Clock genes in *Dictyostelium*

Deb Bell-Pedersen
July 2007

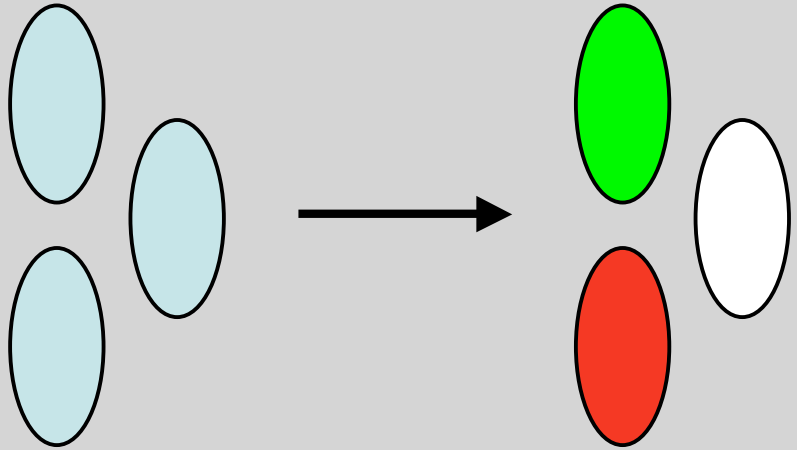
Clock genes

Symmetry-breaking, using math to elucidate a developmental biology problem, and connecting this to a medical problem

Richard Gomer
July 2007

- **Symmetry-breaking in a population**
- **Forming groups of n cells**
- **Using some of what we learned to develop new medical therapeutics**

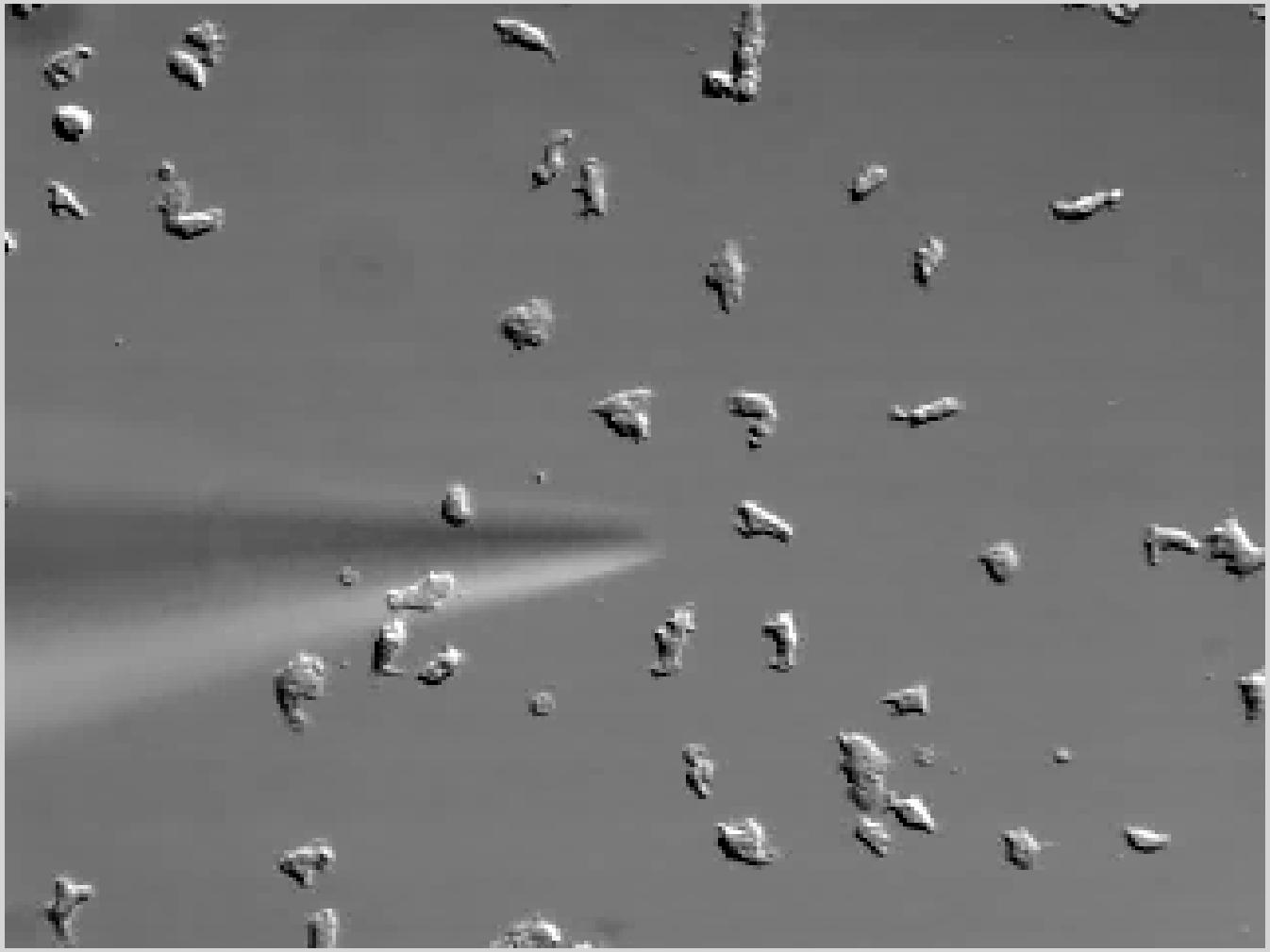
Symmetry breaking



Dictyostelium

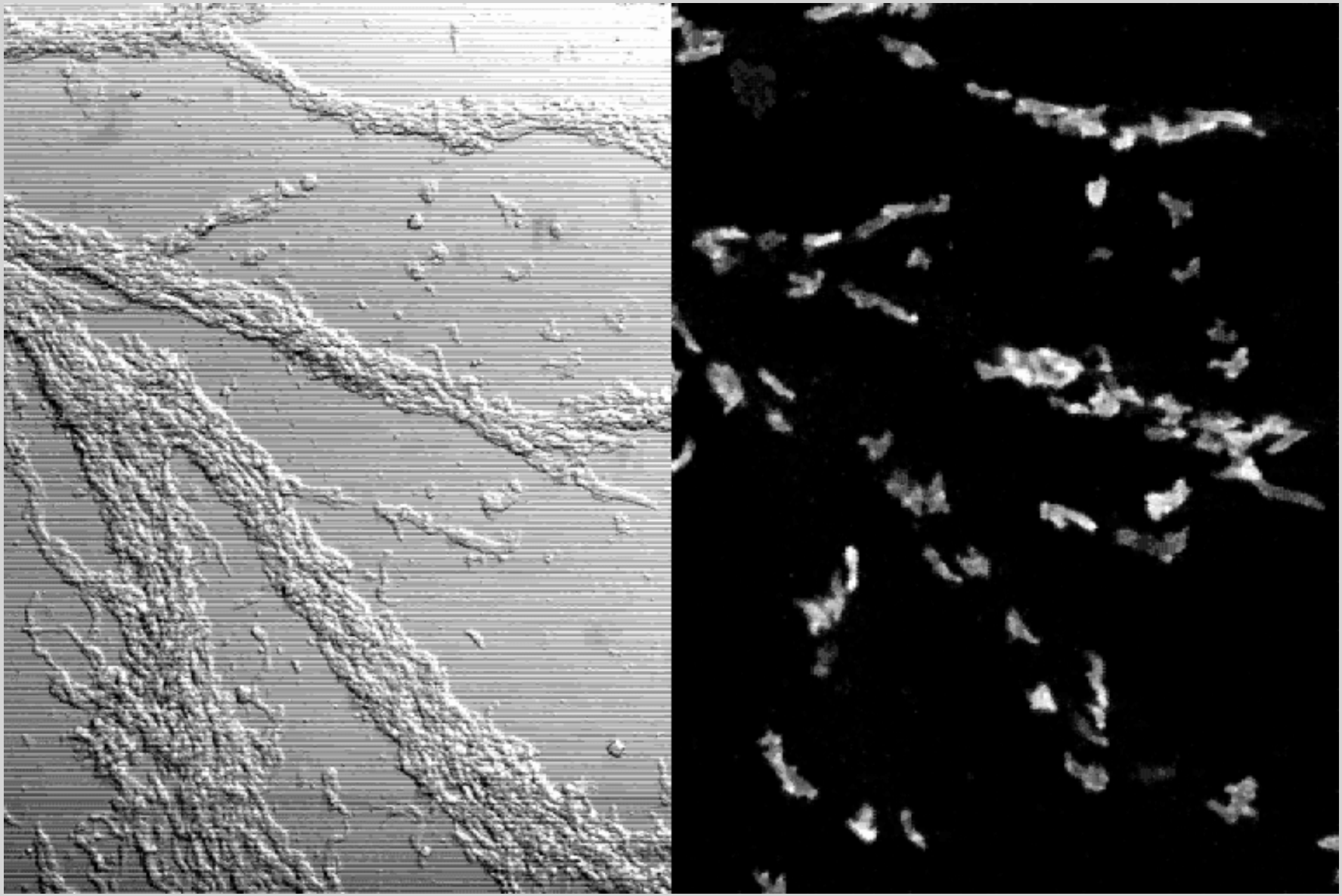
- **unicellular amoebae that lives on soil, eats bacteria, and increases its number by fission**
- **easy to work with**
- **starved cells form groups of ~20,000 cells**

Dictyostelium



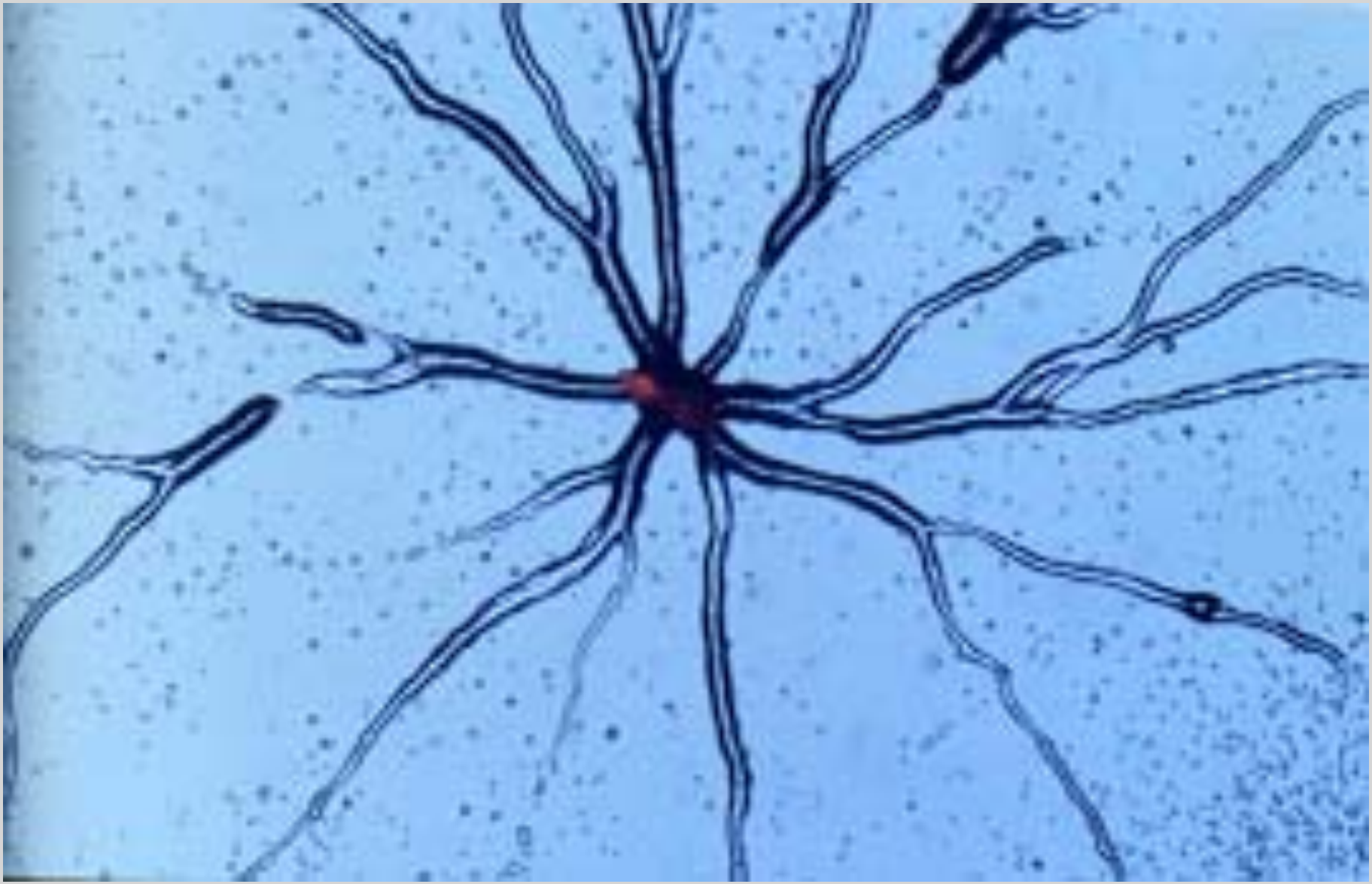
(Rick Firtel, UCSD)

Starving *Dictyostelium* cells

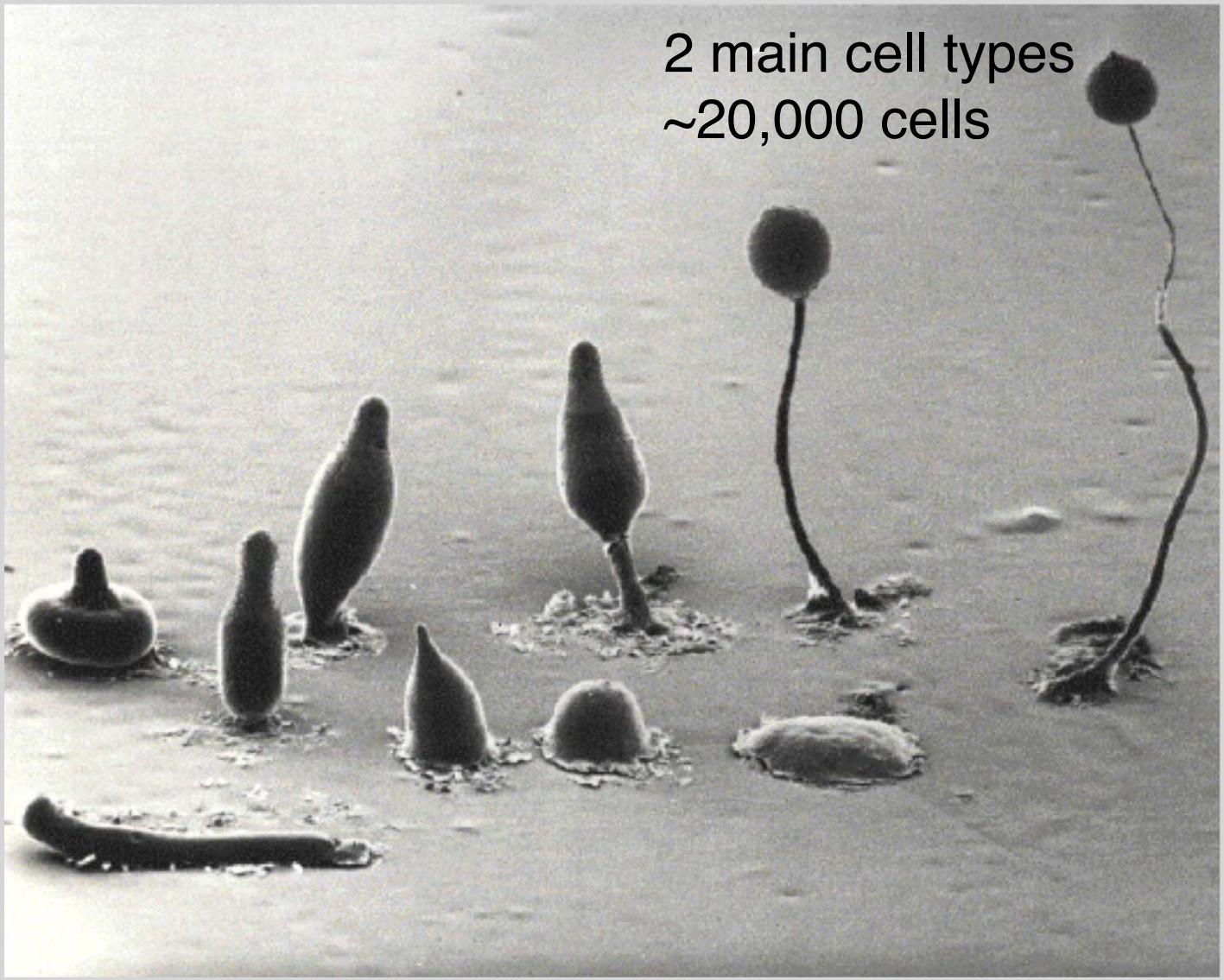


(Dave Knecht, U. of Connecticutt)

