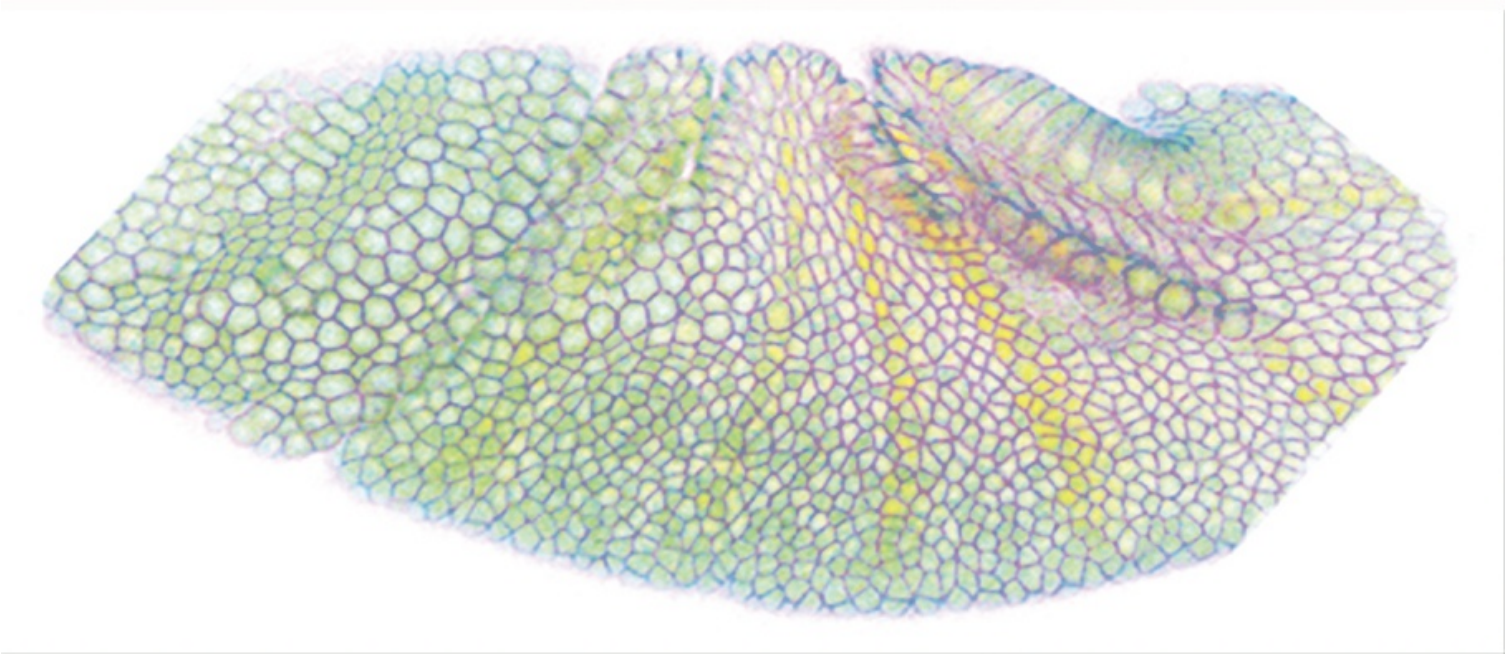
Mechanics of Morphogenesis



Lecture 2: Adhesion: from affinity to thermodynamics models

Thomas Lecuit
chaire: Dynamiques du vivant

COLLÈGE DE FRANCE
1530

Summary- tissue organisation and plasticity

- *Organisation:*
  - Cells adopt morphologies and configurations that tend to approach minimal surface energy
  - Reflects balance between:
    -hydrostatic/turgor pressure
    -cortical tension
    -cell walls/cortex stiffness
    - cell-cell adhesion

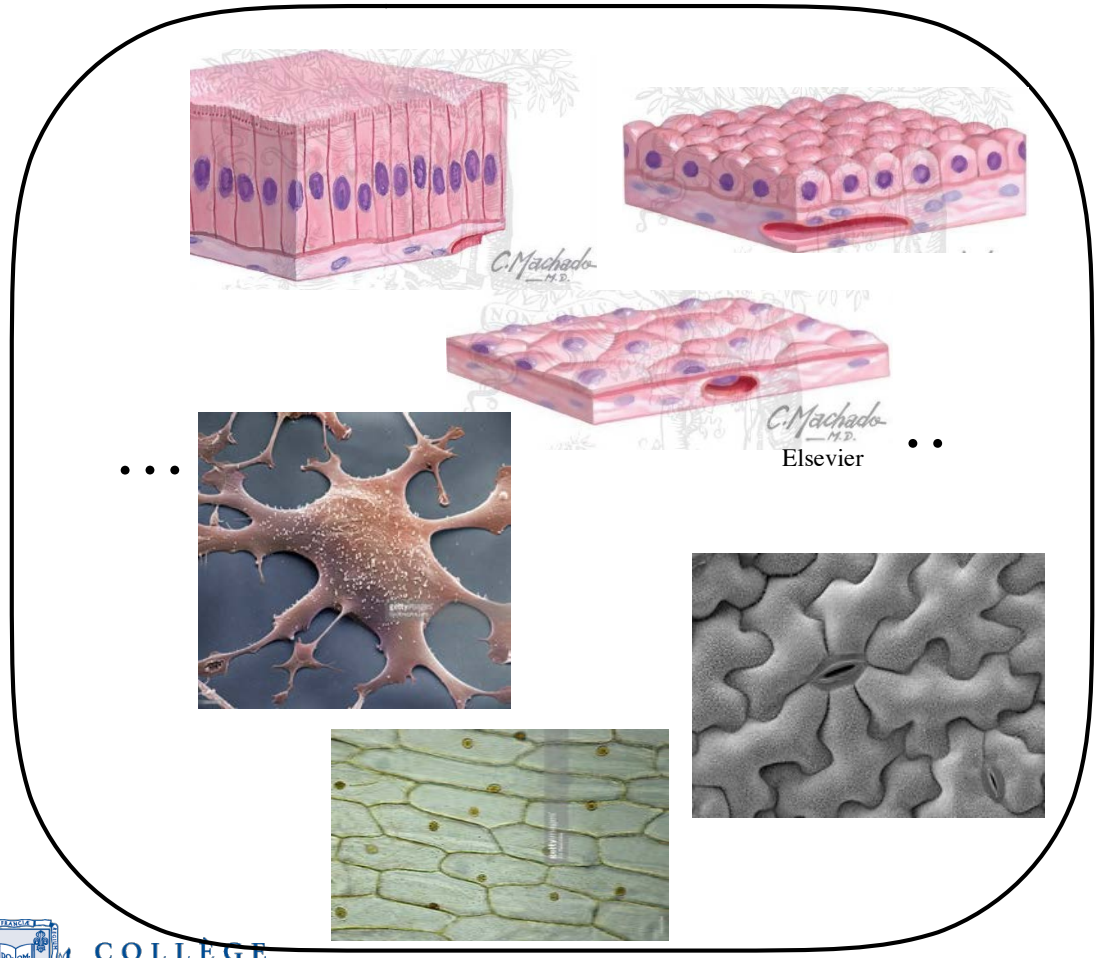
- *Dynamics:*
  - Cell connectedness varies so tissues can be modelled as gaz, viscoelastic fluids or elastic solids.
  - Reflects differences in cell-cell adhesion
  - Cell shape changes and cell movements are driven by active contractile systems in animals and wall remodelling in plants
  - Cell-cell adhesion resists active remodelling and maintains tissue cohesion under stress.

Mechanics of Cell shape - Geometry & Dynamics

- Cells « morpho-space » is a huge multidimensional space
- Genetic/biochemical space of regulatory interactions is also very large

◇ How can we reduce the dimensionality of these spaces?

◇ How many parameters for description of and control over cell shape?



- Quantitative Theory:

Physical parameters

↑ ↓

Biological parameters

Cell shape and Surface Tension

On Growth and Form. chapter V

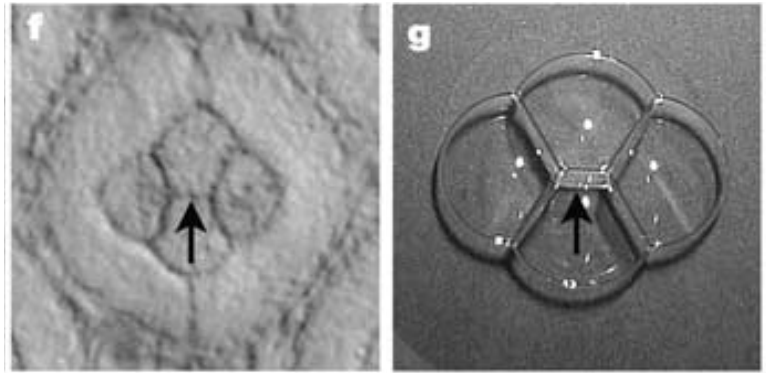
d'Arcy W Thompson, *On Growth and Form*, 1917

- Thermodynamic description: near equilibrium/quasi-static

  > « Justified » by separation of time scales between molecular and cellular processes?

- Minimisation of surface energy E
- Minimisation of surface S
- *Surface tension* $E = \lambda . S$

  λ: amount of work done per unit of surface change



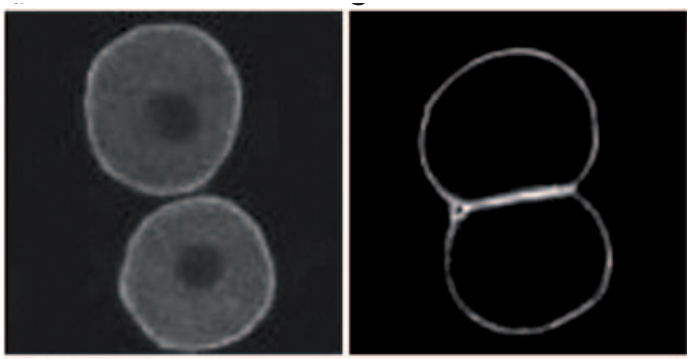
Hayashi T & Carthew R, *Nature*, 431:647 (2004)

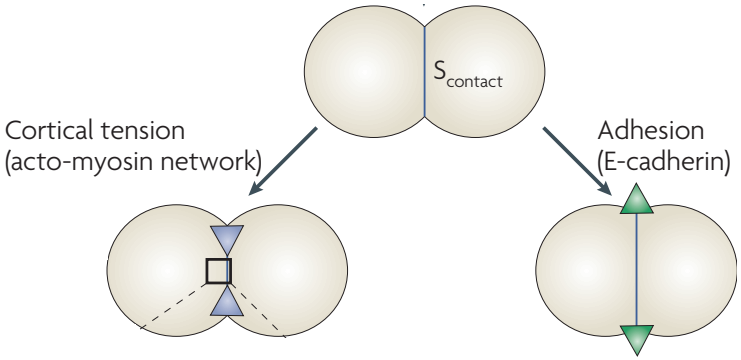
Cell shape and Surface Tension

- Surface tension $E = \lambda . S$

λ: amount of work done per unit of surface change

$\lambda = f(\text{Adhesion}, \text{Tension})$





Lecuit T and Lenne PF, *Nature Rev. Mol. Cell Biol.*, 8:633 (2007)

Adhesion in multicellular organisms

1. Affinity and Adhesion: a specificity problem
2. Adhesion: a thermodynamic model
3. The molecular framework of adhesion
4. Evolutionary origin of adhesion mechanisms
5. Adhesion as an active mechanism
6. Adhesion and *dissipation*

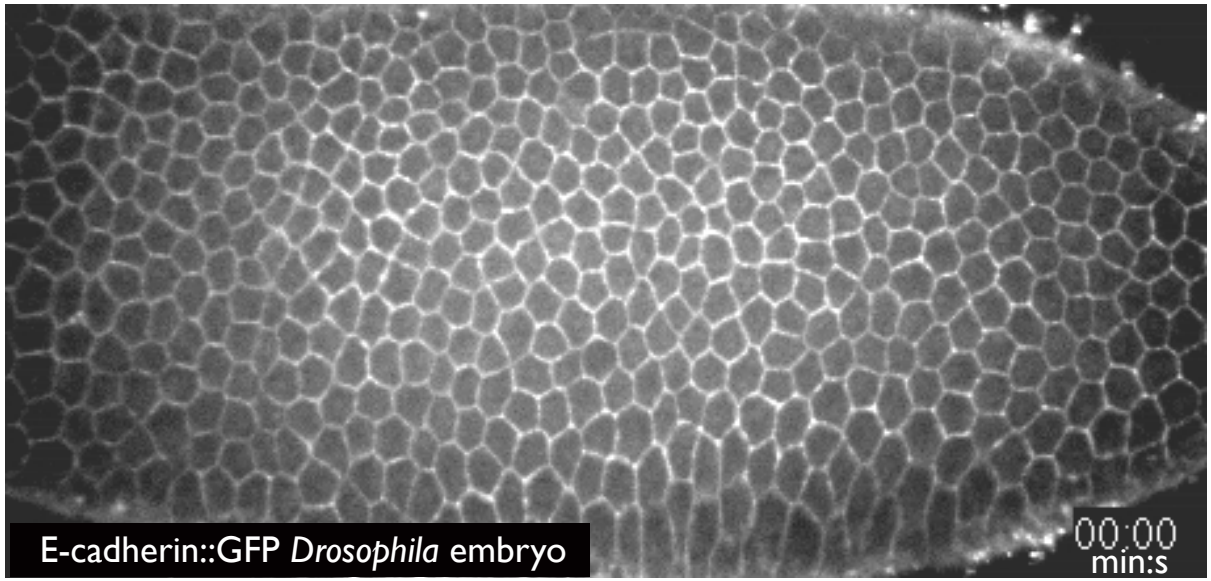
Adhesion in multicellular organisms

- Remarkable process at the cross road of :
  - **Evolution:**
    - *How did multicellular organisms arise?* Principles of cell collective behaviours.
  - **Developmental Biology:**
    - *How do organisms acquire specific shapes?* From aggregates to functional forms.
  - **Cell Biology:**
    - *What are the molecular underpinnings of cell-cell interactions and association?* Principles of specific multi-molecular couplings.
  - **Biophysics:**
    - *What are the physical basis of cell-cell adhesion?* The forces that hold cells together

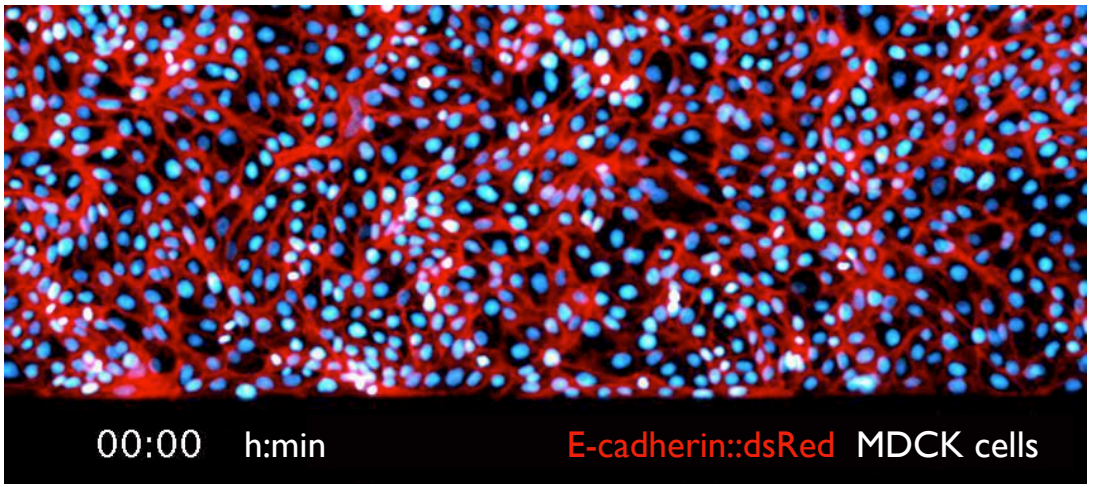
***Explain:* Balance between robust organisation and intrinsic dynamics**

Adhesion in multicellular organisms

- The dynamics of adherent cells



Bertet C., Sulak, L and Lecuit T. *Nature* 429:667. 2004



Cohen DJ, Gloerich M and Nelson WJ. *PNAS*. 113:14698. 2016

Adhesion in multicellular organisms

1. Affinity and Adhesion: a specificity problem

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5. Adhesion as an active mechanism

6. Adhesion and *dissipation*

Regeneration and Cell Aggregation

ON SOME PHENOMENA OF COALESCENCE AND REGENERATION IN SPONGES[1]

BY

H. V. WILSON

University of North Carolina
Chapel Hill, N. C.
October 29, 1907

THE REGENERATION OF SPONGES (MICROCIONA) FROM DISSOCIATED CELLS

H. V. WILSON AND J. T. PENNEY
University of North Carolina

Mechanical dissociation of sponge cells
Spontaneous re-association and formation of new sponges

Interpreted in terms of de-differentiation and re-differentiation

Does not favour the idea that differentiated cells sort according to origin

Henry van Wilson
1863-1939

Cell Aggregation: Cell selective binding

*STUDIES ON CELL AGGREGATION: DEMONSTRATION OF MATERIALS WITH SELECTIVE CELL-BINDING ACTIVITY**

By A. A. Moscona

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CHICAGO, AND MARINE BIOLOGICAL LABORATORY, WOODS HOLE, MASSACHUSETTS

