

Challenges in Morphogenesis and Tissue Engineering.

"Opportunities in Biological Physics" Workshop
March 4, 2007

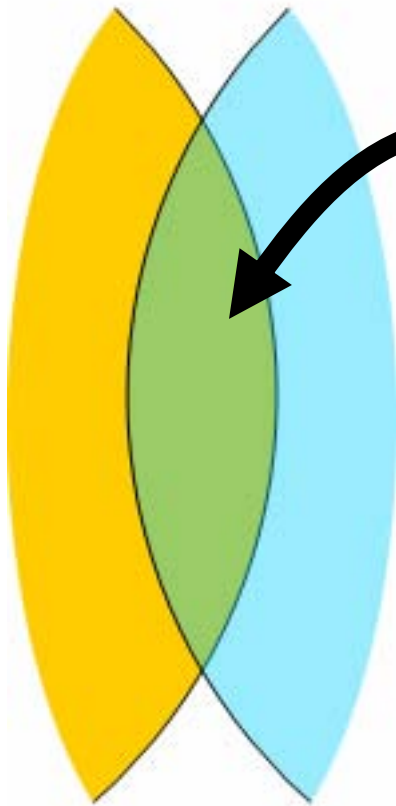
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University of Pittsburgh

Tissue Engineers

- Forward Engineering
- Translational research
- Apply fundamental "laws"
- Build artificial structures
- ... combine with cultured cells.
- Produce clinically useful device

Developmental Biologists

- Reverse Engineering
- Basic research
- What are fundamental "laws"?
- Gene function
- Establishment of pattern
- Elaboration of structure (morphogenesis)



Shared Interests:

1. Reverse / Forward Engineering
Tissues and Organs.
2. Bioimaging
3. Cell and Molecular Biology

Reverse / Forward Engineering Tissues and Organs.

Functional Challenges to Being Multicellular:

Many cellular/physiological processes happen on surface areas

- gas and heat exchange
- nutrient uptake / waste disposal
- secretion
- infection
- evaporation

Area to Volume Ratio Looks Bad for "Large" Organisms

- single cell ~ 10 μm diameter
- vertebrates ~ 100 cm in diameter
(A/V 10,000 times less than single cell)

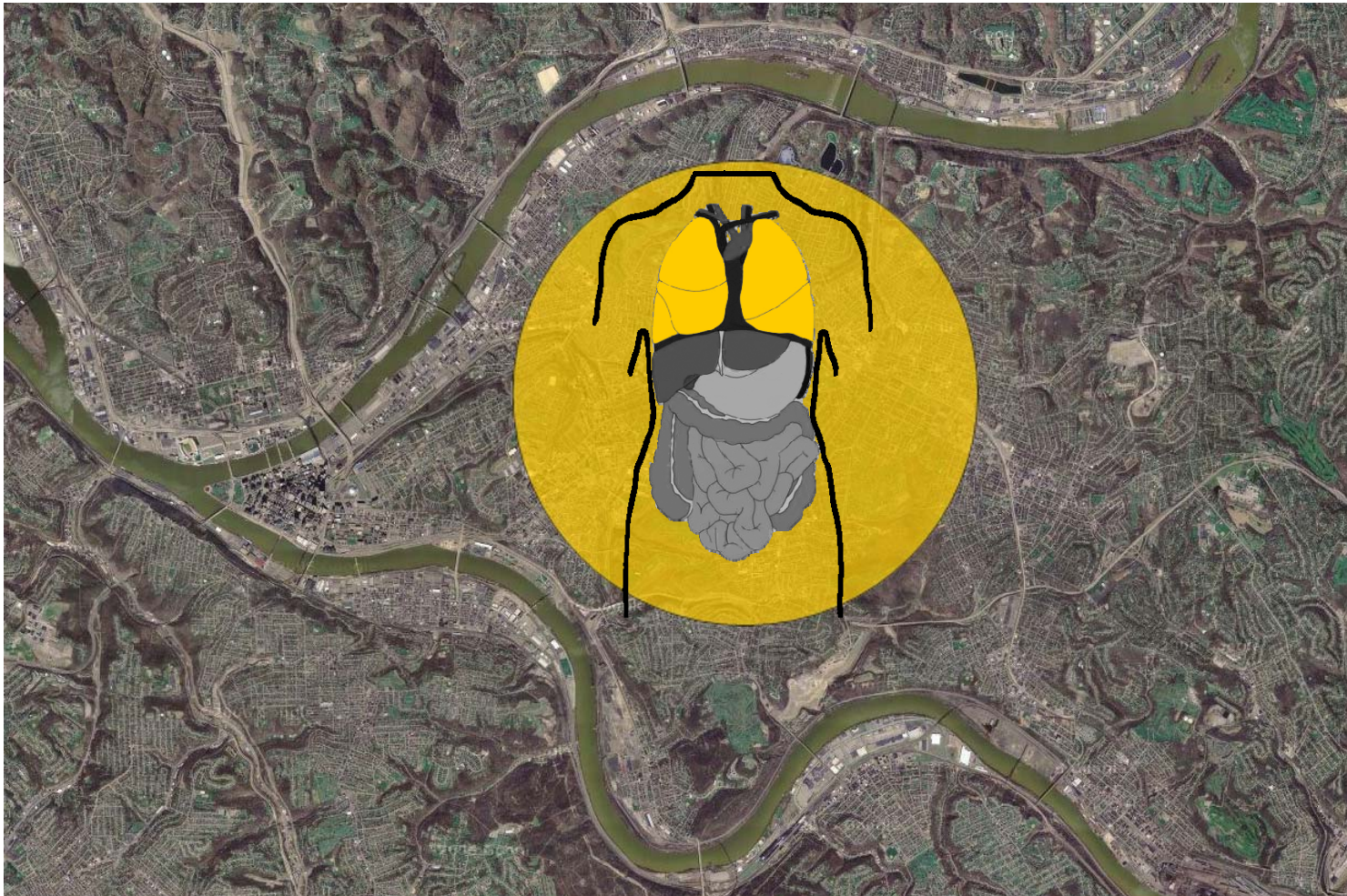
Pittsburgh: 150 km², population 350,000 people



