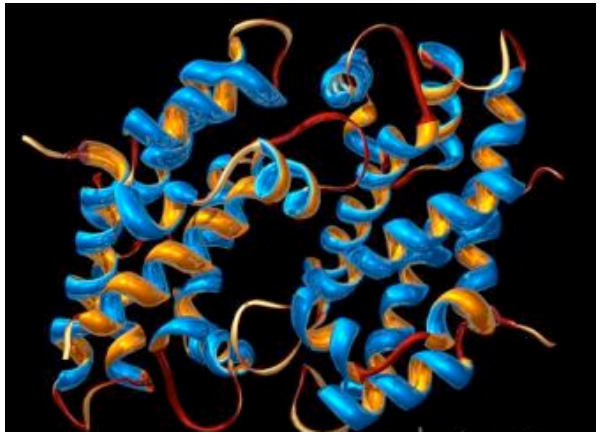
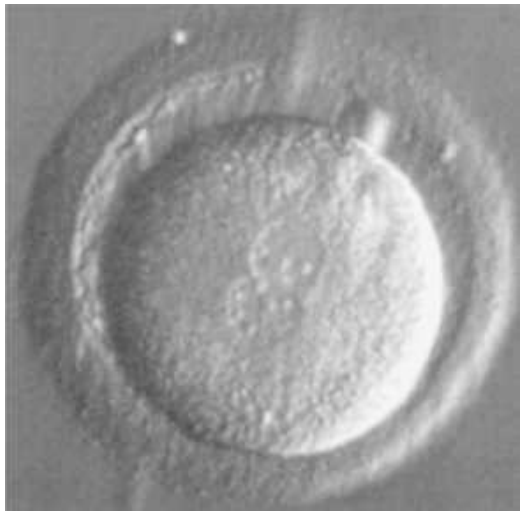
Self-organization and emergence as a central principle of human development.

How do (relatively) simple things like these:



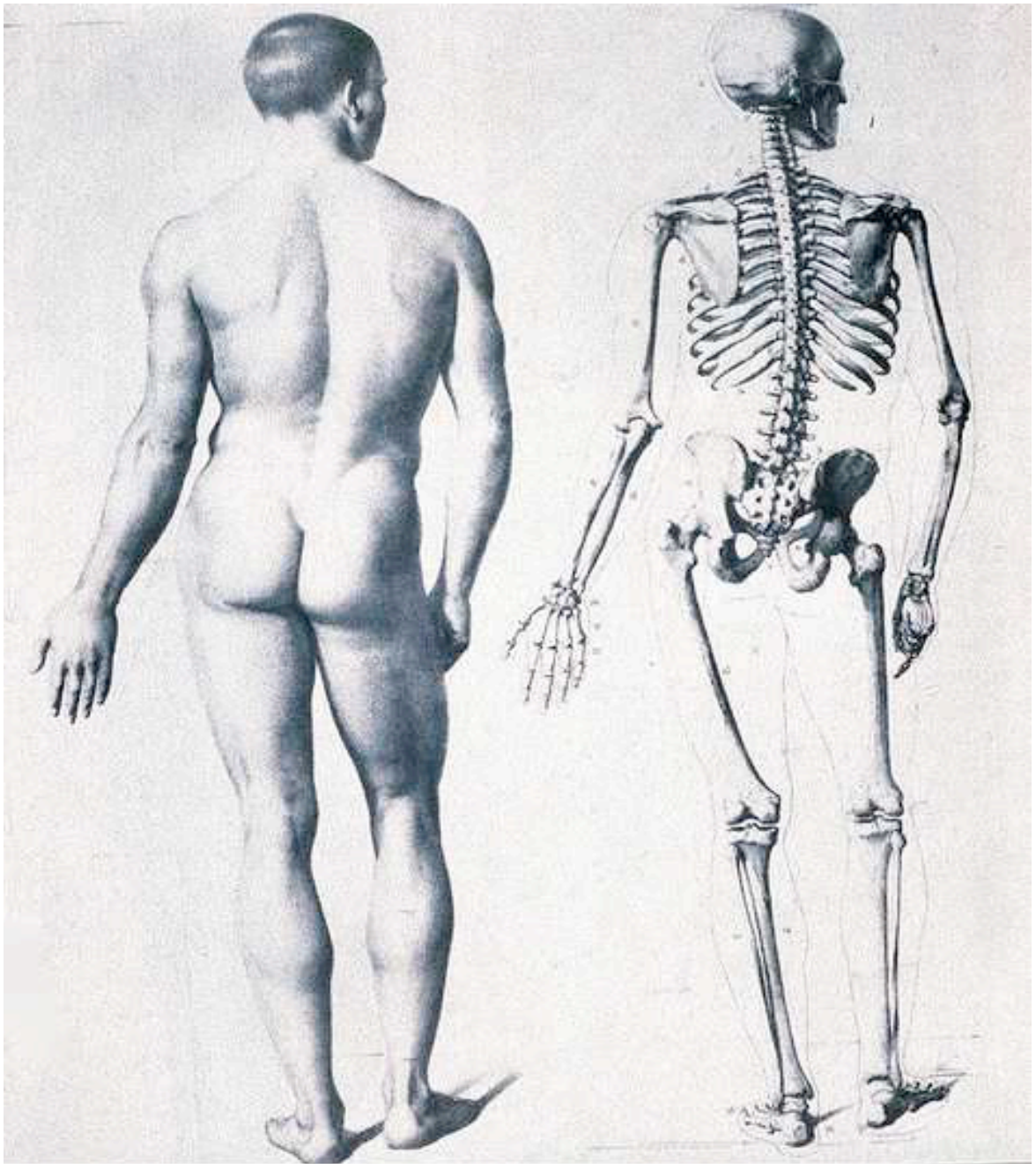
Produce (relatively) sophisticated beings like these:

?

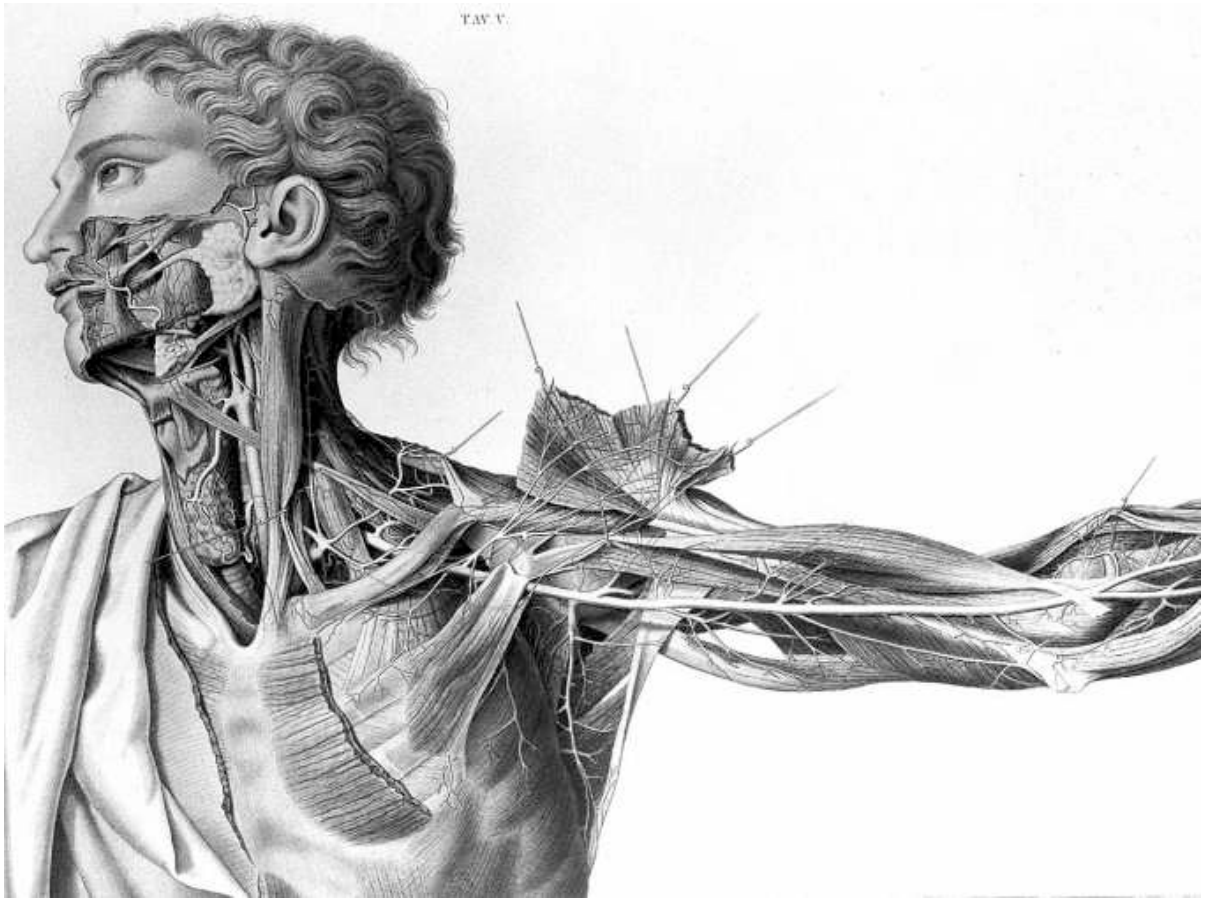


Not only are there many cells, but they are arranged in a complicated way...

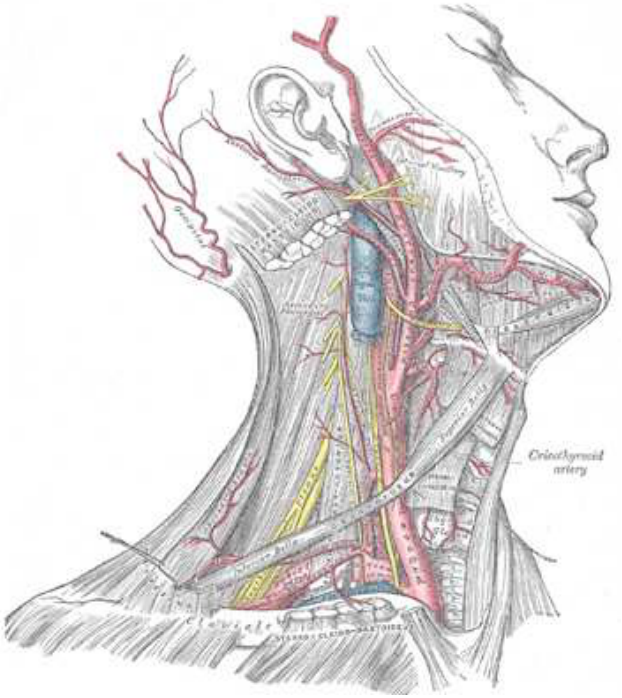
… at whole-body scales...



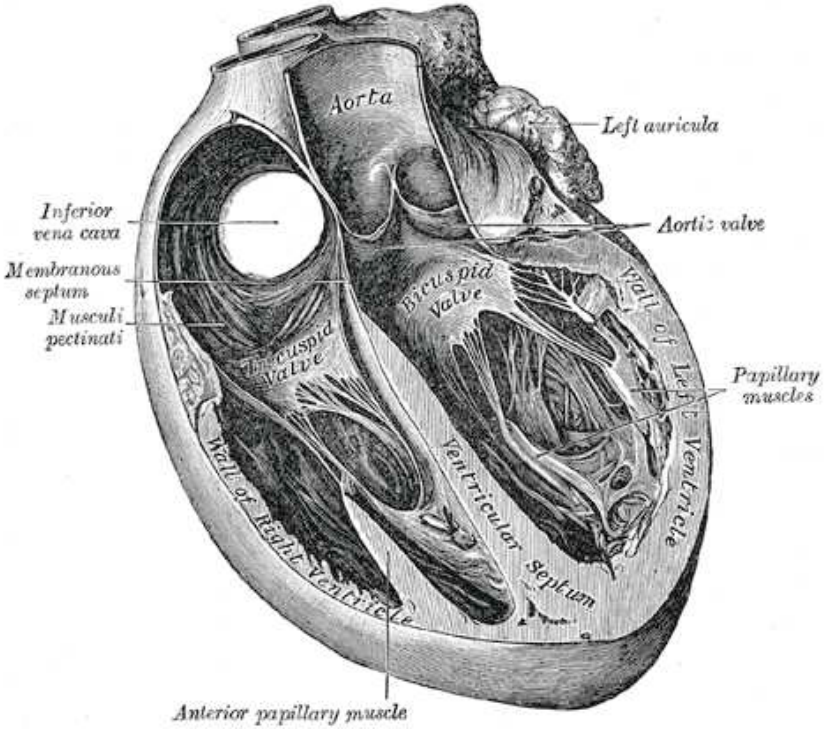
Drawing: Julian Fau. CC Wikimedia commons

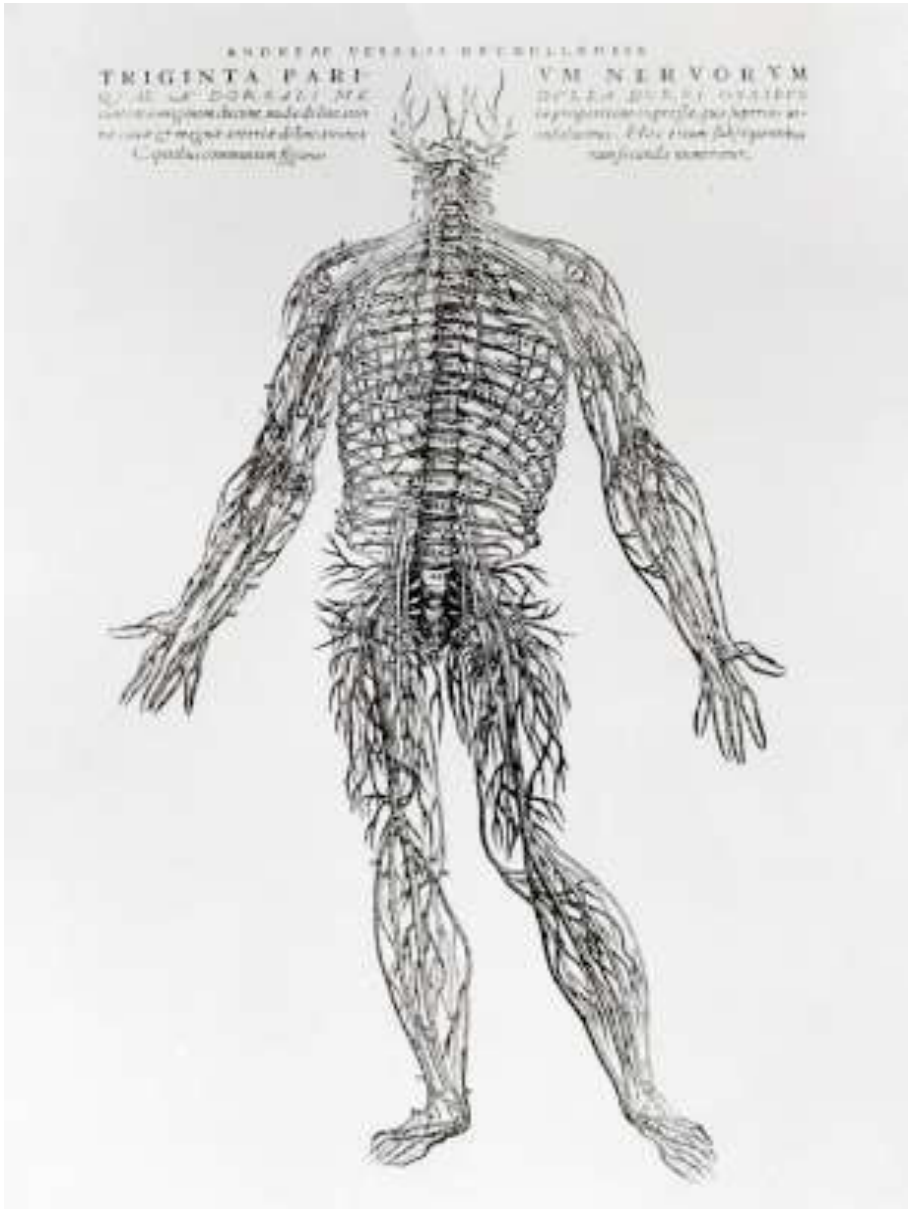


… at the level of major body parts

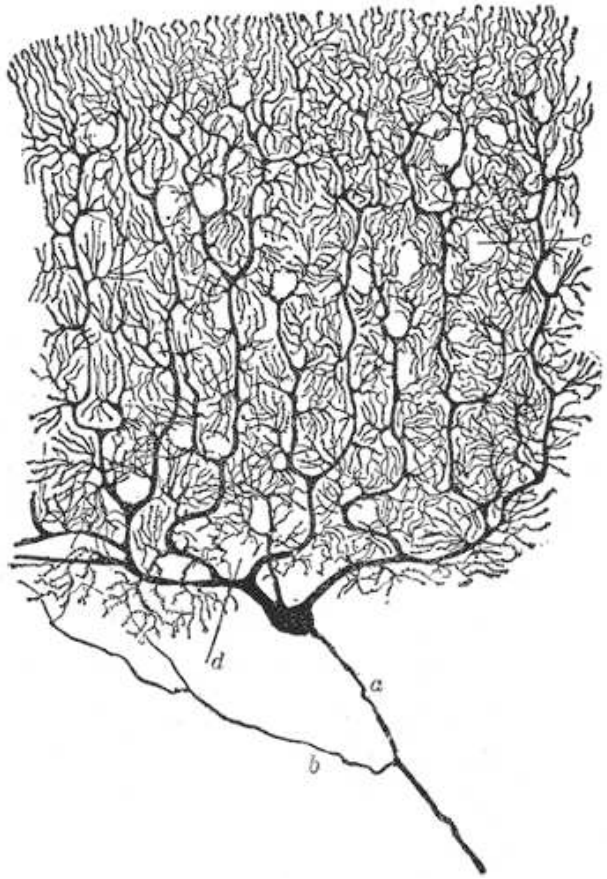


… at organ and organ system level



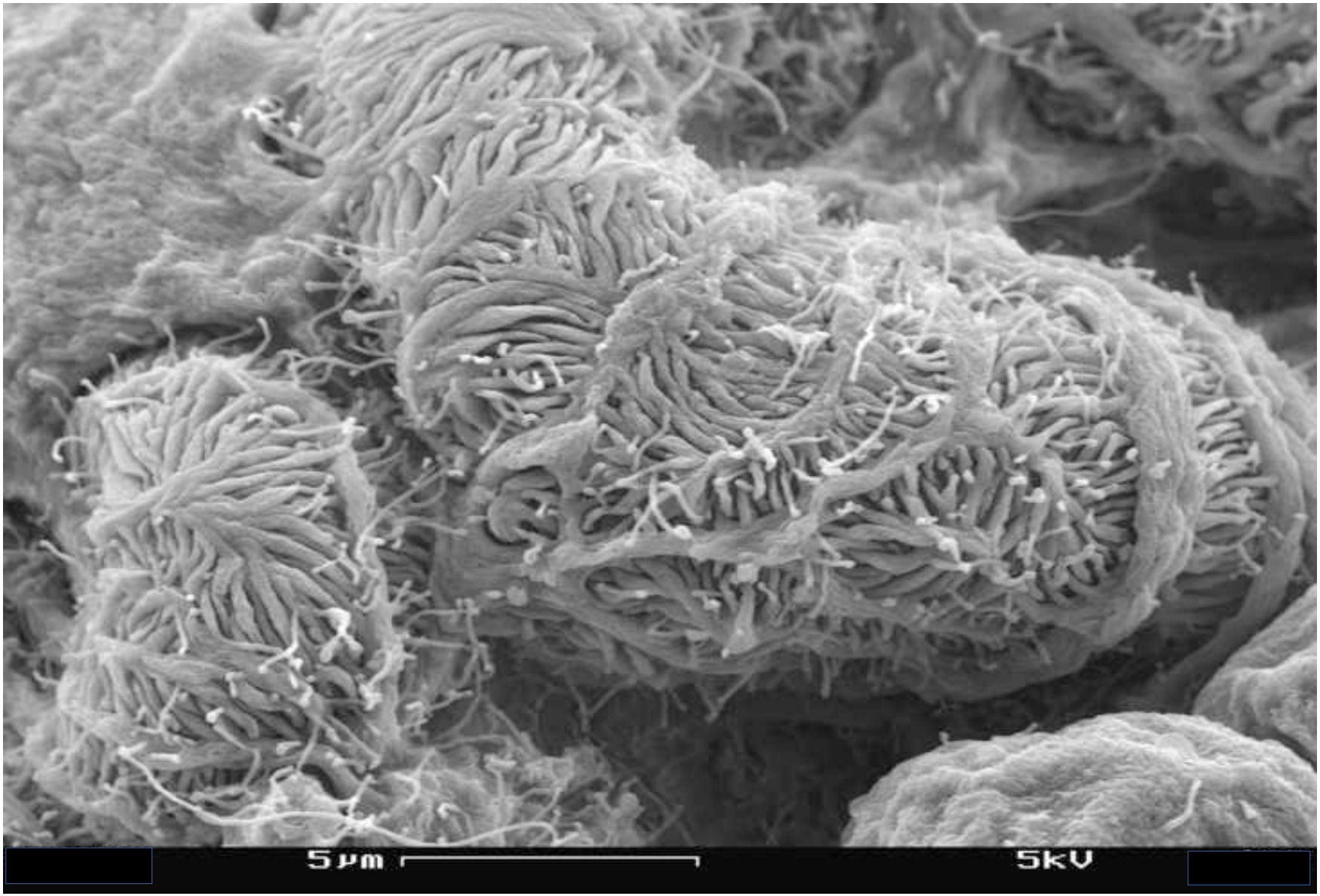


(Spinal nerves)



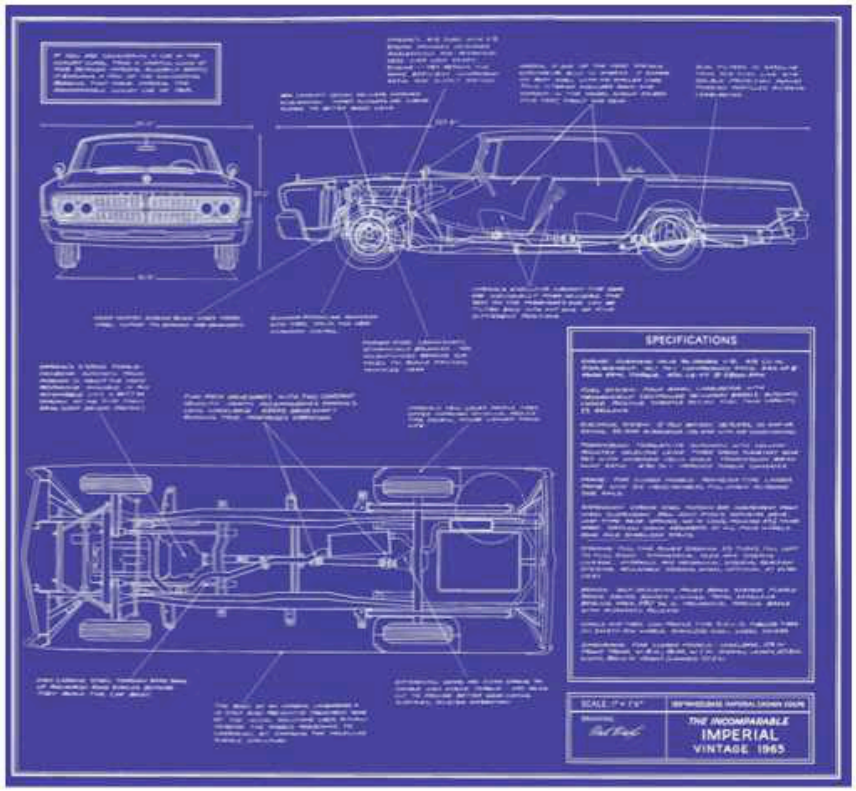
This is just one brain cell, making connections with thousands of others. We have over a billion of them.

The detail keeps up even to microscopic scales.
(This is the filter – 'glomerulus' - of a kidney)

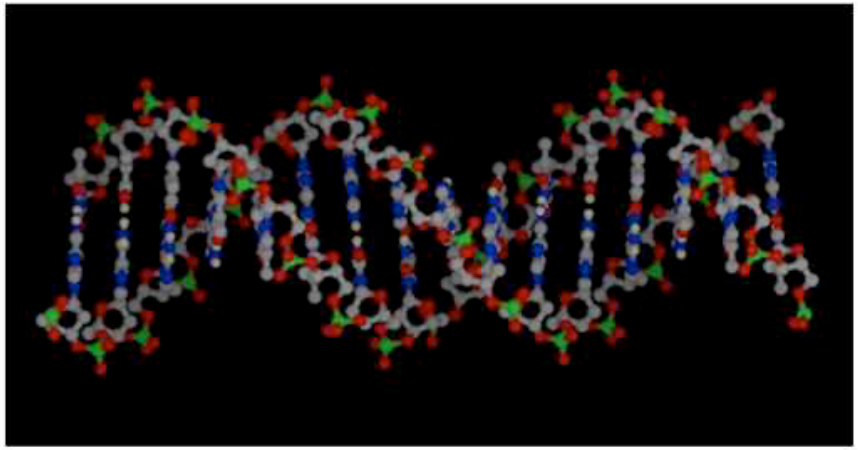


Pic credit “Secret Disc” CC Wikimedia Commons

The metaphor of "genetic blueprint" is not helpful (for at least two reasons)



These are not the same types of transformation!

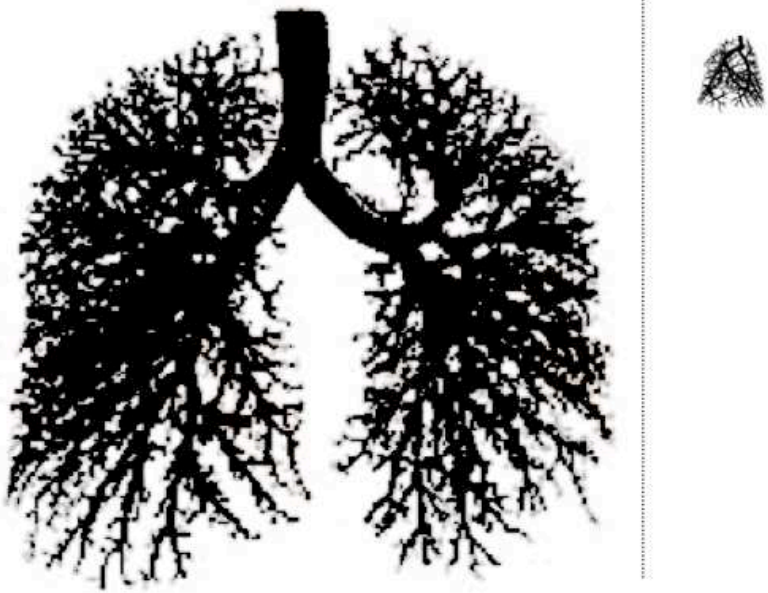


(a) (b)

Figure 2

Fingerprints from a pair of twins. Right thumbs of (a) TX004 and (b) TX005.

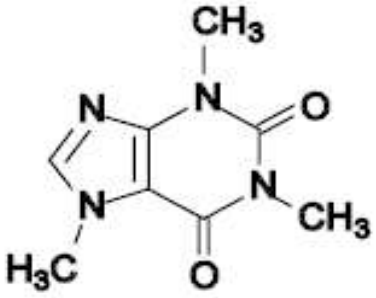
Lung



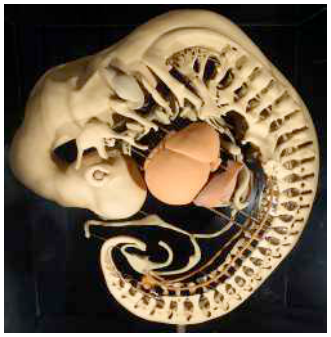
Human Rat

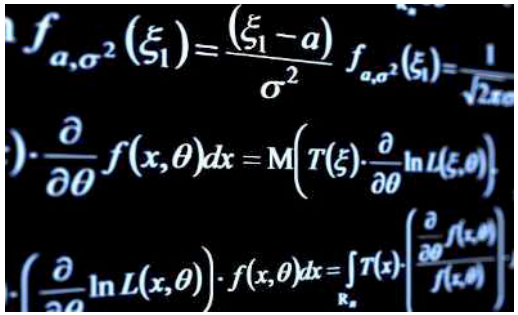
Paediatricians

Biologists



Chemists



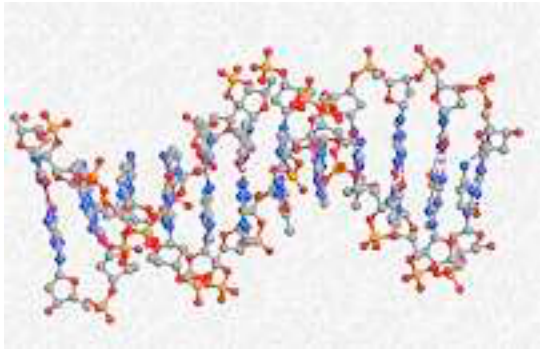


Mathematicians

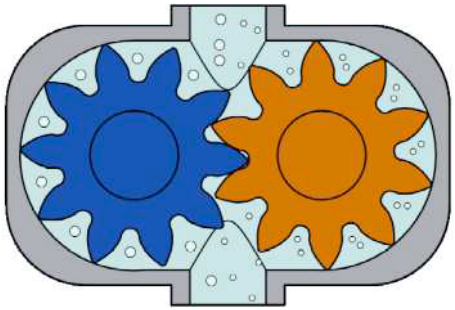
Computer scientists

Robotics engineers

Images: csgroup8, Vergilius Eremite, Rocketmagnet, Van Klamm David Ludwig,, all CC Wikimedia Commons



Genes build (molecular) machines



The machines build the child

Image: , Anjelandro Porto, Ajsmen91 CC Wikimedia commons

Self-organization

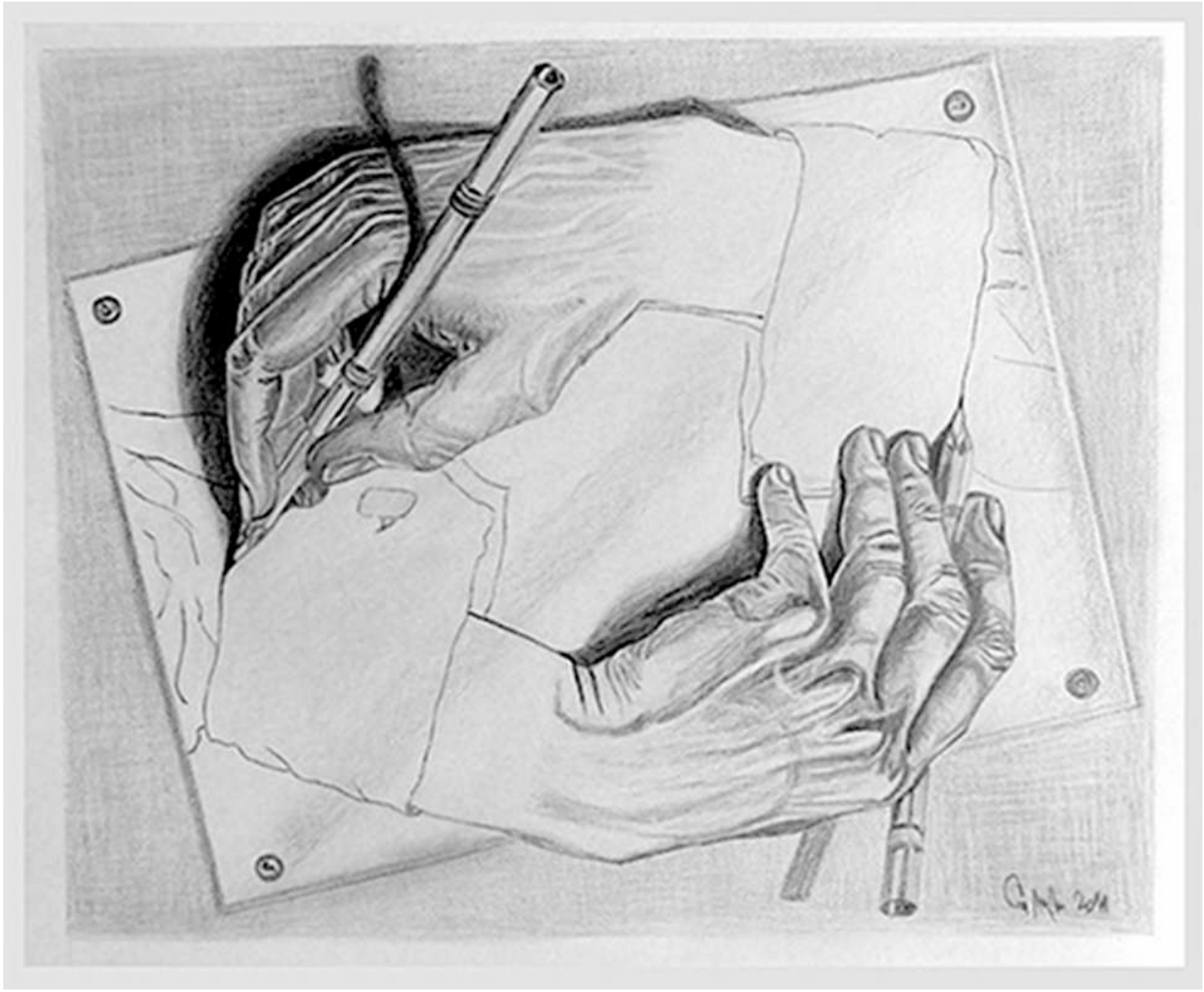


Image credit: Gabriella Meyermann, after M.C.Escher. CC.

So, how can things as simple as molecules do so much?

