UNIT 3.2

Cells and Spaceflight Hardware

Presented by:
Dr. Nancy Searby

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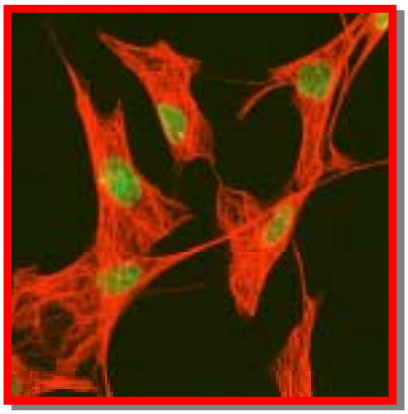


Cells and Spaceflight Hardware

Dr. Nancy Searby

Vocabulary:

- Prokaryote
- Eukaryote
- Confluent

Purpose:

To become familiar with the unique requirements cells place on spaceflight hardware and to learn from existing and planned spaceflight cell culture hardware.

Objectives:

To understand:

a) The diversity of cell types of interest to the space program

b) Environmental conditions cells need

c) Other considerations

d) Operations for cell experiments

e) Supporting well-controlled experiments

f) Current approaches to cell biology hardware

g) Beyond basic culture capabilities

Why Study Cells in Space?

NASA Strategic Plan

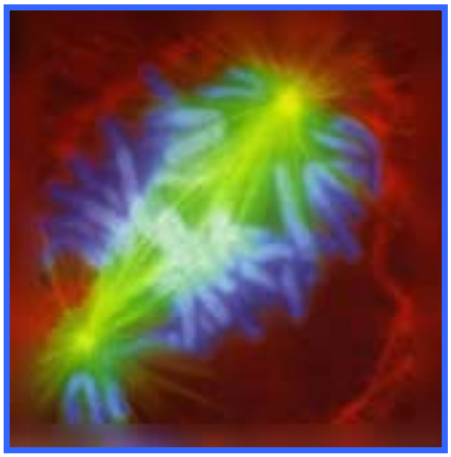
"What is the fundamental role of gravity in vital biological systems in space?"

Absence of Gravity Can Only be Tested in Spaceflight

System Level		**Component Level**
Complex		*Simpler*
Animals		Animal Cells
Plants		Plant Cells
		Unicellular Organisms

Critical Questions for ISS Cell Research

Determining the fundamental role of gravity in vital biological systems in space is one of six science and research areas that provide the philosophical underpinning for why NASA exists.

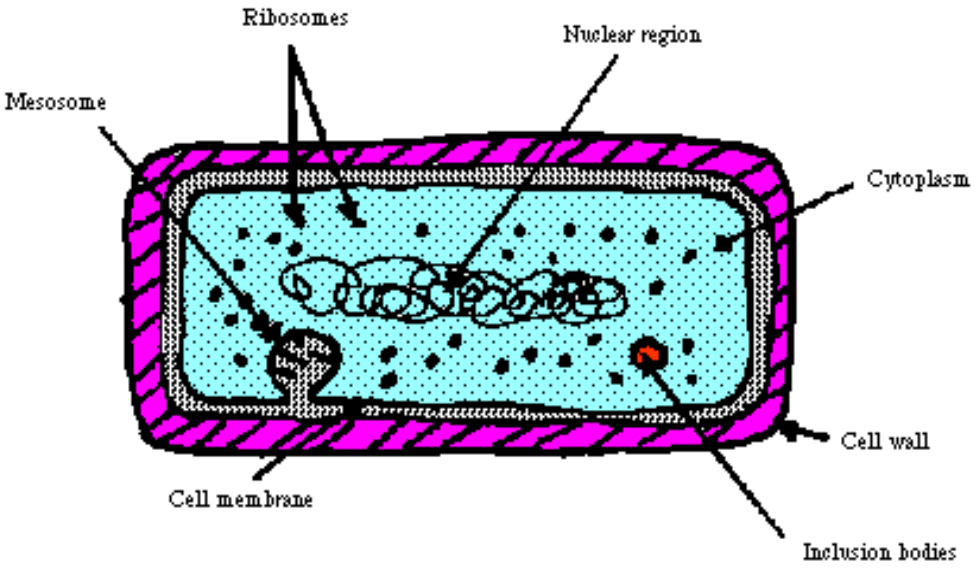


- Can single cells sense gravity?
- Do cells sense gravity directly or indirectly?
- How do cells transduce altered gravity to a cellular response?
- How are cell-cell and cell-surface contacts in multi-cellular systems affected by microgravity?