GoogleMaps, 2007

Pittsburgh: 150 km², population 350,000 people

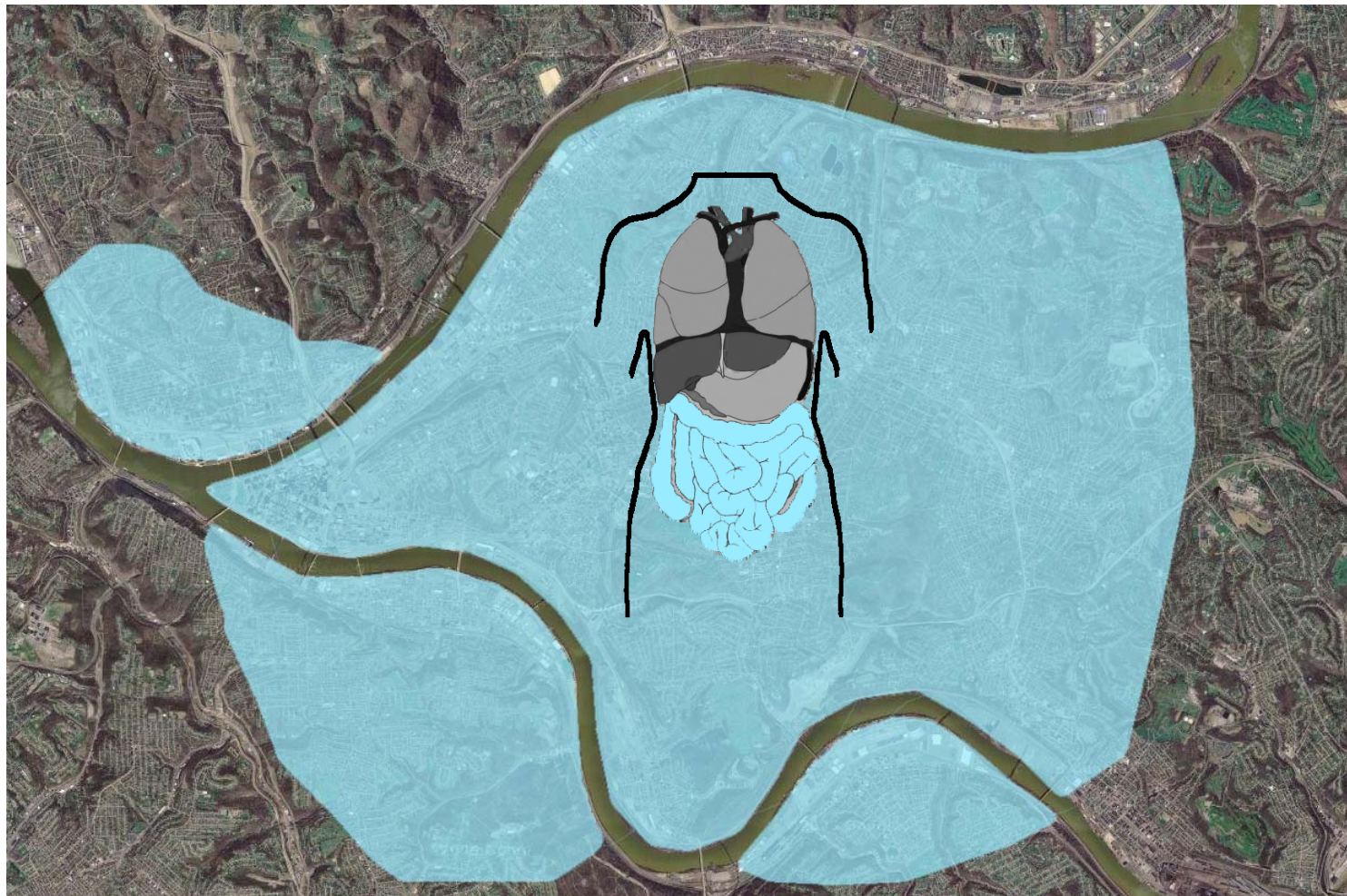
Surface Area of Lungs (~ 100 m² per person) : 35 km²



Pittsburgh: 150 km², population 350,000 people

Surface Area of Lungs (~ 100 m² per person) : 35 km²

Surface Area of Intestines (~ 300 m² per person) : 105 km²



The Challenge:

How do you make functional tissues with large areas from simple balls of cells?

Tissue Engineer's Solution:

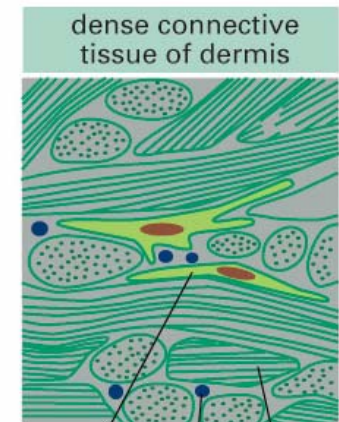
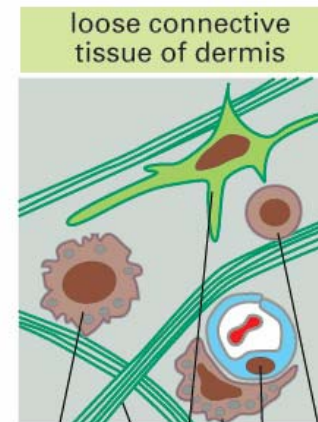
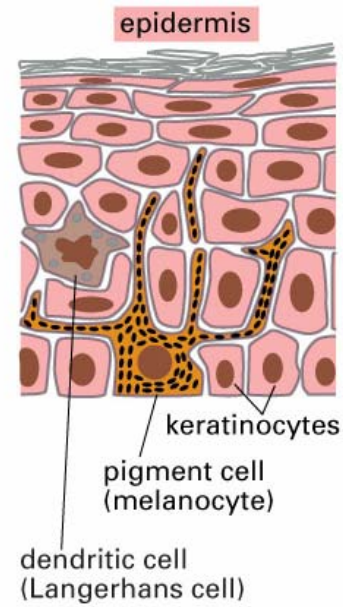
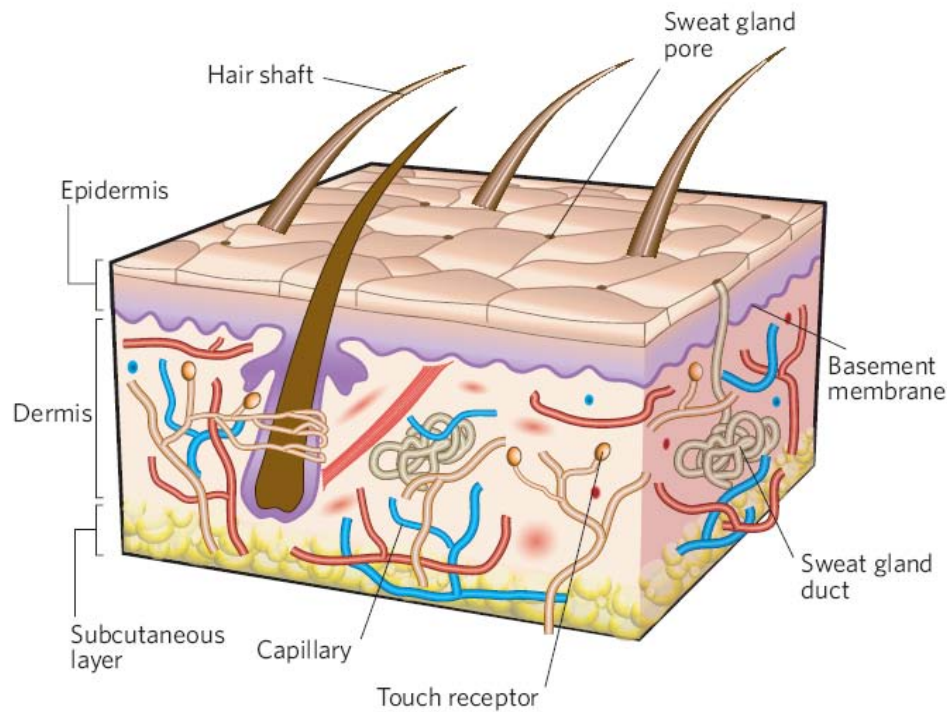
1. Tissue-engineered Skin.

Embryo's Solution:

1. Gastrulation.

2. Branching morphogenesis.

Human Skin



collagen fiber mast cell lymphocyte fibroblast endothelial cell forming capillary macrophage

fibroblast collagen fiber elastic fiber

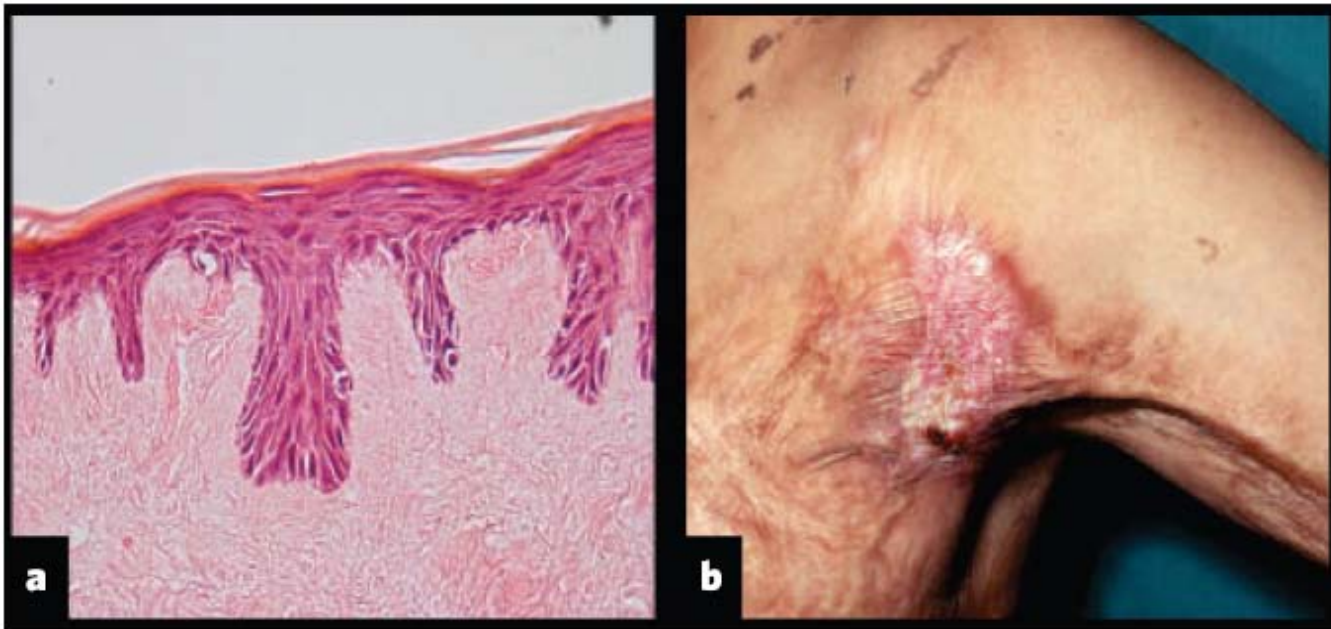
S. MacNeil (2007) Nature, "Progress and opportunities for tissue-engineered skin."