Communicated by George W. Beadle, April 2, 1963

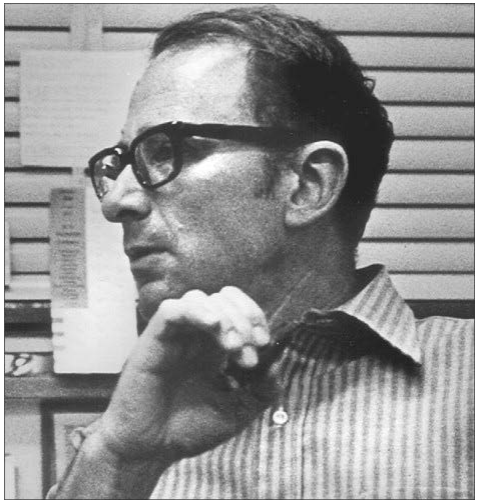
Moscona AA. *PNAS*, 49: 742. 1963

Microciona prolifera

M cells

Haliclona oculata

H cells

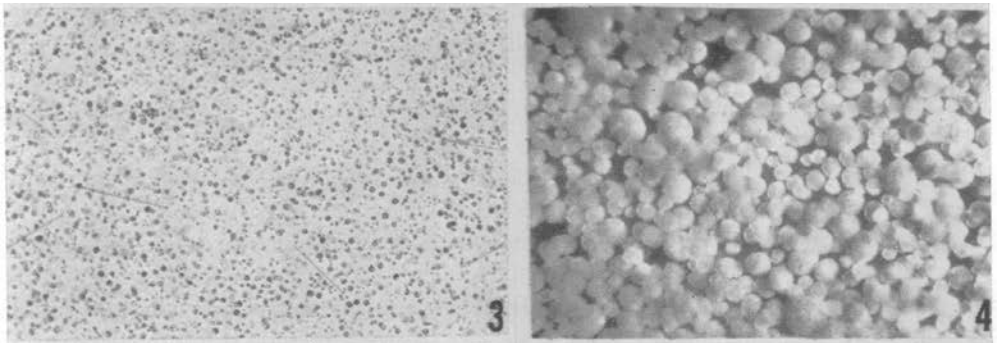


Aron A Moscona in 1972
1921-2009

M and H cells could be tracked based on natural pigmentation of cells

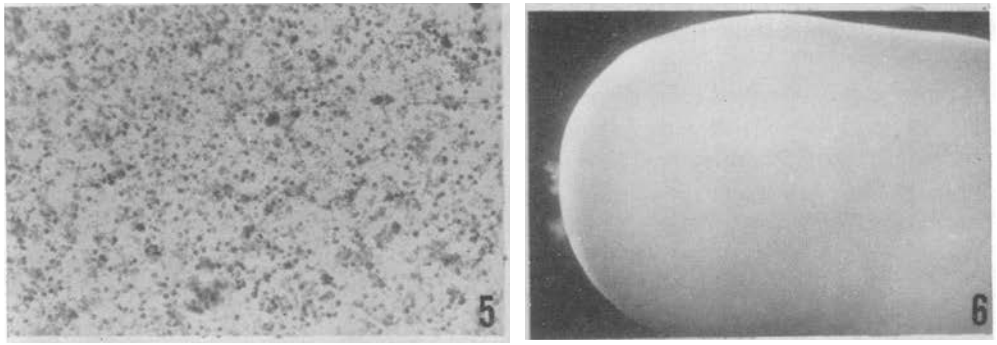
Cell Aggregation: Cell selective binding

Chemical dissociation (no Ca2+) of M cells



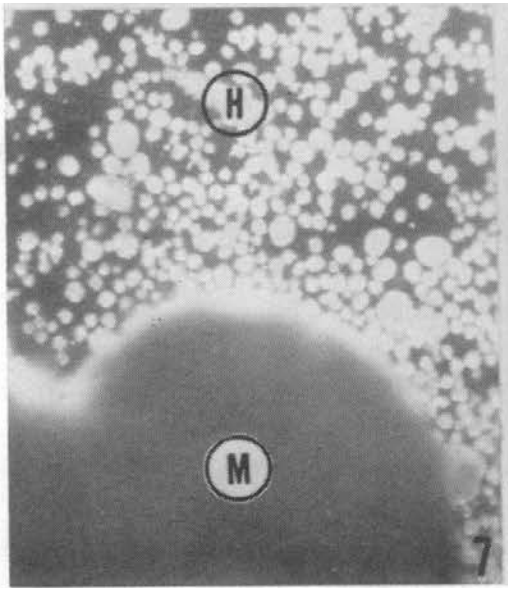
+Ca2+ @ 24°C
t=0

+Ca2+ @ 24°C
t= +24h

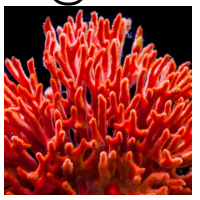


+Ca2+ @ 5°C
t= +24h

+M cell-binding activity
+Ca2+ @ 5°C t= +24h



+M cell-binding activity
+Ca2+ @ 5°C t= +24h



- Re-aggregation of chemically dissociated cells requires:
  -Ca2+
  -High temperature
  -Cell surface binding activity

- The cell surface binding activity shows species specificity

M cell-binding activity:
prepared from dissociation buffer at 0°C, and centrifugation.

Cell Aggregation: Cell selective binding

Cell Aggregation: Properties of Specific Cell-Ligands and Their Role in the Formation of Multicellular Systems

A. A. Moscona

Department of Biology, University of Chicago, Chicago, Illinois, 60637, and Woods Hole Marine Biological Laboratory, Woods Hole, Massachusetts

DEVELOPMENTAL BIOLOGY 18, 250–277 (1968)

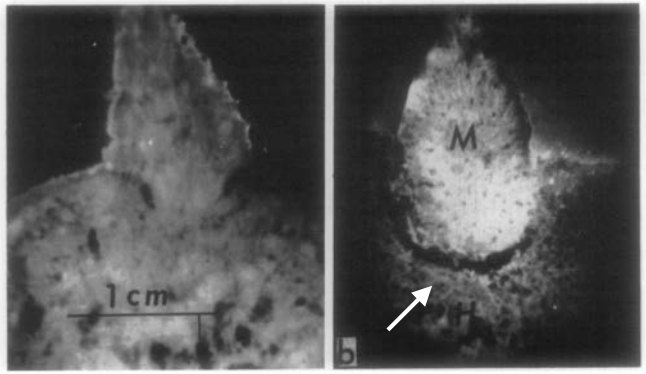


FIG. 1. Sections through 12-hour grafts: (a) *Microciona* (M) on M; note fusion of graft with host; (b) M on *Haliclona* (H); note separation of graft from host cells.

1. **Aggregation of cells and grafting experiments show similar selectivity**

2. Aggregation M Factor shows **specific activity:**
   - activity is specifically depleted in « used medium »
   - still active on formaldehyde fixed cells (not metabolically inactivated)

3. Aggregation M Factor: insensitive to DNAse, RNAse, Trypsin, collagenase, hyaluronidase etc
   sensitive to Pronase (non specific exo/endo peptidase) and a-Amylase (glycosidase)
   > **Surface Glycoprotein**

4. Antiserum against M Factor:
   > inactivates M Factor and induces *Microciona* cell agglutination selectively
   > **Antigenic specificity of M-Factor ligand(s) reflects aggregative affinities of cells**

Cell Aggregation: Molecular theory of specificity

THE PROBLEM OF SPECIFICITY IN GROWTH AND DEVELOPMENT*

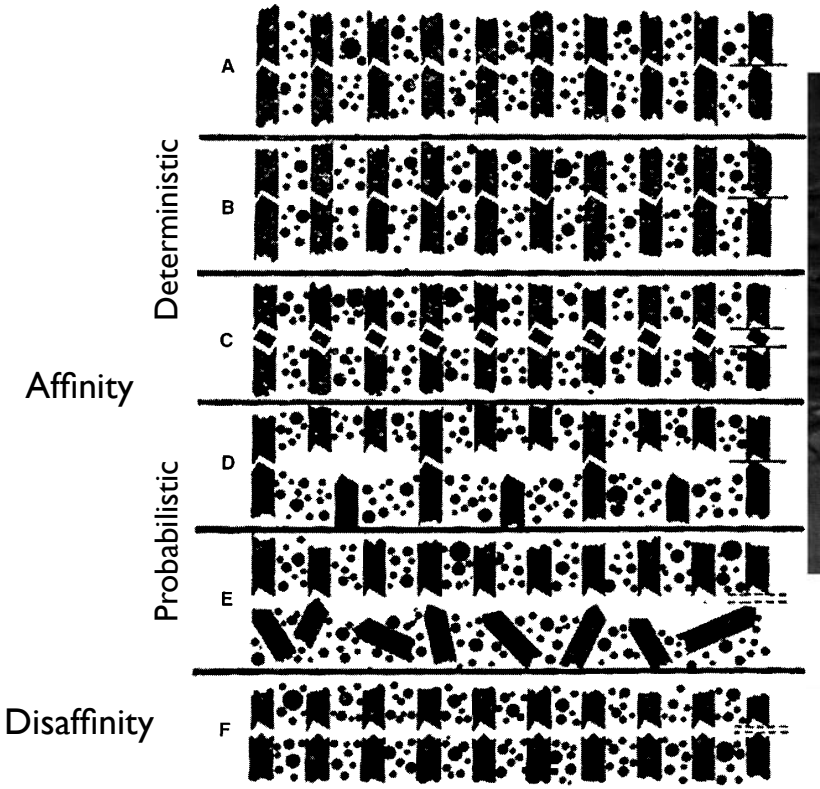
PAUL WEISS

YALE JOURNAL OF BIOLOGY AND MEDICINE 1947

- Interpret cellular affinities in terms of molecular structure and organisation
- Specificity: correspondance and mutual fitting between 2 properties
  Can be resolved in terms of molecular theory

This past discussion leans heavily on current concepts of immunochemistry, particularly those developed by Pauling.[24] It may be premature to tie the phenomena with which we have been dealing too closely to the antigen-antibody model. Rather than trying to force all biological specificity into the immunological compartment, we might have to consider the latter as merely a special case of a more universal biological principle, namely, *molecular key-lock configuration as a mechanism of selectivity,* whether involving enzymes, genes, growth, differentiation, drug action, immunity, sensory response, or nervous co-ordination.

Being contact relationships, they can easily be conceived of as products of intermolecular forces, and their specificity as the result of steric conformances, that is, fittingly interlocking configurations of the molecular species to either side of the surface of contact.* These relations will have to be viewed as dynamic rather than static, and as statistical rather than rigidly fixed; that is to say, as the bonds in question are presumably incessantly made and broken, the rate and frequency of these events are as instrumental in determining the degree of specificity attained as are the nature and arrangement of the molecular groups involved.



Paul A Weiss
1898-1989

Cell Aggregation: adhesiveness of cell membranes

SIGNIFICANCE OF THE CELL MEMBRANE IN EMBRYONIC PROCESSES

By JOHANNES HOLTFRETER*
Biology Department, University of Rochester, Rochester, N. Y.
Annals: New York Academy of Sciences, 709-760. 1948

Based mainly upon observations on amphibian material, the attempt has been made to show that many embryological phenomena may be better understood if we take into consideration the properties and functions of the interfacial membranes which separate the cells from each other and from the external medium. While all cells are furnished with a liv-

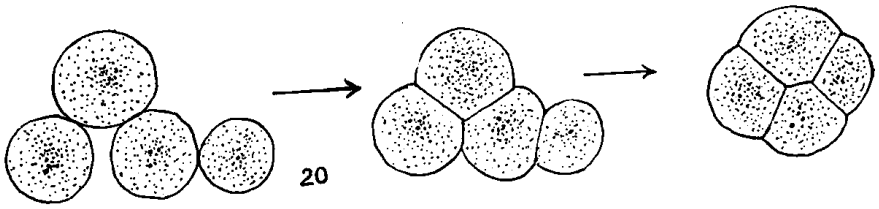


FIGURE 20. Aggregation of myelin vesicles in a 10^{-2}M solution of $CaCl_2$.

The direction of cellular migration, and the histotypical groupings and regroupings exhibited by the various types of cells in a developing organism, appear to be controlled by a selective adhesiveness of the cell membrane, which varies with the developmental stage and with the kind of cells involved. Cellular adhesiveness depends both on the chemical constitution of the contacting cell surfaces and on the composition of the immersion fluid. From the observed antagonistic effects of hydrating

Johannes Holtfreter , *circa* 1950
1901- 1992

With progressive differentiation, there arise cell-specific differences of adhesiveness which are reflected in the display of histotypical patterns of aggregation, disaggregation, migration, and recombination of the various cell strains (Holtfreter, 1939, 1944). It is not known whether these manifestations of a selective adhesiveness result from a molecular lock-and-key mechanism of the naked cell surfaces (Weiss, 1941) or from the interference of specific cementing substances, which may either exudate from the contacting cells themselves or be furnished by the external fluid.

> Explicit reference to Paul Weiss molecular theory of specificity

The Concept of Tissue Affinity

Johannes Holtfreter , *circa* 1950
1901- 1992

J. Holtfreter. Gewebeaffinität, ein Mittel der embryonalen Fortbildung
Arch. Exp. Zellforschung., 23: 169-209. 1939

The Concept of Tissue Affinity

A STUDY OF THE MECHANICS OF GASTRULATION

PART II

JOHANNES HOLTFRETER

Department of Zoology, McGill University, Montreal

J. Exp. Zool. 1943

The shape of the cells in an aggregate is largely a function of their relative position, and of tissue affinity. In a homologous combination, invading endoderm or mesoderm cells stretch perpendicular to the surface and interlock with those of the substratum. In heterologous combinations, an aggregate of like cells tends to keep together and to establish a smooth surface in contact with unlike cells.

Rana pipiens

- Grafting experiments:

Homologous combination
endoderm/endoderm

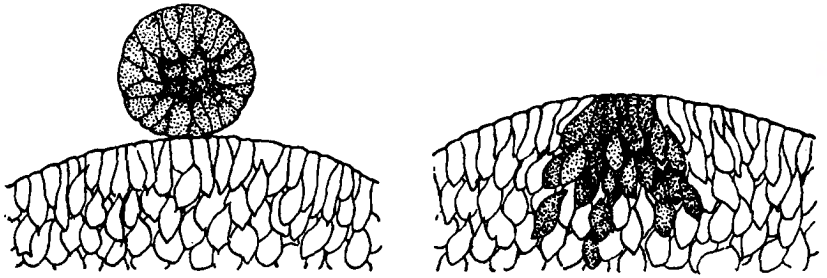


Fig. 17 An aggregate of uncoated endoderm is incorporated into an endodermal substratum but forms no groove.

Heterologous combination
ectoderm/endoderm

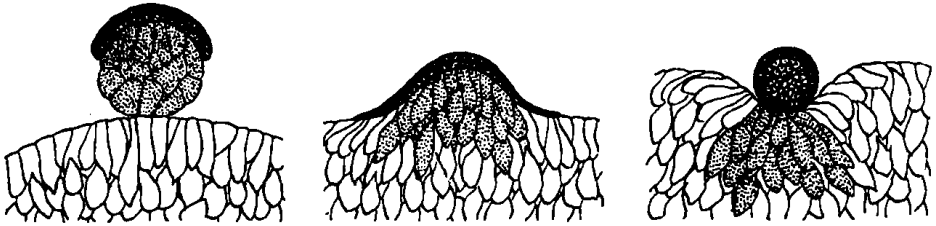


Fig. 21 An endodermal graft covered by ectoderm invaginates into endoderm; the ectoderm spreads at first, then becomes isolated.

The Concept of Cell Affinity

DIRECTED MOVEMENTS AND SELECTIVE ADHESION OF EMBRYONIC AMPHIBIAN CELLS [1]

PHILIP L. TOWNES [2] AND JOHANNES HOLTFRETER
Departments of Biology and Anatomy, The University of Rochester, Rochester, New York

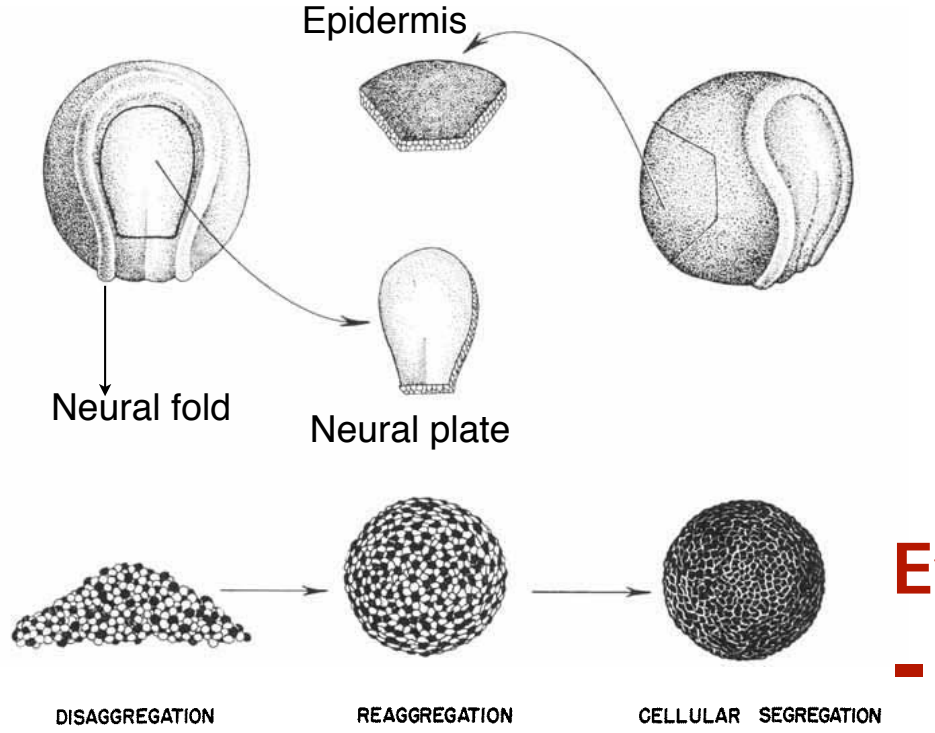


Fig. 10 A piece of the medullary plate and a piece of prospective epidermis are excised and disaggregated by means of alkali. The free cells are intermingled (epidermal cells indicated in black). Under re-adjusted conditions the cells re-aggregate and subsequently segregate so that the surface of the explant becomes entirely epidermal.