John Conway's Game of Life

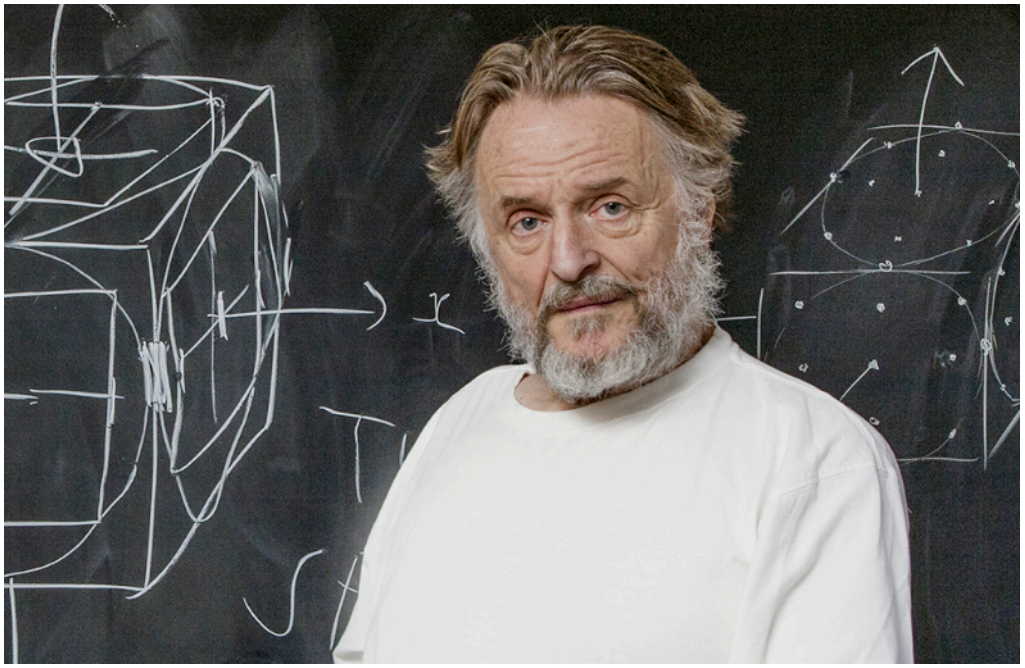
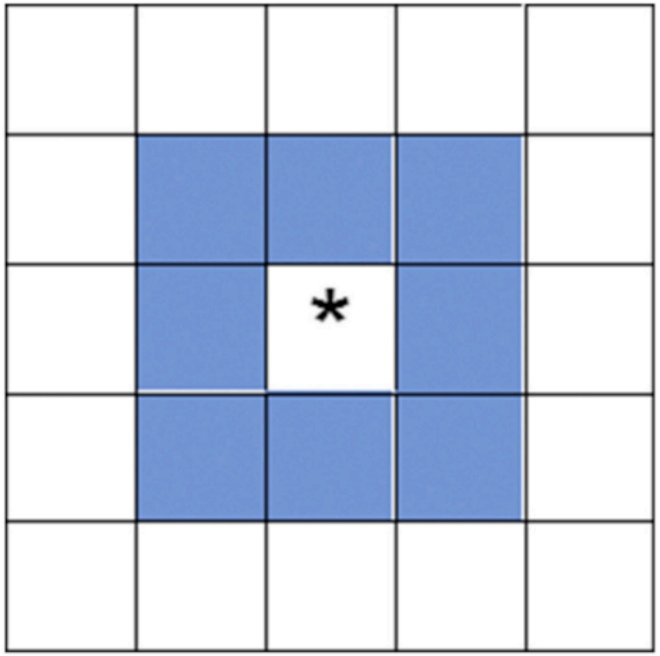
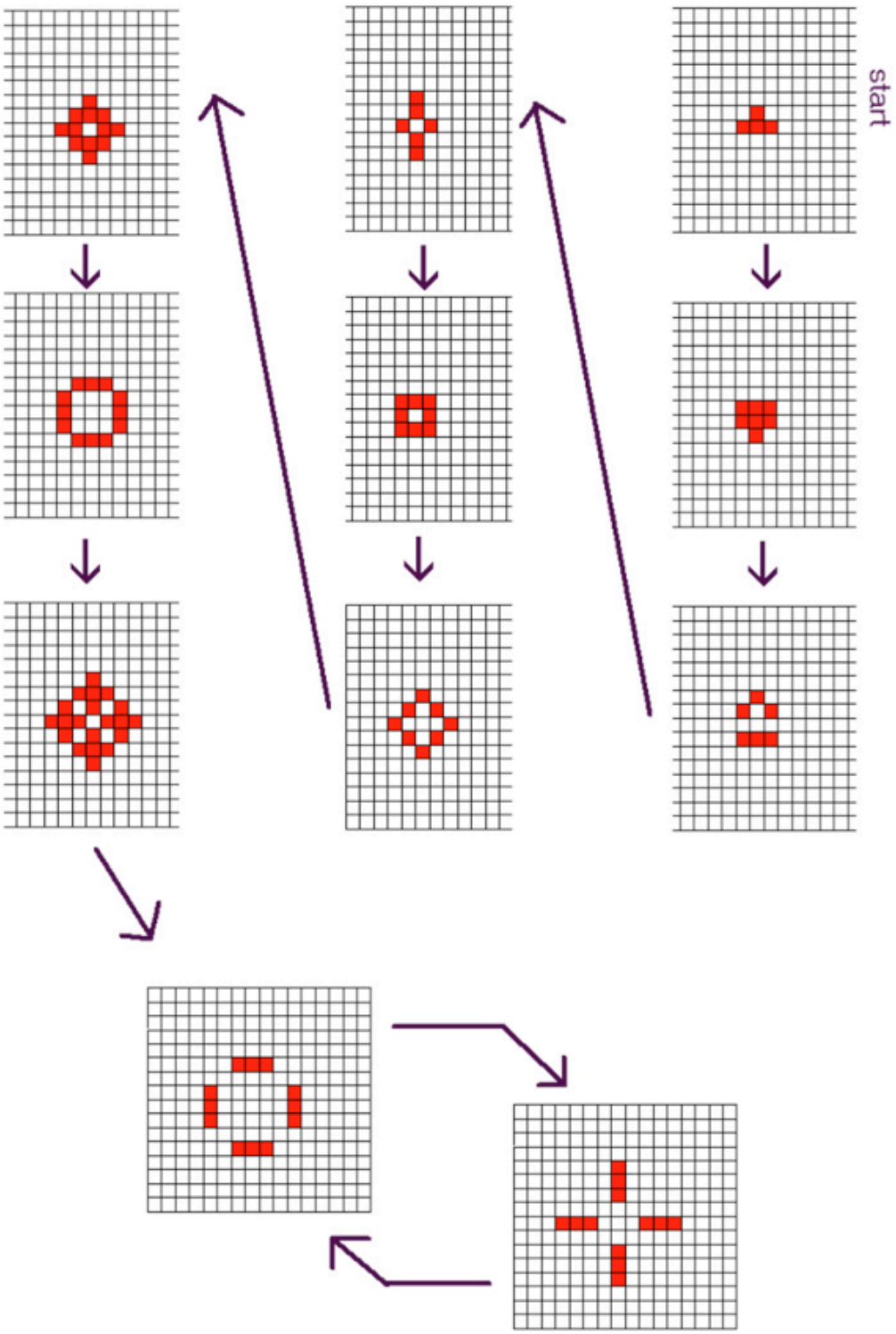


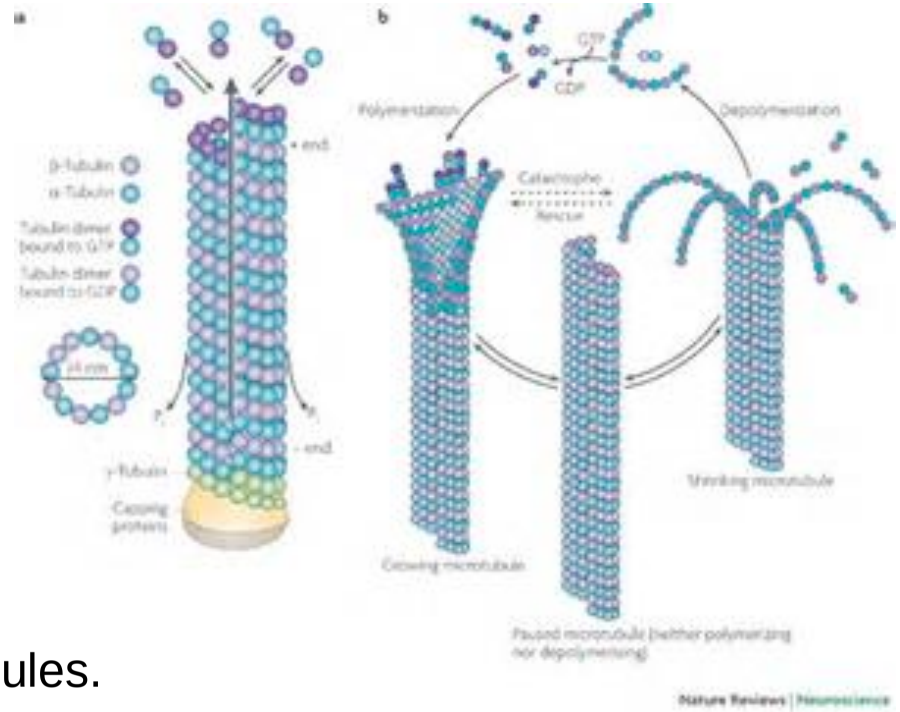
Photo credit: Princeton University, Office of Communications, Denise Applewhite



1. A cell that has fewer than two neighbours dies from lack of trophic support.
2. A cell that has four or more neighbours dies from overcrowding (for example, through build up of toxins).
3. If exactly three cells are neighbours of an empty location, one of them divides so that one daughter stays where the mother was and the other occupies the previously-empty location (since mothers and daughters are instantly equivalent, it makes no difference which of the cells is considered to have divided).


start

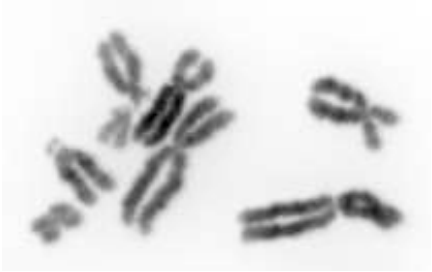
These are our elementary building-blocks, that obey kind-of elementary rules.



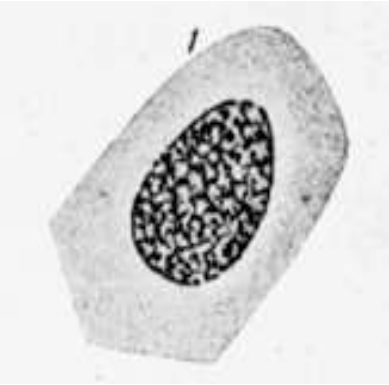
Microtubules.

But it clearly cannot just be crystallization, because microtubules have to end up in the right place to do something useful.

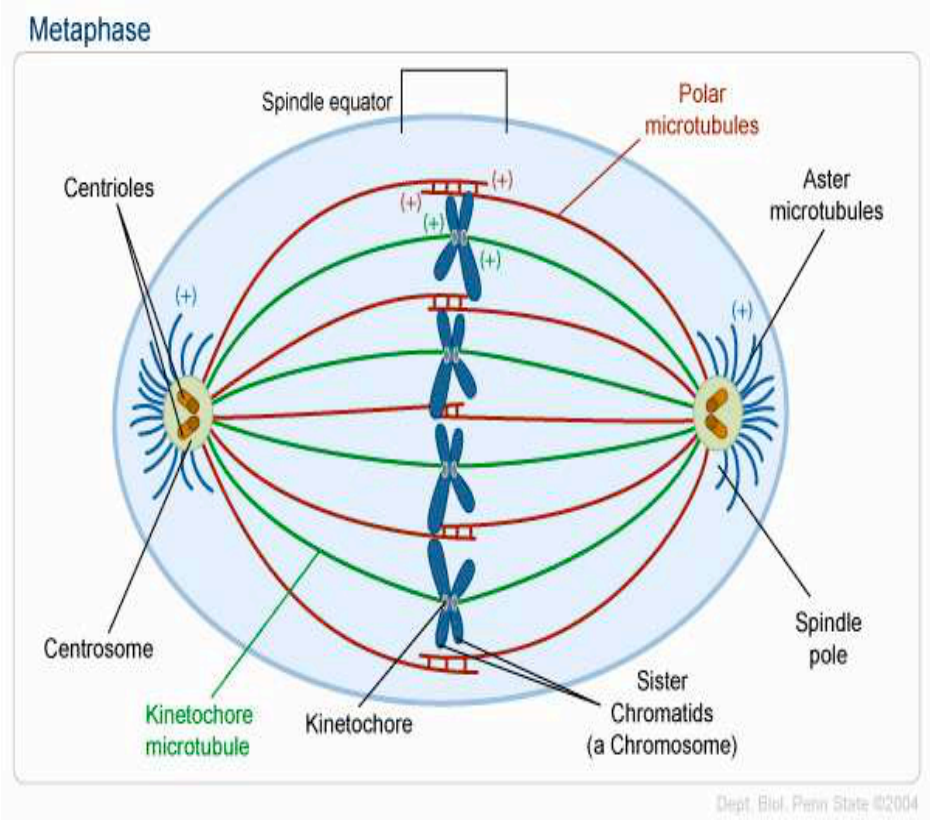
To divide, cells have to:

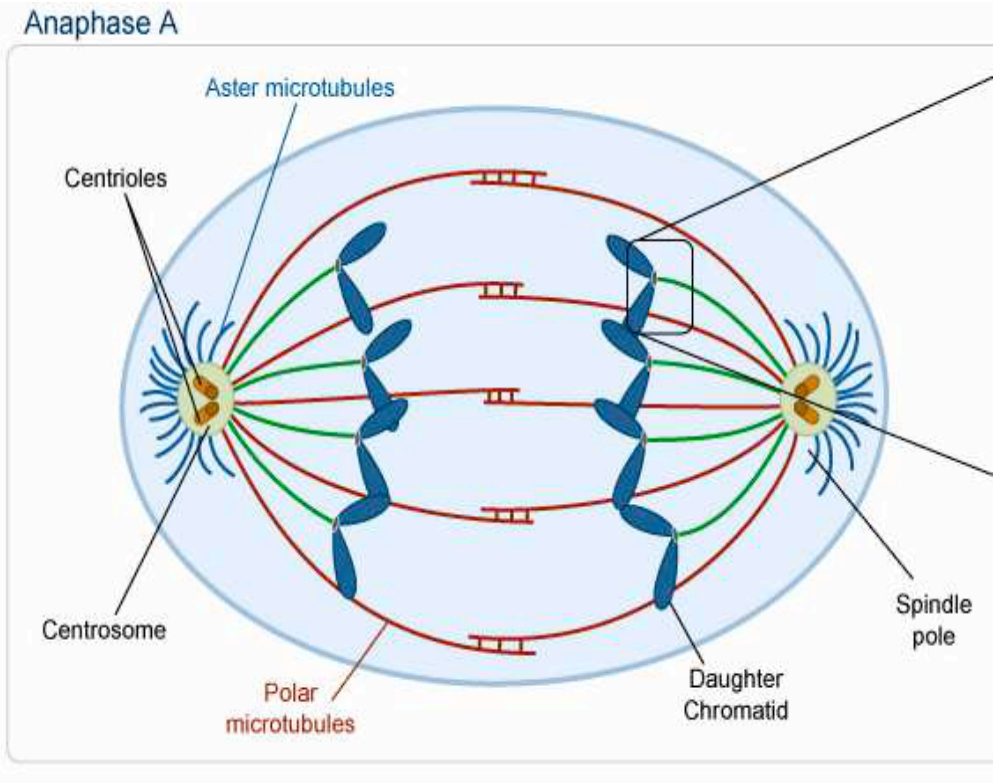


- Copy their chromosomes
- Move one copy of each chromosome to each of the new cells.
- Separate the new cells



Pulling copied chromosomes to daughter cells – the spindle apparatus





Paired copies line up in the middle of the cell

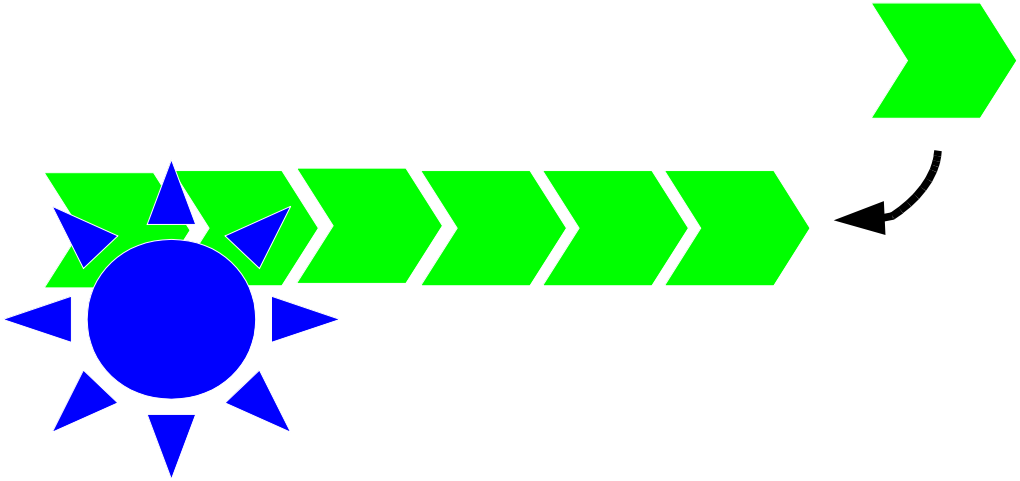
And, once all are lined up, they are allowed to separate and travel along the spindle.

Two problems:

How does the spindle find them?

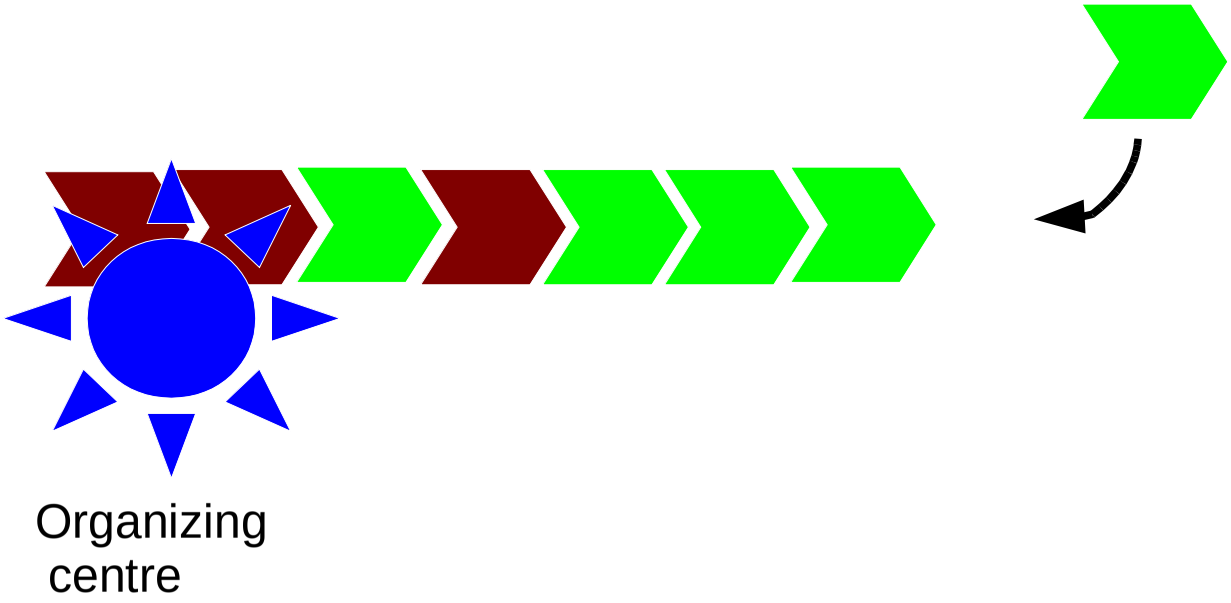
How does the cell know when all are ready for the separation phase to happen?

Microtubules



Organizing centre

Microtubules



Microtubules

Organizing centre

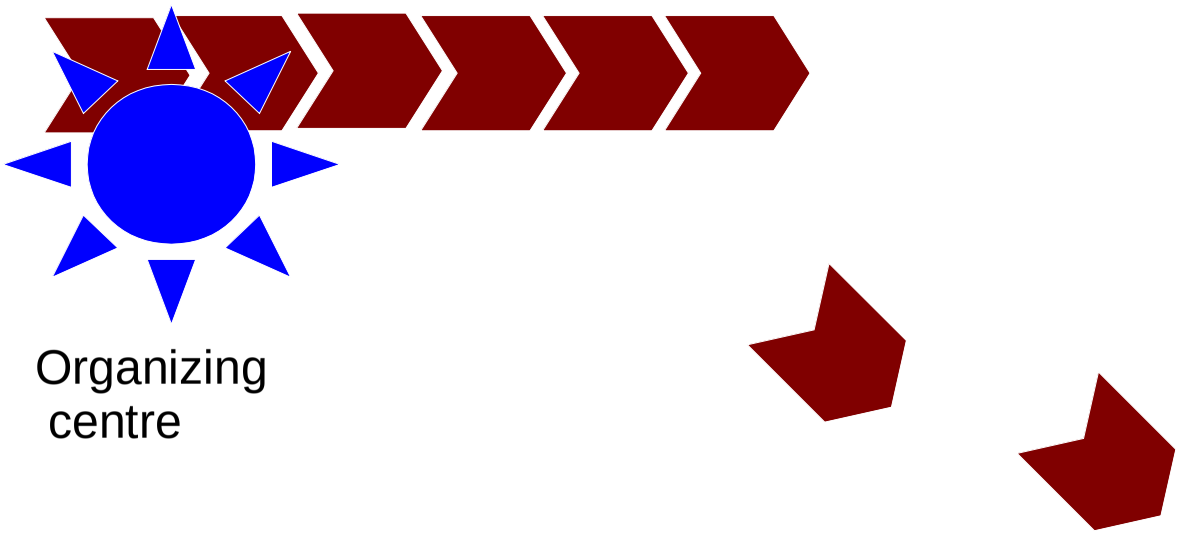
Microtubules

Organizing centre

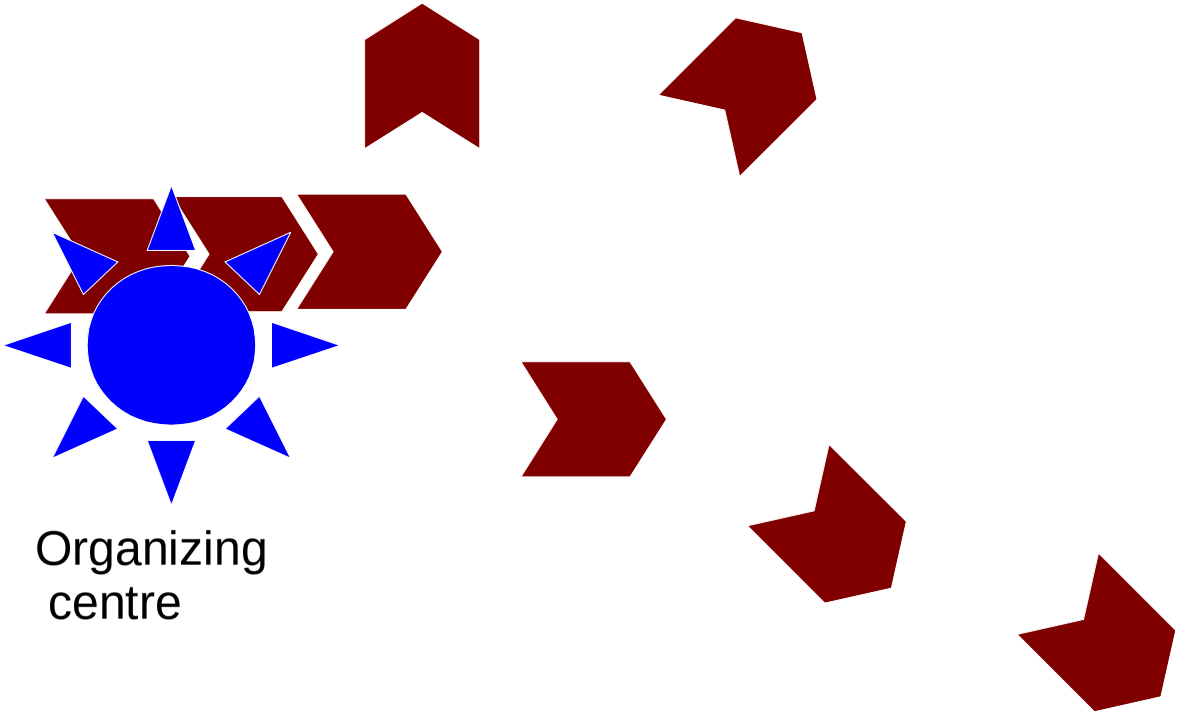
Microtubules

Organizing centre

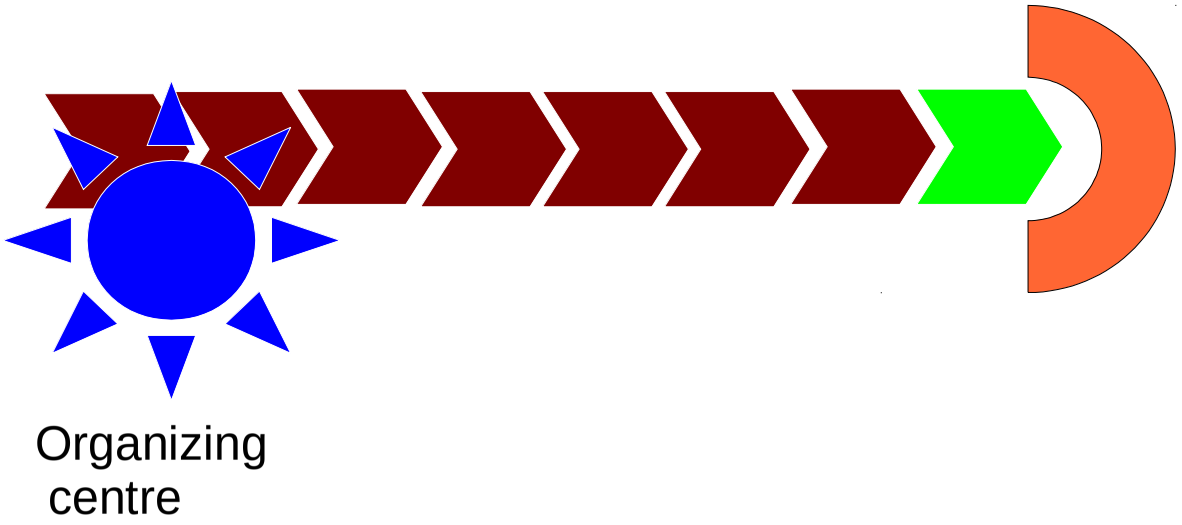
Microtubules



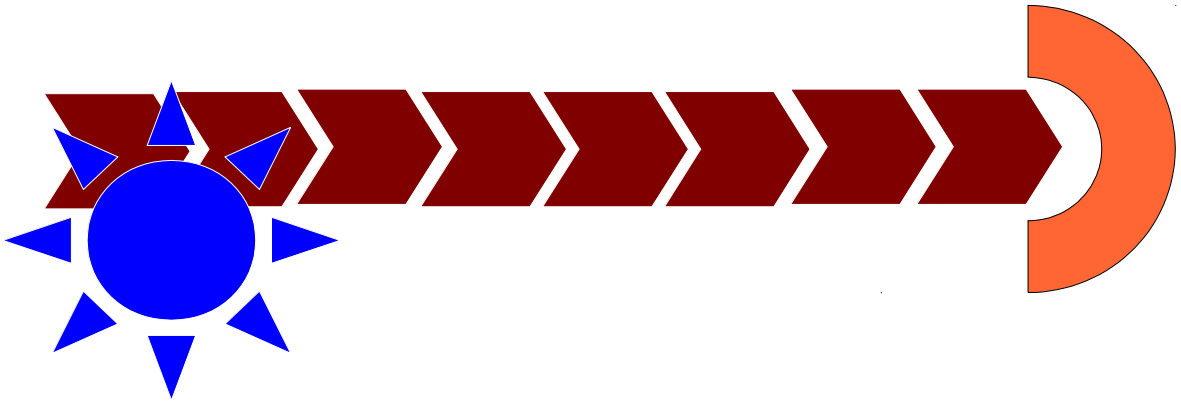
Microtubules



Microtubules can be stabilized by special proteins



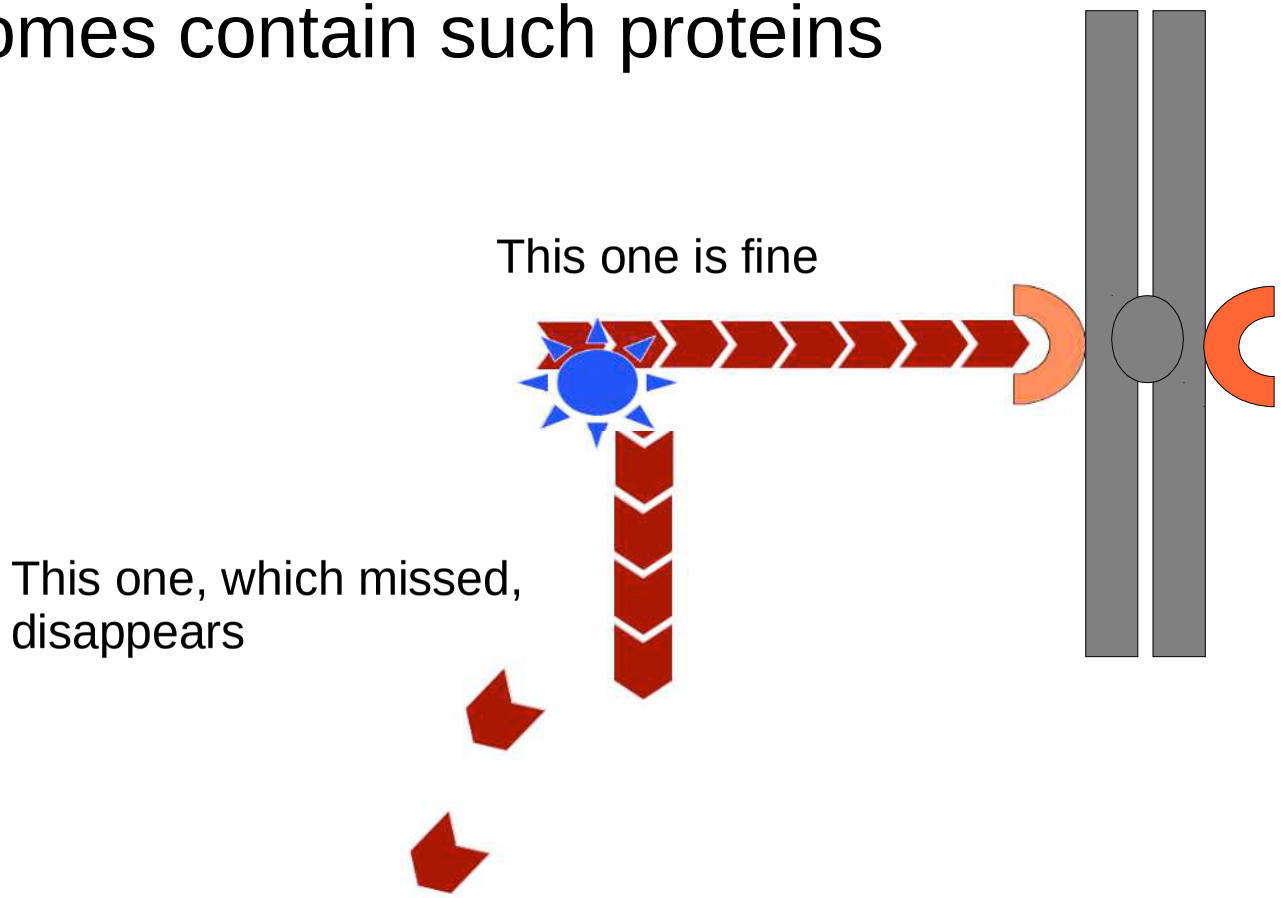
Microtubules can be stabilized by capping proteins



Organizing centre

This is still OK

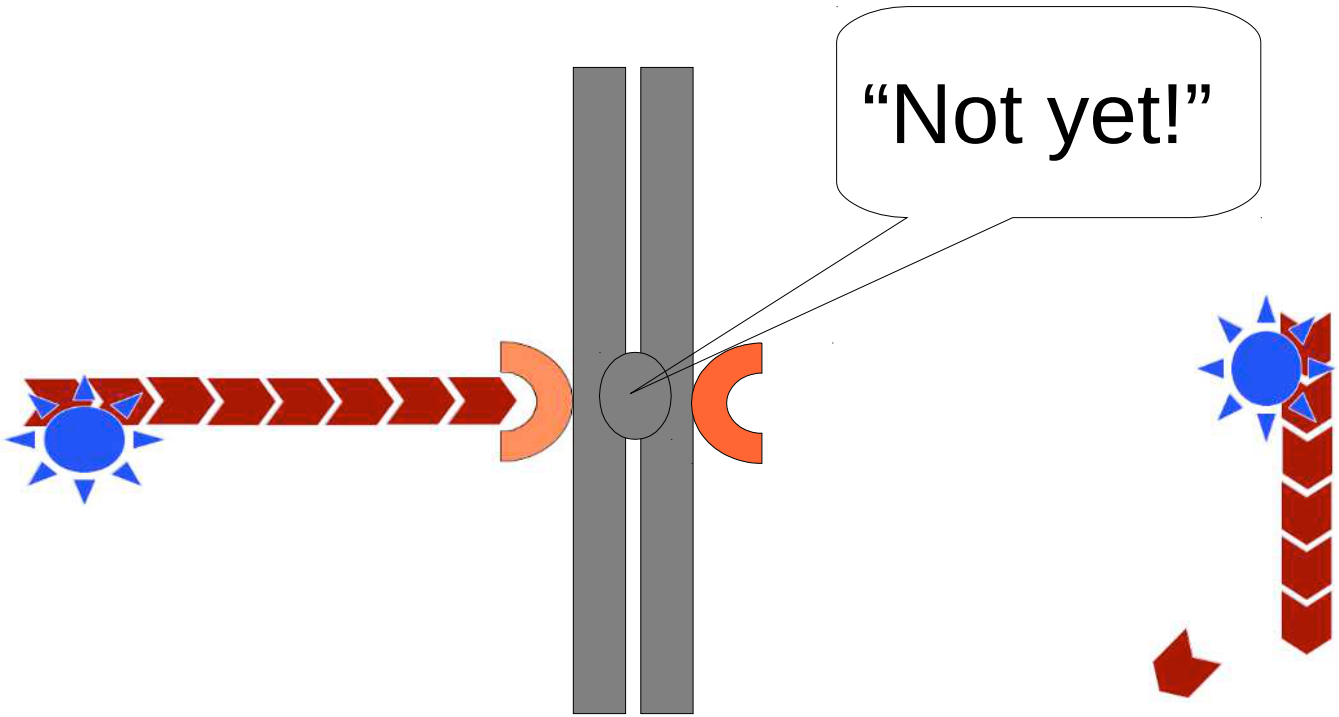
Chromosomes contain such proteins

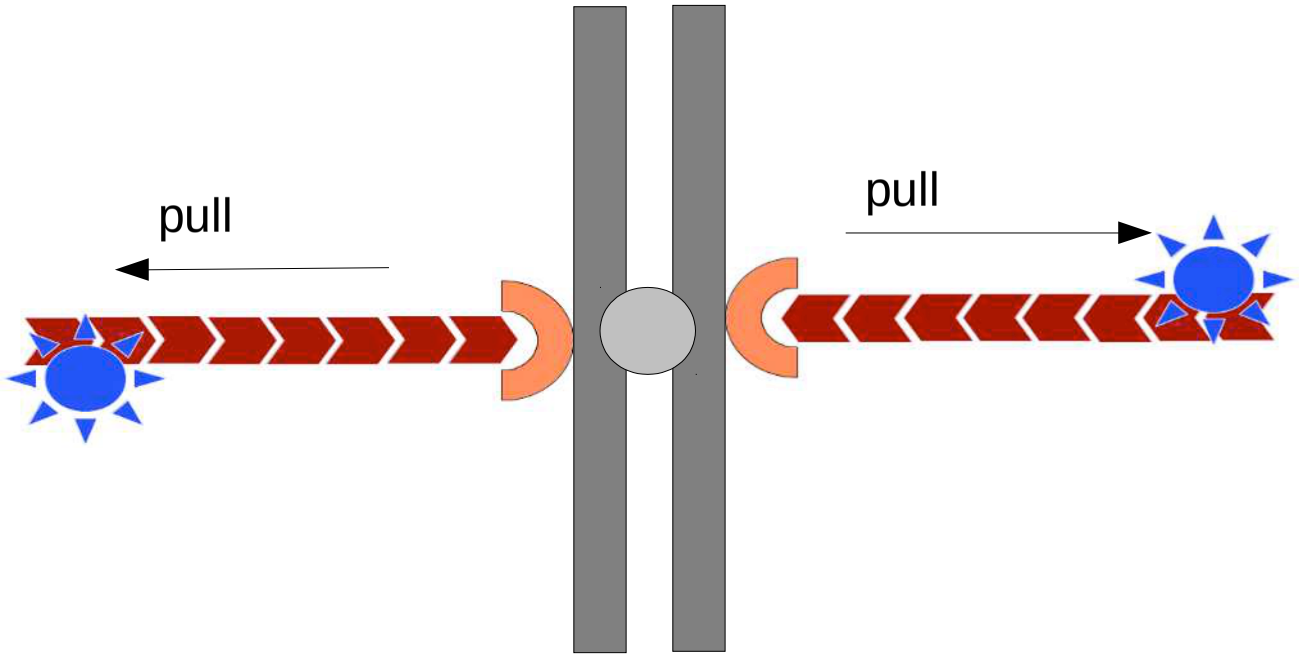


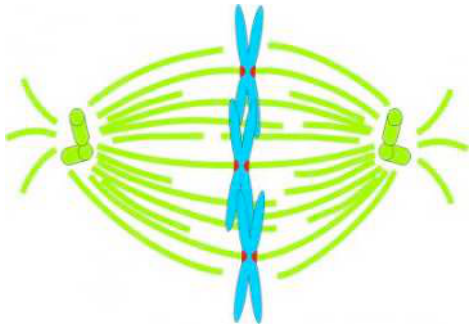
So, if the centres send out microtubules randomly, only those that find chromosomes survive. The system therefore manages to connect microtubules to chromosomes without knowing where they were.

