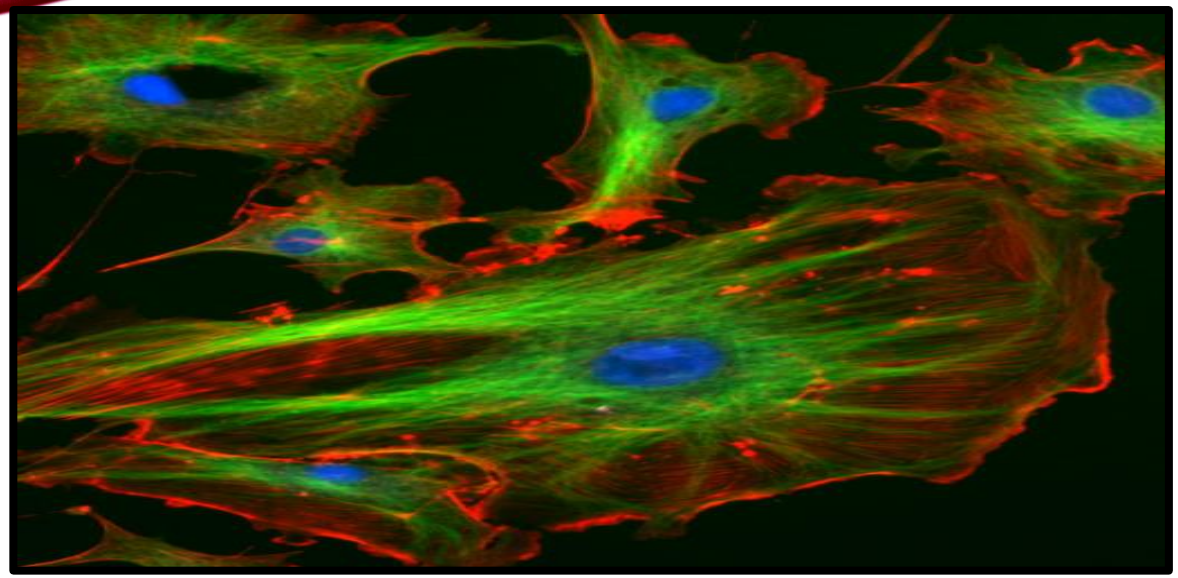


CELLULAR BIOLOGY

Experiments and Applications

CONTACT & RESOURCES

- Course Instructor Information

  Kristen Johnson, PhD.

Email: krisjohnson@unomaha.edu

Office Hours: via zoom by appointment

Matthew Northam, PhD.

Email: mnortham@unomaha.edu

TA: Missy Briardy



- Campus Resources
  - Accessibility Services Center
  - UNO counseling center
  - UNO Criss library
  - Tutoring services
    - Math-science learning center
    - Writing center

COURSE OBJECTIVES

1. Given lectures, primary literature, and in-class discussion, students will demonstrate the ability to recognize, interpret, and apply concepts, such as: cell adhesion, proliferation, differentiation, cell signaling, and gene expression.

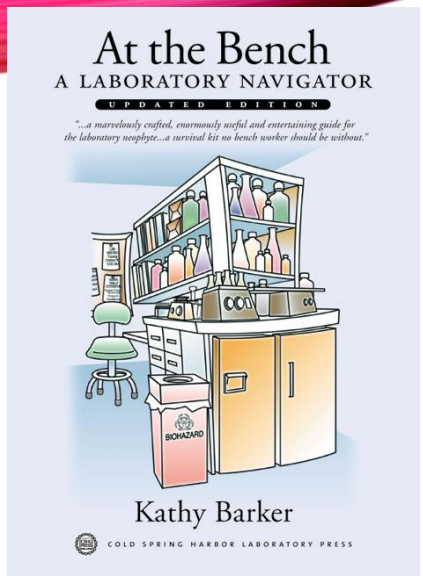
2. Through the care of human cell cultures, students will demonstrate proficiency in basic techniques in cell culture - including aseptic technique, cell line maintenance, characterization, microscopy, and cryopreservation.

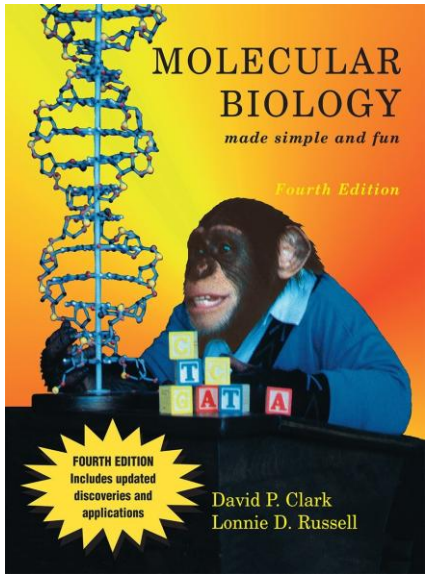
3. By investigating gene expression in response to drug treatments, students will build the skills necessary to perform basic techniques at the molecular level in cell biology – including RNA isolation, and detection of nucleic acids by quantitative PCR.

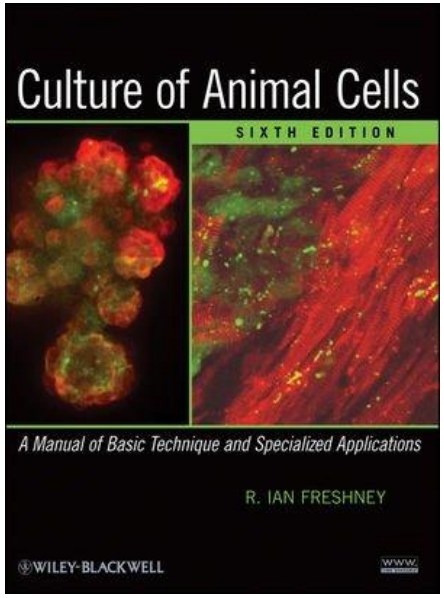
4. Through the completion of writing assignments and formal presentations using their own laboratory work, students will demonstrate the ability to communicate and interpret findings in biological sciences.

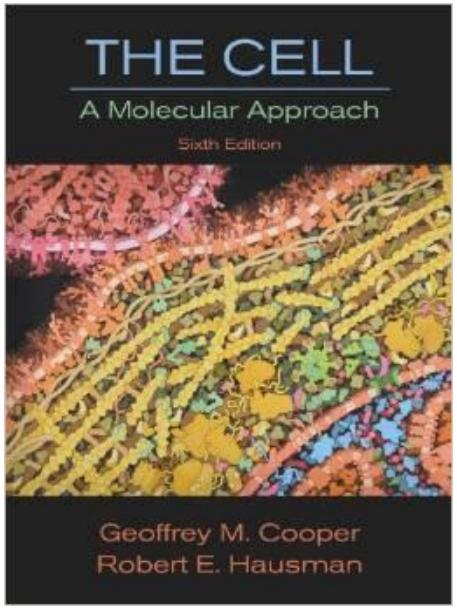
Prerequisites-BIOL 2140, 3020 and CHEM 2210 or 2250

COURSE MATERIAL









- No required textbook for this course.
- Primary literature will be posted weekly. Bi-weekly quizzes will cover the assigned literature.
- A calculator
- A lab notebook or binder.

SYLLABUS

- The Syllabus File can be downloaded from Canvas.
- We will skim through the policy and schedule now but:
  - Read the full syllabus on your own.
  - Keep in mind the schedule is subject to change
  - Due dates and other details are posted on each Assignment in Canvas.

LECTURE AND LAB POINTS

Assessment		
Concept Quizzes (bi-weekly)	**70 points (10 pts each)**	**18%**
Paper Quizzes (bi-weekly)	60 points (10 pts each)	15%
Lab Skills Assignments	110 points total	27%
Pipette exercise-wk1	*14 points*	
Cell Observations	*5 points*	
Experimental Results (5pts/lab)	***55 points***	
Completion of Daily Activities	***26 points***	
Primer Design Exercise	*10 points*	
Cytotoxicity Project	150 points total	37%
Proposals/Prep/In-class discussion	*40 points*	
Compound Selection 10 pts		
Primer Selection 10pts		
Cytotoxicity Protocols 10pts		
Final Exp Proposal 10pts		
Final Paper	***65 points***	
Final Presentation	***45 points***	
Group Participation (Based on Peer Evals)	**10 points**	3%
Total	400 points	100%

WEEKLY LECTURE SCHEDULE

- Lecture Quizzes Post Thursday, Due following Tuesday
- Lab Homework Due dates posted
  - Typically, you will have 1 week to complete an assignment following the acquisition of data.

LAB SCHEDULE

Monday or Thursday 1-3:50pm

- Each lab will have pre-lab material
  - Demonstration
  - Protocol/Instructions
  - The above material will be posted to Canvas, Thursday prior

- Some appointments will be needed for hood work.
  - Appointments posted weekly or all at once?

POLICY

- Late work penalty of 10% per day
- No make-up work (Quizzes, Exams) for inexcusable reasons
- Accommodation needs, such as more time on tests, must be communicated to me by you at least 24hrs prior
- Points back?

QUESTIONS?

FINAL PROJECT-WHAT DO YOU WANT TO STUDY?

- Drug discovery really began with all natural products (i.e penicillin), as apposed to synthetic compounds.
- Why choose natural cures?
  - the emergence of antibiotic resistant microbes, hypersensitive reaction, and implications of disease connections with synthetic products
- Many natural cures are not FDA approved or regulated,
  - Warning labels are not posted, concentrations for uses are not on the bottle





FINAL PROJECT-WHAT DO YOU WANT TO STUDY?

- Tea Tree Oil
- Taurine
- Sodium Fluoride
- Caffeine
- Manuka Honey
- Juglone
- Peppermint Oil
- Elderberry Juice
- Glyphosate
- Phthalates
- BPA

New ideas are welcome

We will work through this project beginning Week 6
Start thinking creatively now.

FINAL PROJECT EXPERIMENTS

- Cyto-toxicity: Assessed by Trypan Blue and Alamar Blue
- Gene Expression Response: Assessed by Quantitative PCR of Reverse Transcribed RNA
- Any Experiment of your design
  - Limitation Cost and Cell Type

You will plan just about everything about this project, from how we make stock solutions, choose controls, and dispose of material.