Streams of cells can coalesce into groups

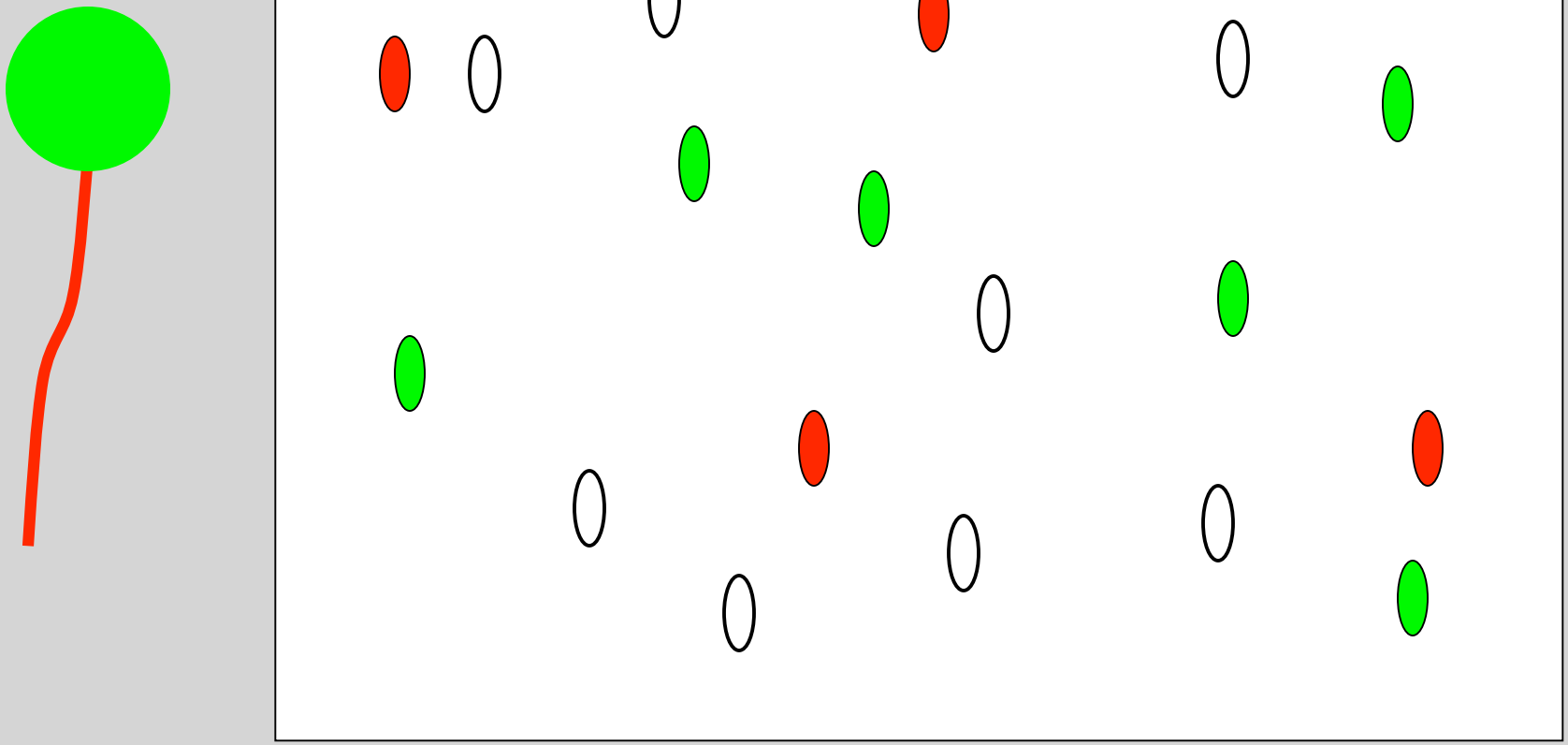


Development of fruiting bodies



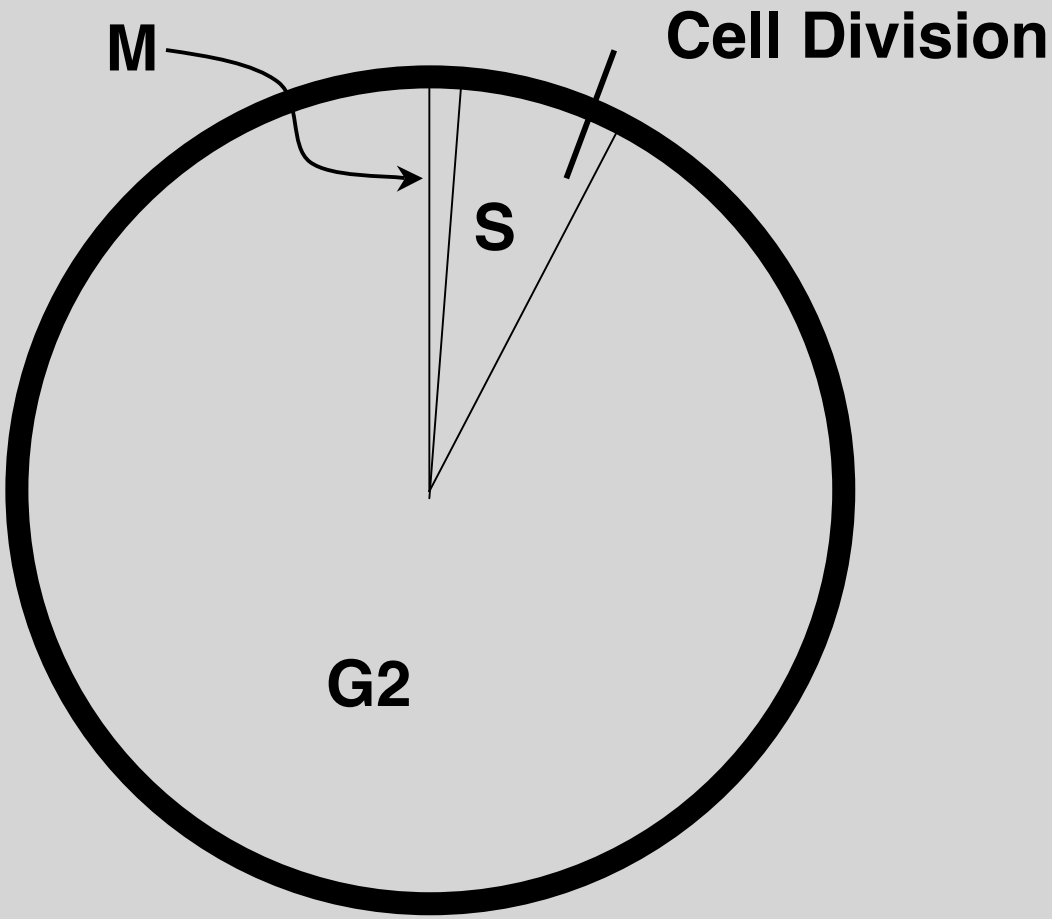
(Mark Grimson, Texas Tech)

Cell-autonomous cell-type choice/ differentiation/ diversity

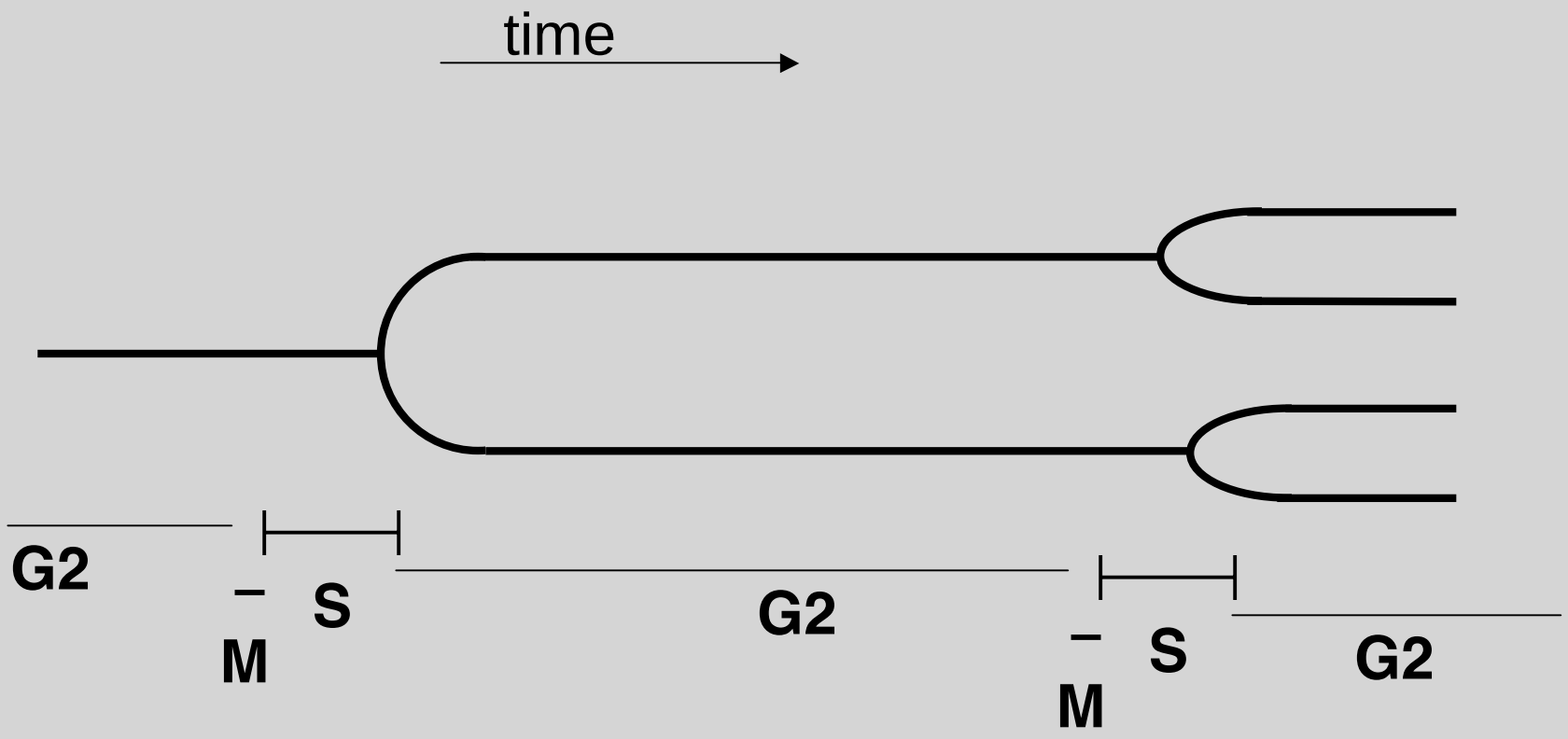


Cells at low cell density can become different cell types

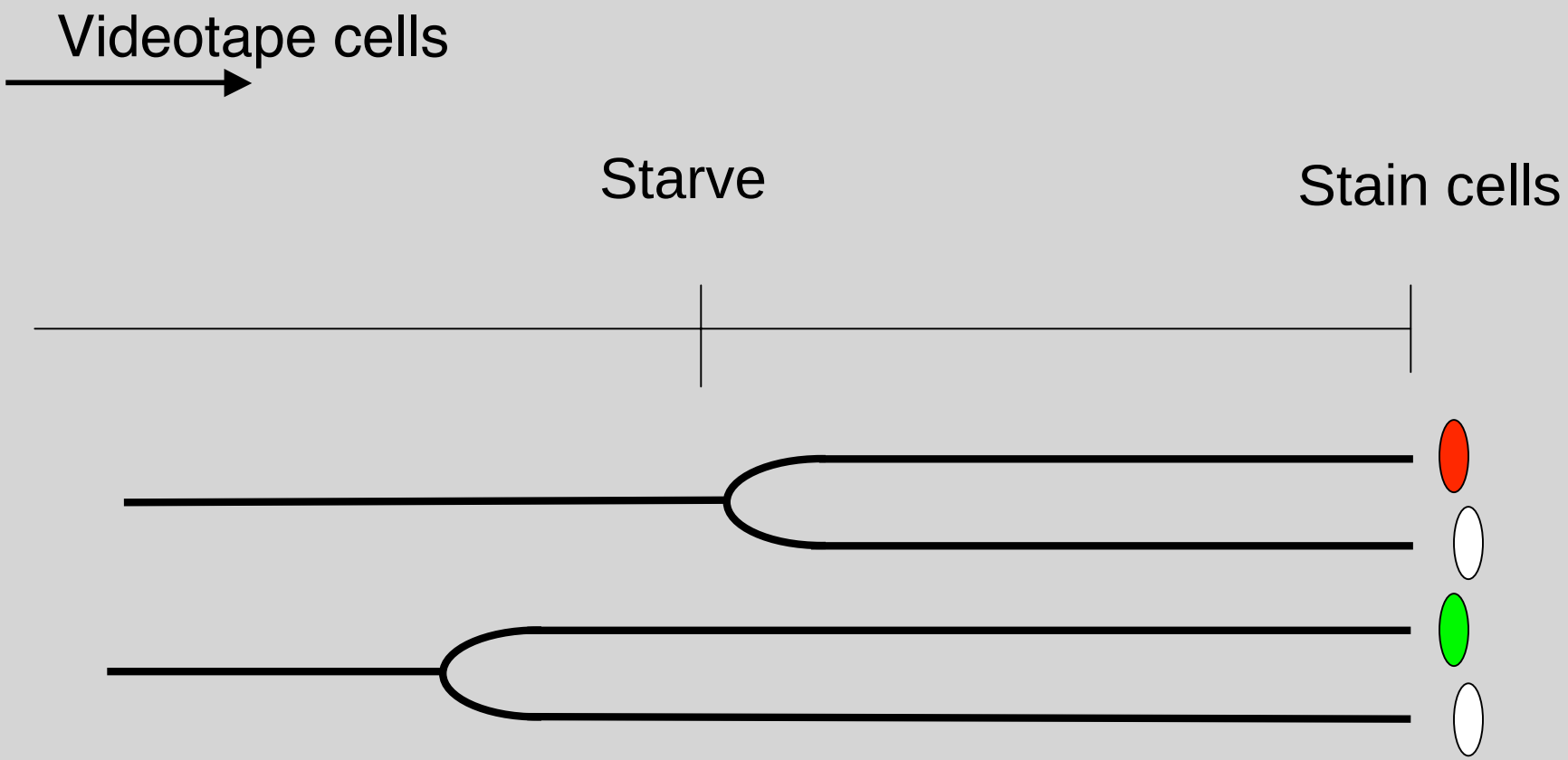
Phases in the *Dictyostelium* cell cycle



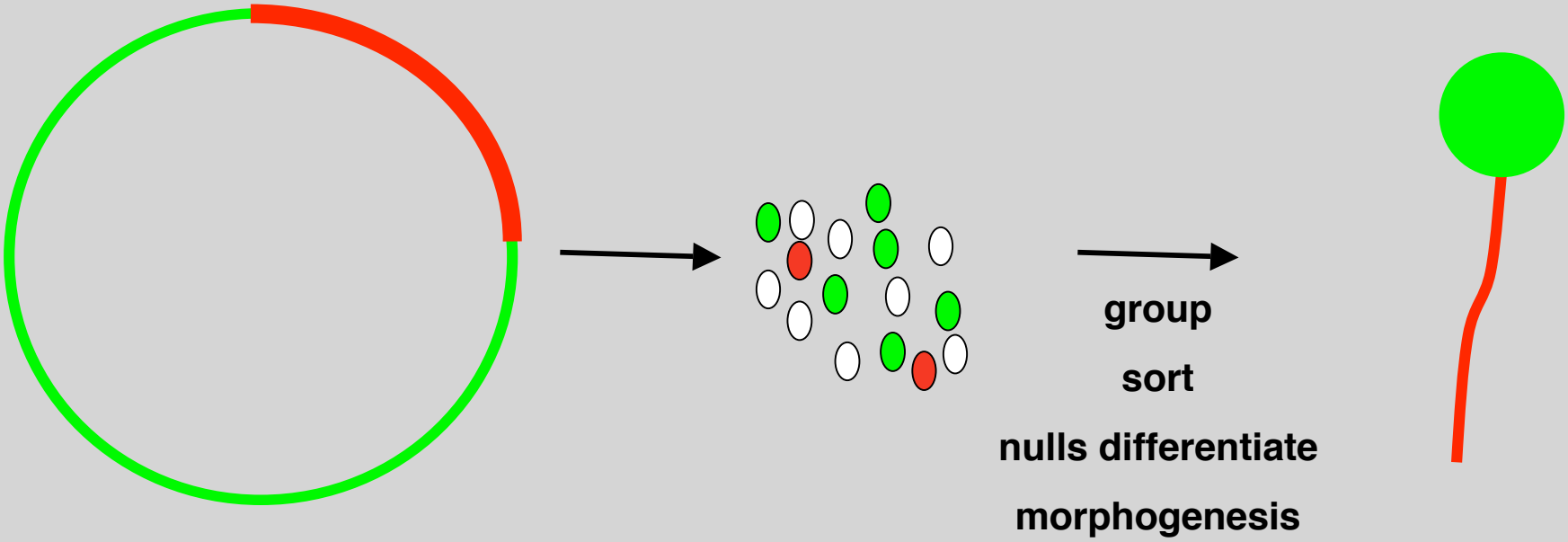
Unrolling the circle



Correlating lineage and fate



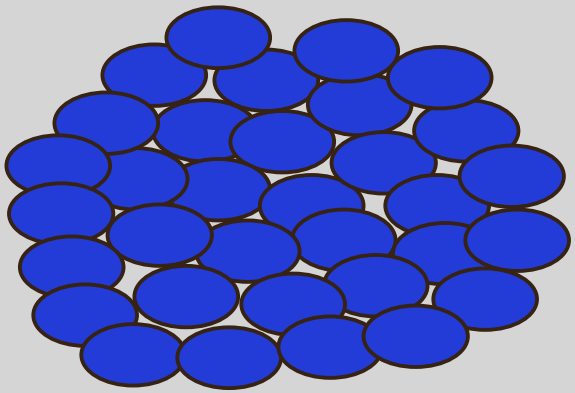
The musical chairs cell type choice mechanism- an easy way to break symmetry



1) Sister cells are different
2) Event (starvation) causes cells to see what phase of the cell cycle they happen to be in, and this regulates their fate

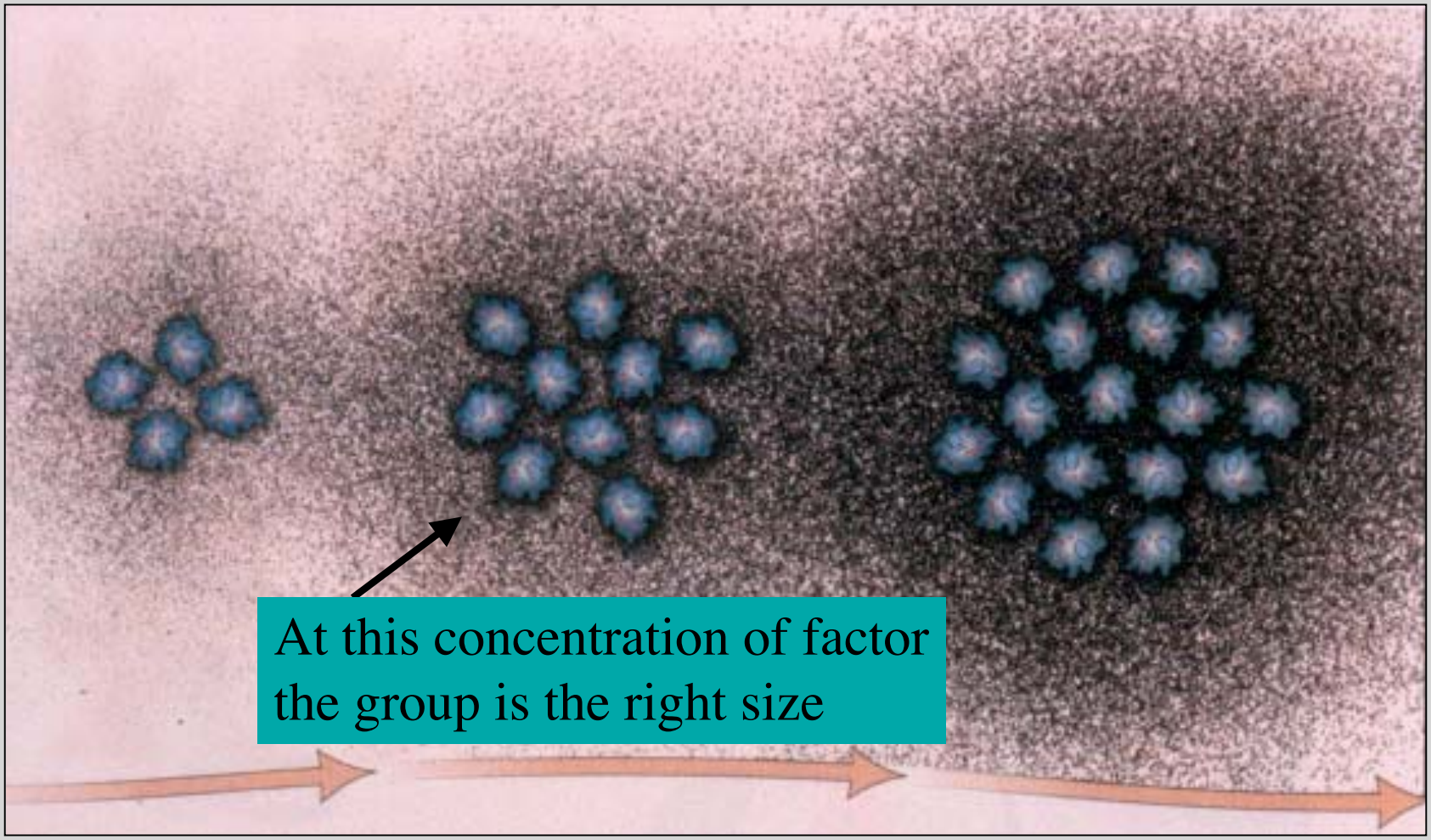
- **Symmetry-breaking in a population**
- **Forming groups of n cells**
- **Using some of what we learned to develop new medical therapeutics**

Cell number counting



n=?