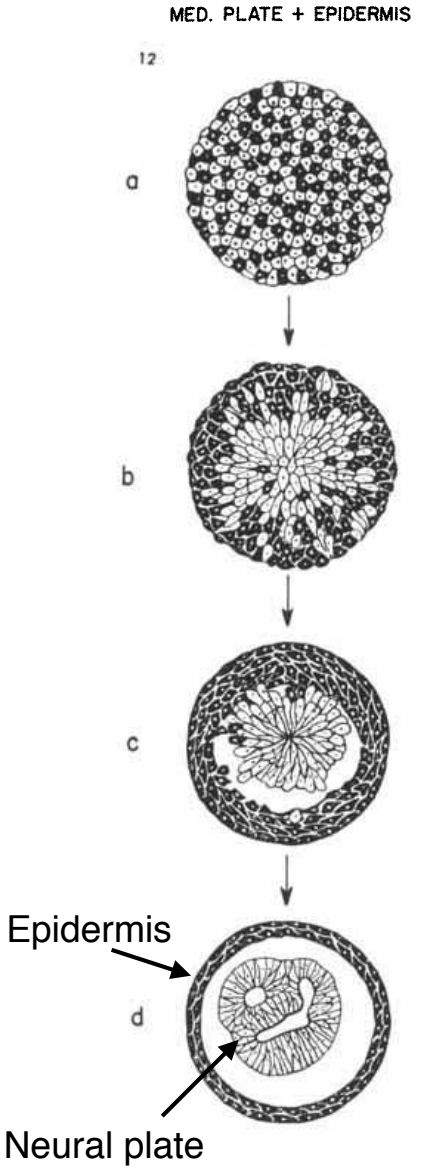
Ambystoma punctatum

Rana pipiens

Evaluate the contribution of:
- **cell motility**
- **cell-cell adhesiveness**

Townes P. and Holtfreter J. *J. Exp. Zool.* 53-120. 1955

The Concept of Cell Affinity



Observations:

- Rapid **cell aggregation**
- Elongation of prospective neural cells
- *Centripetal* **migration** of prospective neural cells
- *Centrifugal* migration of epidermal cells
- **Sorting** of distinct cell populations according to identity
- **Clear separation** between two tissues

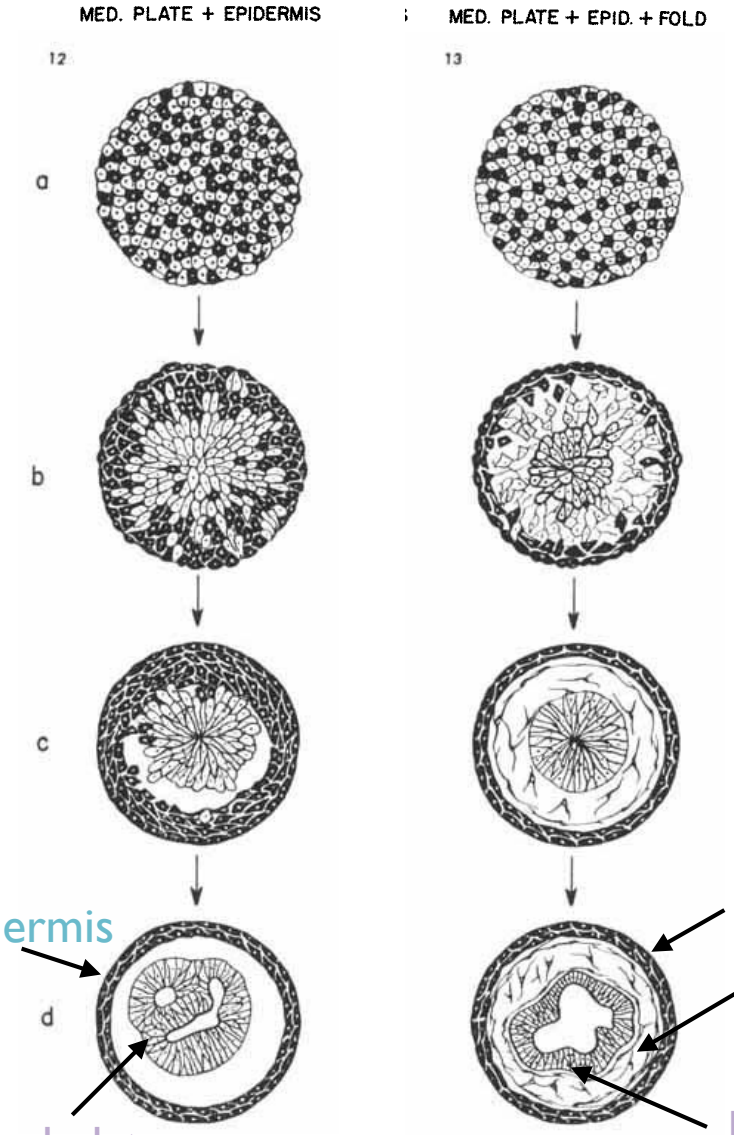
Interpretation:

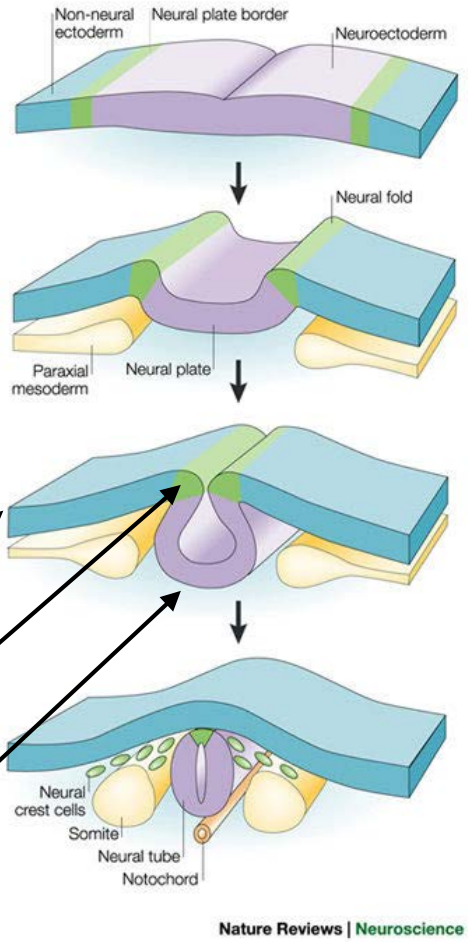
- **Cell-type specific movement**

- 2 forms of **cell adhesion:**
  - not specific at early stages
  - specific at late stages, following prolonged contact

Townes P. and Holtfreter J. *J. Exp. Zool.* 53-120. 1955

The Concept of Cell Affinity





Laura S. Gammill & Marianne Bronner-Fraser, 2003

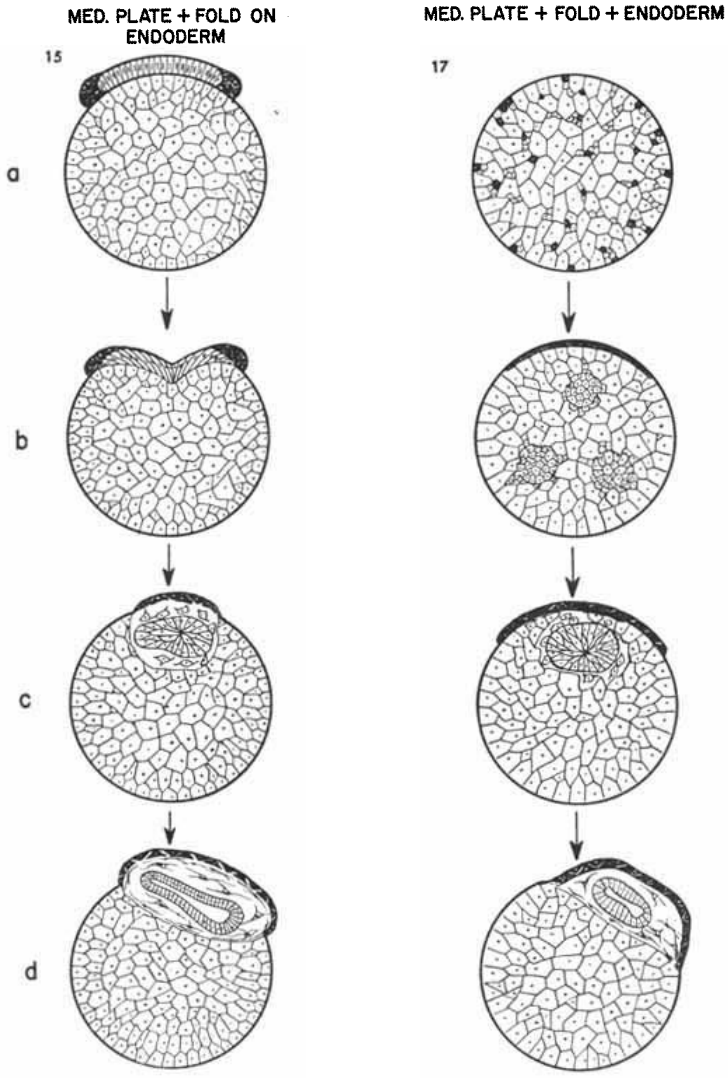
Epidermis

Neural fold

Neural plate

Townes P. and Holtfreter J. *J. Exp. Zool.* 53-120. 1955

Tissue movement and Cell sorting



1. Cells tend to sort out and re-organise in configurations that ressemble the normal embryonic development

2. Tissue movement of 2 kinds:
   - **Spreading**
   - **Invagination**

Townes P. and Holtfreter J. *J. Exp. Zool.* 53-120. 1955

Forces driving Tissue Movement

Argues against role of:

- Cell division and growth
- Tension of outer layer (cortex)
  - *viscous gel layer* characterised by WH. Lewis (42): plasmagel

The relation of the viscosity changes of protoplasm
to ameboid locomotion and cell division.
In: *A Symposium on the Structure of Protoplasm*,
ed. by W. Seifritz, pp. 163-197. 1942

Mechanics of invagination. *Anat. Rec.*, 97: 139-156. 1947

It is inconceivable that epiboly or the formation of such deep cavities as the archenteron could result from curlings due to the contraction of any kind of surface structure. At any rate, single cells, or uncoated cell masses, move into the depth just as well as does a layer of coated cells.

  - elastic coat characterised by J. Holtfreter (43)

Properties and functions of the surface coat in Amphibian embryos
J. Exp. Zool., 93:251-323.

  - May coordinate, but not cause/direct invagination/epiboly.

Conclusions:

-Invagination is driven by some sort of cell locomotior

as proposed earlier, J. Holtfreter. A study of the mechanics of gastrulation. *J. Exp. Zool.* 1943

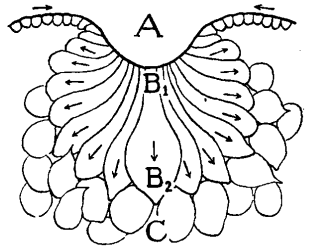


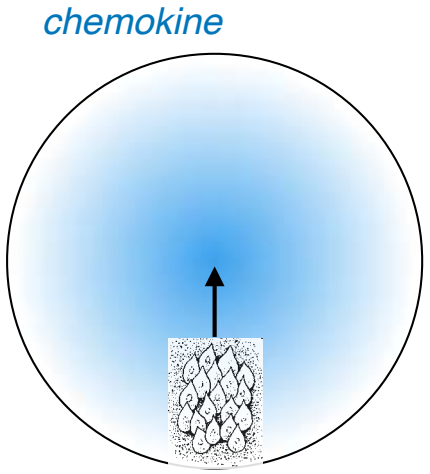
Fig. 24 Diagram illustrating the mechanics of invagination.

Townes P. and Holtfreter J. *J. Exp. Zool.* 53-120. 1955

Forces driving Cell Sorting

1. Active, directed cellular motility:

- Cell-type specific tendency to migrate outward or inward
- Cells respond uniquely to an inside-outside gradient
- Interpreted as akin to a gradient of interfacial tension



They suggested that if the blastocoel, or the interior of a cell aggregate in general, contains surface tension-lowering substances, invagination may be due to a kind of cytotactic reaction of the proximal cell surfaces to a gradient of interfacial tension between inside and outside of the embryo. The cells or cell masses would move toward the surface tension-lowering gradient comparable to the engulfing of certain kinds of oil drops by a sea urchin egg.

see L. Rhumbler 1927
MJ. Kopac & R. Chambers 1937
J. Holtfreter 1943

- **Requires labile, unspecific intercellular adhesive bonds and mobile molecules**

In morphogenesis, the forces controlling directed movements must overcome those of cell adhesion. It becomes evident once more that the molecular bonds involved in these early, temporary and indiscriminate cell adhesions are extremely labile and as such not comparable to the bonds involved in antigen-antibody reactions.

Townes P. and Holtfreter J. *J. Exp. Zool.* 53-120. 1955

Forces driving Cell Sorting

2. Passive, cellular adhesiveness:

21. Early adhesion:

Cell motility resisted by indiscriminate, weak adhesive bonds

22. Late, tissue-specific cell-cell adhesion:

- can vary in degree/strength

It is the fact that adhesiveness between different tissues may vary in *degree*. Dissection of a fresh neurula shows for instance that the prechordal mesoderm is much less firmly attached to the medullary plate than is the notochordal tissue.

- Molecular interpretation:

> **Key-lock mechanism**

based on Pauling's theory
that strength of chemical bonds proportional to complementariness of molecules (P.Weiss, 47)

> **Labile bonds and molecular mobility**

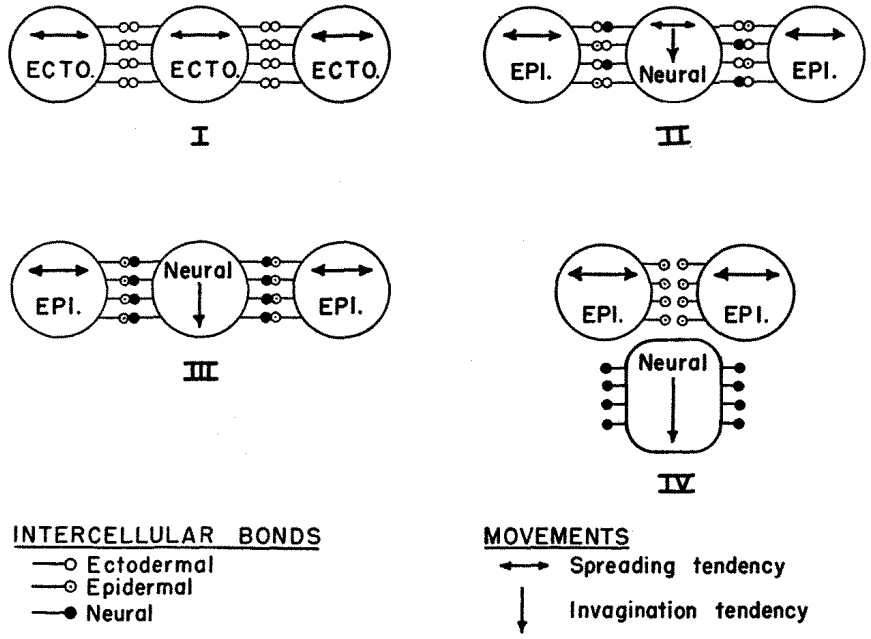
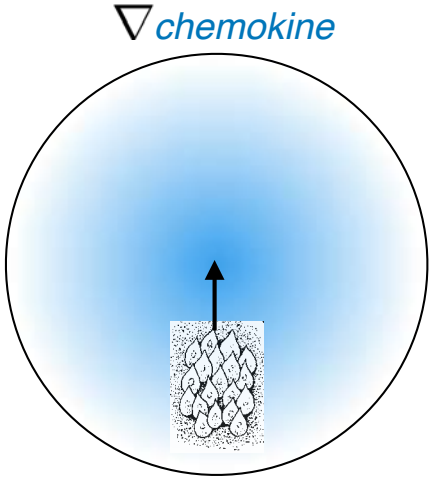


Fig. 27 Schematic representation of changes in intercellular bonds and migration tendency leading to segregation of medullary and epidermal cells (or tissues).

Townes P. and Holtfreter J. *J. Exp. Zool.* 53-120. 1955

Forces driving Cell Sorting

1. Central role of cell-specific directed motility
2. Gradient of chemokine
3. Suggests possible role of surface tension at cellular level
4. Cell-specific adhesion stabilises final configuration



5. In consequence of directed movements, the different cell types in a composite aggregate are sorted out into distinct homogeneous layers, the stratification of which corresponds to the normal germ layer arrangement. The tissue segregation becomes complete because of the emergence of a selectivity of cell adhesion

- Uncouples motility and adhesion
- Uncouples adhesion and surface tension

Townes P. and Holtfreter J. *J. Exp. Zool.* 53-120. 1955

Adhesion in multicellular organisms

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The Differential Adhesion Hypothesis

Malcolm Steinberg
1930-2012

Reconstruction of Tissues by Dissociated Cells

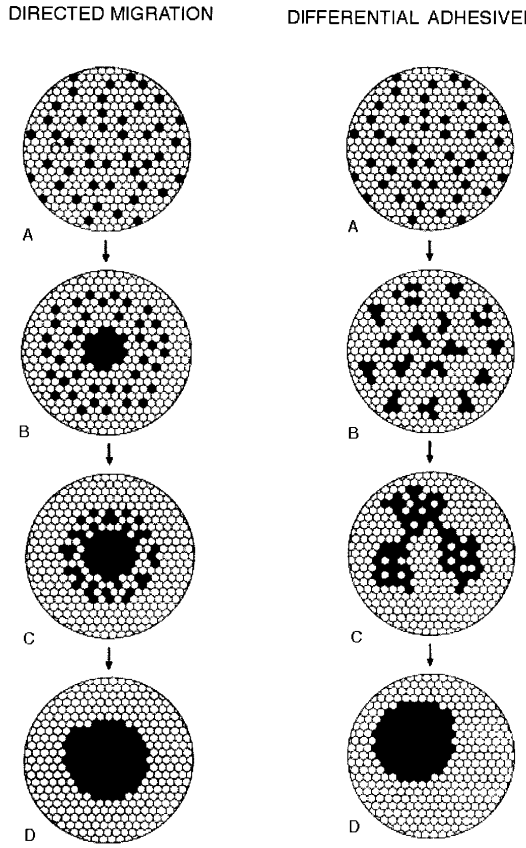
Some morphogenetic tissue movements and the sorting out of embryonic cells may have a common explanation.