Jacapo Werther CC

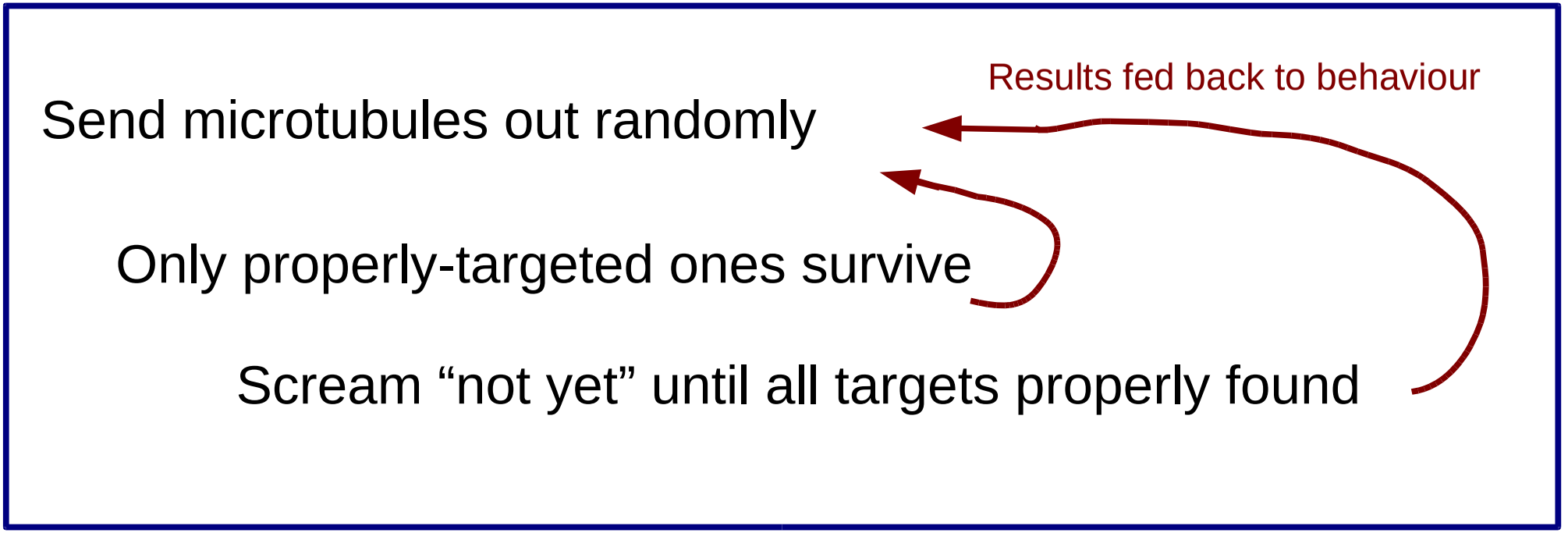
Problem 2: knowing when to stop waiting


“Not yet!”


pull
pull



So, “dumb” protein machines did something 'clever' at scales far larger then themselves through this process:



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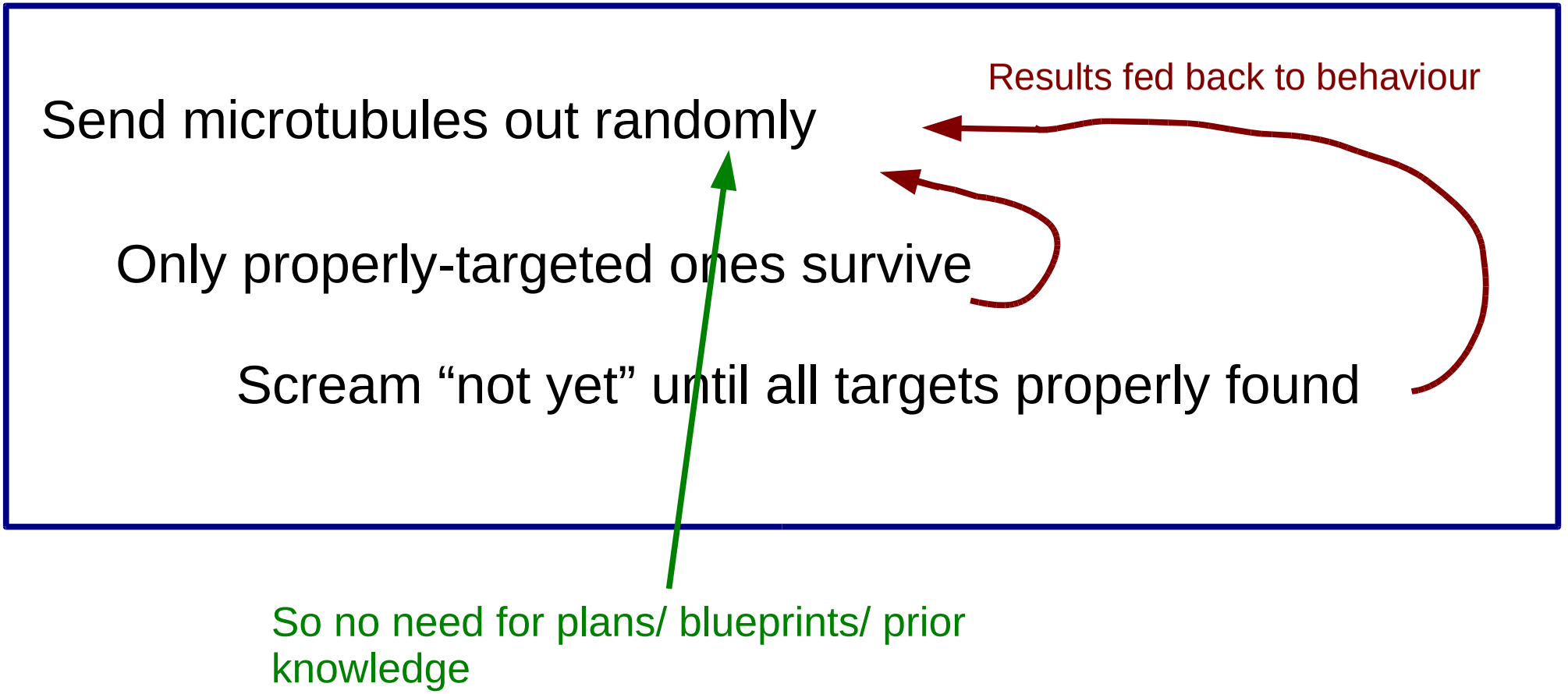

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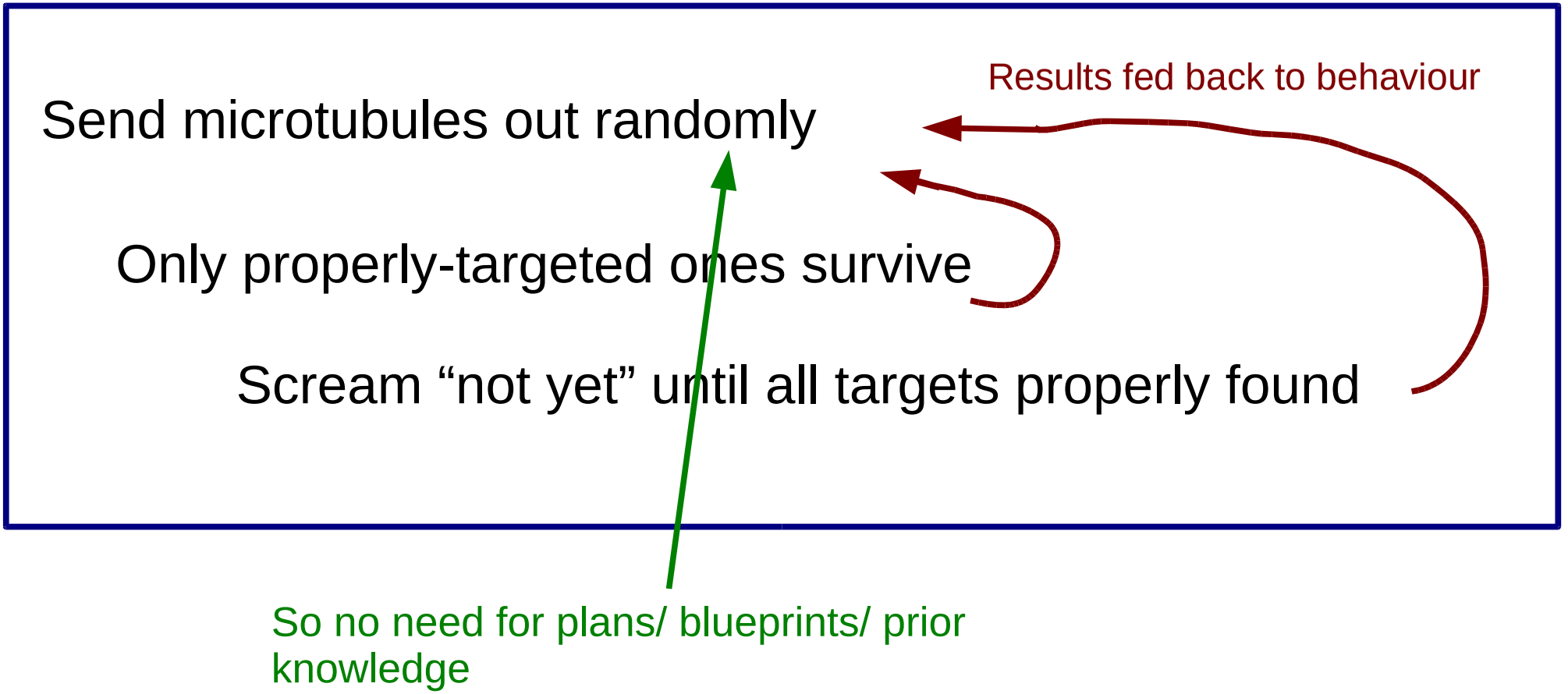
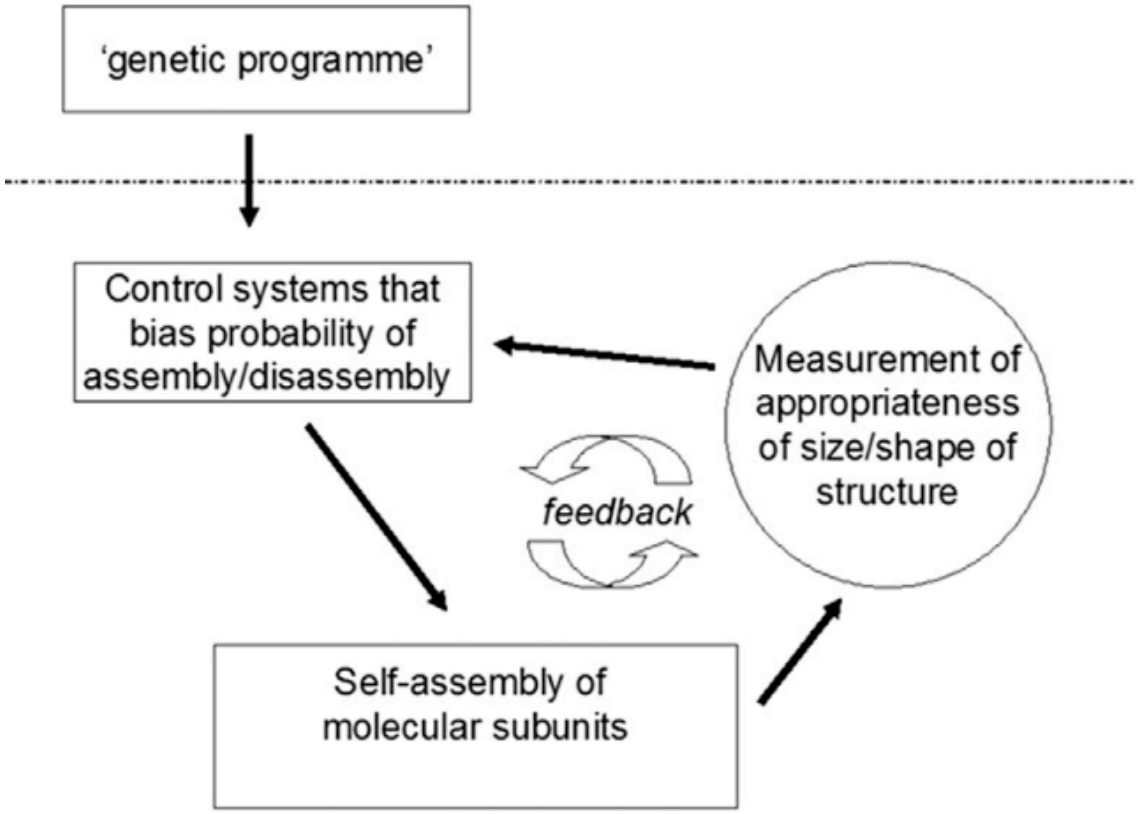
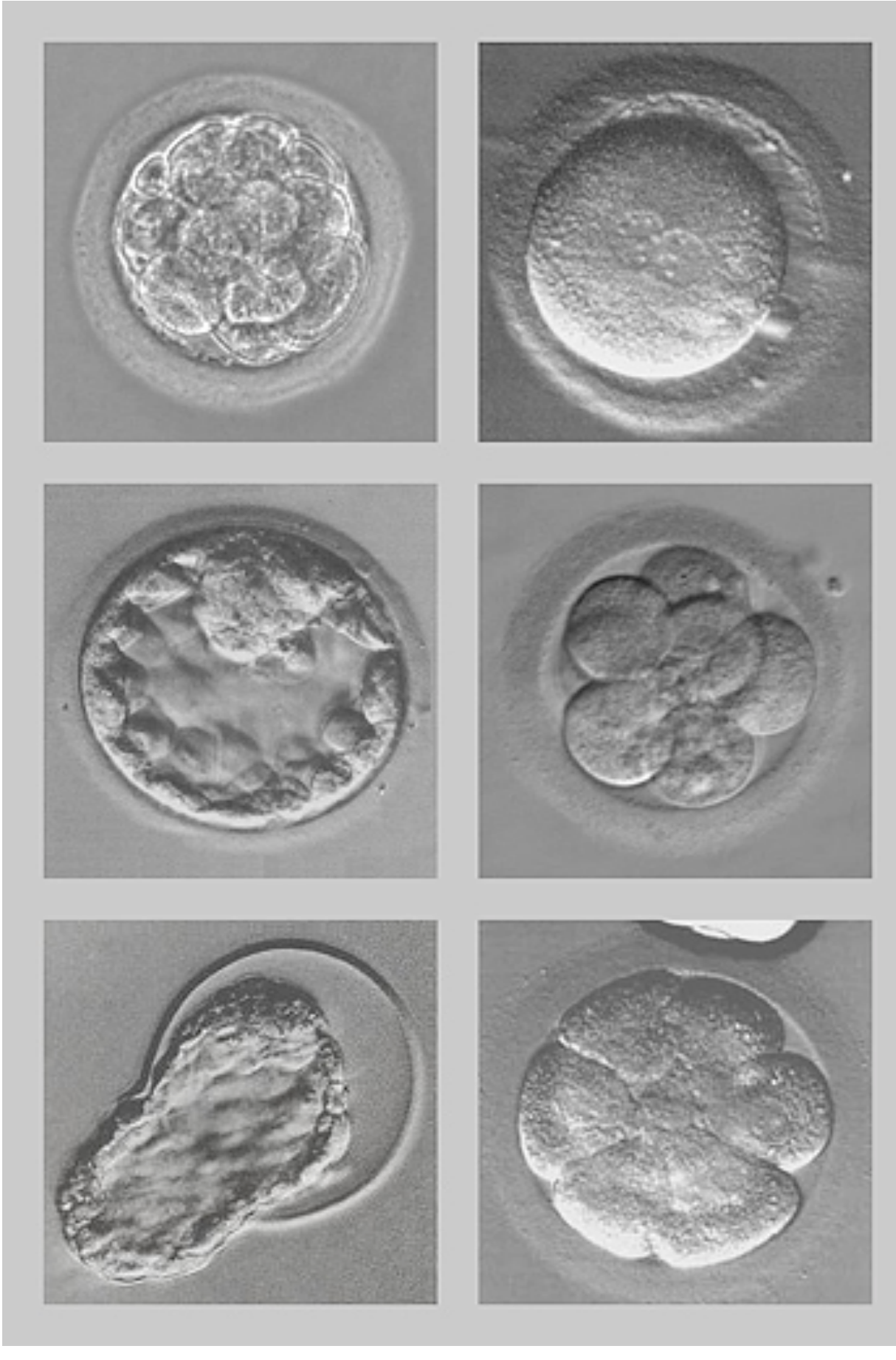
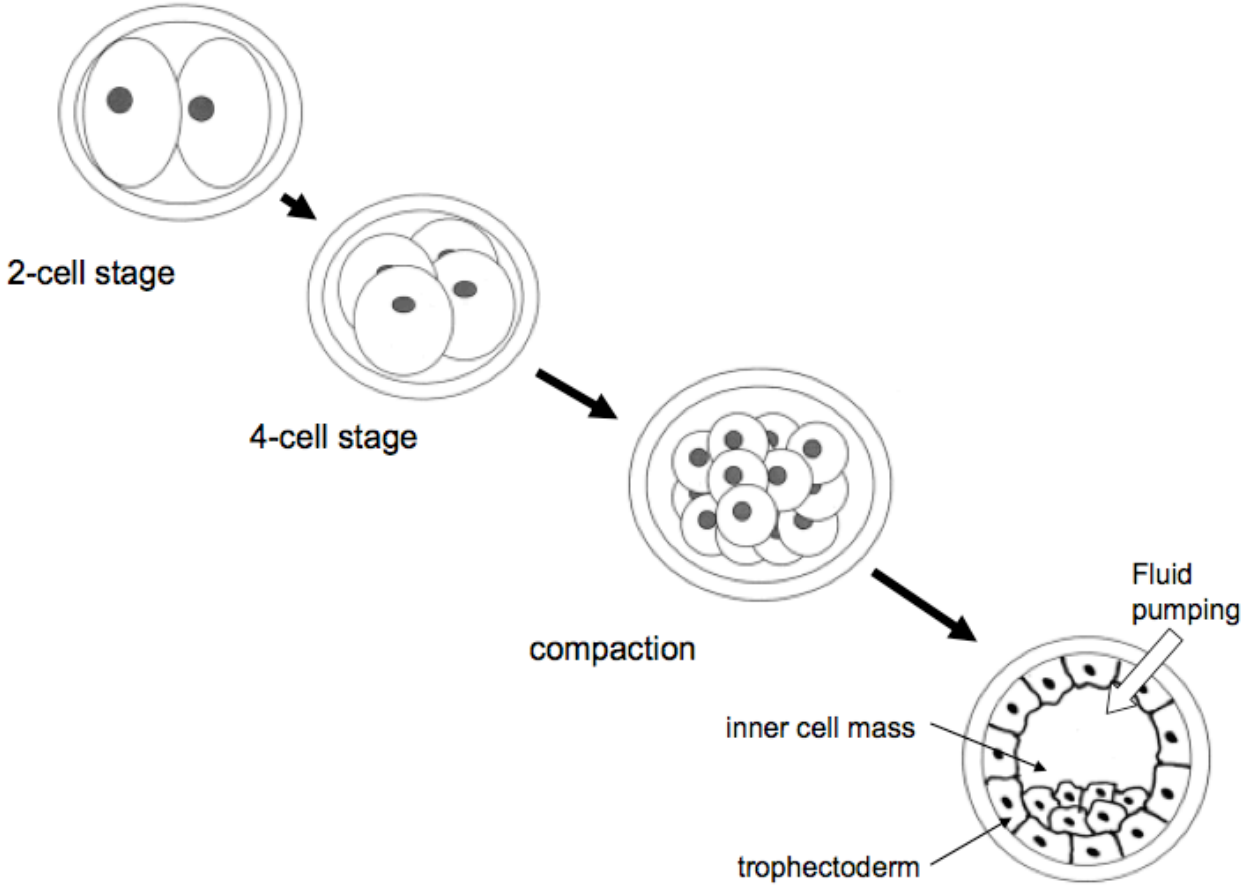


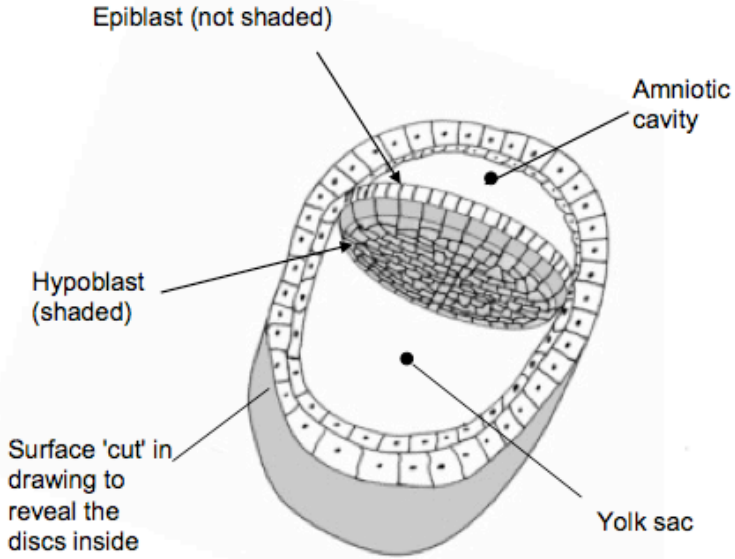
… and this self-organizing system is used again and again as we make our 100 billion cells.


'genetic programme'
Control systems that bias probability of assembly/disassembly
Measurement of appropriateness of size/shape of structure
feedback
Self-assembly of molecular subunits

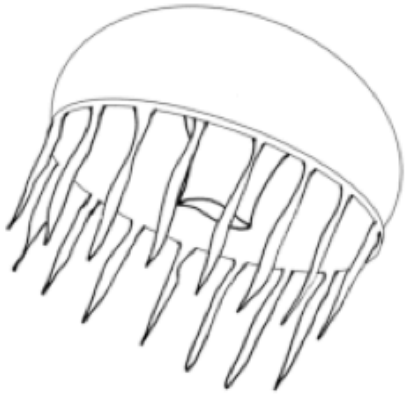
Early human embryos: all the cells are the same.



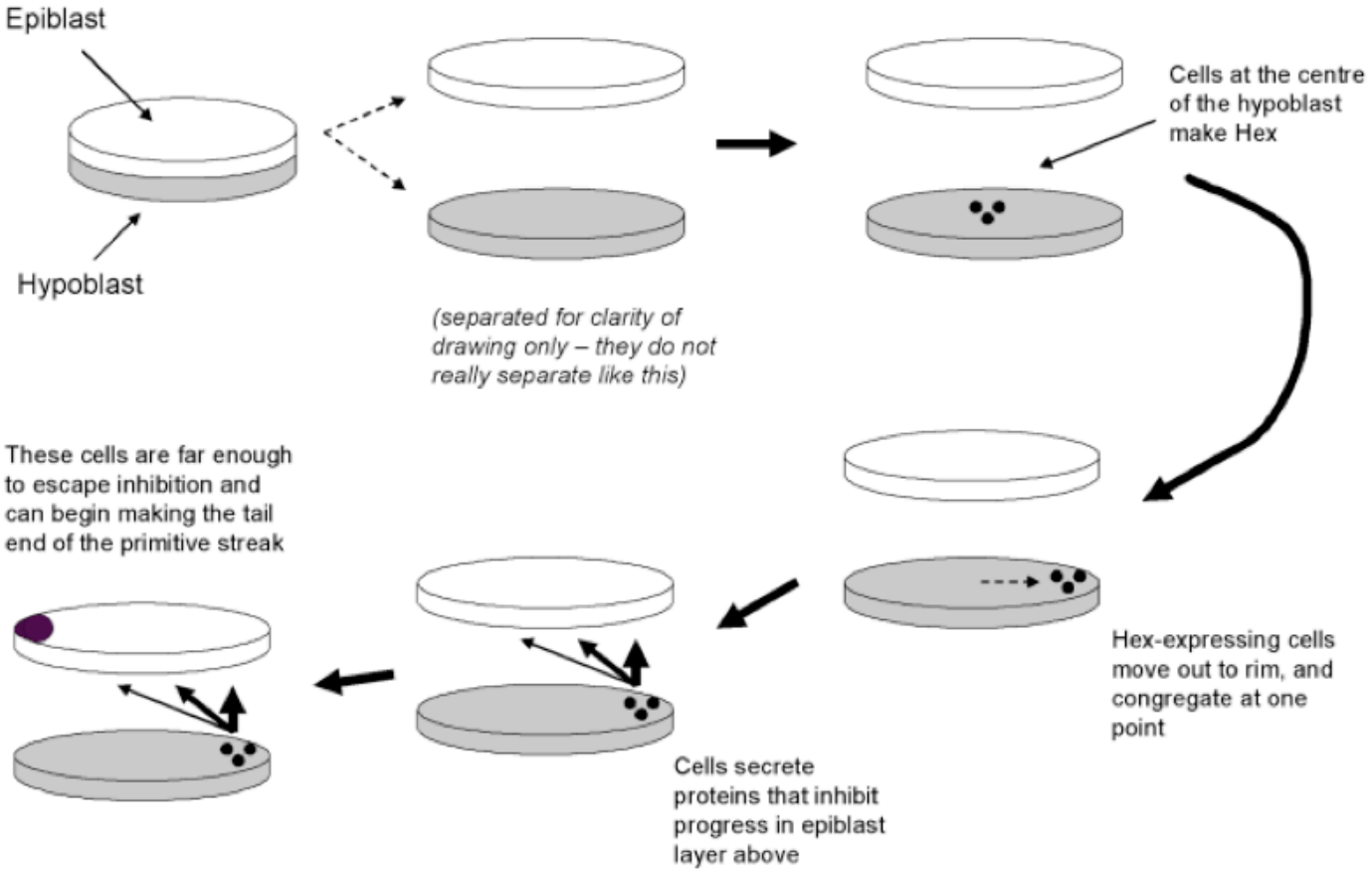

2-cell stage
4-cell stage
compaction
Fluid
pumping
inner cell mass
trophectoderm

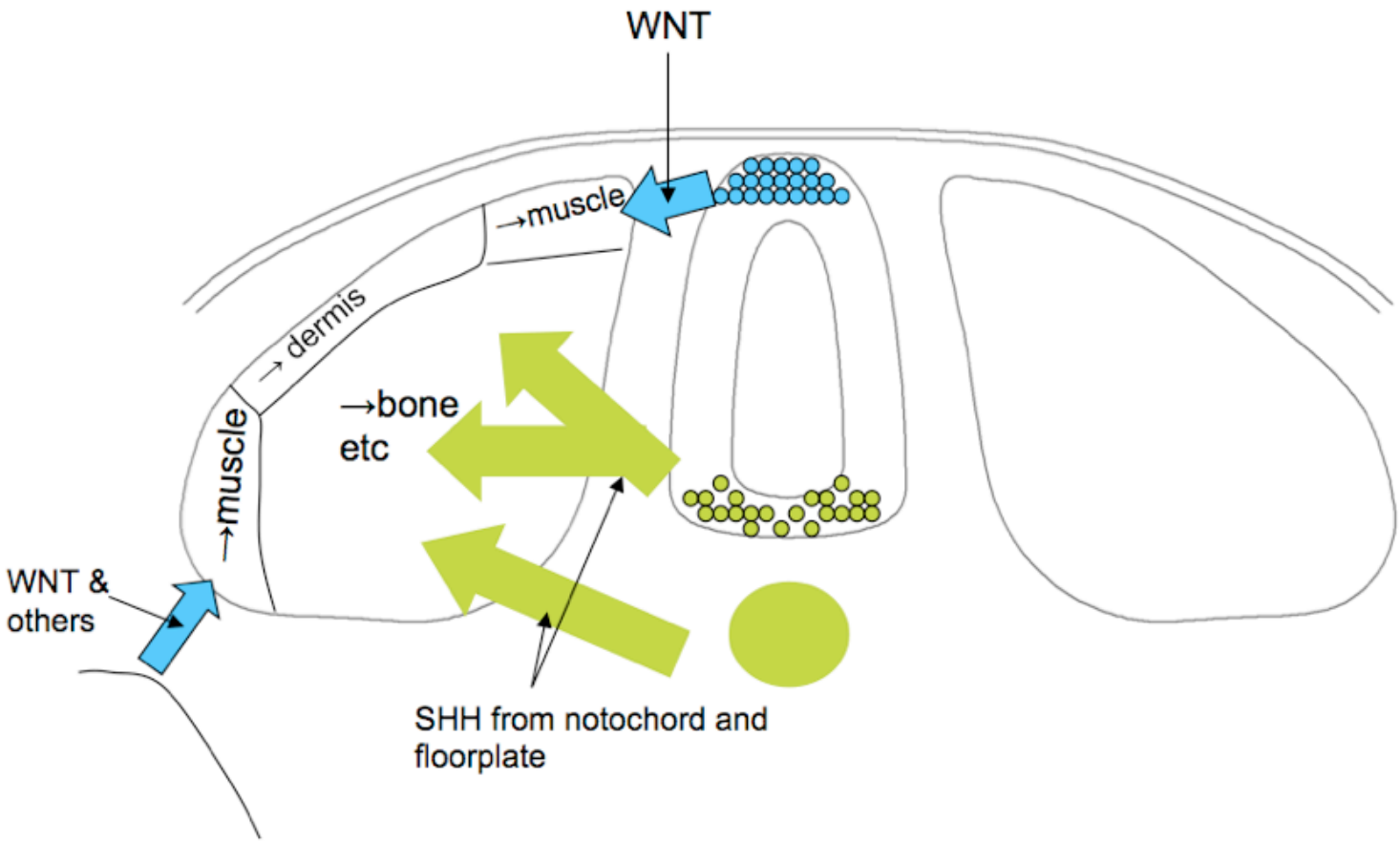


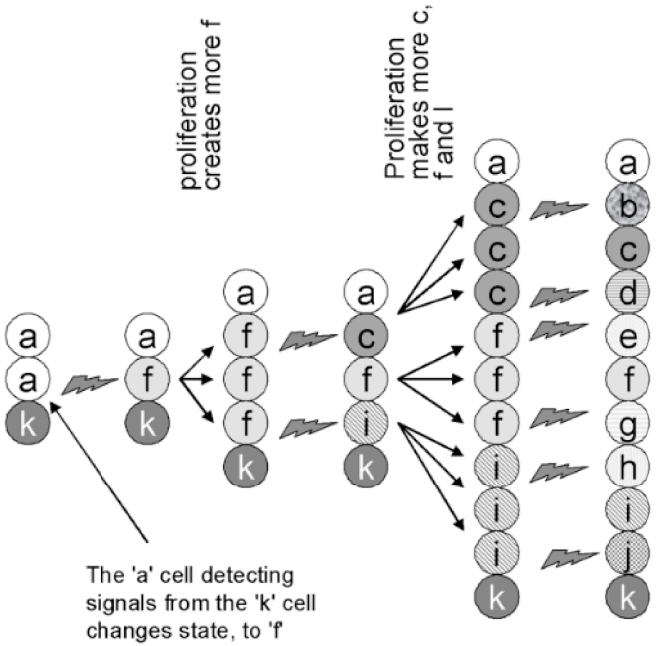
Human embryo at 9 days



Jellyfish (for comparison of symmetry)


Epiblast
Hypoblast
(separated for clarity of drawing only – they do not really separate like this)
Cells at the centre of the hypoblast make Hex
Hex-expressing cells move out to rim, and congregate at one point
Cells secrete proteins that inhibit progress in epiblast layer above
These cells are far enough to escape inhibition and can begin making the tail end of the primitive streak


WNT
→muscle
→ dermis
→muscle
→bone
etc
WNT &
others
SHH from notochord and
floorplate



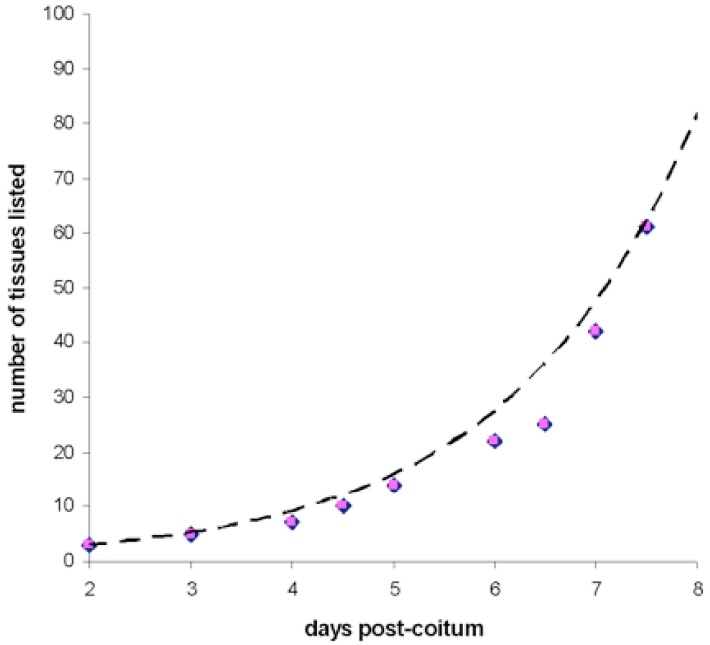
matters

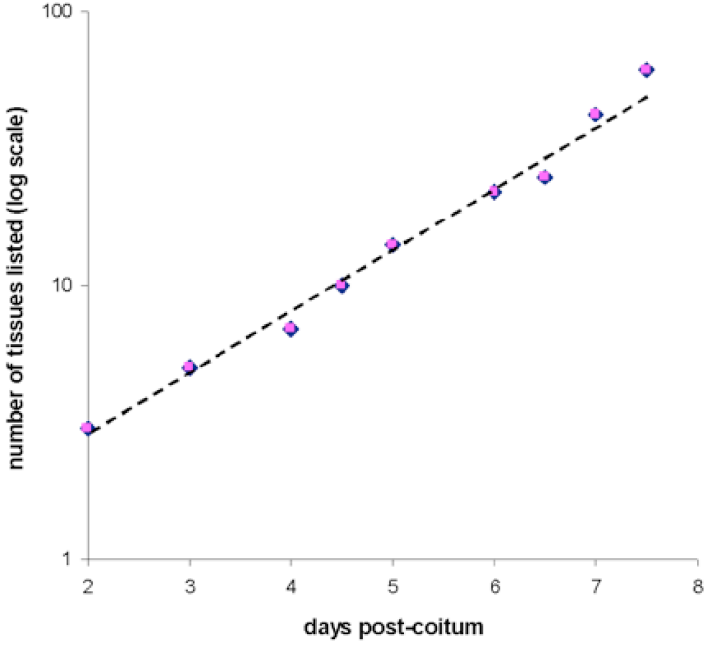
Anatomical complexity is acquired at an exponential rate during mouse embryonic development