A possible way to sense cell number



Chalones

Experiments starting in 1950's postulate existence of factors secreted by cells in a tissue that slow proliferation of the cells to regulate tissue size

Largely unknown except for myostatin and leptin

Implicated in tumor dormancy

Identification could lead to immediate way to inhibit tumor and metastasis growth

Using shotgun antisense to find size regulation mutants in *Dictyostelium*

WT

smlA^{-}

1 mm

Looking for secreted factors

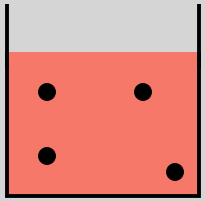
1) Make conditioned media

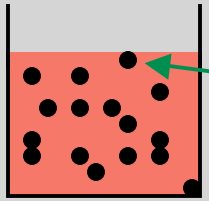
WT cells

Mutant cells

cells

2) Collect conditioned media





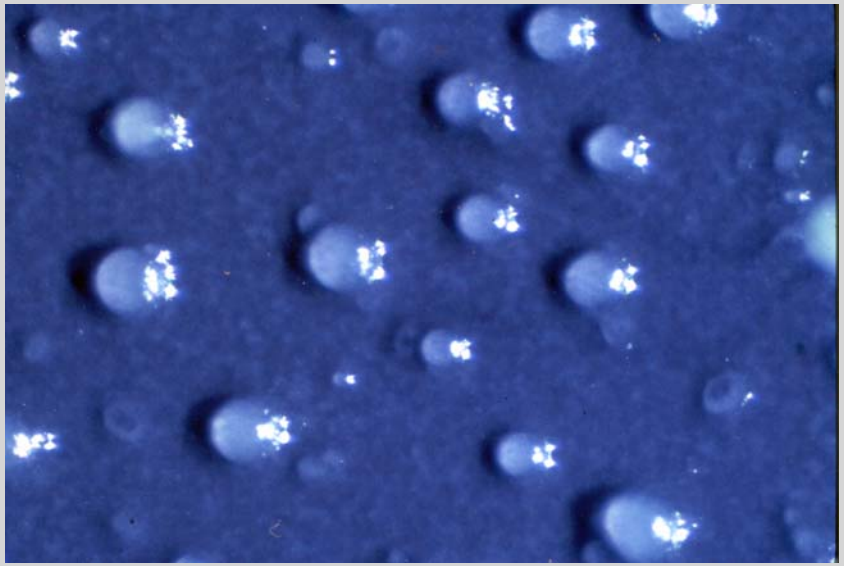
Factor oversecreted by mutant

3) Culture WT cells in conditioned media

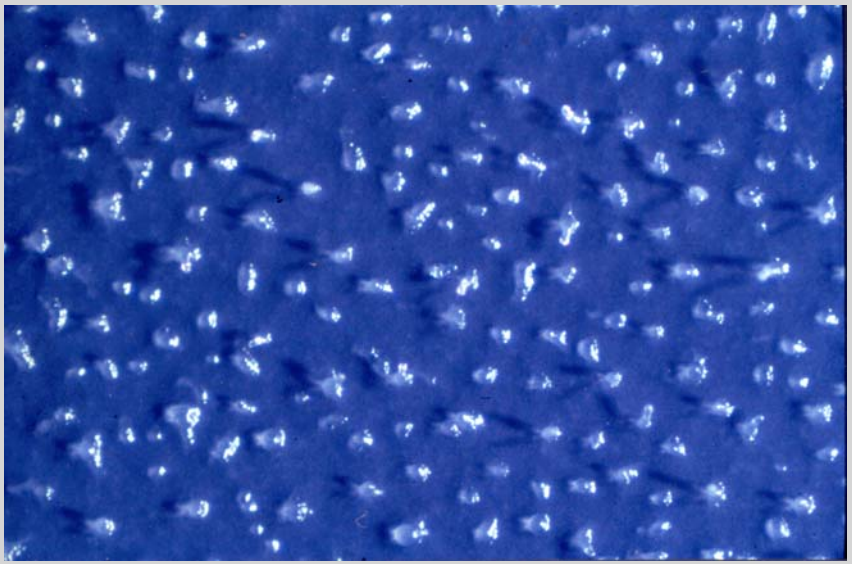
The exudate from *smlA*$^{-}$ cells causes WT cells to form small groups

WT cells developed on:

WT conditioned medium



***smlA*$^{-}$ conditioned medium**



1 mm

Hypothesis:

- cells secrete a factor
- the factor somehow limits group size
- *smlA*$^{-}$ cells oversecrete the factor

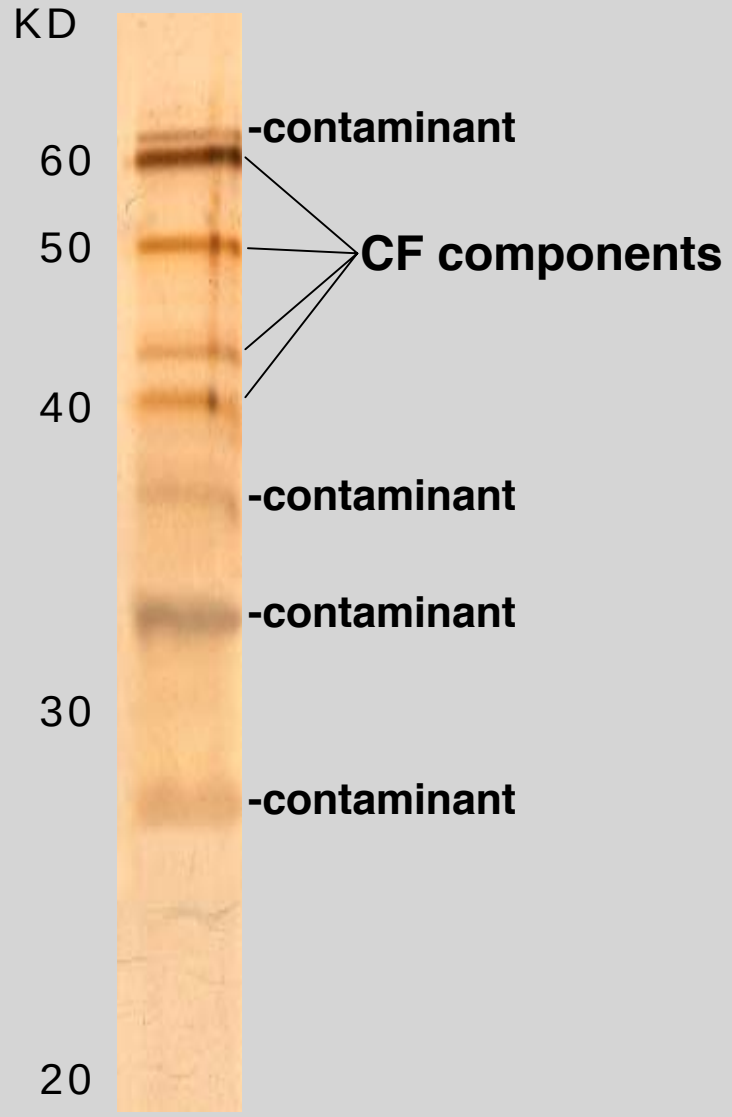
  this excessively limits group size

-what is it?
-as cells secrete it, won't the concentration keep building up?
-Does it fit a diffusible cell-number counting factor model?
-if you knock it out, will you get big groups?

Partially purified counting factor

Purification of the activity that reduces the size of groups formed by WT cells

After
-ion exchange
-hydroxylapatite
-native gel purification



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-what is it? A 450 kDa complex

-as cells secrete it, won't the concentration keep building up? No

-Does it fit a diffusible cell-number counting factor model?

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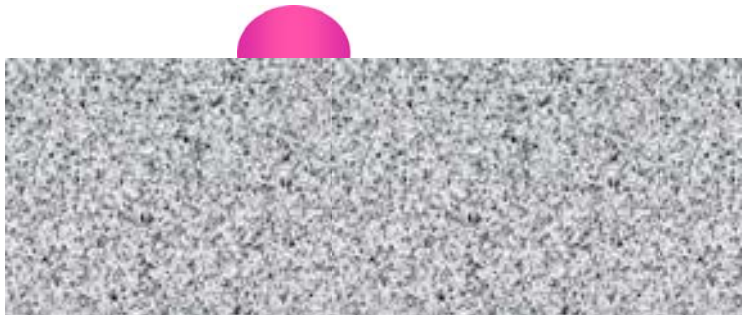
Calculating the concentration of a factor secreted by a cell sitting on dirt or agar



$$C = 2\Phi\left(\frac{1}{2\sqrt{\pi D}}\right)^3 \int_0^{\tau} t^{-3/2} e^{-\left(r^2/4Dt\right)} dt$$

where the diffusion coefficient $D=kT/6\pi\eta R$
η is the viscosity
R is the particle radius
Φ is the flux from the source
τ is the time since the source began secreting
r is the distance from the source

This cannot be solved in closed form, but can be converted to a series

$$\text{As } \tau \longrightarrow \infty$$

$$C = \frac{\Phi}{2\pi^{3/2}\mathrm{Dr}} \sqrt{\pi} = \Phi/2\pi\mathrm{Dr}$$

Its easy to correct for diffusion in dirt (water with loosely packed macroscopic particles)

$$D' \sim D/2.5$$

Calculating Φ from the purification of counting factor

CM is made from cells starved at 5 x 10^6 cells/ ml starved for 20 hours

		Activity			Protein			
Material	Volume, ml	Units per ml	Units	%	μg/ml	μg	%	Specific activity, units/ μg
Whole CM	150	1	150	100	14	2100	100	.07
Ion exchange fractions	8	5	40	26	60	480	23	.08
Hydroxyapatite fractions	5	5	25	16	30	150	7	.16
Gel elution purification	0.66	30	20	13	3	2	0.1	10

With 2 μg, 13% yield, and assuming no degradation, Φ~120 molecules of CF secreted/ cell/ minute

Having receptors binding the secreted factor reduces the free concentration

$$C' = C\,\frac{\Phi t - B}{\Phi t}$$

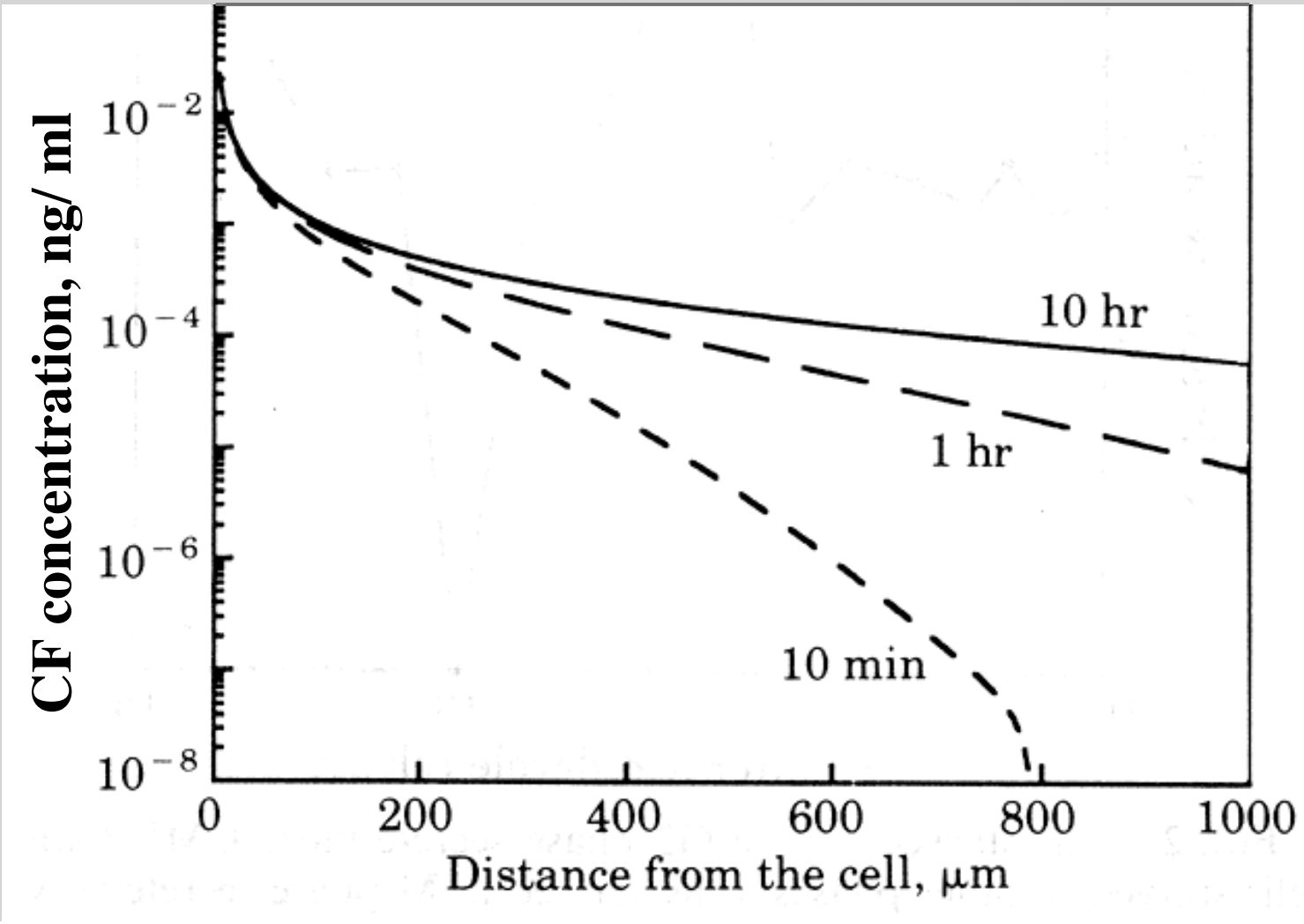
where Φt is the number of molecules the cell secreted, and B is the number of molecules it bound

From classical receptor kinetics $B = \dfrac{R_T}{\dfrac{K_D}{C'} + 1}$

so

$$C' = \frac{C - K_D - R_T C/\Phi t + \sqrt{(C - K_D - R_T C/\Phi t)^2 + 4K_D C}}{2}$$