2 August 1963, Volume 141, Number 3579 Malcolm S. Steinberg

SCIENCE

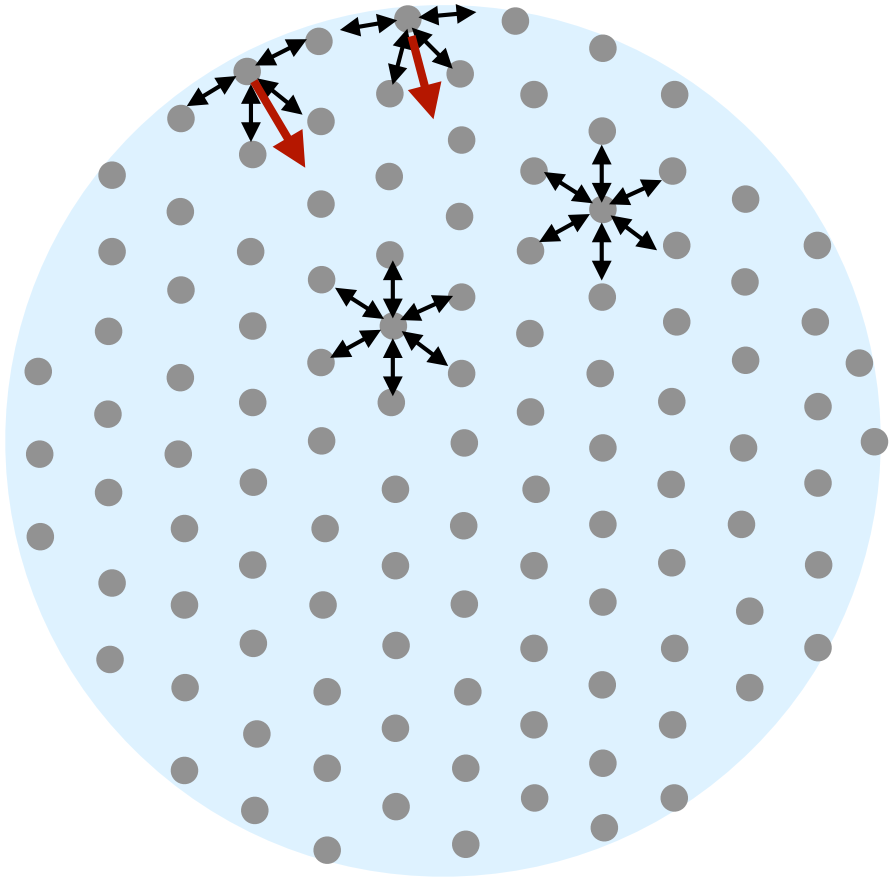
Holtfreter:
- directed motility

Steinberg:
- random motility and selective stabilisation by adhesion
- based on quantitative differences in adhesion
- unrestricted by qualitative specificity
- cells in tissue are like molecules in a fluid



Steinberg MS. *Science*. 141:401-408. 1963

Interfacial Energy or Surface Tension



Interfacial Energy: J/m^2

- maximisation of intermolecular forces minimises the free energy
- anisotropic forces at interface associated with increased interfacial energy compared to bulk
- Free energy minimisation causes surface minimisation
- It is the amount of reversible work to change the surface $dE = k.dS$

Surface tension: N/m

- derives from free energy difference between interface and bulk
- consequence of net inward intermolecular force at interface

A thermodynamic view of Adhesion

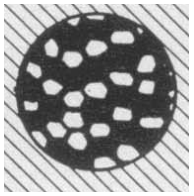
1. Cells in a tissue are akin to molecules in a fluid
(though recognises that cell movement is active: « active fluid »)

2. Free Energy minimisation of liquid drop/cell aggregate predicts its organisation at thermodynamic equilibrium

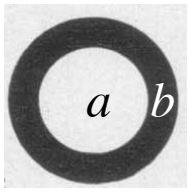
3. Tissue interfacial free energies arises from cellular adhesive interactions

4. Work of adhesion: work done in the realisation of adhesion over unit area
(between 2 phases)
Work of cohesion: (within single phase)

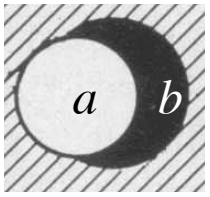
$$W_{ab} \geqq \frac{W_a + W_b}{2}$$ no-sorting



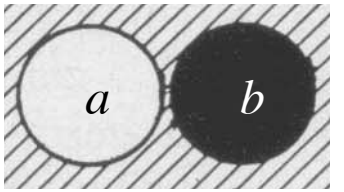
$$W_{ab} < \frac{W_a + W_b}{2}$$ sorting



or



or



$W_{ab} \geqq W_b$
$W_a > W_{ab}$

$W_a \geqq W_b > W_{ab}$

Steinberg MS. *Science*. 141:401-408. 1963

$W_{a,b}$ or W_{ab}

a: strongly cohesive unit
b: weakly cohesive unit

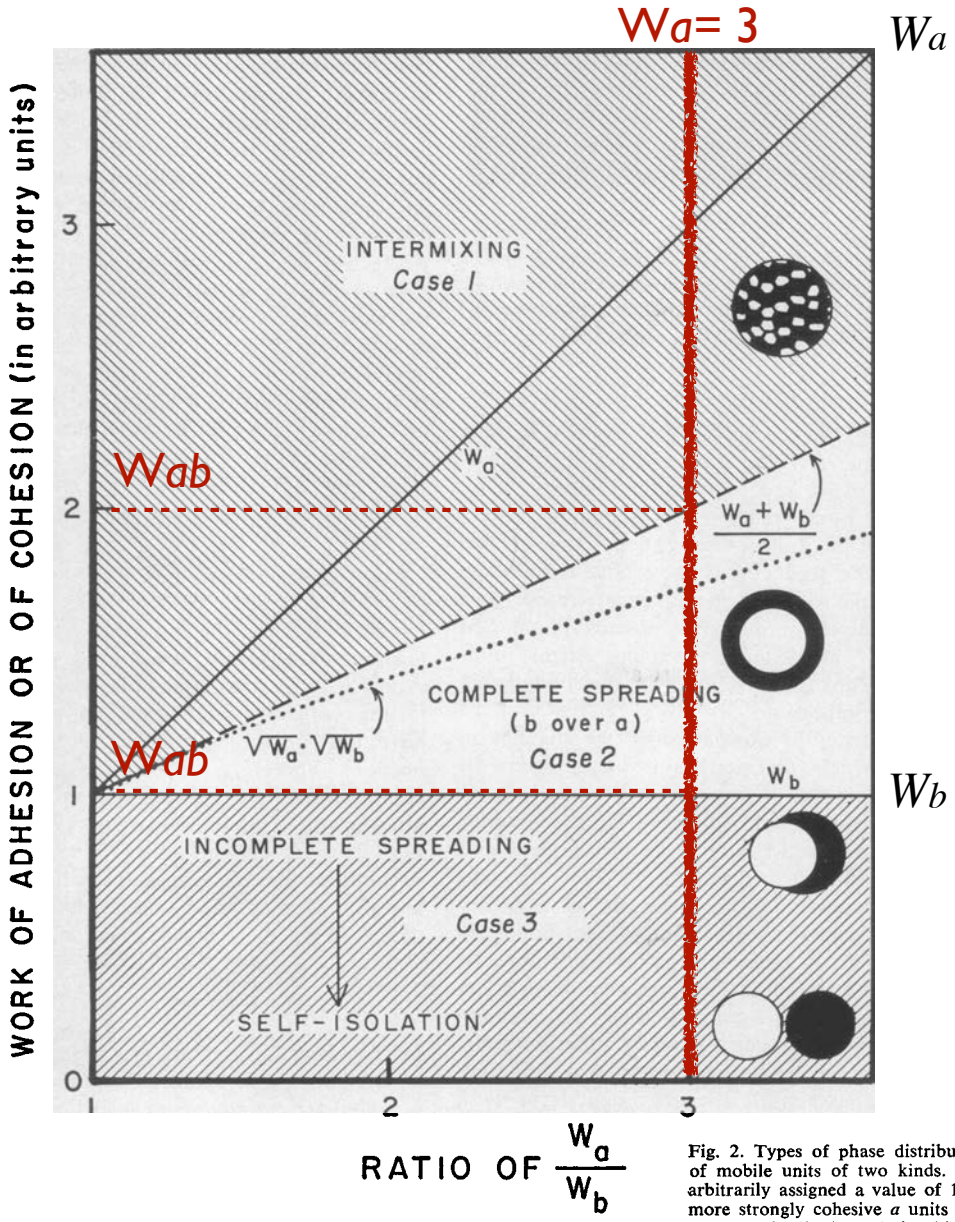


Fig. 2. Types of phase distribution, at equilibrium, in coherent populations consisting of mobile units of two kinds. The work of cohesion of the weakly cohesive *b* units, arbitrarily assigned a value of 1, is given by the line W_b. The work of cohesion of the more strongly cohesive *a* units is denoted by W_a. The diagram is used as follows. For any set of adhesive relationships, that vertical line is drawn which passes through the calculated value of W_a/W_b as read on the abscissa. The work of adhesion of *a* units to *b* units (W_{ab}), as read on the ordinate, is then entered upon this line. The background shading at this point indicates the distribution of the *a* and *b* phases for this system at thermodynamic equilibrium. *Example*: If $W_a = 3$, with W_b defined as 1, then $W_{ab} = 2.1$ would yield intermixing; $W_{ab} = 1.5$ would yield complete coverage of *a* by *b*; and $W_{ab} = 0.5$ would yield incomplete coverage of *a* by *b* (see *24*). The intersection of the vertical line with the dotted line $(W_a)^{1/2}$ $(W_b)^{1/2}$ marks the value of W_{ab} which would be generated in the model system devoid of adhesive specificity, as described in the text. [Modified from Steinberg (*30*)]

Steinberg MS. *Science*. 141:401-408. 1963

Model without adhesive specificity

Assumptions: - Unique molecular species involved in adhesion
- Different average densities in cells a and b

If the frequency of adhesive sites per unit area on the surfaces of cells a and b is designated f_a and f_b, respectively, the probability of apposition of sites in the cell pairs a-a, b-b, and a-b is given by $(f_a)^2$, $(f_b)^2$, and $(f_a)(f_b)$ for the respective cases. Introducing the proportionality constant k, we may write the equations

$$W_a = k\,(f_a)^2 \qquad (7)$$

$$W_b = k\,(f_b)^2 \qquad (8)$$

$$W_{ab} = k\,(f_a)(f_b) \qquad (9)$$

$$W_{ab} = \sqrt{W_a} \cdot \sqrt{W_b}$$

Following the convention that $W_a \geqq W_b$, we obtain

$$f_a \geqq f_b \qquad (10)$$

$$\downarrow$$

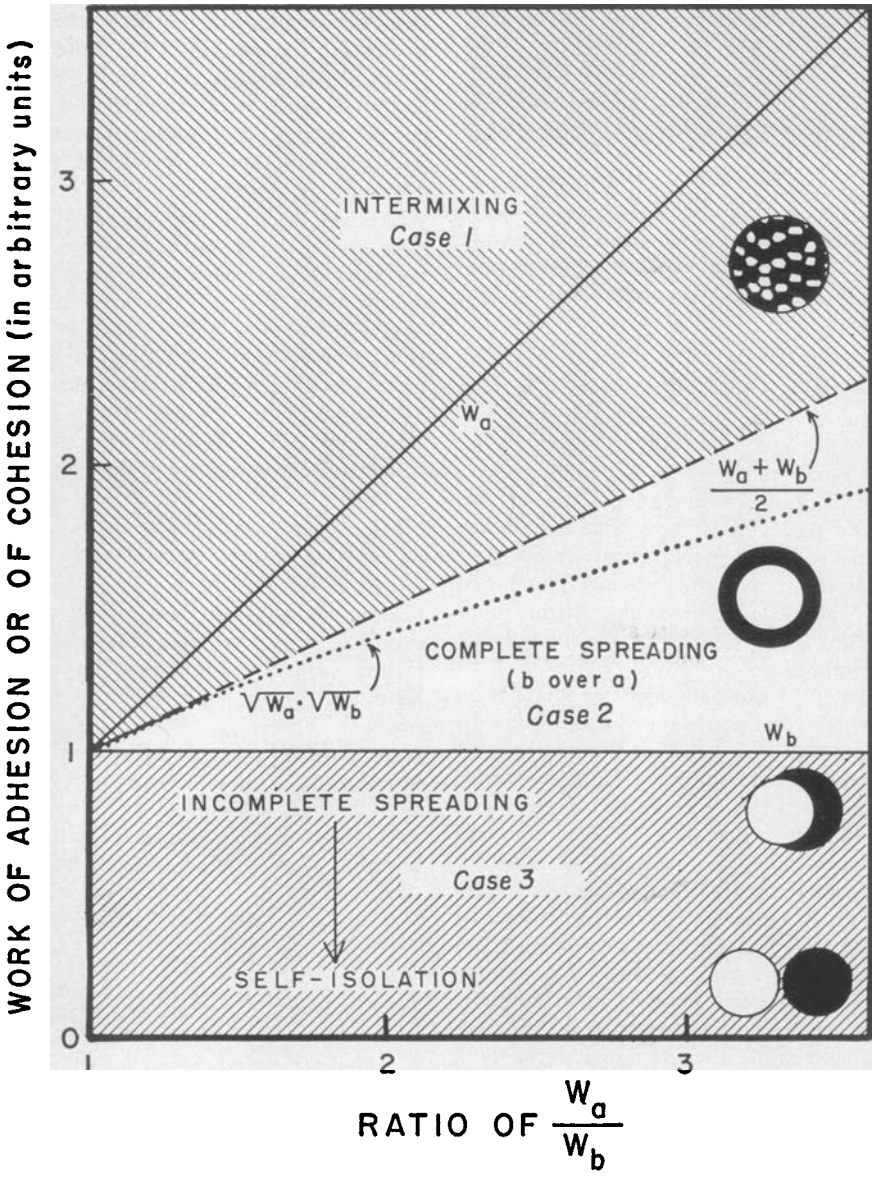
$$\frac{(f_a)^2 + (f_b)^2}{2} \geqq (f_a)(f_b) \geqq (f_b)^2 \qquad (13)$$

thus, $$\frac{W_a + W_b}{2} \geqq W_{ab} \geqq W_b$$

>>Accurately predicts envelopment behaviour

Steinberg MS. *Science*. 141:401-408. 1963

Model without adhesive specificity



No adhesive specificity

$$W_{ab} = \sqrt{W_a} \cdot \sqrt{W_b}$$

Steinberg MS. *Science*. 141:401-408. 1963

Complexity: biological heterogeneity/specificity
Simplicity: physical quantitative parameter

While the *adaptedness* brought about through evolution appears complex, the *adaptiveness* which makes evolution possible is born of simplicity. The entire genetic code (and more) is expressible with an alphabet containing only four elements. It would appear that a not inconsiderable amount of the "information" required to produce, through morphogenetic movement, the anatomy of a body part may be expressed in a code whose sole element is quantity: more versus less. There is, I think, reason to expect that as more realms of biological specificity yield to analysis, their most impressive feature may be the simplicity of the terms in which specificity—information, if you will—can be expressed (*34*).

Steinberg MS. *Science*. 141:401-408. 1963

Holfreter & Steinberg

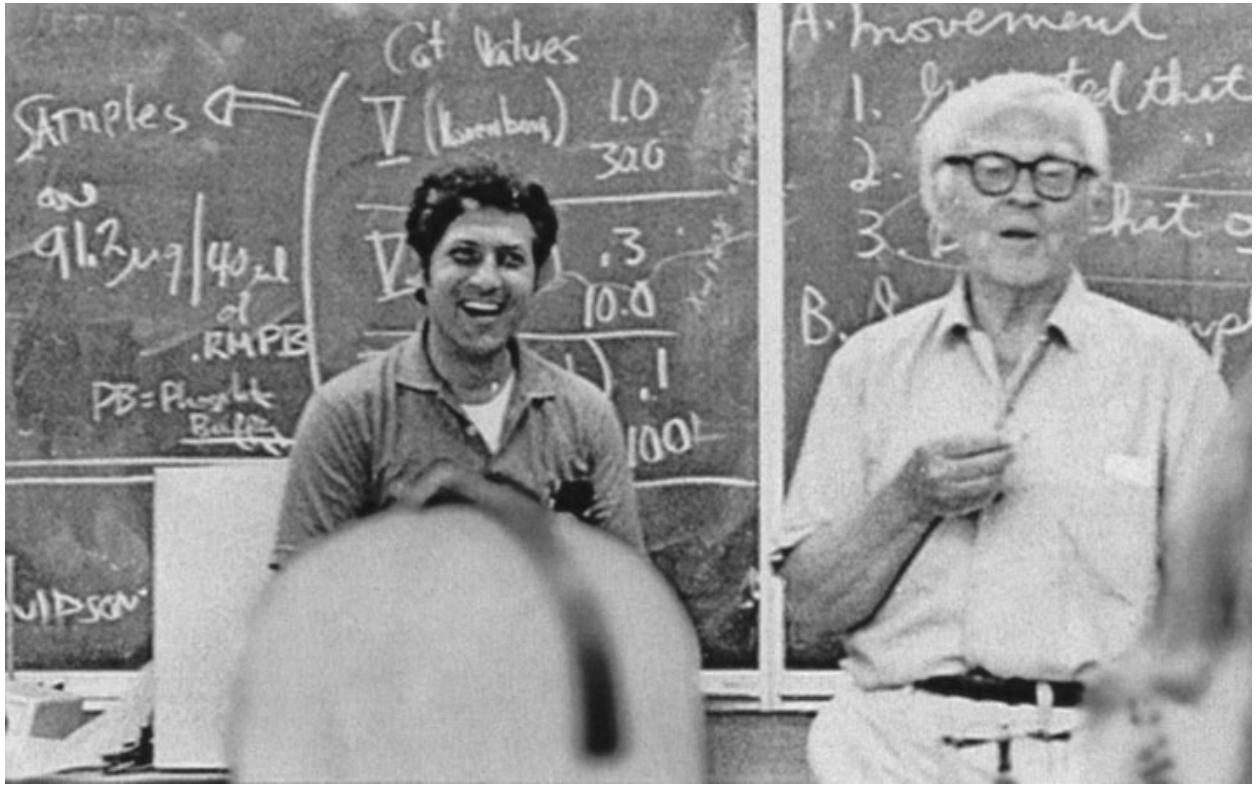


Fig. 2. Hans Holtfreter pronouncing his disagreement with the experimental evidence of Mal Steinberg (shown here laughing) for the thermodynamic model of cell sorting. This photograph was taken at the embryology course at the Marine Biology Laboratory at Woods Hole, 1971.

from Steinberg & Gilbert *J. Exp. Zool.* 2004, about Townes & Holtfreter *J. Exp. Zool.* 1955

Evidence supporting the D.A.H.