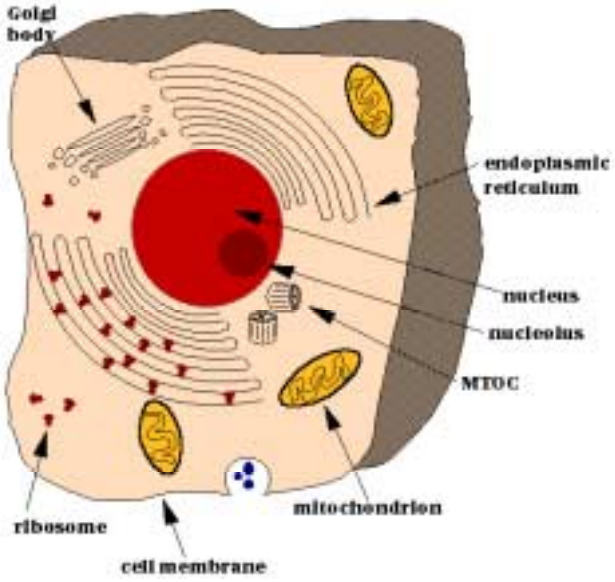
Cellular Diversity

Two types of cells:

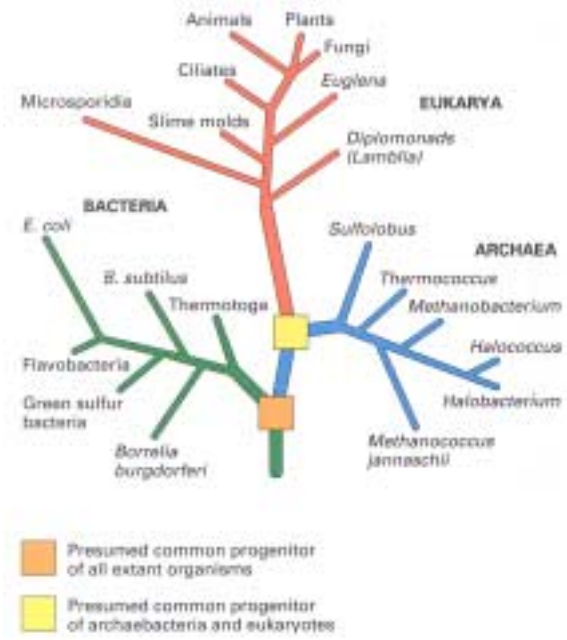
Prokaryotic cells (~1 μm) lack a defined nucleus
"true" bacteria
archaebacteria



Eukaryotic cells (~10s μm) contain a membrane-limited nucleus



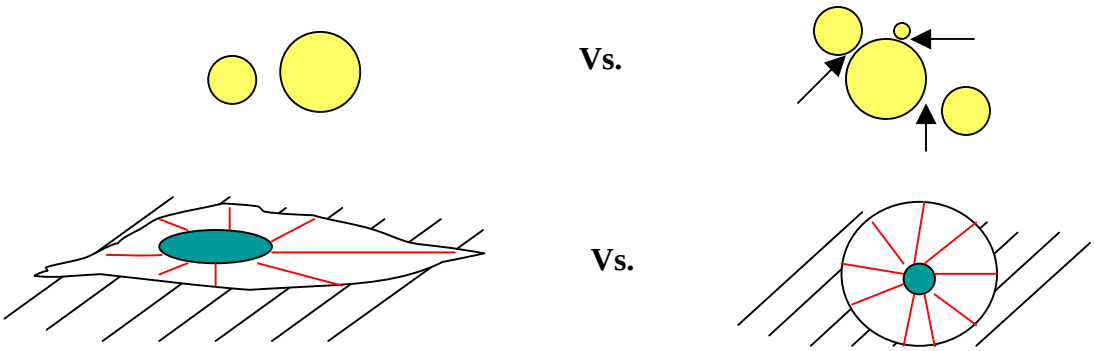
Cellular Diversity as Shown on a Phylogenetic Tree