Diffusion of a secreted factor from a cell sitting on dirt or agar



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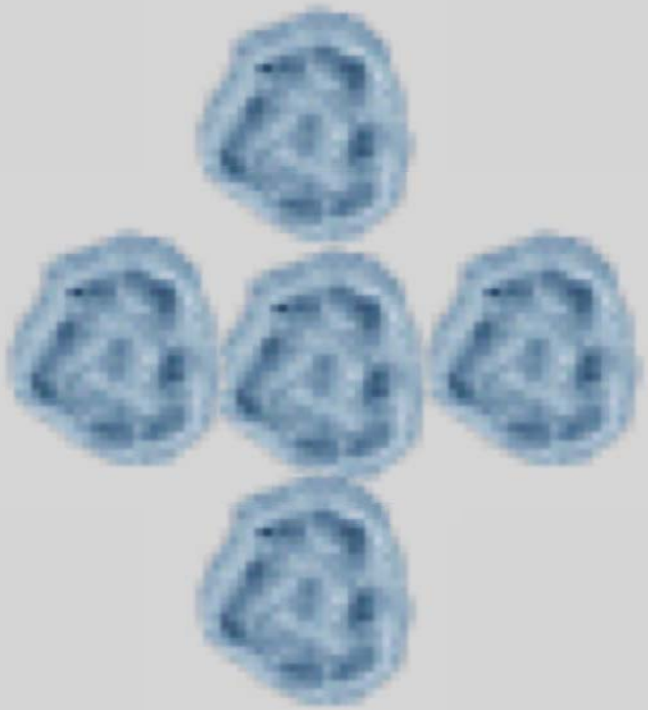
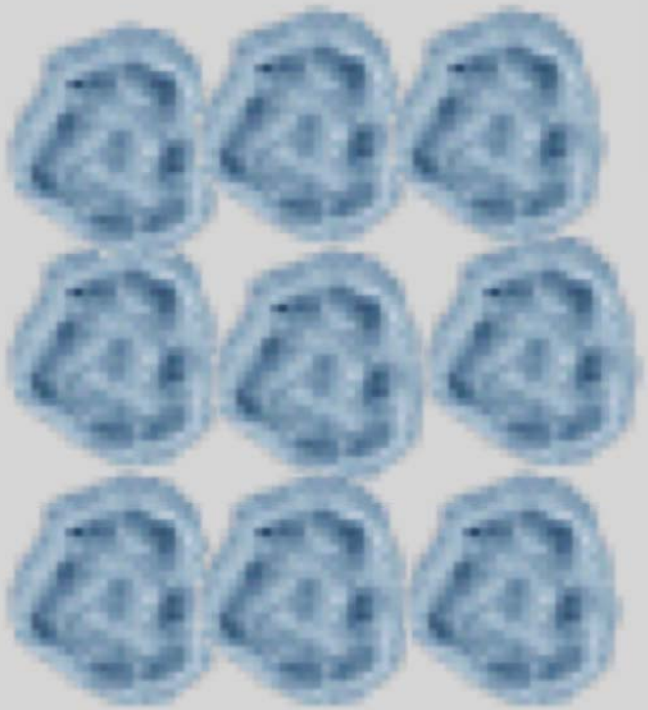
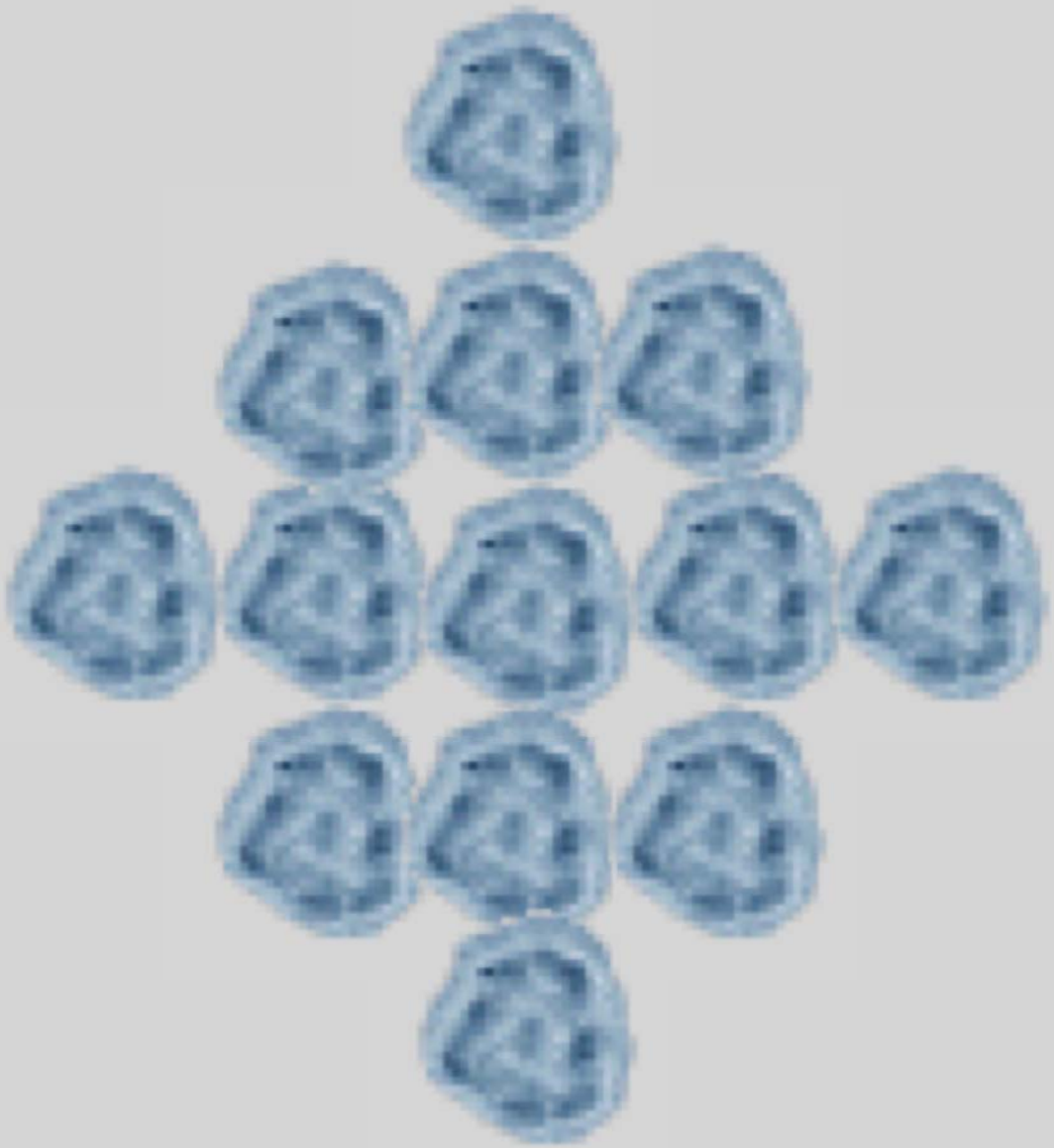
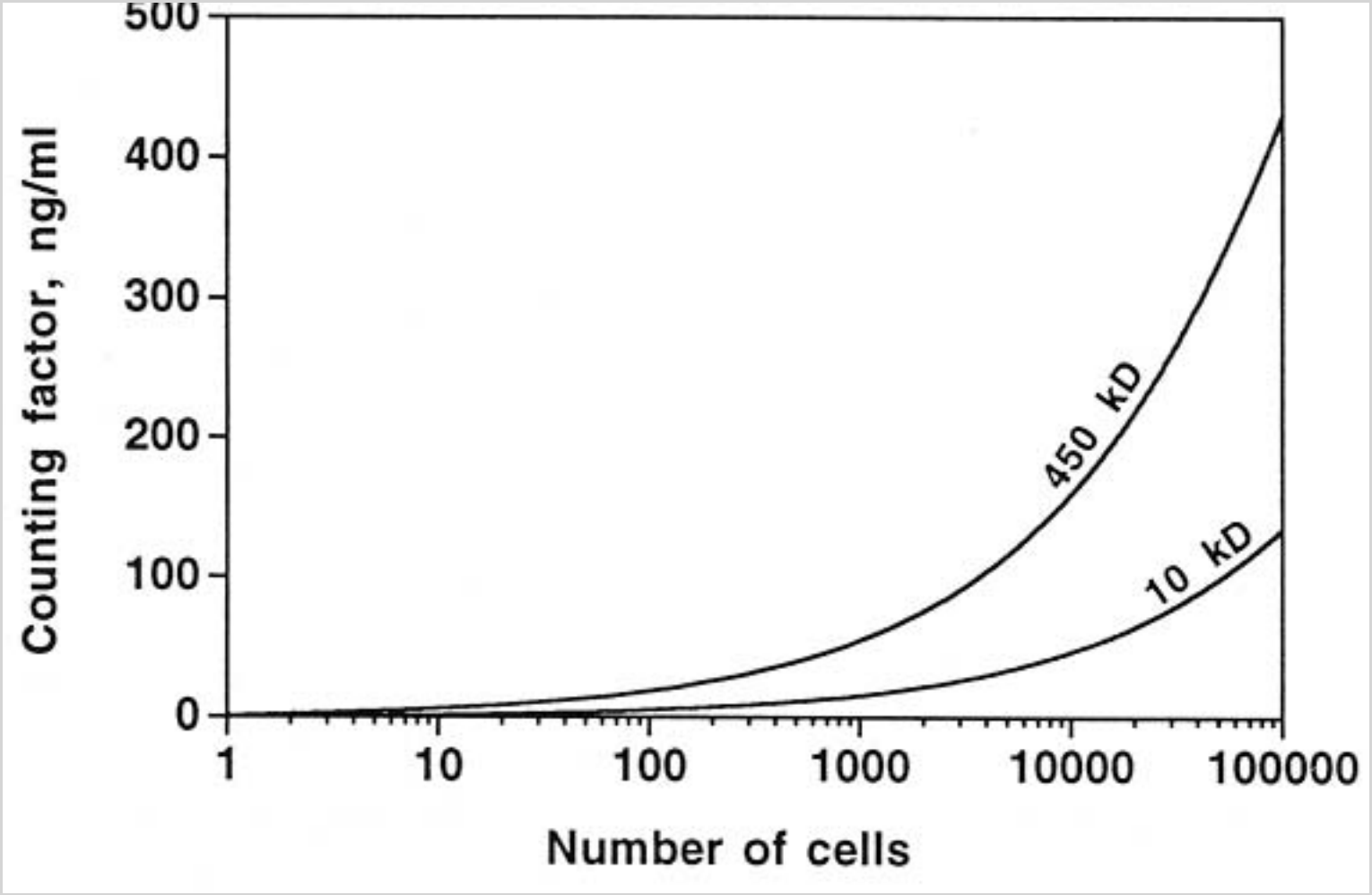






The CF concentration increases with the number of cells in a group



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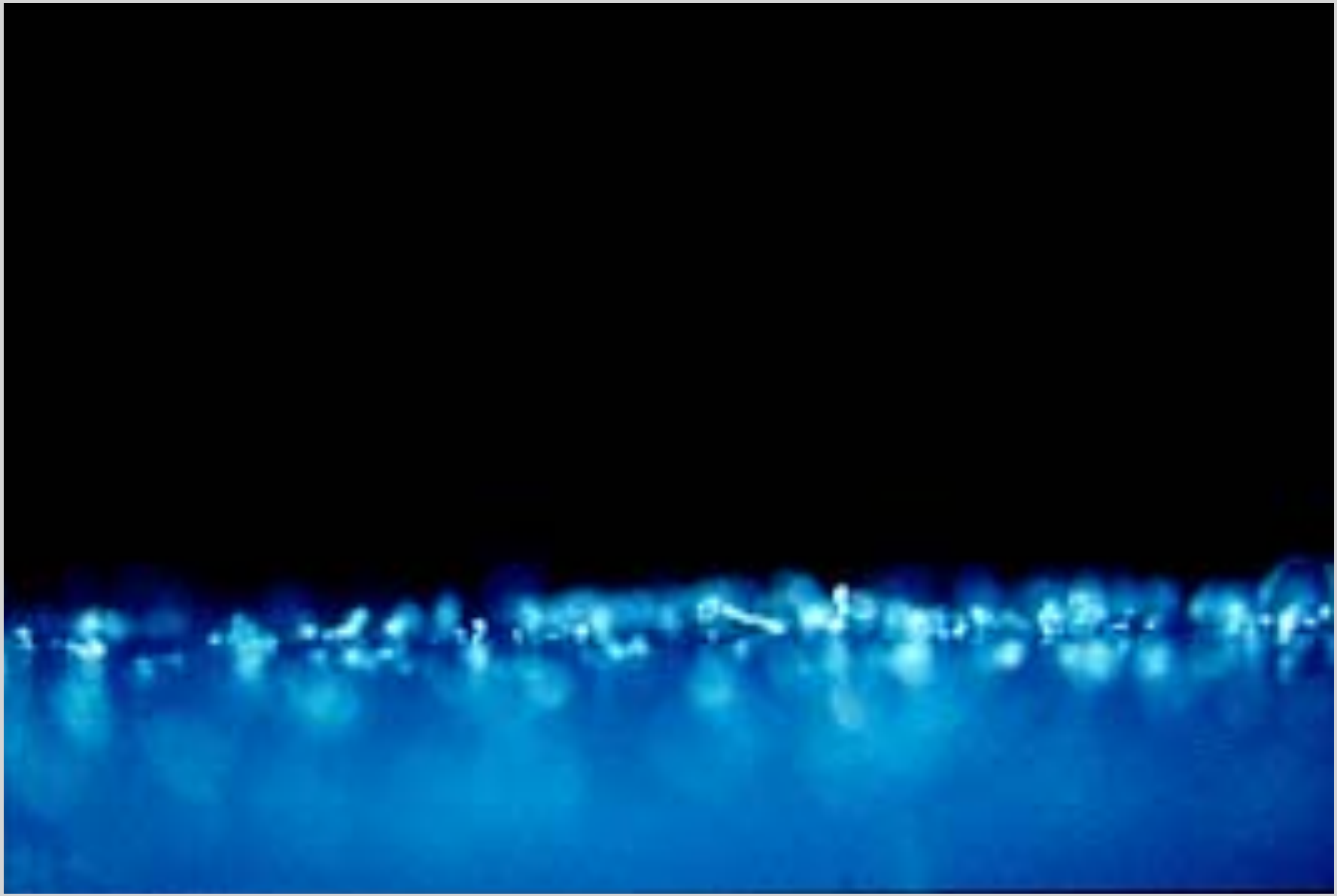
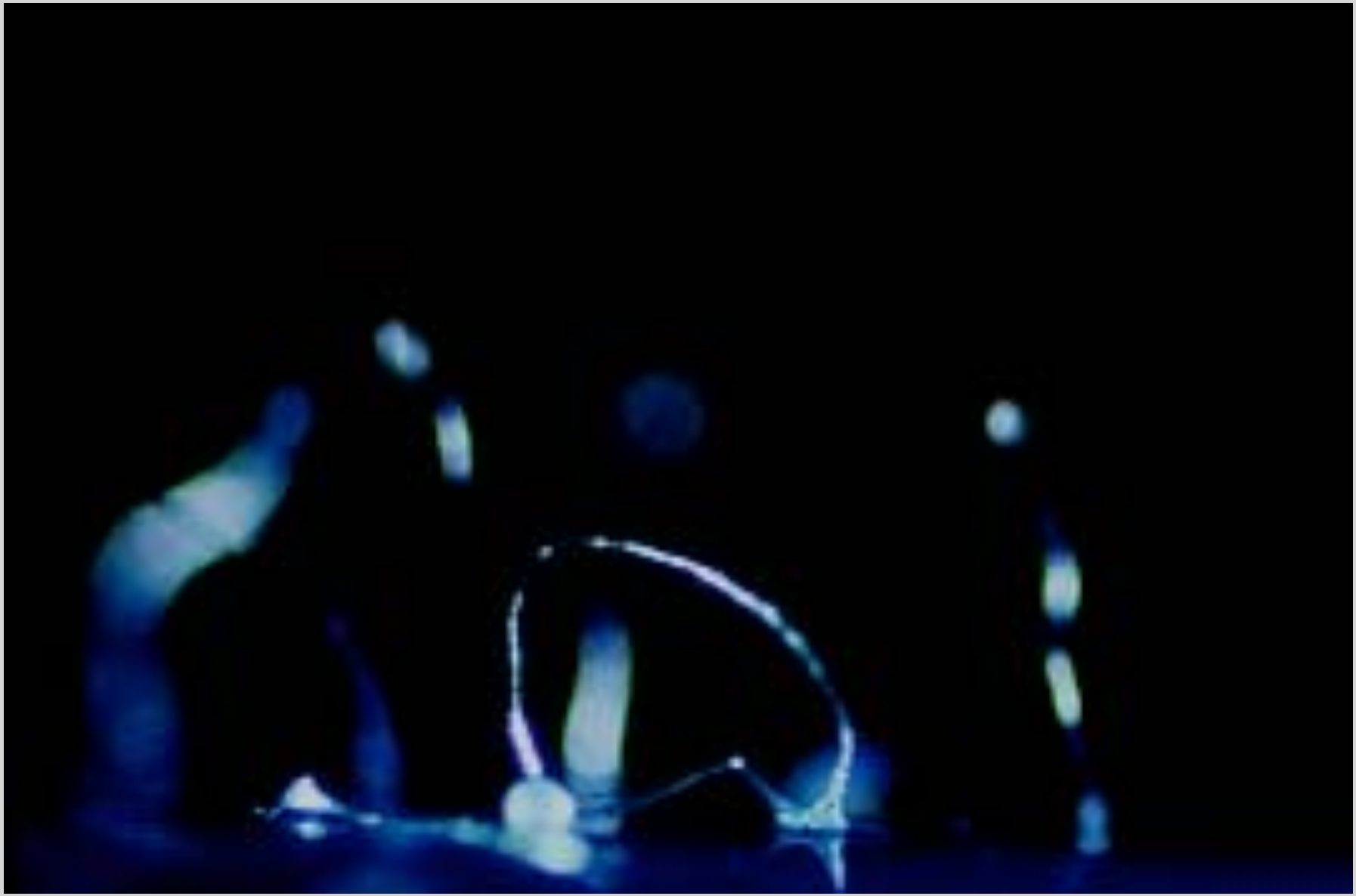
smlA^{-}



1 mm

WT

countin¯



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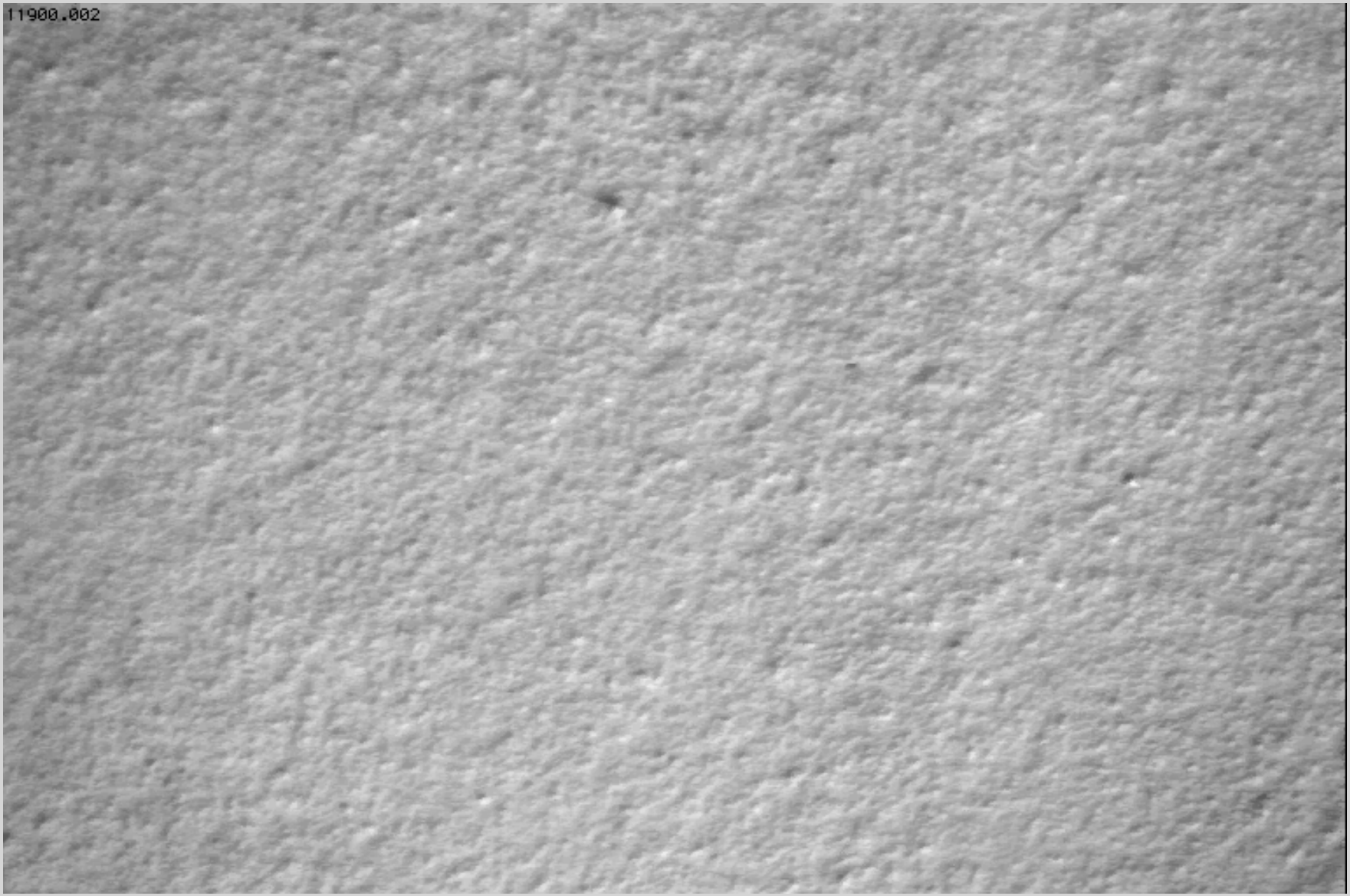
CF is a factor secreted by aggregating cells that regulates group size

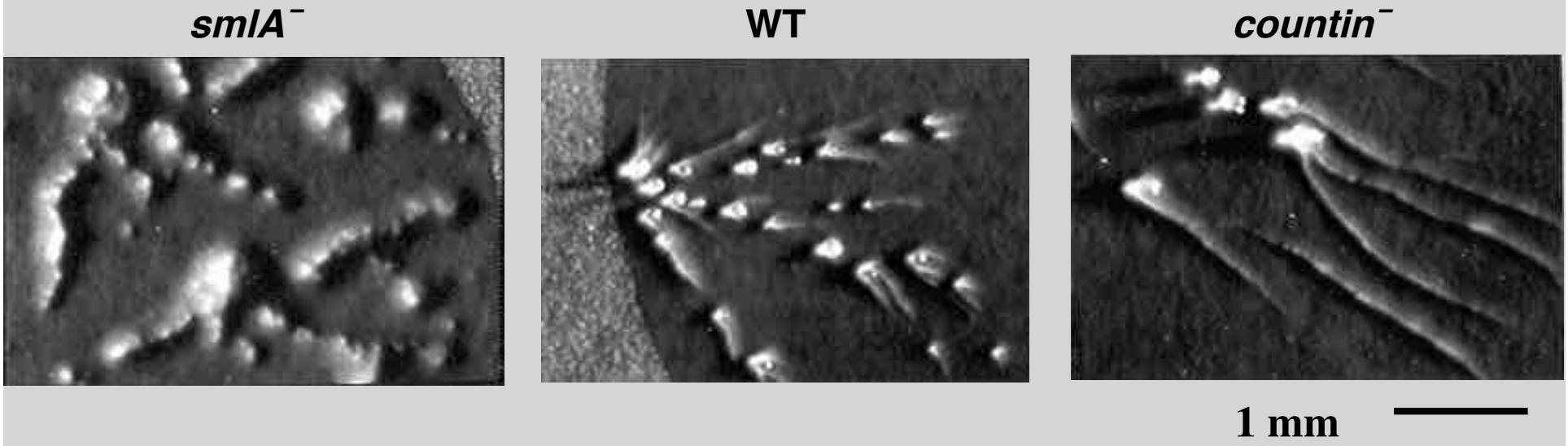
What does it do to individual cells to keep groups at ~ 20,000 cells?