1. Cell sorting follows a transitive hierarchy:

> Follows a quantitative cell parameter

If A sorts out internal to B, and B sorts out internal to C
then A sorts out internal to C

Steinberg MS. *J. Exp. Zool. 173:395-434*. 1970

2. Final configuration is independent of initial conditions

Explants engulfment and sorting of dissociated cells converge on same outcome.
Steinberg MS. *J. Exp. Zool. 173:395-434*. 1970

3. Resistance to compression (cohesion) scales with sorting behaviour

If Cell aggregate A resists more to centrifugation (i.e. that is more cohesive) than B, then A sorts internal to B.
Phillips & Steinberg *P.N.A.S. 64:121*. 1969

- But does not prove that surface-tension like property is cell-cell adhesion

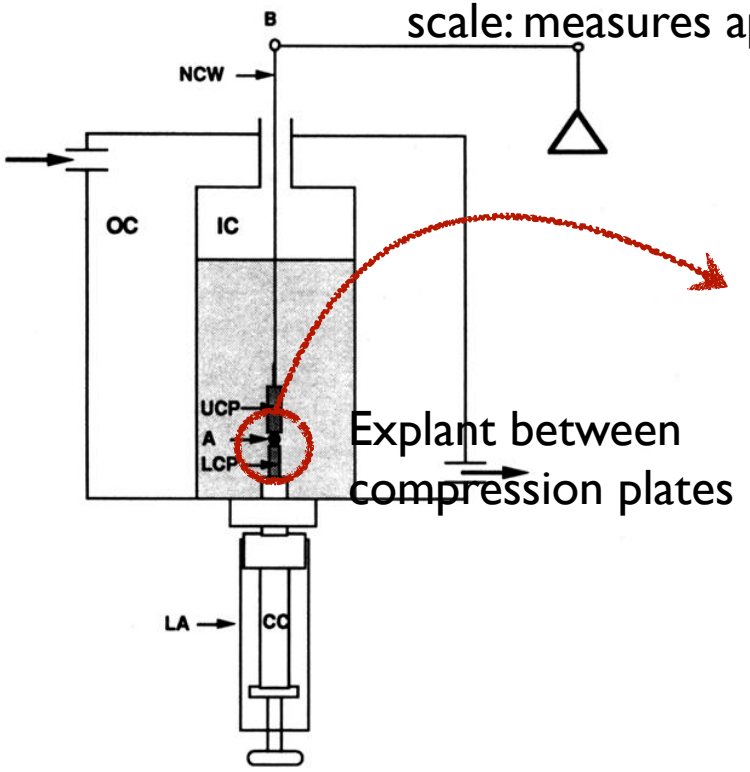
Measurement of tissue surface tension

VOLUME 72, NUMBER 14 PHYSICAL REVIEW LETTERS 4 APRIL 1994

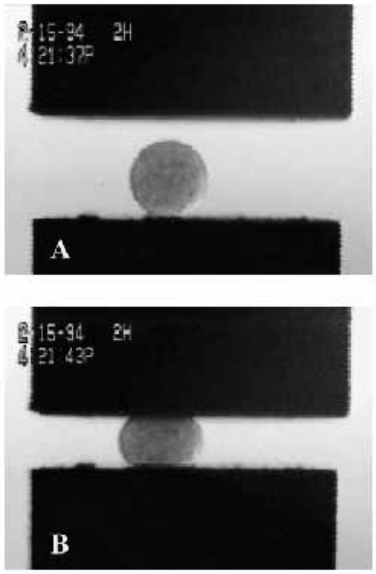
Liquid Properties of Embryonic Tissues: Measurement of Interfacial Tensions

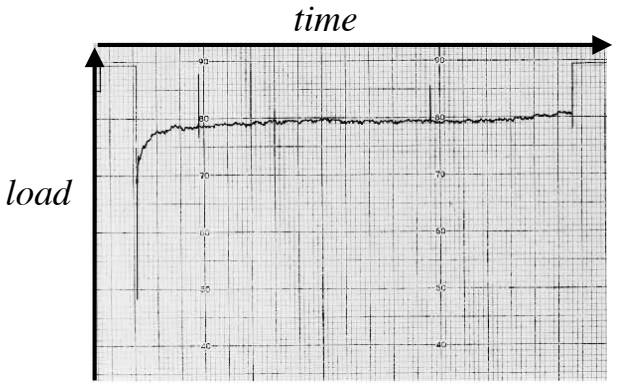
Ramsey A. Foty,[1] Gabor Forgacs,[2] Cathie M. Pfleger,[1] and Malcolm S. Steinberg[1,*]

scale: measures apparent load F



Explant between compression plates





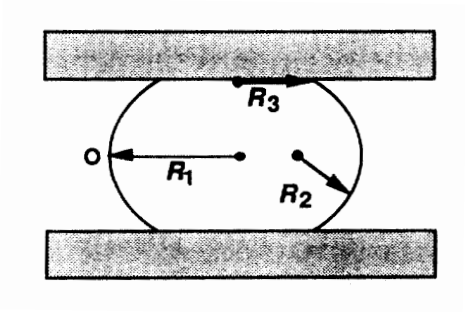
Foty RA. et al, and Steinberg MS. *PRL*. 72:2298. 1994

Measurement of tissue surface tension

VOLUME 72, NUMBER 14 PHYSICAL REVIEW LETTERS 4 APRIL 1994

Liquid Properties of Embryonic Tissues: Measurement of Interfacial Tensions

Ramsey A. Foty,[1] Gabor Forgacs,[2] Cathie M. Pfleger,[1] and Malcolm S. Steinberg[1,*]



Surface tension $\sigma = \frac{F}{\pi R_3^2}\left(\frac{1}{R_1}+\frac{1}{R_2}\right)^{-1}$.

Measurement of surface tension from F and geometry ($R1$, $R2$, $R3$) of explant at equilibrium

Surface tension predicts sorting behaviour

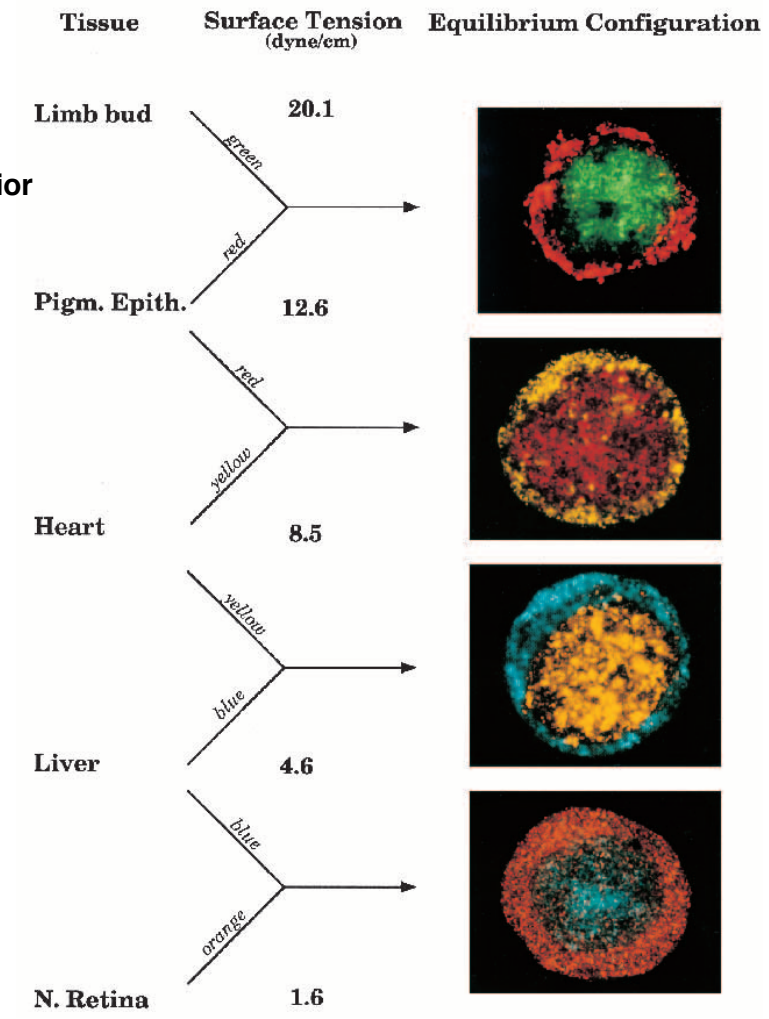
Development 122, 1611-1620 (1996)

Surface tensions of embryonic tissues predict their mutual envelopment behavior

Ramsey A. Foty[1], Cathie M. Pfleger[1,†], Gabor Forgacs[2] and Malcolm S. Steinberg[1,*]

Tissue	Number of aggregates	Number of compressions	σ ± s.e.m. (dyne/cm)
Limb bud mesoderm	12	17	20.1±0.5
Pigmented epithelium	13	21	12.6±0.4
Heart	12	16	8.5±0.2
Liver	14	22	4.6±0.1
Neural retina	11	22	1.6±0.1

mN/m



Is cell sorting caused by differences in the work of adhesion?

- Differences between cell aggregates and liquids:

1. Cells are « active particles ». Aggregates are thermodynamically open systems, The final configuration need not reflect minimisation of adhesive free energy.

2. Adhesion is much more than « close range attraction ». The forces that attract cells are not necessarily the same as those that hold cells together.

   Adhesion does not simply arise from H-bonds, van der Waals forces, electrostatic interactions etc.

3. The work of adhesion need not be the same as the work of de-adhesion.

   If there is a *maturation* of adhesion *after* cells are brought into contact (*i.e.* due to cells being active systems) the breakage of adhesive bonds is not the simple reverse of their formation.
   (see Townes and Holtfreter 1955)

4. Adhesion molecules are not distributed uniformly and are mobile units.

   Surface and adhesion are not linearly scaling with one another.

A.K. Harris, *J. Theor. Biol.* (1976) 61:267

Adhesion in multicellular organisms

1. Affinity and Adhesion: a specificity problem

2. Adhesion: a thermodynamic model

3. The molecular framework of adhesion

4. Evolutionary origin of adhesion mechanisms

5. Adhesion as an active mechanism

6. Adhesion and *dissipation*

The Discovery of Cell-Cell Adhesion Molecules

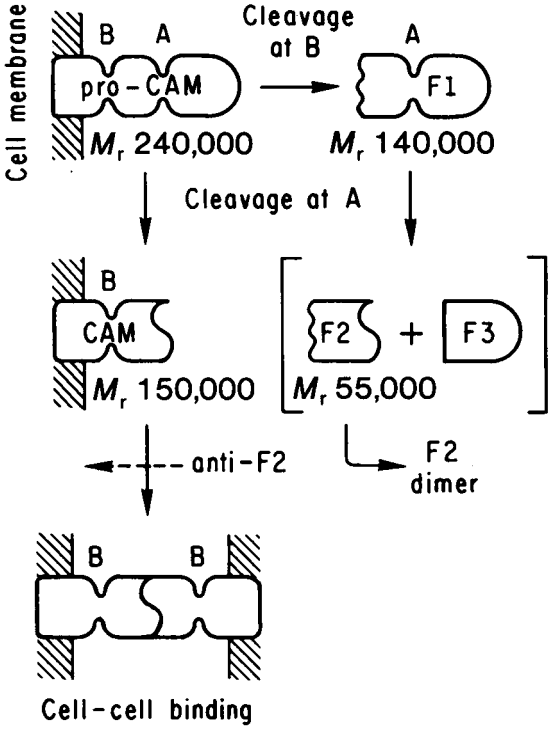
Mechanisms of adhesion among cells from neural tissues of the chick embryo

(cell–cell binding/brain and retinal cells/cell surface proteins/proteolytic activation)

URS RUTISHAUSER, JEAN-PAUL THIERY, ROBERT BRACKENBURY, BEN-AMI SELA, AND GERALD M. EDELMAN

Proc. Nat. Acad. Sci. USA
Vol. 73, No. 2, pp. 577–581, February 1976
Cell Biology

Hypothesised Cell Adhesion Molecule (CAM)
CAM-CAM interaction mediates adhesion
Proteolytic cleavages of CAM and Antibody against F2 blocks cell adhesion



Gerald Edelman in 1972
1929-2014

The Discovery of Cell-Cell Adhesion Molecules

Adhesion among Neural Cells of the Chick Embryo

I. AN IMMUNOLOGICAL ASSAY FOR MOLECULES INVOLVED IN CELL-CELL BINDING*

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 252, No. 19, Issue of October 10, pp. 6835–6840, 1977
Printed in U.S.A.

ROBERT BRACKENBURY,‡ JEAN-PAUL THIERY,§ URS RUTISHAUSER, AND GERALD M. EDELMAN
From The Rockefeller University, New York, New York 10021

Trypsinised cells re-aggregate
Fab (antigen binding) fragment against these cells prevents re-aggregation
Cells release proteins that block Fab fragment activity

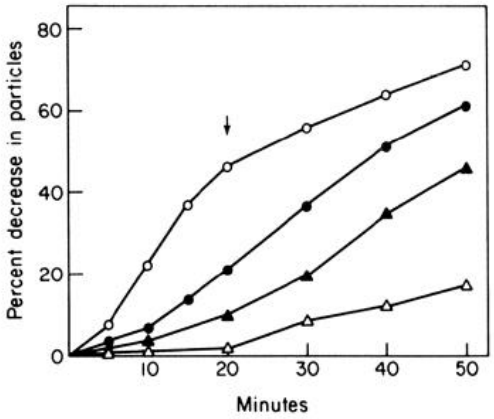
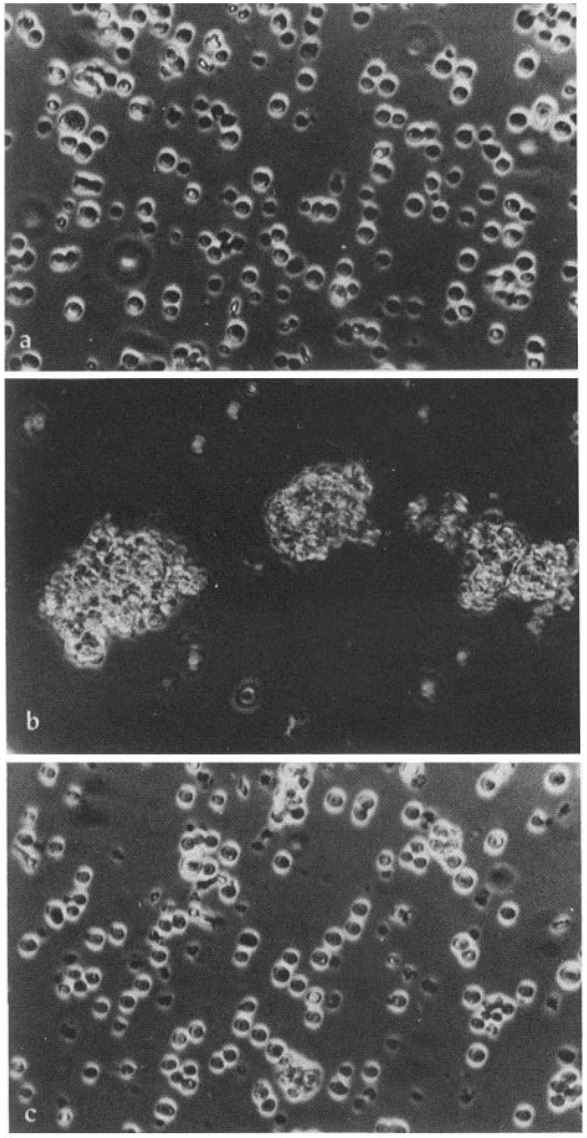


FIG. 2. Aggregation of retinal cells from 10-day-old chick embryos detected as the rate of decrease in total particle number. ○——○, in the presence of 1 mg of Fab′ from unimmunized rabbits; ●——●, ▲——▲, and △——△, in the presence of 1 mg of Fab′ from three different rabbits immunized with retinal cells from 10-day-old chick embryos. The *arrow* indicates the time at which the rate of aggregation was routinely measured in assays.



t=0

t=30 min

t=30 min
+ anti-R10 Fab fragment

FIG. 1. Aggregation of retinal cells from 10-day-old chick embryos. *a*, cells prior to aggregation; *b*, aggregates produced after incubation for 30 min at 37°; *c*, aggregation for 30 min at 37° in the presence of anti-R10 Fab′.

The Discovery of Cell-Cell Adhesion Molecules

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 252, No. 19, Issue of October 10, pp. 6841-6845, 1977
Printed in U.S.A.