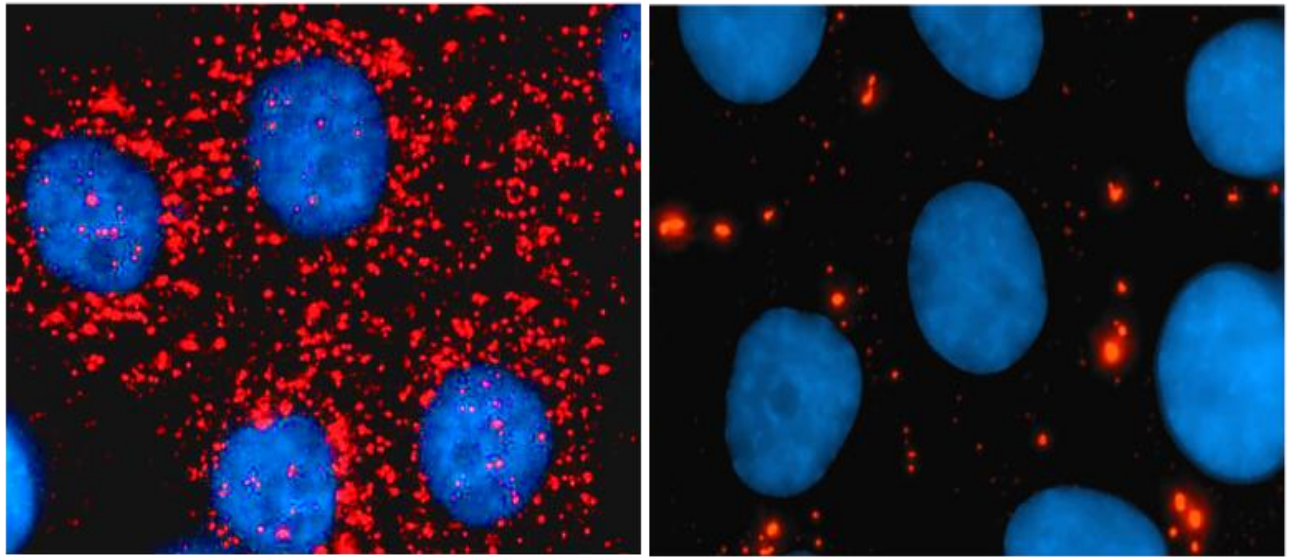
Instructions for Laboratory Notebooks



Flexible choice of notebook, but I recommend a Binder to organize.

WHAT DO CELL BIOLOGISTS DO ANYWAY?

Cell biology is the study of **cell** structure and function, and it revolves around the concept that the **cell** is the fundamental unit of life. Focusing on the **cell** permits a detailed understanding of the tissues and organisms that **cells** compose.



HISTORY – CULTURE OF ANIMAL CELLS

1964-69 – Rabies, mumps, and Rubella vaccines in WI-38 human lung fibroblasts (Wiktor et al., 1964; Sokol et al., 1968)

1975 – Hybridomas – monoclonal antibodies (Kohler & Milstein, 1975)

1983 – Immortalization by SV40 (Huschtscha & Holliday, 1983)

1980-87 – Development of many specialized cell lines

1998 – Tissue engineered cartilage (Aigner et al., 1998)

1998 – Culture of human embryonic stem cells (Thomson et al., 1998)

2000 – Human Genome Project (Dennis at al., 2001)

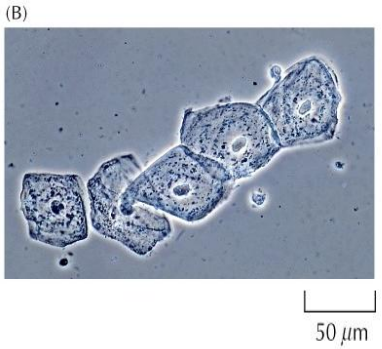


FURTHER APPLICATIONS

1. **Model systems**
   - Basic cell biology and biochemistry
   - Interactions between disease-causing agents and cells

2. **Toxicity testing**
   - Effects of new drugs, cosmetics, chemicals on many cell types, particularly kidney and liver cell lines

3. **Cancer research**
   - Study the characteristics of cancer cells
   - Mechanisms that can induce normal cells to become cancer-causing cells



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 1.25**

© 2013 Sinauer Associates, Inc.

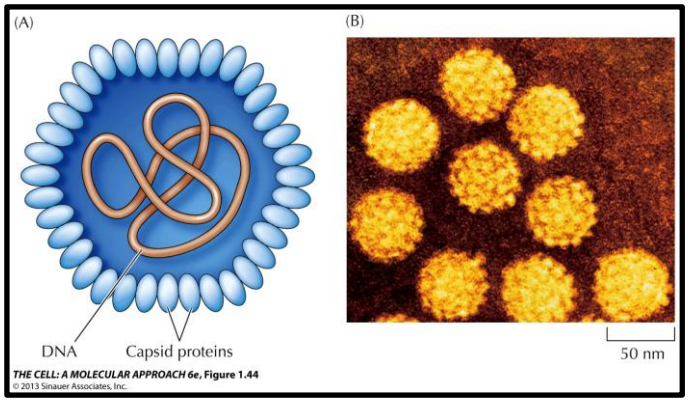
FURTHER APPLICATIONS



4. **Virology**
   - Clinical detection and isolation of viruses
   - Vaccine development

5. **Cell-based manufacturing**
   - Vaccines
   - Proteins (insulin, monoclonal antibodies, hormones, etc.)
   - Tissue engineering

6. **Genetic counseling**
   - Amniocentesis vs
     Blood Draw from your arm



FURTHER APPLICATIONS

7. **Genetic engineering**
   - Transfection of cultured cells to study expression of genes or produce large quantities of a desired protein

8. **Drug screening and development**
   - Cytotoxicity assays and high throughput screening

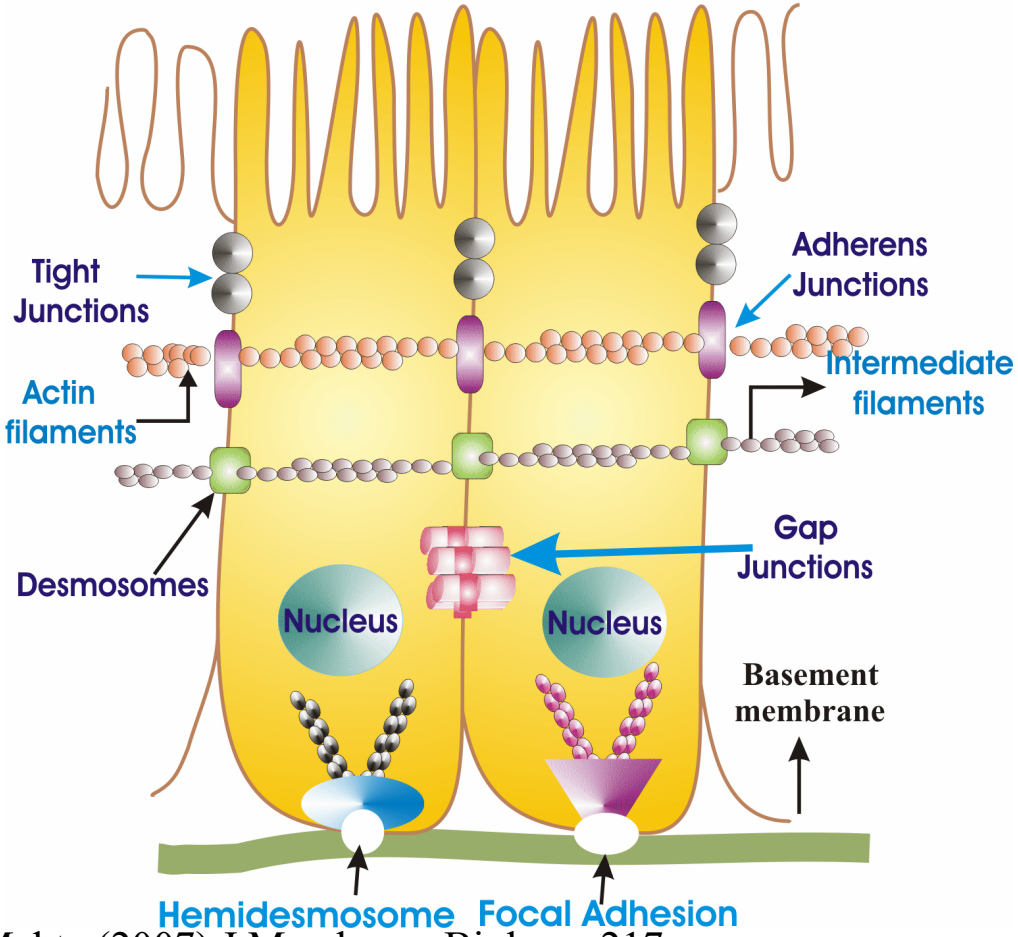
And much, much more…

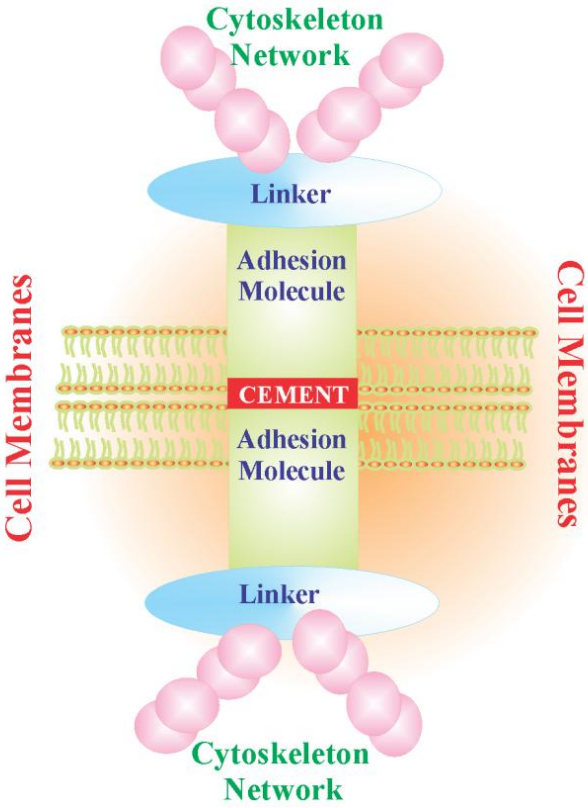


DR. JOHNSON'S WORK- DISSECTING A MECHANISM

- Roberts BJ, **Johnson KE**, Saowapa J, Svoboda RA, JohnsonKR, Wahl III KJ (2014) *Palmitoylation of Plakophilin is Required for Desmosome Assembly.* Submitted to J. Cell Science
- **Johnson KE**, Mitra S, Katoch P, Kelsey LS, Johnson KR, Mehta PP (2013) *Phosphorylation on Ser-279 and Ser-282 of Connexin43 Regulates Endocytosis and Gap Junction Assembly in Pancreatic Cancer Cells.* Mol Biol Cell. 24(6): 715-33.
- Kelsey L, Katoch P, **Johnson KE**, Batra SK, Mehta PP (2012) *Retinoids Regulate the Formation and Degradation of Gap Junctions in Androgen-Responsive Human Prostate Cancer Cells.* PLoS ONE 7(4): e32846. doi:10.1371/journal.pone.0032846.
- Govindarajan R, Chakraborty S, **Johnson KE**, Falk M.P, Wheelock MJ, Johnson KR, Mehta PP (2010) *Assembly of Connexin43 into Gap Junctions is Regulated Differentially by E-cadherin and N-cadherin in Rat Liver Epithelial Cells.* Mol Biol Cell. 21: 4089-4107.
- Mitra S, Annamalai L, Chakraborty S, **Johnson K**, Song XH, Batra SK, Mehta PP (2006) *Androgen-regulated Formation and Degradation of Gap Junctions in Androgen-responsive Human Prostate Cancer Cells.* Mol Biol Cell. 17(12): 5400-16.
- Chakraborty, S., Mitra, S., **Knudtson, K.**, and Mehta, P.P. *E-cadherin Differentially Regulates the Fate of Connexin43 and Connexin32 in A431D Cells.* Abstract for American Society for Cell Biology, Annual Meeting. San Francisco, December 11-12, 2005.
- Govindarajan, R., Annamalai, L., Chakraborty, S., **Knudtson, K.**, and Mehta, P.P. *Ubiquitylation and endocytosis of connexin43 in rat liver cells.* Abstract for American Society for Cell Biology, Annual Meeting. San Francisco, December 11-15, 2003.