Lodish, Molecular Cell Biology, 2000

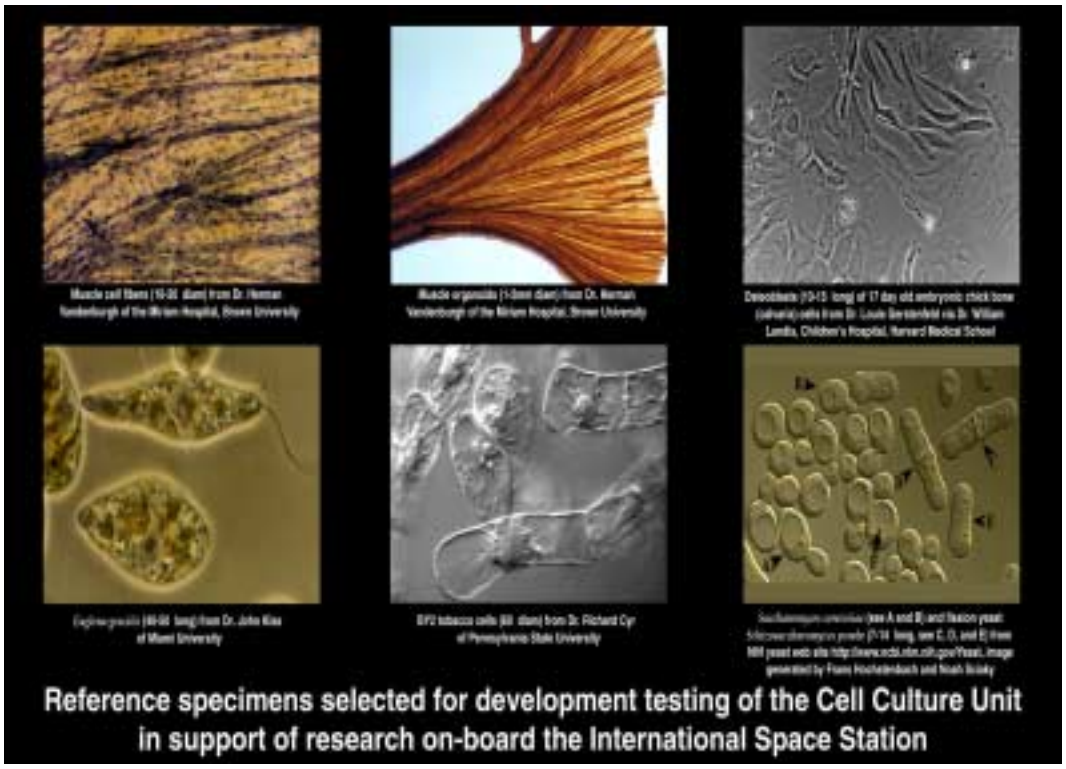
Do cells respond to gravity?

- Gravity sensing cells - plant cells, cells of the vestibular system
- Potentially gravity sensing cells - yeast bud formation, cytoskeleton of mammalian cells



- Prokaryotes (bacteria) - not presumed to be gravity sensing - but respond to the altered microenvironment (lack of convection, buoyancy)
- Results from previous space flight studies:
  - Plant cell amyloplasts are centrally clustered
  - Yeast bud formation alterations
  - Mammalian cell gene expression and morphology altered
  - Bacterial growth rates, antibiotic resistance changes

Examples of Cellular Diversity



Cellular Diversity - Cells to Test Hardware

Cell Type	Hardware Driver	Spaceflight Interest
Muscle cells C2C12 murine myoblasts	adherent cell line	relevant to spaceflight muscle atrophy
Muscle tissue Bioartificial muscles (Dr. Vandenburgh)	tissue culture	relevant to spaceflight muscle atrophy
Bone cells primary osteoblasts	adherent primary cell	relevant to spaceflight bone loss
Euglena gracilis	requires light motile cell	Cell motility, gravi- vs. light- tropism
Plant cells BY2 tobacco cell line	produce profuse extracellular matrix	large mutant pool
Yeast *S. cerevisiae*	Produces lots of gas (CO_2)	model organism, complete genome unknown

What does a cell need to grow?

