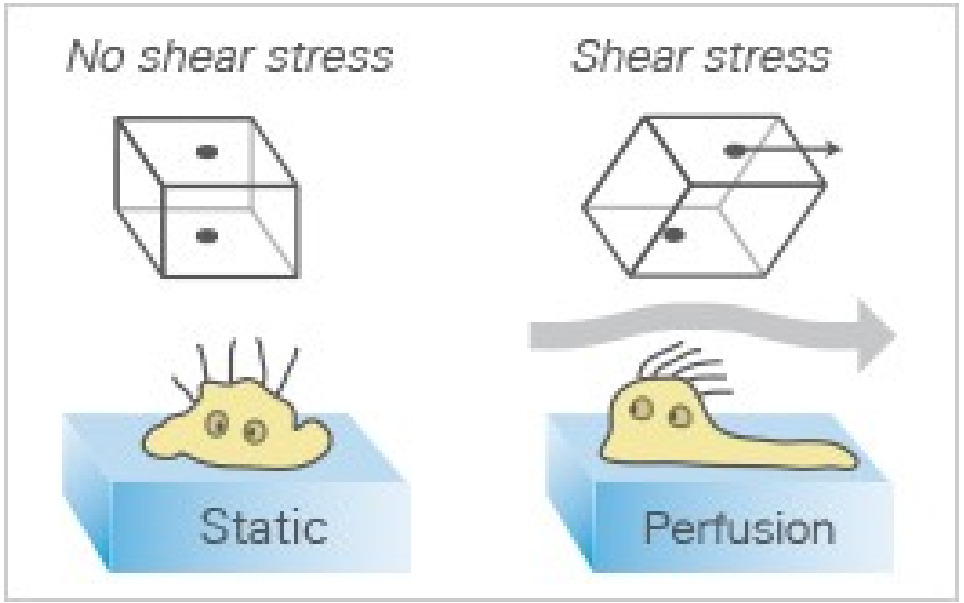
Vývojová biologie

The role of physical phenomena on development



Tomáš Bárta
tbarta@med.muni.cz

Contents

Mechanosensing in embryogenesis

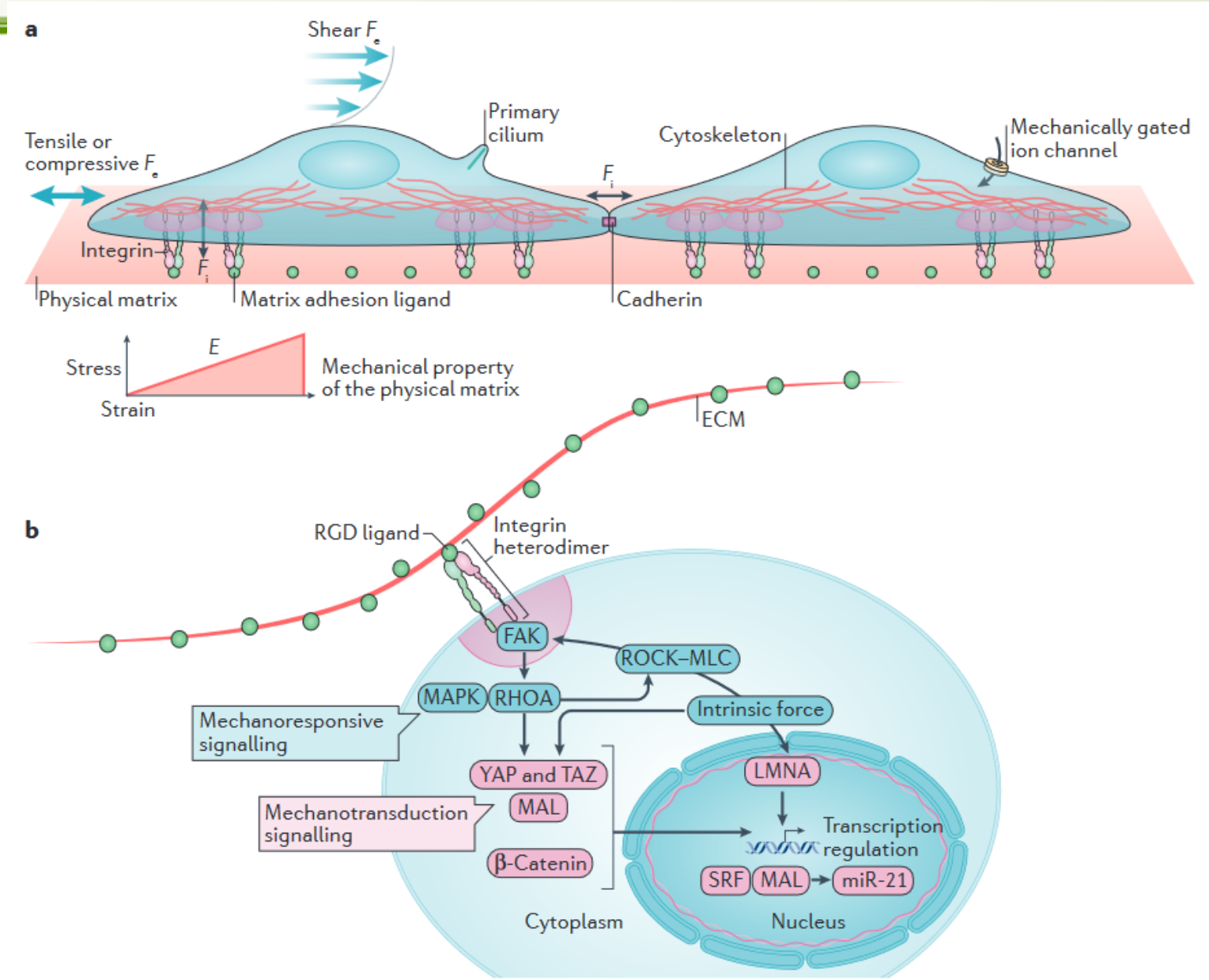
- Mechanical force is an invisible but ubiquitous part of biological systems.
- Forces such as gravity and osmotic pressure set physical limits for the body's plane. At the same time, the cells of the embryo use these forces to create the complex shapes that we find in the animal kingdom.
- The mechanical forces generated by living cells at the molecular level have a great impact on embryogenesis.
- The direct result of the action of force is the movement that occurs during the separation of chromosomes, the migration of cells or the folding of tissues.
- A less direct, but equally important effect of force is the activation of mechanosensitive signaling, which allows cells to explore their mechanical surroundings and communicate with each other over short and long distances = > mechanical forces are a way of communicating
-

Mechanosensing in embryogenesis

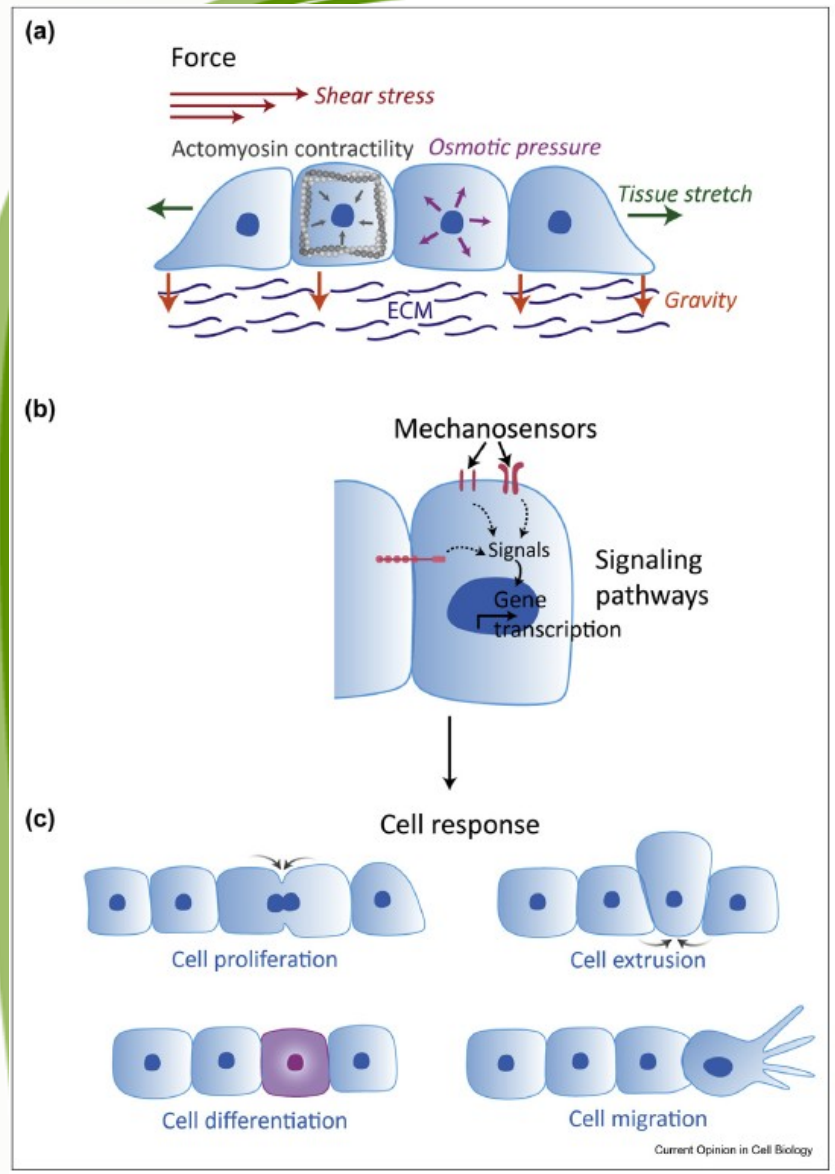
- The ability of cells to "perceive" mechanical signals and convert them into biochemical signals.

- **And how/what does it "perceive"?**
- Ion channels
- Primary cilium
- Integrins
- Cadherins
- Actin/Myosin cytoskeleton
- Notch
- Growth factor receptors

Mechanosensing in embryogenesis



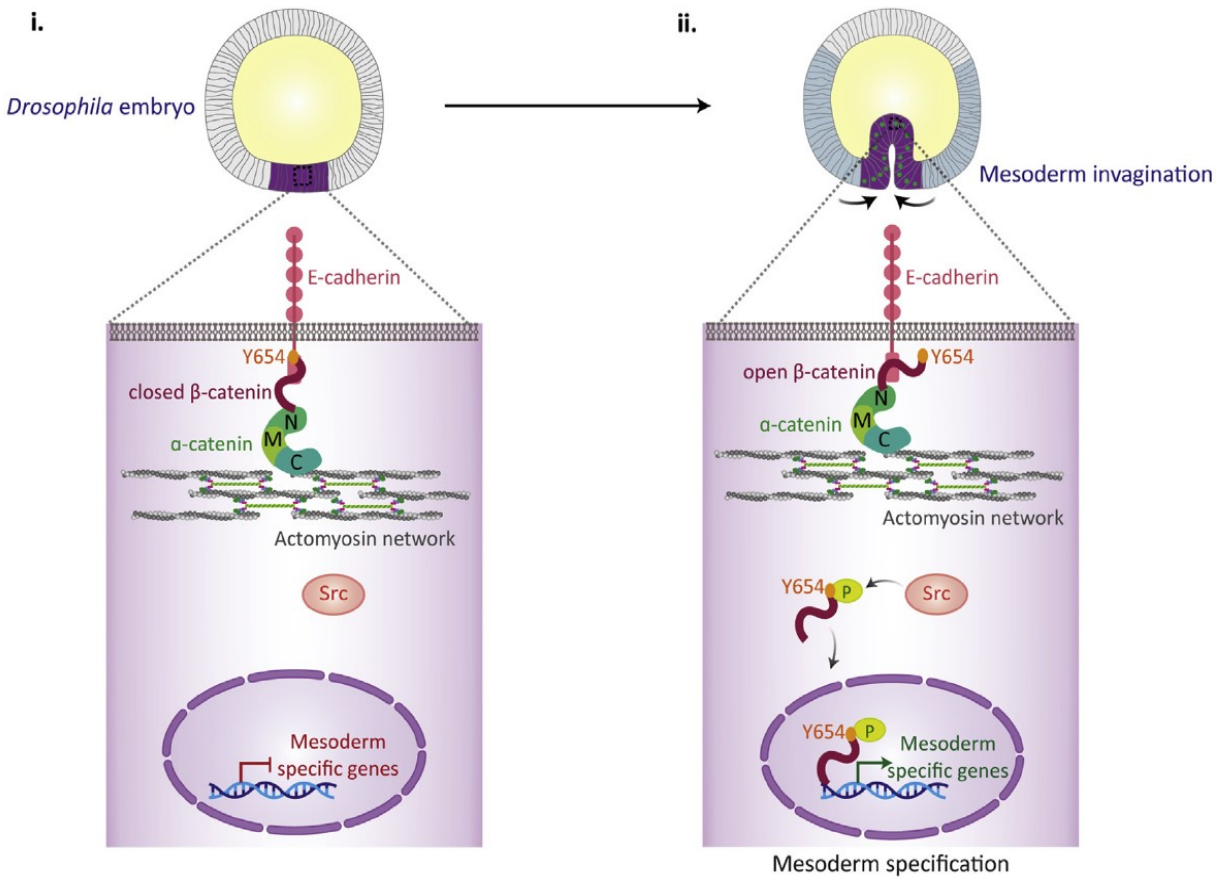
Mechano reception in embryogenesis



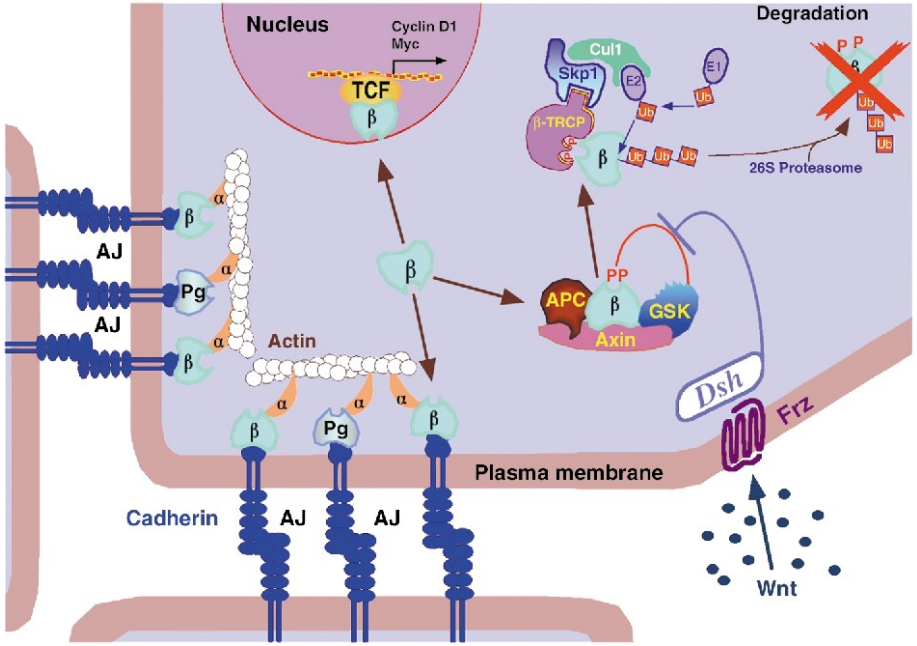
- Many mechanical forces act on the cell:
  - Intrinsic: osmotic pressure, contractility of actin and myosin
  - External: shear stress, gravity, stretching
- Forces are perceived and interpreted by mechanosensors (adhesive molecules, ion channels), which then lead to a change in gene expression.
- This leads to the cell reaction -> a change in cell/tissue morphology

https://doi.org/10.1016/j.ceb.2020.08.007

Mechanosensing in embryogenesis – Cadherin/β-catenin – gastrulation and induction of mesoderm



Link to previous lecture

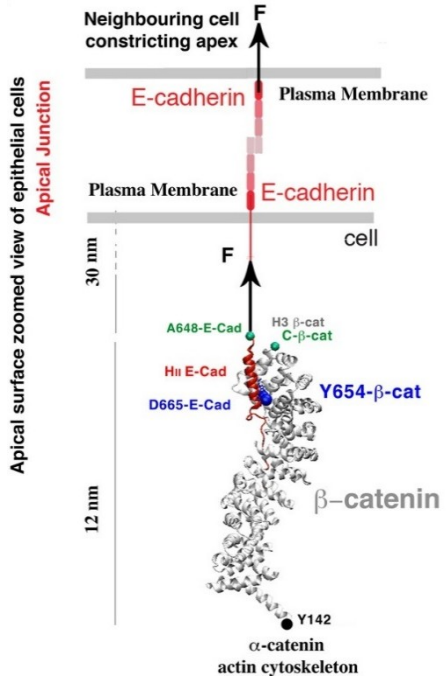


- During the gastrulation, there is a movement of cells = > the action of mechanical forces.
- These forces activate the β-catenin pathway, which contributes to the mesoderm specification by activating the expression of genes that specify the mesoderm.

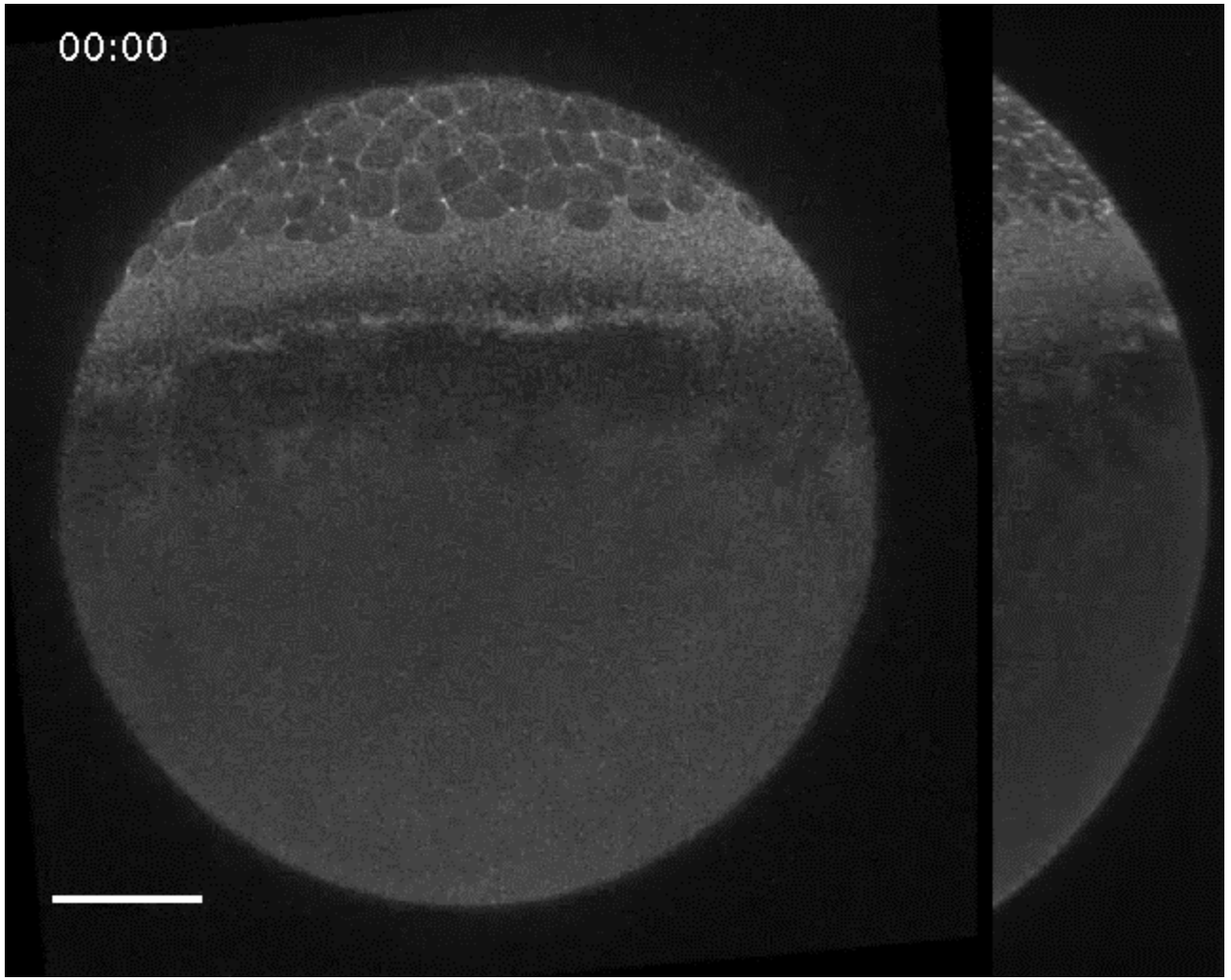
https://doi.org/10.1016/j.ceb.2020.08.007
Current Opinion in Cell Biology

Mechanosensing in embryogenesis – Cadherin/β-catenin – gastrulation and induction of mesoderm

- β-Catenin is a primary molecule in mechanoreception
-
- in the nucleus as a transcription factor (regulates gene expression)
- but also structural role in adherens junctions (link between E-cadherin and cytoskeleton)
- Cadherin-catenin-actin "axis" is under constant mechanical pressure not only due to the internal contractility of actin. An external mechanical stimulus acts on the intercellular contacts and increases this pressure, resulting in a sense of mechanical pressure (1 nm).