Cell Junctions and Gap Junctions

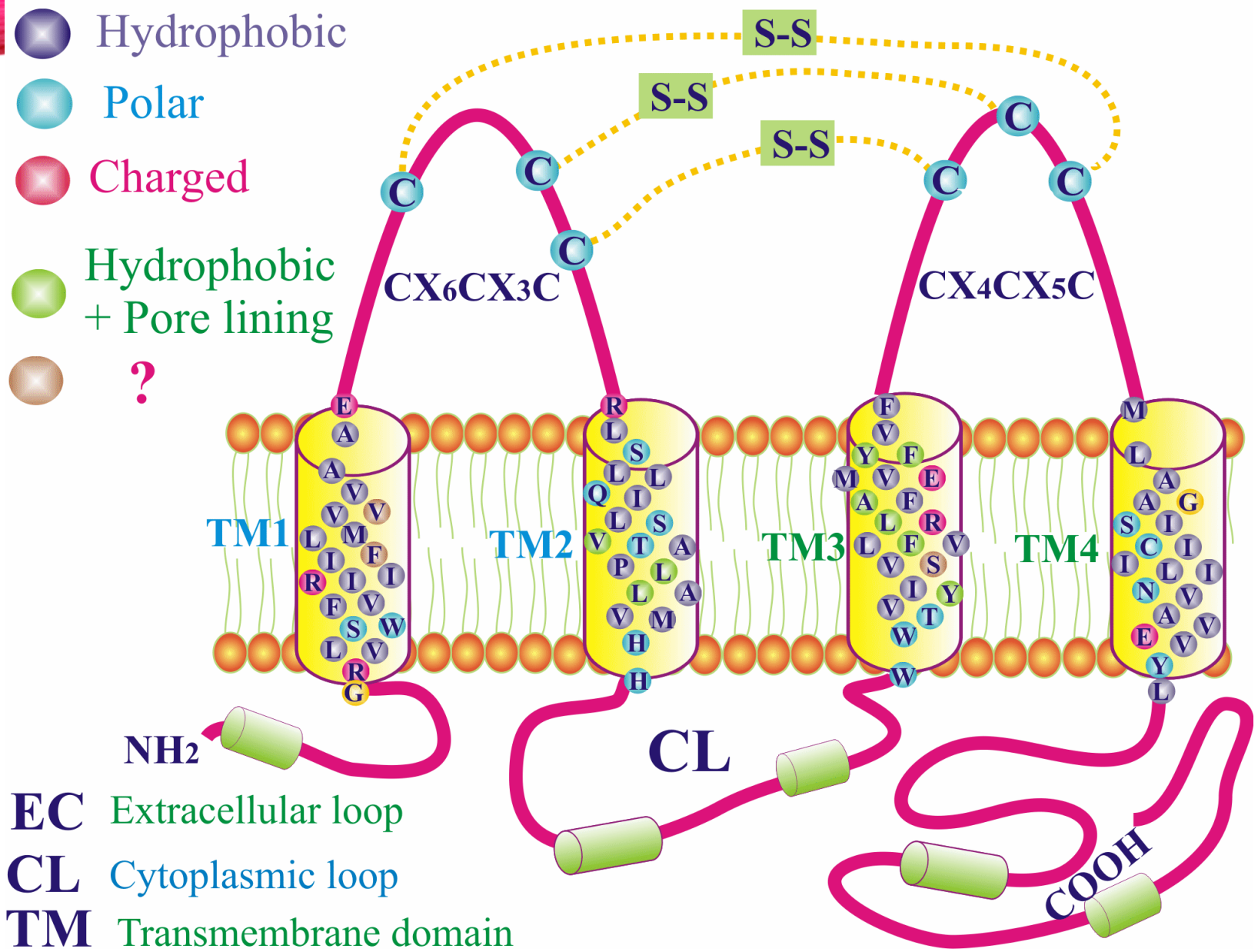




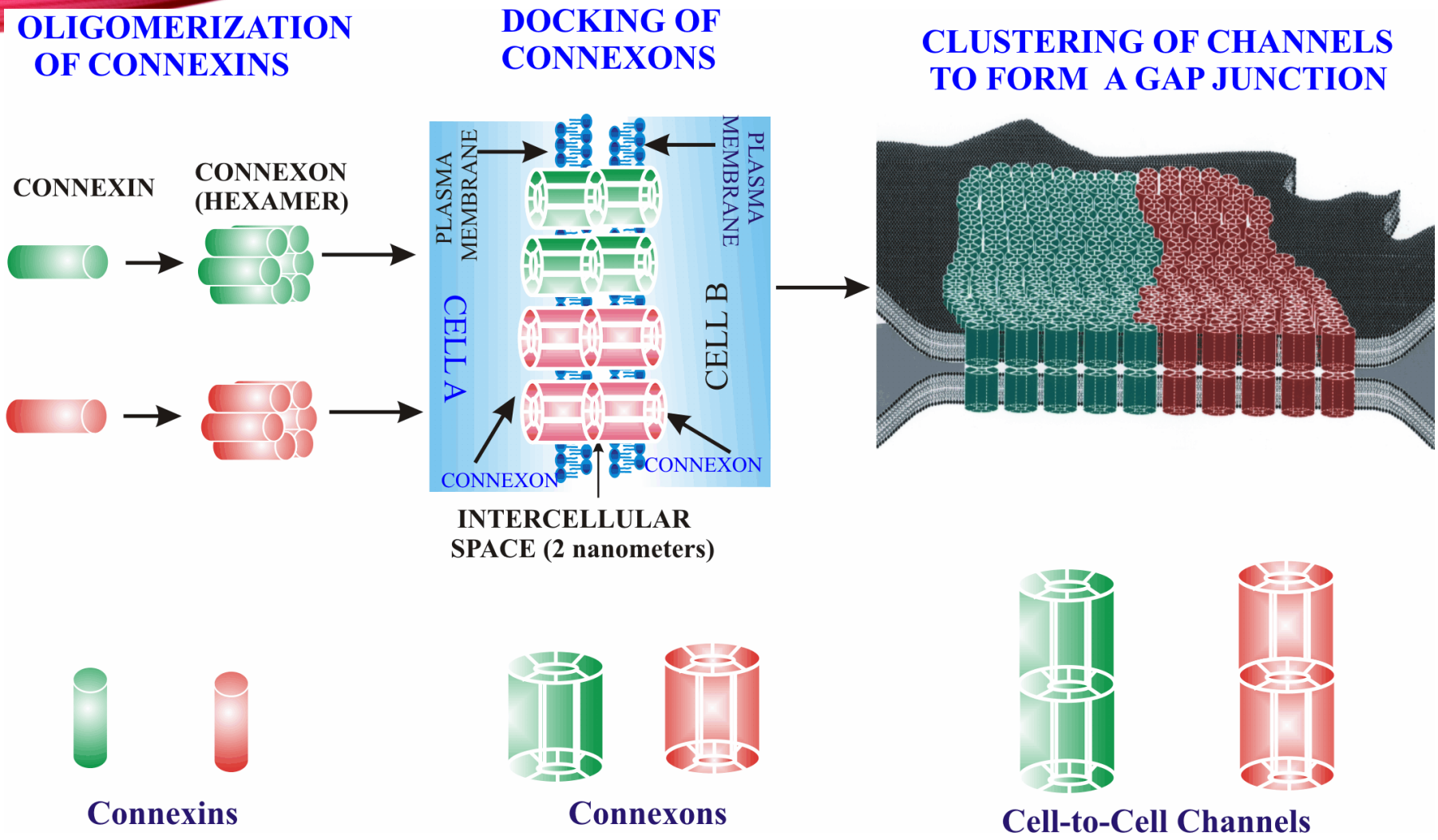
Mehta (2007) J Membrane Biology, 217

Gap junctions are clusters of cell-cell channels. The channels permit the leak-proof passage of small molecules of less than 1500 Da.