Adhesion among Neural Cells of the Chick Embryo

II. PURIFICATION AND CHARACTERIZATION OF A CELL ADHESION MOLECULE FROM NEURAL RETINA*

(Received for publication, April 19, 1977)

JEAN-PAUL THIERY,‡ ROBERT BRACKENBURY,§ URS RUTISHAUSER, AND GERALD M. EDELMAN
From The Rockefeller University, New York, New York 10021

The Discovery of Cell-Cell Adhesion Molecules

FUNCTIONAL CORRELATION BETWEEN CELL ADHESIVE PROPERTIES AND SOME CELL SURFACE PROTEINS

MASATOSHI TAKEICHI

THE JOURNAL OF CELL BIOLOGY · VOLUME 75, 1977 · pages 464-474

Masatoshi Takeichi

Adhesion is calcium dependent
Calcium protects against trypsinisation

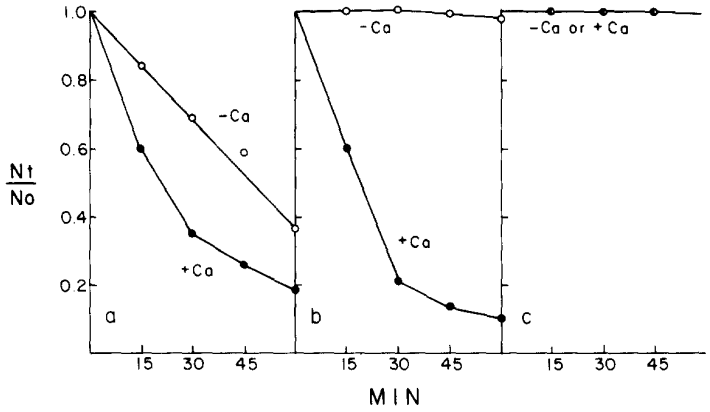


FIGURE 1 Aggregation of cells dissociated with 1 mM EDTA (*a*), 0.01% trypsin + 0.01 mM Ca^{2+} (*b*), and 0.01% trypsin + 1 mM EDTA (*c*). Medium for aggregation contains 1 mM Ca^{2+} (●—●), or no divalent cation (○—○).

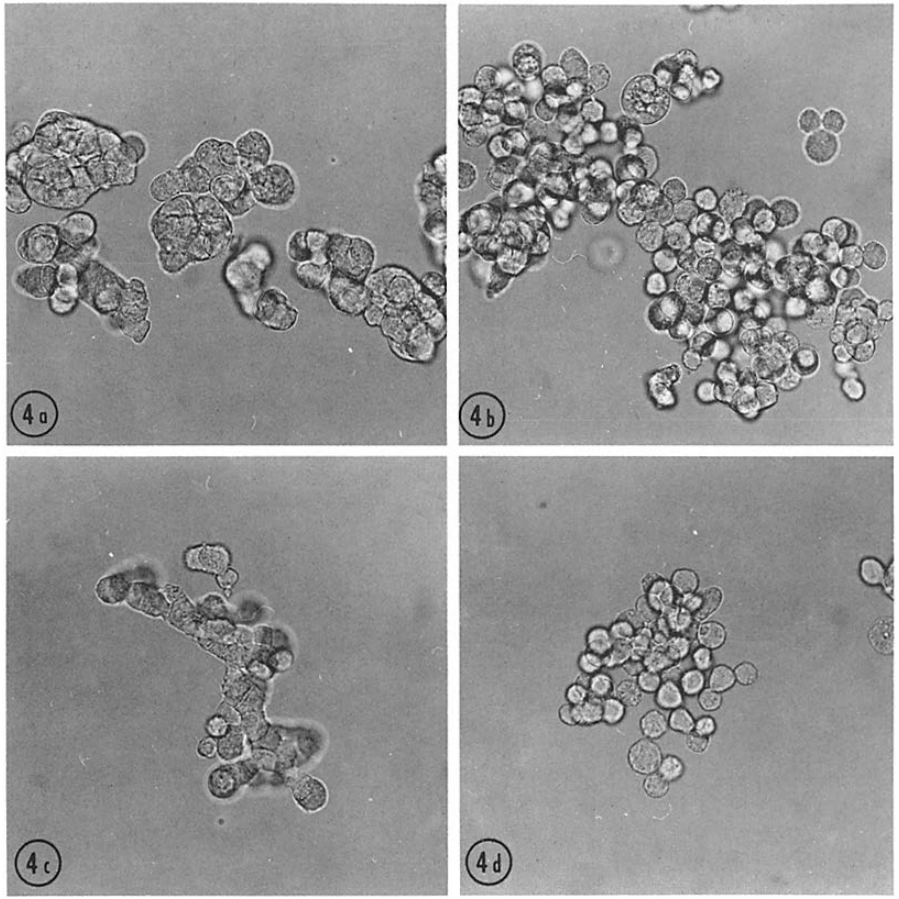


FIGURE 4 Photomicrographs of cell aggregates. E-cells in 1 mM Ca^{2+} (*a*), in no divalent cations (*b*), and in 1 mM Mg^{2+} (*d*); TC-cells in 1 mM Ca^{2+} (*c*). Cells were incubated for 60 min at 37°C. × 360.

Cells dissociated with EDTA and re-aggregated in different conditions

The Discovery of Cell-Cell Adhesion Molecules

Surface antigen in early differentiation

(preimplantation embryo/mouse/embryonic development/teratocarcinoma)

R. KEMLER, C. BABINET, H. EISEN, AND F. JACOB

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 10, pp. 4449–4452, October 1977
Cell Biology

Discovery of Fab fragment against F9 EC line that blocks compaction.

François Jacob
1920-2013

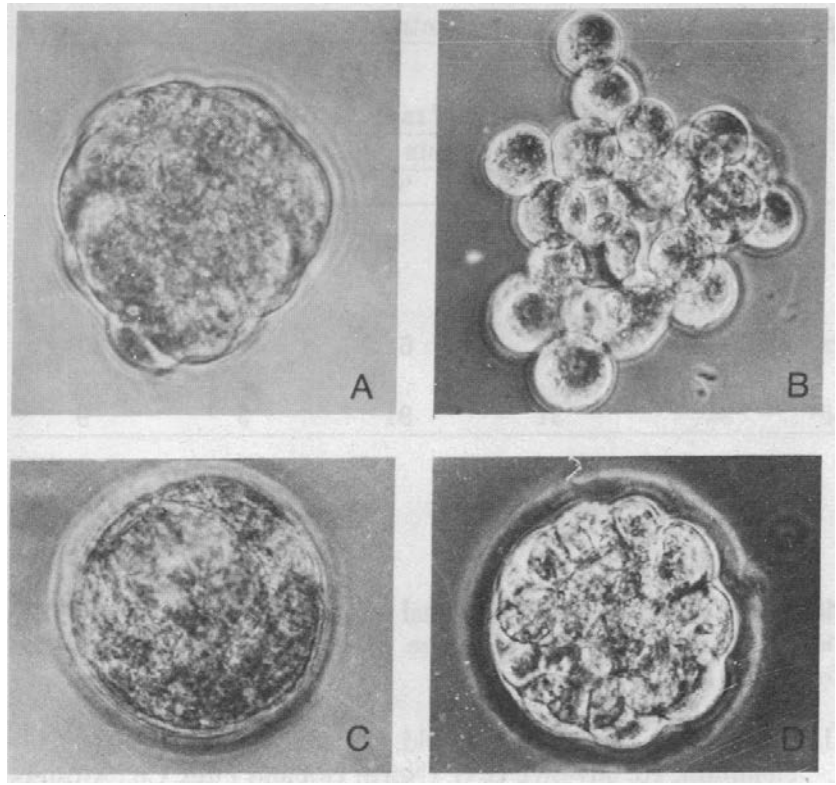
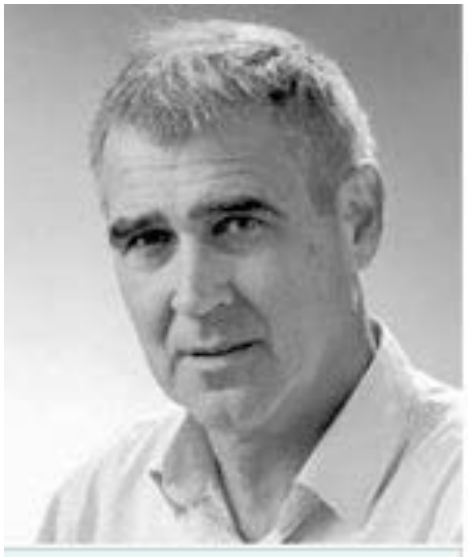
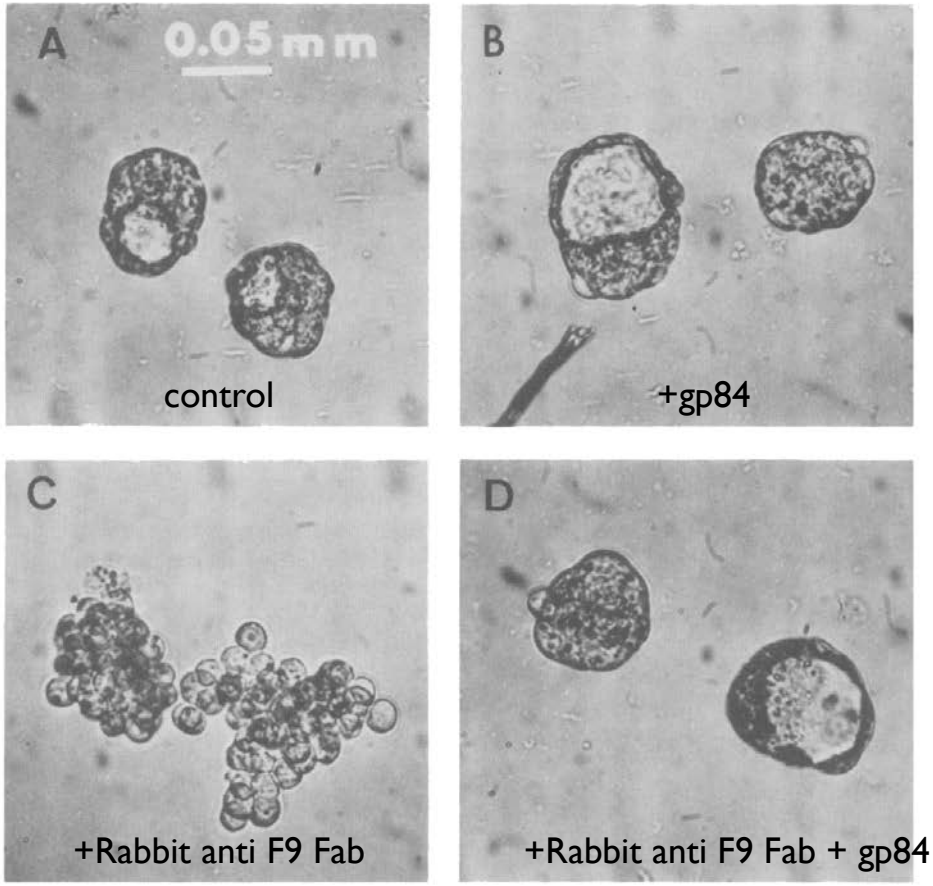


FIG. 1. Two-cell embryos with (*C* and *D*) and without (*A* and *B*) zona pellucida were grown in Whitten's medium (*A* and *C*) and Whitten's medium with rabbit anti-F9 Fab, 1/20 (*B* and *D*). (×400.)



Rolf Kemler

The Discovery of Cell-Cell Adhesion Molecules



Cell, Vol. 21, 927–934, October 1980, Copyright © 1980 by MIT

A Cell Surface Glycoprotein Involved in the Compaction of Embryonal Carcinoma Cells and Cleavage Stage Embryos

François Hyafil, Dominique Morello, Charles Babinet and François Jacob

Identification of gp84, a surface glycoprotein that antagonizes the decompaction effect of a Fab fragment of anti-EC IgG.

Figure 4. Compaction of Preimplantation Embryos in the Presence of Fab and gp84

8-cell precompaction embryos [72 hr post-human chorionic gonadotrophin (HCG)] were cultured in Whitten's medium containing (A) no addition; (B) F9 tumor-derived Fab target inhibitory material (gp84) dialyzed against Whitten's medium; (C) rabbit anti-F9 Fab; (D and E) both anti-F9 Fab and gp84. Pictures were taken 96 hr (A, B, C, D) or 111 hr (E) post-HCG; at 111 hr post-HCG the following structures were formed: (A) 19 blastocysts (17 of which expanded as in E), 1 degenerated blastocyst; (B) 24 blastocysts (21 expanded), 1 degenerated; (C) 20 grape-like structures with at least 40 cells per embryo; (D) 21 blastocysts (19 expanded), 1 degenerated.

The Discovery of Cell-Cell Adhesion Molecules

Cell, Vol. 26, 447–454, November 1981 (Part 1), Copyright © 1981 by MIT

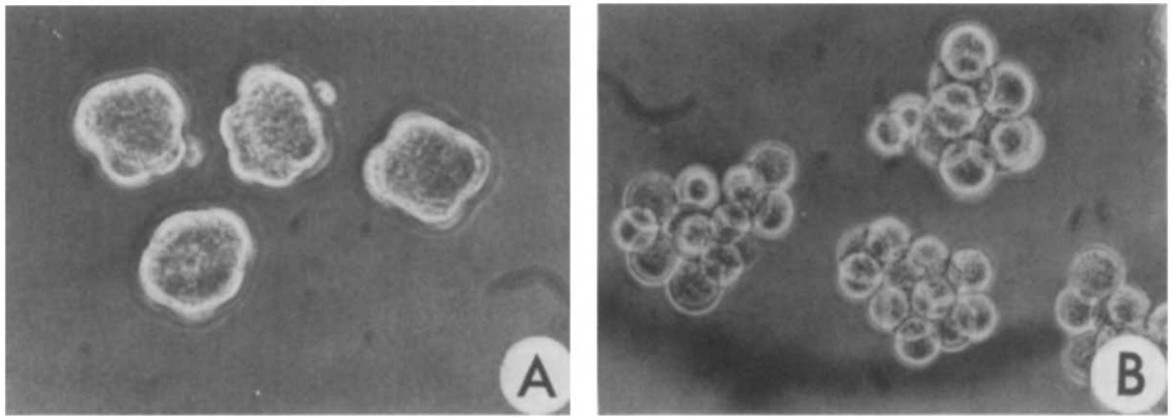
Cell–Cell Interactions in Early Embryogenesis: A Molecular Approach to the Role of Calcium

François Hyafil, Charles Babinet and François Jacob

1981: Naming of Uvomorulin (UM).

Purification of anti-UM antibody.

Effect of Ca2+ on compaction interpreted as change in **Uvomorulin conformation.**



+CaCl2 +no calcium

Decompaction induced by anti-EC IgG that recognises a specific cell surface glyvoprotein called uvomorulin. Ca2+ induces conformational change of Uvomorulin.

>Evidence: Ca2+ protects effect of trypsinization on Uvomorulin

The Discovery of Cell-Cell Adhesion Molecules

The EMBO Journal vol.4 no.13A pp.3393–3398, 1985

Identification of a putative cell adhesion domain of uvomorulin

Dietmar Vestweber and Rolf Kemler

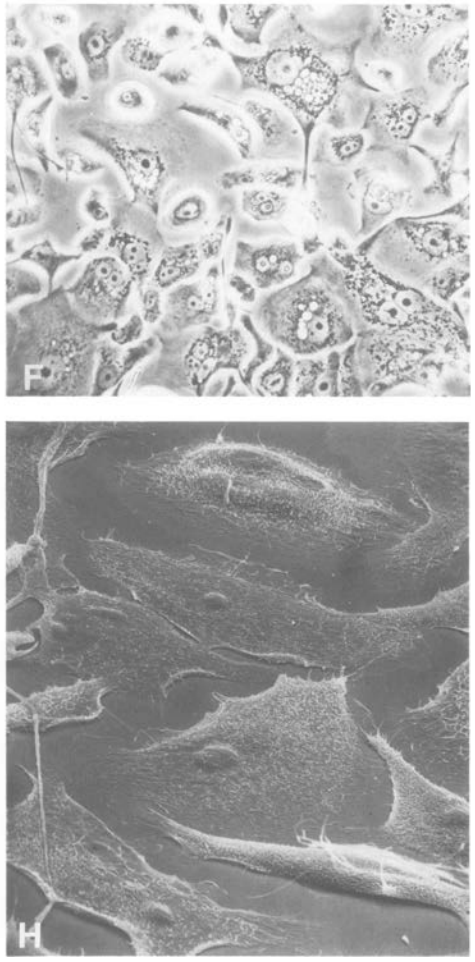


Fig. 1. Effect of monoclonal antibody DECMA-1 on cell-cell interaction.