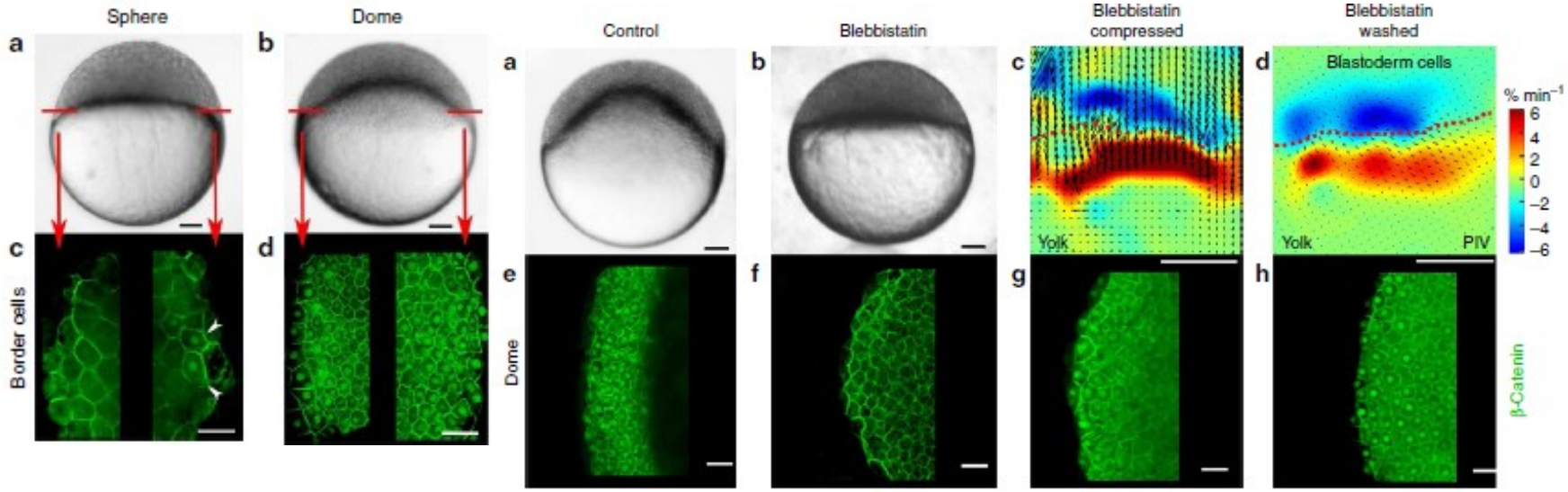


Mechanosensing in embryogenesis – Cadherin/β-catenin – gastrulation and induction of mesoderm



Mechanosensing in embryogenesis – Cadherin/β-catenin – gastrulation and induction of mesoderm

- Blockage of gastrulation movements (genetic, pharmacol.) leads to inhibition of mesodermal genes – repeated action of mechanical forces saves the phenotype and expression of mesoderm. Genes
- Feedback for signal pathways.



Blebbistatin = specific non-muscle myo-II inhibitor

ARTICLE
Received 21 Jun 2013 | Accepted 24 Oct 2013 | Published 27 Nov 2013
DOI: 10.1038/ncomms3821 OPEN

Evolutionary conservation of early mesoderm specification by mechanotransduction in Bilateria

Thibaut Brunet[1,†,*], Adrien Bouclet[1,*], Padra Ahmadi[1], Démosthène Mitrossilis[1], Benjamin Driquez[1],

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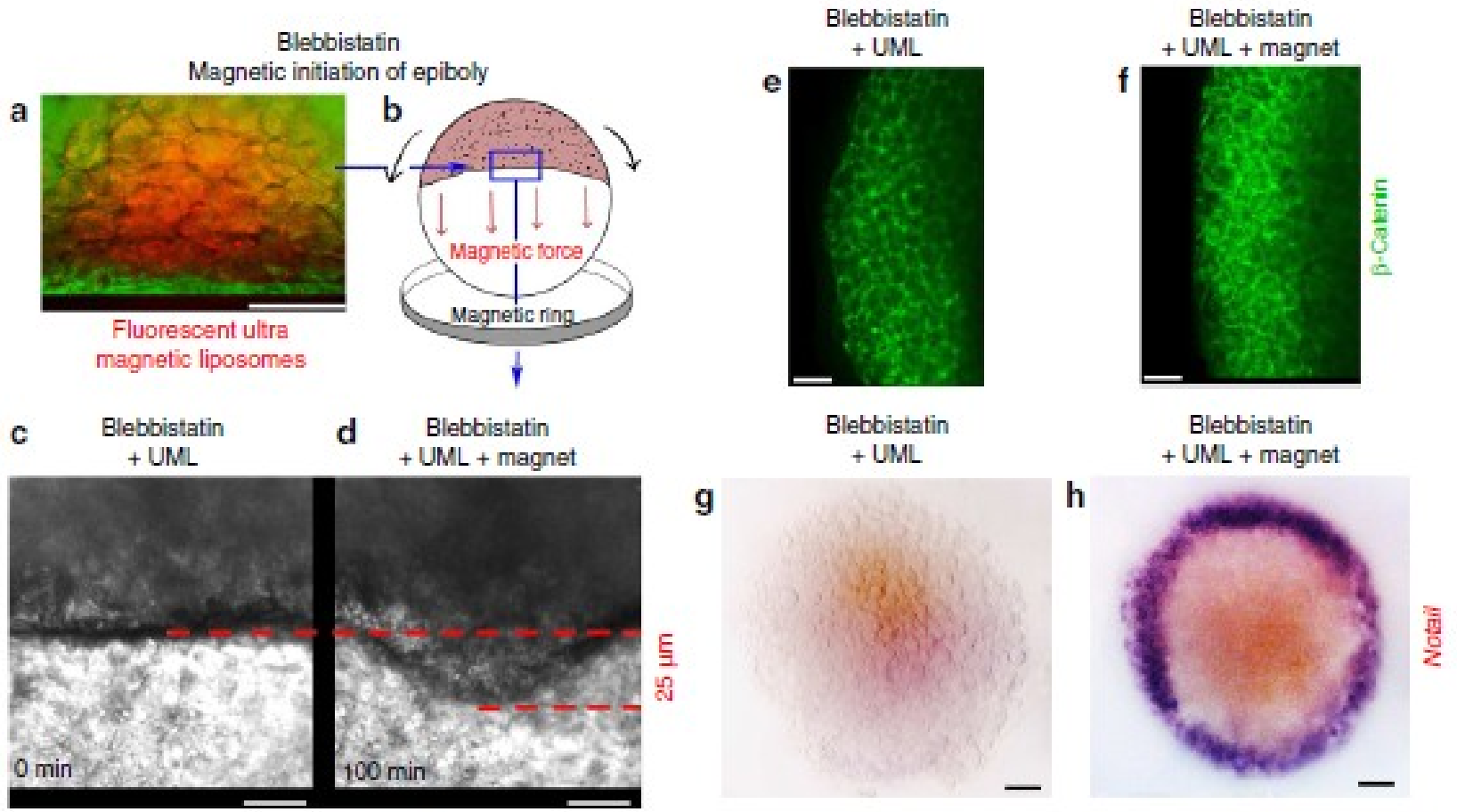

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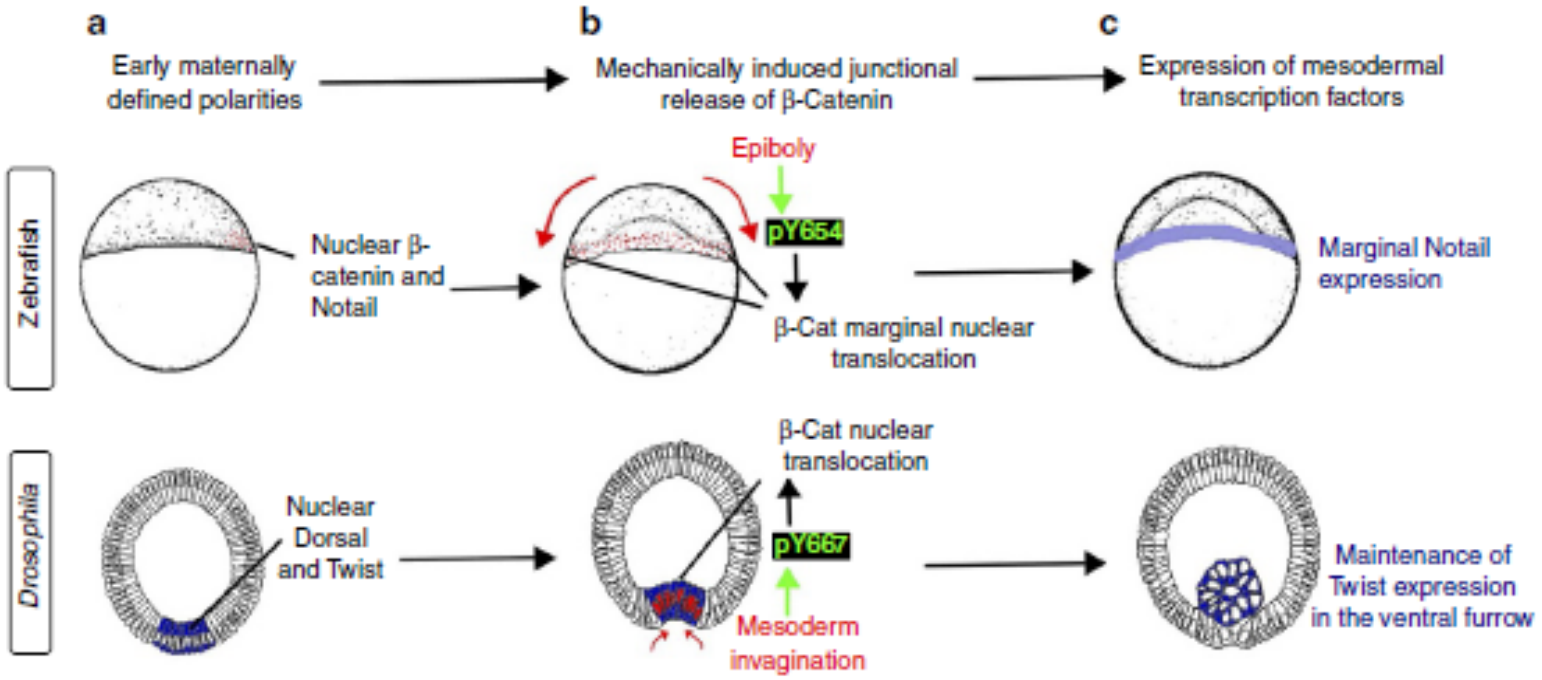

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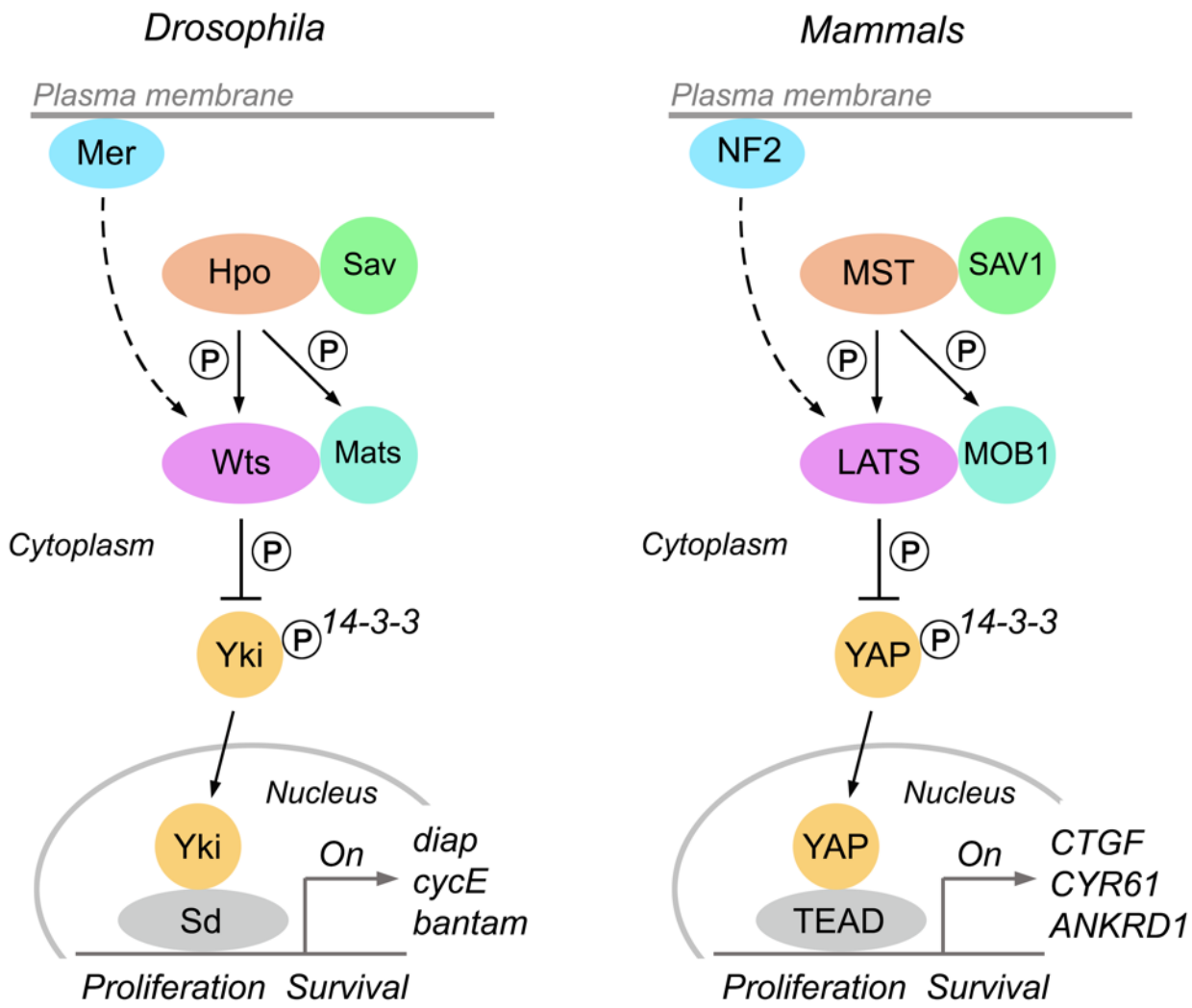
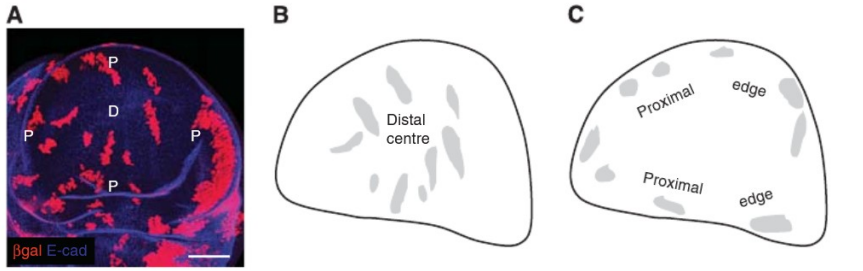
Intercellular mechanosensing and tissue growth - Hippo

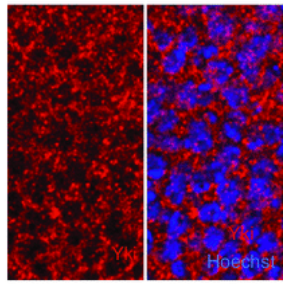
Hippo not only in size control



Intercellular tension and tissue growth - Hippo

- ECM stiffness, cell density, cytoskeleton pressure affect YAP/Yki localization
- Molecular mechanism is unclear

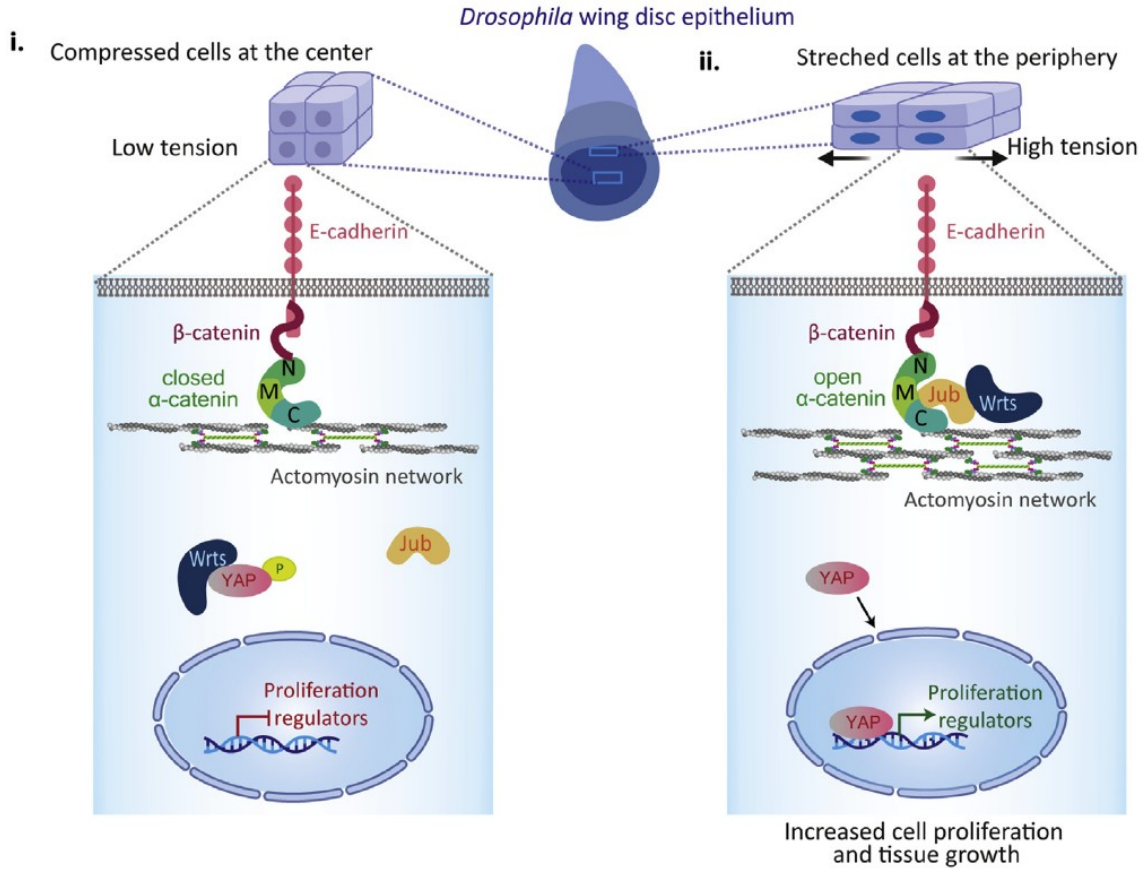




Differential proliferation rates generate patterns of mechanical tension that orient tissue growth