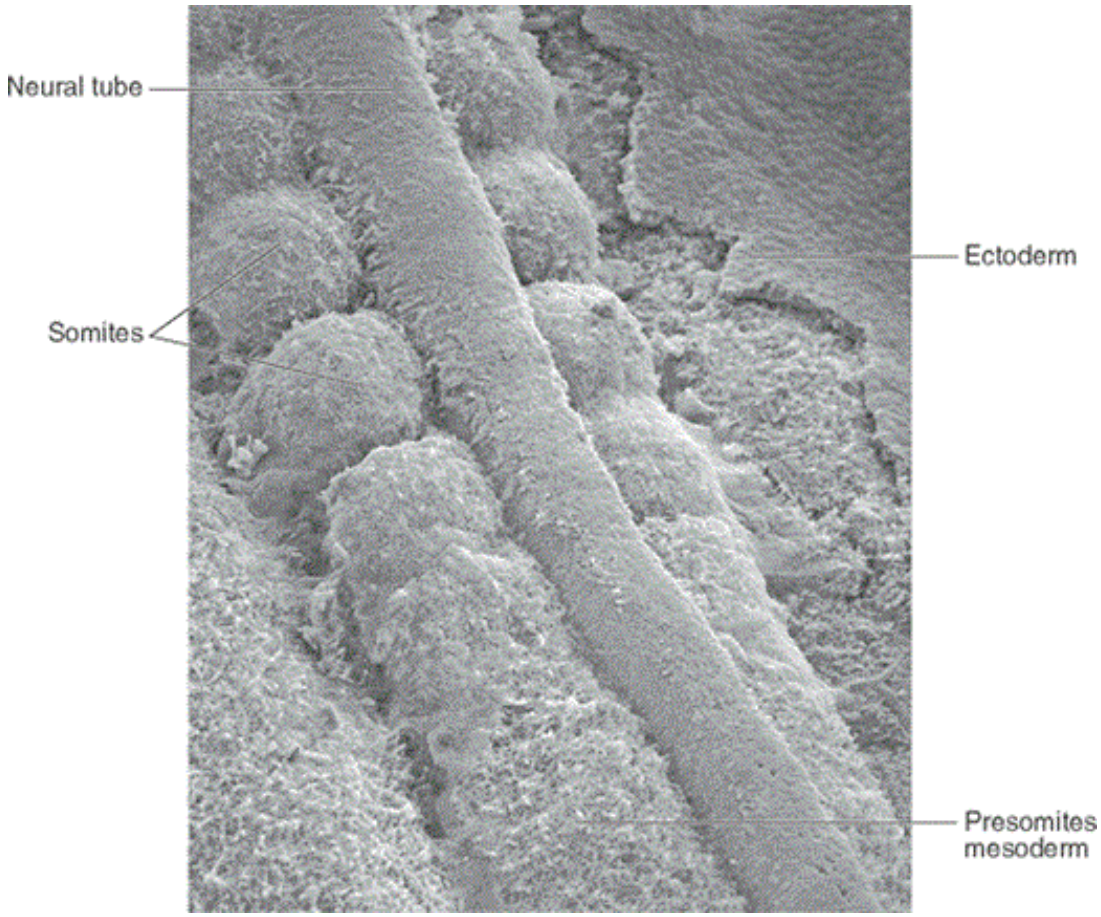
Correspondence
jamie.davies@ed.ac.uk

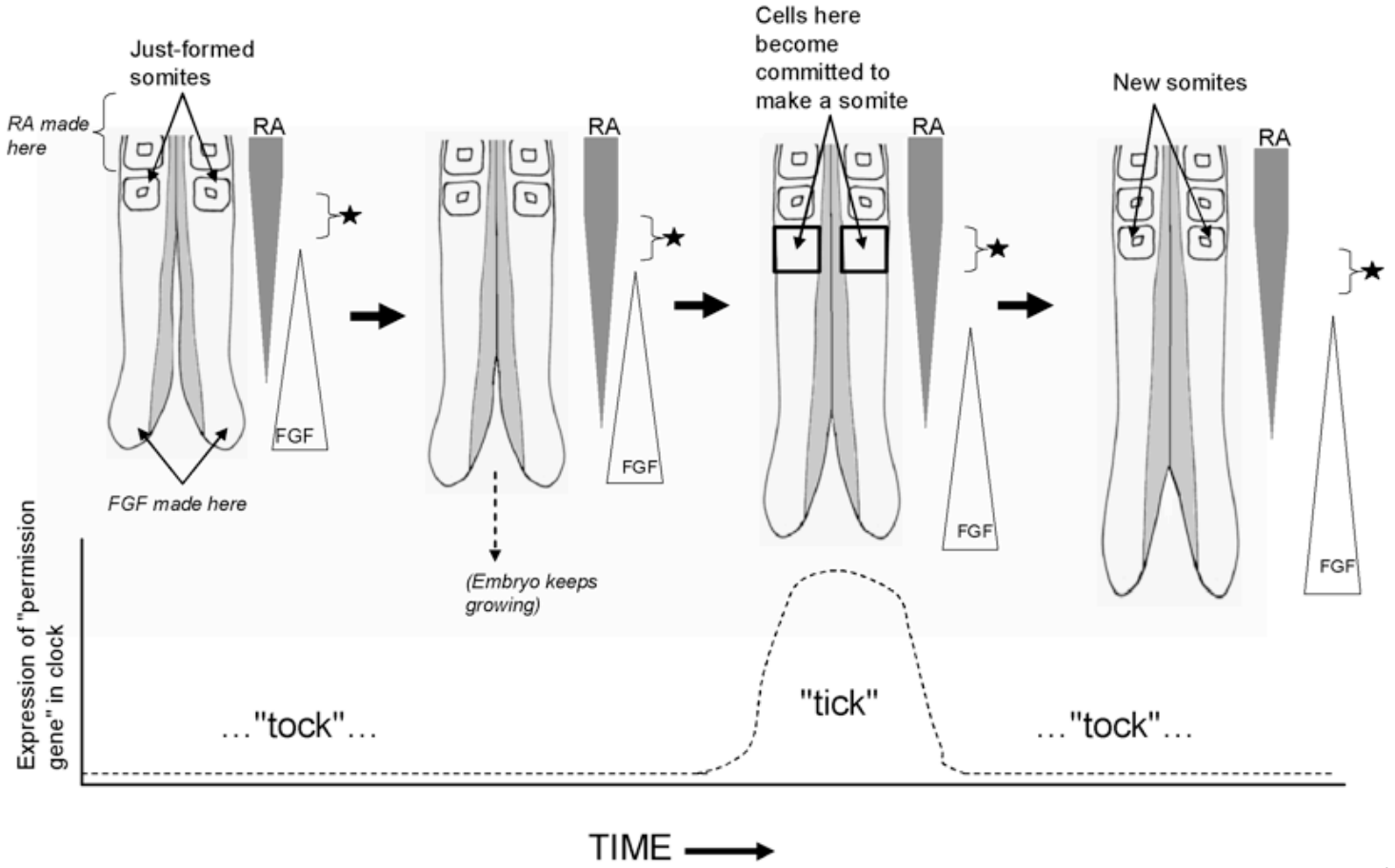
Jamie A Davies
Centre for Integrative Physiology, University of Edinburgh

Mouse data






Neural tube
Somites
Ectoderm
Presomites
mesoderm


Just-formed somites
RA made here
RA
FGF
FGF made here
(Embryo keeps growing)
Cells here become committed to make a somite
New somites
Expression of "permission gene" in clock
…"tock"…
"tick"
…"tock"…
TIME

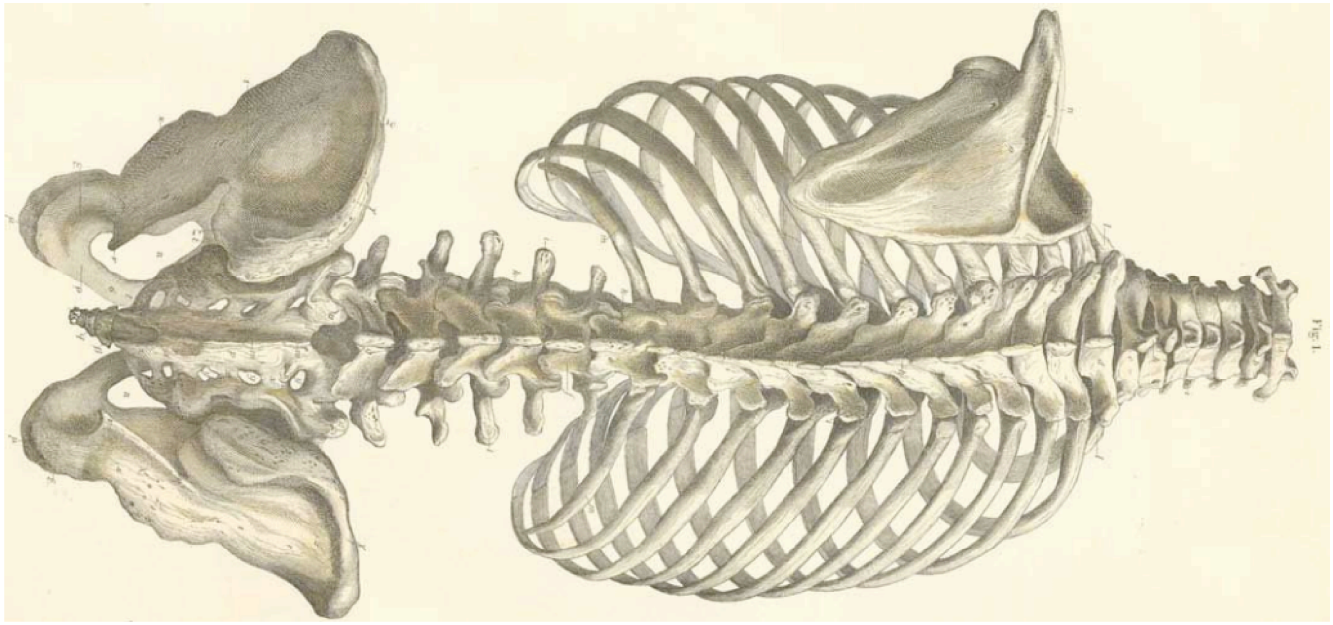
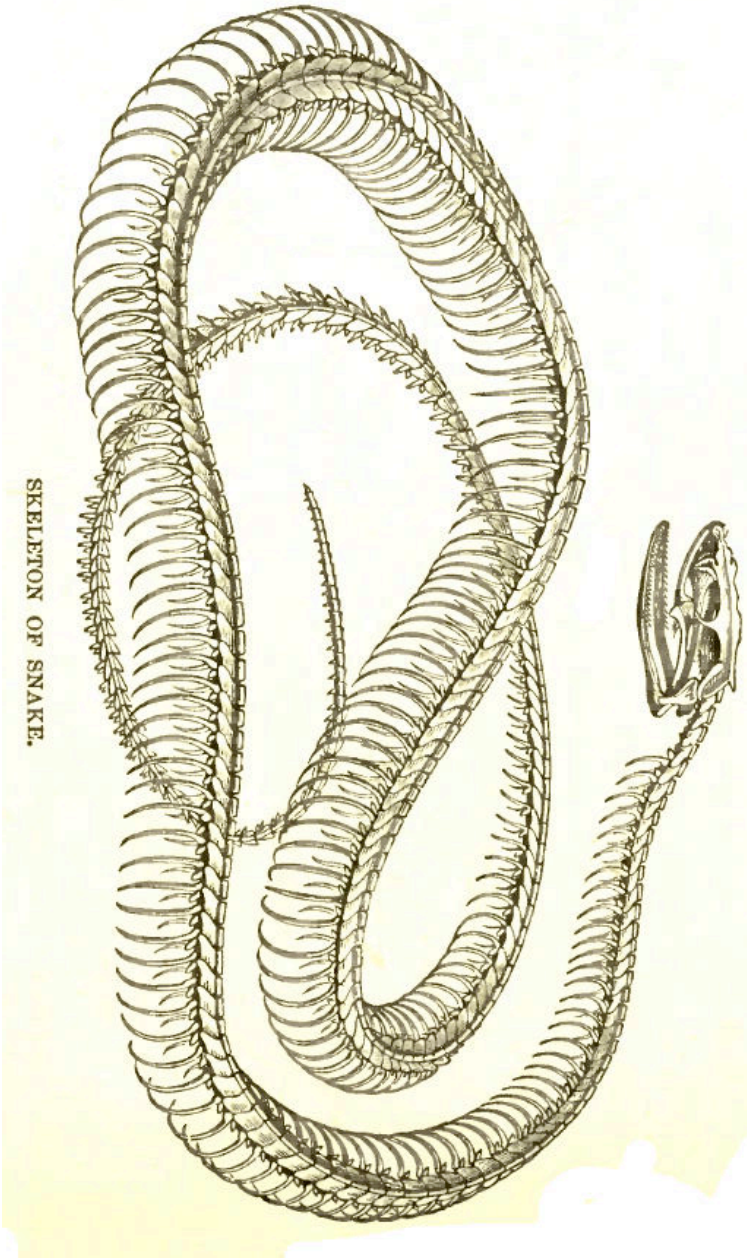


Fig. 1



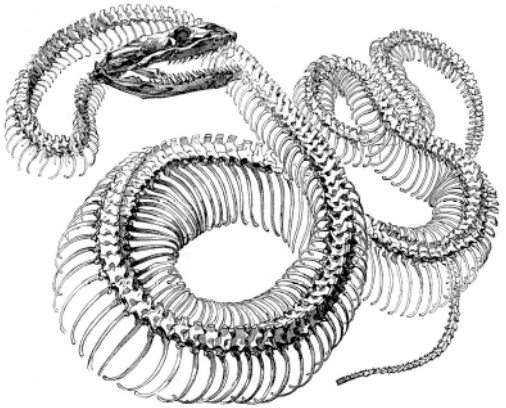
SKELETON OF SNAKE.

Vol 454|17 July 2008|doi:10.1038/nature07020 nature

LETTERS

Control of segment number in vertebrate embryos

Céline Gomez[1], Ertuğrul M. Özbudak[1], Joshua Wunderlich[1], Diana Baumann[1], Julian Lewis[2] & Olivier Pourquié[1,3]



Cell Rep. 2013 Jan 31;3(1):1-7. doi: 10.1016/j.celrep.2012.11.012. Epub 2012 Dec 7.

Accelerating the tempo of the segmentation clock by reducing the number of introns in the Hes7 gene.

Harima Y[1], Takashima Y, Ueda Y, Ohtsuka T, Kageyama R.

⊕ Author information

Abstract

Periodic somite segmentation is controlled by the cyclic gene Hes7, whose oscillatory expression depends upon negative feedback with a delayed timing. The mechanism that regulates the pace of segmentation remains to be determined, but mathematical modeling has predicted that negative feedback with shorter delays would give rise to dampened but more rapid oscillations. Here, we show that reducing the number of introns within the Hes7 gene shortens the delay and results in a more rapid tempo of both Hes7 oscillation and somite segmentation, increasing the number of somites and vertebrae in the cervical and upper thoracic region. These results suggest that the number of introns is important for the appropriate tempo of oscillatory expression and that Hes7 is a key regulator of the pace of the segmentation clock.

PMID: 23219549 DOI: 10.1016/j.celrep.2012.11.012

[Indexed for MEDLINE] **Free full text**

Proc Natl Acad Sci U S A. 2013 Nov 12;110(46):E4316-24. doi: 10.1073/pnas.1308811110. Epub 2013 Oct 22.

Transcript processing and export kinetics are rate-limiting steps in expressing vertebrate segmentation clock genes.

Hoyle NP[1], Ish-Horowicz D.

Author information

Abstract

Sequential production of body segments in vertebrate embryos is regulated by a molecular oscillator (the segmentation clock) that drives cyclic transcription of genes involved in positioning intersegmental boundaries. Mathematical modeling indicates that the period of the clock depends on the total delay kinetics of a negative feedback circuit, including those associated with the synthesis of transcripts encoding clock components [Lewis J (2003) Curr Biol 13(16):1398-1408]. Here, we measure expression delays for three transcripts [Lunatic fringe, Hes7/her1, and Notch-regulated-ankyrin-repeat-protein (Nrarp)], that cycle during segmentation in the zebrafish, chick, and mouse, and provide in vivo measurements of endogenous splicing and export kinetics. We show that mRNA splicing and export are much slower than transcript elongation, with the longest delay (about 16 min in the mouse) being due to mRNA export. We conclude that the kinetics of mRNA and protein production and destruction can account for much of the clock period, and provide strong support for delayed autorepression as the underlying mechanism of the segmentation clock.

KEYWORDS: RNA export; RNA splicing; mRNA processing; somites; transcriptional delays

PMID: 24151332 PMCID: PMC3831944 DOI: 10.1073/pnas.1308811110

BUT

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Published in final edited form as:
Science. 2014 Feb 14; 343(6172): 791–795.
Published online 2014 Jan 9. doi: 10.1126/science.1247575

PMCID: PMC3992919
NIHMSID: NIHMS567392
PMID: 24407478

Somites Without a Clock

Ana S. Dias,[#1] Irene de Almeida,[#1] Julio M. Belmonte,[2] James A. Glazier,[2] and Claudio D. Stern[1,†]

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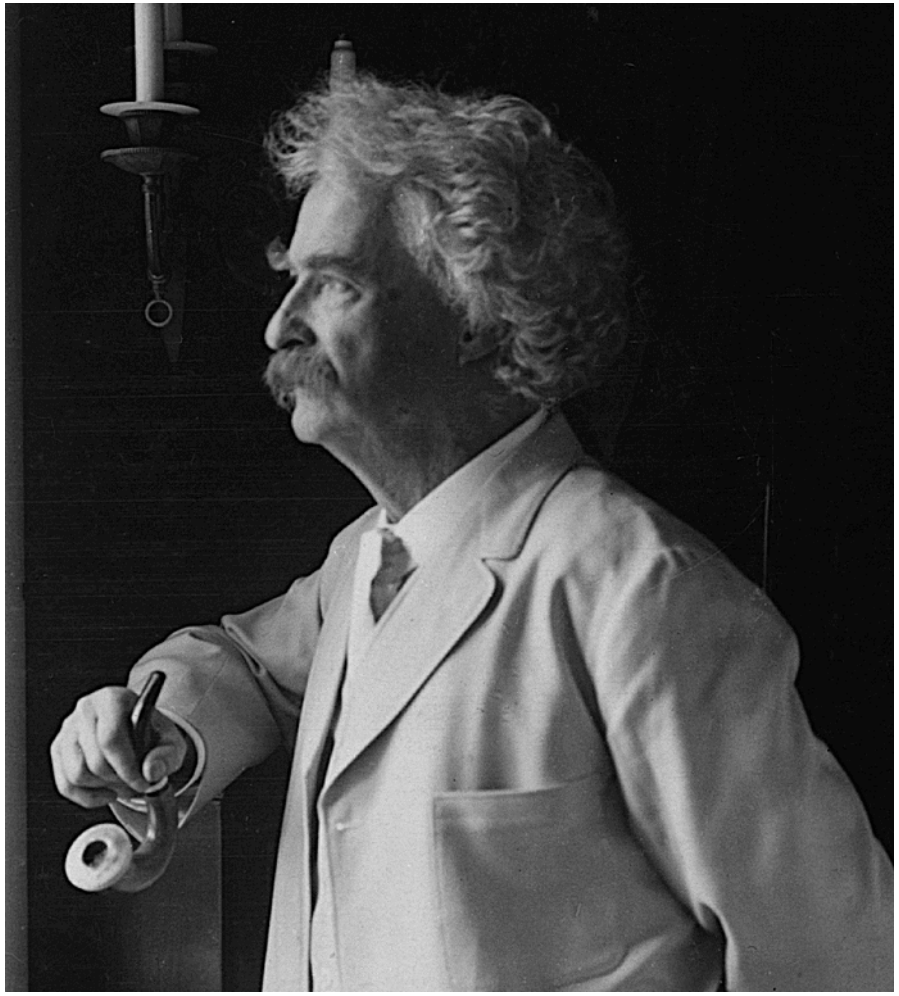
Abstract

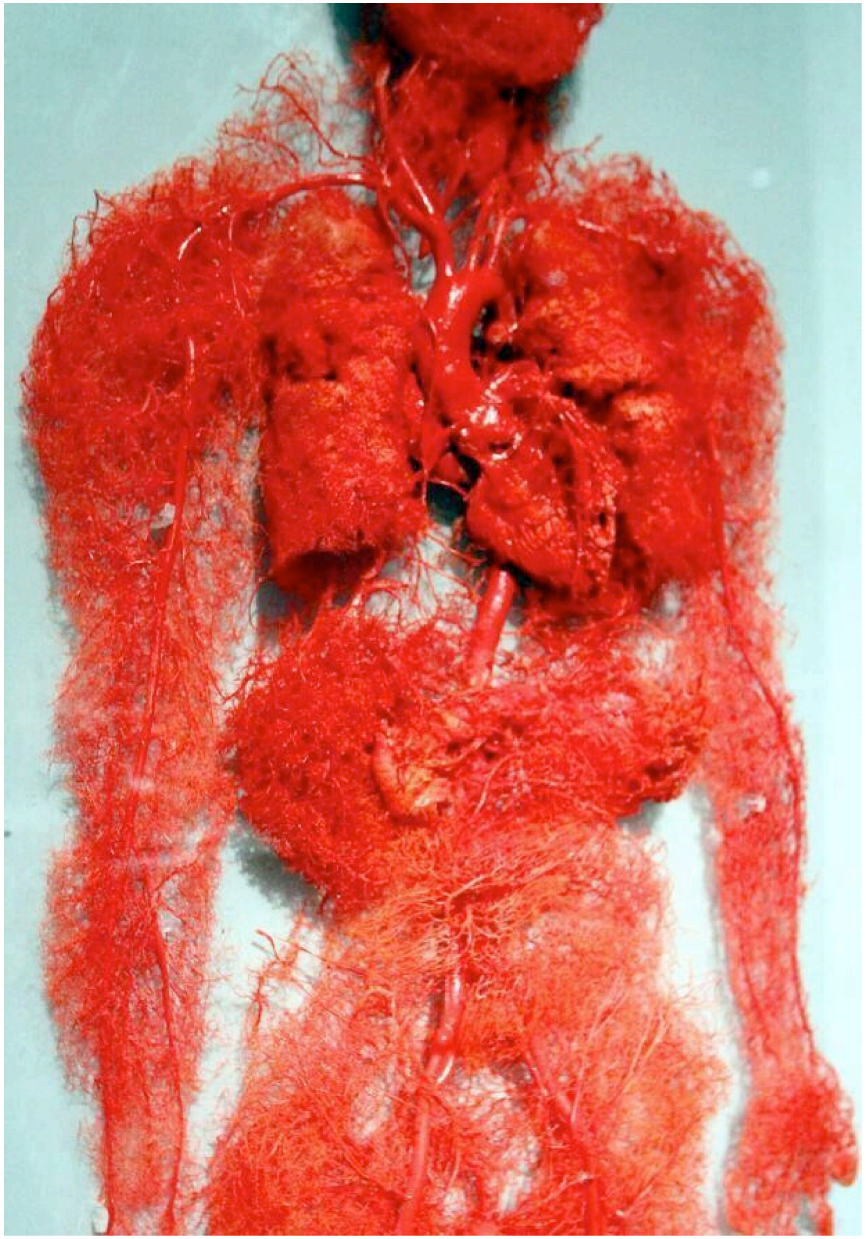
Go to:

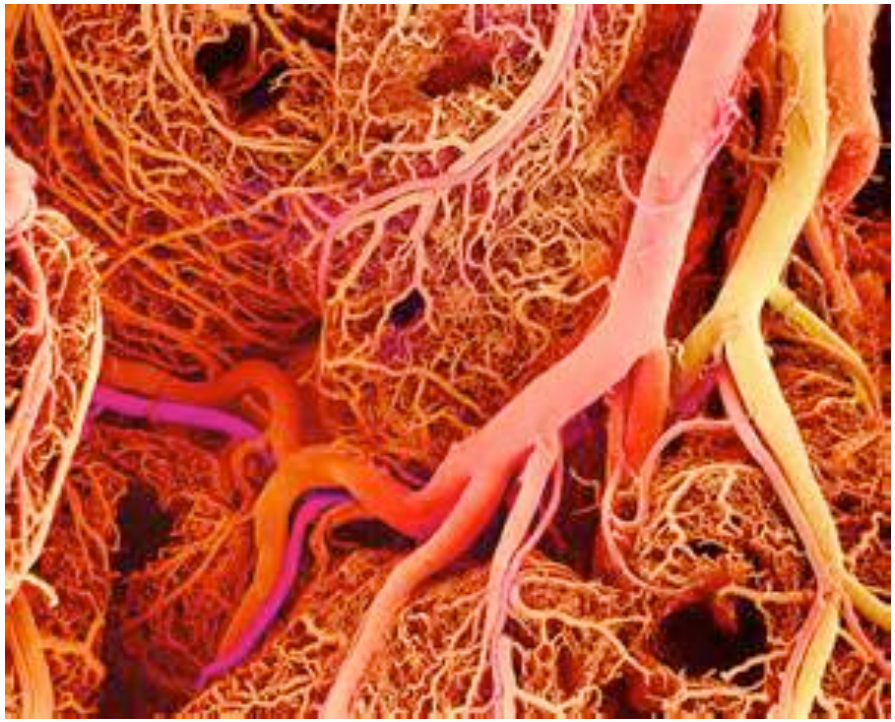
The formation of body segments (somites) in vertebrate embryos is accompanied by molecular oscillations (segmentation clock). Interaction of this oscillator with a wave traveling along the body axis (the clock-and-wavefront model) is generally believed to control somite number, size, and axial identity. Here we show that a clock-and-wavefront mechanism is unnecessary for somite formation. Non-somite mesoderm treated with Noggin generates many somites that form simultaneously, without cyclic expression of Notch-pathway genes, yet have normal size, shape, and fate. These somites have axial identity: The Hox code is fixed independently of somite fate. However, these somites are not subdivided into rostral and caudal halves, which is necessary for neural segmentation. We propose that somites are self-organizing structures whose size and shape is controlled by local cell-cell interactions.

Researchers have already cast much darkness on the subject, and if they continue their investigations, we shall soon know nothing at all about it.



Blood supplies provide a good example of self-organization



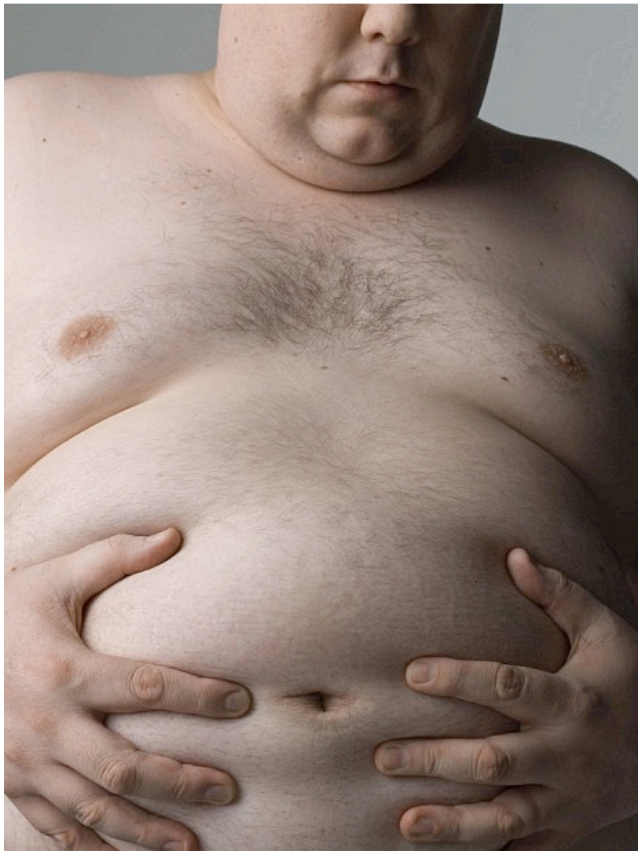


The blood supply has to serve an unpredictable tissue mass:

Credits: Adam Charlton

The blood supply has to serve an unpredictable tissue mass:

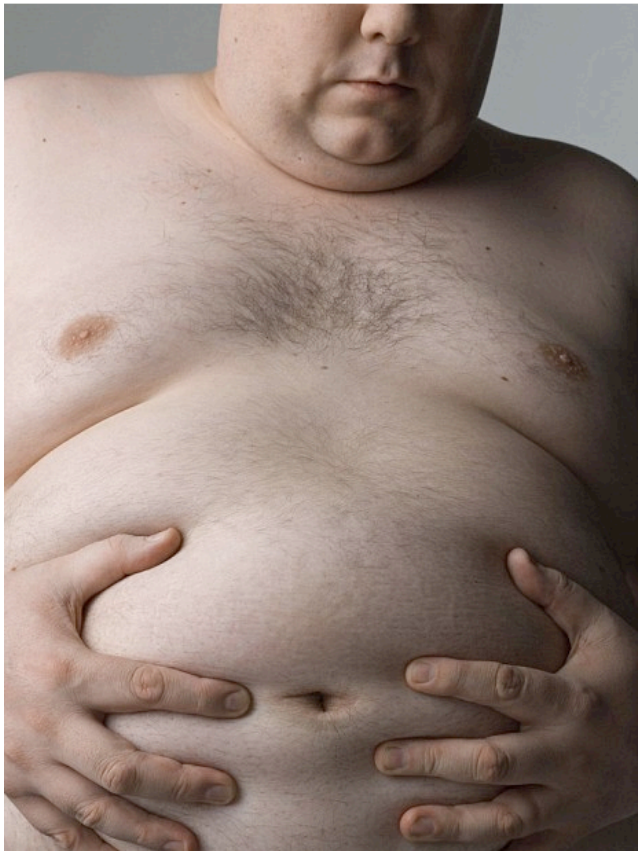
Credits: Adam Charlton



Daily Mail

The blood supply has to serve an unpredictable tissue mass:

Credits: Adam Charlton

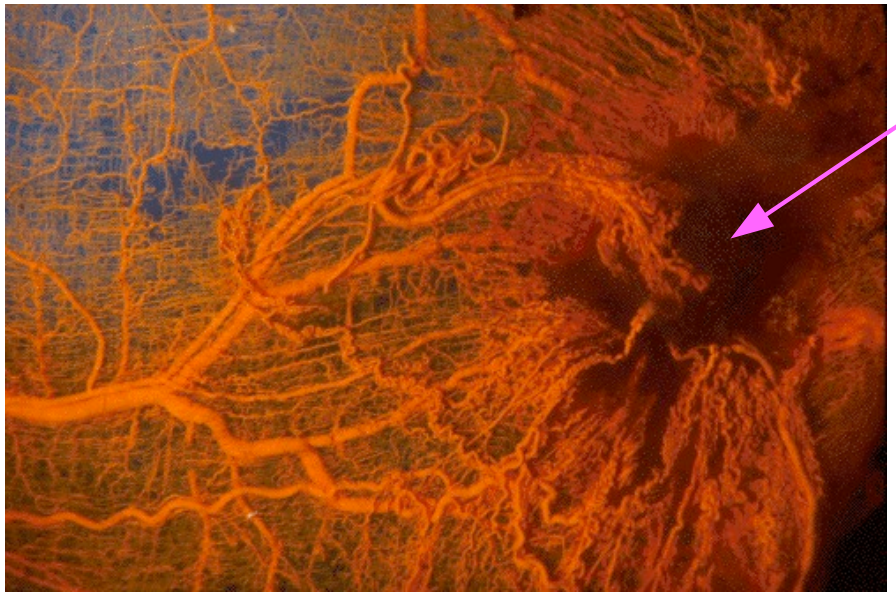


Daily Mail

Tom Adriaenssen, Wikipedia Commons

Tumours too:

This one's 'benign' in the usual medical sense


Growing tissue

Clearly this cannot be from an advanced 'blueprint'

How does it happen?

Blood vessel cell

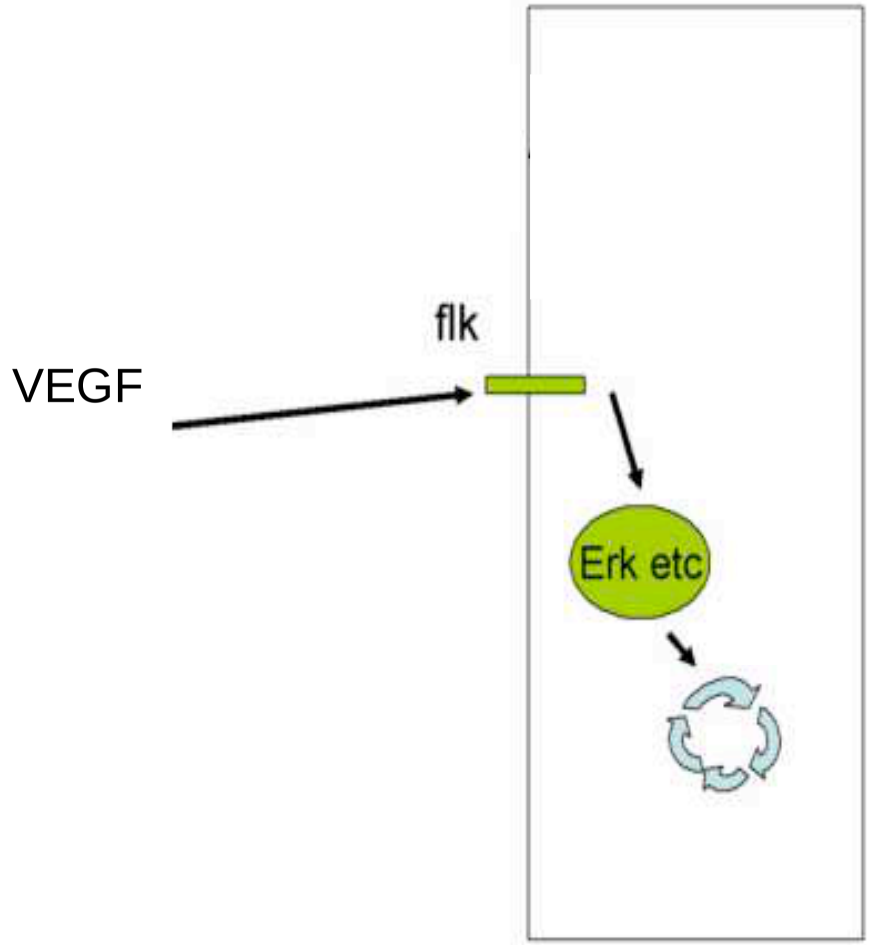


Image from Davies JA (2013) Mechanisms of Morphogenesis 2nd Ed.