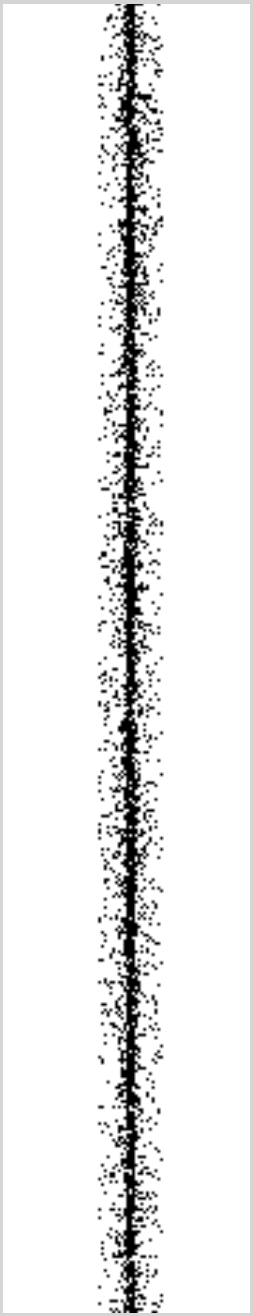
High CF levels cause streams to break



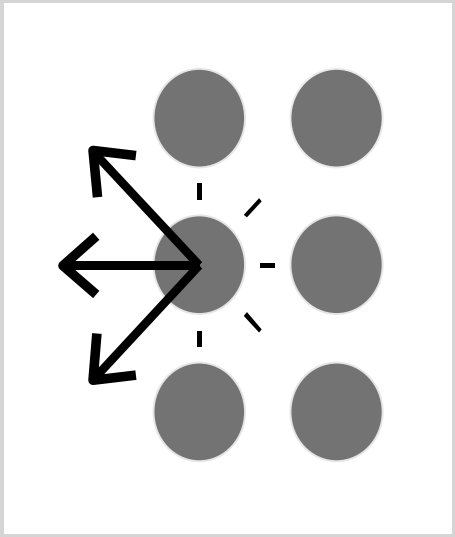
Disrupting CF activity prevents breakup

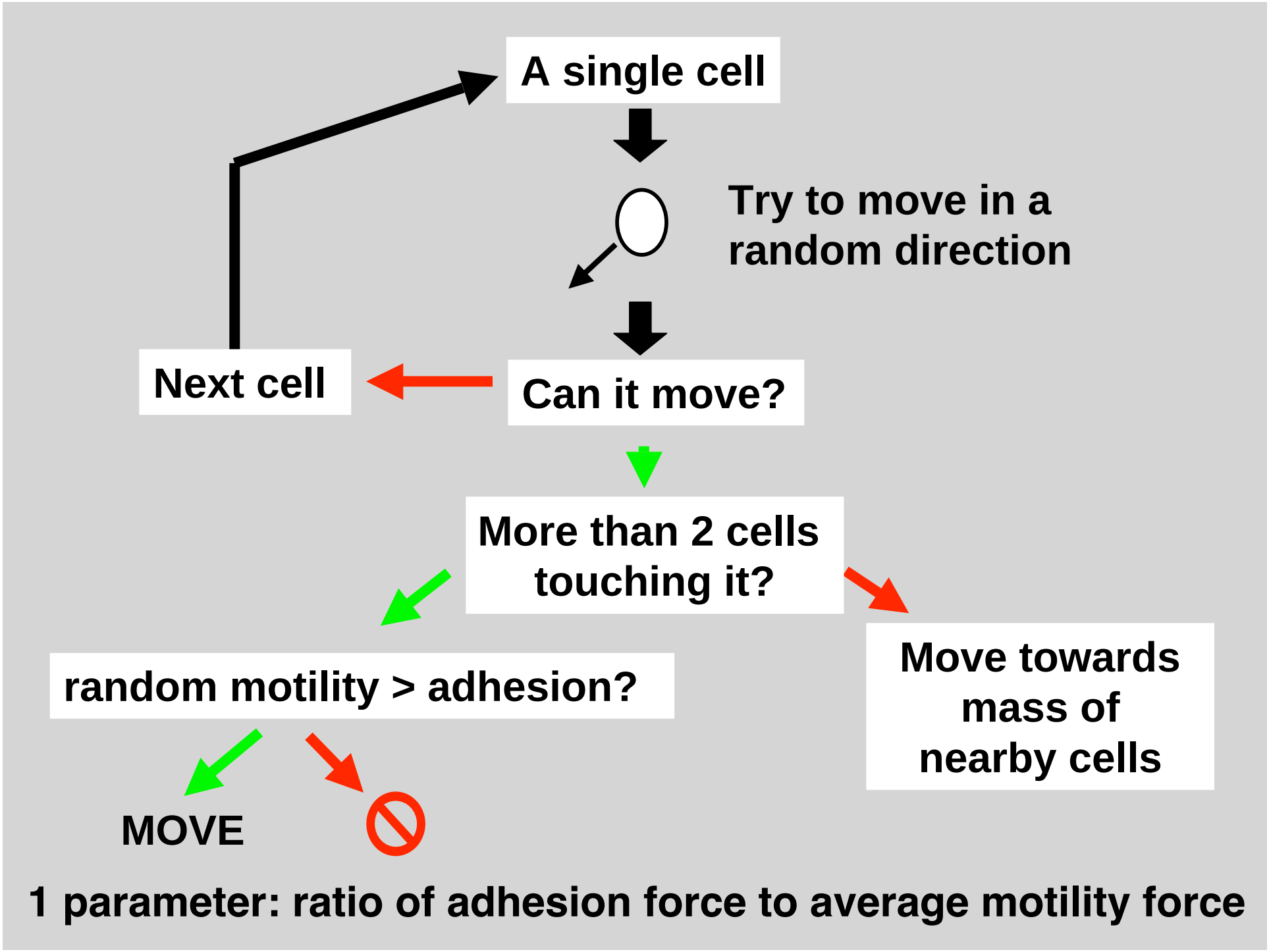
CF affects group size by altering stream breakup





Using a computer simulation to see what might cause a stream to break up

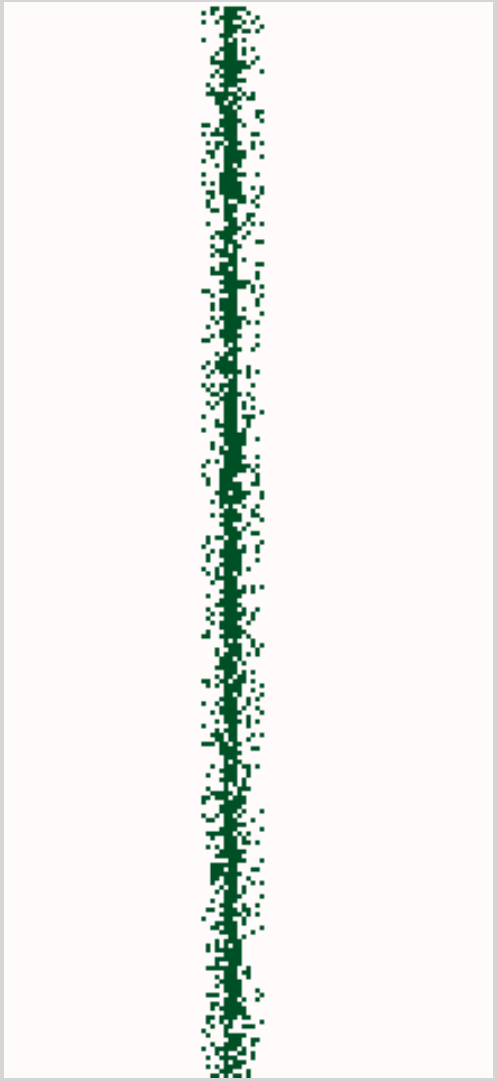




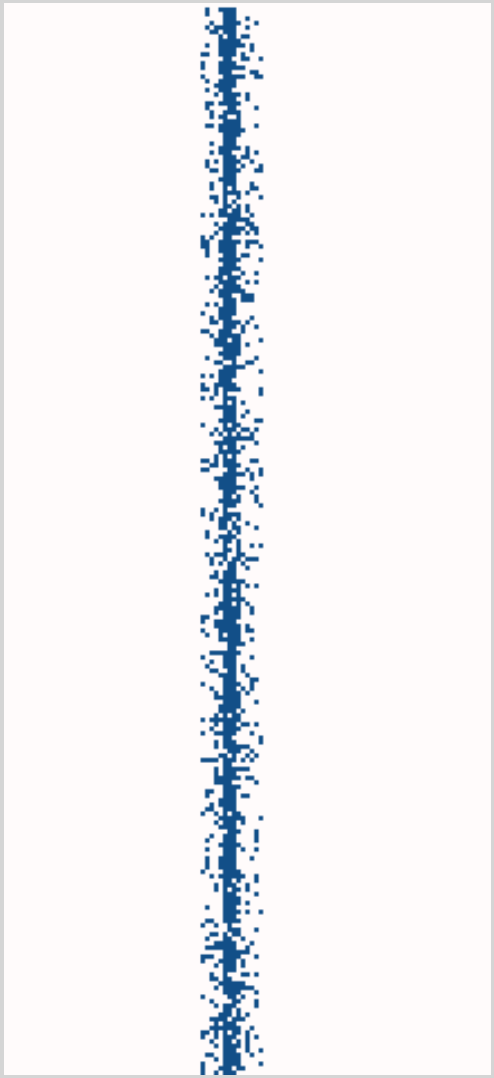
1 parameter: ratio of adhesion force to average motility force

Computer simulation of streams breaking up

High Adhesion



Low Adhesion



Computer simulation of streams breaking up

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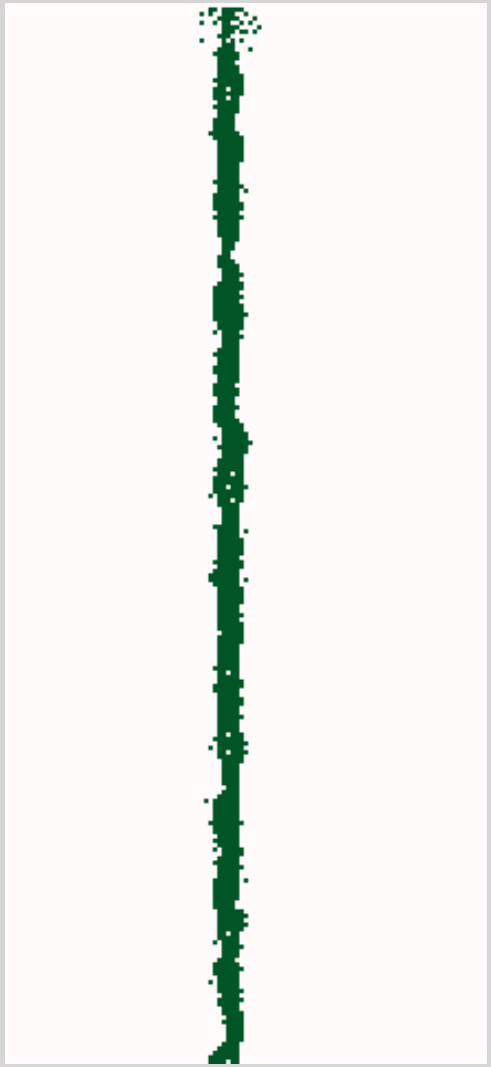
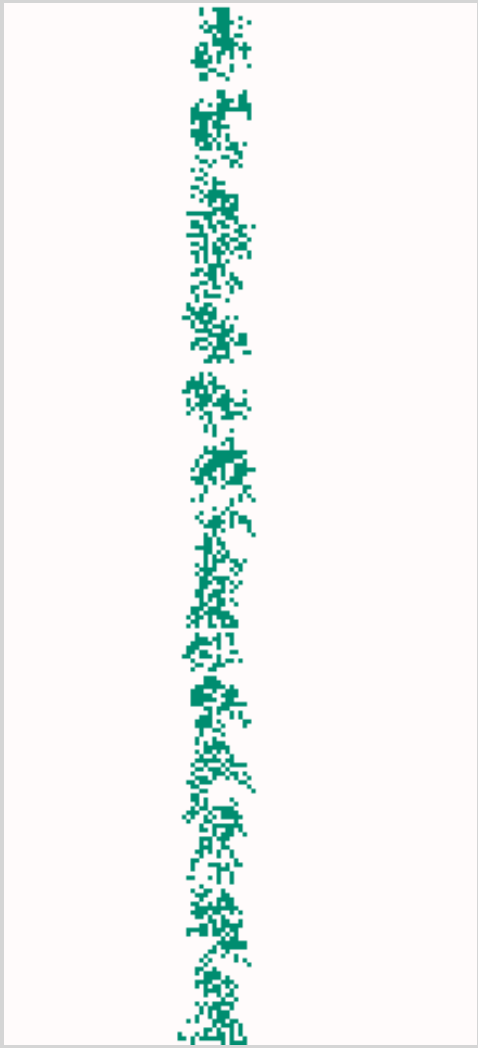
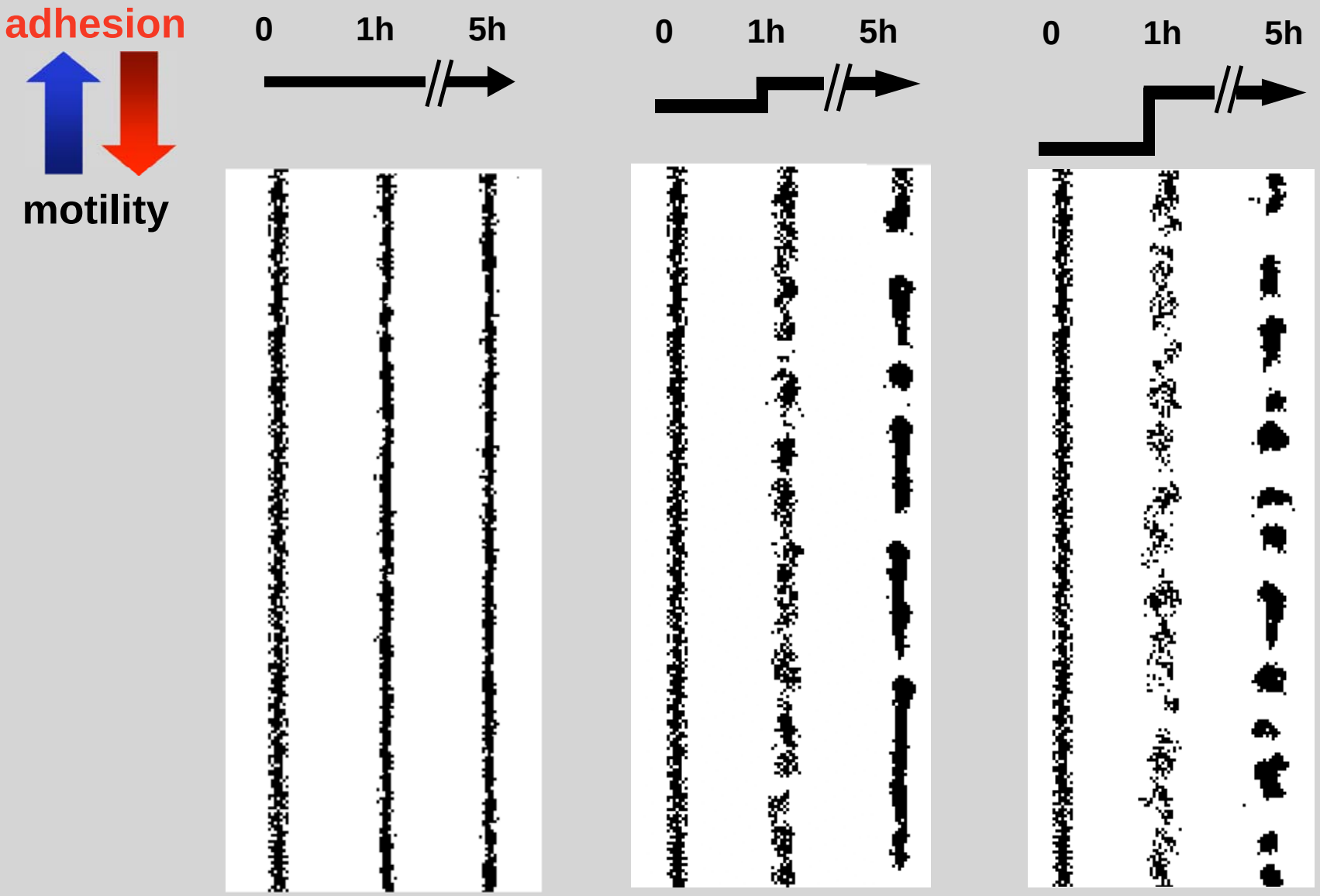