Yanlan Mao[1,5], Alexander L Tournier[2,5,*], Andreas Hoppe[3], Lennart Kester[1], Barry J Thompson[4] and Nicolas Tapon[1,*]

(Lecuit and Le Goff, 2007). I development, the orientation major influence on the shape a



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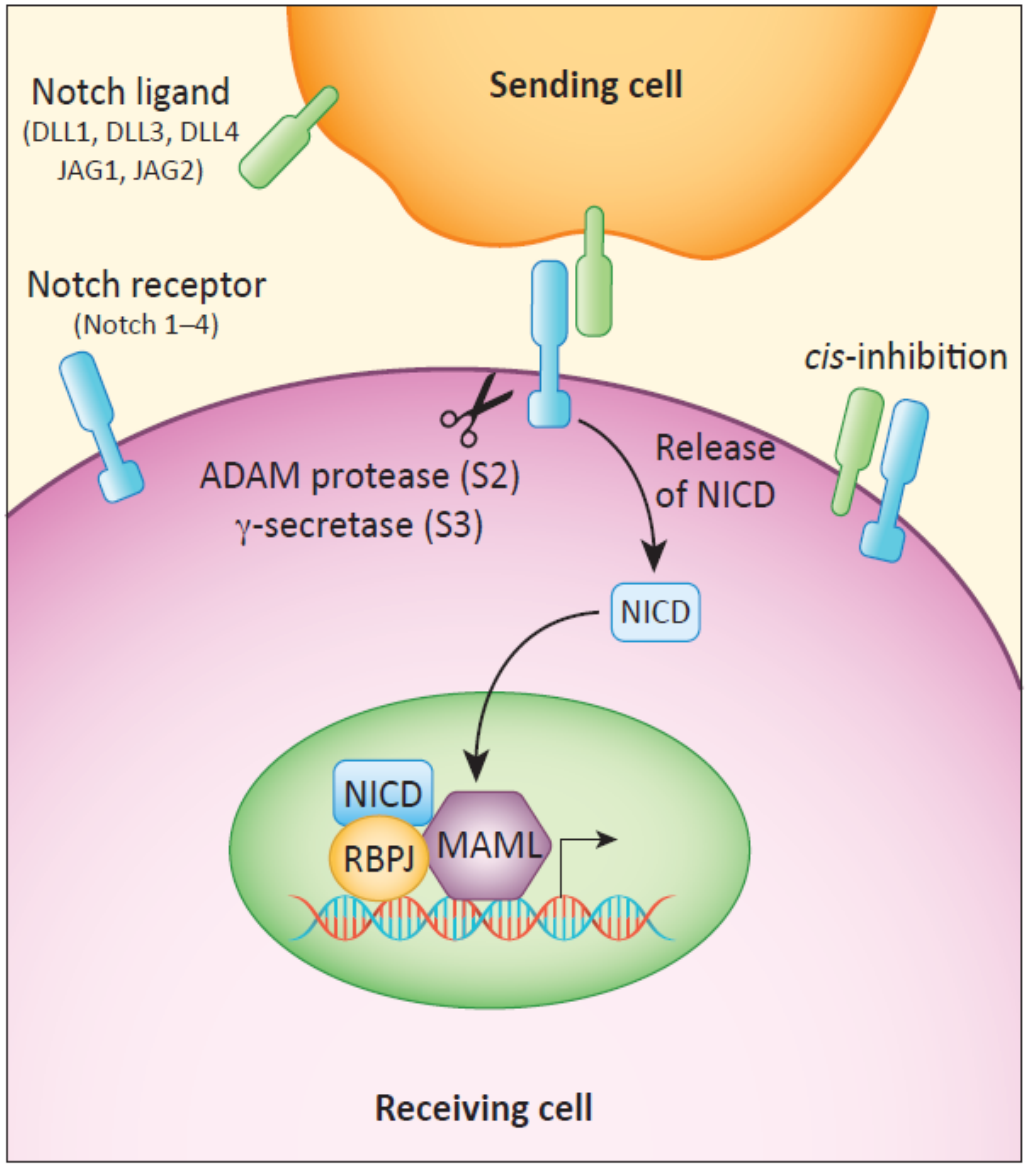
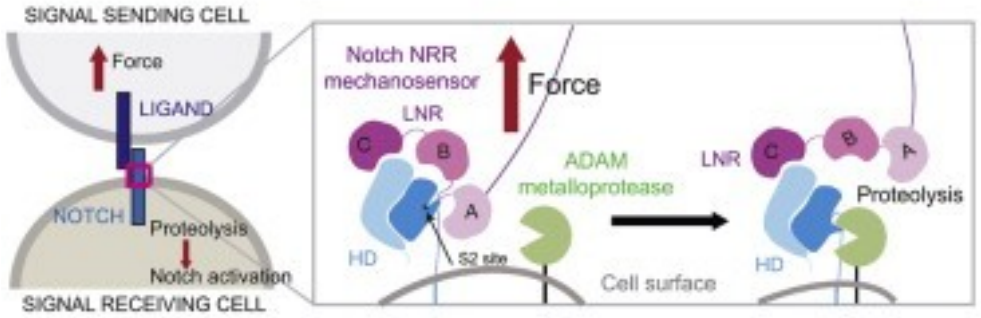
Current Opinion in Cell Biology

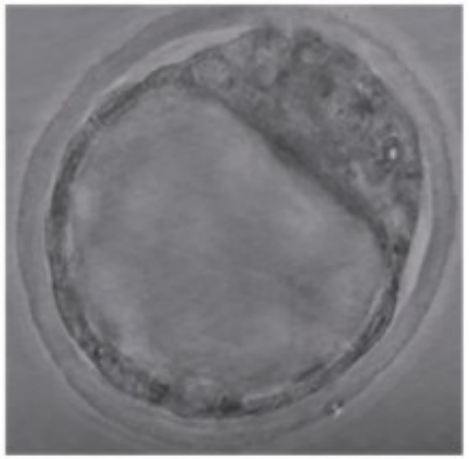
NOTCH


Notch ligand
(DLL1, DLL3, DLL4
JAG1, JAG2)
Sending cell
Notch receptor
(Notch 1–4)
cis-inhibition
ADAM protease (S2)
γ-secretase (S3)
Release
of NICD
NICD
NICD
RBPJ
MAML
Receiving cell


SIGNAL SENDING CELL
Force
LIGAND
NOTCH
Proteolysis
Notch activation
SIGNAL RECEIVING CELL
Notch NRR
mechanosensor
LNR
Force
ADAM
metalloprotease
S2 site
Cell surface
HD
Proteolysis

NOTCH

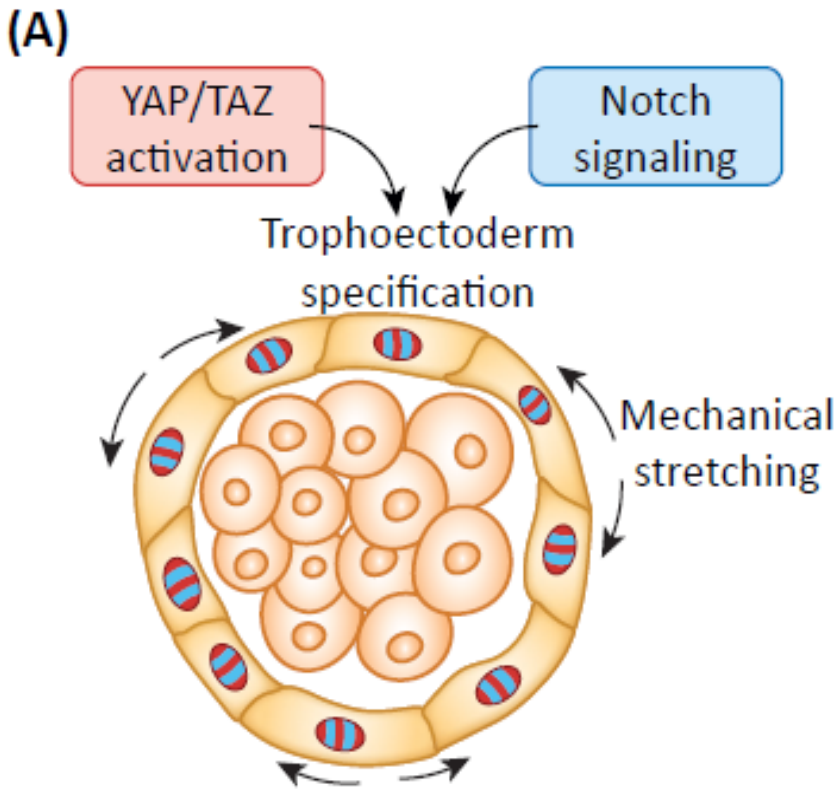
- After fertilization, the mammalian zygote produces about 100 cells within about 4 days.



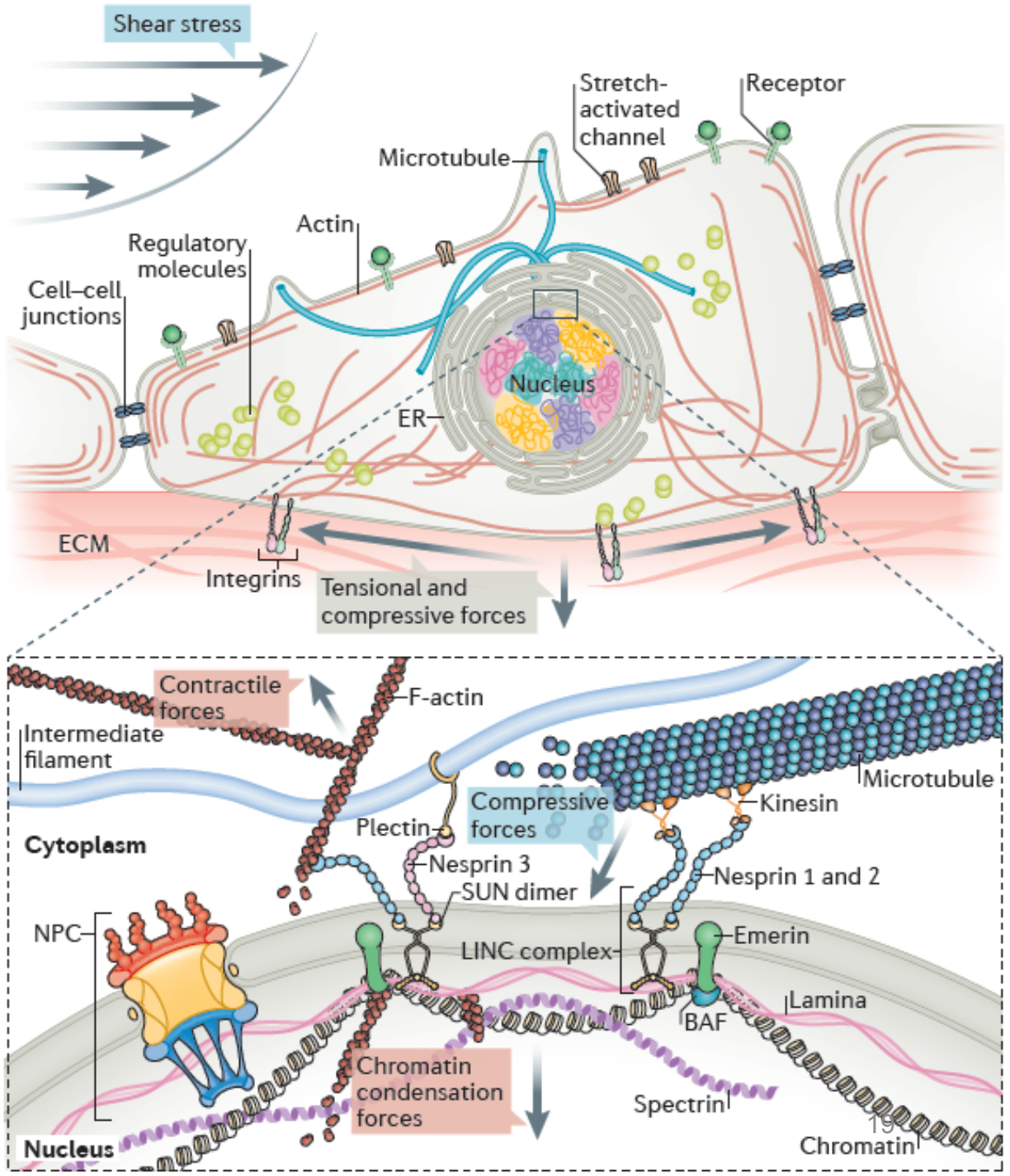
Embryoblast (ICM): Oct4, Nanog, Sox2
Trophectoderm (TE): CDX2

- These factors determine individual cell lines, but how did the individual lines were generated?
- The position of blastomeres in the embryo. Different polarity and adhesion of cells.

NOTCH


(A)
YAP/TAZ activation
Notch signaling
Trophoectoderm specification
Mechanical stretching

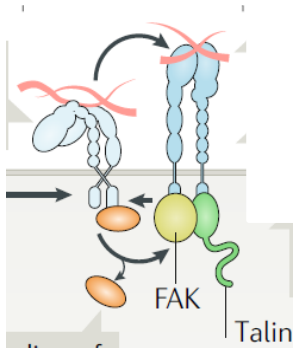
Integrins

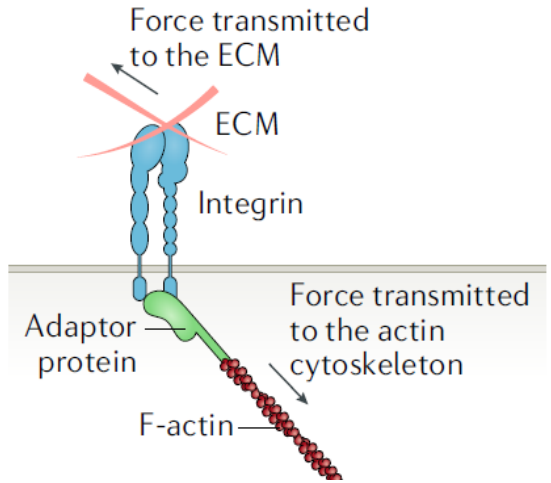


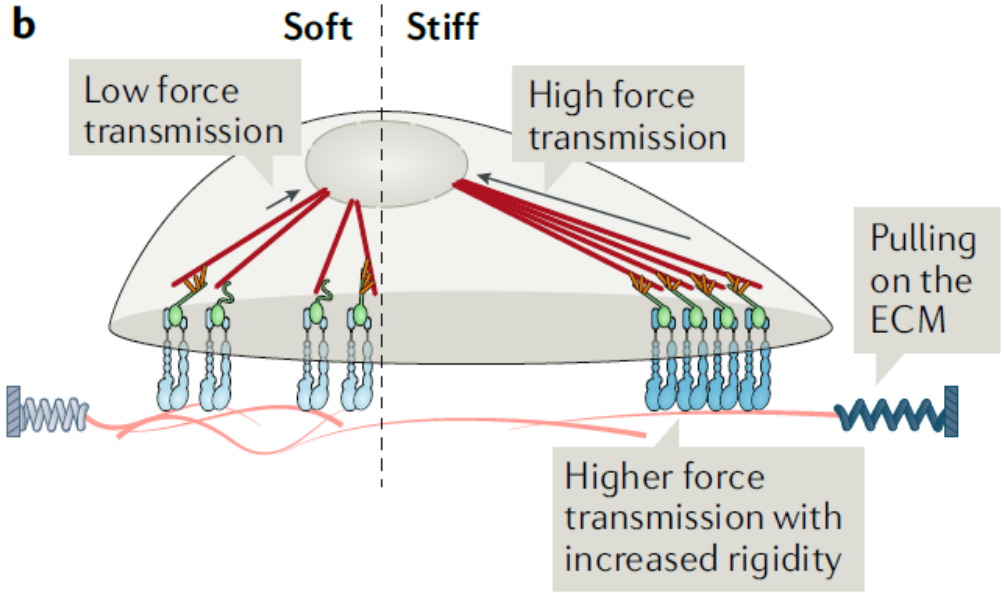
Regulation of genome organization and gene expression by nuclear mechanotransduction

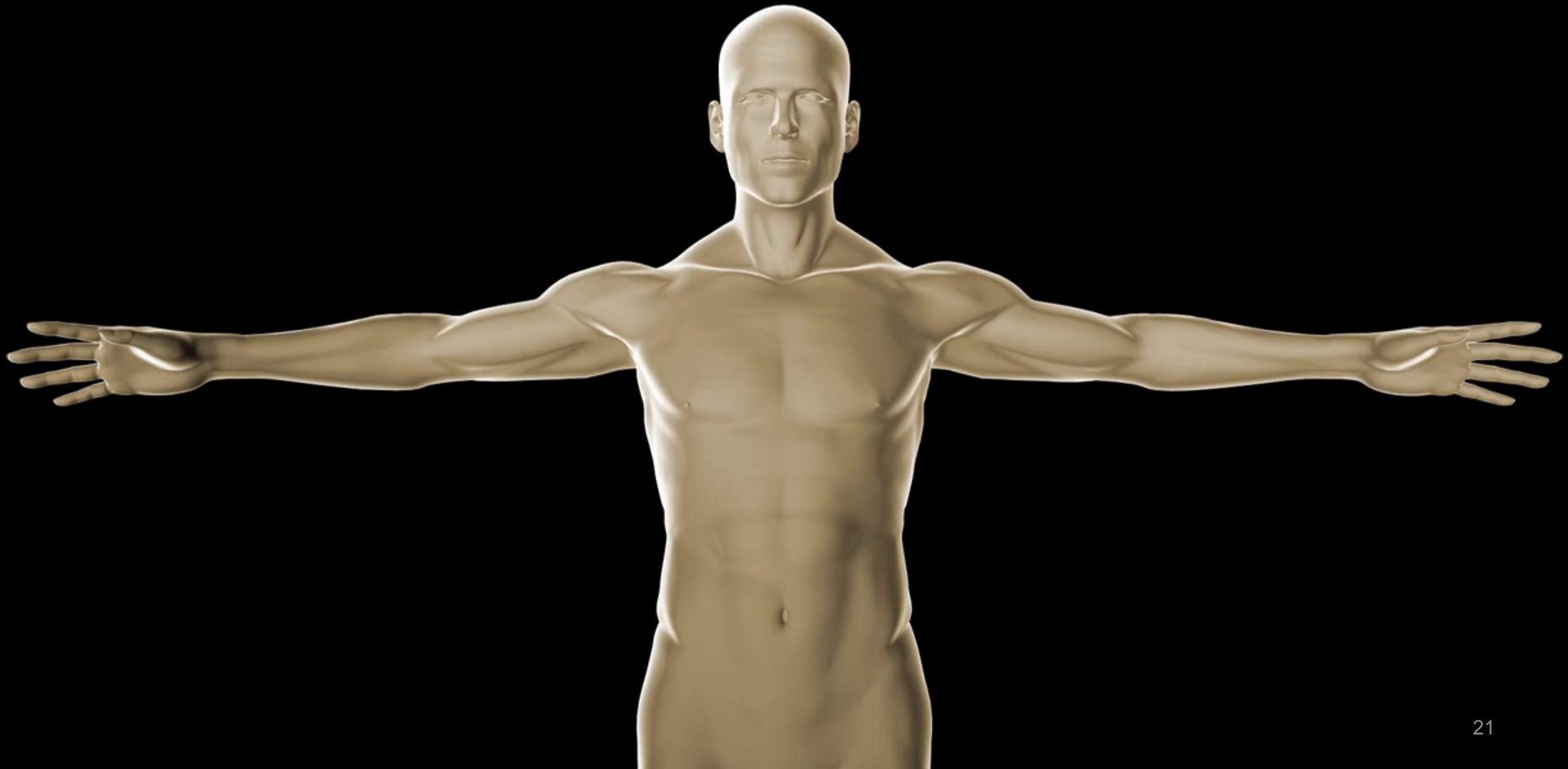
Caroline Uhler[1] and G. V. Shivashankar[2,3]