Tissue cell

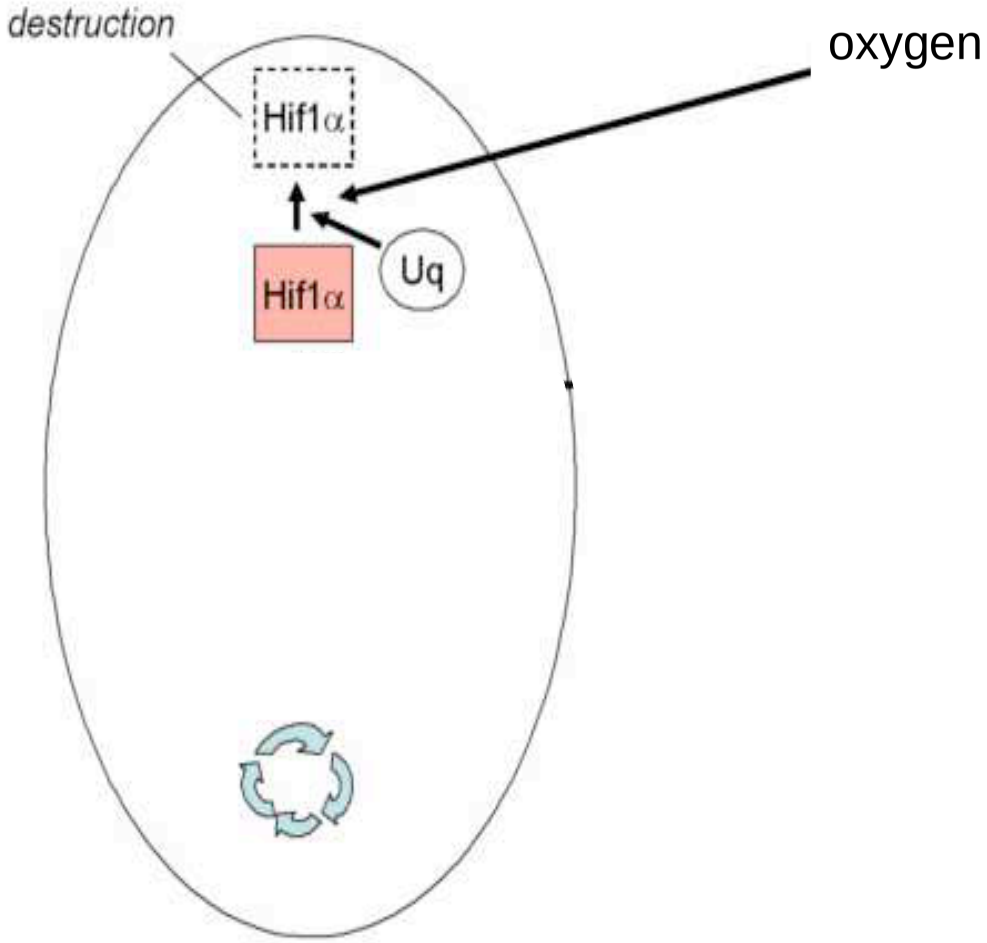


Image from Davies JA (2013) Mechanisms of Morphogenesis 2nd Ed.

Tissue cell

(no/ low oxygen)

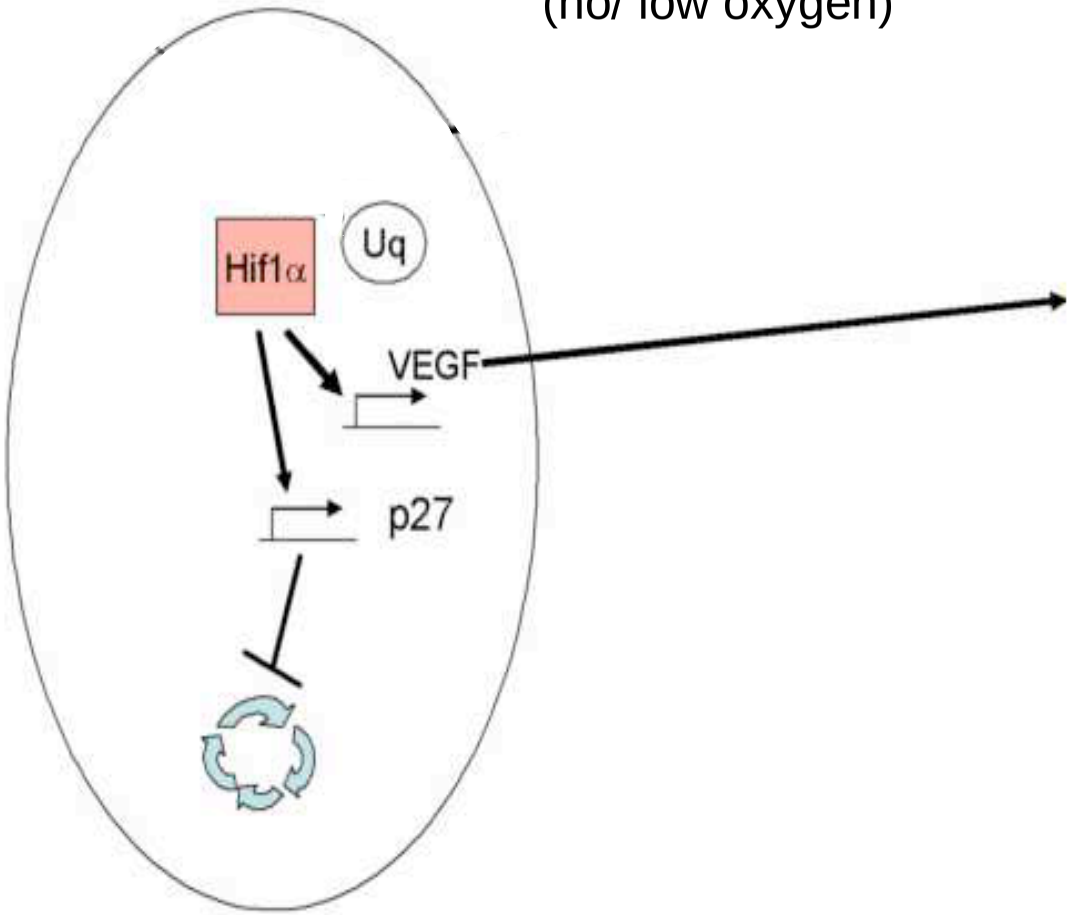


Image from Davies JA (2013) Mechanisms of Morphogenesis 2nd Ed.

Tissue cell

Blood vessel cell

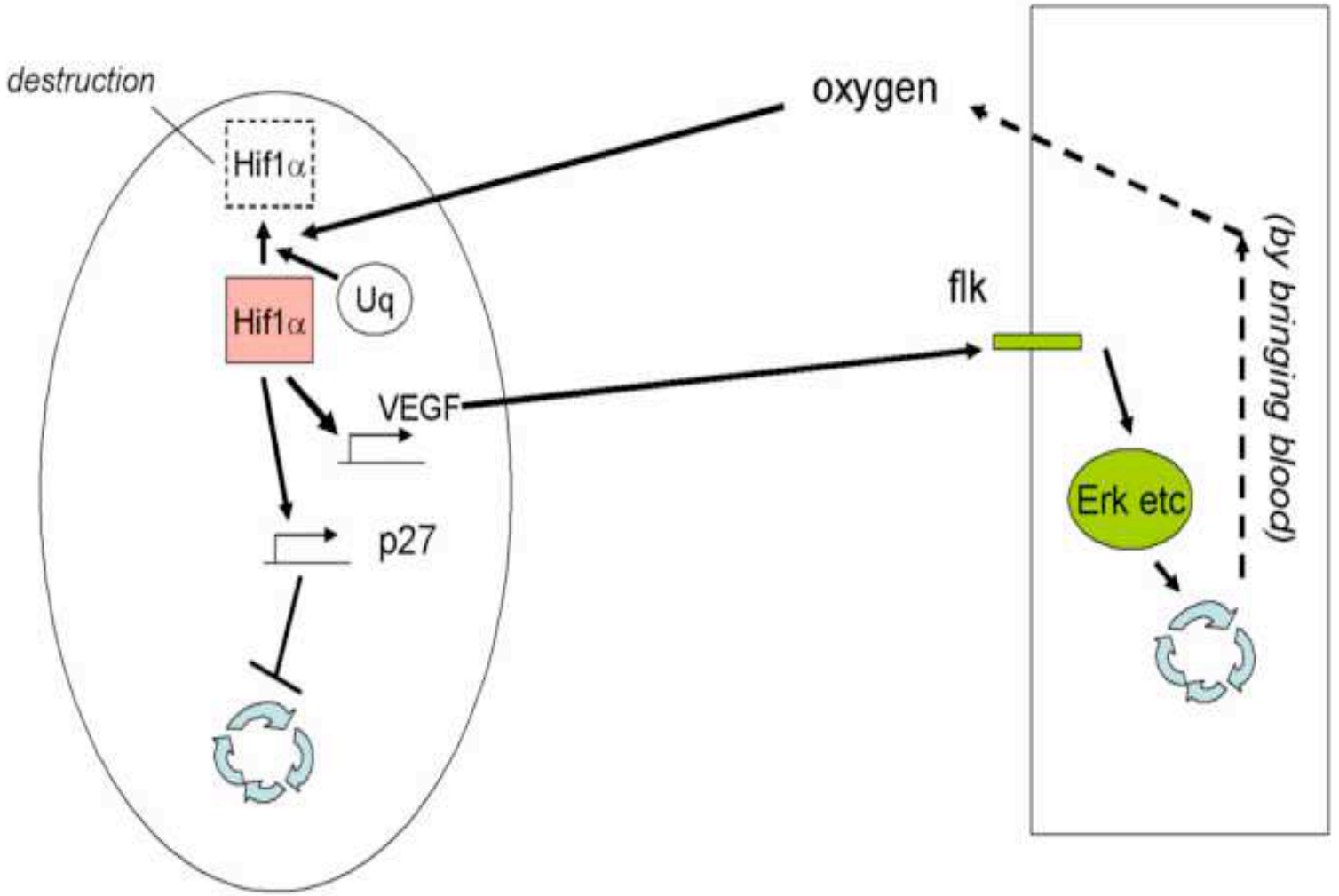
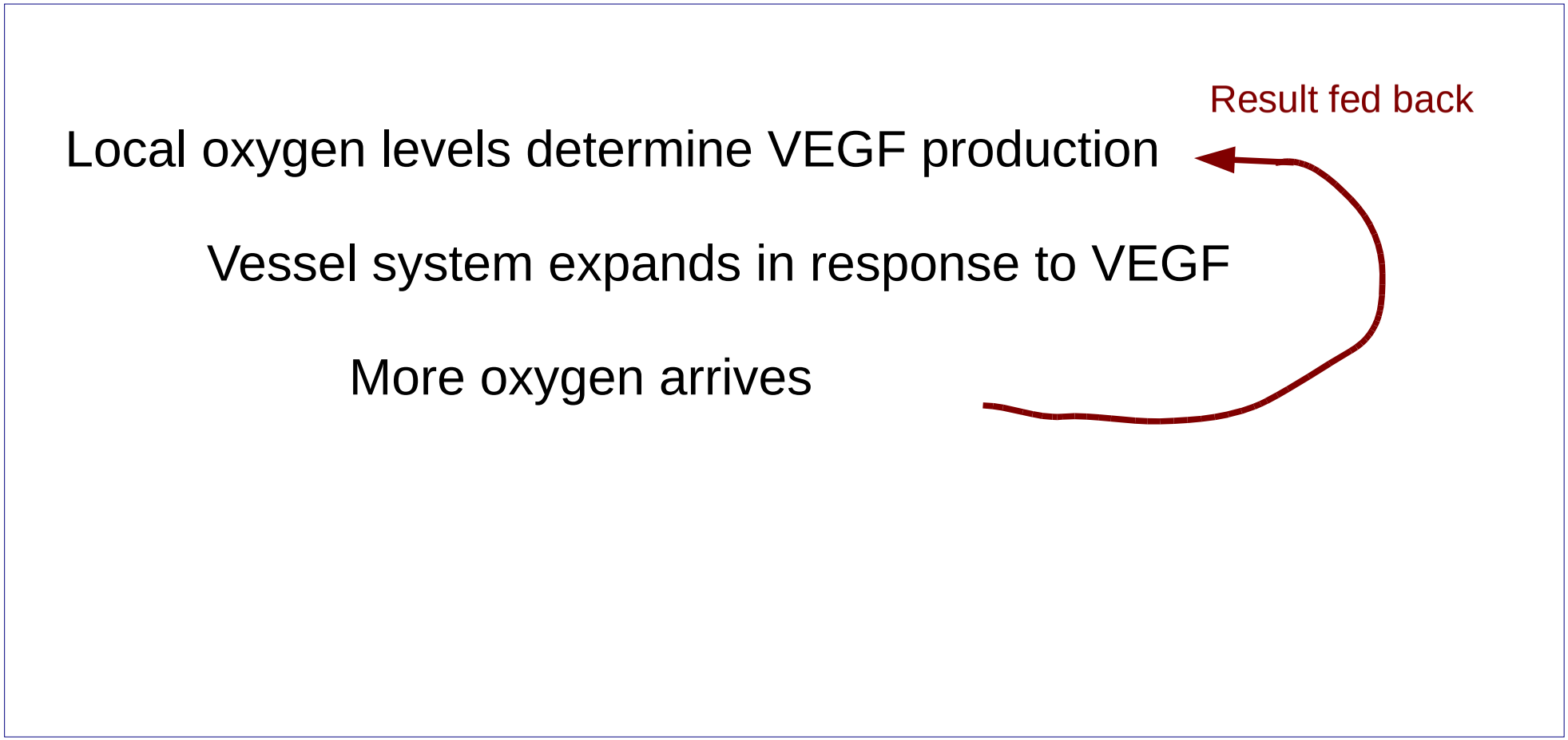
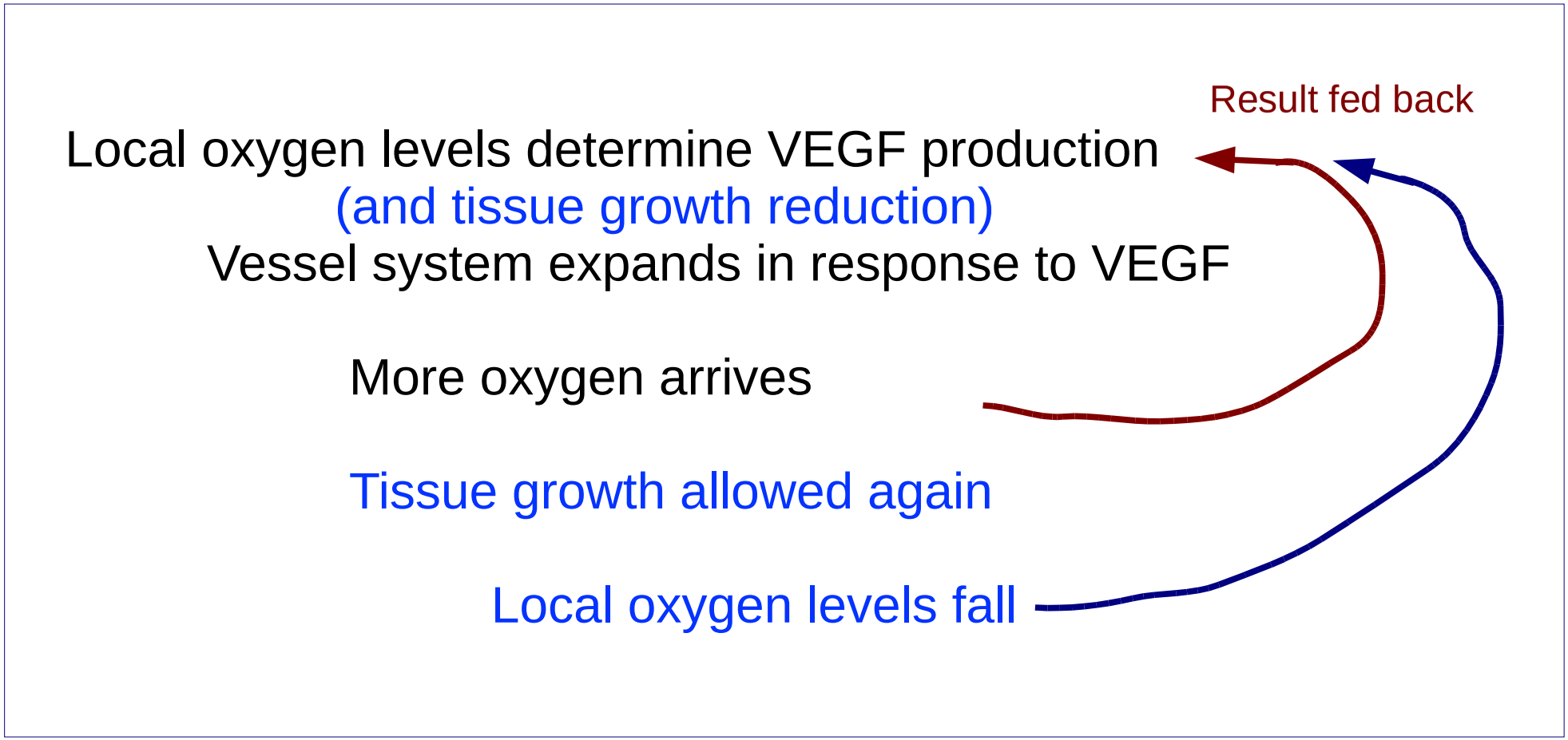


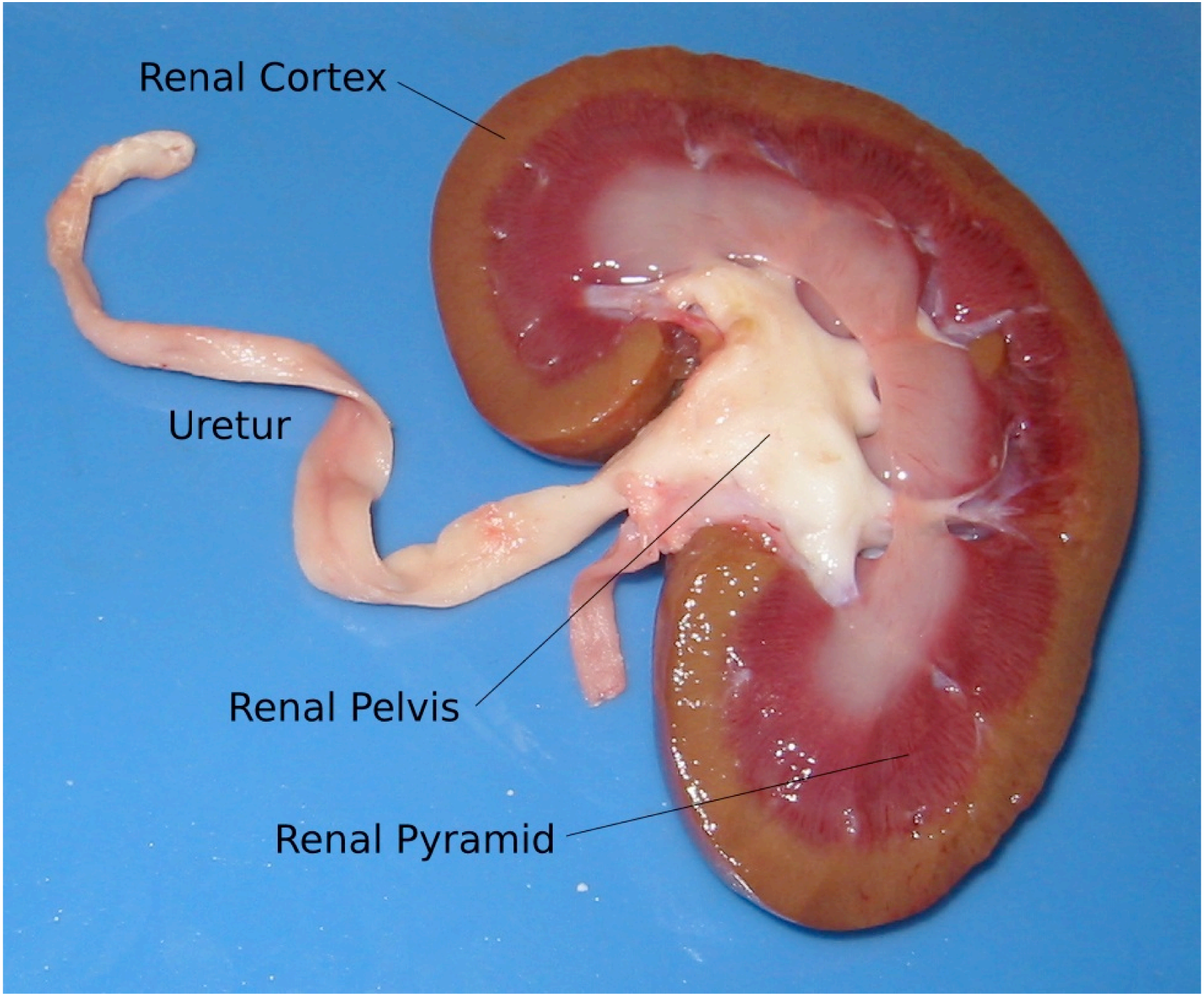
Image from Davies JA (2013) Mechanisms of Morphogenesis 2nd Ed.

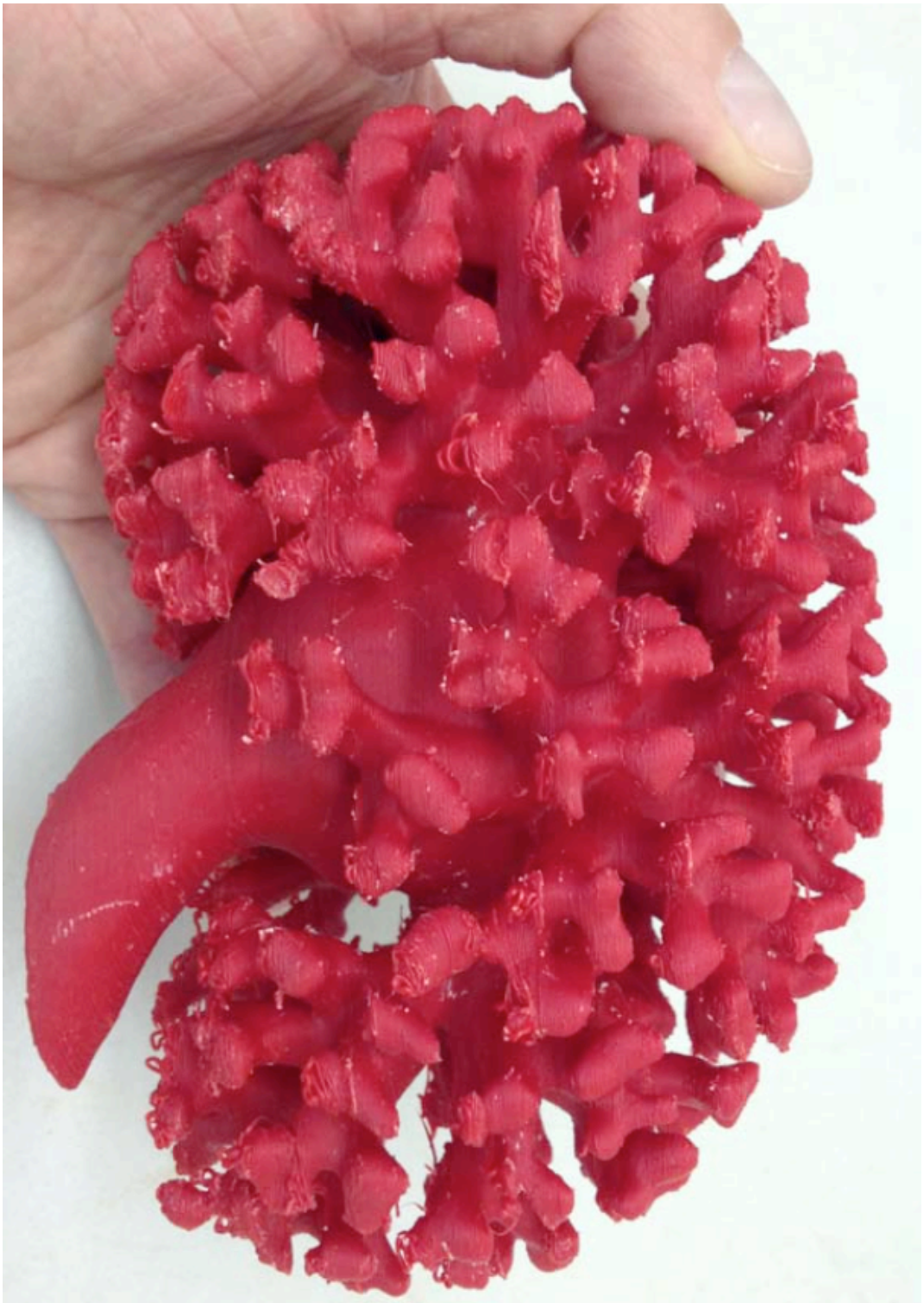
Similar formal structure:



Similar formal structure:




Renal Cortex
Uretur
Renal Pelvis
Renal Pyramid



A model of self-loathing:

Model:

UB secretes a substance called 'horrid' – a noise component is also added

UB tips always grow in the direction of least [horrid]

UB branches (1 tip → 2 adjacent ones) if [horrid] < threshold

(everything is sensed only locally, in the pixels immediately adjacent to each cell's own pixel)

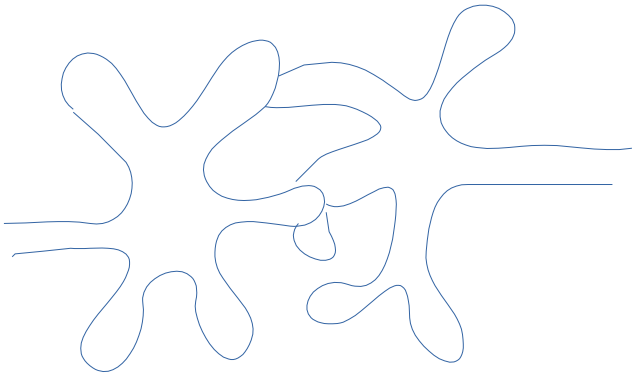
Anatomy [Horrid]

Experimental method:

Culture kidneys aimed at one another

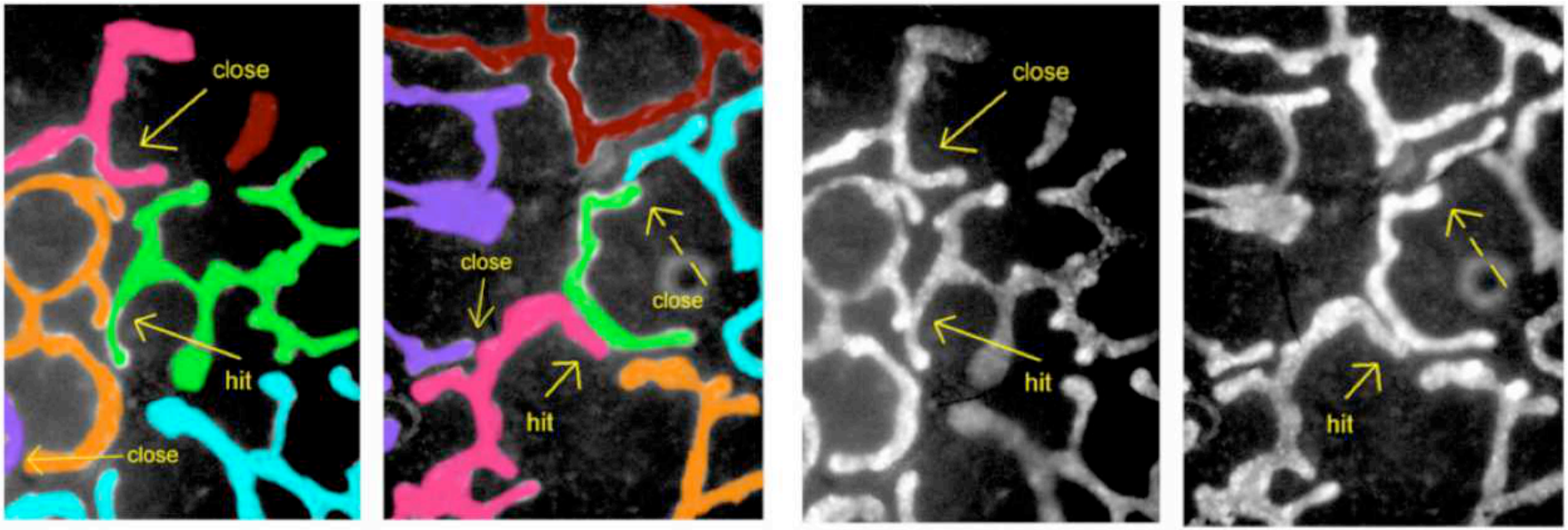
Add various test drugs (ideally ones that hit whole classes of signalling molecules)

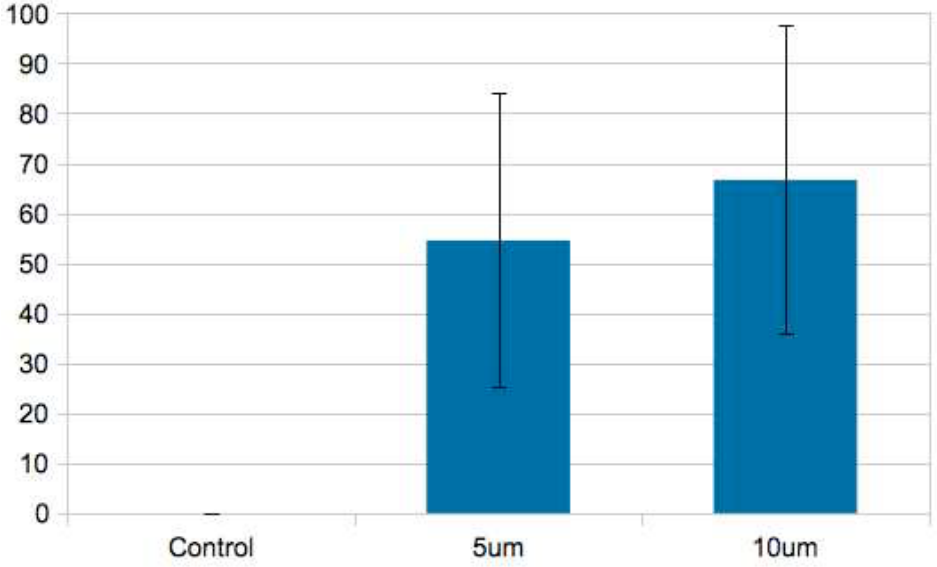
Hope to see a crash.



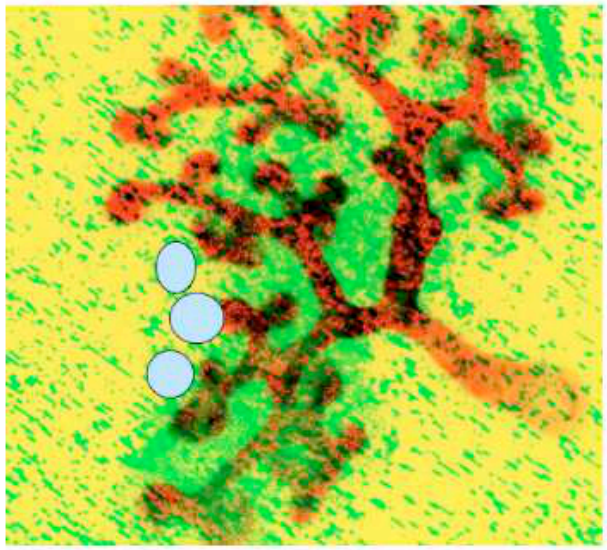
?