CONNEXIN TOPOLOGY

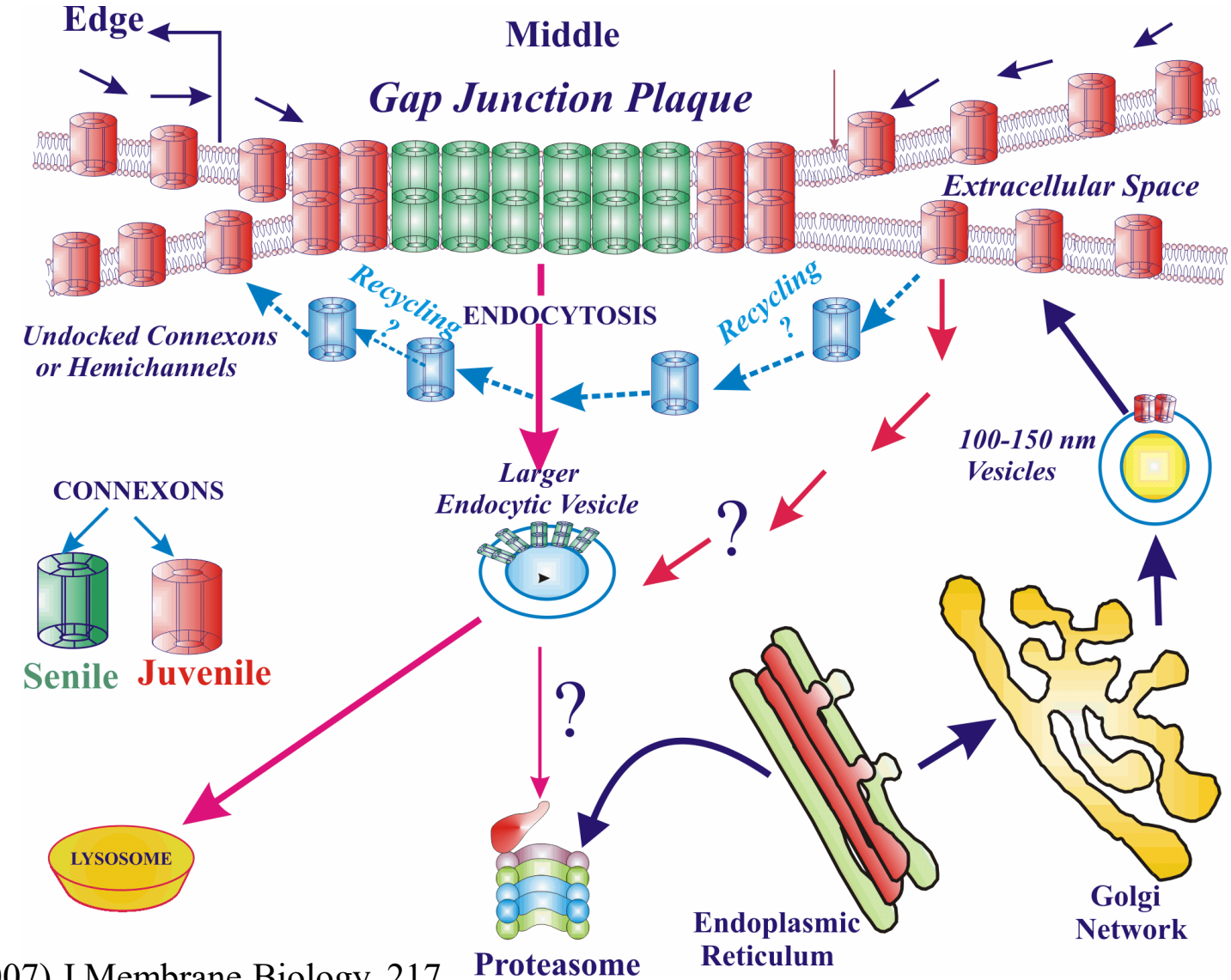


Assembly of Gap Junctions



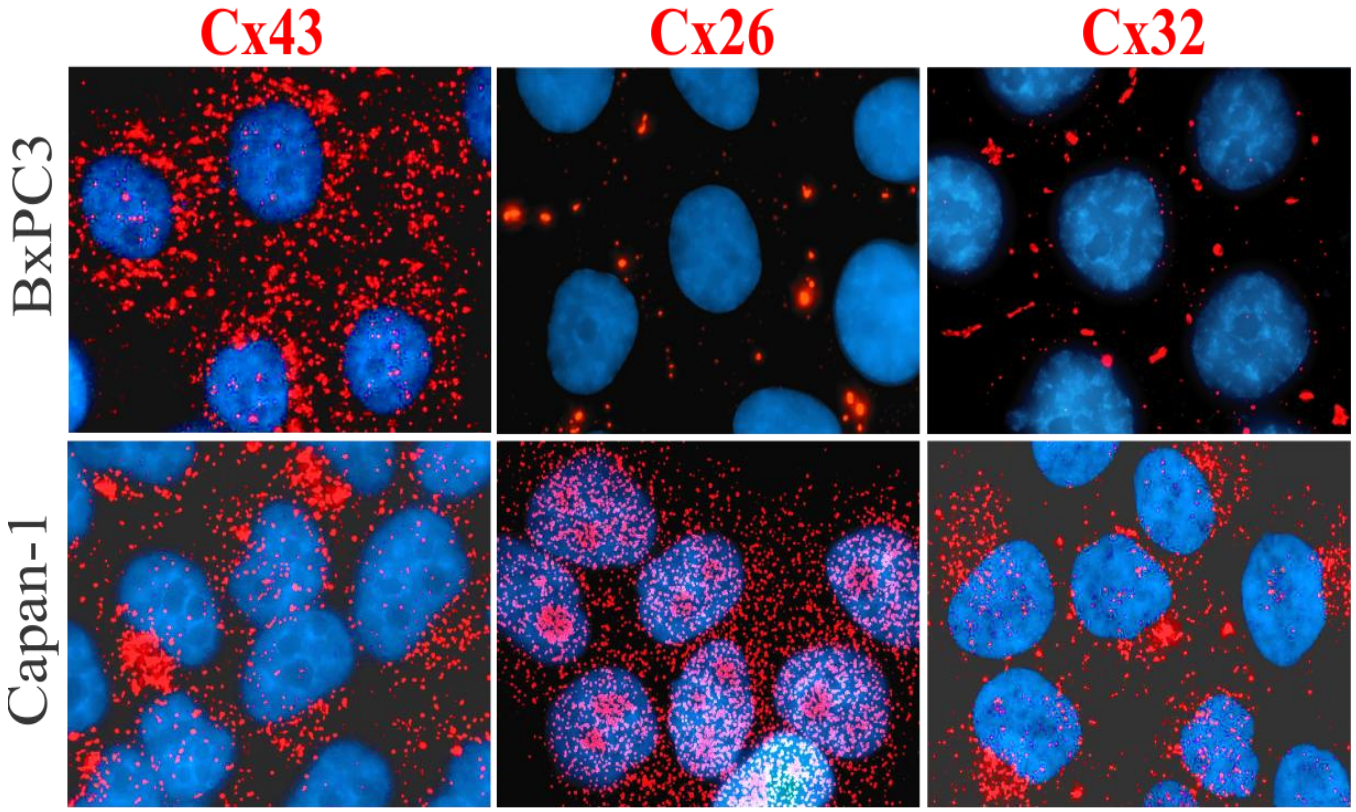
Mehta (2007) J Membrane Biology, 217

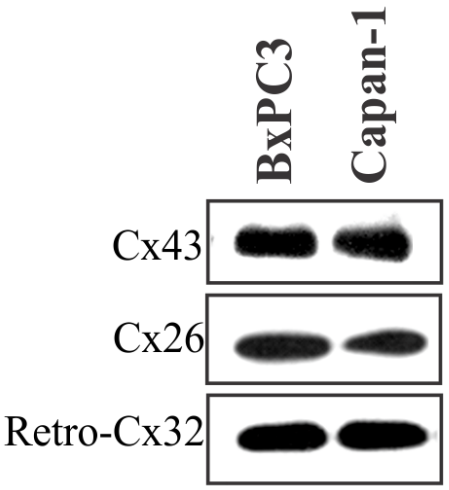
How are Gap Junctions Assembled and Disassembled?



Mehta (2007) J Membrane Biology, 217

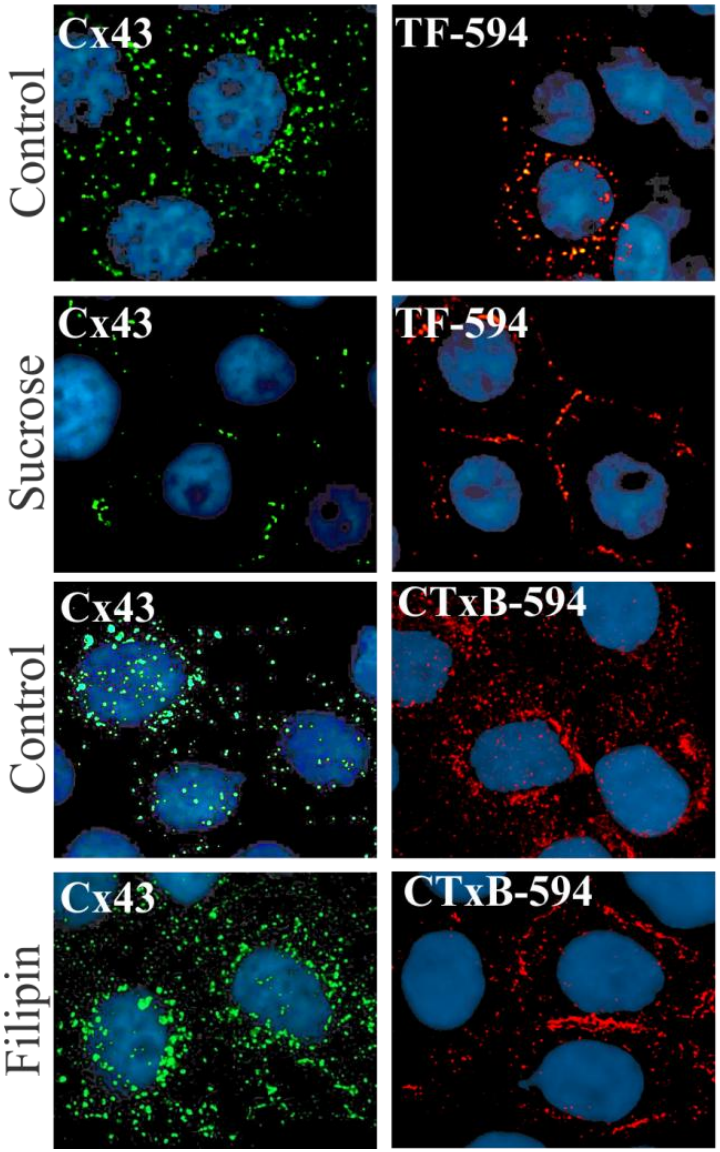
Differential Assembly of Pancreatic Connexins