Integrins

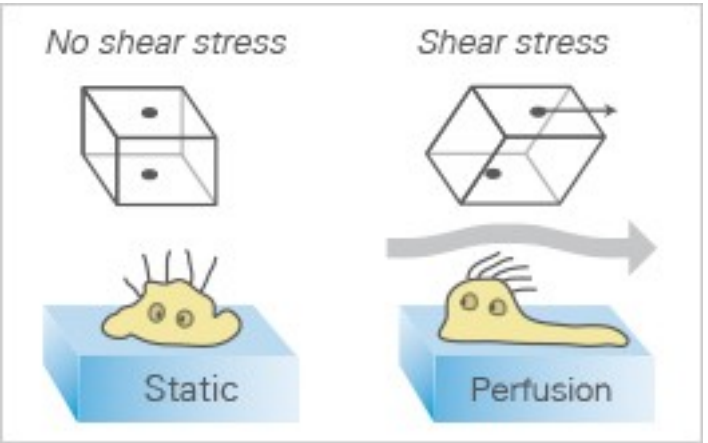

FAK
Talin


Force transmitted
to the ECM
ECM
Integrin
Adaptor
protein
Force transmitted
to the actin
cytoskeleton
F-actin


b
Soft
Stiff
Low force
transmission
High force
transmission
Pulling
on the
ECM
Higher force
transmission with
increased rigidity

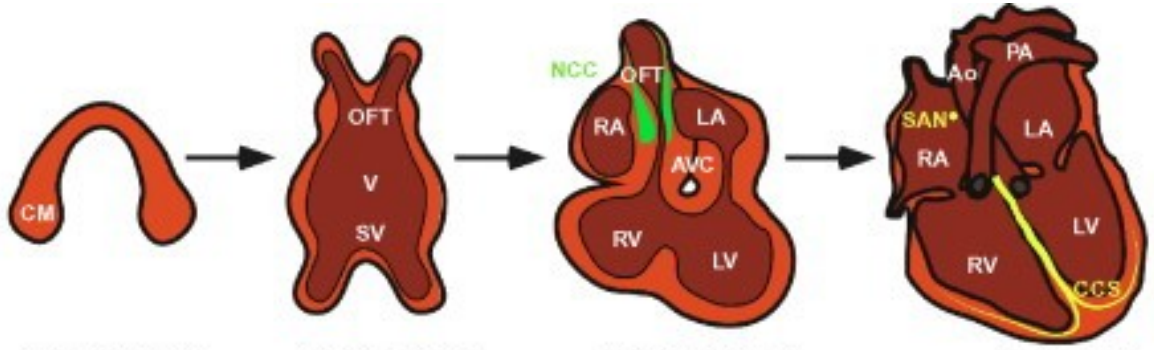


Shear forces



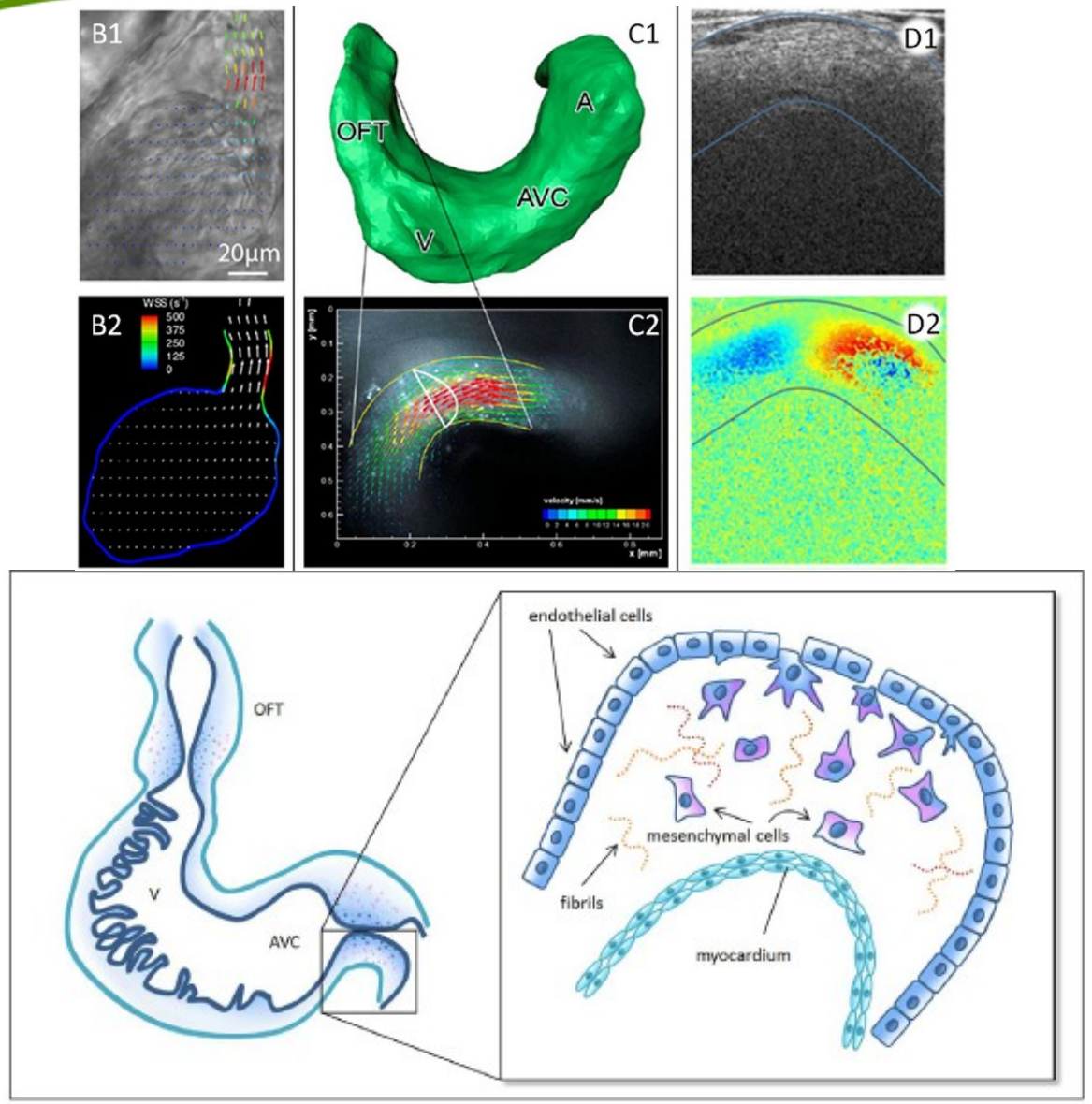
Shear forces and heart development

- The heart begins its development as a tube and gradually develops into a multi-chamber apparatus.
- But in the course of development, it constantly draws blood.
- Blood pressure exerts shear forces on endothelial cells.
- Shear forces are perceived by endothelial cells = > affects their organization and physiology.



Stage	Cardiac crescent	Linear heart tube	Looped heart tube	Mature heart
Human development	Day 15	Day 20	Day 30	Day 40-birth
Mouse development	E7.5	E8.0	E10–12	E14–18
Key events	Myocyte differentiation Migration to the midline	Bidirection contractions	Looping morphogenesis Addition of SHF progenitors Cushion formation Neural crest addition Ventricular septation begins	Conduction system OFT septation Cushion remodeling

Shear forces and heart development

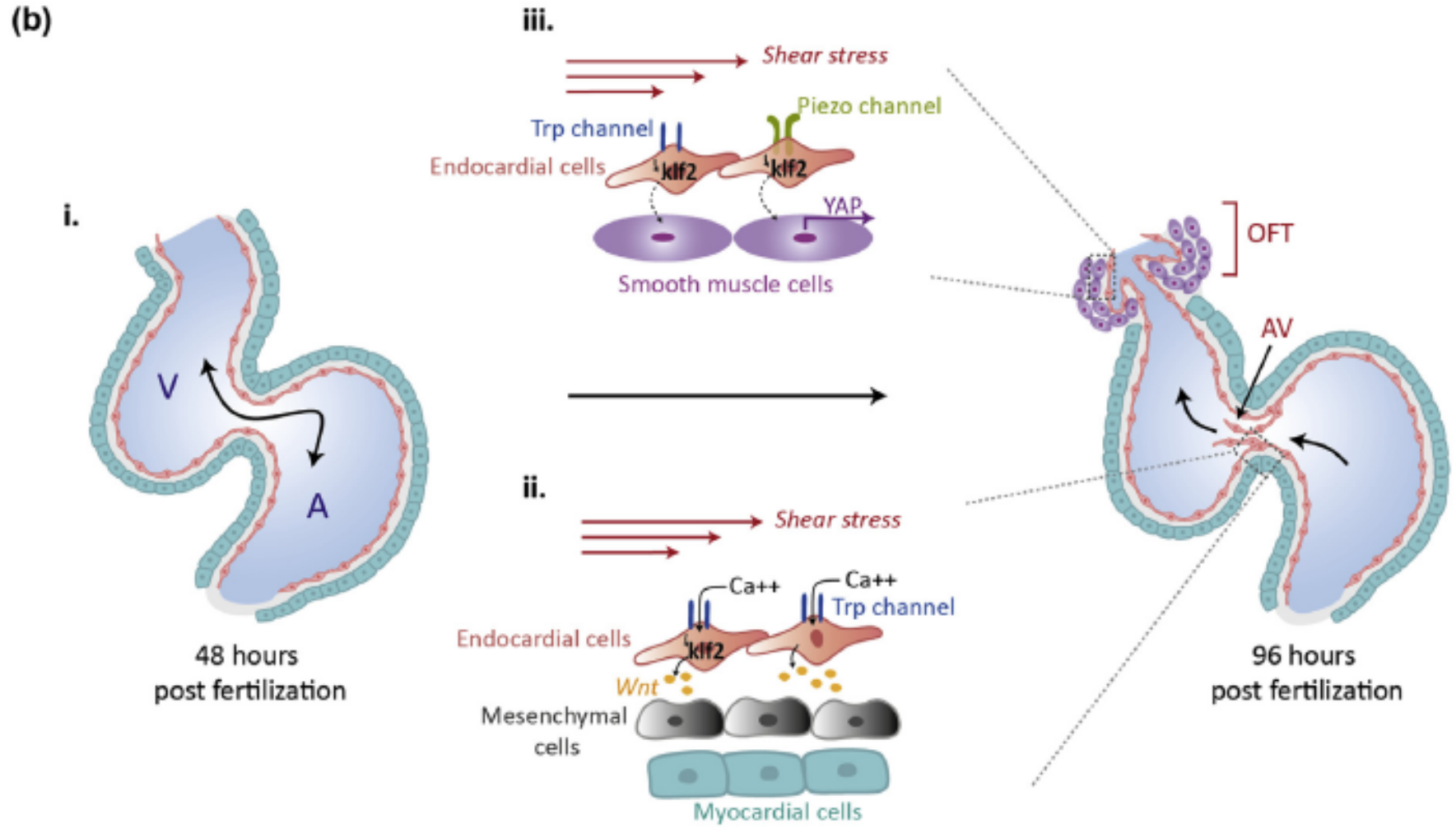


Influence of blood flow on cardiac development

Katherine Courchaine [1], Graham Rykiel [1], Sandra Rugonyi*

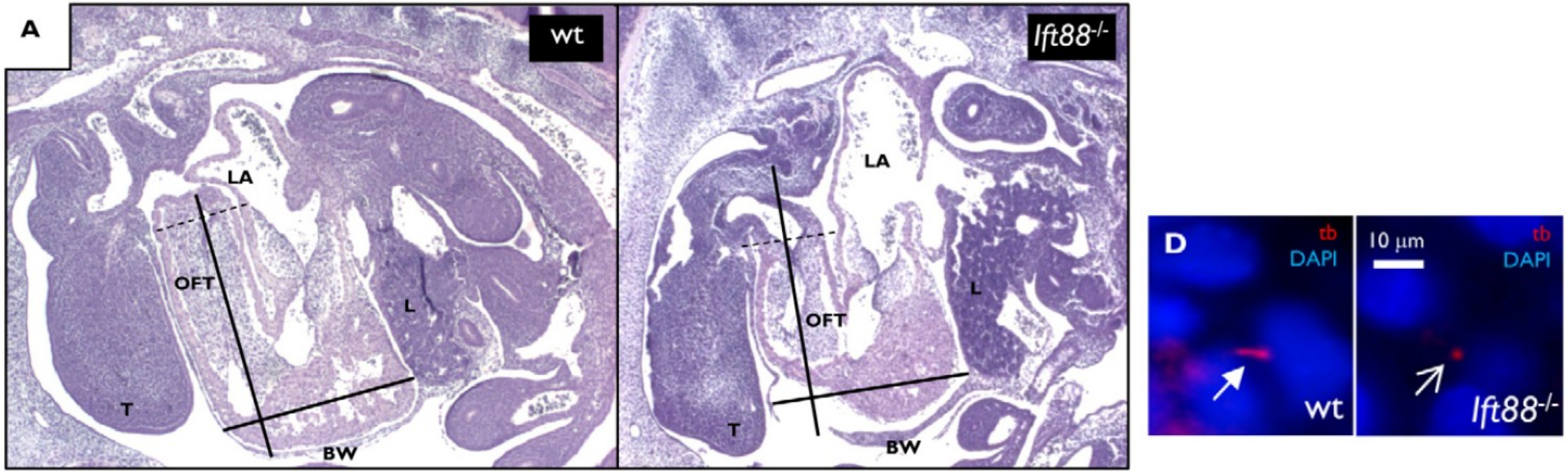
Biomedical Engineering, School of Medicine, Oregon Health & Science University, Portland OR, USA

Shear forces and heart development



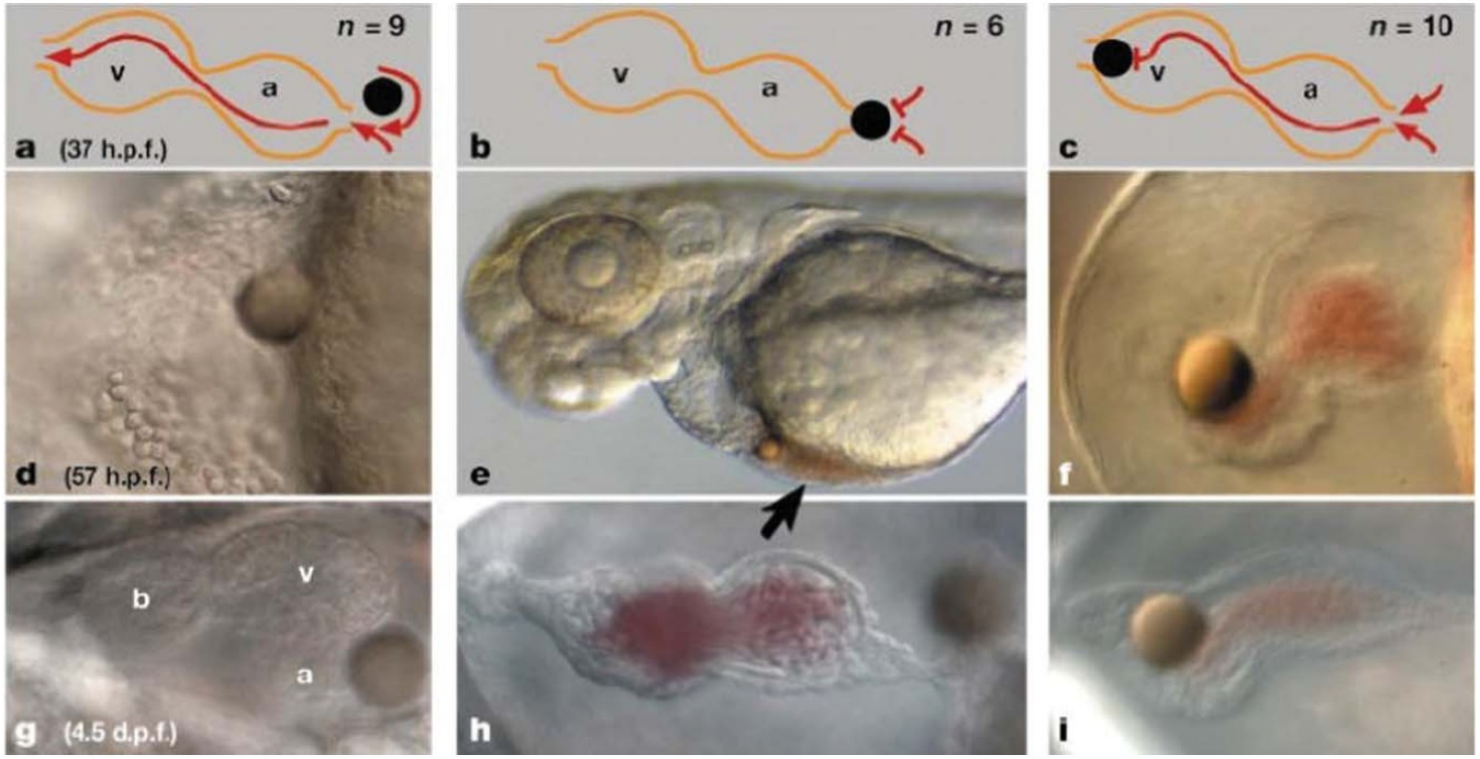
Shear forces and heart development

Disruption of shear forces (genetically, surgically, change in viscosity) leads to disorders in valve development



The development of heart valves therefore depends on the perception of shear forces by mechanosensitive channels in endothelial cells, thus determining the right place for valve development and activation of the corresponding genes.