Effect of blocking antibody on cell organisation

Localisation of CAM at cell contacts

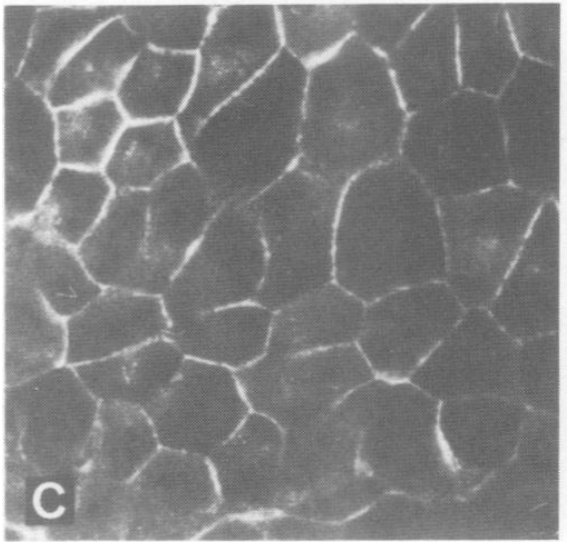


Fig. 3. Indirect immunofluorescence tests with DECMA-1

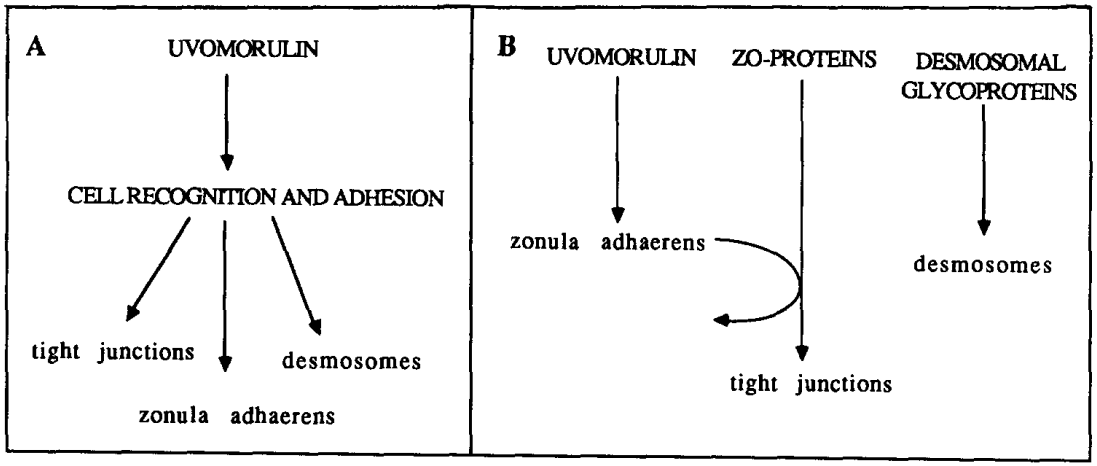
The Discovery of Cell-Cell Adhesion Molecules

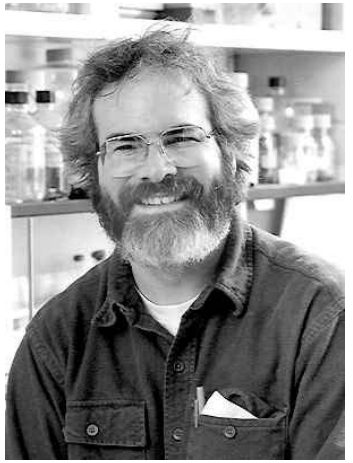
The Role of the Cell Adhesion Molecule Uvomorulin in the Formation and Maintenance of the Epithelial Junctional Complex

Barry Gumbiner, Bruce Stevenson,* and Ann Grimaldi

Department of Pharmacology and Cell Biology Program, University of California, San Francisco, California 94143; and *Department of Biology, Yale University, New Haven, Connecticut

The Journal of Cell Biology, Volume 107, October 1988 1575–1587





Barry Gumbiner

The Discovery of Cell-Cell Adhesion Molecules

Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA

Akira Nagafuchi, Yasuaki Shirayoshi, Kenji Okazaki, Kunio Yasuda & Masatoshi Takeichi*

Department of Biophysics, Faculty of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606, Japan

NATURE VOL. 329 24 SEPTEMBER 1987

These results provide the first direct evidence that E-cadherin is essential for intercellular adhesion. They also show that expression of a single class of peptide is sufficient to cause Ca^{2+}-dependent cell-cell adhesion.

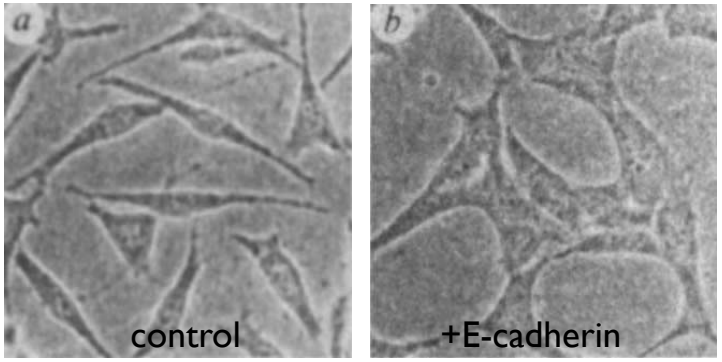


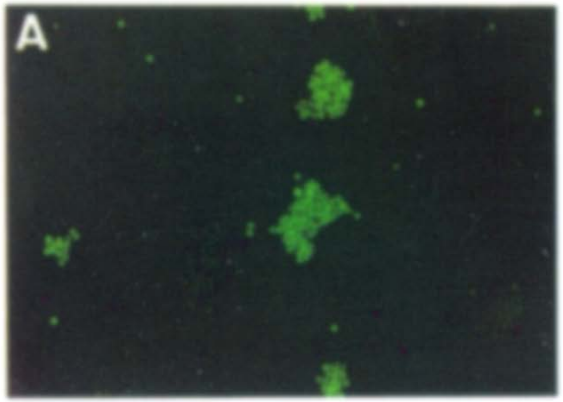
Fig. 3 Photomicrographs of untransformed L (*a* and *c*) and transformed EL8 (*b* and *d*) cells. *a*, *b*, Phase-contrast microscopy, ×125;

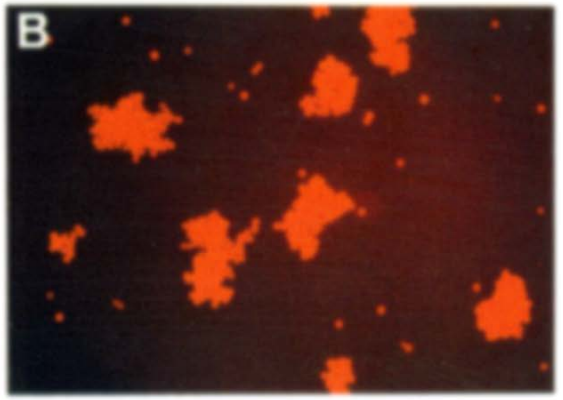
Selective aggregation and sorting by *different* Cadherins

Cell, Vol. 54, 993–1001, September 23, 1988
Akinao Nose, Akira Nagafuchi,
and Masatoshi Takeichi
Department of Biophysics
Faculty of Science
Kyoto University

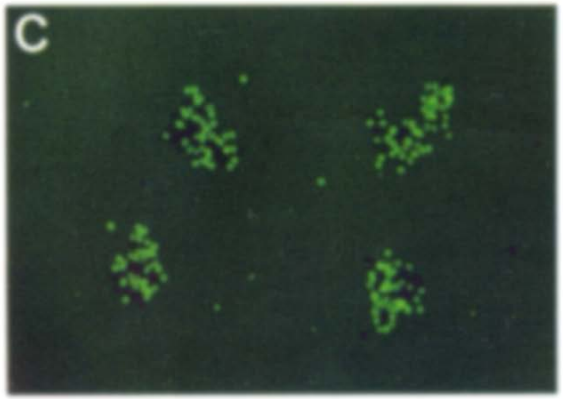
All cells

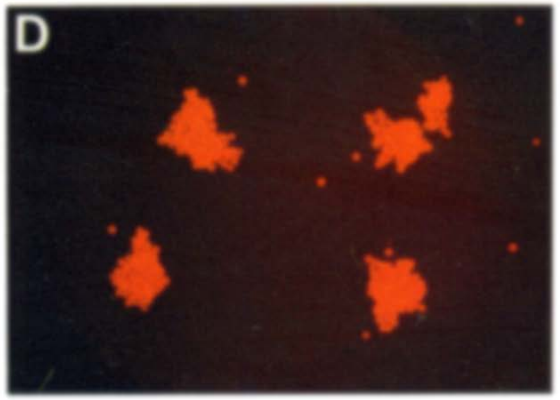
E-cadherin cells
+ P-cadherin cells

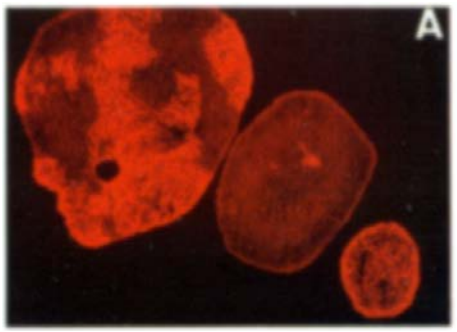


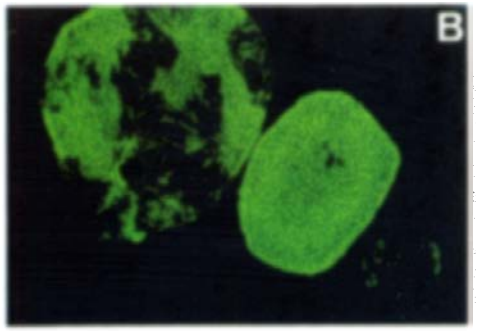


E-cadherin cells
+ E-cadherin cells





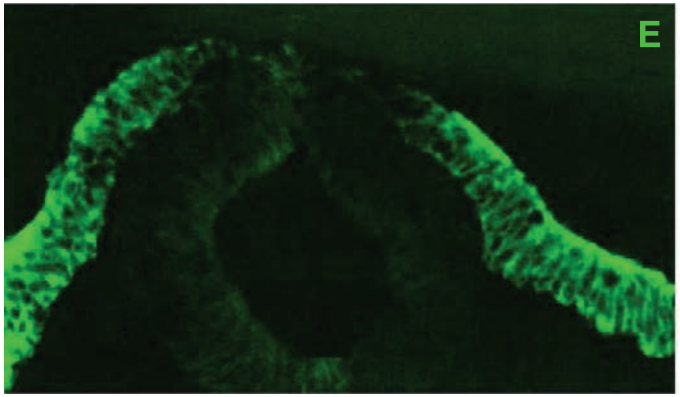




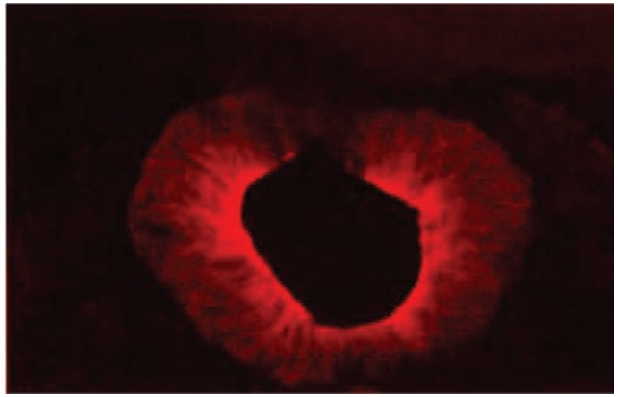
E-cadherin cells
+ P-cadherin cells

Differential expression of cadherins in different tissues

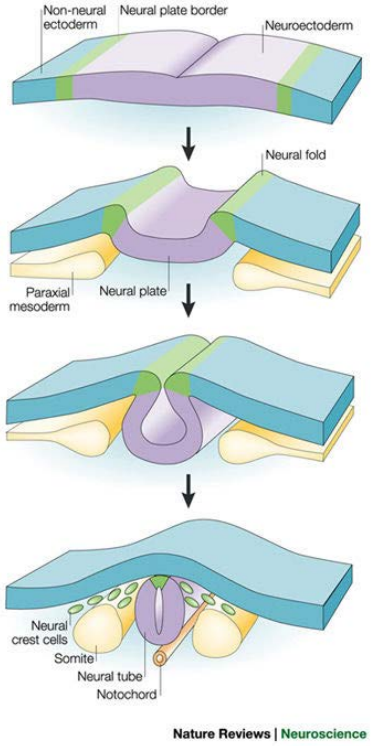
E-cadherin



N-cadherin



K. Hatta & M. Takeichi

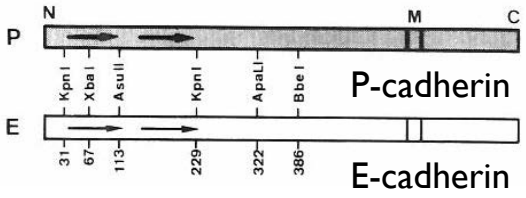


Laura S. Gammill & Marianne Bronner-Fraser, 2003

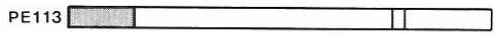
The determinants of *specificity* in Cadherins

Akinao Nose, Katsumi Tsuji, and Masatoshi Takeichi

Cell, Vol. 61, 147–155, April 6, 1990,

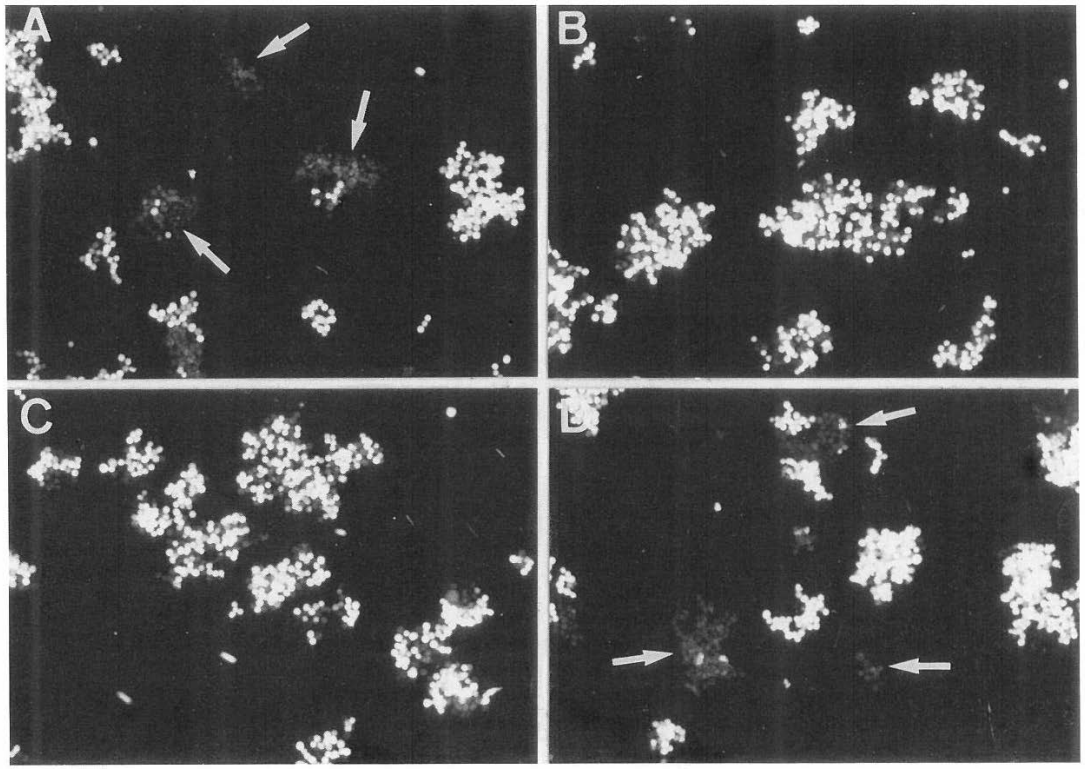


« Dark » cells express:



Fluorescent cells express:

E-cadherin P-cadherin

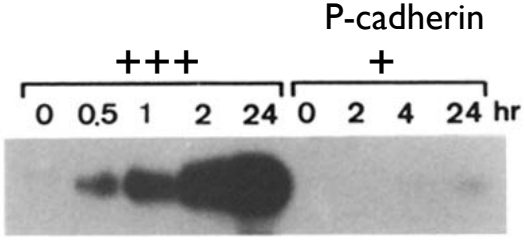


Cell sorting mediated by *quantitative* differences in expression of cadherin

MALCOLM S. STEINBERG*† AND MASATOSHI TAKEICHI‡

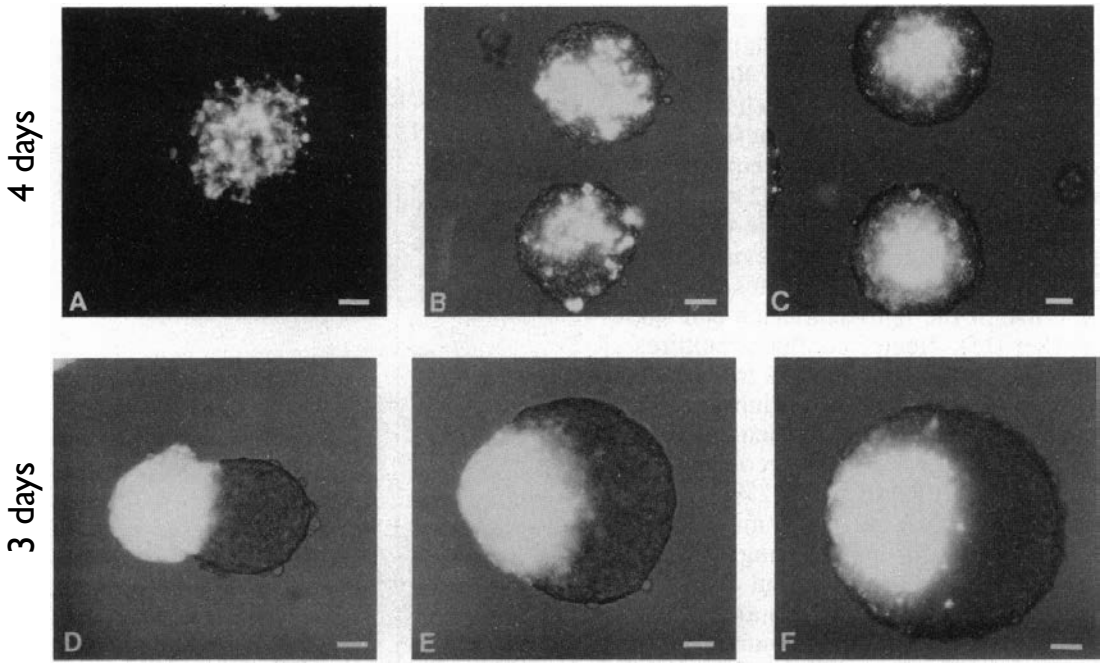
Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 206–209, January 1994
Developmental Biology

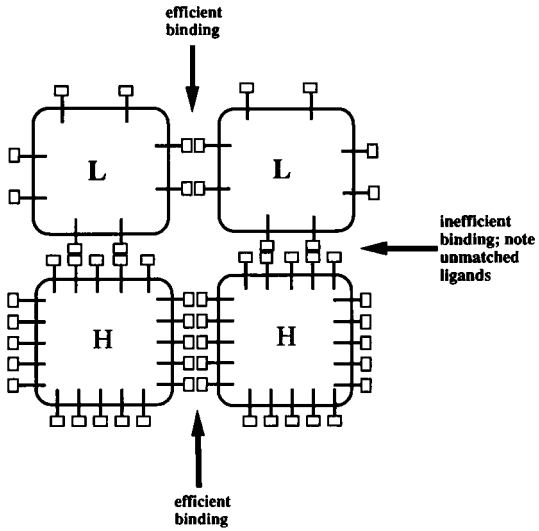
P-cadherin +++ (fluorescent cells)
P-cadherin + (dark cells)