Tissue-engineered Skin

The need for organs and tissues far exceeds the supply...

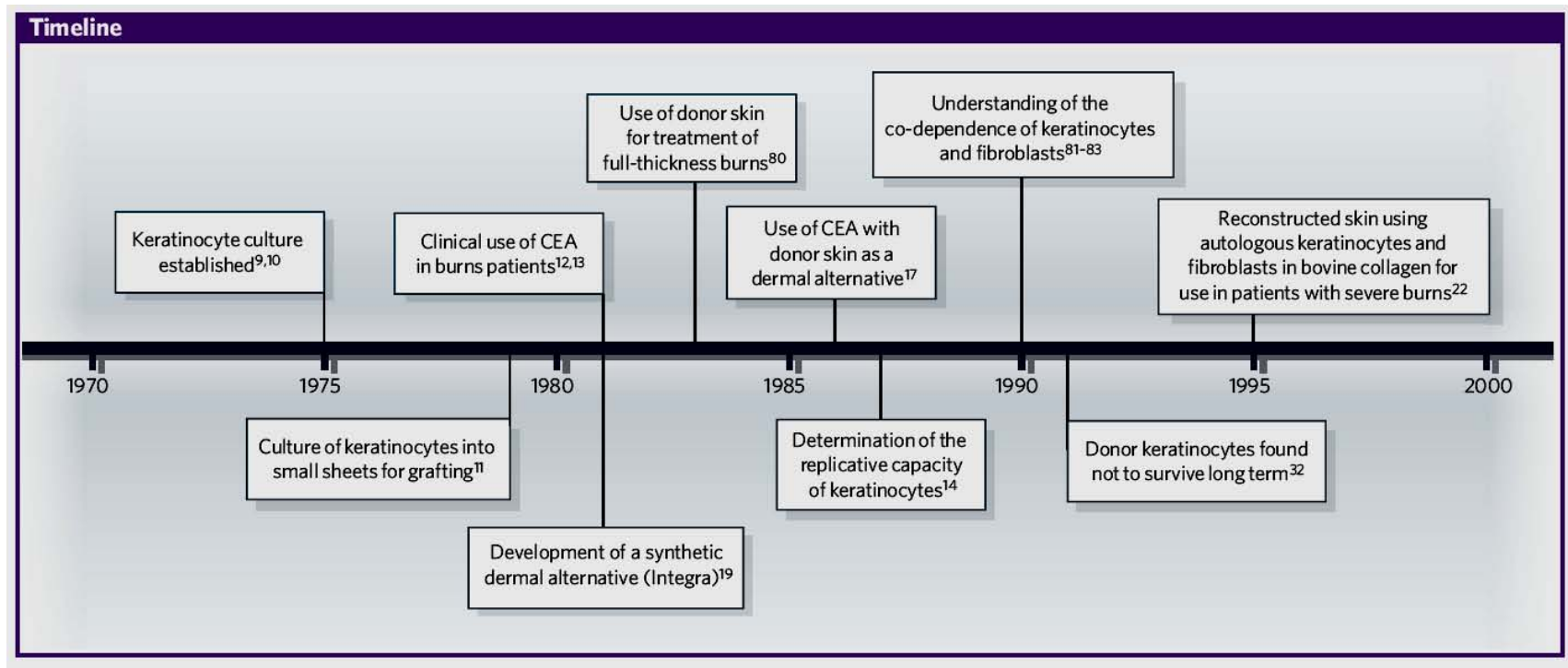
Engineered skin is used to:

- restore the barrier function of damaged or burned skin (prevent infection and reduce evaporation)
- augment wound healing
- ameliorate healing defects such as contractures



S. MacNeil (2007) Nature, "Progress and opportunities for tissue-engineered skin."

Forward-engineering approaches created tissue-engineered skin.



S. MacNeil (2007) Nature, "Progress and opportunities for tissue-engineered skin."

Tissue-engineered Skin

Scaffolds for tissue engineered skin can come from the synthetic materials, cadavers, or pigs.

Cells for engineered skin can come from the patient or other human donors.

In addition to creating an engineered tissue that mimics the native tissue, tissue engineers face challenges to make a "product" that...

- ... is simple to use,
- ... does not harm the patient, and
- ... actually works to cure problem.

Products:

Epidermis/Dermis combined: Apligraf, Orcel, Cambrex

Epithelial Cover: Epicel, Myskin, Epidex, CellSpray

Dermal Replacement: Donor Skin, Integra, Alloderm, Transcyte, Permacol

The Challenge:

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Tissue Engineer's Solution:

1. Tissue-engineered Skin.

Embryo's Solution:

1. Gastrulation.

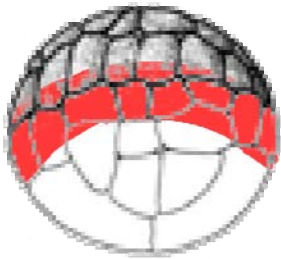
--- Doubling the Surface Area

2. Branching morphogenesis.

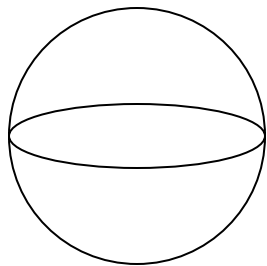
--- Multifold Expansion of the Area

What is gastrulation?

