.
- Semi-autonomous organelles (mitochondria, plastids) can make some proteins due to own genes, translation process.
- Most proteins imported, but many lipids made independently of nuclear genome.

Importing into Mitochondria, Fig 8.47

- Have 4 sites for proteins:
- outer, inner membranes, intermembrane space, matrix.
- Where membranes seem to fuse are TOM and TIM insersion sites
- Requires ATP

Importing into Plastids, Fig 6.2

- Have 6 targets: outer, inner membranes, inner membrane space, matrix, thylakoid membranes, thylakoid lumen.
- Have analogous targeting as mitochondria.
- Transit peptides at N terminous is removed at docking
- Thylakoid transfer domain revealed after transit peptide is removed.

The Cytoskeleton

- Microtubules
- Microfilaments
- Intermediate filaments
- Also motor proteins and other associated proteins that integrate system

Functions of the Cytoskeleton, Fig 9.1

- Structure and support
- Intracellular transport
- Contractility and motility
- Spatial organization

Microtubules, Figs 9.8, 9.9

- Hollow, long, tubular
- Found in mitotic spindle, cilia, flagella, cytoplasmic tracks, sub plasma membrane.
- Longitudinal rows are protofilaments (13/MT) alpha, beta subunits.
- Beta is + end-- fast growing, alpha is - end--slow growing
- “Grows” on + end
- MAPs give stability