Low --> High Adhesion

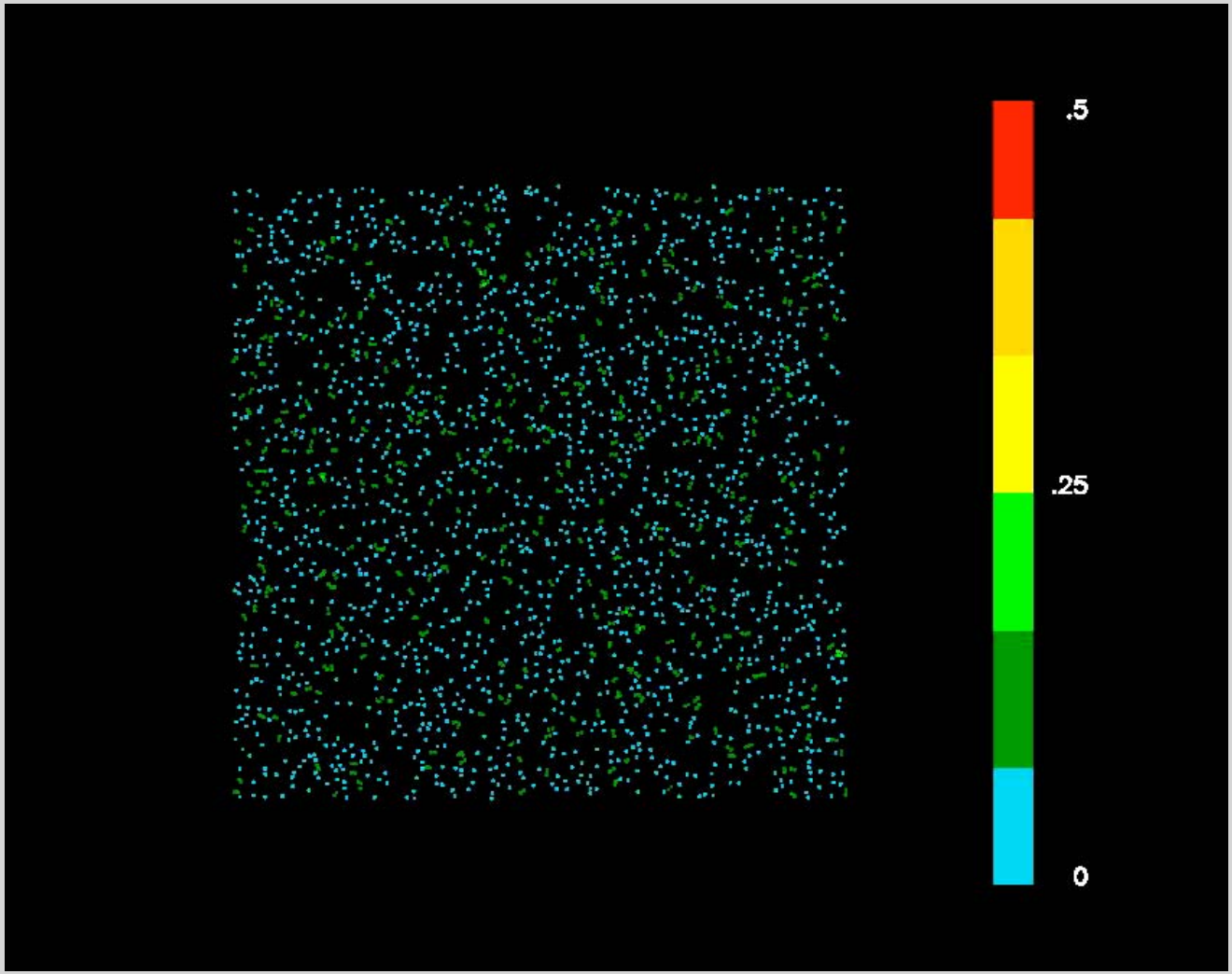


A decrease in adhesion force or an increase in cell motility increases the number of aggregates



Simulation with a diffusible factor

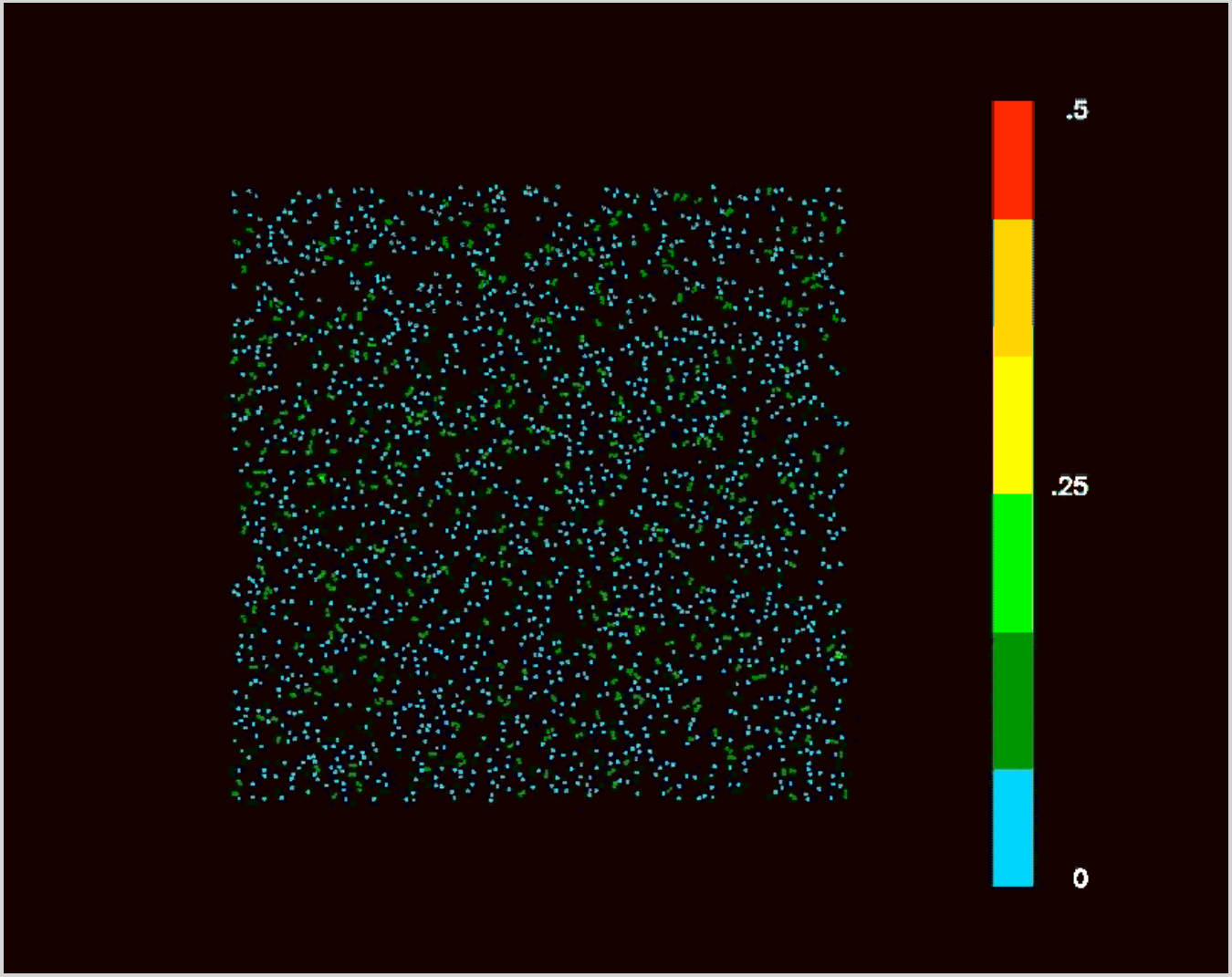
- decreasing cell-cell adhesion
- increasing random motility



John Dallon
BYU

Simulation with no factor, and

- high cell-cell adhesion
- low random motility



John Dallon
BYU

We observed what the computer simulations predicted

CF → random motility → Stream breakup

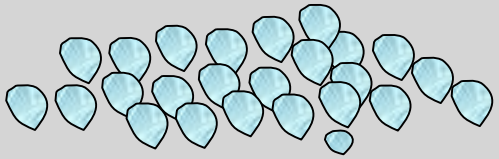
CF → adhesion ⊣ Stream breakup

Few cells

[CF] is low

adhesion

motility



Many cells

[CF] is high

adhesion

motility

Stream stays together

Stream breaks up

Cell-number counting using a secreted factor works if the factor, upon leaving the group

- **Diffuses**
- **Absorbs**
- **Adsorbs**
- **Breaks down**

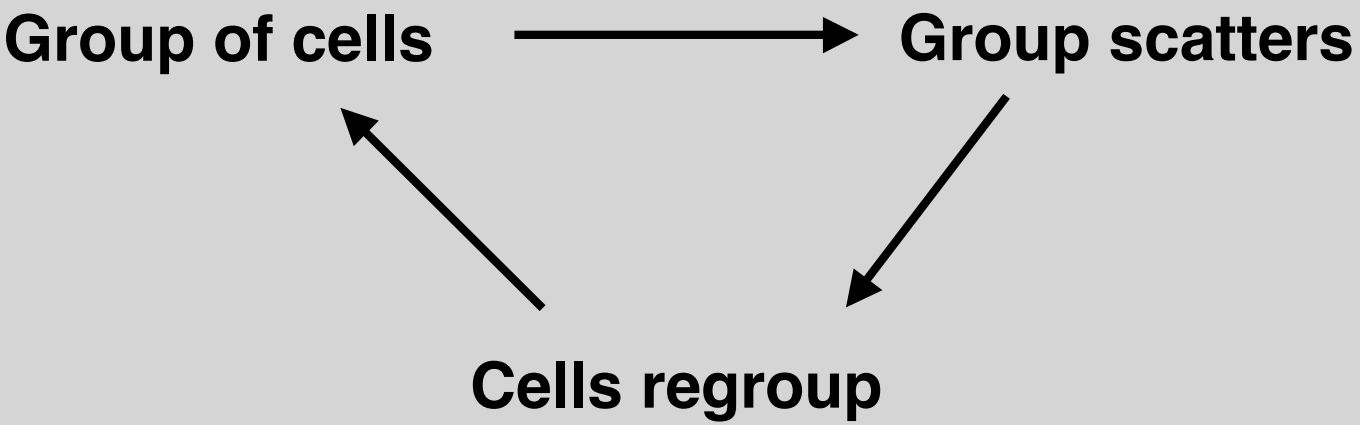
Switching source and sink also allows cell-number counting

If the factor is diffusing into the group from the surrounding tissue/environment

And if the cells in the group act as a sink

Then few cells --> high concentration in the group
many cells --> low concentration

Under some conditions, this system can oscillate

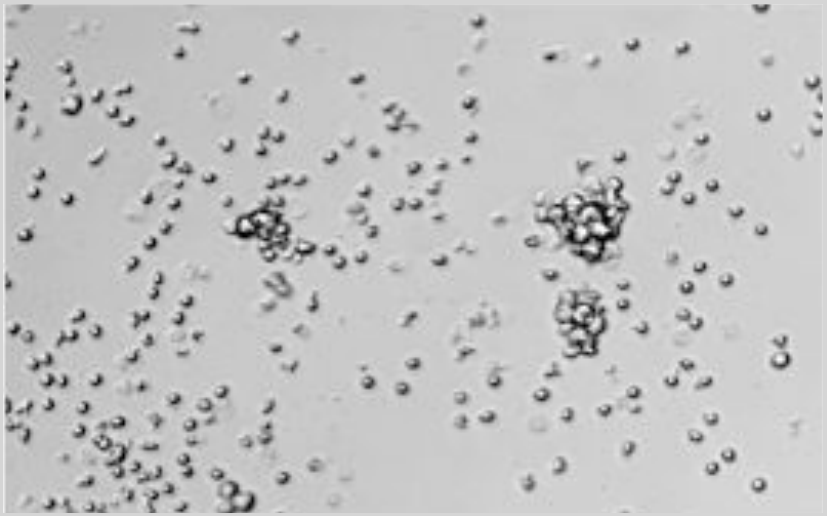


- **Symmetry-breaking in a population**
- **Forming groups of n cells**
- **Using some of what we learned to develop new medical therapeutics**

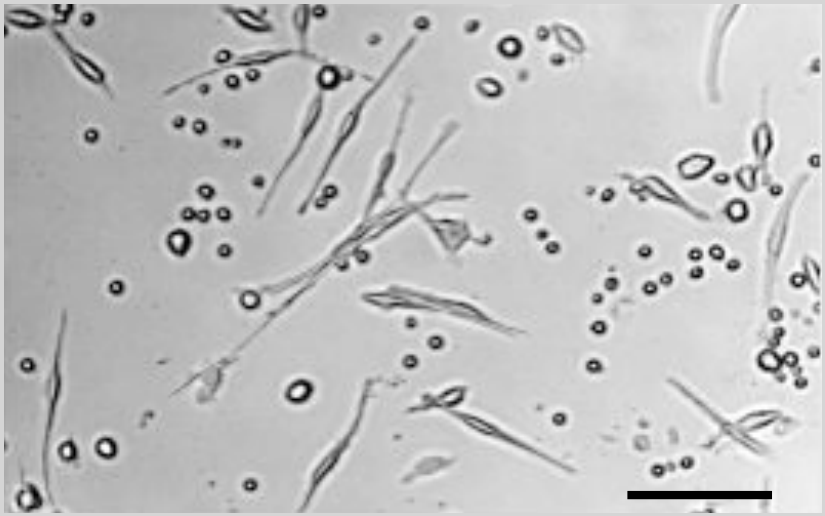
We started to look for cell-density (quorum) sensing factors secreted by human white blood cells

To collect secreted factors, we put human peripheral blood mononuclear cells in serum-free medium

Human peripheral blood mononuclear cells can differentiate into elongated cells within 3 days



1% serum



serum free

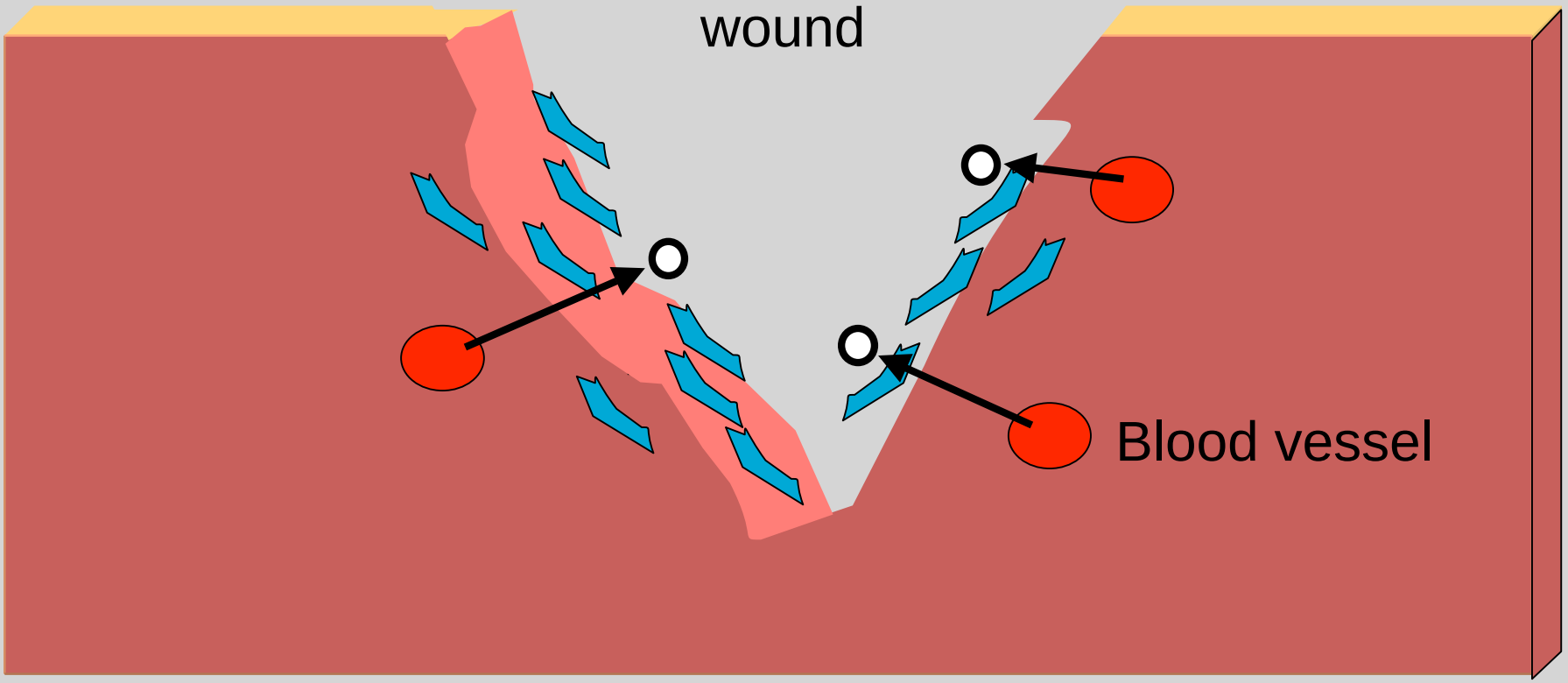
0.1 mm

Thus there is a factor in serum that inhibits fibrocyte differentiation

Introduction: Wound healing

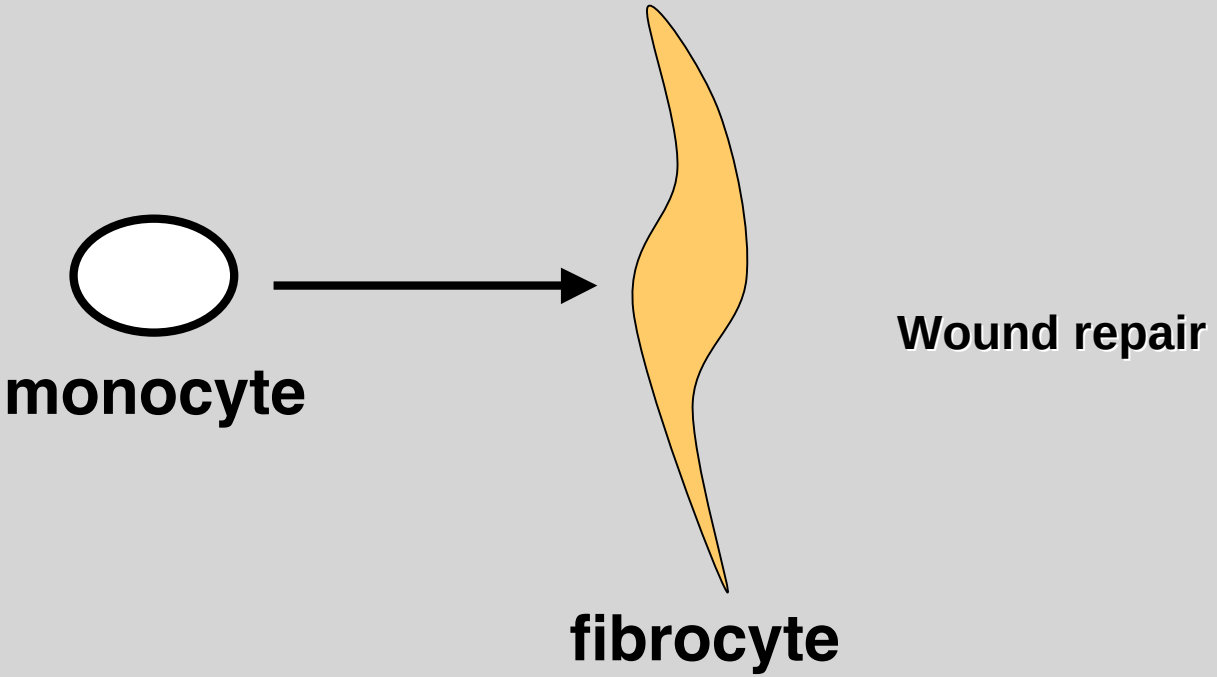
Fibroblasts at wound edge proliferate

Circulating monocytes leave the blood, enter the wound, and differentiate into fibroblast-like cells