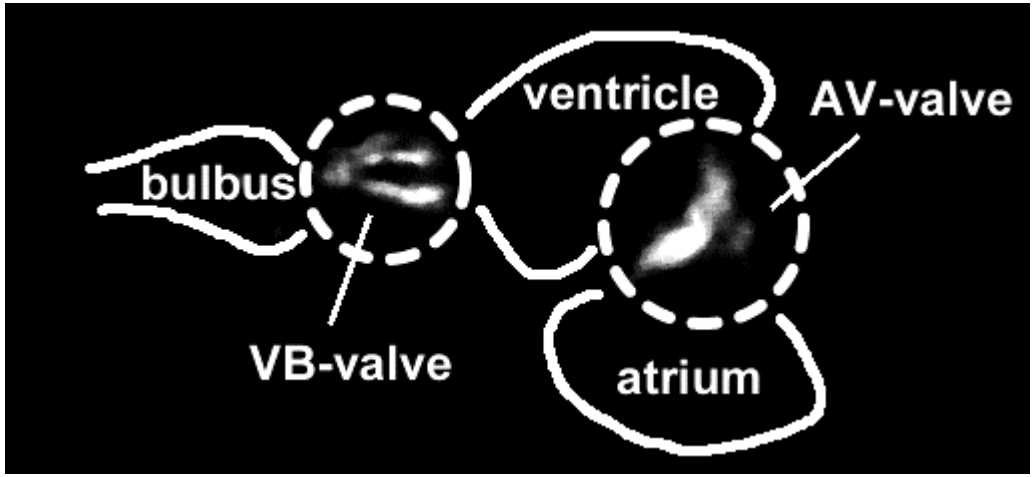
Shear forces and heart development

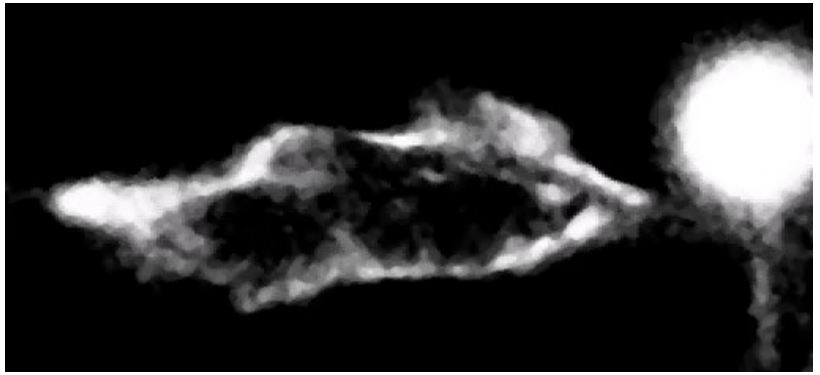


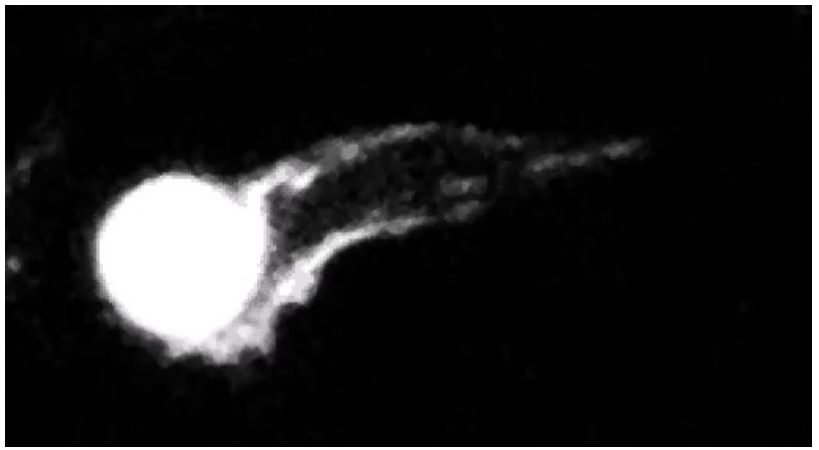
Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis

Jay R. Hove*†, Reinhard W. Köster†‡, Arian S. Forouhar*, Gabriel Acevedo-Bolton*, Scott E. Fraser‡ & Morteza Gharib*

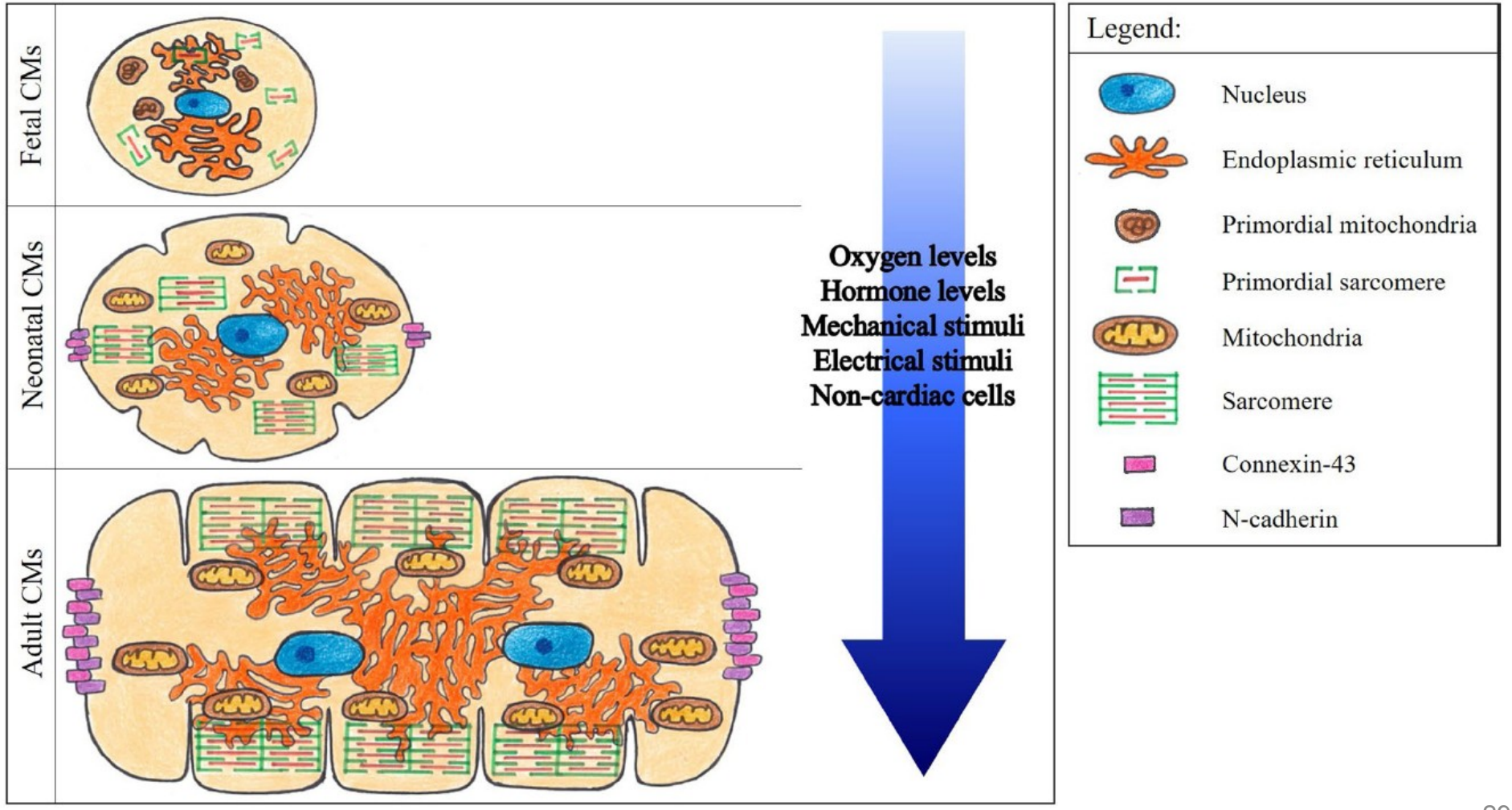
Shear forces and heart development

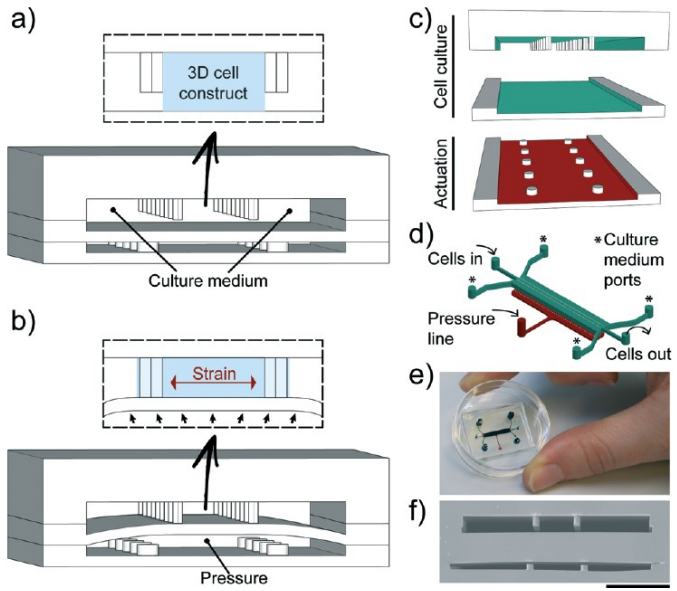


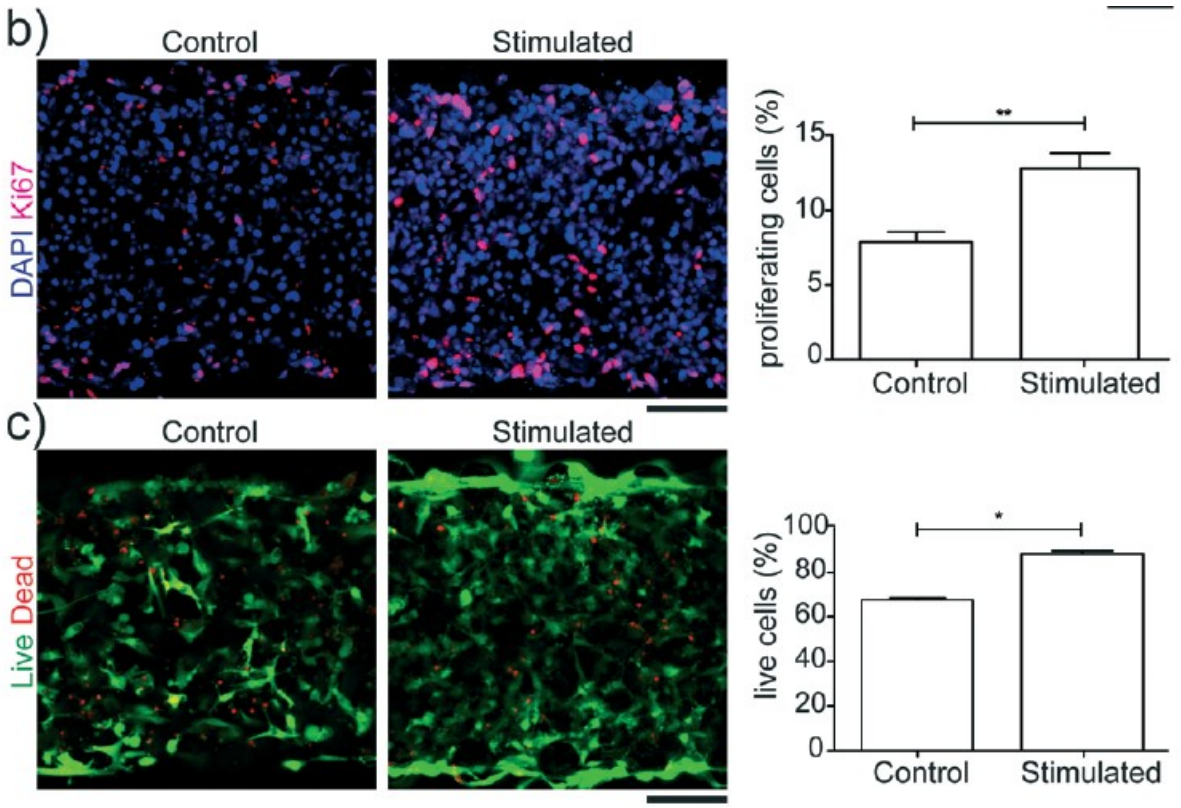




Food for thought







Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues†‡

Anna Marsano,*[a] Chiara Conficconi,§[ab] Marta Lemme,§[ab] Paola Occhetta,[b] Emanuele Gaudiello,[a] Emiliano Votta,[b] Giulia Cerino,[a] Alberto Redaelli[b] and Marco Rasponi*[b]

Lab on a Chip

Video related to research article appearing in *Lab on a Chip*

Anna Marsano, Chiara Conficconi, Marta Lemme, Paola Occhetta, Emanuele Gaudiello, Emiliano Votta, Giulia Cerino, Alberto Redaelli and Marco Rasponi

"Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues"

Read the article at
http://pubs.rsc.org/en/Content/ArticleLanding/2015/LC/C5LC01356A

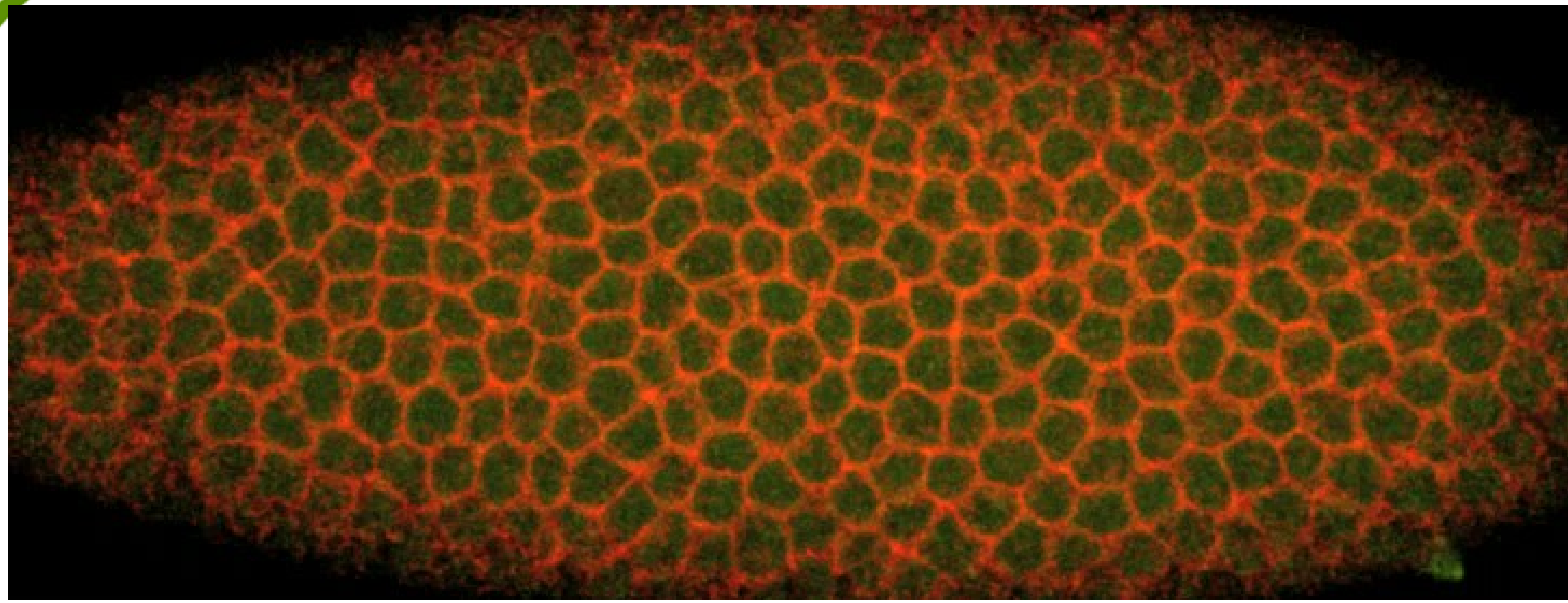
Mechanical forces and morphogenesis

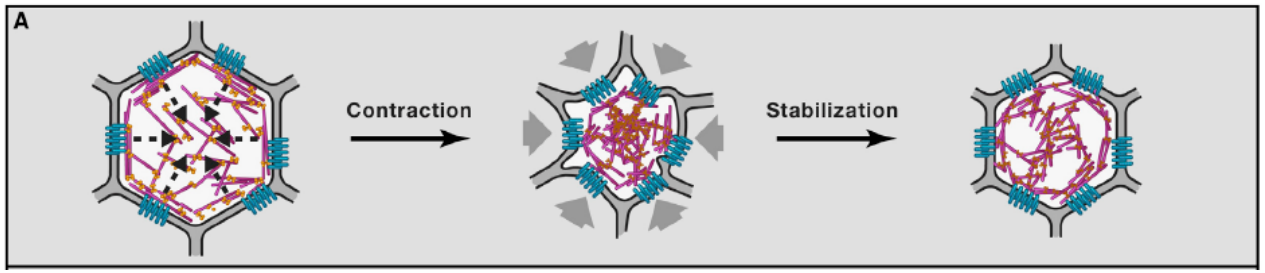
- During the development of mechanical forces cause changes in the shape, size, number and position of cells, which is accompanied by a change in gene expression => impact on morphogenesis.
- All of these cellular processes that lead to a change in tissue shape are a form of force between individual cells, normally mediated by intercellular adhesion.

This force is generated through:

- Actin
- Polymerization of micro-domes
- Osmotic pressure
- Molecular motors - Myosin

Mechanosensing in embryogenesis – gastrulation and induction of mesoderm





Forces in Tissue Morphogenesis and Patterning

Carl-Philipp Heisenberg[1,*] and Yohanns Bellaïche[2,*]
[1]Institute of Science and Technology Austria, 3400 Klosterneuburg, Austria
[2]Institut Curie, CNRS UMR3215, INSERM U934, 75248 Paris Cedex 05, France
*Correspondence: heisenberg@ist.ac.at (C.-P.H.), yohanns.bellaiche@curie.fr (Y.B.)
http://dx.doi.org/10.1016/j.cell.2013.05.008

Mechanical forces and morphogenesis

- Changes in the cytoskeleton are transmitted to neighboring cells and the ECM through the interconnection of the cytoskeleton to adhesive molecules that provide cell-to-cell, cell-ECM interaction (cadherins, integrins).
- Actin-myosin contraction and interaction mediated by cadherins are basic and evolutionarily conserved mechanisms that generate and transmit forces for the formation of morphogenesis (see differential adhesion hypothesis/equlibrium state in ECM and cell adhesion – all forces in equilibrium, the state of lowest energy).