Alk Inhibitor data

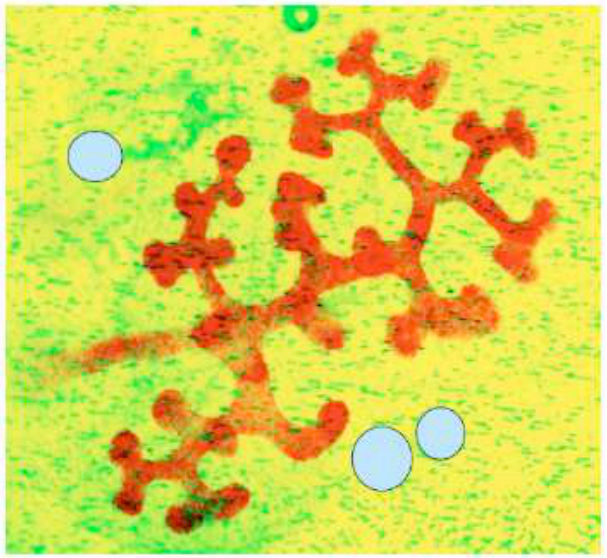




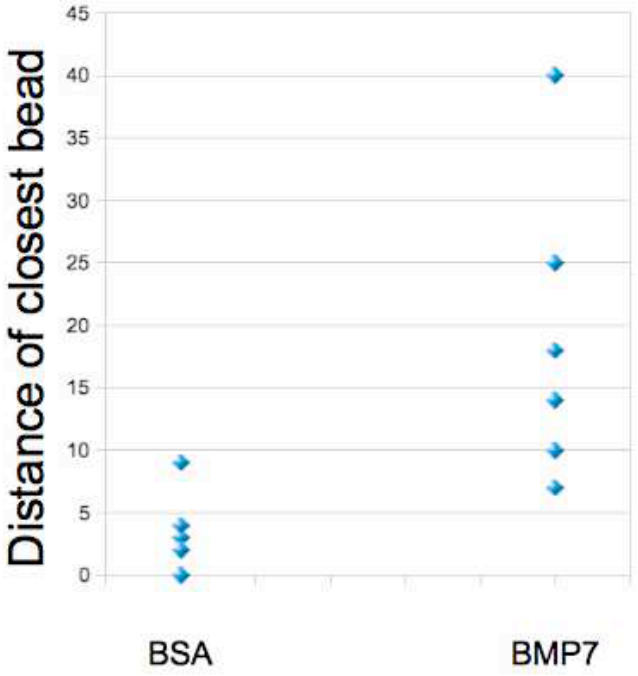
Experimental sources of BMP7



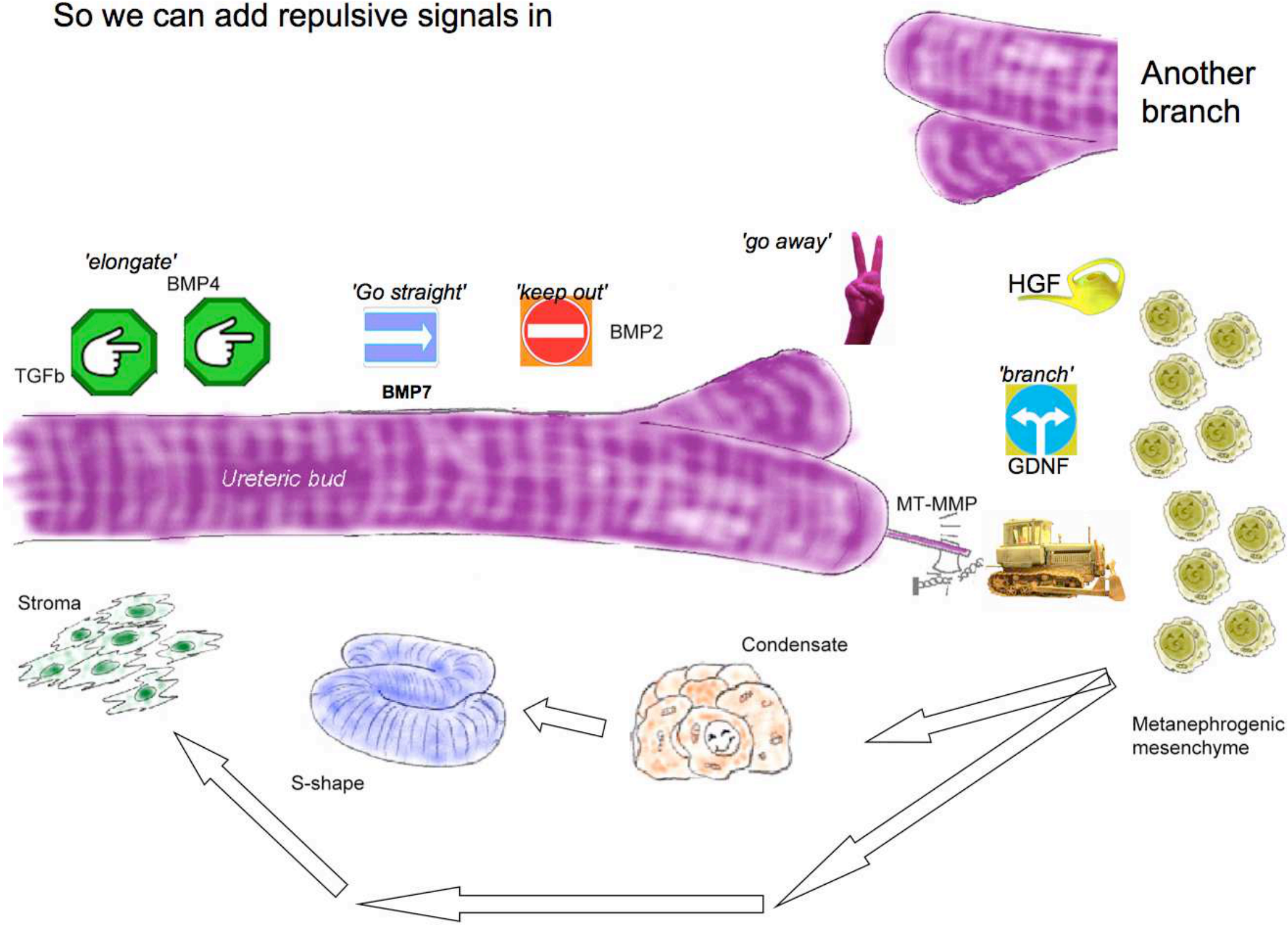
BSA beads



BMP7 beads

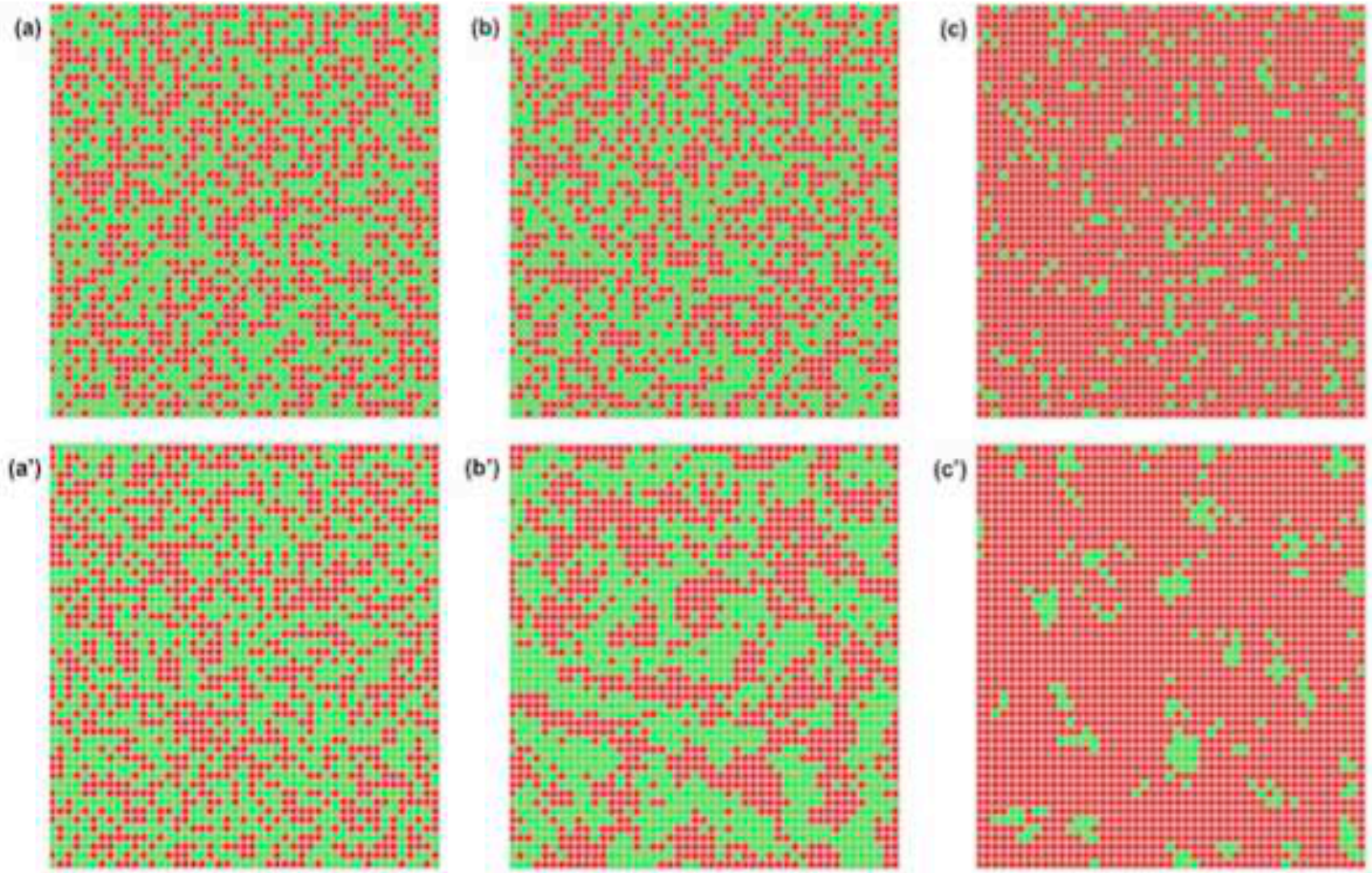


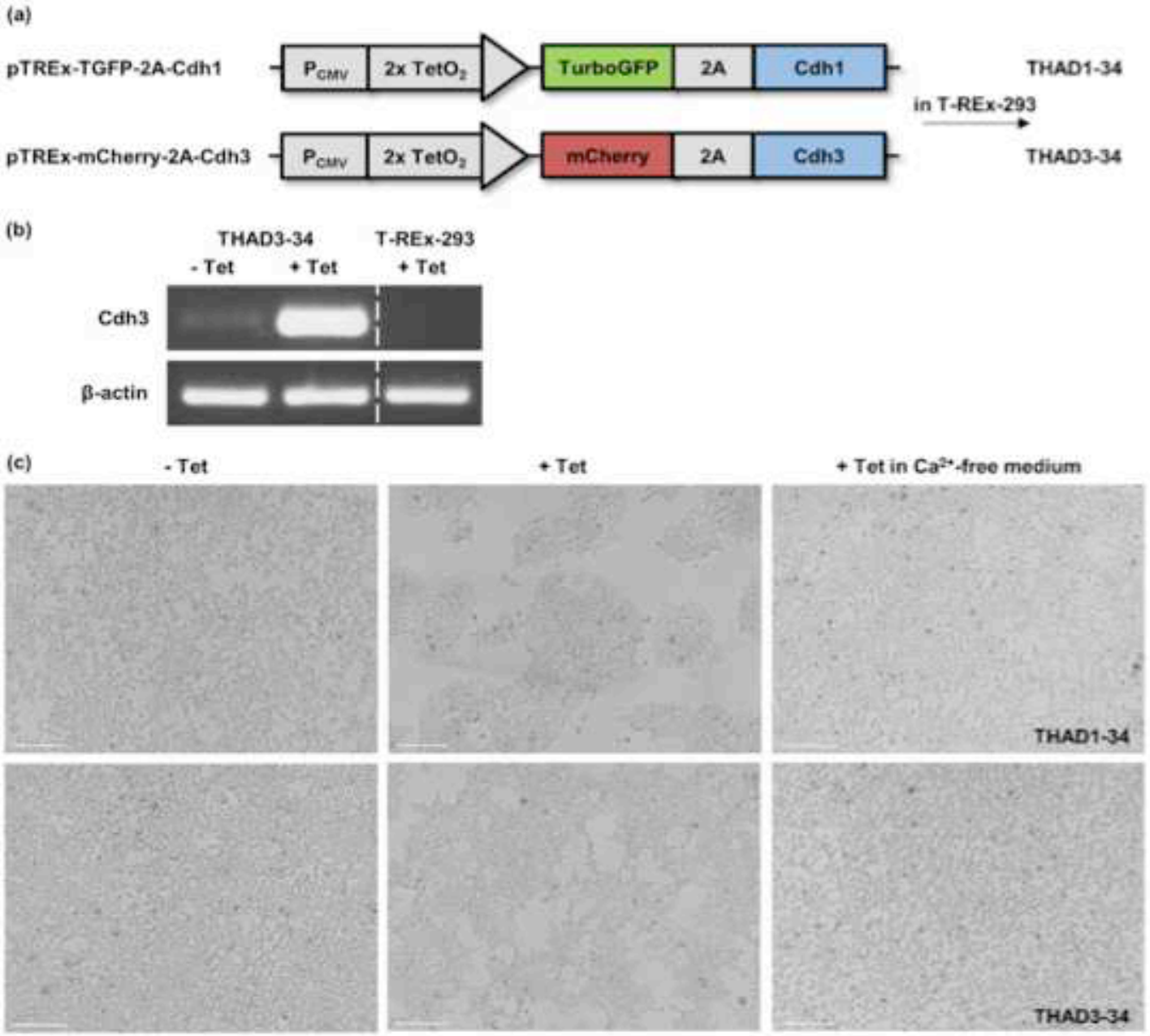
So we can add repulsive signals in

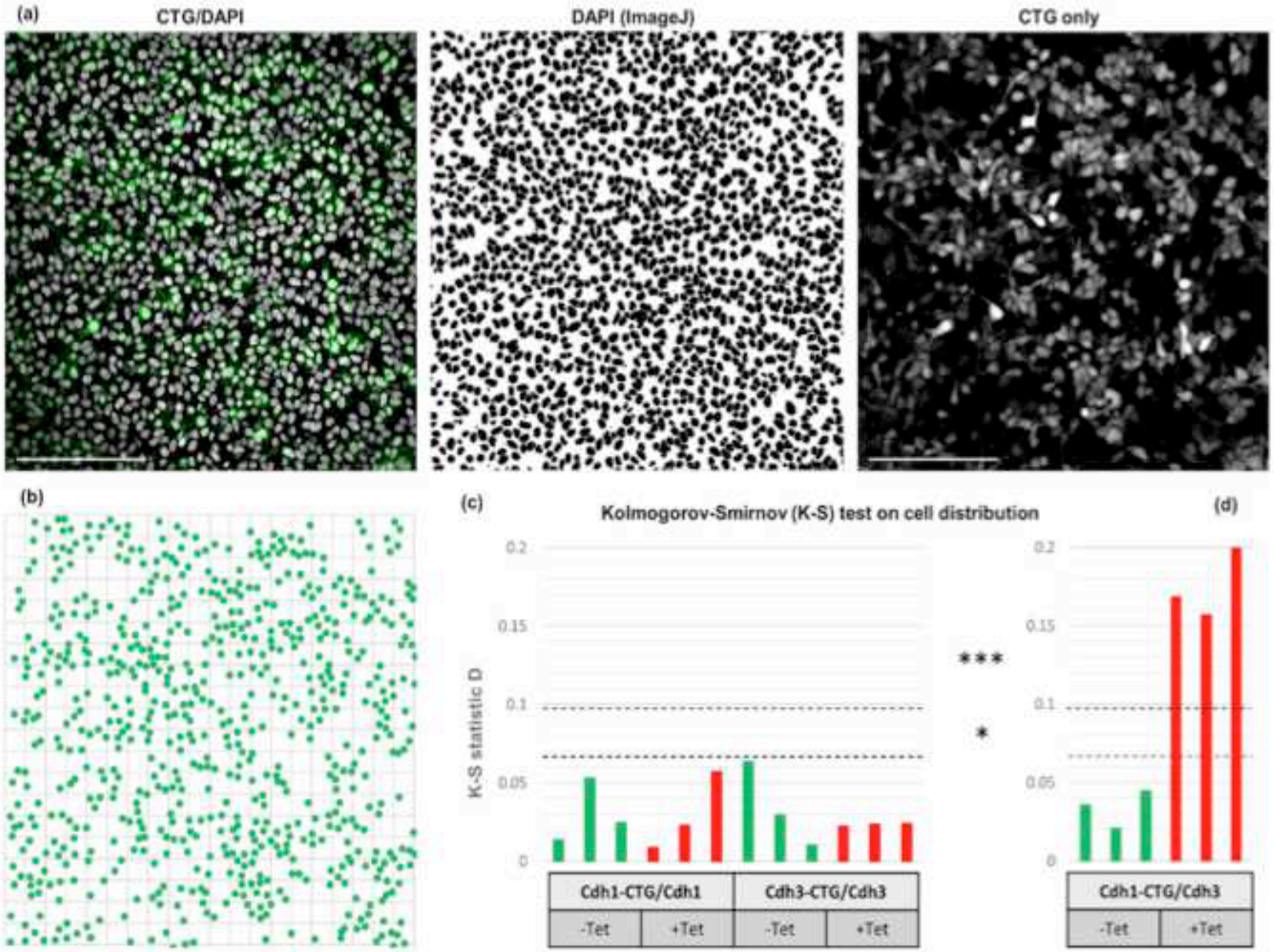


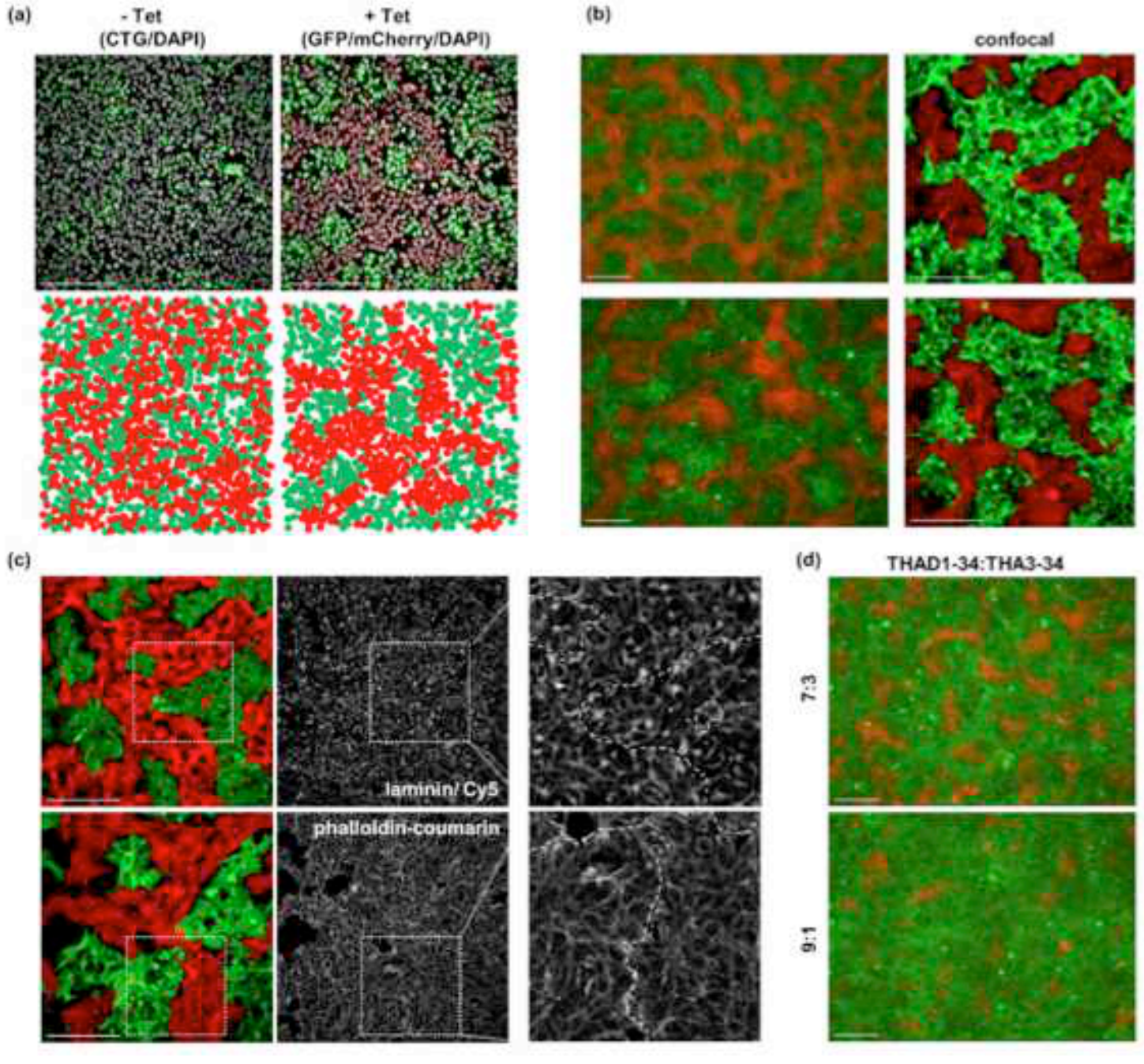
Synthetic biology: teaching human cells new ways of patterning:

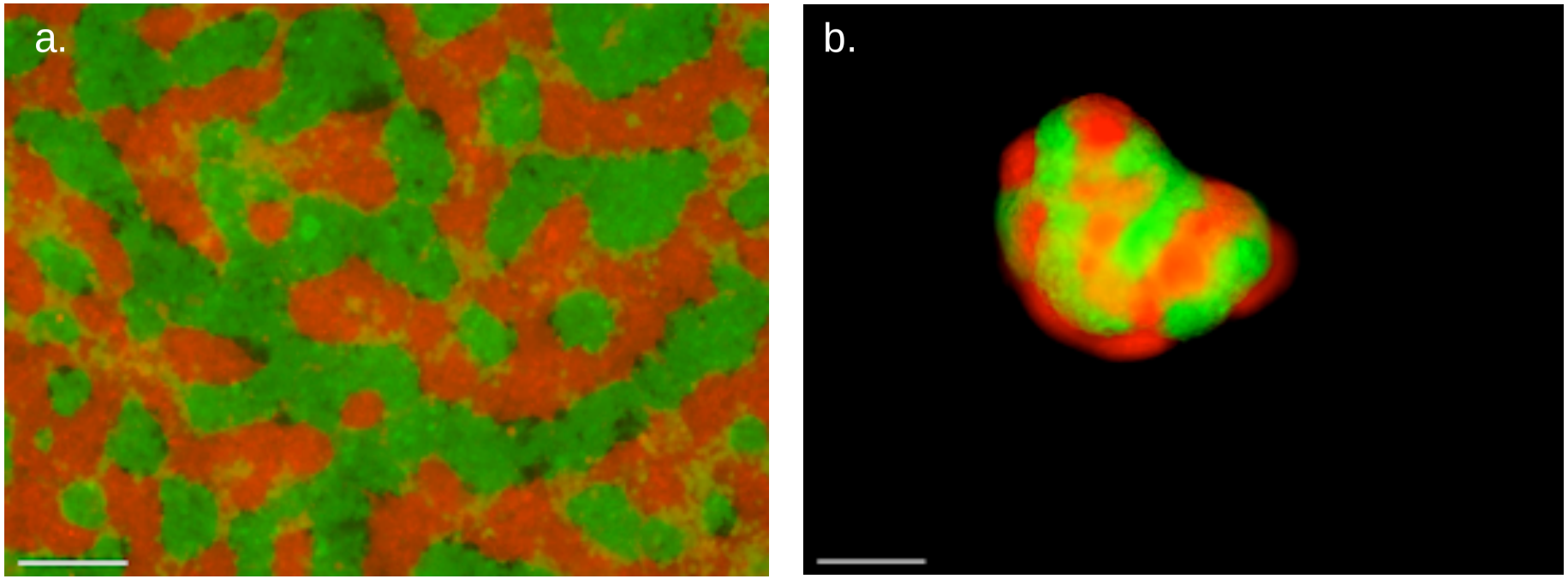
Phase separation:

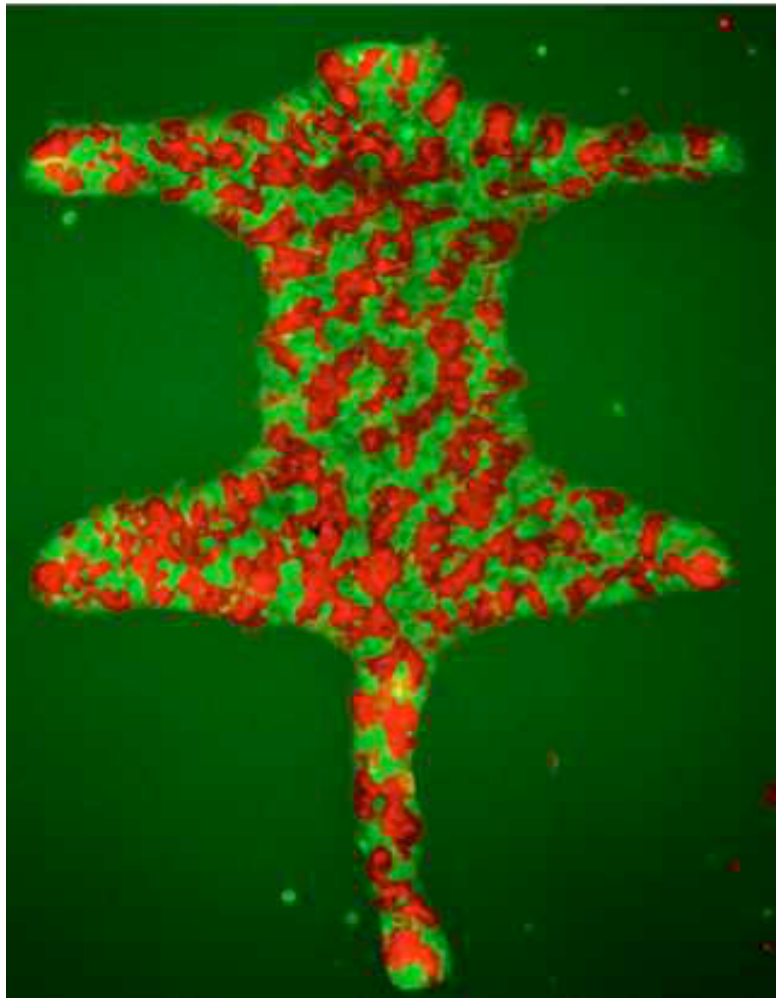

(a)
(b)
(c)
(a')
(b')
(c')
50/50 mix, AA=BB=AB
50/50 mix, AA>BB>AB
90/10 mix, AA>BB>AB

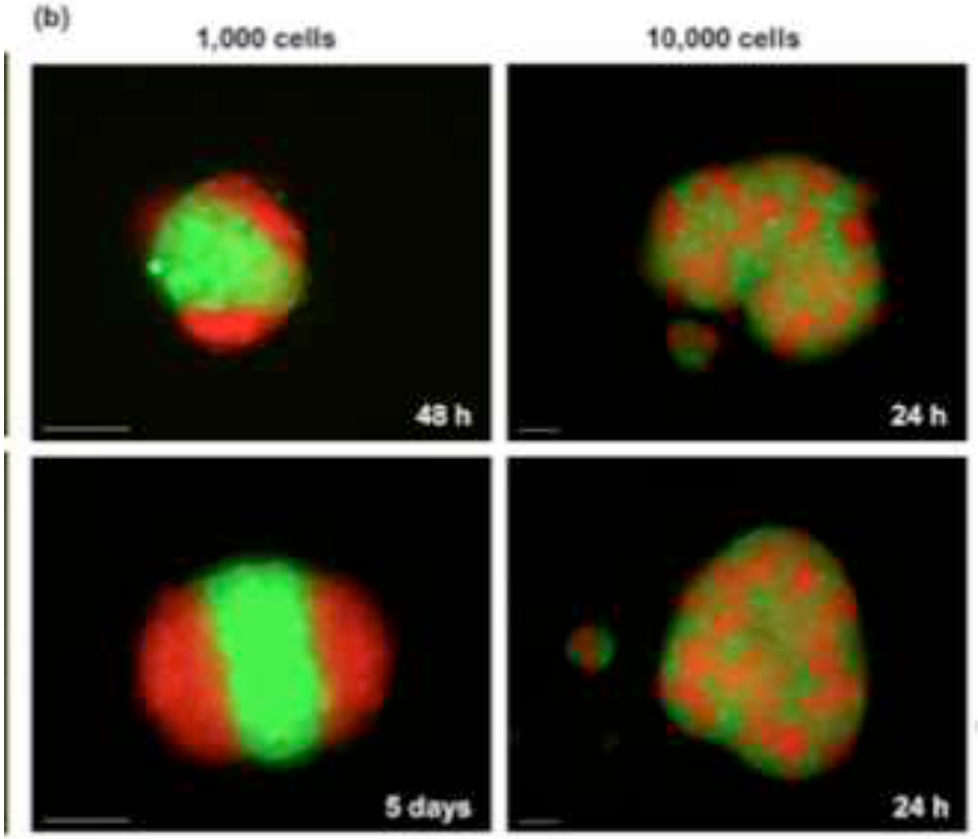

(a)
pTREx-TGFP-2A-Cdh1
P CMV
2x TetO2
TurboGFP
2A
Cdh1
THAD1-34
in T-REx-293
pTREx-mCherry-2A-Cdh3
P CMV
2x TetO2
mCherry
2A
Cdh3
THAD3-34
(b)
THAD3-34
T-REx-293
- Tet
+ Tet
+ Tet
Cdh3
β-actin
(c)
- Tet
+ Tet
+ Tet in Ca2+-free medium
THAD1-34
THAD3-34


(a)
CTG/DAPI
DAPI (ImageJ)
CTG only
(b)
(c)
Kolmogorov-Smirnov (K-S) test on cell distribution
K-S statistic D
0.2
0.15
0.1
0.05
0
Cdh1-CTG/Cdh1
Cdh3-CTG/Cdh3
-Tet
+Tet
-Tet
+Tet
***
*
(d)
0.2
0.15
0.1
0.05
0
Cdh1-CTG/Cdh3
-Tet
+Tet

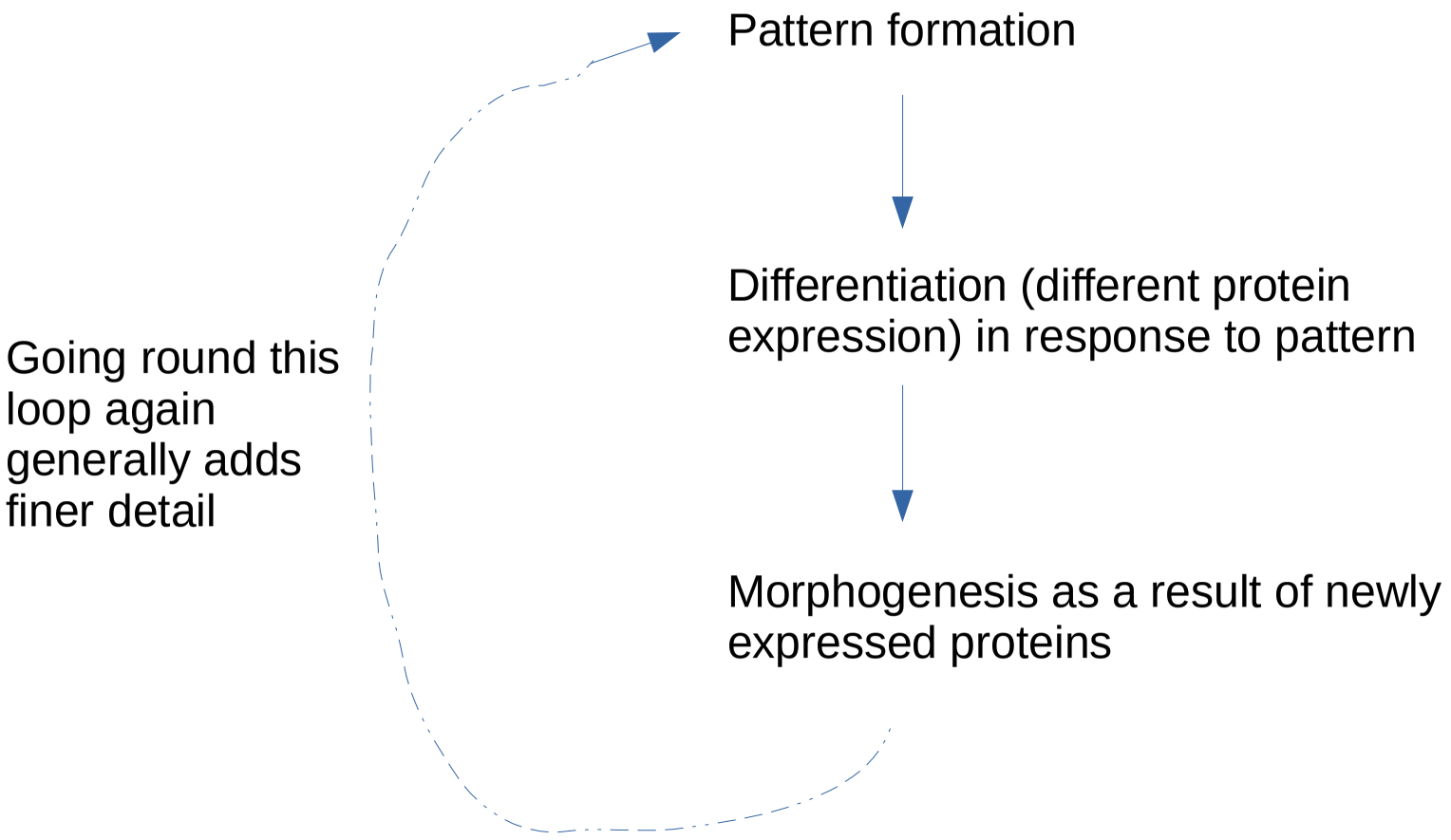

(a)
- Tet
(CTG/DAPI)
+ Tet
(GFP/mCherry/DAPI)
(b)
confocal
(c)
laminin/ Cy5
phalloidin-coumarin
(d)
THAD1-34:THA3-34
7:3
9:1


a.
b.



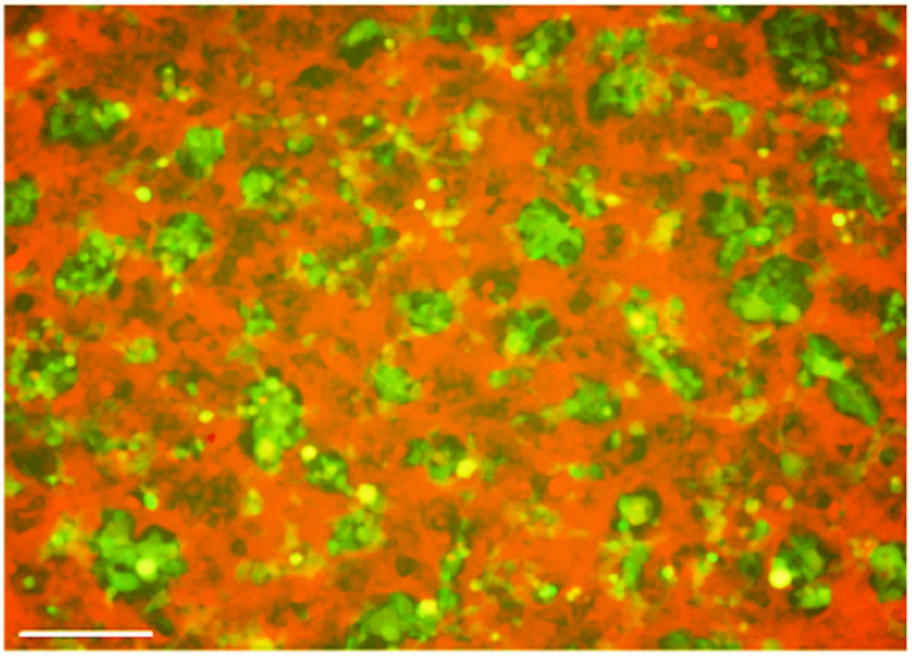

(b)
1,000 cells
10,000 cells
48 h
24 h
5 days
24 h

Animal development generally works like this :

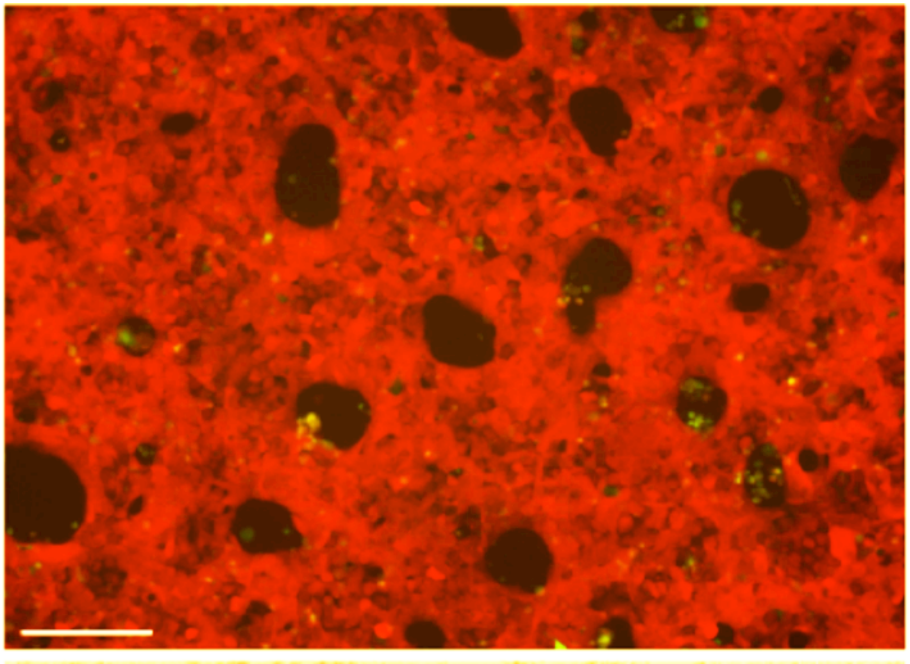


The green cells have a tamoxifen-inducible apoptosis system in it:

First pattern, then induce the death.