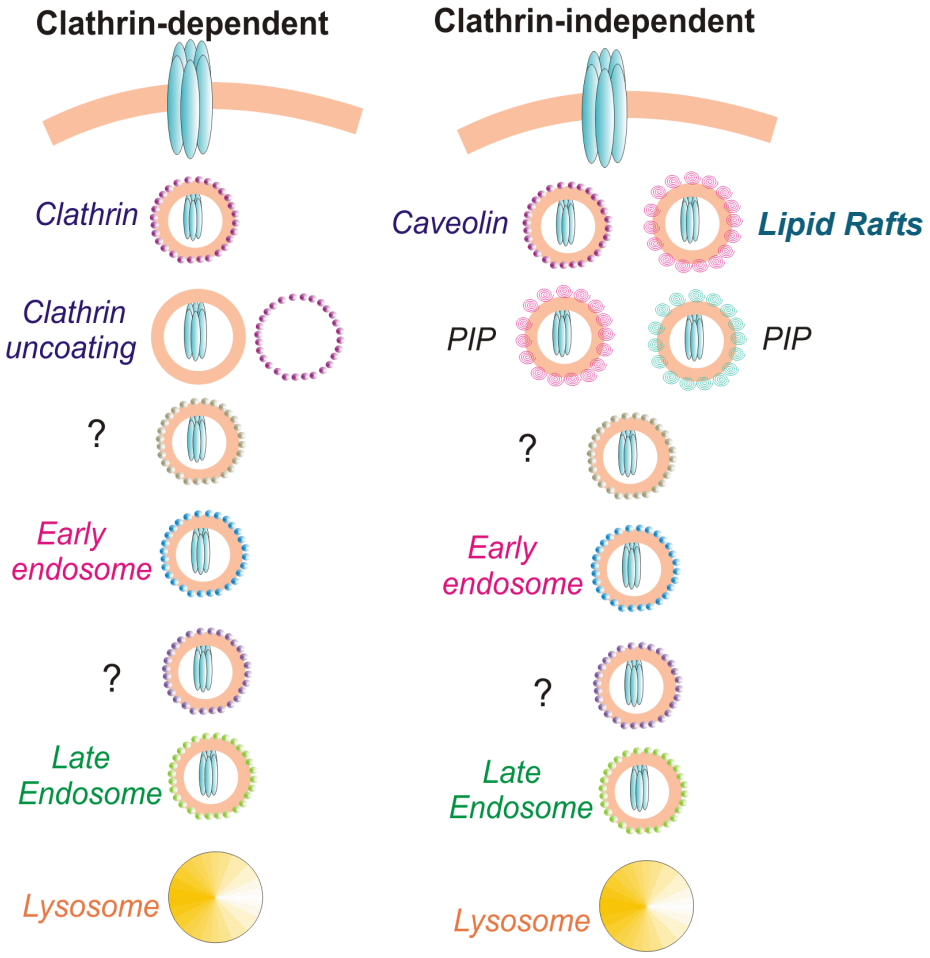




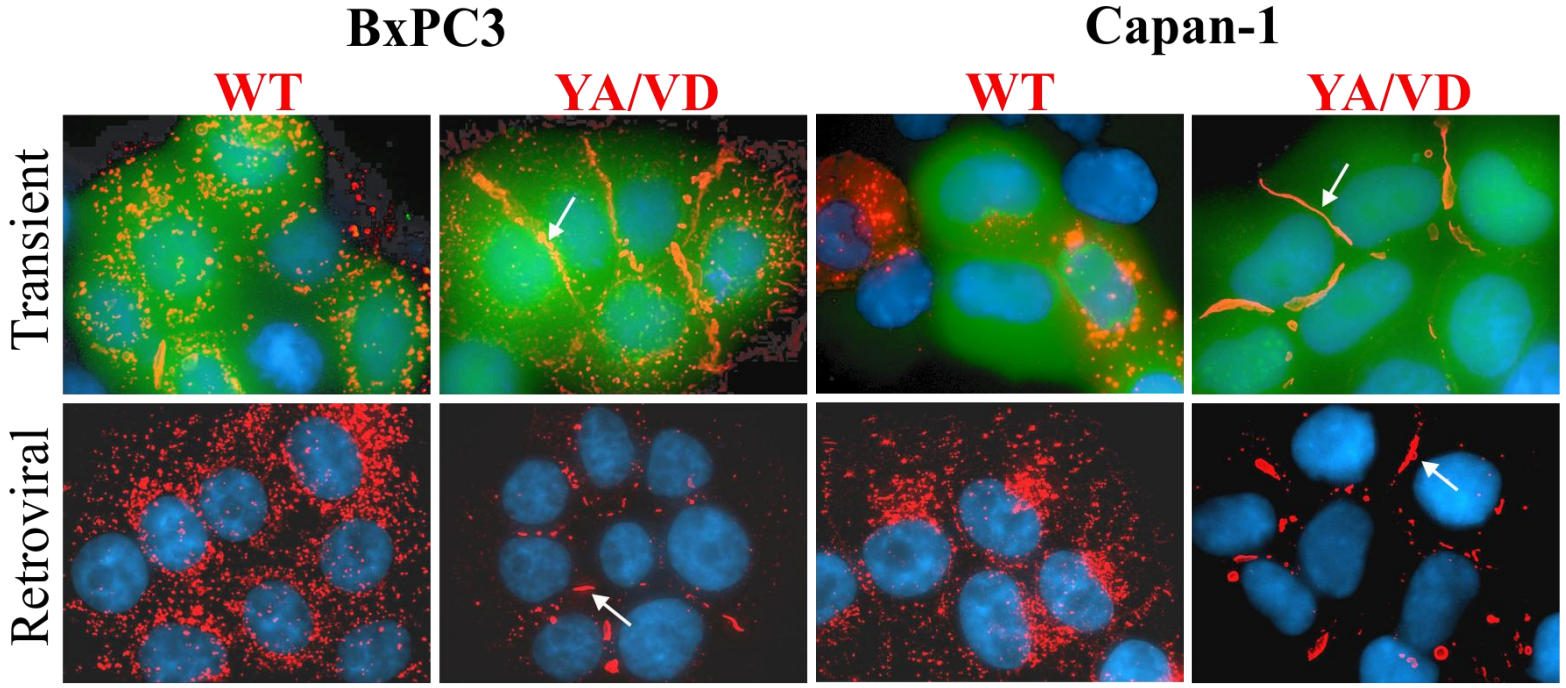
The assembly of Cx43 is selectively impaired in BxPC3, but not in Capan-1 cells

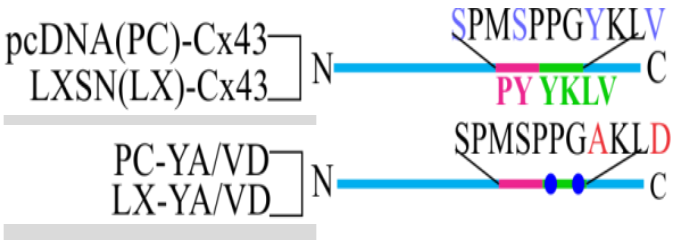
THE CLATHRIN-MEDIATED PATHWAY REGULATES INTRACELLULAR CX43

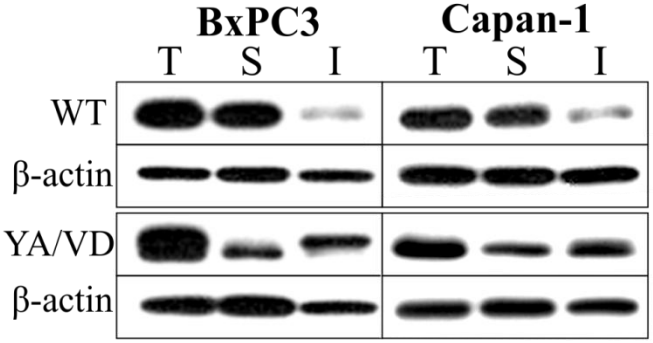




DEFECTIVE ASSEMBLY OF CX43 IS RESTORED UPON EXPRESSION OF A SORTING-MOTIF MUTANT





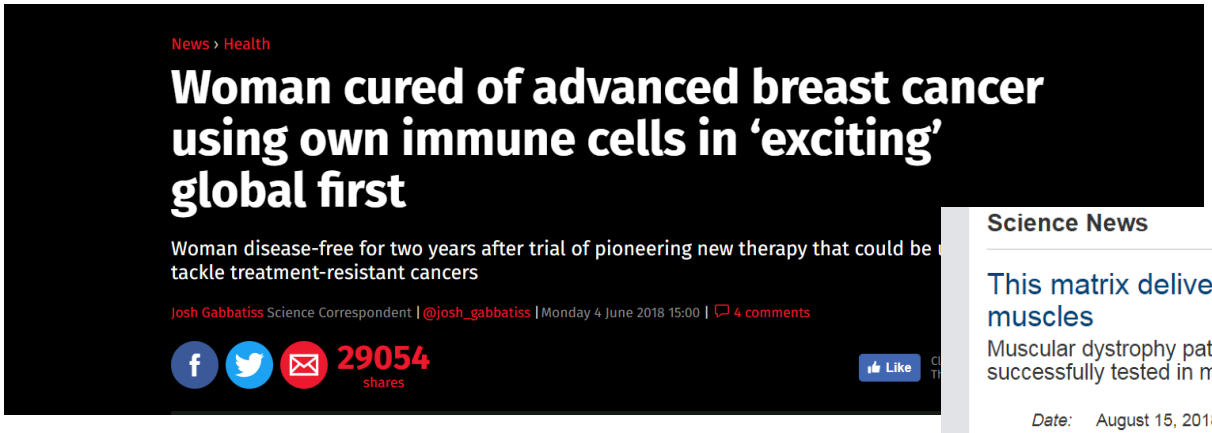


CURRENT INTERESTS

- The motility of cells expressing mutants of p120 in the presence of glucose or taurine
- Comparison of the cytotoxicity of pancreatic cancer cells expressing an assembly-competent Cx43 mutant vs assembly incompetent Cx43.
- Differential expression of kinases in communication competent pancreatic cancer cells vs incompetent cells.
- Effects of essential oils on wound healing and cellular communication.

FUSE, Honors projects and Biotechnology internships

LET'S EXPLORE WHAT IS NEW IN CELL BIO



News › Health

Woman cured of advanced breast cancer using own immune cells in 'exciting' global first

Woman disease-free for two years after trial of pioneering new therapy that could be t
tackle treatment-resistant cancers

Josh Gabbatiss Science Correspondent | @josh_gabbatiss | Monday 4 June 2018 15:00 | 4 comments

29054
shares

Like



Science News

from research organizations

This matrix delivers healing stem cells to injured elderly muscles

Muscular dystrophy patients could someday also benefit from this hydrogel successfully tested in mice

Date: August 15, 2018

Source: Georgia Institute of Technology

Summary: Muscles of the elderly and of patients with Duchene muscular dystrophy have trouble regenerating. A new nanohydrogel with muscle stem cells has boosted muscle growth in mouse models while protecting the stem cells from immune reactions that usually weaken or destroy them.

Share:

RELATED TOPICS

Health & Medicine

- Stem Cells
- Immune System
- Skin Cancer

Plants & Animals

FULL STORY

2 Extra Credit Opportunities:
Extra Credit 1-Due Friday of Week 8
Extra Credit 2-Due Friday of Week 15

USING A CELL MODEL SYSTEM WE CAN ANSWER MANY QUESTIONS.

WHAT QUESTIONS DO YOU HAVE? WHAT ARE YOUR INTERESTS IN CELL BIOLOGY?

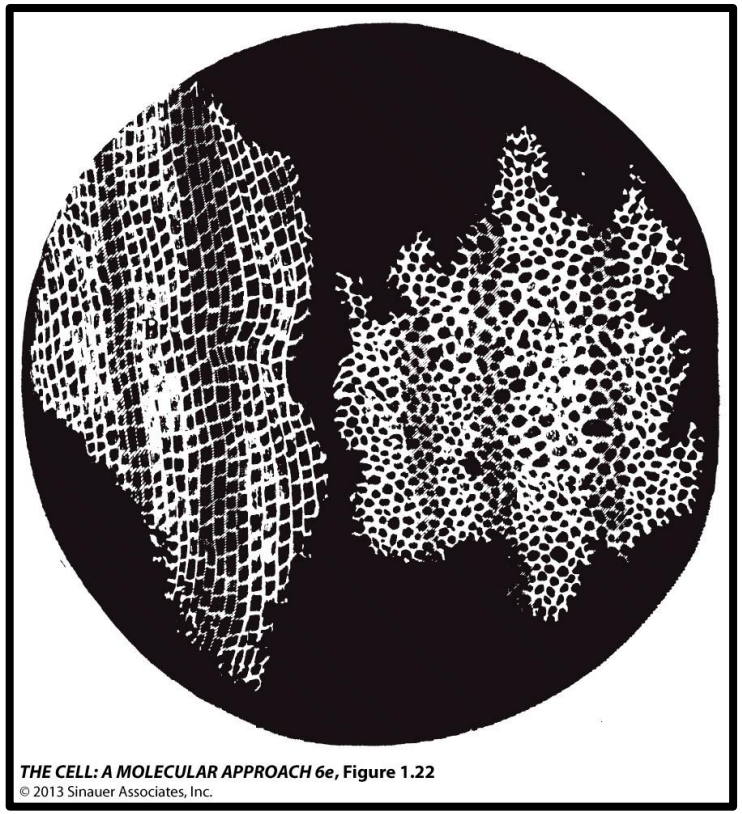
PLEASE TURN IN YOUR “TELL ME ABOUT YOURSELF” PAGE