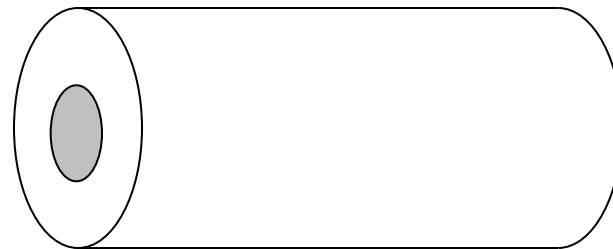
blastula



tadpole

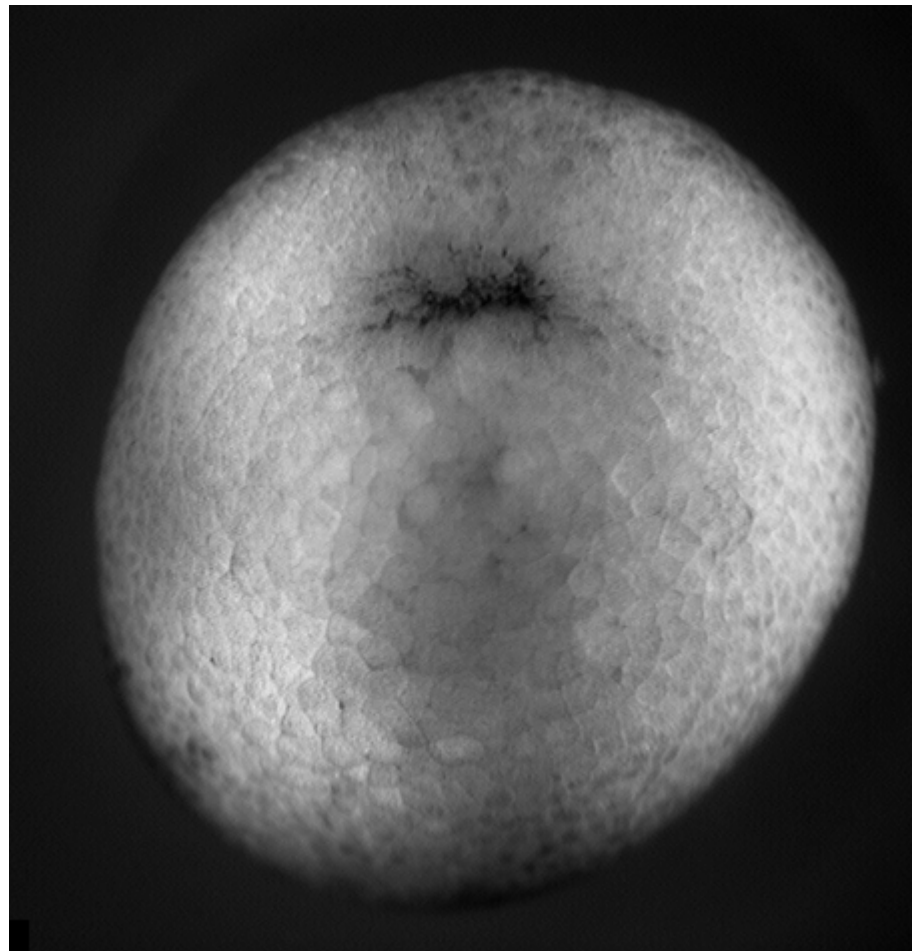
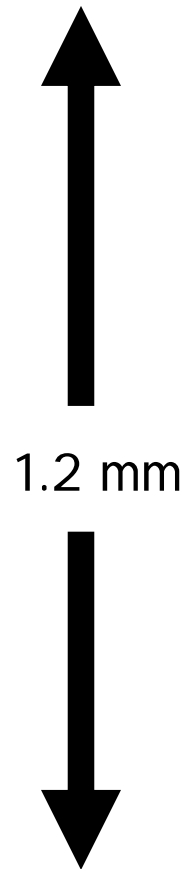


ball



tube

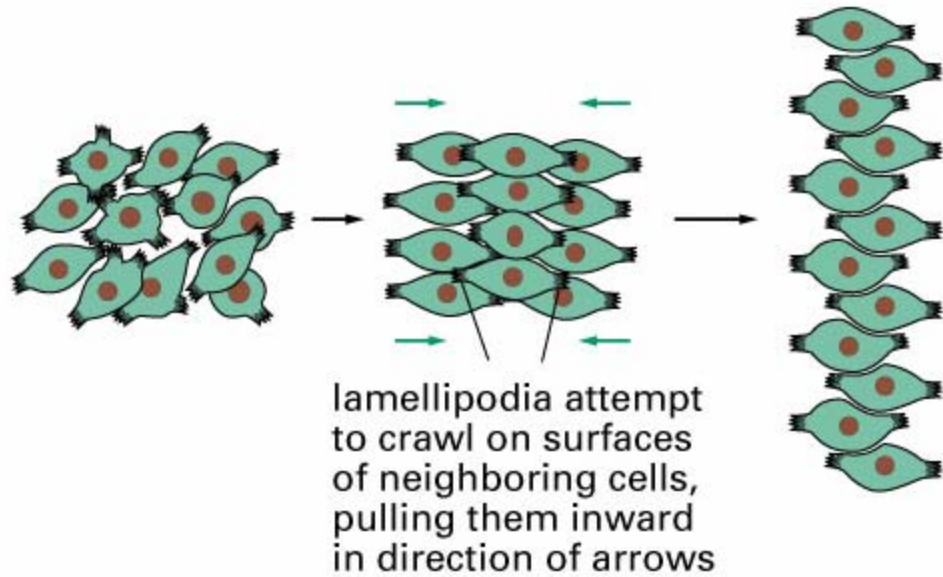
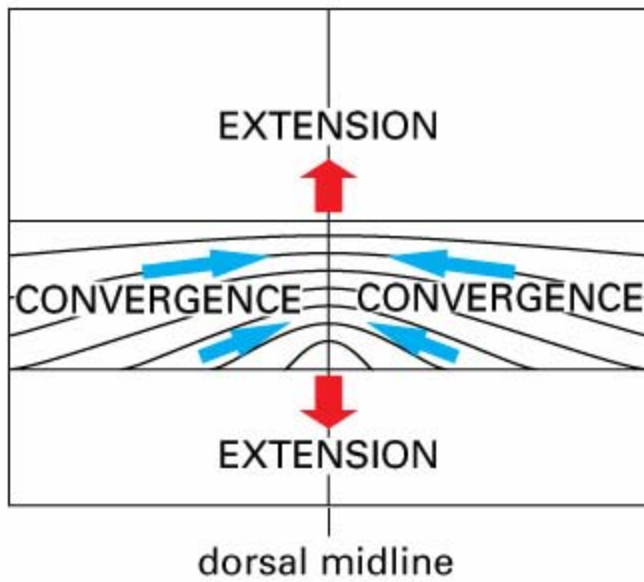
Gastrulation and Axis Elongation



14 hours elapsed time

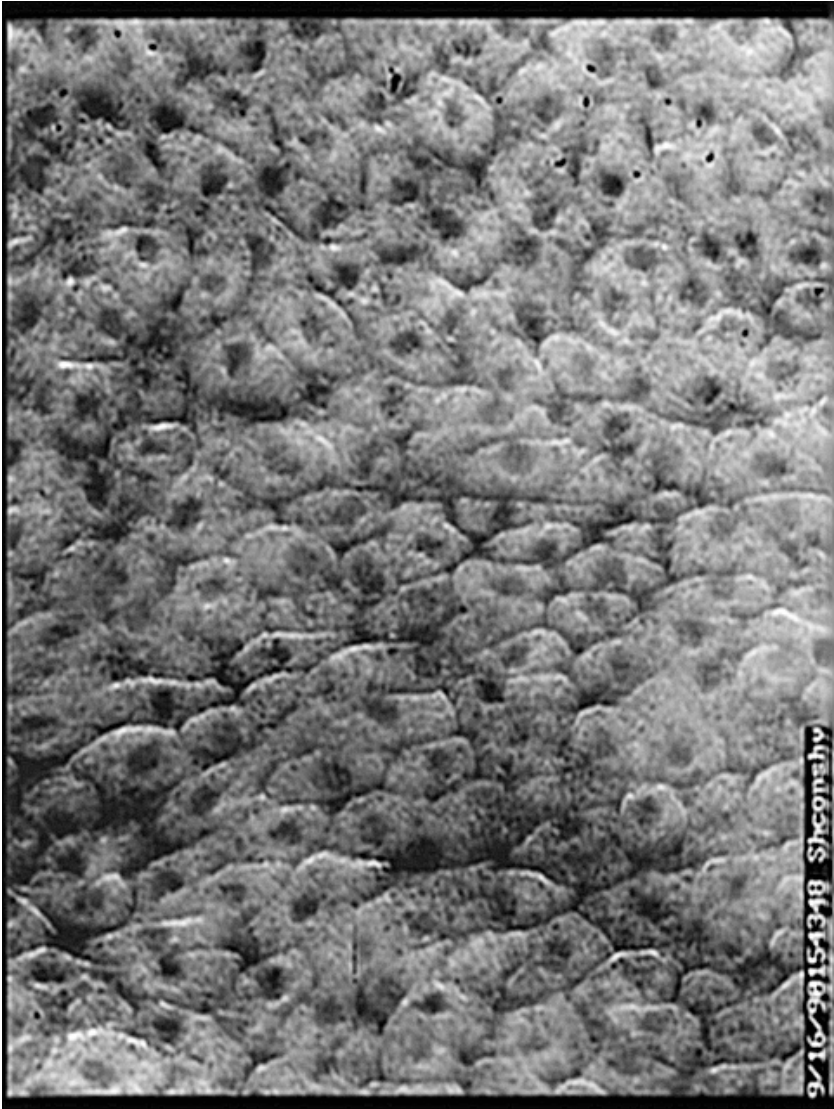
Dave Shook, U. Virginia

Shape changes in the embryo are due to directed cell movements.