- Nutrients and regulatory molecules from culture medium (pH 5.6 - 7.4)
- Temperature Control (15 - 37°C)
- Gas Exchange
- Cell attachment surface or means of stirring

Cell Environmental Conditions

Parameter	C2C12	Avian Obs	Avian Organoids	Tobacco	Euglena	Yeast
Temperature	37°C	37°C	37°C	26°C	23°C	25°C
Gas	5% Co_2, air	5% Co_2, air	5% Co_2, air	air	air	air
Estimated 0_2 consumption rate	0.05 μmol / 10^6 cells/hr	0.1 μmol / 10^6 cells/hr	0.1 μmol / 10^6 cells/hr			0.3 μmol / 10^6 cells/hr
Medium pH	7.2 – 7.4	7.2 – 7.4	7.2 – 7.4	5.0	3.5	5.5
Cell #	6 x 10^3 cells/cm^2	6.5 x 10^3 cells/cm^2	N/A	1 x 10^5 cells in 10 ml	5 x 10^4 cells/ml	5 x 10^5 cells/ml

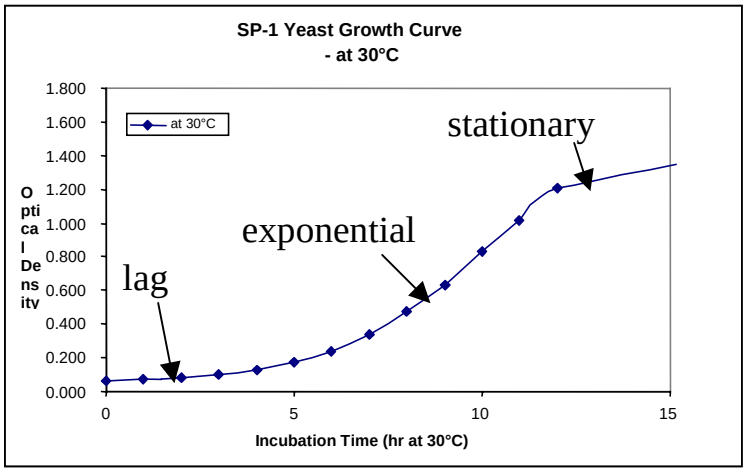
Estimated oxygen consumption: 10^8 cells over 30 days

- Respiration Rates:
    - Chick tibia: 0.035 μmol $O_2/10^6$ cells/hr
    - Hybridoma: 0.35 μmol $O_2/10^6$ cells/hr
    - E. coli: 3 μmol $O_2/10^6$ cells/hr
- Estimation of total oxygen consumption:
  For 10^8 Hybridoma cells:
  0.35 μmol $O_2/10^6$ cells/hr* 10^8 Cells*(24 hr/day*30 days) = 0.025 mol O_2
- Estimation of volume of air required:
  0.025 mol O2*(1 mol air/0.21 mol O2)* 22.4 L/mol = 2.7 L air

Materials Issues

- Adherent cells require hydrophilic surfaces with surface charge close to water (70 dyn/cm)
    - Polystyrene, untreated; 30 dyn/cm
    - Polystyrene, tissue culture; 60 dyn/cm
    - Glass
- Need acceptable chemical compatibility
- Needs to be cleanable - autoclave, ethanol/uv, gamma sterilization
- Optically clear at desired wavelengths
- Permeability if needed

Cell Experiment Operations



- Extend culture life by subculturing
    - Suspension cell growth
    - Adherent cell growth
        - Contact inhibited
        - Non-contact inhibited
- Provide additives
- Take samples
    - Suspended cells
    - Cell-free media
- Preserve cells and samples
    - Chemical fixation
        - Aldehydes; e.g., glutaraldehyde, formaldehyde
        - Methanol
        - RNA preservatives; e.g., RNAlater
    - Cold
        - 4ºC (refrigerator)
        - -20ºC (freezer)
        - -80ºC (deep freezer)
        - -183ºC (cryogenic freezer)

What do cells need to grow *in space*?