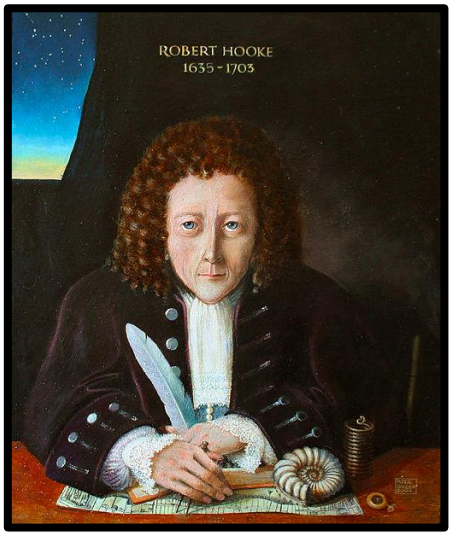
Cellular Biology and Culture: History and Applications

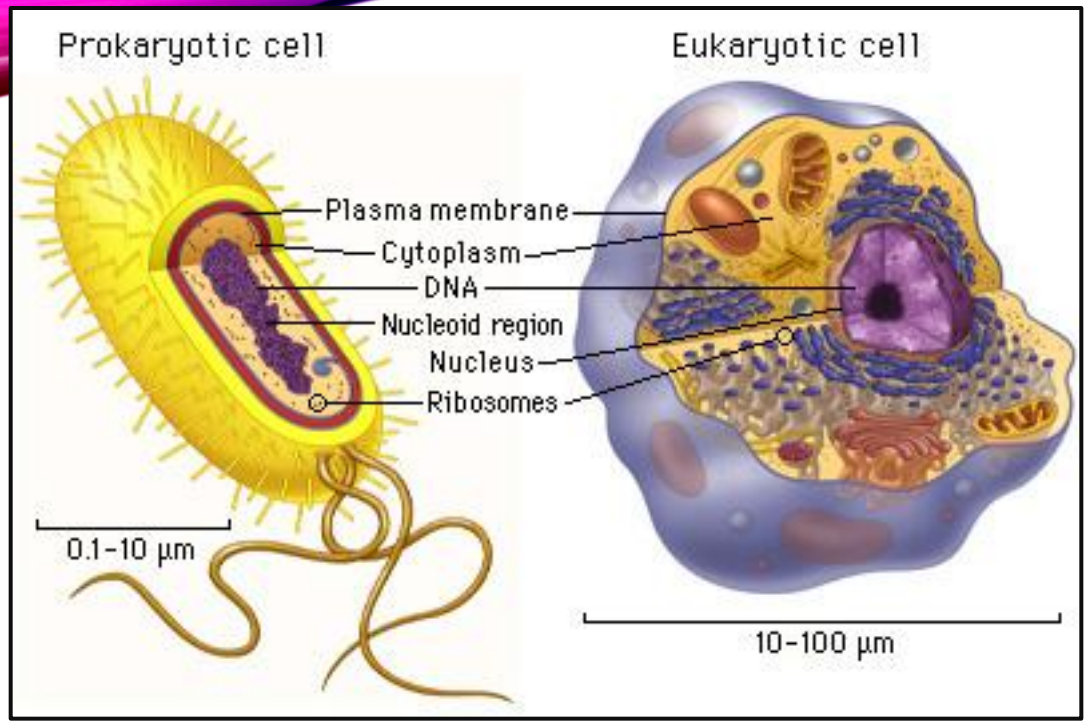
CELLS: BUILDING BLOCKS OF LIFE

Robert Hooke 1665 “Micrographia”



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 1.22**
© 2013 Sinauer Associates, Inc.





Prokaryotic	Eukaryotic
Small cells (< 5um)	large cells (> 10um)
always unicellular	often multi cellular
no nucleus instead has a 'naked loop' called a nucleoid	always has nucleus with linear DNA (chromosomes) and histones
no membrane bound organelles	membrane bound organelles
ribosomes are small (70s) s = **svedberg** unit of measure of the size of organelles.	ribosomes are large (80s)
no mitochondria	mitochondria present
cell division by binary fission	cell division by mitosis or meiosis
reproduction mainly asexual however there are some non meiotic forms of gene exchange such as **conjugation**.	reproduction asexual or sexual
many metabolic pathways, fermentation, nitrogen fixation and photosynthesis.	common form of respiration involving organic molecules like sugar.

CELLS AS EXPERIMENTAL MODELS

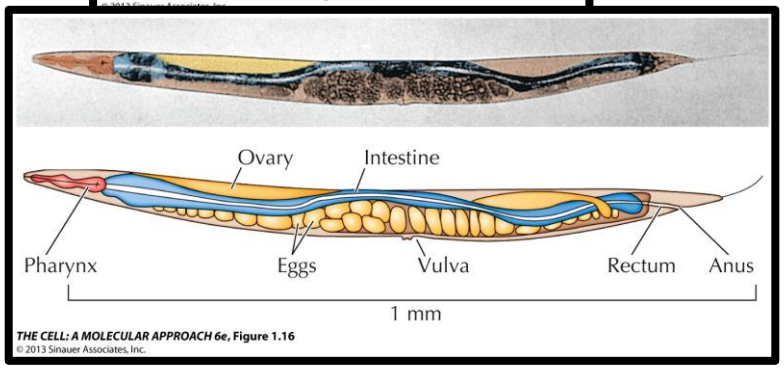
- **Bacteria (*E. coli*)**
  - Model for fundamental biochemistry and molecular biology
  - 4.6 Mbp, 4,300 genes

- **Yeasts (*Saccharomyces cerevisiae*)**
  - Simplest eukaryotes
  - 12 Mbp, 6,000 genes

- **Nematode (*C. elegans*) and fruit fly (*Drosophila melanogaster*)**
  - Simple multicellular organisms
  - model for animal development and cell differentiation

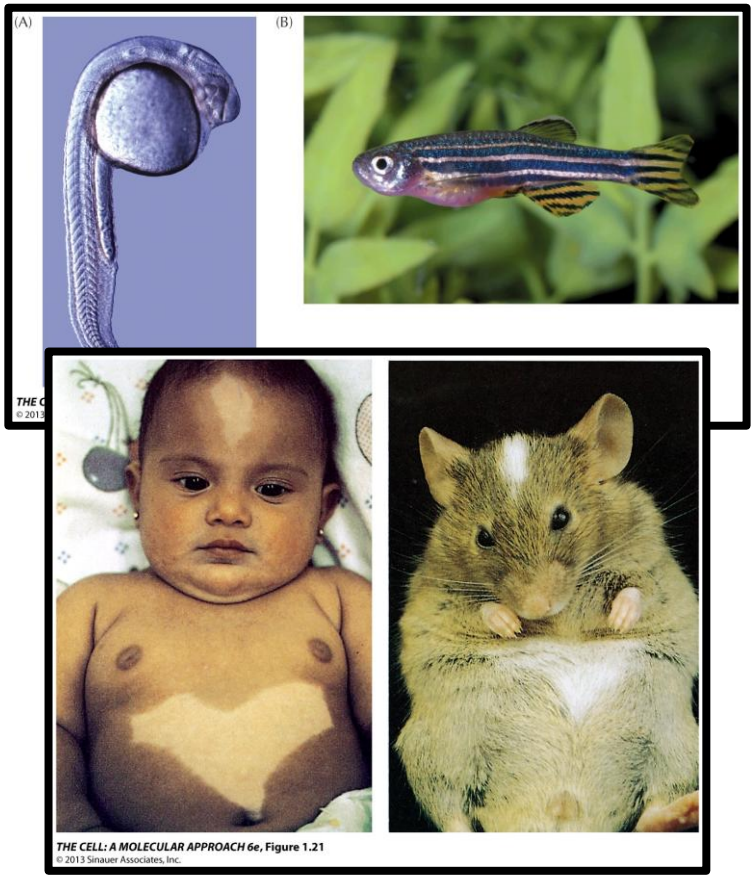
- **Plant (*Arabidopsis thaliana*)**
  - Simple genome (125 Mbp), easily grown in lab setting





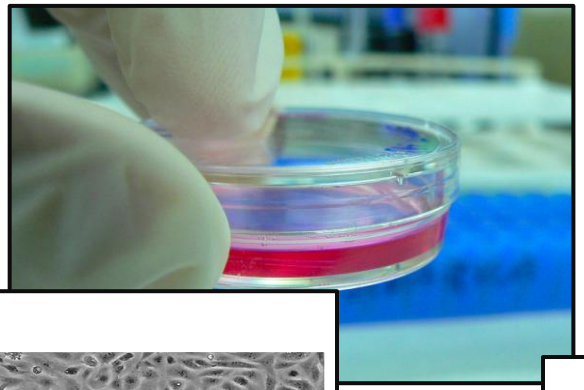
CELLS AS EXPERIMENTAL MODELS

- **Frog (*Xenopus laevis*) and Zebrafish**
  - •Models for early vertebrate development
- **Mice**
  - •Genetically engineered animals allow study of mutations in context of entire animal
  - •Often a great model for humans
- **Humans**
  - •3 Bbp, 20,000-25,000 genes
  - •Grow isolated cells in culture, including specialized cells

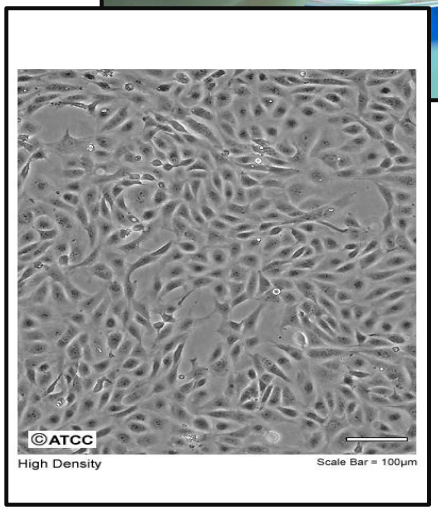


CELL CULTURE

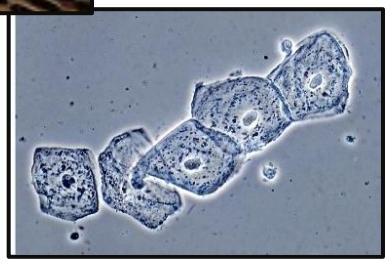
The complex process by which cells are grown under controlled conditions, generally outside of their natural environment.











HISTORY – CULTURE OF ANIMAL CELLS

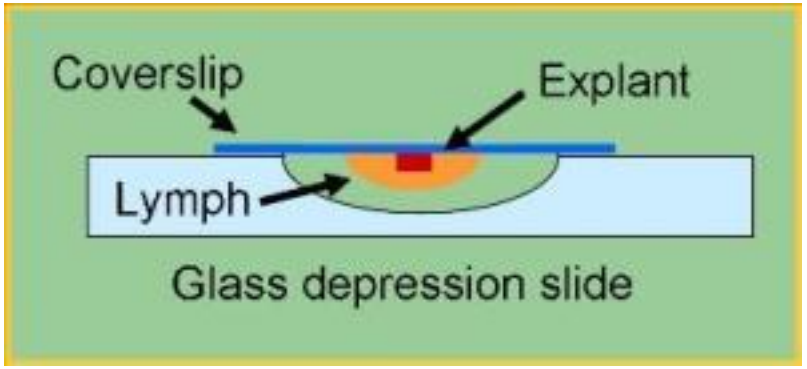
1907 – The birth of tissue culture: Ross Granville Harrison "The outgrowth of the nerve fiber as a mode of protoplasmic movement"

1907-1950s – Alexis Carrel frontier tissue culture and the famous chicken heart cultures that "supposedly" survived 35 years.