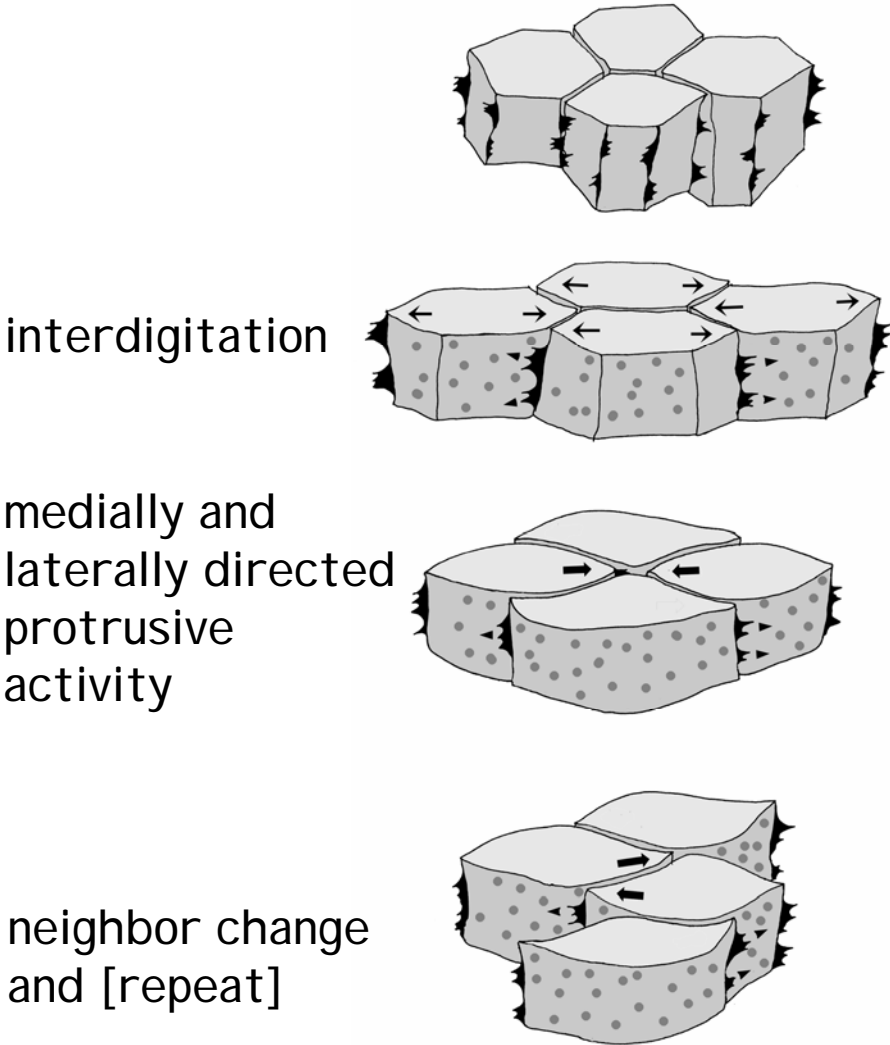


Molecular Biology of the Cell, 4th Edition.

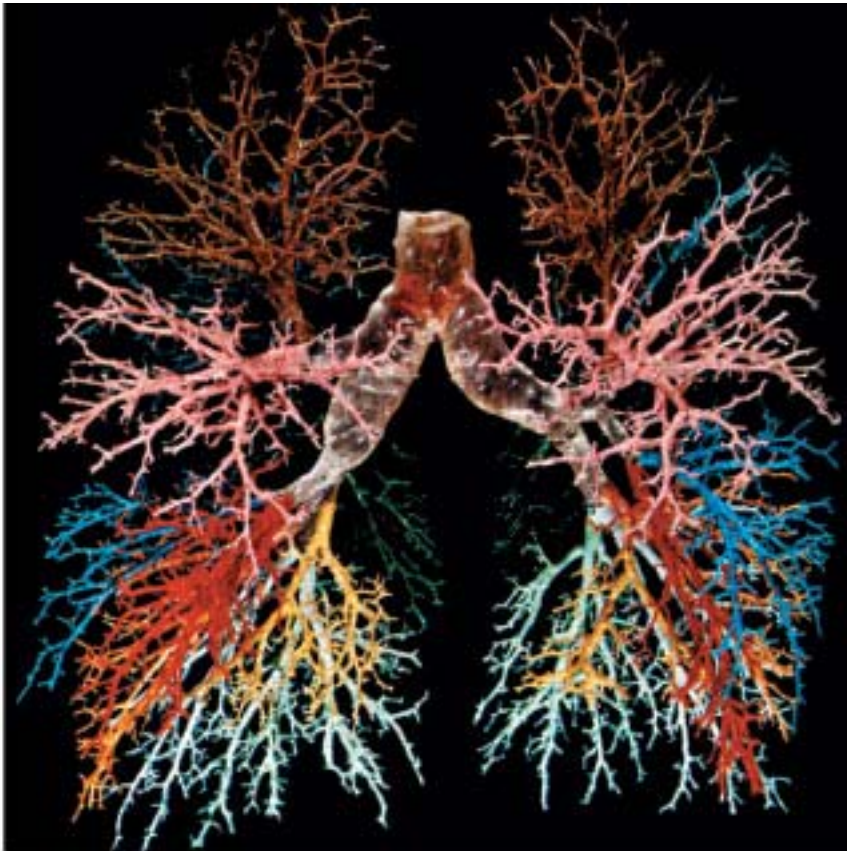
Shape changes in the embryo are due to directed cell movements.



John Shih 8 hours elapsed time



More complex movements of organogenesis begin where the process of gastrulation leaves off...