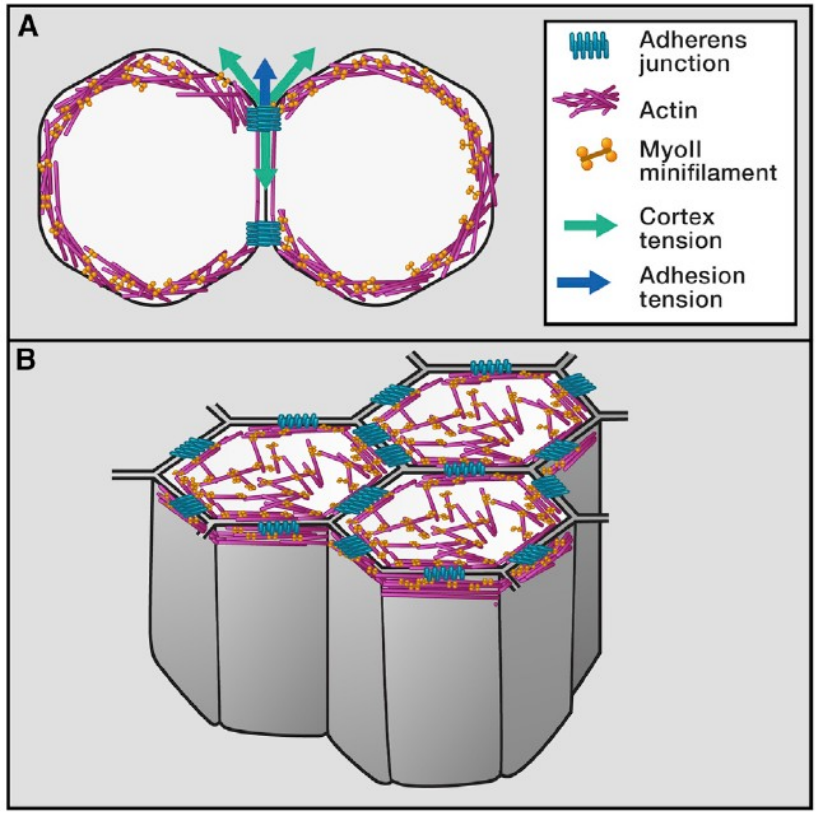
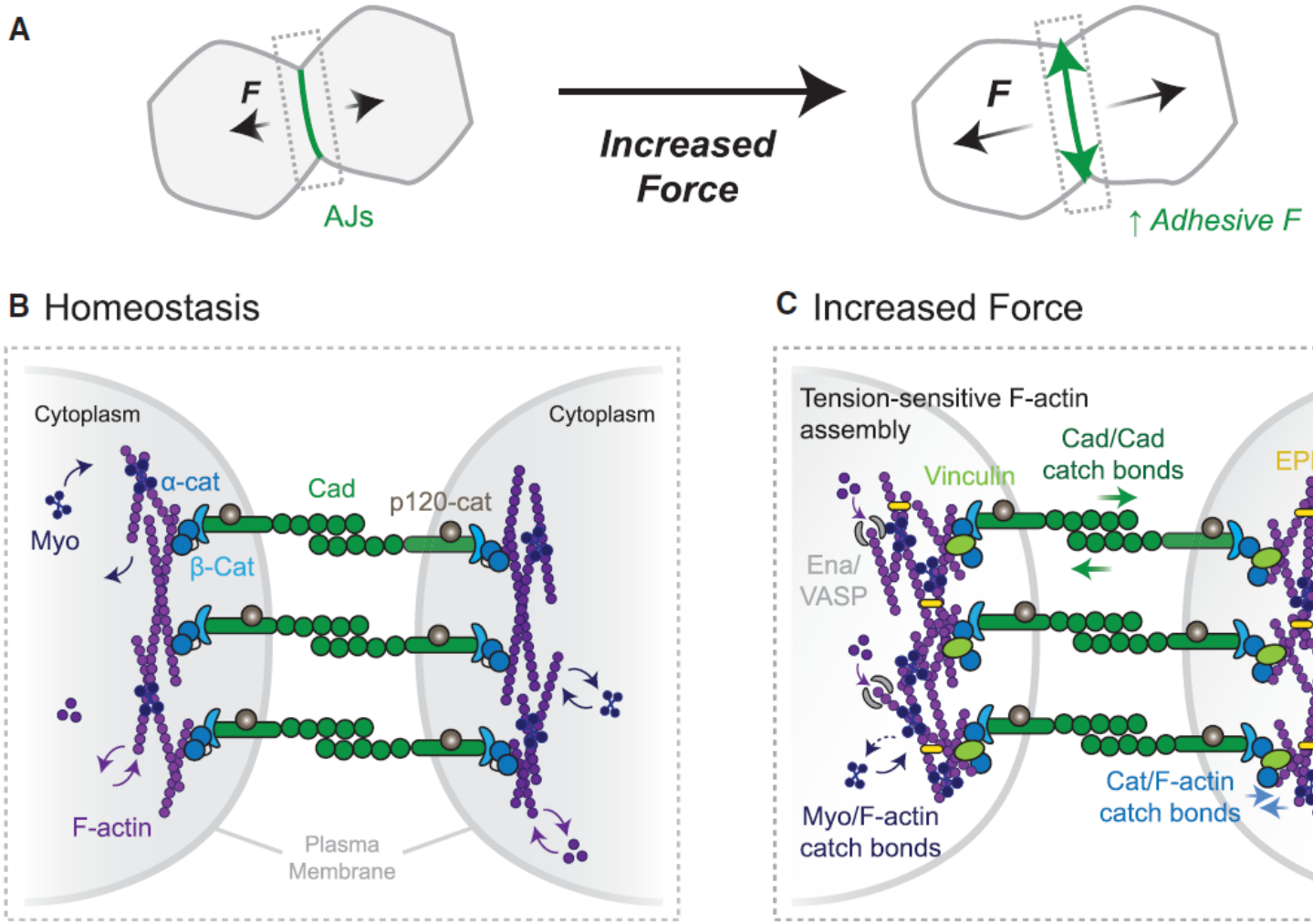


Figure 1. Self-Organization of Cells at Steady State Determined by Actin-Myosin Contractility and Cell Adhesion

(A) Upon cell-cell contact, the contacting cells change their shape in response to mechanical forces associated with actin-myosin contractility (green arrow) and adhesion (blue arrow).

(B) In epithelial tissues, adhesive contacts and the actin-myosin network are organized in belt-like structures at the apical domain of the cell. At steady state, the arrangement of epithelial cells at their apex is determined by actin-myosin contractility and cell-cell adhesion.

Mechanical forces and morphogenesis



Mechanical Force-Driven Adherens Junction Remodeling and Epithelial Dynamics

Diana Pinheiro[1,2,3] and Yohanns Bellaïche[1,2,*]
[1]Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, 75248 Paris Cedex 05, France
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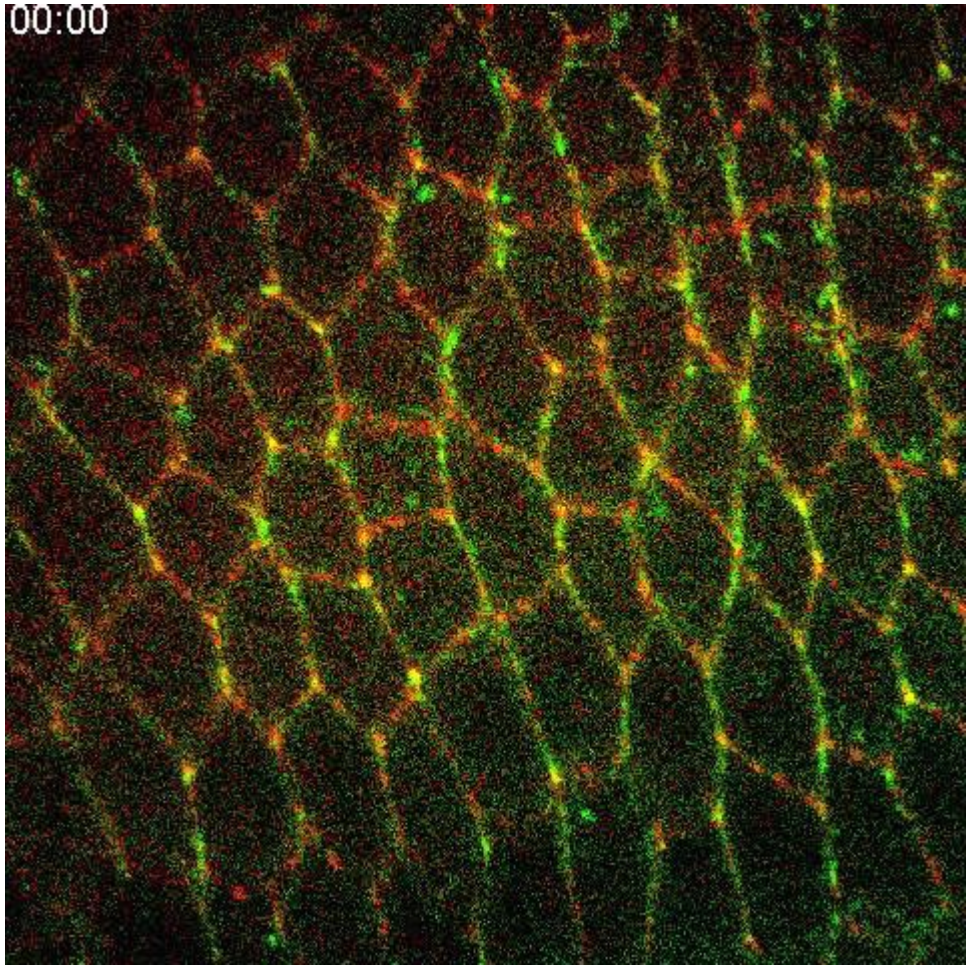
https://doi.org/10.1016/j.devcel.2018.09.014

Mechanical forces and morphogenesis



Mechanical forces – cell differentiation and proliferation

- The ability of cells to perceive external mechanical forces affects tissue size and architecture not only by changing their adhesive and cytoskeletal organization, but also by influencing their differentiation.

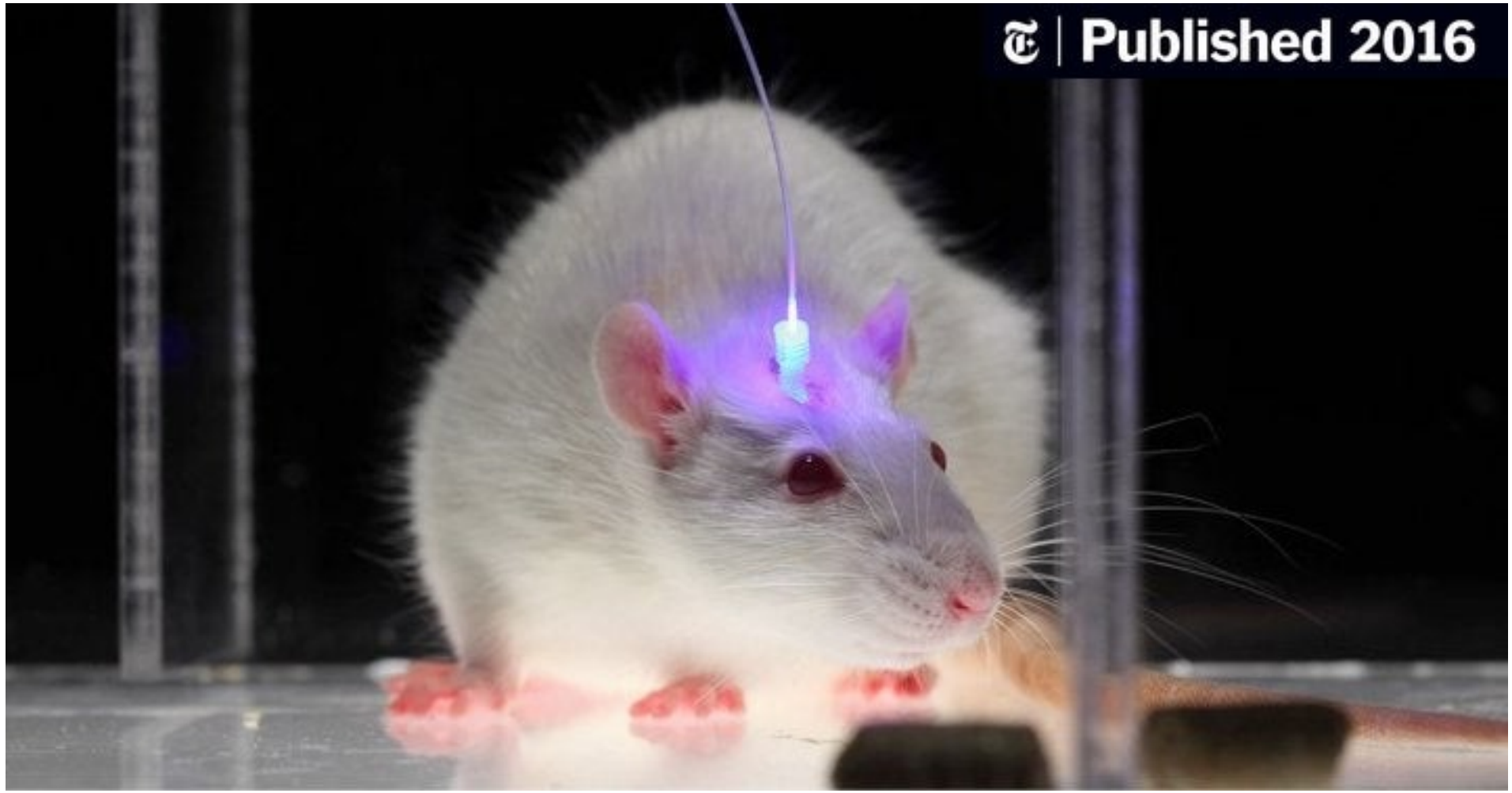
BONUS: Vibrational cues alter developmental timing

Agalychnis callidryas

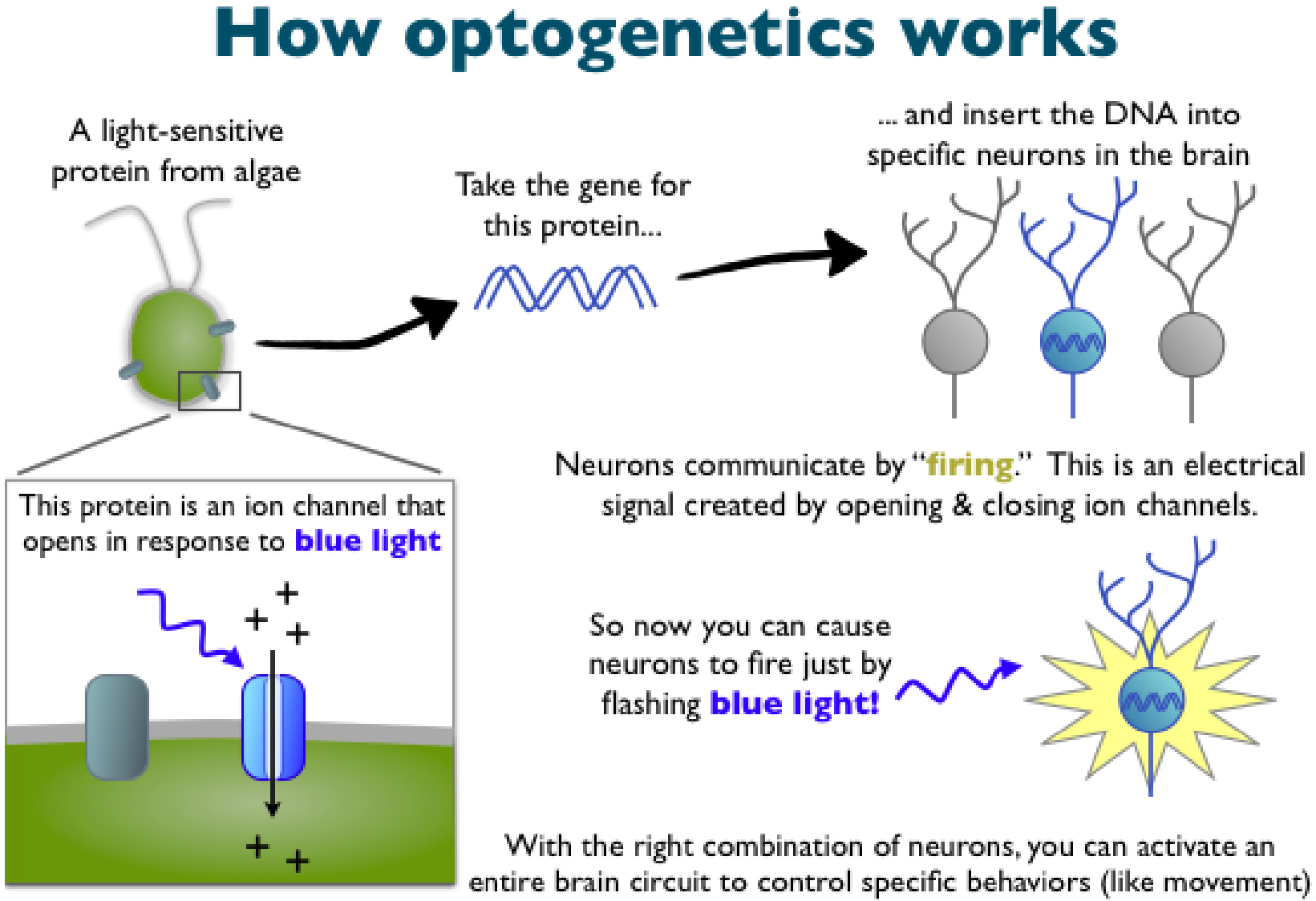
- It lays eggs on the leaves, which are located above the water
- Normal development lasts 7 days.
- In the case of a predator attack, larvae hatch (within seconds!) if the eggs have about 5 days of development.
- Signaling an attack of eggs by a predator is through vibration
- This leads to the production of enzymes that disrupt the shell
-


How do embryos escape from danger?
Kristina L. Cohen, Marc A. Seid & Karen M. Warkentin