Photos courtesy of NCI Visuals Online; source: G. Terry Sharrer, Ph.D. National Museum of American History, unknown photographer.



HISTORY – CULTURE OF ANIMAL CELLS

1948 – Development of first cloned cell strain, L929, which was isolated by capillary cloning from mouse L-cell fibroblasts.

1952 – George Otto Gey established first continuous human cell line, HeLa – cells harvested from the cervical carcinoma of Henrietta Lacks.

1950s – Development of defined media and wide use of antibiotics in media.





ADVANTAGES OF TISSUE CULTURE

1. **Control of the environment**
   - Just like in any good experiment, we want to control as many external factors as possible, to minimize any confounding influences
   - We easily control the physiochemical environment (pH, temperature, osmolality, dissolved gases)
   - More difficult to control the physiological environment (hormone and nutrient concentration) but becoming more feasible with the development of serum-free media and cloned matrix constituents

2. **Characterization and homogeneity of samples**
   - In contrast to tissue samples, cell cultures assume a homogenous constitution
   - Cell cloning allows for further homogeneity
   - Easily performed assays with virtually identical samples at each subculturing

ADVANTAGES OF TISSUE CULTURE

3. Economy, scale and mechanization

- Easily control cell exposure to reagents without complications such as reagent metabolism and bioavailability *in vivo*
- Multi-well plating and robotics allows for large scale studies with minimal time and expense

***4. In vitro* modeling of *in vivo* conditions**

- Screening tests of reagents in cell culture avoid much of the legal, moral and ethical questions of animal experimentation
- Allows for tight regulation of the concentration, duration and metabolic state of experimental compounds
- Advances in histotypic and organotypic models allows for even more accurate replication of *in vivo* conditions

LIMITATIONS OF TISSUE CULTURE

1. **Expertise**
   - Sterile handing and environmental control
   - Skill of operator to identify problems such as microbial contamination or cross-contamination (stock authentication)
2. **Quantity**
   - Requires expensive equipment and advanced training for a relatively small amount of tissue



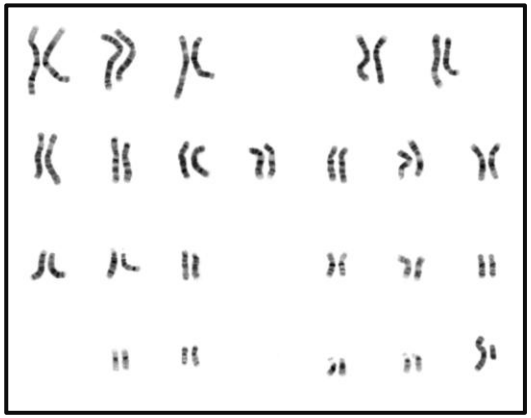
LIMITATIONS OF TISSUE CULTURE

3. Origin of cells

- Long history of cross-contamination, errors in stock control and overgrowth of undifferentiated cells leading to misidentification of cell lines
- Requires technology to authenticate each cell line used

4. Instability

- Genetic and phenotypic instability of cell lines, resulting in large part from their unstable aneuploid chromosomal constitution, leads to heterogeneity and variability from one passage to the next





CELL CULTURE IS SIMPLY NOT THE SAME AS IN VIVO

• Loss of the three-dimensional structure and of the mixed cell population means cell interactions characteristic of the histology of the tissue of origin are lost

• Culture environment also lacks systemic components involved in homeostatic regulation in vivo – hormones, nervous influence, oxygen tension

• Cannot accurately replicate the in vivo effects of pharmacokinetics, metabolism, and organ or systemic responses in a in vitro model



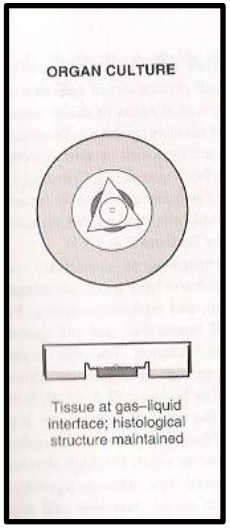
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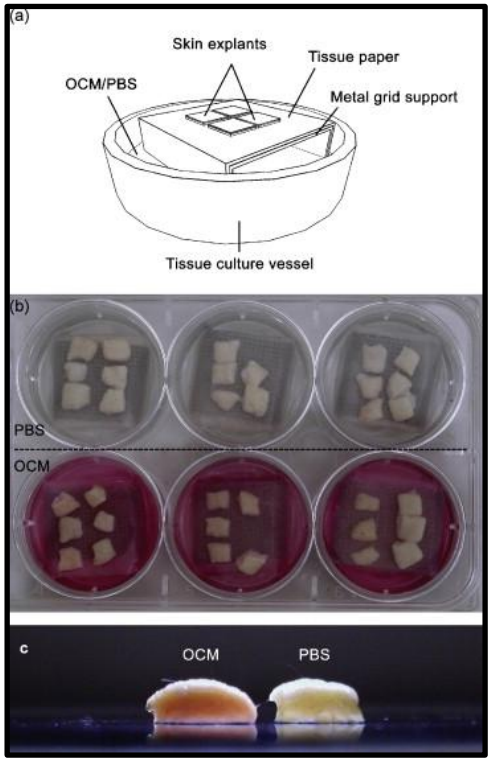
TYPES OF TISSUE CULTURE

1. **Organ Culture – architecture of the tissue in vivo is retained**

   - Source: embryonic organs, adult tissue fragments
   - Effort: high
   - Characterization: easy, by histology
   - Propagation: not possible
   - Homogeneity: high inter-sample variation



Culture of Animal Cells, 6th Ed (Wiley-Blackwell)

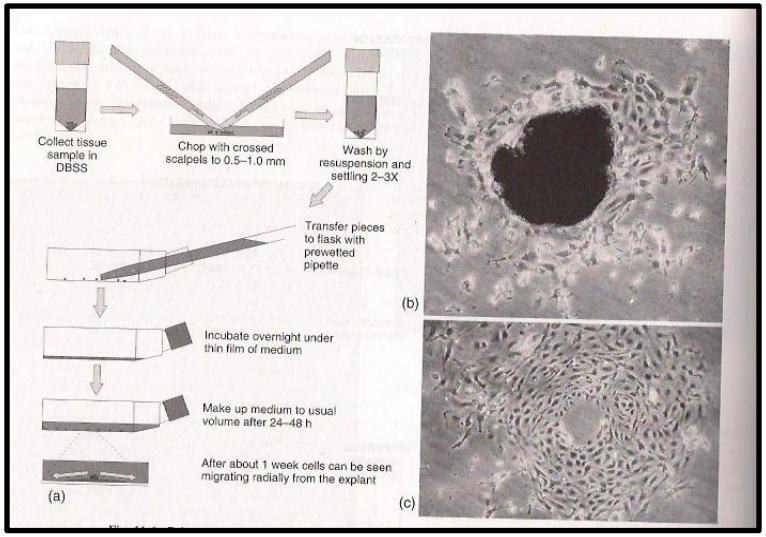


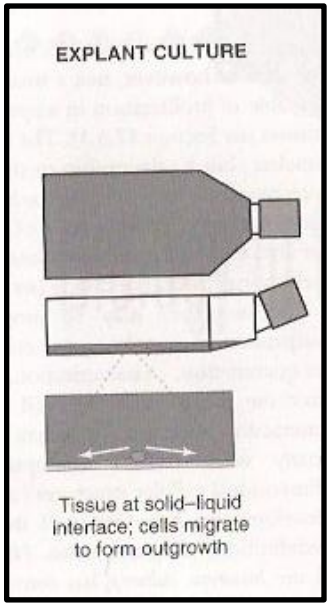
Development of an ex vivo human skin model for intradermal vaccination: tissue viability and Langerhans cells behavior. *Vaccine* (2009)

TYPES OF TISSUE CULTURE

2. Primary Culture – a fragment of tissue which is dissociated

- Source: tissue fragment; by explant or enzymatic dissociation
- Effort: moderate
- Characterization: cytology and markers
- Propagation: possible from outgrowth
- Homogeneity: high inter-sample variation



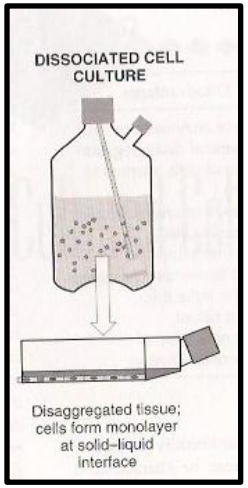


Culture of Animal Cells, 6th Ed (Wiley-Blackwell)

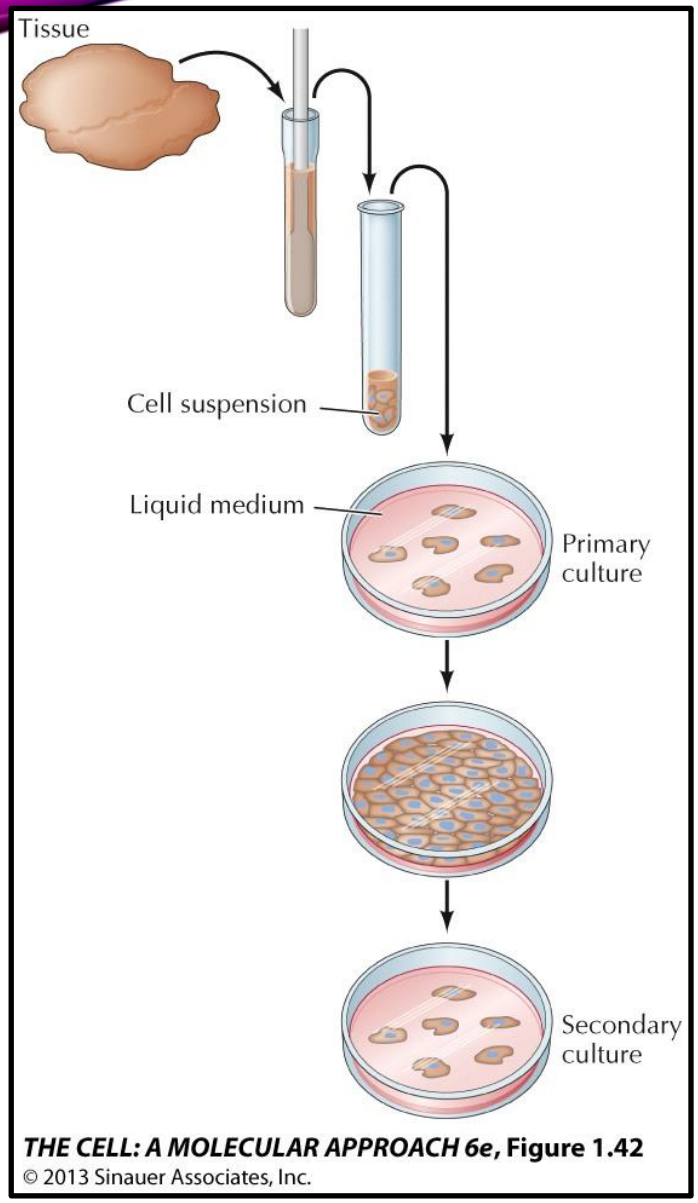
TYPES OF TISSUE CULTURE

3. Cell Culture – dispersed cells originating from a primary culture

- Source: disaggregated tissue, primary culture, propagated cell line
- Effort: low
- Characterization: biochemical, molecular, immunological and cytological assays
- Propagation: standard procedure. May be finite or continuous
- Homogeneity: low inter-sample variation