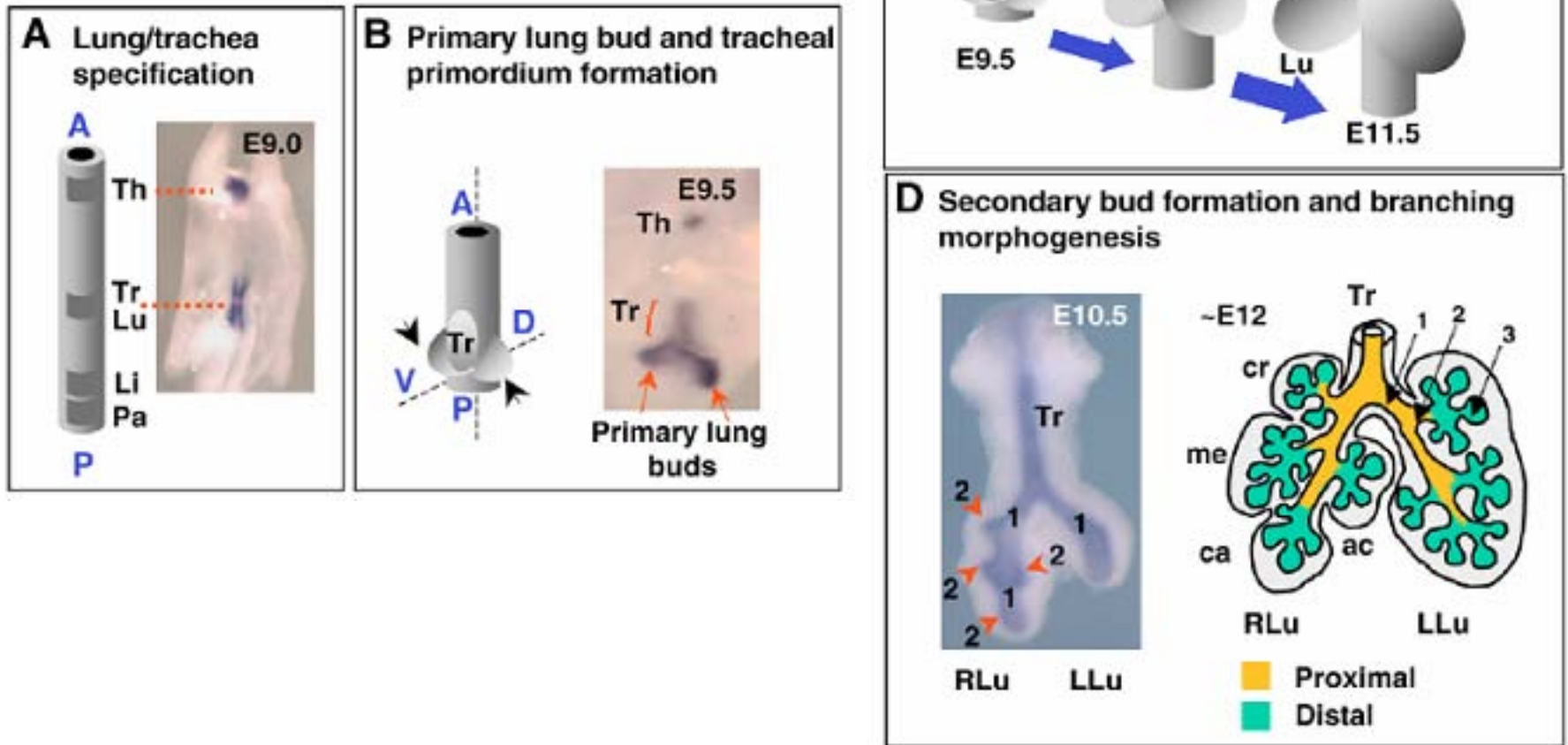


... consider branching morphogenesis during lung formation.

In addition to coordinated cell movement there is:

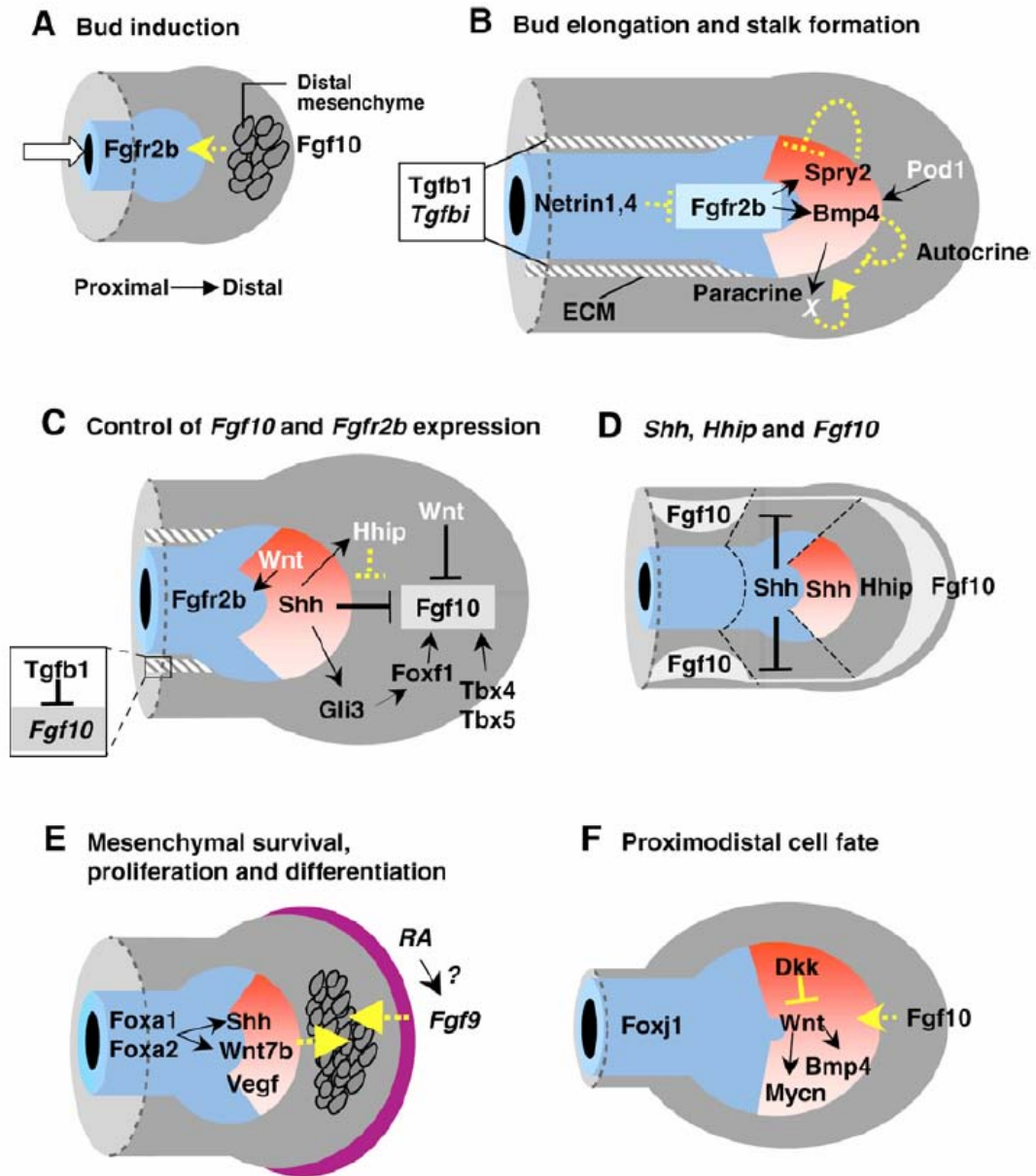
- cell division
- cell growth
- cell-cell communication
- new gene expression

Laying out the architecture of the developing lung from the "inner" tube produced during gastrulation.



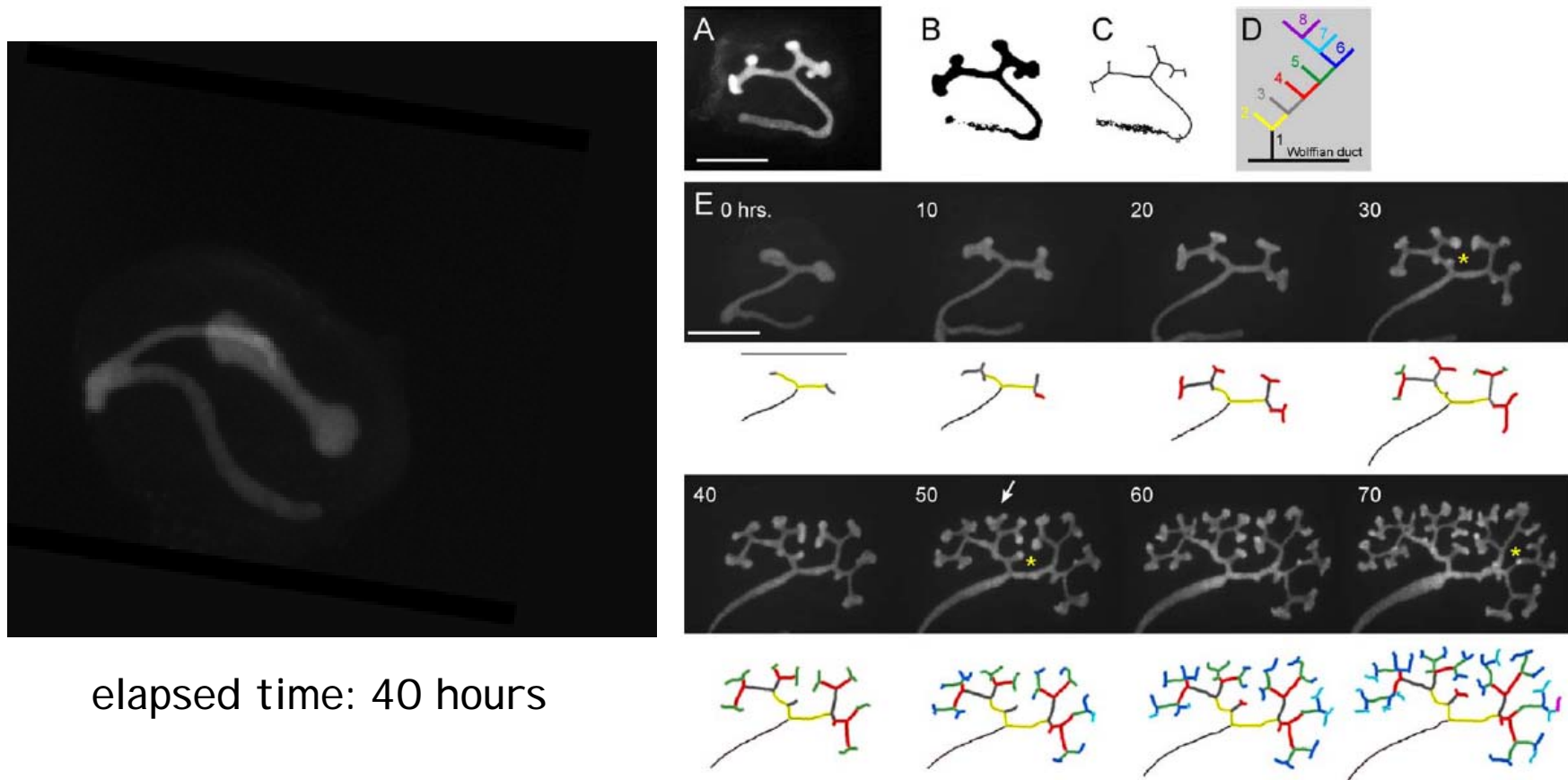
Cardoso and Lu (2006). Development, "Regulation of early lung morphogenesis: questions, facts and controversies."