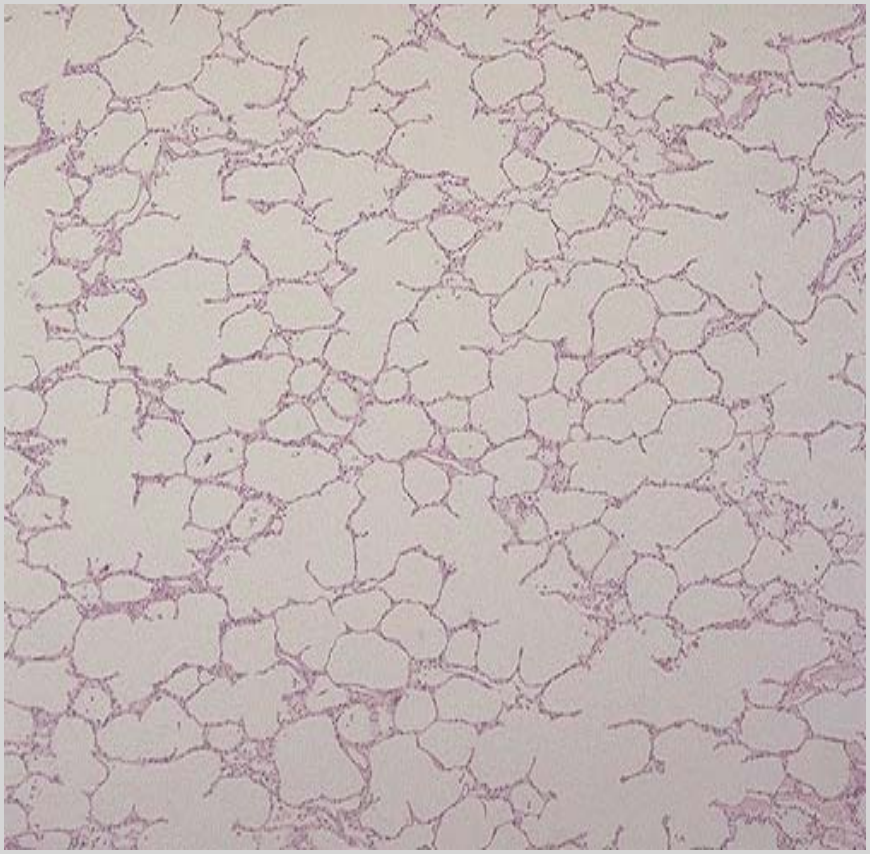
Introduction: Fibrocytes

- **Fibrocytes:** monocyte-derived cells that express markers of both hematopoietic cells and stromal cells

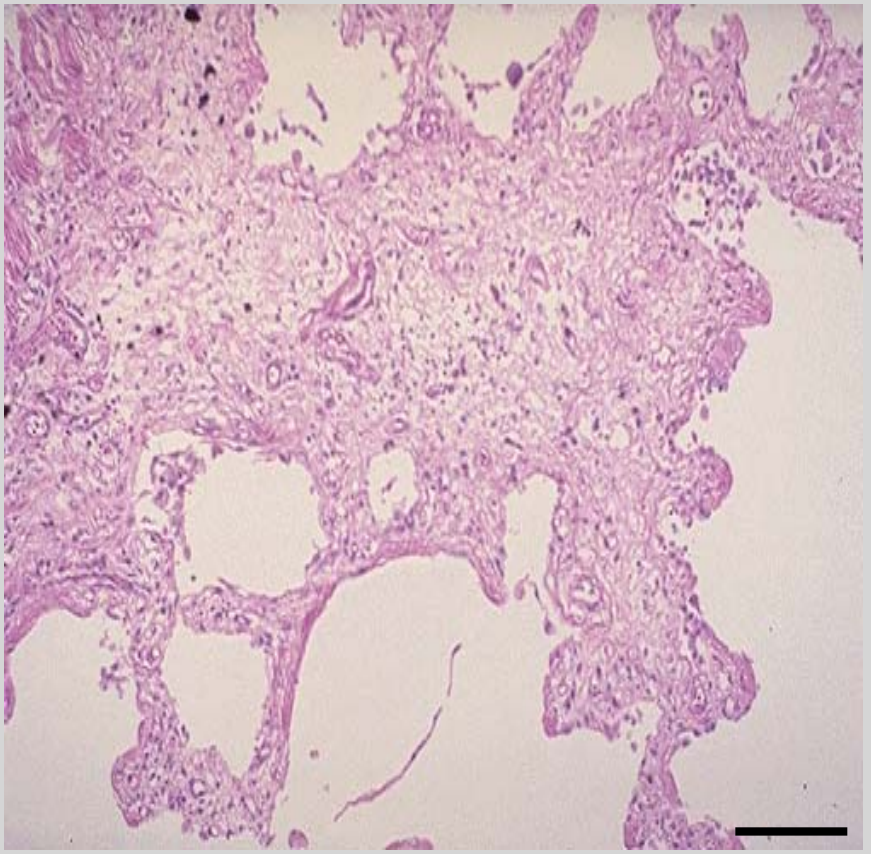


- **Good:** Circulating fibrocyte precursors migrate to injury sites and mediate wound healing

Fibrosing diseases are internal scar tissue



Normal lung



Fibrotic lung

Histology = healing wound

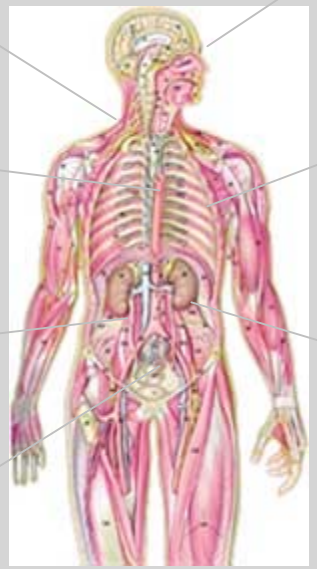
Examples of fibrosing diseases

Skin
- Hypertrophic scarring
- Scleroderma

Cardiac
- Congestive cardiomyopathy
- Post MI scarring
- Restenosis/ intimal hyperplasia

Gastrointestinal
- Liver cirrhosis
- Primary biliary cirrhosis
- Sclerosing cholangitis

GYN
- Fibroids
- Endometriosis



Ocular
- Trabeculectomy surgery
- Corneal refractive surgery

Pulmonary
- Idiopathic Pulmonary Fibrosis
- Chronic asthma
- Mild asthma
- Neonatal bronchopulmonary dysplasia

Renal
- Diabetic nephropathy
- Renal fibrosis
- immune complex disease, HIV nephritis, Lupus nephritis, other

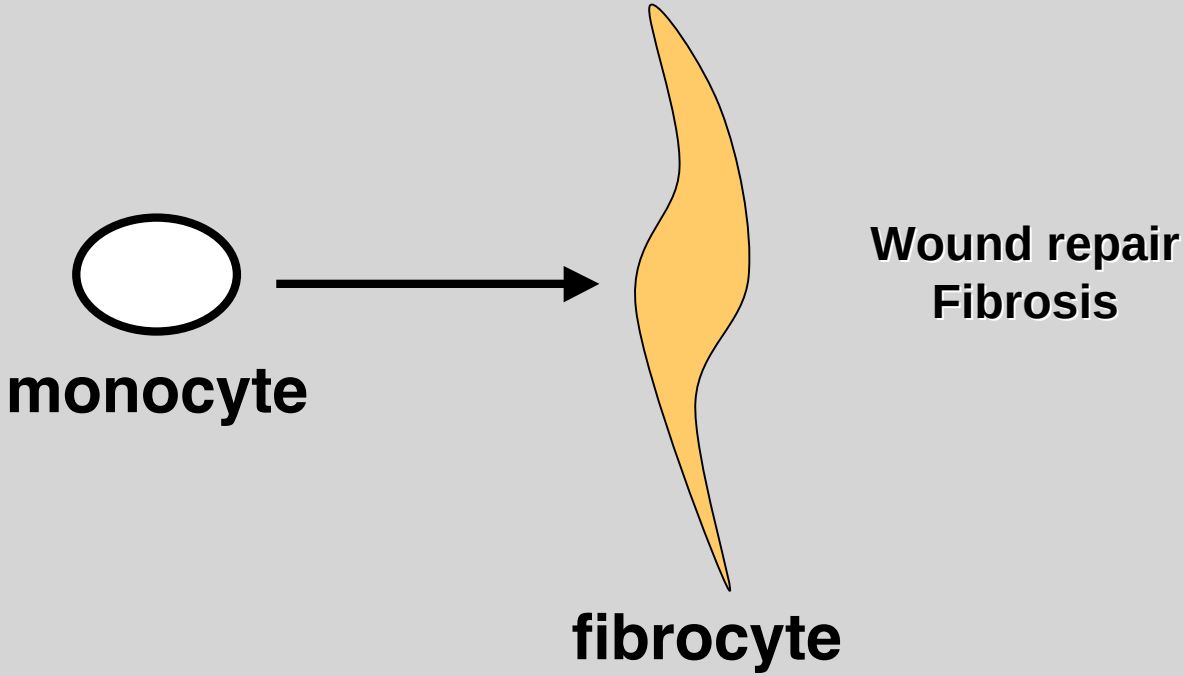
Other
- Radiation fibrosis
- Post-surgical scarring (neuro, ortho, GYN)

Fibrocytes involved

No FDA- approved therapy for fibrosis

Introduction: Fibrocytes

- **Fibrocytes:** monocyte-derived cells that express markers of both hematopoietic cells and stromal cells

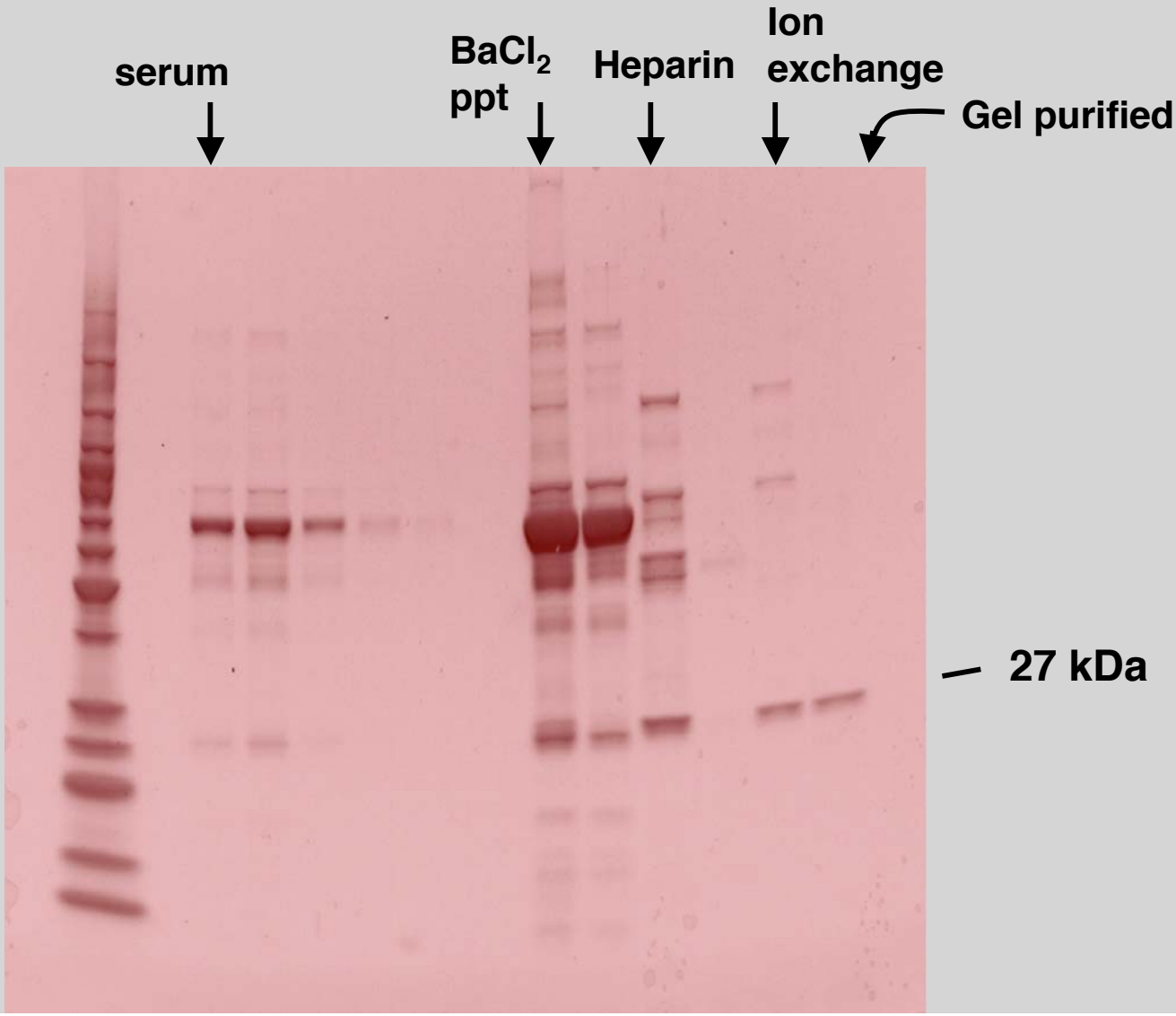


- **Good:** Circulating fibrocyte precursors migrate to injury sites and mediate wound healing

- **Bad:** Fibrocytes are implicated in fibrotic disorders

Nothing was known about what regulates this differentiation

Purification of the activity in serum that inhibits fibrocyte differentiation

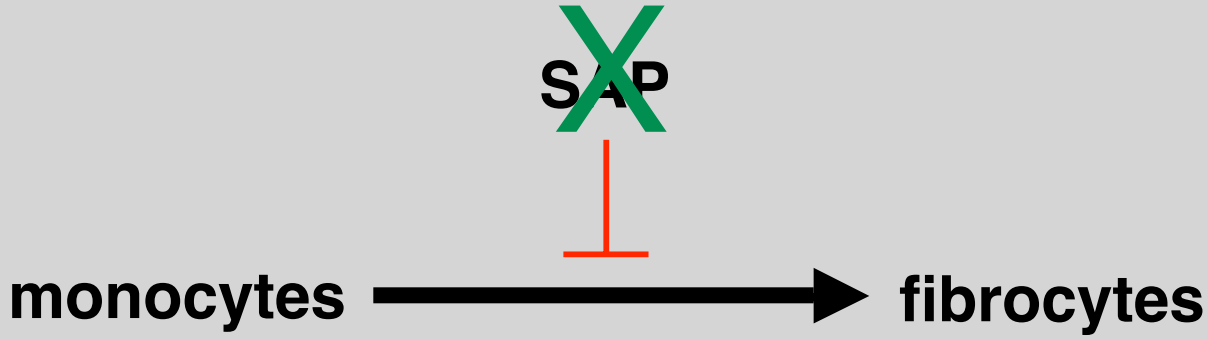


The factor that inhibits fibrocyte differentiation:

MW and peptide sequences match Serum Amyloid P (SAP)

- made by liver, circulates in blood
- linker to help cells bind DNA, bacteria, polysaccharides

Manipulating fibrocyte differentiation

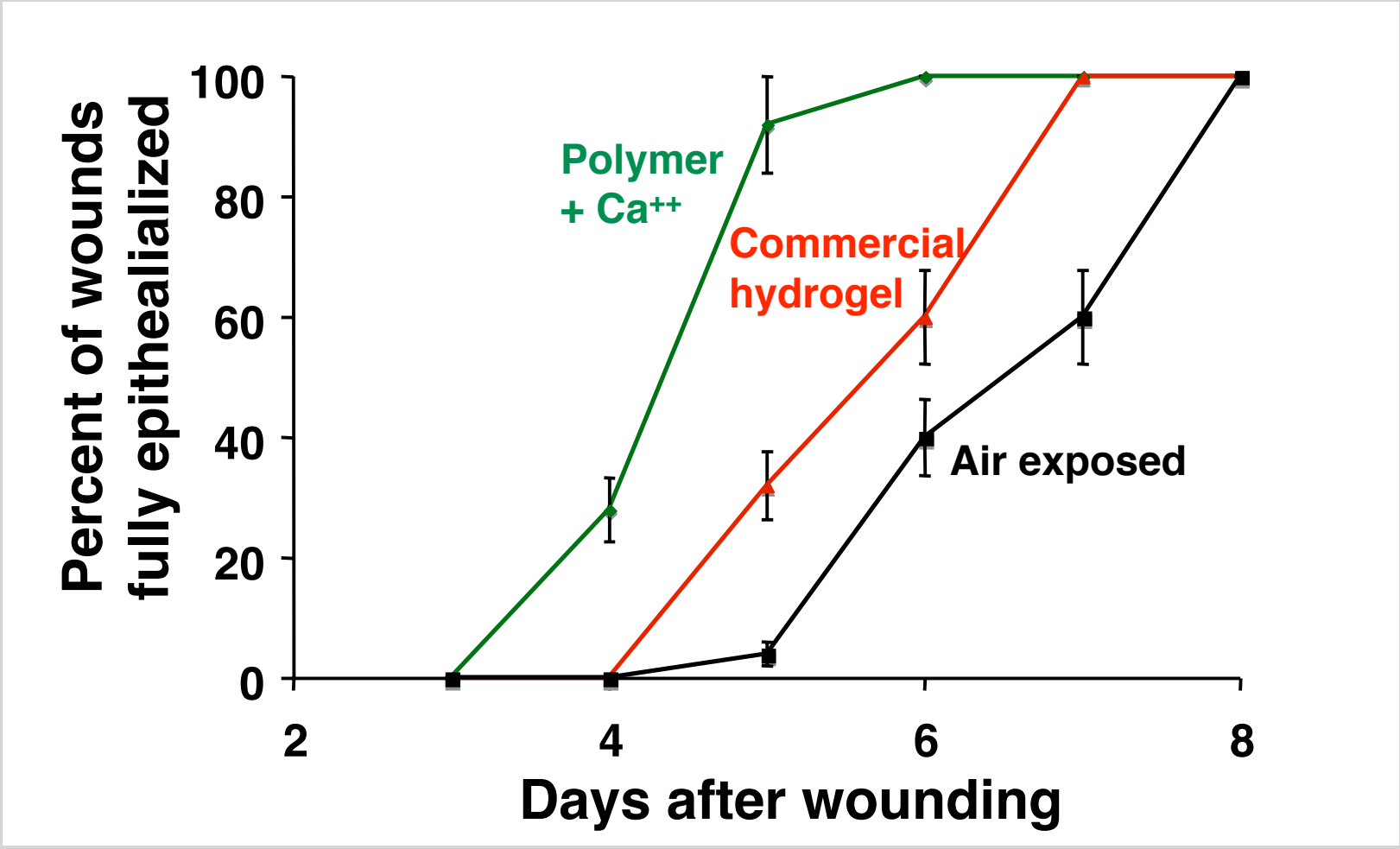


Possibilities:

- **Improve wound healing by removing SAP**
- **Prevent fibrosis by giving SAP**

SAP binds very strongly to a non-toxic biopolymer in the presence of Ca^{++} (other groups working to purify SAP found this)

Depleting SAP speeds wound healing in pigs



Stephen Davis, U. Miami

Manipulating fibrocyte differentiation

Possibilities:

- **Improve wound healing by removing SAP**
- **Prevent fibrosis by giving SAP**

Testing SAP as a therapeutic for pulmonary fibrosis in rats

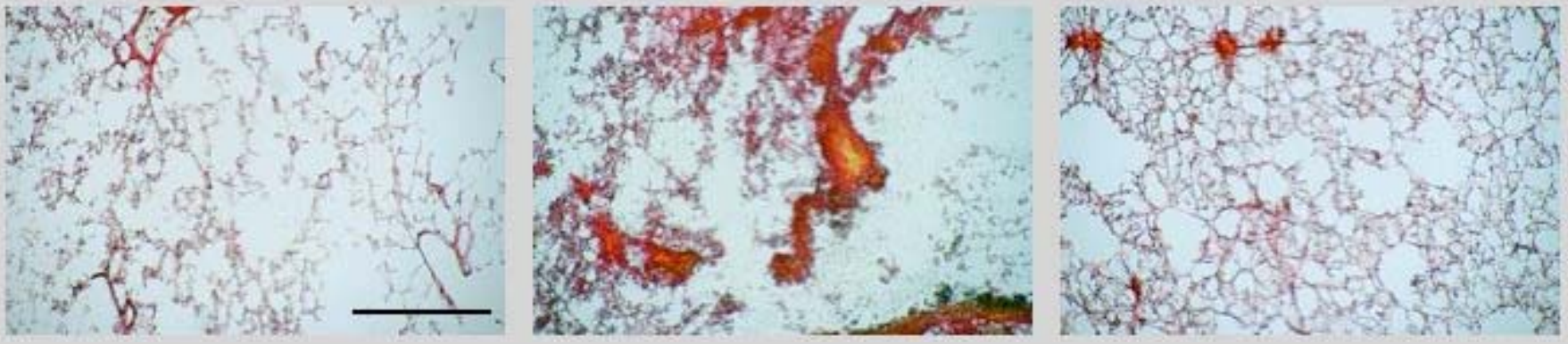
Bleomycin (frontline chemotherapeutic for testicular cancer) causes pulmonary fibrosis

Intratracheal injection in rats causes pulmonary fibrosis in 14 days

Can we prevent this fibrosis with IV injections of SAP (double serum SAP every 2 days for 9 days)?

SAP treatments reduce fibrosis after bleomycin exposure

Saline control **Bleomycin only** **Bleomycin + SAP**



0.5 mm

Picosirius red to label collagen

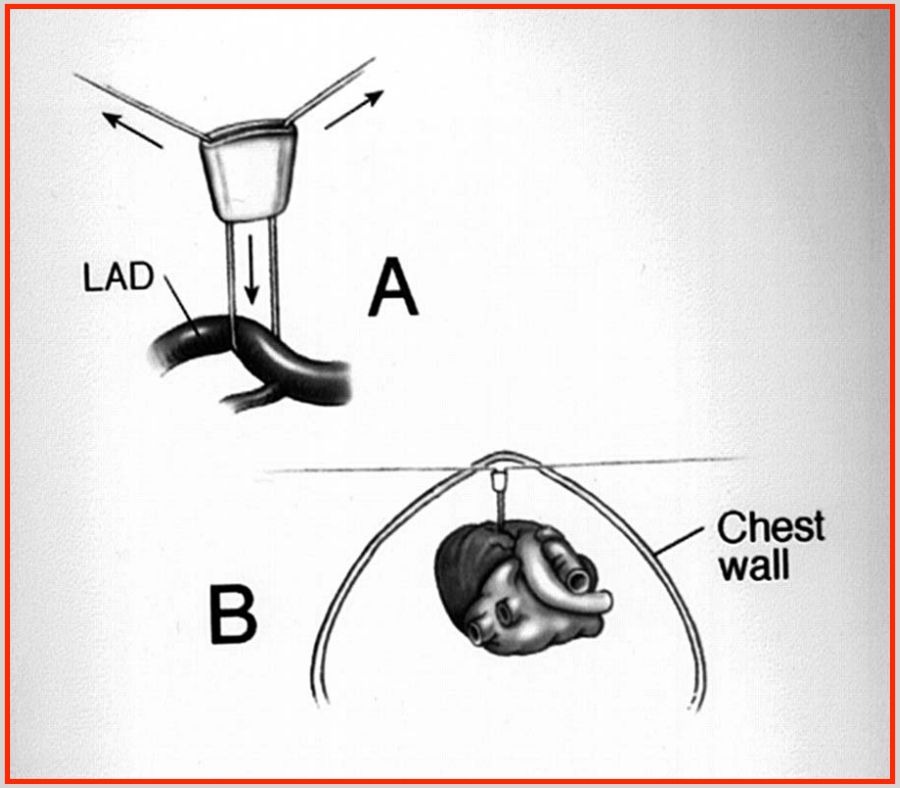
Can SAP prevent cardiac fibrosis?

Mouse Cardiac Fibrosis Model

•Mimics Congestive Heart Failure

- **Place a suture loosely around the left anterior descending coronary artery**
- **Wait 1 week**
- **Tighten the suture for 15 minutes/ day for 5 days**
- **Induces cardiac fibrosis**

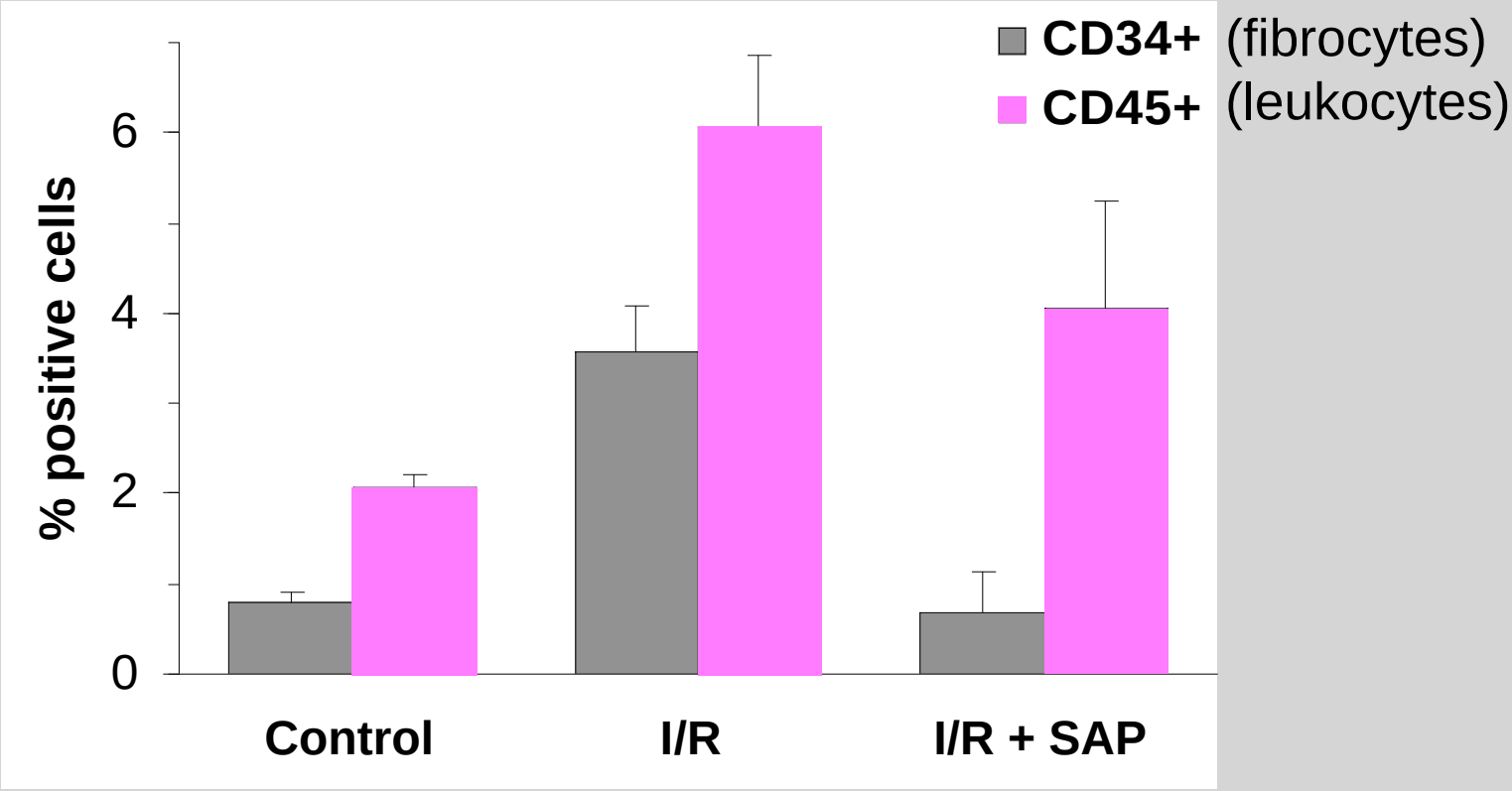
- **Rx: daily injections of murine SAP; if fully absorbed, doubles ambient level**
- **Assay: histology and count fibrocytes by flow cytometry**



Mark Entman, Baylor College of Medicine

SAP injections reduce the number of fibrocytes in the ischemic heart

After 5 days of ischemia/ reperfusion (I/R), sacrifice, dissociate heart cells, and do flow cytometry, staining for fibrocytes (CD34) and leukocytes (CD45)



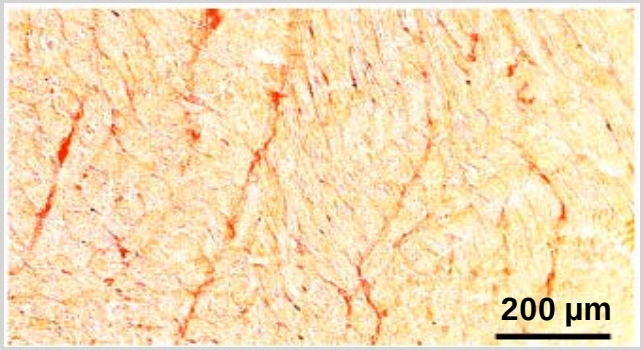
Mark Entman, Baylor College of Medicine

SAP prevents cardiac fibrosis

Histology

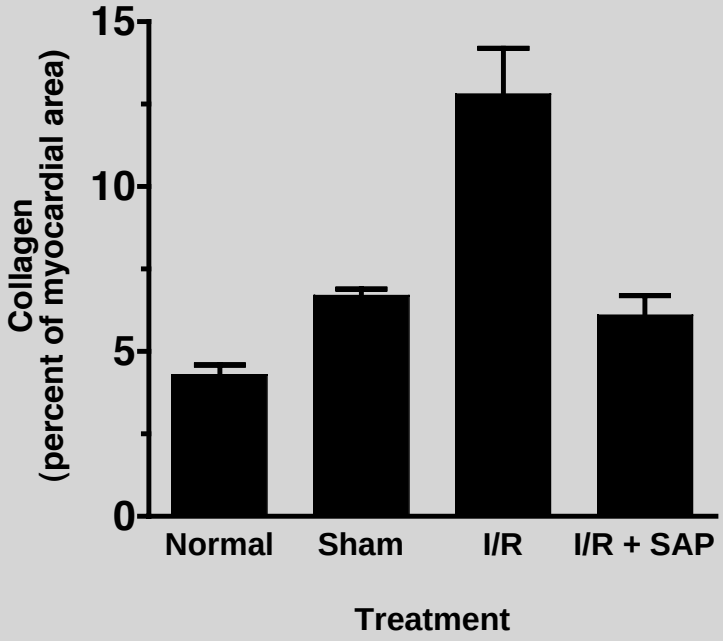
I/R

I/R+SAP



Picrosirius red to label collagen

Collagen content assay

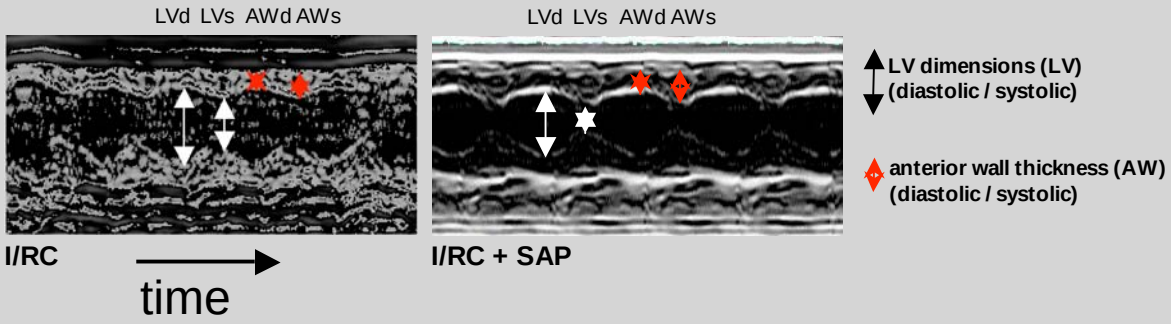


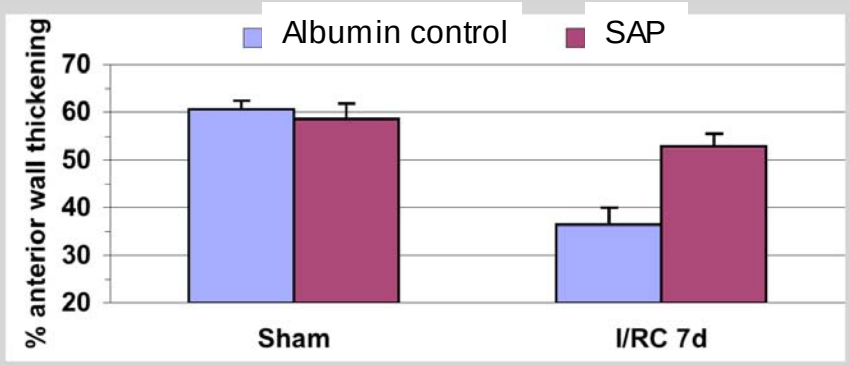
Mark Entman, Baylor College of Medicine

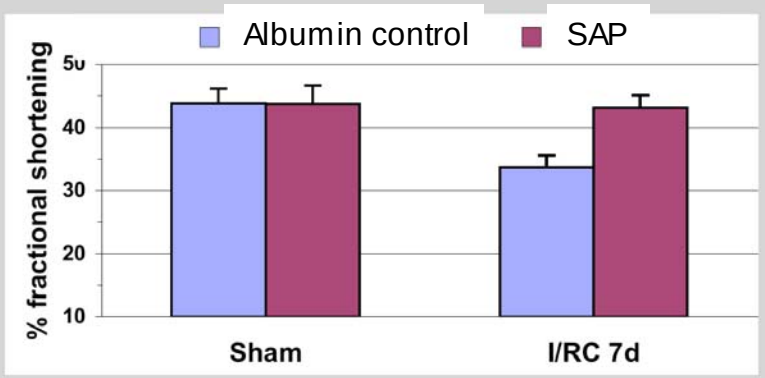
SAP prevents cardiac fibrosis-induced dysfunction

2D directed M-mode echocardiography

% anterior wall thickening = (AW systolic – AW diastolic) / AW diastolic * 100%
% fractional shortening = (LV diastolic – LV systolic) / LV diastolic * 100%

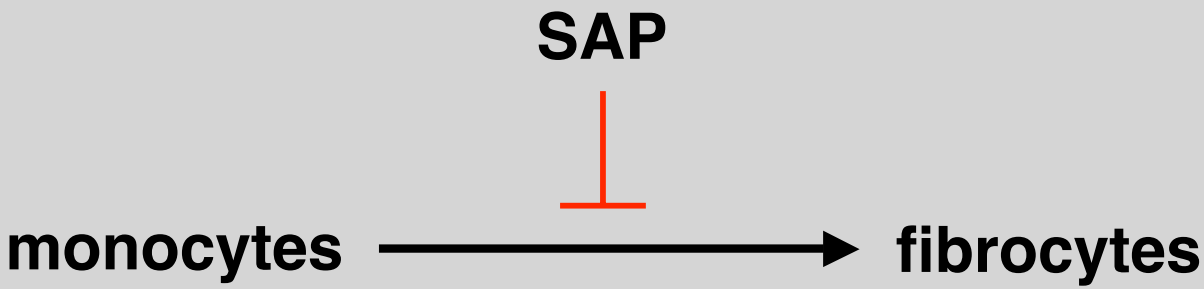






Mark Entman and Sandra Haudek, Baylor College of Medicine

Summary



- SAP injections reduce fibrosis in 3 models
  - pulmonary fibrosis in rats
  - pulmonary fibrosis in mice
  - cardiac fibrosis in mice

- Local SAP injections also effective in mouse wounds, possibly also effective in Red Duroc scarring

- Conversely, a wound healing dressing that binds SAP speeds wound healing in rats, and is better than a current standard wound dressing in pigs

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Wany Jang	CF regulating G6Pase
Yitai Tang	CF regulating PI3K
Michelle Coldiron	CF pathway
Kevin Houston	AprA second site suppressors

<u>Collaborators</u>

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Leland Fan (Texas Children's)	**patient serum samples**
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