Culture of Animal Cells, 6th Ed (Wiley-Blackwell)



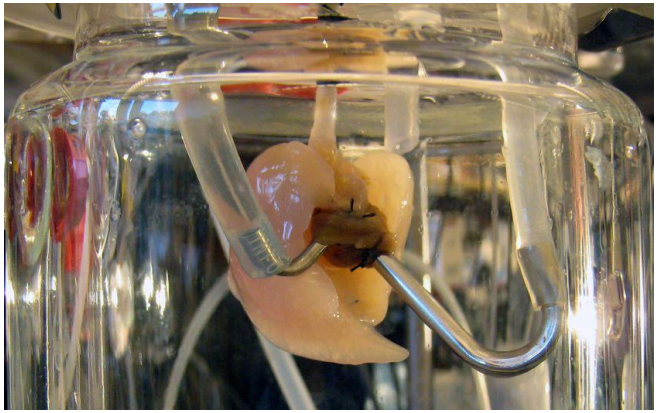
***THE CELL: A MOLECULAR APPROACH 6e*, Figure 1.42**

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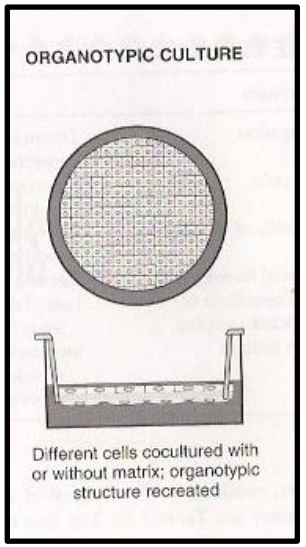
TYPES OF TISSUE CULTURE

4. Organotypic or Histotypic Culture

- Source: primary culture or cell lines
- Effort: moderate
- Characterization: histology, confocal microscopy, MRI
- Propagation: only after dissociation
- Homogeneity: low intersample variation

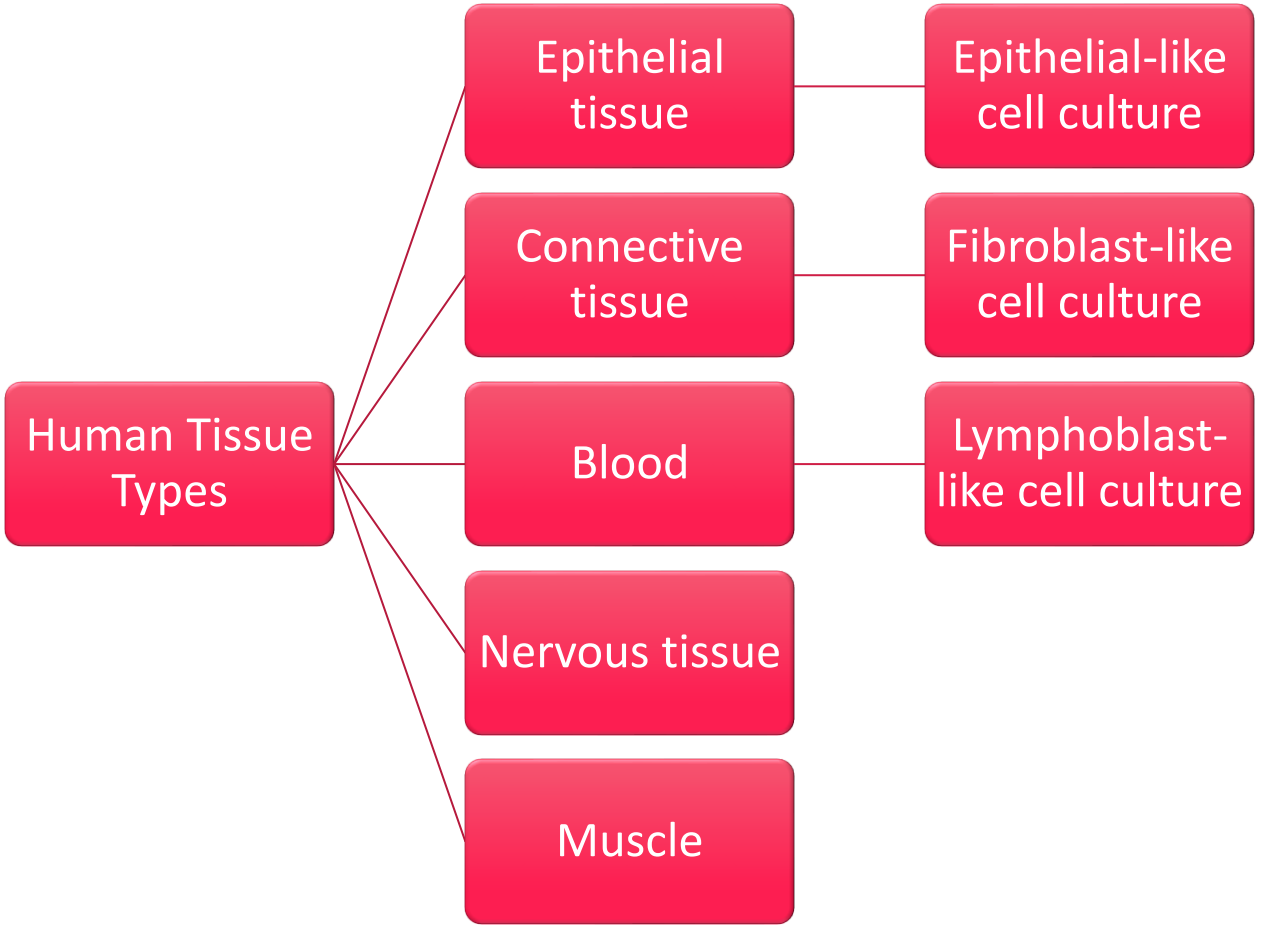


Regeneration and orthotopic transplantation of a bioartificial lung. *Nature* (2010)

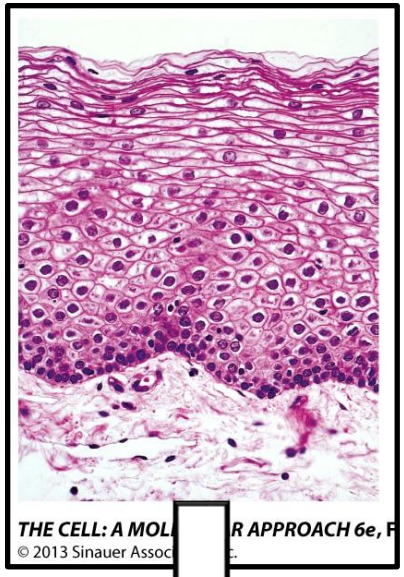


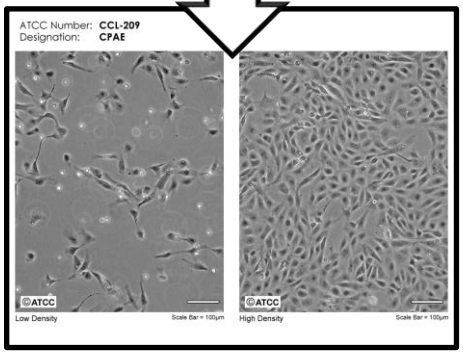
Culture of Animal Cells, 6th Ed (Wiley-Blackwell)

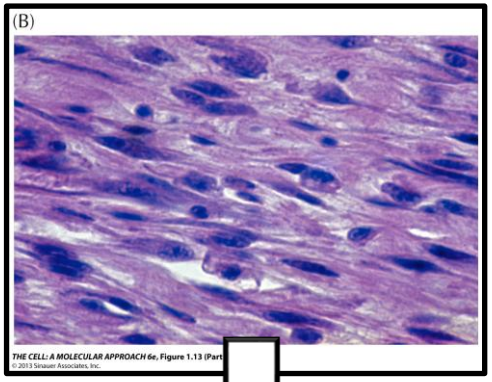
TYPES OF CELLS IN CULTURE

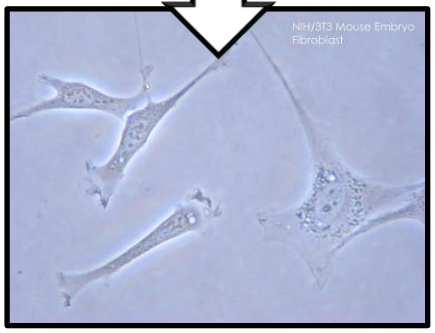


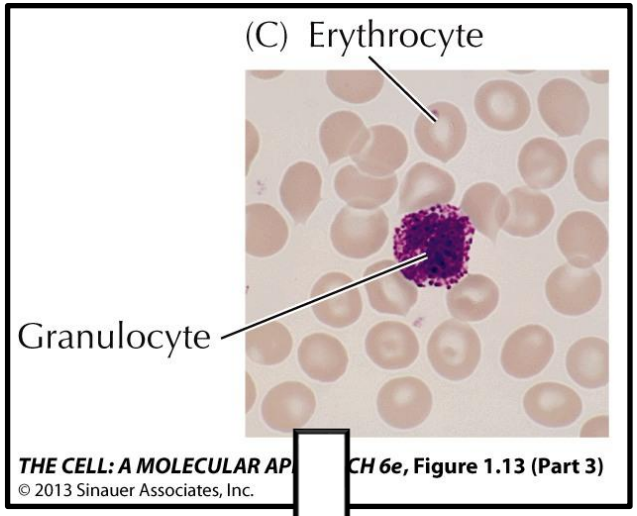
TYPES OF CELLS IN CULTURE

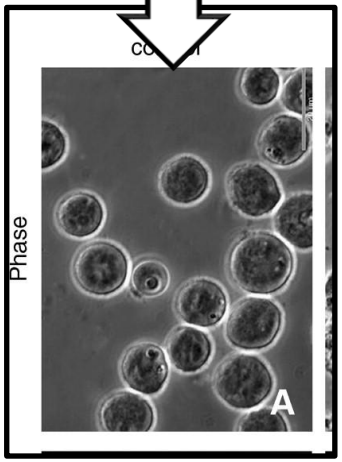












RECOMMENDED READING