Multiple steps of induction, elongation, proliferation, differentiation and regulation.



Cardoso and Lu (2006). Development, "Regulation of early lung morphogenesis: questions, facts and controversies."

Branching morphogenesis is a common theme in organogenesis - eg. Renal branching



Watanabe and Constantini (2004) *Dev. Biol.*, "Real-time analysis of ureteric bud branching morphogenesis in vitro."

Progress in Organ-engineering:

Current state is analogous to that in late 1970's.

Culture primary tissues that grow and undergo morphogenesis.

But we do not yet have...

Predictive control of cell behaviors, shape changes, and movements to create tissues.

Control of tissue formation from the establishment of extracellular matrix deposition and remodeling to the creation of polarized 3-dimensional structures.

Grand Challenge: Create "Programmable" 3-D Tissues.

Ability to track and identify cell state and behaviors.

Organs are made of many cell types, each capable of expressing different genes and carrying out different functions.

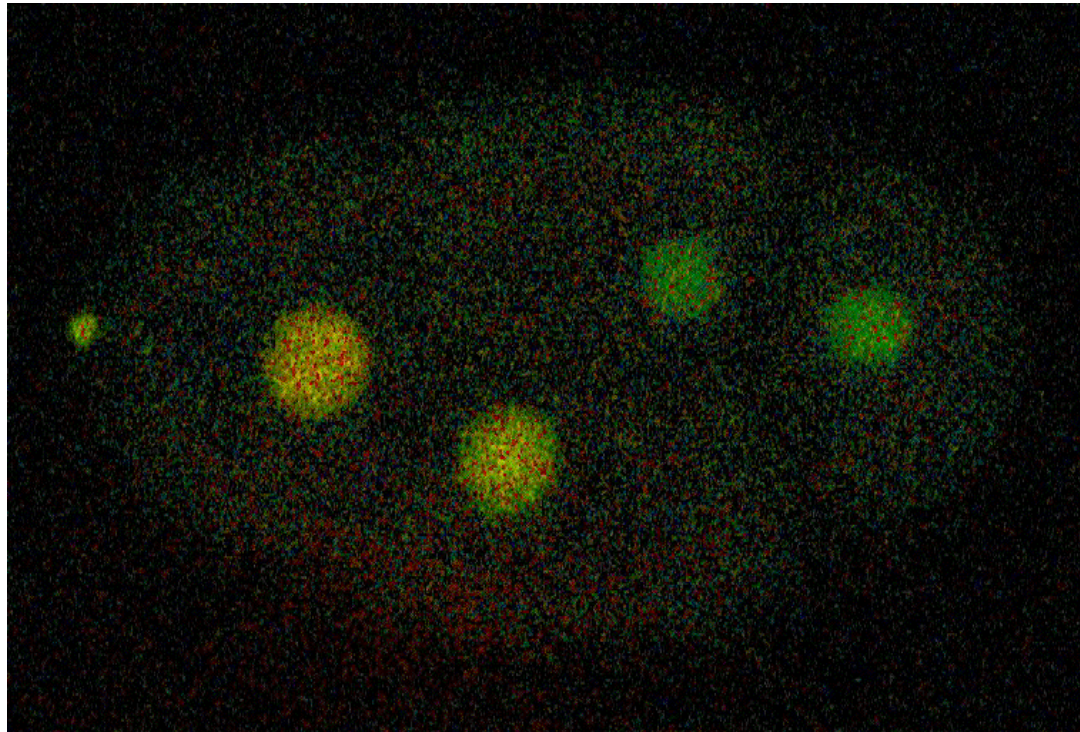
Ability to non-invasively drive cells into specific programs.

Must be able to direct both individual cells and groups of similar cells to the same action.

Must make functional organs!

Grand Challenge: Create "Programmable" 3-D Tissues.

- Ability to track and identify cell state and behaviors.
- Ability to non-invasively drive cells into specific programs.



Auto-tracking and assignment of cell identity up to 350 cell stage in *C. elegans* embryo.

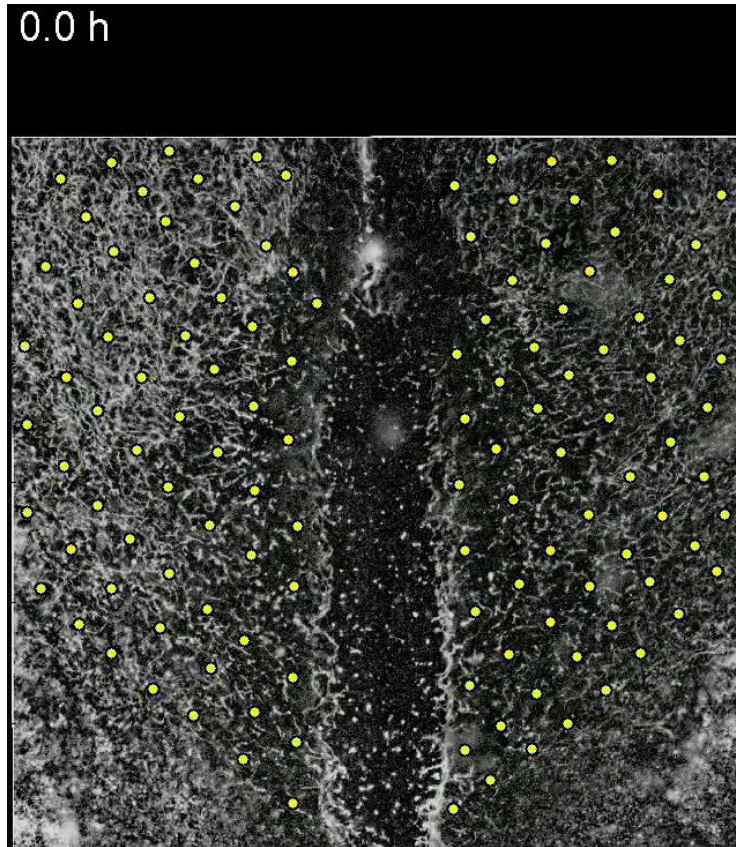
+ 30,000 images
+ processing takes 30'
rather than 2 weeks

Group is adding live reporters of gene expression.

Bao et Waterston (2006) PNAS,
"Automated cell lineage tracing in *Caenorhabditis elegans* "

Grand Challenge: Create "Programmable" 3-D Tissues.