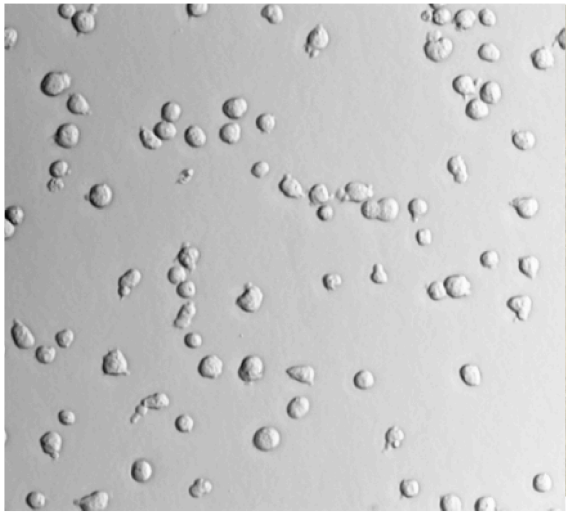
No Tamoxifen

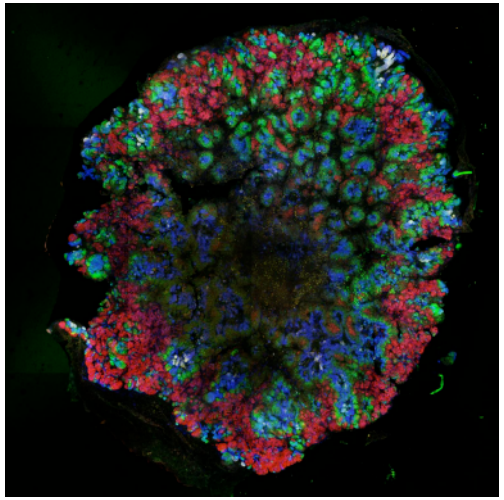


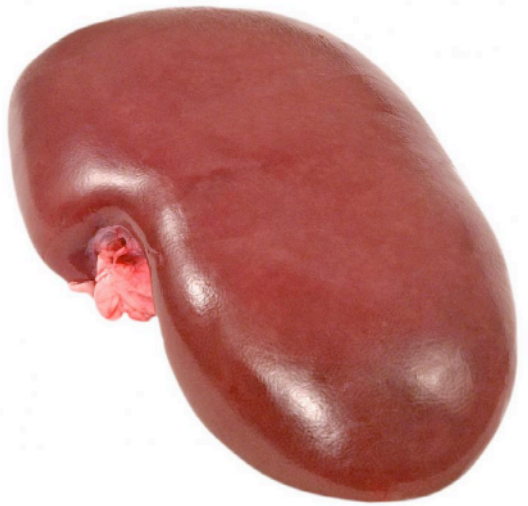
With Tamoxifen

Cachat, Liu, Davies (2017)

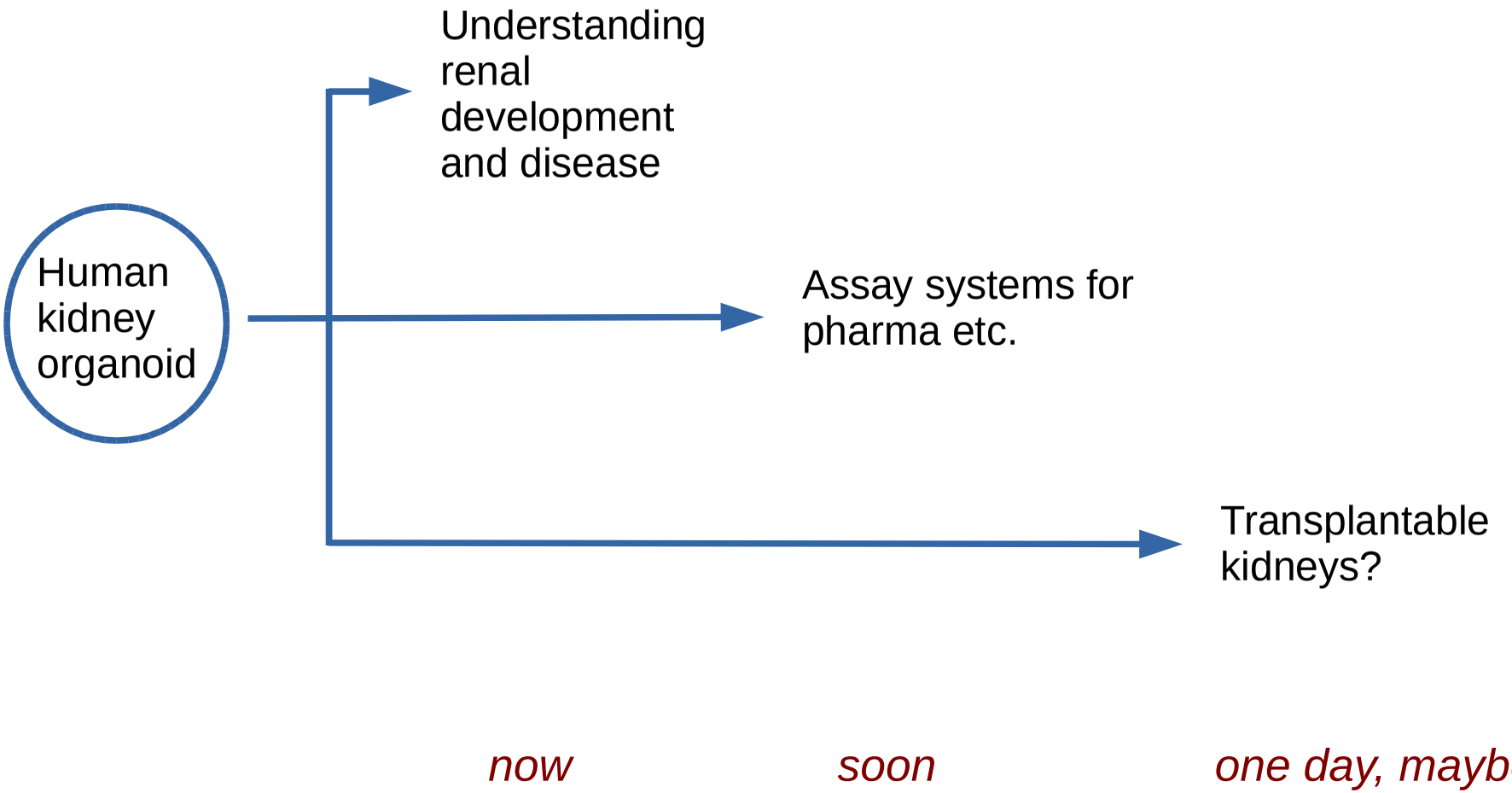
Can we use self-organization for useful purposes?





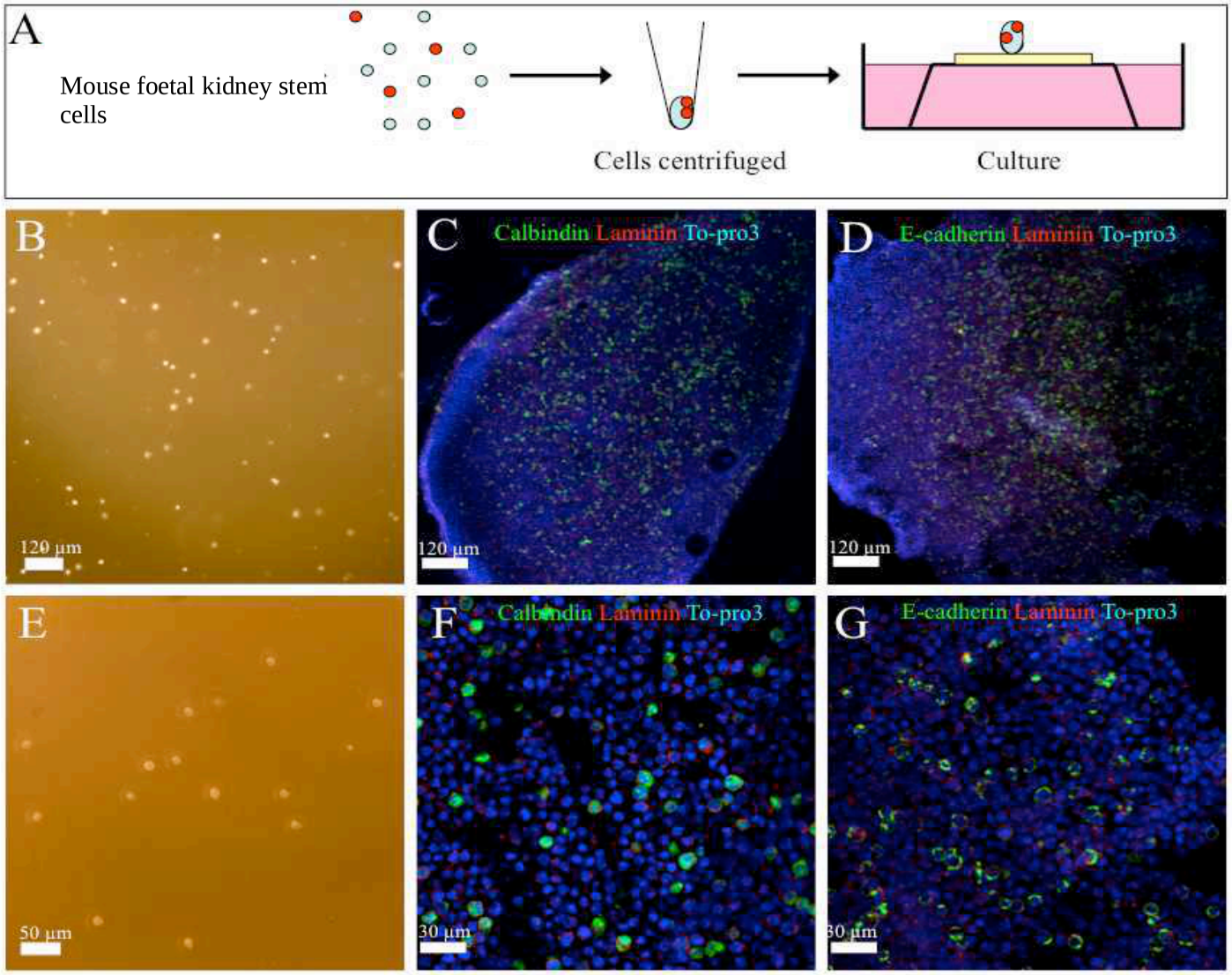


Why make kidney organoids?



A bit of history: our path to the first renal organoids (publ. 2010).

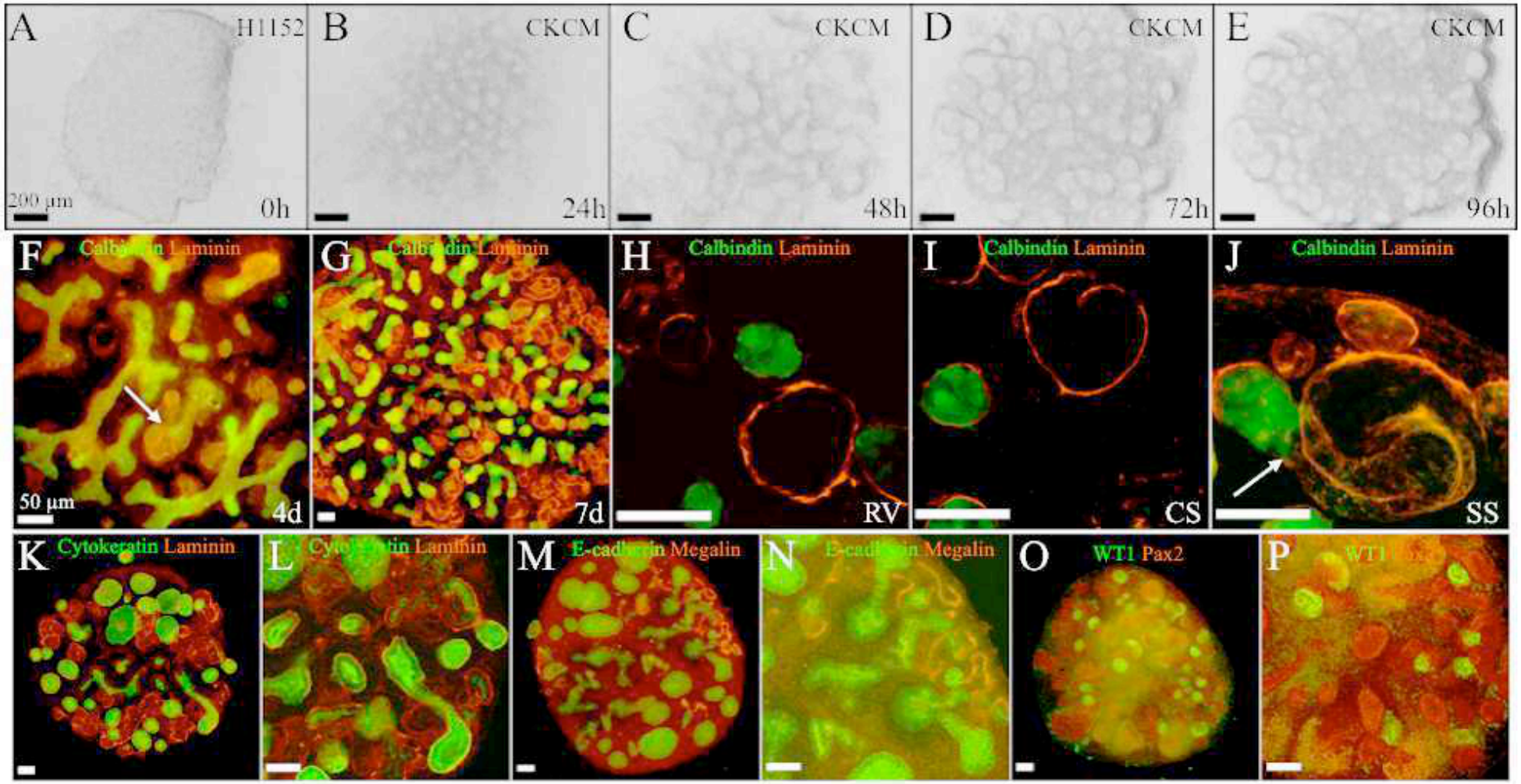
Mathieu Unbekandt



... they died (anoikis)

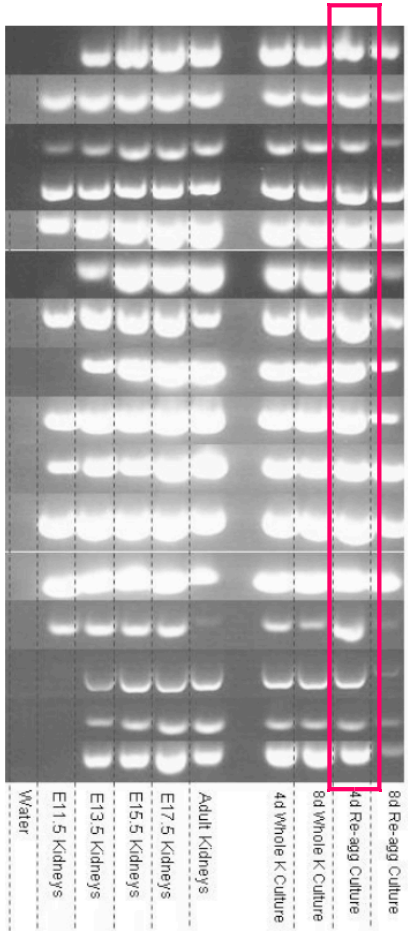
(… sigh)

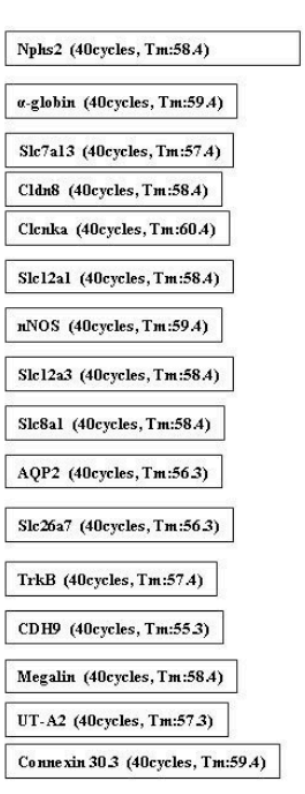
But they got by “with a little help from their friends”



Unbekandt M, Davies JA. *Kidney Int*. 2010 Mar;77(5):407-16.

We seemed to have all of the cell types we expect:





podocytes

Mesangium

(proximal and distal tubules)

desc thick limb

asc thin limb

thick asc limb

macula densa

distal conv tubule

connecting tubule

coll duct princ cell

coll duct intercalated type A

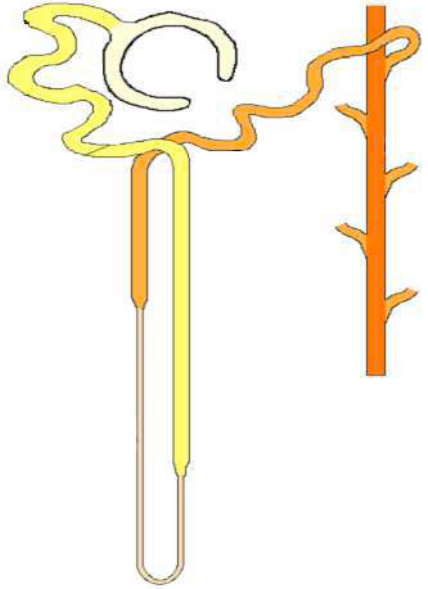
JG cells

stroma

proximal convoluted tubule

desc thin loop

asc thin limb



-ve ctrl

+ve controls

Re-aggregates

White line means 'present'

These are the cell types for which we are testing

National Museums Scotland

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Adva

Image © National Museums Scotland

Description

Mini mouse kidney grown from stem cells
Davies and Dr Melanie Lawrence, Univers

Museum reference

T.2015.135

Collection

History of Science

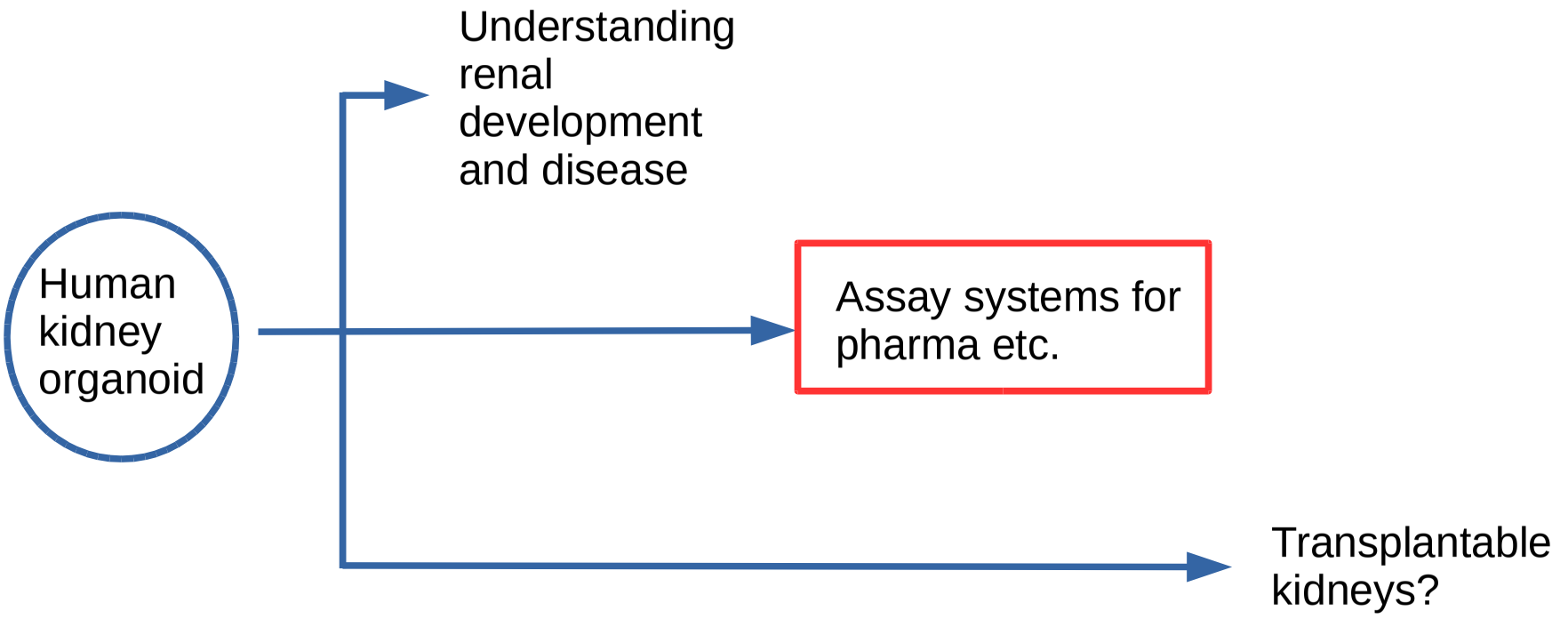
Object name

Kidney

Production information

Davies, Jamie, Dr, 2015 (fl.), Maker
Edinburgh, Midlothian, Scotland, EUROPE
Lawrence, Melanie, Dr, 2015 (fl.)

Why?


Understanding renal development and disease
Human kidney organoid
Assay systems for pharma etc.
Transplantable kidneys?
now
soon
one day, maybe

iPSC-derived renal organoids

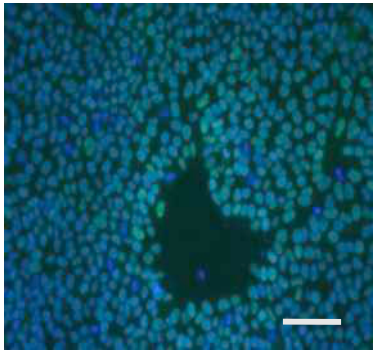
Differentiation protocol and characterisation of renal pseudo-2D tissue

a

Protocol adapted from Takasato et al. 2015

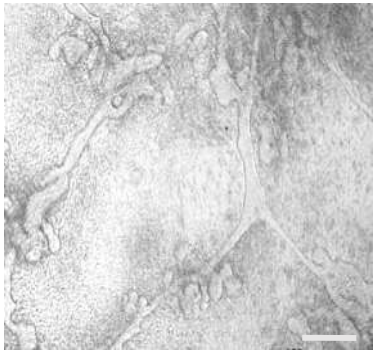
b

OCT3/4, DAPI



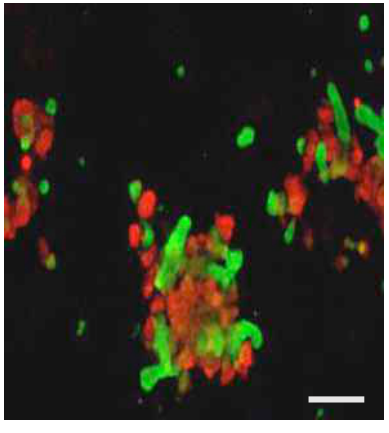
C

Bright field image

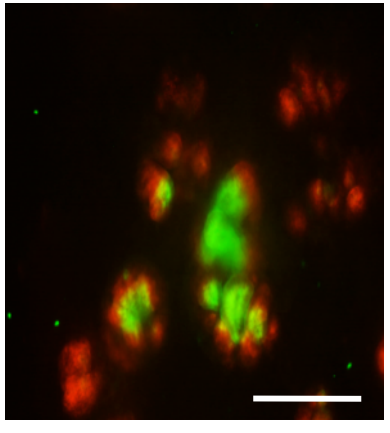


d

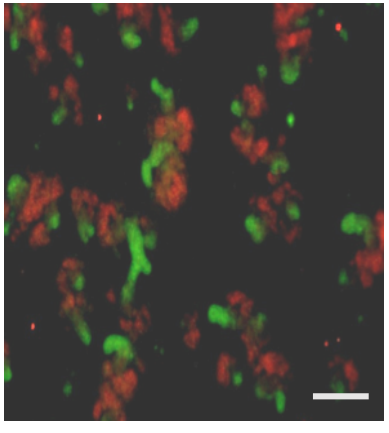
WT1, ECAD



Jagged1, LTL



NPHS1, ECAD



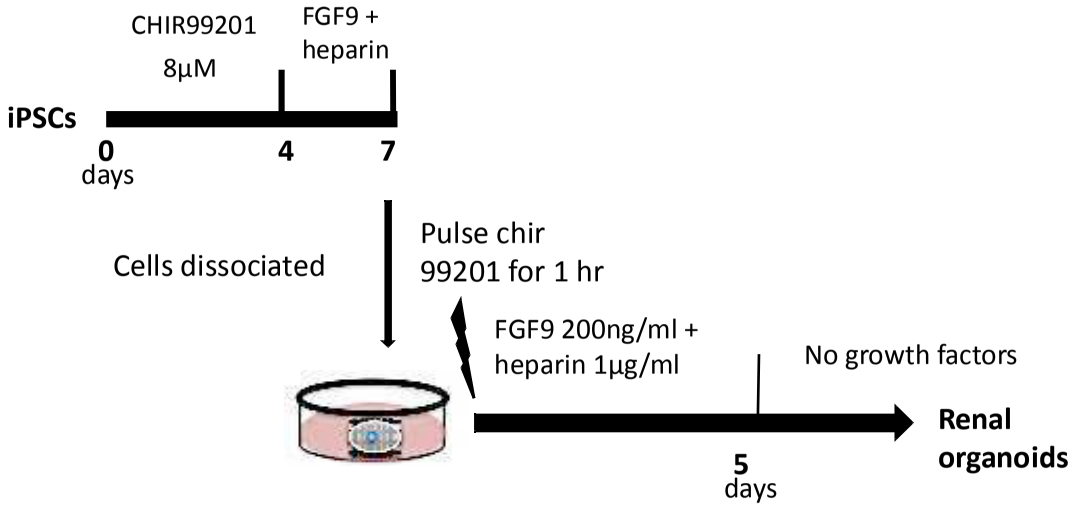
Scale bars = 200µm

iPSC-derived renal organoids

a

Renal organoids

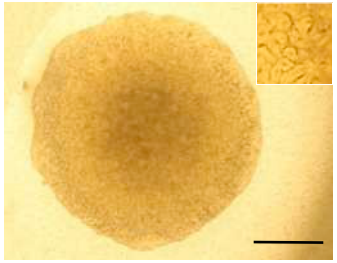
Monolayer differentiation of the iPSCs for 7 days



Cultured at air fluid interface

b

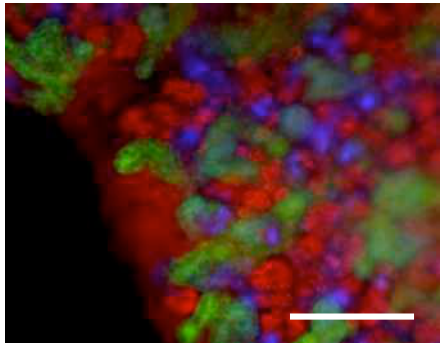
Bright field image



Size marker = 1mm

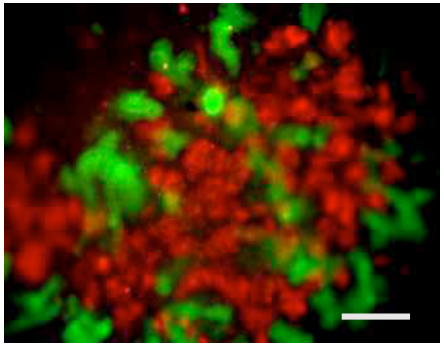
c

WT1, ECAD, LTL



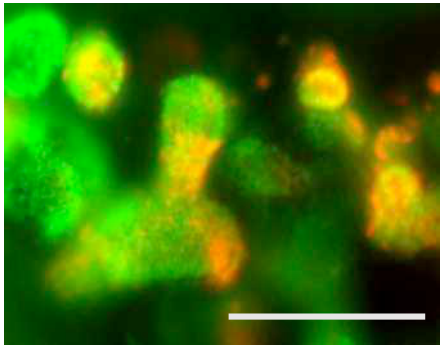
d

NPHS1, ECAD



e

CALB, GATA3



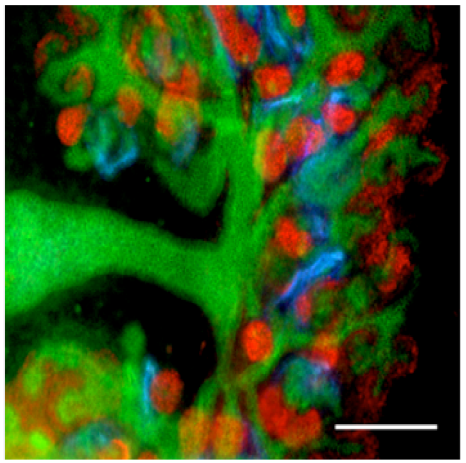
Size markers = 200um

More images of renal human iPS cells

Photo credits: Weijia Liu Mona Elhendawy

a

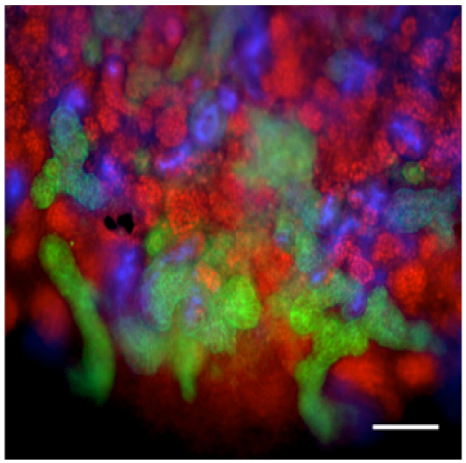
WT1, ECAD, LTL



Control (mouse kidney)

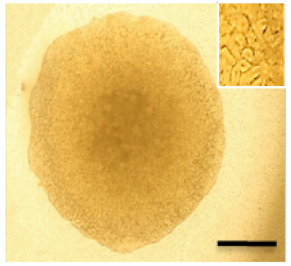
b

WT1, ECAD, LTL



b

Bright field image

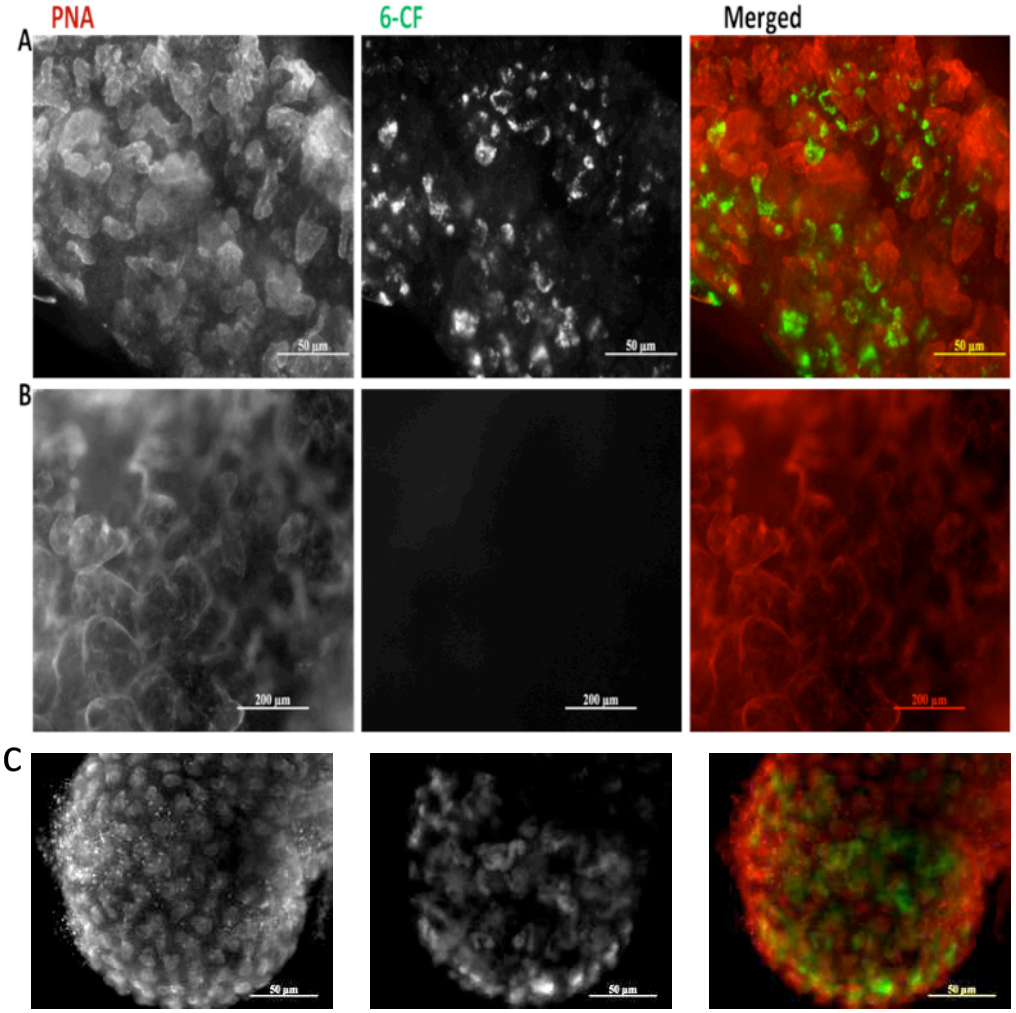


hiPS-derived

Images from the book ‘’Organoids and mini-organs’’.

Expression of organic Anion and cation transporters

6 CF anion uptake test for organoids



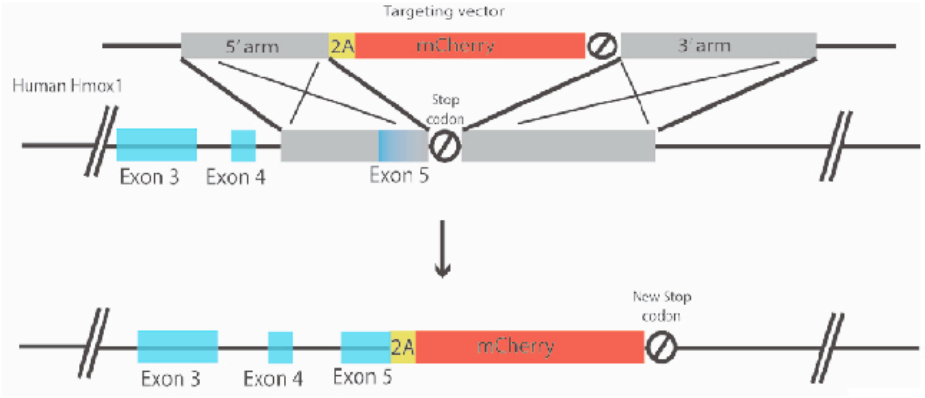
+inhibitor

Control mouse kidney

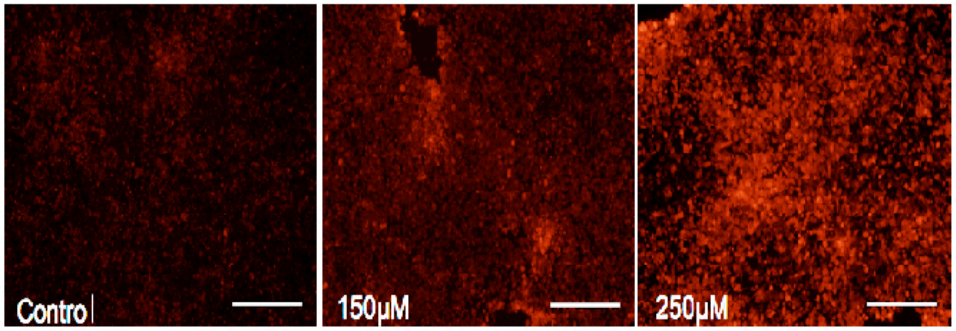
HMOX1-2A-mCherry as a stress reporter.

Melanie Lawrence

a.



b.