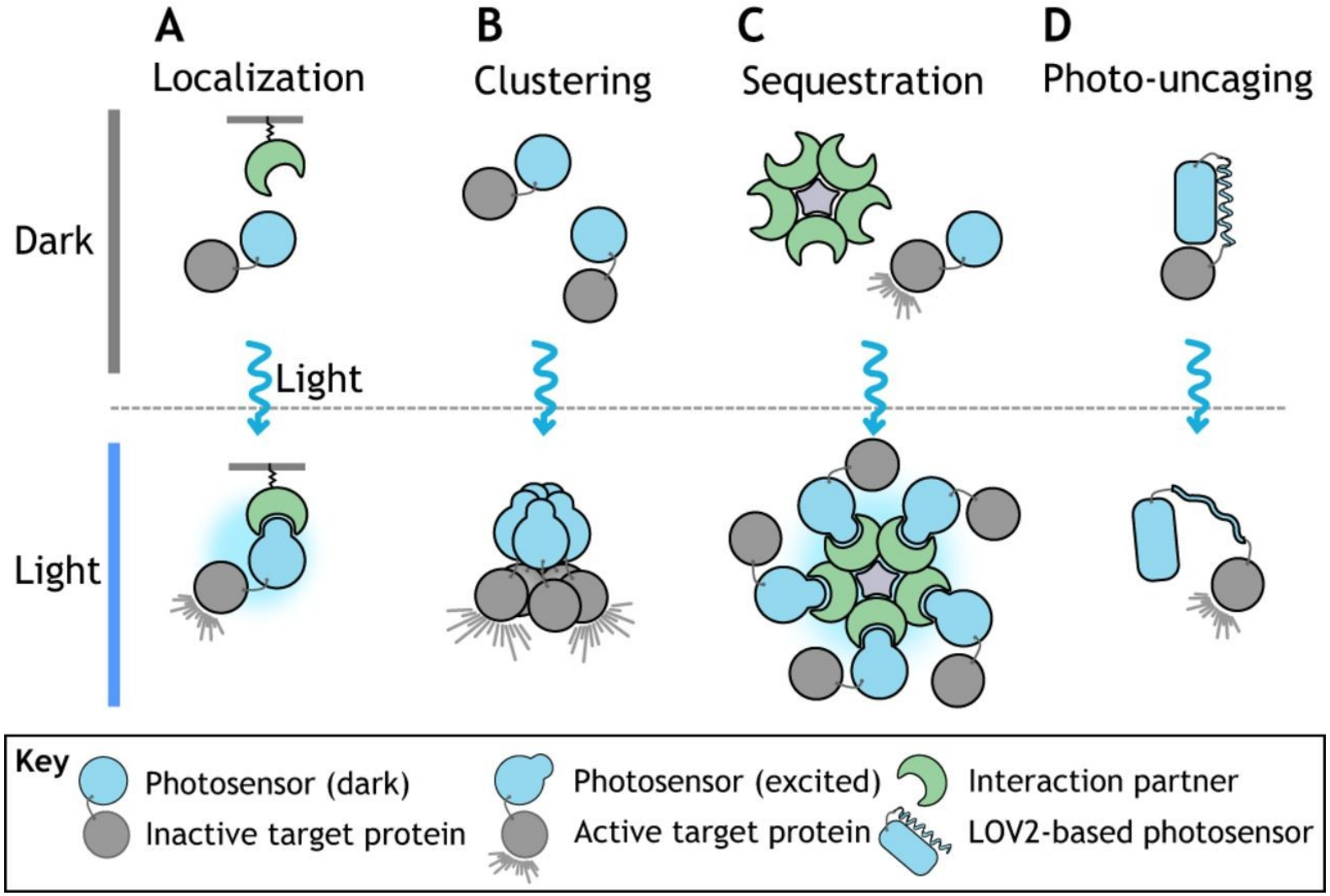
BONUS: optogenetics



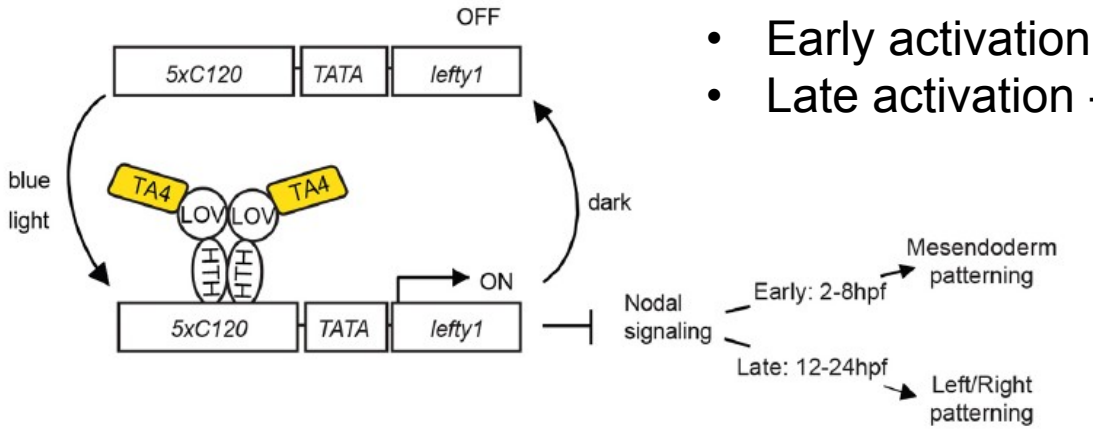
BONUS: optogenetics

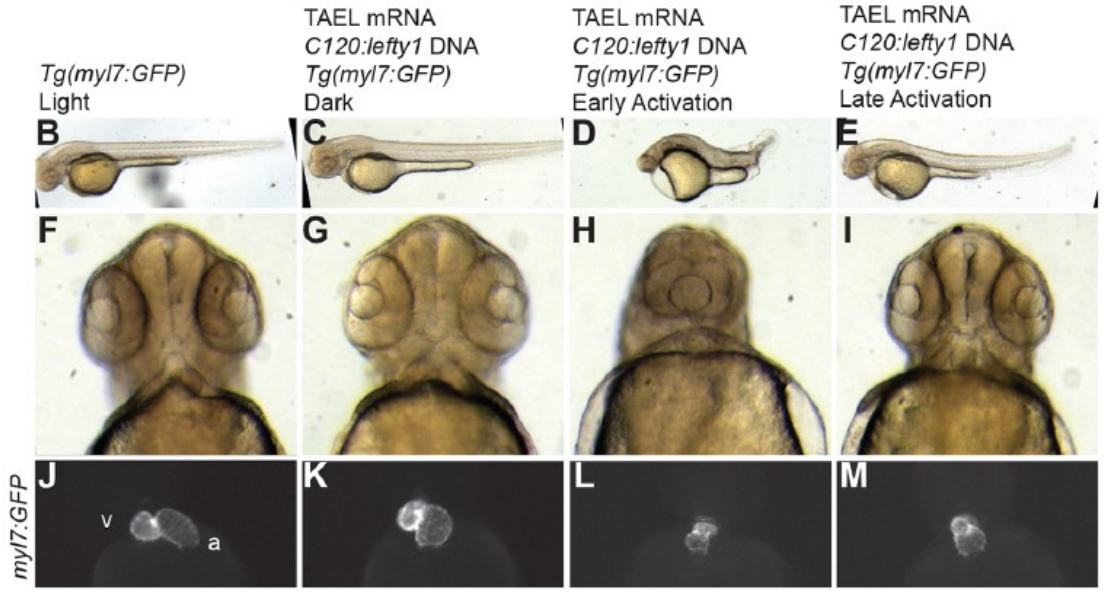


BONUS: optogenetics



BONUS: Optogenetics in Developmental Biology





- Early activation -> shortening of the body and cyclops
- Late activation -> symmetry of the heart

Development (2017) 144, 345-355 doi:10.1242/dev.139238

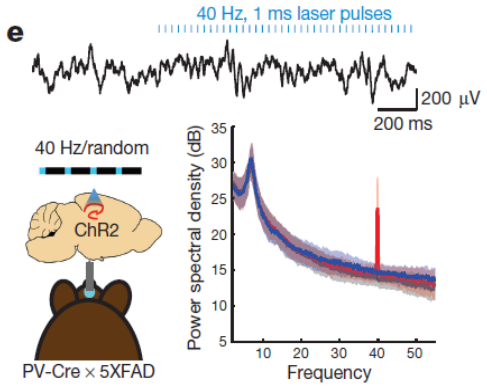
BONUS: optogenetics

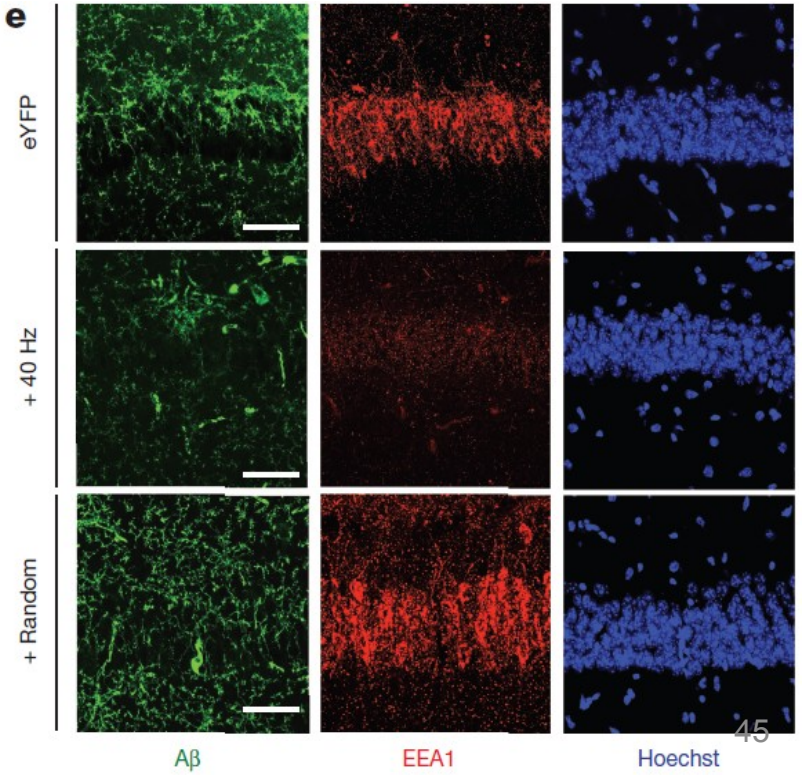
ARTICLE

doi:10.1038/nature20587

Gamma frequency entrainment attenuates amyloid load and modifies microglia

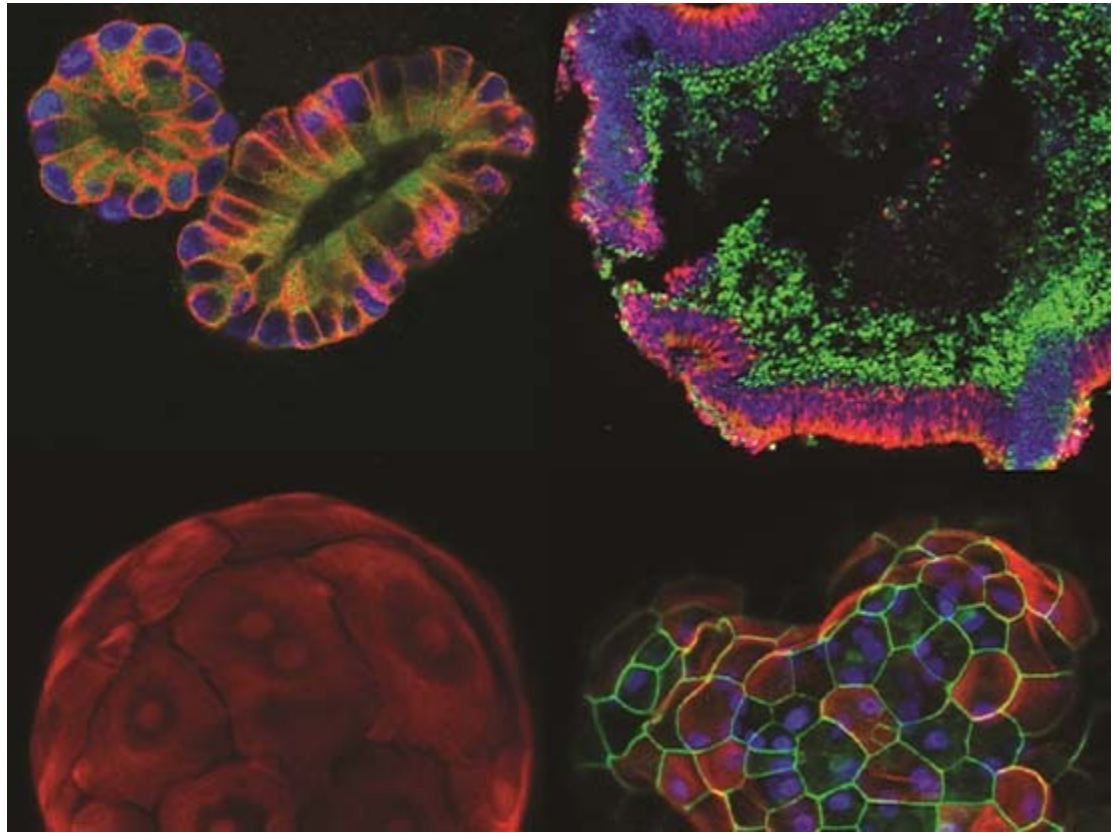
Hannah F. Iaccarino[1,3]*, Annabelle C. Singer[2,3,4]*, Anthony J. Martorell[1,3], Andrii Rudenko[1,3], Fan Gao[1,3], Tyler Z. Gillingham[1,3], Hansruedi Mathys[1,3], Jinsoo Seo[1,3], Oleg Kritskiy[1,3], Fatema Abdurrob[1,3], Chinnakkaruppan Adaikkan[1,3], Rebecca G. Canter[1,3], Richard Rueda[1,3], Emery N. Brown[1,3,5,6], Edward S. Boyden[2,3,4] & Li-Huei Tsai[1,3,7]



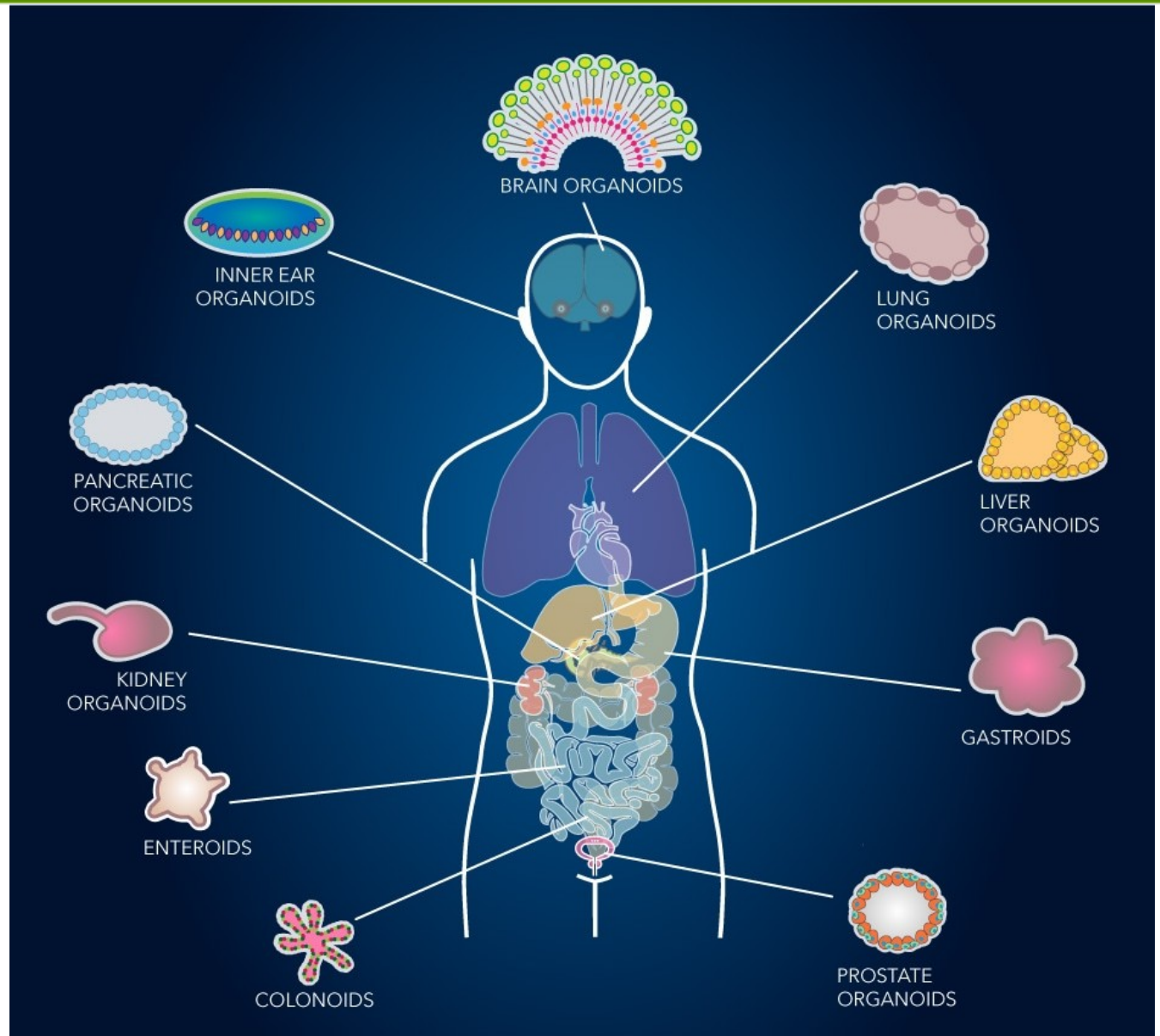


BONUS: organoids for studying the influence of physical phenomena on development and physiology

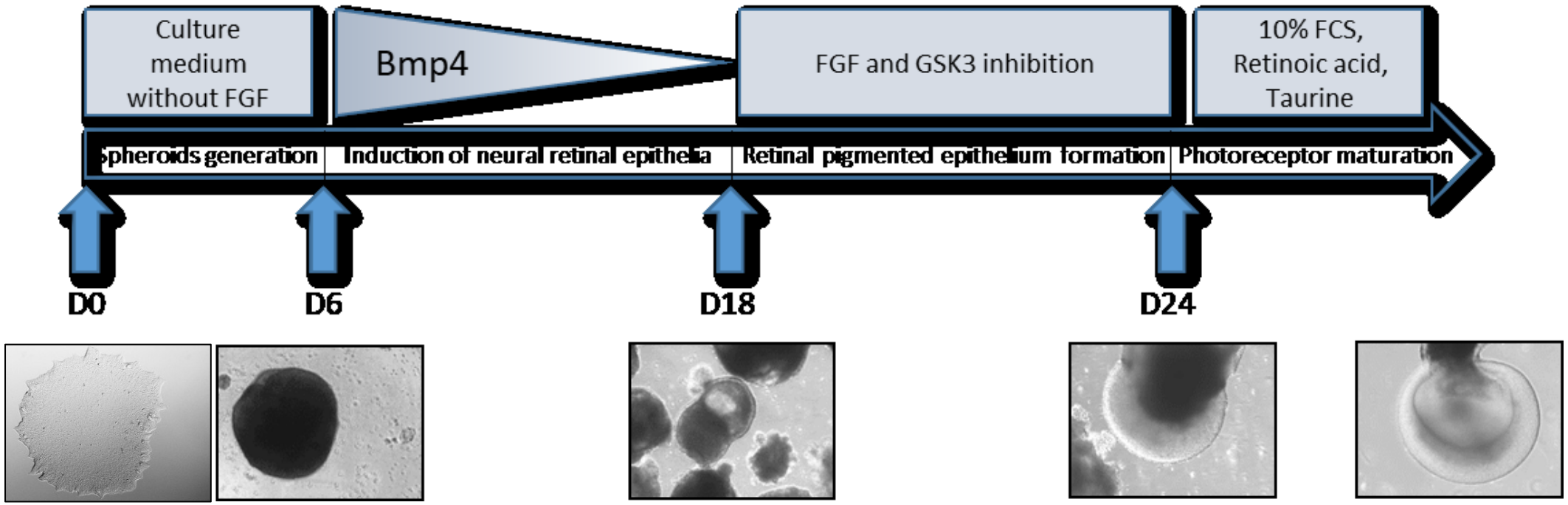
- Organoids are three-dimensional miniatures of organs that have a similar structure and **function** to the organ