- Ability to track and identify cell state and behaviors.
- Ability to non-invasively drive cells into specific programs.



Tracking cell and tissue movements using particle image velocimetry during gastrulation in chick embryos.

+ able to create separate deformation maps of cell and neighboring tissues.

+ calculating strain fields within developing embryo.

Zamir et Rongish (2006) PNAS. "Mesodermal cell displacements during avian gastrulation are due to both individual cell-autonomous and convective tissue movements."

Grand Challenge: Create "Programmable" 3-D Tissues.

- Ability to track and identify cell state and behaviors.
- Ability to non-invasively drive cells into specific programs.

Cell shape change and motility involves a coordinated deployment of components and processes of the cytoskeleton:

- dynamic assembly and disassembly of polymers
- regulation and modification of their structure by associated proteins
- actions of motor proteins among the polymers

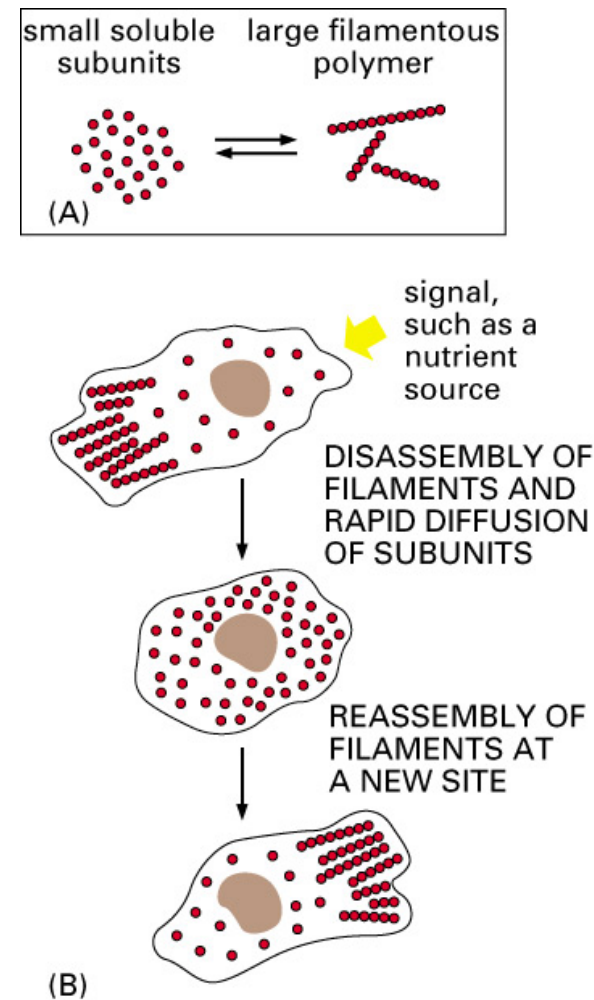
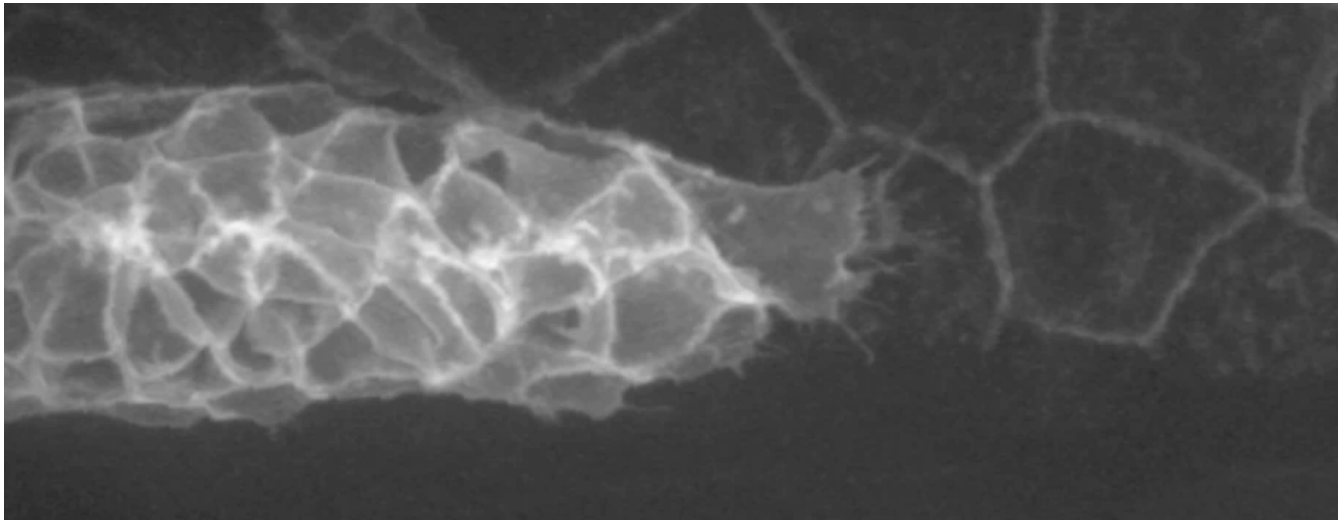
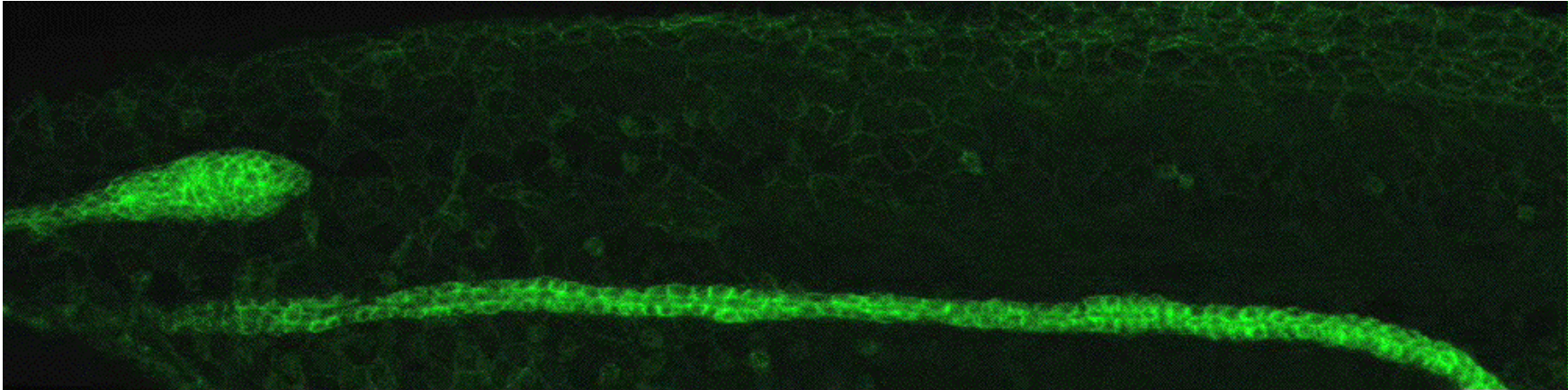


Figure 16-2. Molecular Biology of the Cell, 4th Edition.

Grand Challenge: Create "Programmable" 3-D Tissues.

- Ability to track and identify cell state and behaviors.
- Ability to non-invasively drive cells into specific programs



I identified "protein" tracks that a group of cells follows to build a sensory organ in the zebrafish.

Haas and Gilmour (2006) Dev Cell, "Chemokine Signaling Mediates Self-Organizing Tissue Migration in the Zebrafish Lateral Line."

Grand Challenge: Create "Programmable" 3-D Tissues.

- Ability to track and identify cell state and behaviors.
- Ability to non-invasively drive cells into specific programs.
- Must make functional organs!

1. "Engineering" control of scaffolds, cells, microfluidics.
2. Imaging modalities required to track, control, and assess the processes of organogenesis are overwhelming our capacity to process the information.
3. Quantitative models of the "multi-physics" of organogenesis will be required to assist with the design of programmable tissues just as circuit simulators and finite-element analysis assist the design process today.
4. Imaging and modeling will be central elements in FDA testing and regulatory approval of artificial organs.



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Development

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