Cell Sorting

Envelopment





Quantitative and *Qualitative* determinants of cell sorting

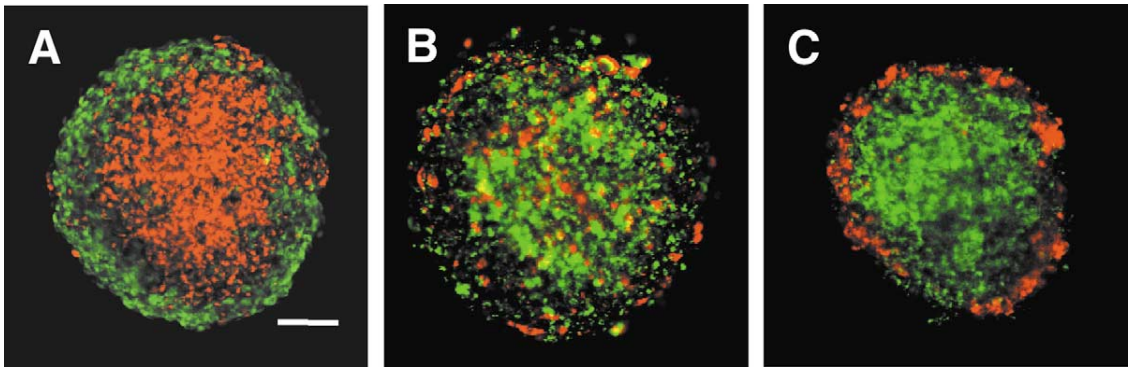
Duke Duguay,[a] Ramsey A. Foty,[a,b] and Malcolm S. Steinberg[a,*]

Developmental Biology 253 (2003) 309–323

1. Little selective adhesion
2. Quantitative differences seem sufficient for sorting

3. **BUT:**
   No direct measurement of bond strength and adhesion strength

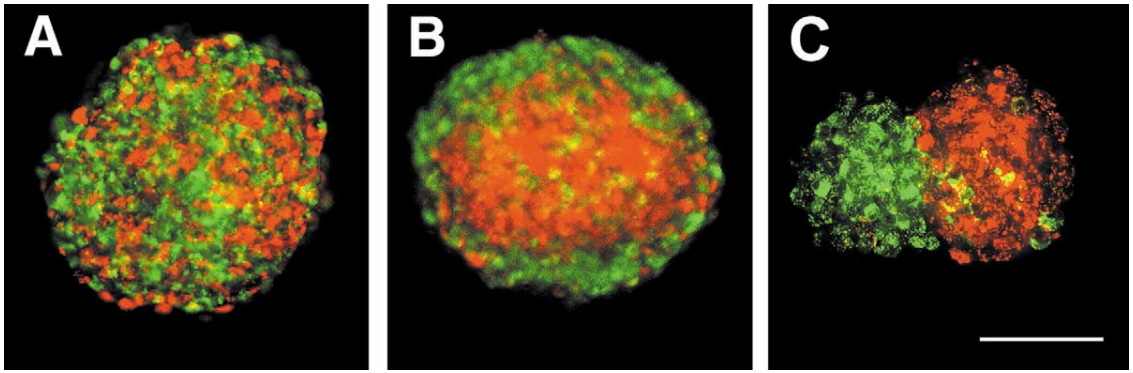
Assumes that [CAM] correlates with adhesion strength



[E-cad] < [P-cad] 2.37 3.55

[E-cad] = [P-cad] 3.55 3.55

[E-cad] > [P-cad] 15.8 3.55 10^4 /cell

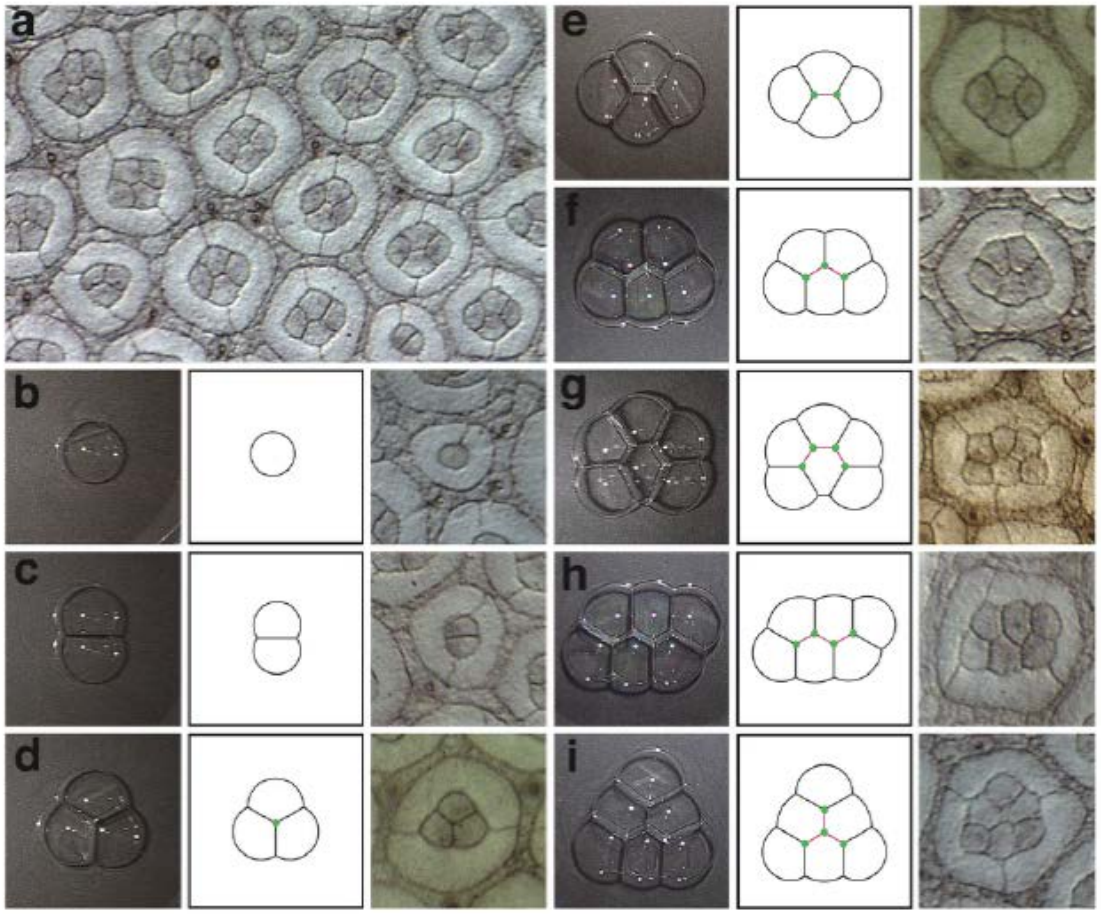


[N-cad] = [N-cad]

[N-cad] < [N-cad]

[B-cad] = [R-cad]

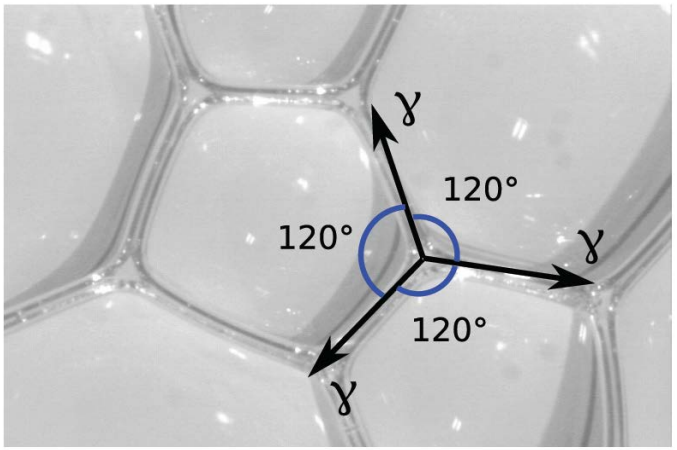
Surface tension: from tissue to cell shape



Surface mechanics mediate pattern formation in the developing retina

Takashi Hayashi[1,2] & Richard W. Carthew[1]

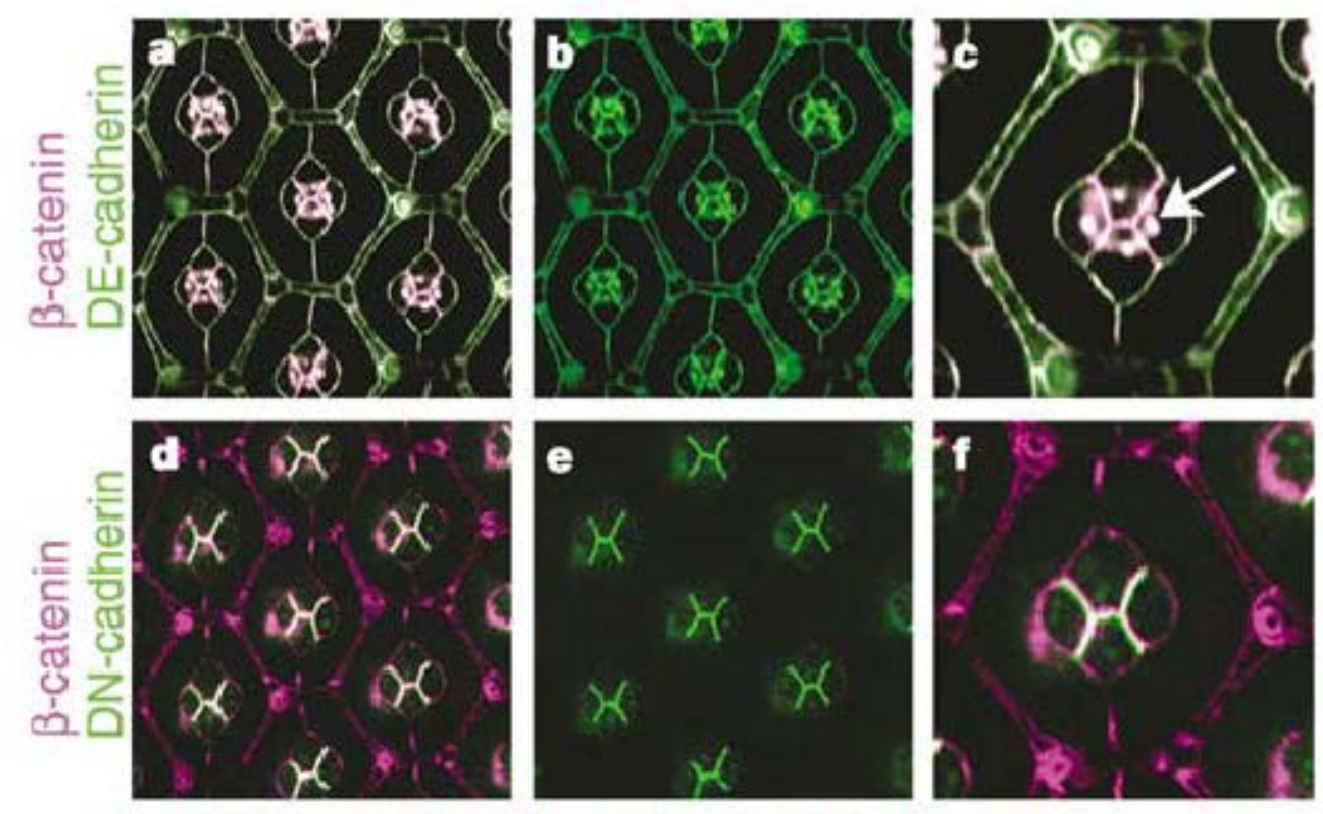
[1]*Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60208, USA*
[2]*Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Tokyo 113-0033, Japan*



Hayashi T. and Carthew R. *Nature*. 431:647. 2004

Surface tension: from tissue to cell shape

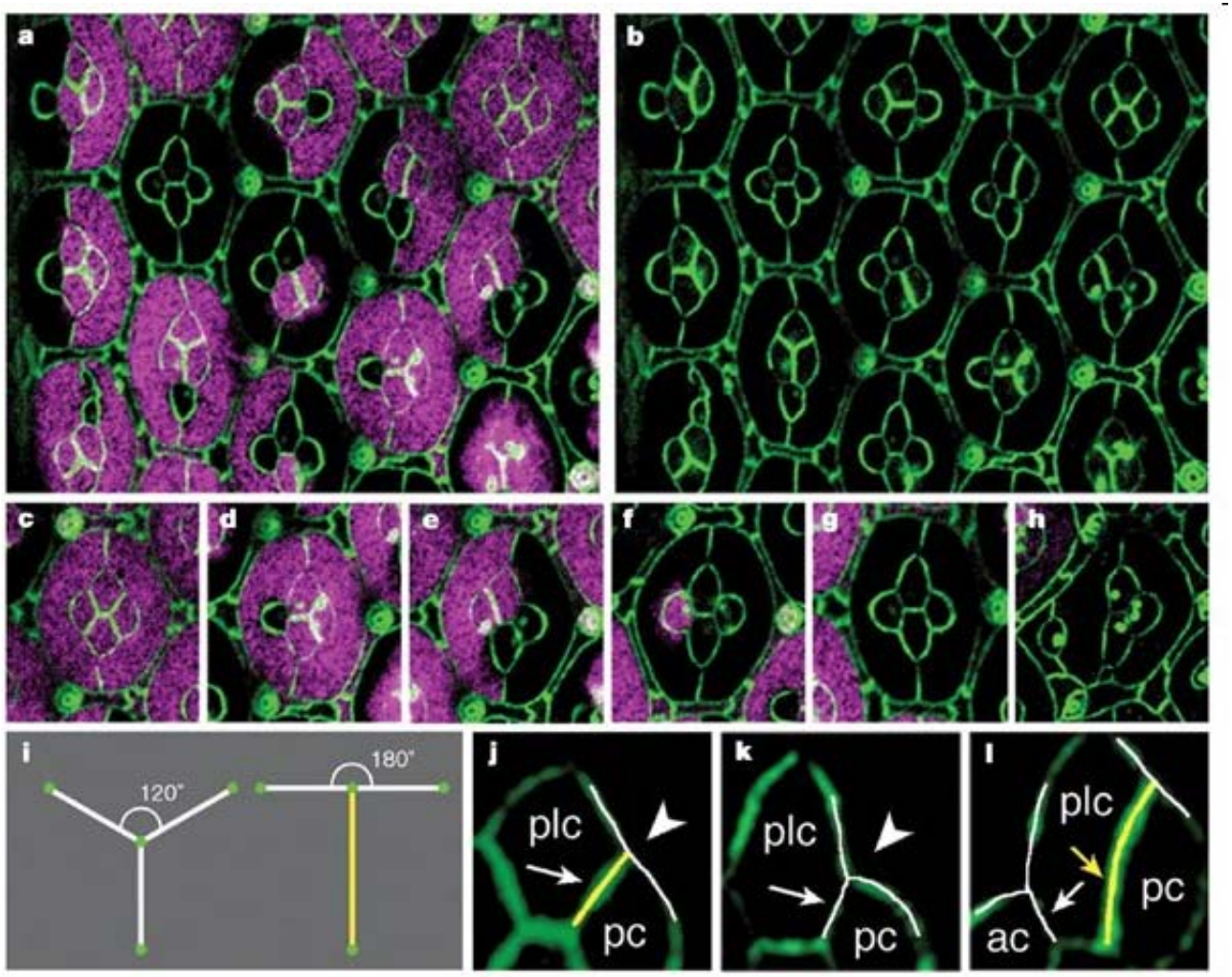
Differential Cadherin expression small cell aggregates: Geometry of contacts



Hayashi T. and Carthew R. *Nature*. 431:647. 2004

Surface tension: from tissue to cell shape

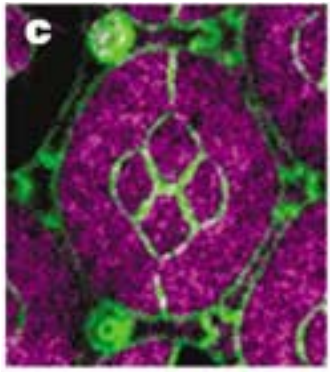
N-cadherin expression dictates cell configurations



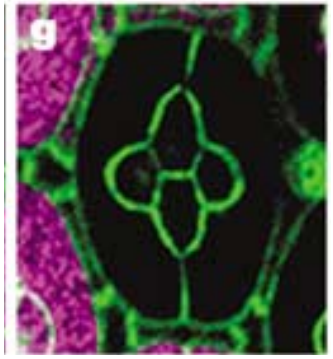
Hayashi T. and Carthew R. *Nature*. 431:647. 2004

Surface tension: from tissue to cell shape

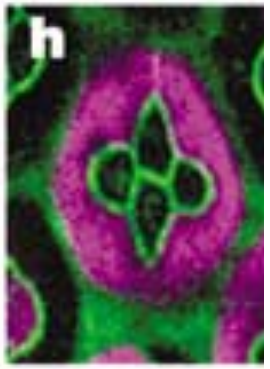
Relative N-cadherin expression dictates cell patterns



wild-type cells



N-cadherin mutant cells



N-cadherin over expressing cells

Hayashi T. and Carthew R. *Nature*. 431:647. 2004

Conclusions

- Adhesion captures the notion of selective/specific aggregation
- Cell sorting phenomena and tissue envelopment behaviours initially interpreted from standpoint of selective migration (Holtfreter)
- The « differential adhesion hypothesis » (DAH) proposes a purely quantitative description and prediction of cell/tissue behaviours based on surface tension of tissues modelled as fluids approaching thermodynamic equilibrium.
- The discovery of cell adhesion molecules offers an apparent validation of the DAH.
- Discussion of DAH by A. Harris: link between cell surface property dependent on CAMs and reversible work of adhesion?

Conclusions

Prochain cours: 7 Novembre 2017