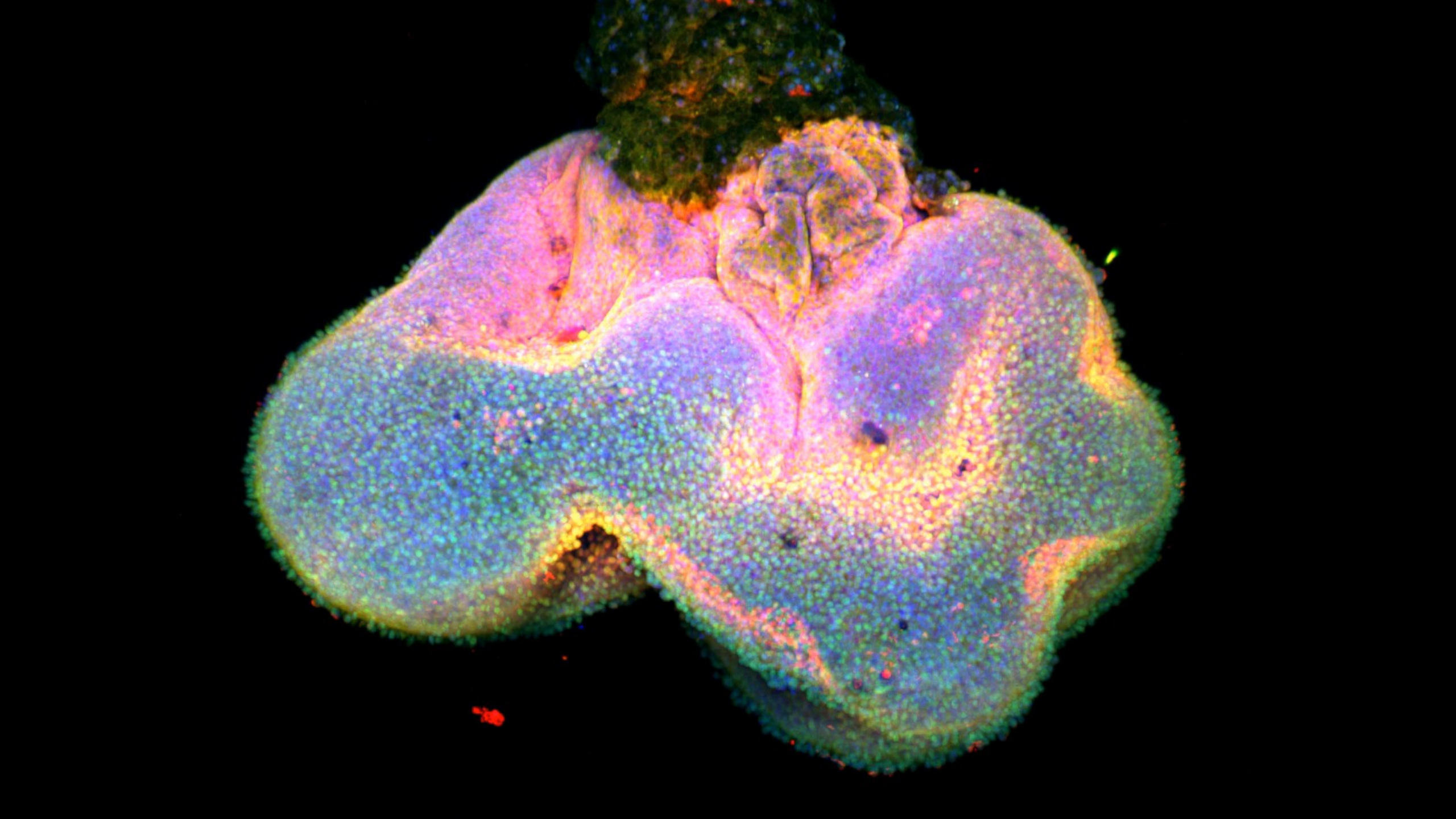


BONUS: organoids for the study of physical phenomena on development and physiology



BONUS:


Culture medium without FGF
Bmp4
FGF and GSK3 inhibition
10% FCS, Retinoic acid, Taurine
spheroids generation
Induction of neural retinal epithelia
Retinal pigmented epithelium formation
Photoreceptor maturation
D0
D6
D18
D24



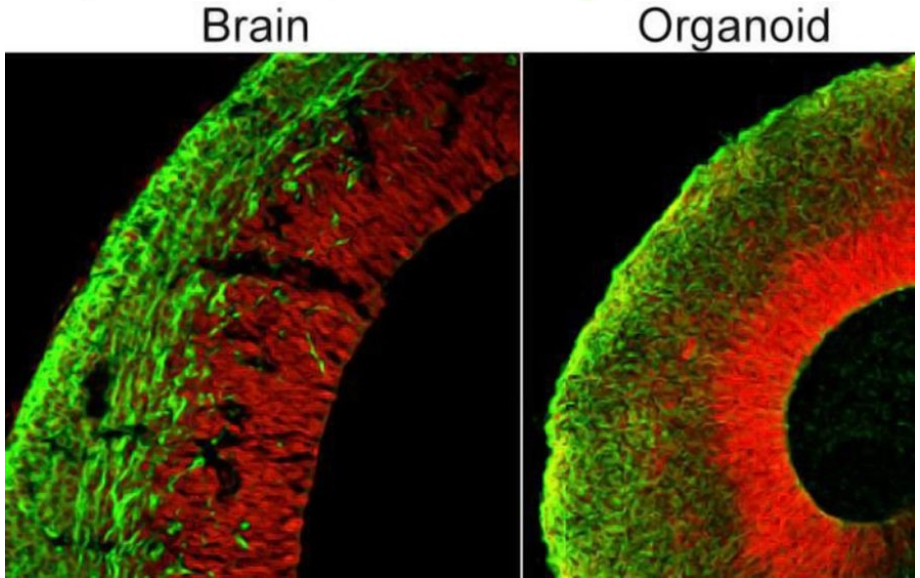
communications biology

ARTICLE

https://doi.org/10.1038/s42003-021-02719-5 OPEN

Induction of inverted morphology in brain organoids by vertical-mixing bioreactors

Dang Ngoc Anh Suong[1,2], Keiko Imamura[1,2,3], Ikuyo Inoue[1,3], Ryotaro Kabai[4], Satoko Sakamoto[4], Tatsuya Okumura[4], Yoshikazu Kato[5], Takayuki Kondo[1,2,3], Yuichiro Yada[1,2], William L. Klein[6], Akira Watanabe[4] & Haruhisa Inoue[1,2,3,7]✉



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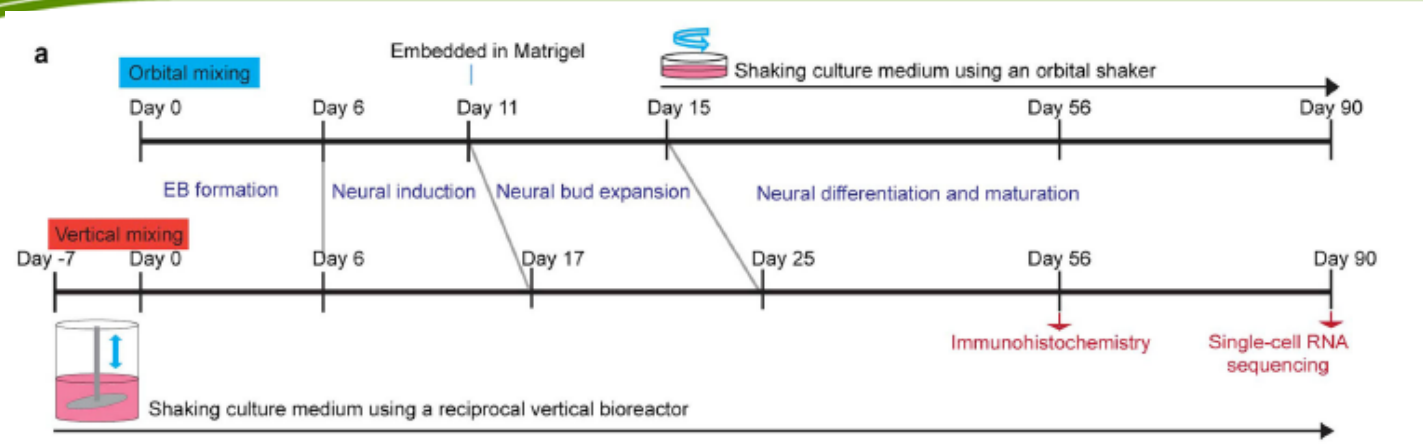
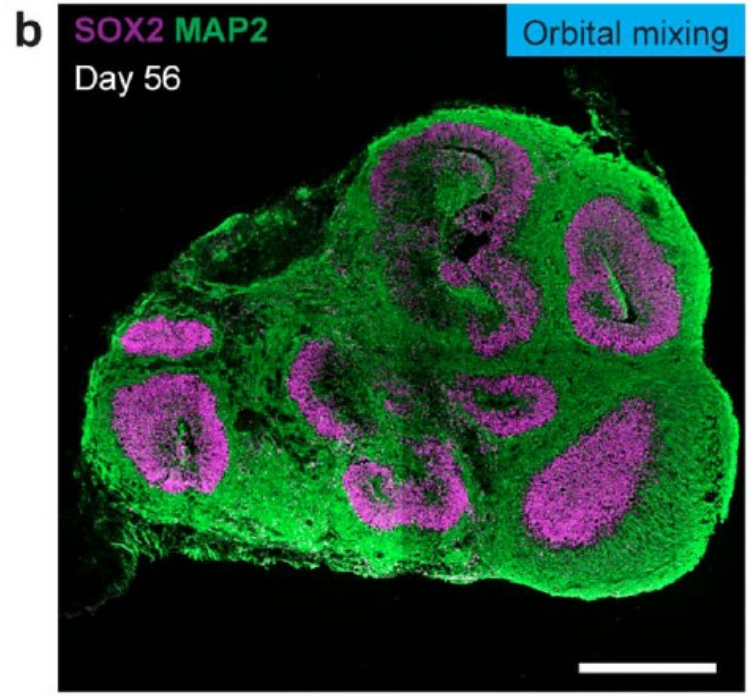
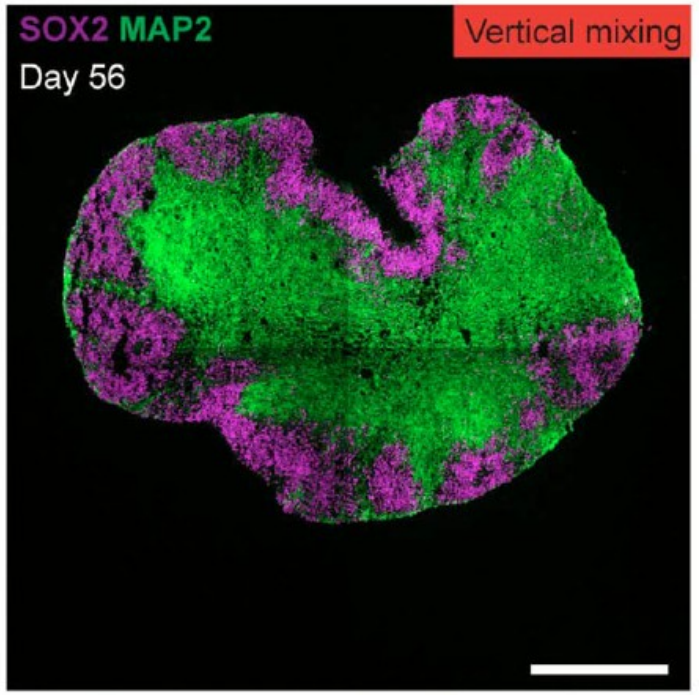
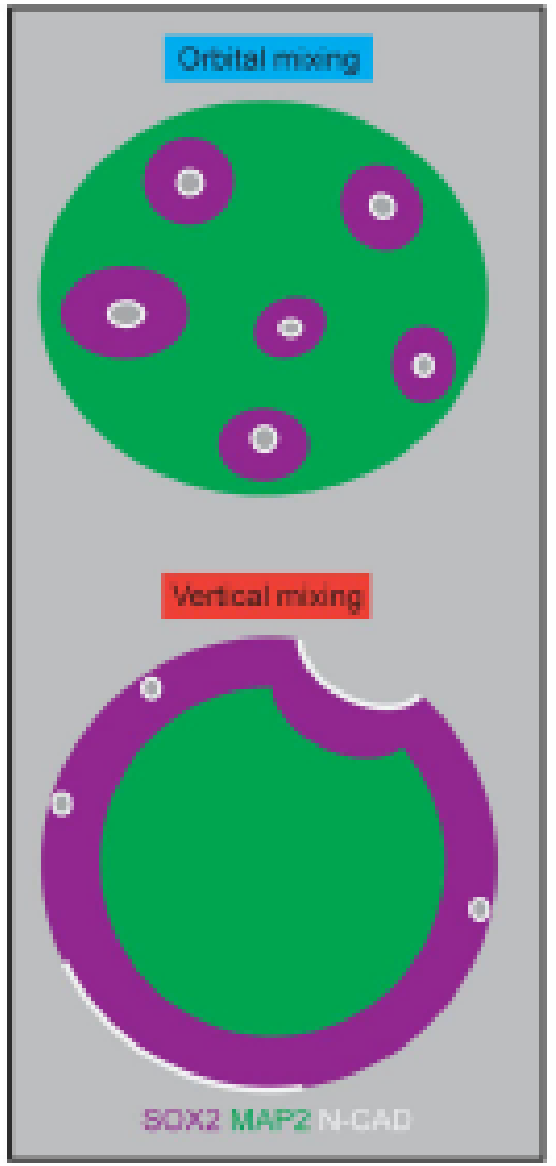
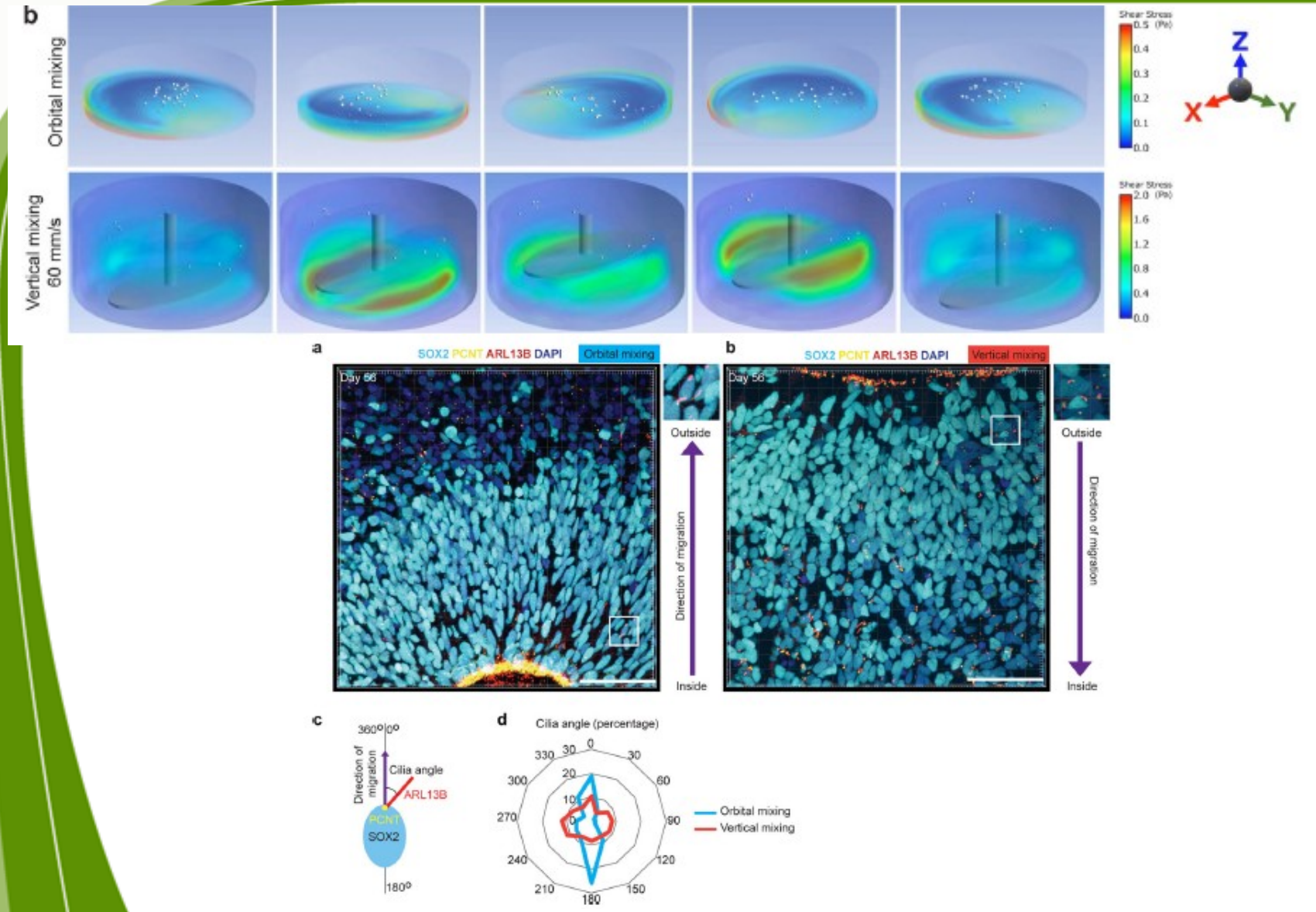

a
Orbital mixing
Embedded in Matrigel
Shaking culture medium using an orbital shaker
Day 0
Day 6
Day 11
Day 15
Day 56
Day 90
EB formation
Neural induction
Neural bud expansion
Neural differentiation and maturation
Vertical mixing
Day -7
Day 0
Day 6
Day 17
Day 25
Day 56
Day 90
Immunohistochemistry
Single-cell RNA sequencing
Shaking culture medium using a reciprocal vertical bioreactor

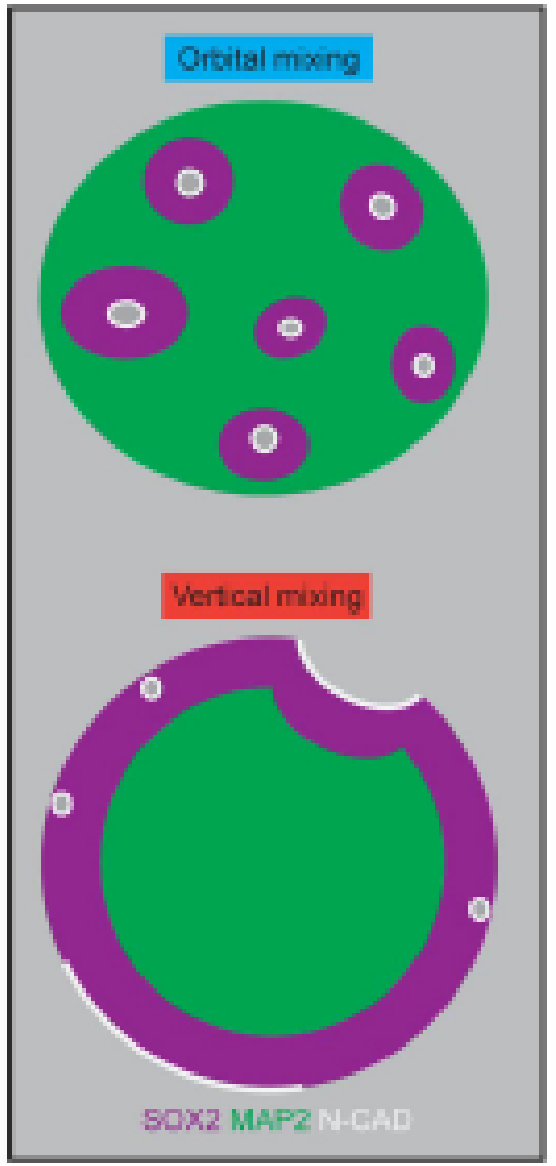

b
SOX2 MAP2
Orbital mixing
Day 56


c
SOX2 MAP2
Vertical mixing
Day 56


Orbital mixing
Vertical mixing
SOX2 MAP2 N-CAD

BONUS:


b
Orbital mixing
Vertical mixing
60 mm/s
Shear Stress
Z
X
Y
a
SOX2 PCNT ARL13B DAPI
Orbital mixing
Day 56
Outside
Inside
Direction of migration
b
SOX2 PCNT ARL13B DAPI
Vertical mixing
c
Direction of migration
Cilia angle
ARL13B
PCNT
SOX2
d
Cilia angle (percentage)
Orbital mixing
Vertical mixing


Orbital mixing
Vertical mixing
SOX2 MAP2 N-CAD

Conclusion and questions

- How does the cell perceive mechanical forces?
- The role of Cadherins and Integrins in mechno reception? And how does it work?
- Hippo signal pathway and perception of mechanical force.
- How do shear forces affect the development of the heart?
- The importance of mechanoreception during gastrulation

Thank you for your attention

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