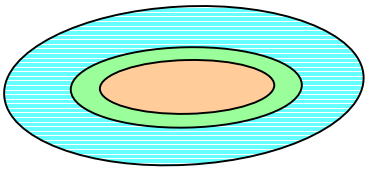
In space….

- No natural convection
- Gas exchange is limited by diffusion
- Liquid-air interfaces must be avoided

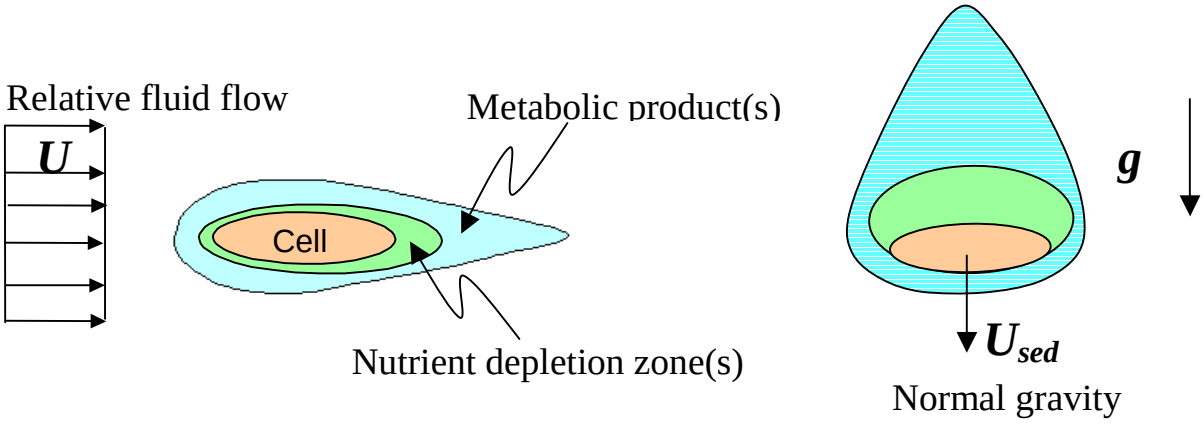
Comparison of solute field around a cell subject to fluid flow, 1g, or microgravity

A living cell consumes nutrients from and emits metabolic products to its local environment. This gives rise to local enrichment/depletion of the product/nutrient fields, which depends on:

- the rate of metabolic processes,
- relative motion between the cell and the surrounding fluid
- gravity



Microgravity



In general, cells are heavier than the surrounding fluid; the metabolic products are typically slightly lighter than the bulk fluid.

Current approaches to cell biology hardware: from simple to complex*

*See supplement

- No gas exchange - test tube, sealed container
- Passive gas exchange - silicone bags
- Active gas exchange - perfusion-based systems

Conclusions

- Cells place unique requirements on spaceflight hardware.
- Diverse cell types need a range of environmental conditions.
- When planning to support cell experiments, consider all operations and support of control experiments.
- Important to learn from existing and planned spaceflight cell culture hardware.
- Great demand to extend next generation capabilities to be able to measure cell responses in flight.

Reference Materials

Literature:

Representative Spaceflight Facilities and hardware for cell culture research, use coordinated by the International Space Life Sciences Working Group (ISLSWG), revised Feb. 2003.

Freshney, R.I. Culture of Animal Cells: A Manual of Basic Technique, 3rd ed.. Wiley-Liss, Inc., New York, NY, 1994.

Lodish, H., Molecular Cell Biology, W.H. Freeman and Co., New York, NY, 2000.

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