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Melanie Lawrence

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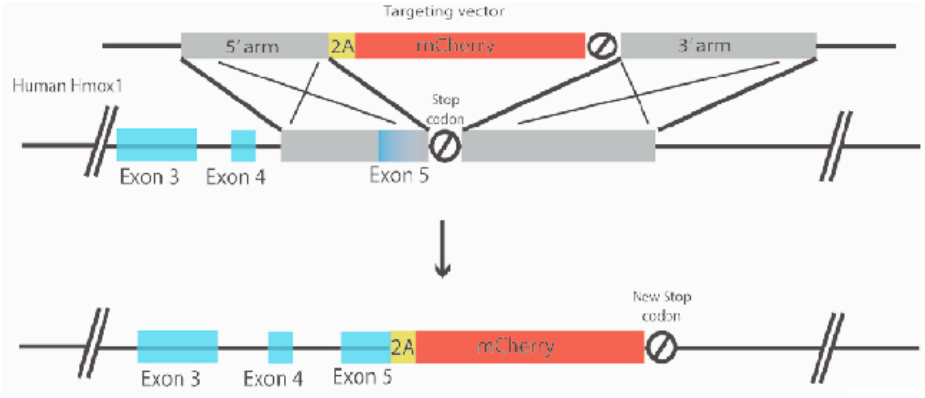

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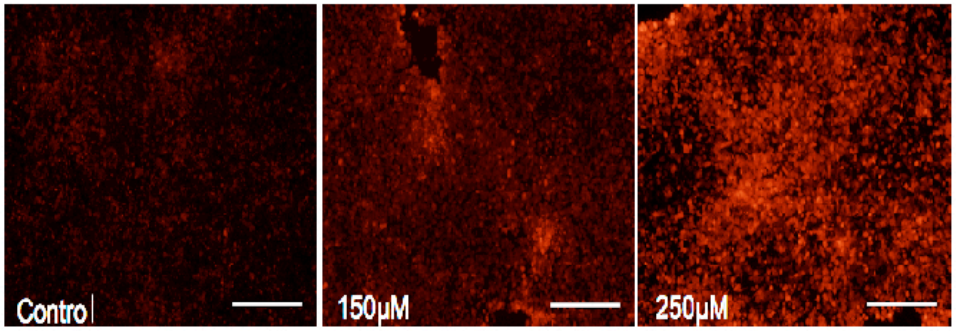
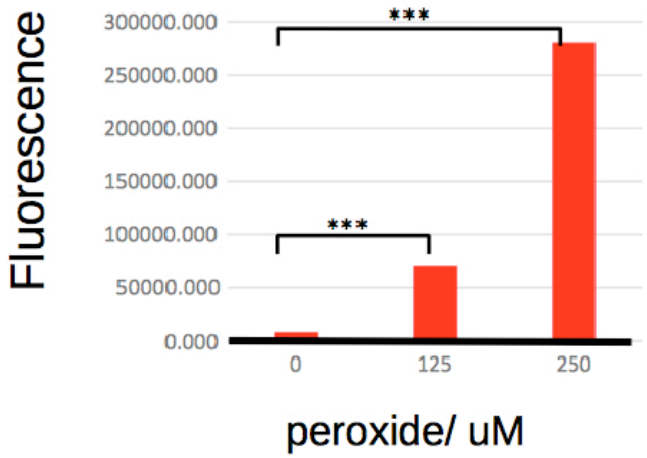


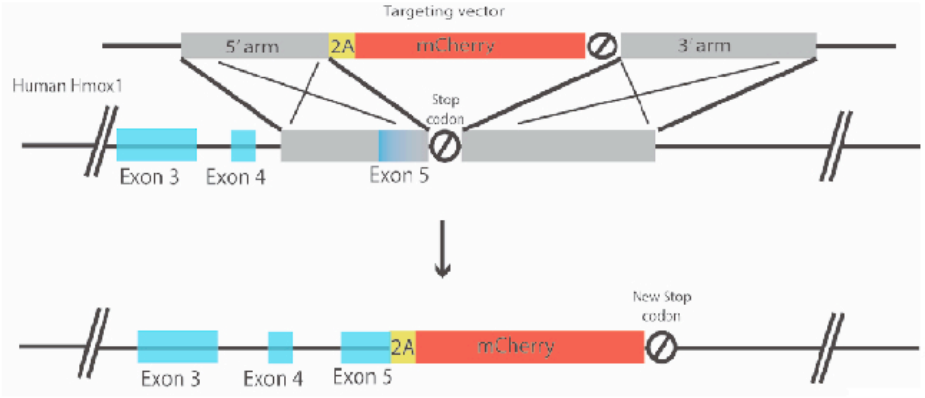
c.



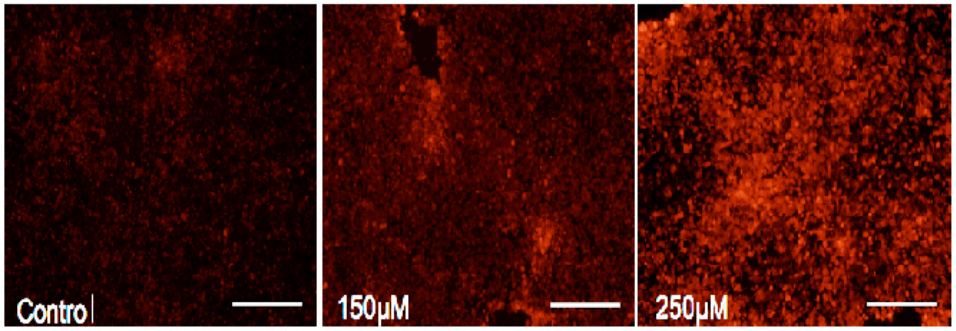
HMOX1-2A-mCherry as a stress reporter.

Melanie Lawrence

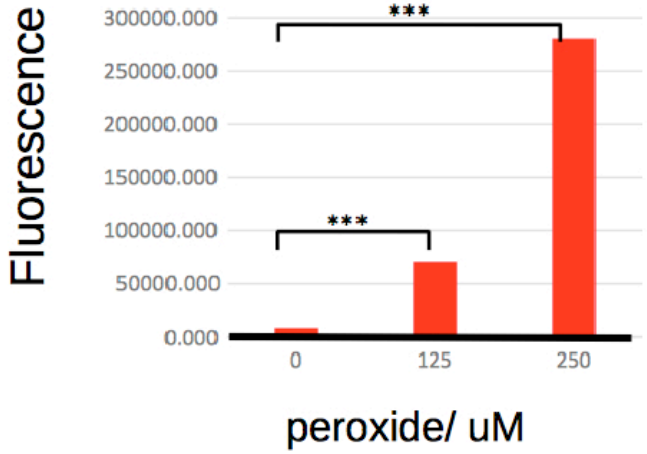
a.

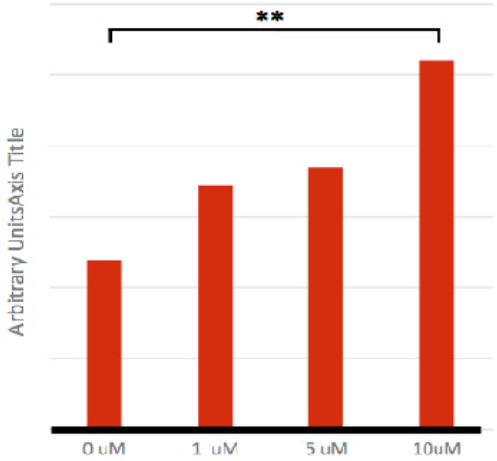


b.



c.





HMOX1-2A-mCherry as a stress reporter.

Melanie Lawrence

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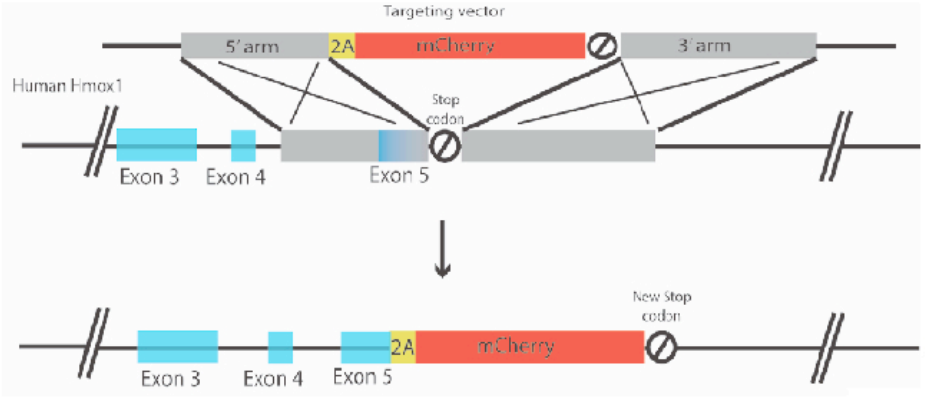

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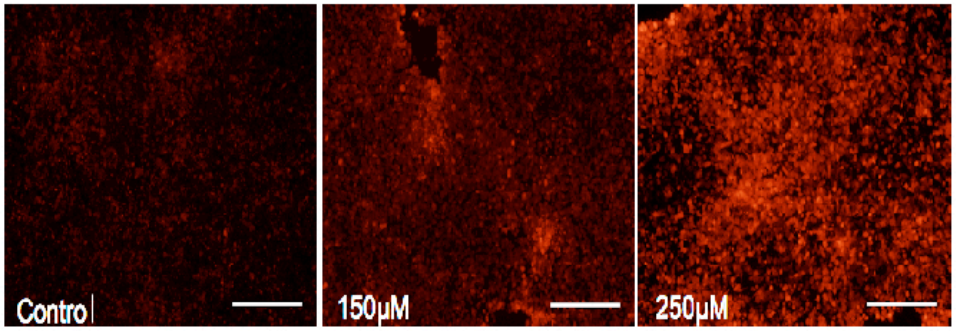

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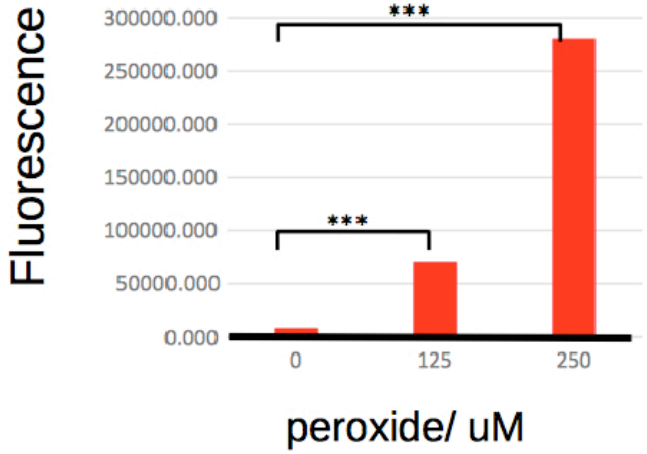
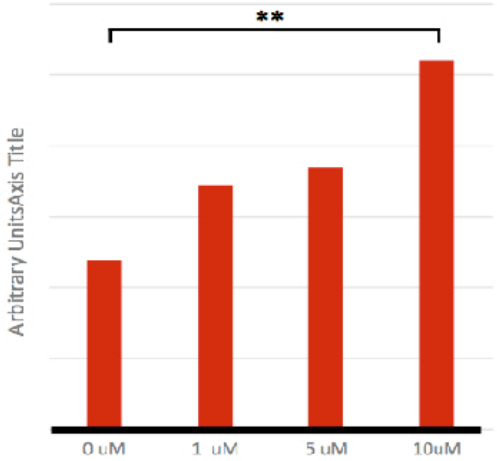
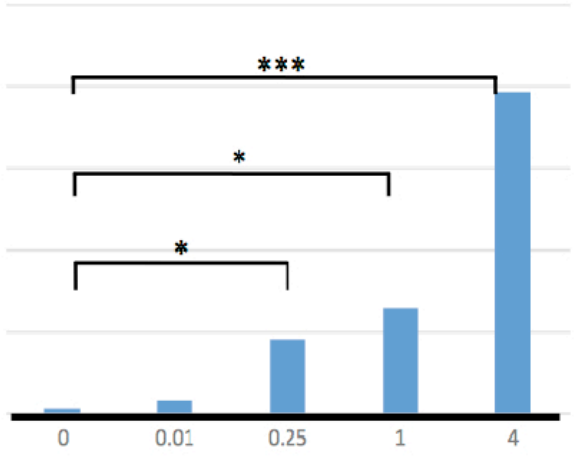
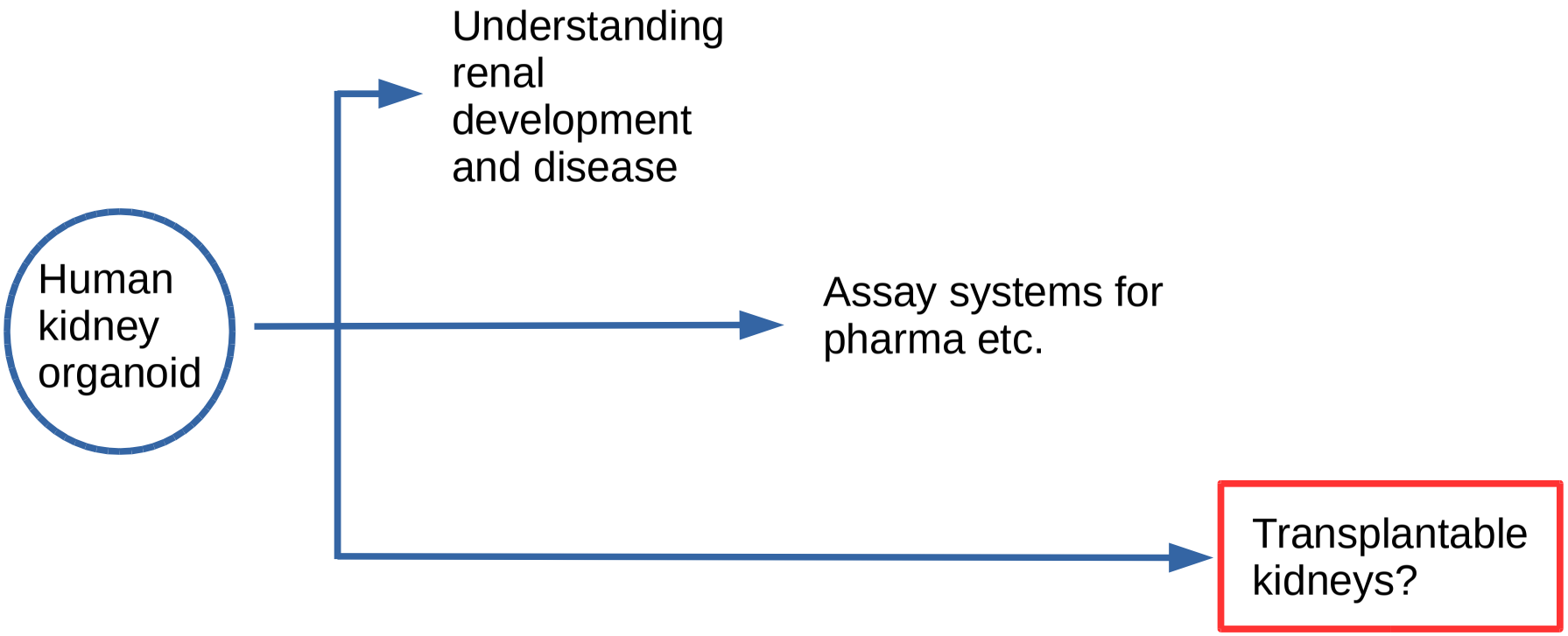
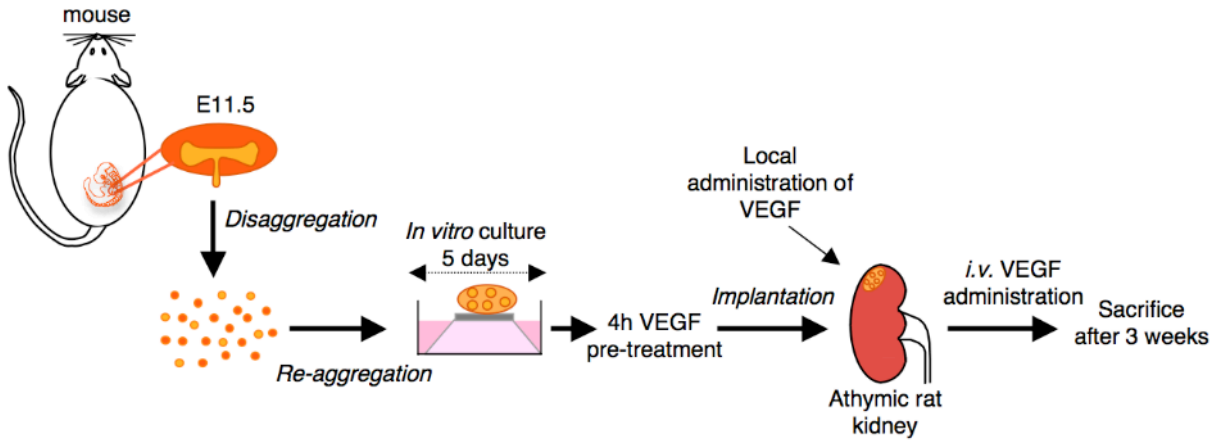




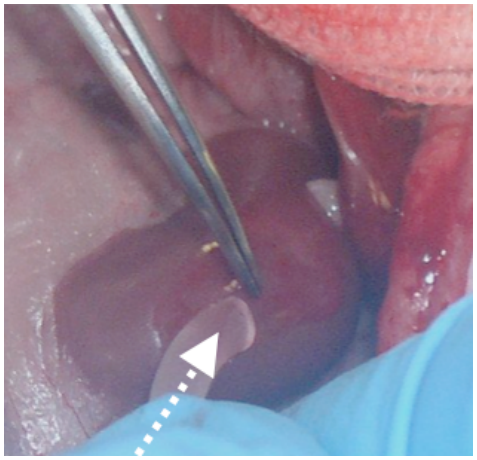



Understanding renal development and disease
Human kidney organoid
Assay systems for pharma etc.
Transplantable kidneys?
now
soon
one day, maybe

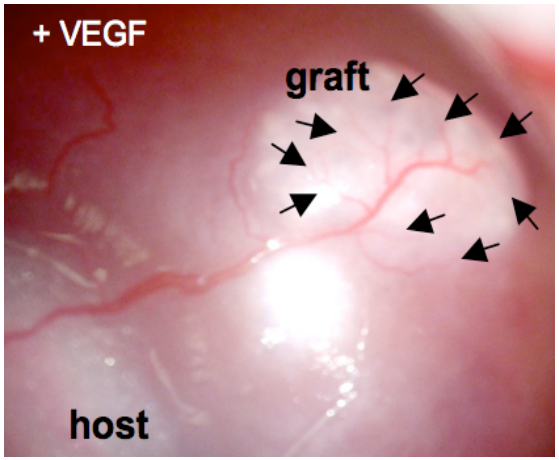
With our colleagues in Italy, we tested mouse organoids in rats:



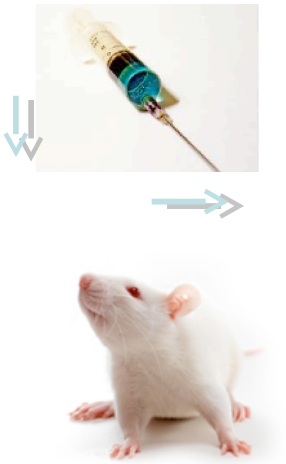
Grafting



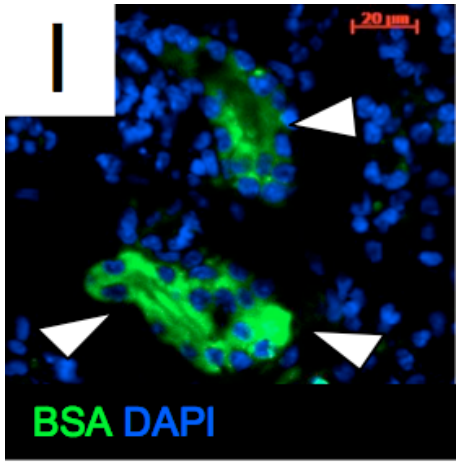
After some weeks



i.v. injection of fluorescent SA



SA turns up in prox tubule



Xinariset al. (2012) *J Am. Soc Neprhol* 2012 Nov;23(11):1857-68.

This work has recently been repeated (by others) with human organoids:

Stem Cell Reports. 2018 Mar 13;10(3):751-765. doi: 10.1016/j.stemcr.2018.01.041. Epub 2018 Mar 1.

Renal Subcapsular Transplantation of PSC-Derived Kidney Organoids Induces Neo-vasculogenesis and Significant Glomerular and Tubular Maturation In Vivo.

van den Berg CW[1], Ritsma L[2], Avramut MC[3], Wiersma LE[4], van den Berg BM[4], Leuning DG[4], Lievers E[4], Koning M[4], Vanslambrouck JM[5], Koster AJ[3], Howden SE[6], Takasato M[7], Little MH[6], Rabelink TJ[4].

Stem Cell Reports. 2018 Mar 13;10(3):766-779. doi: 10.1016/j.stemcr.2018.01.008. Epub 2018 Feb 8.

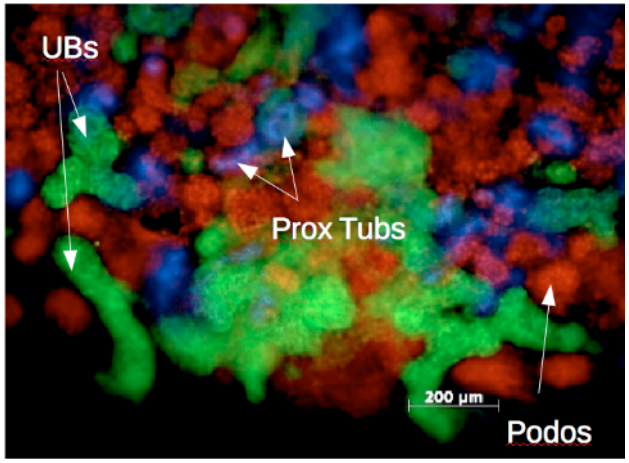
Generation of Functioning Nephrons by Implanting Human Pluripotent Stem Cell-Derived Kidney Progenitors.

Bantounas I[1], Ranjzad P[2], Tengku F[1], Silajdžić E[1], Forster D[3], Asselin MC[3], Lewis P[1], Lennon R[4], Plagge A[5], Wang Q[1], Woolf AS[2], Kimber SJ[6].

But there is a big problem with organoids:

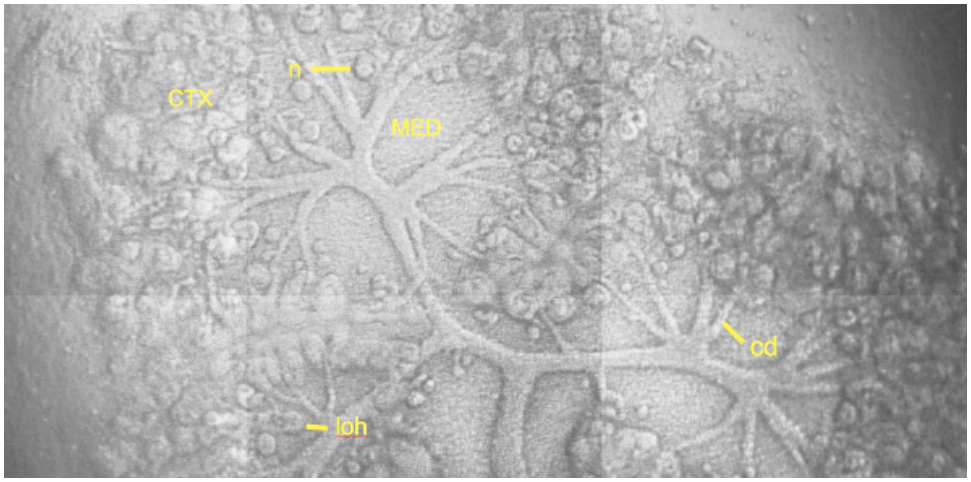
Photo credits: Weijia Liu Mona Elhendawy

This…



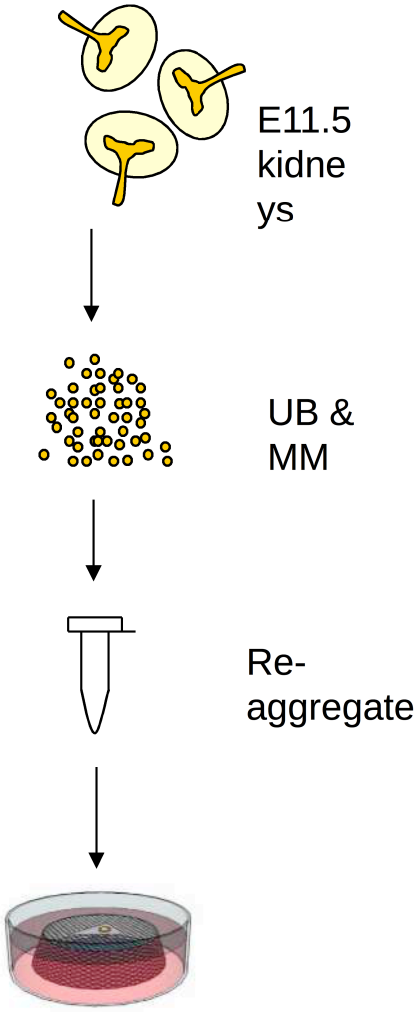
Renal organoid from human iPSC

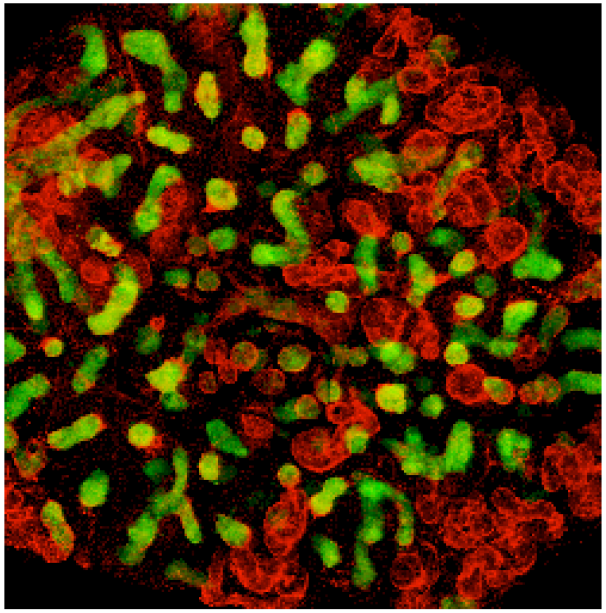
isn't this



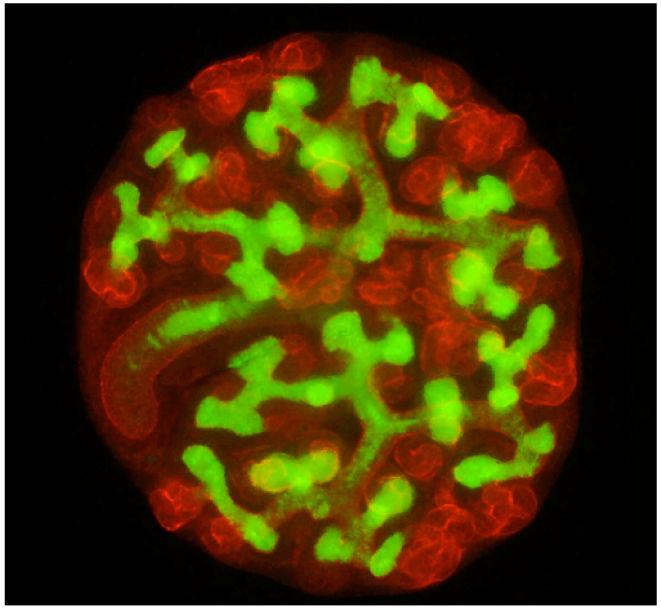
Organ culture

Self-organization has limits, good for micro-anatomy but not macro-.





engineered

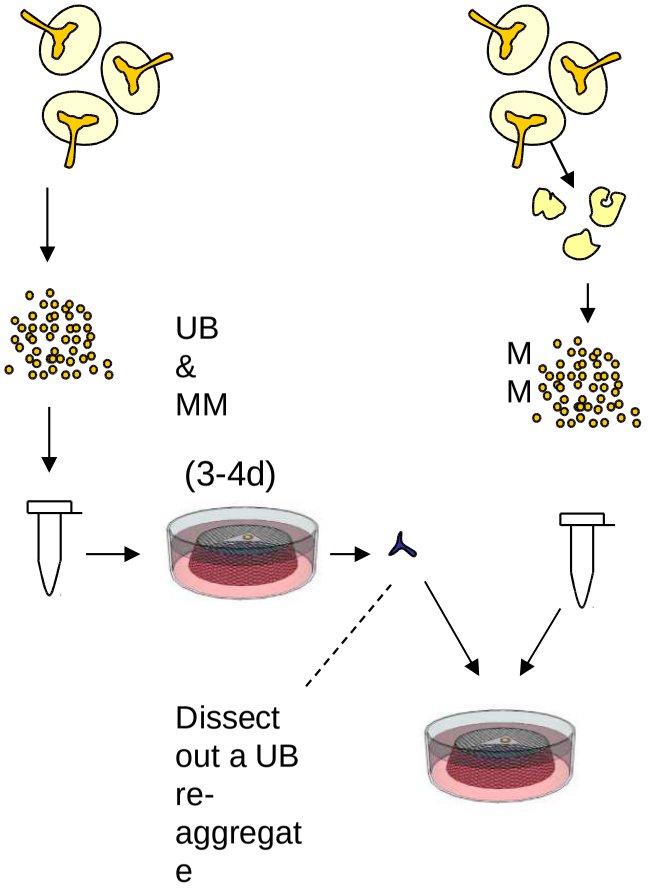


natural (1 tree)

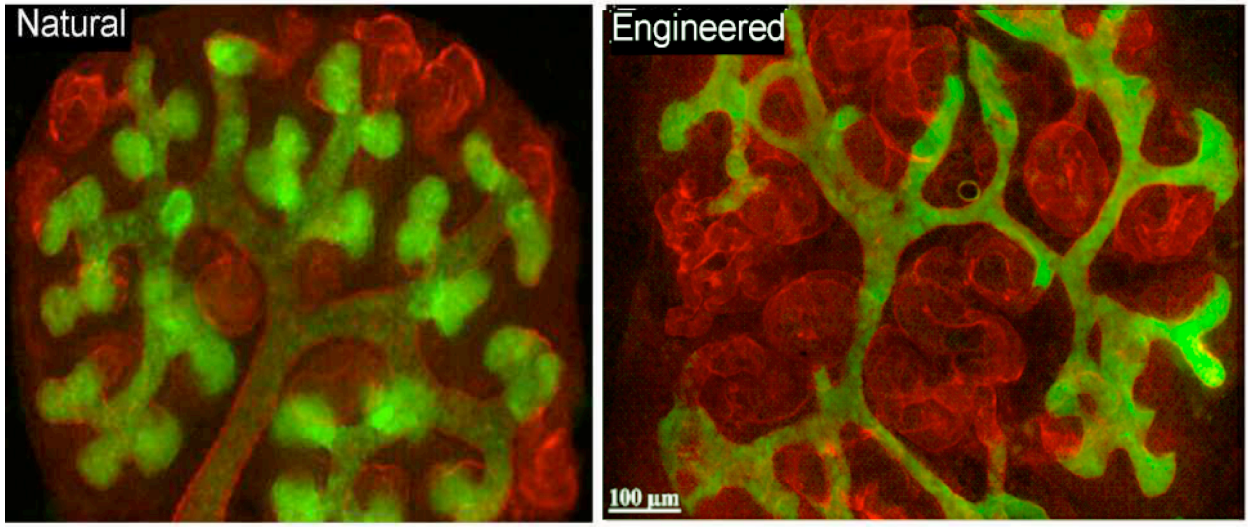
Unbekandt & Davies 2010

That's a problem: a kidney only makes sense if it has one urine collecting duct system.

A serial reaggregation system can yield a kidney based around a single collecting duct system.



Veronika Ganeva.

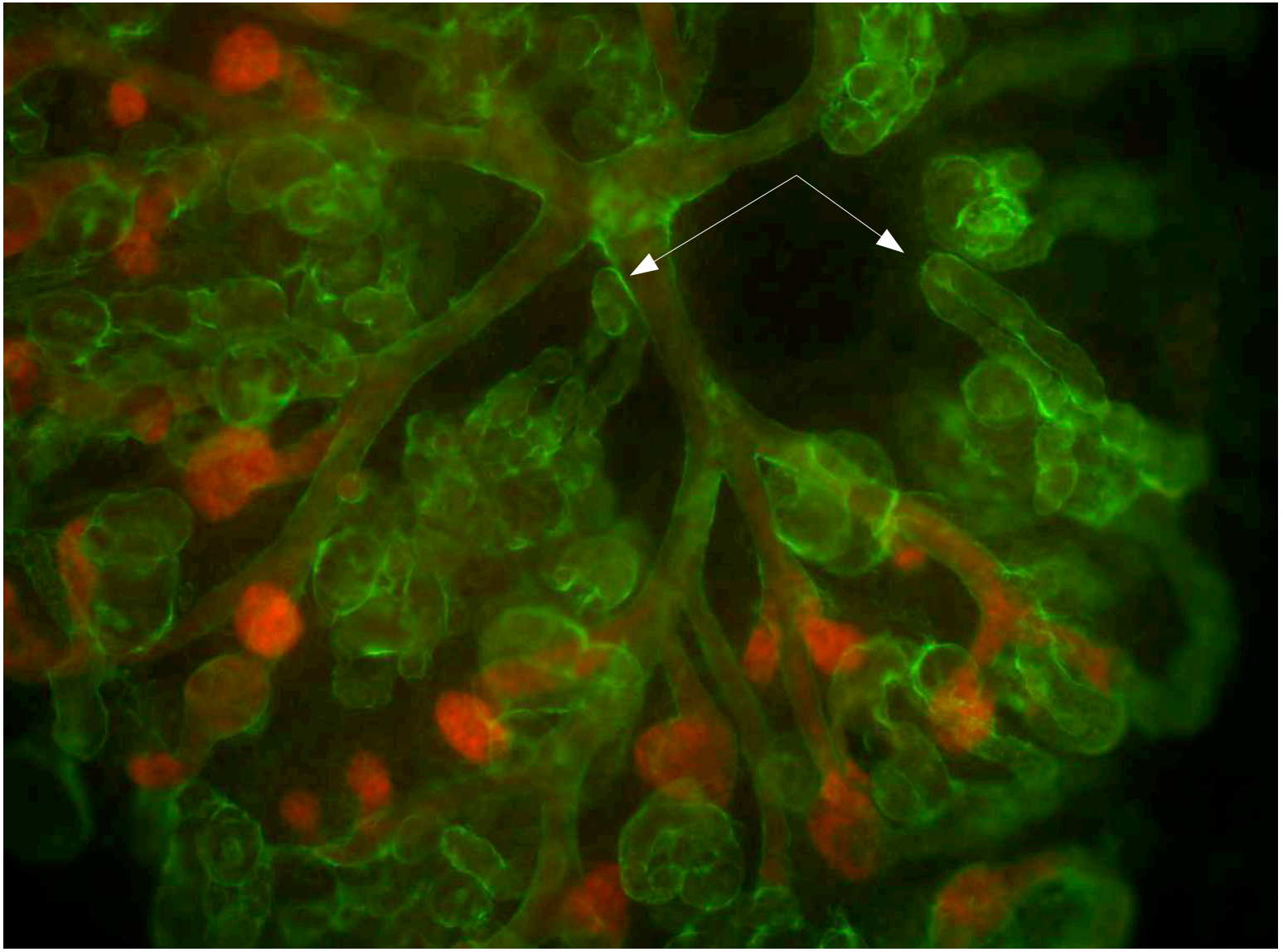


1 tree!

Ganeva V, Unbekandt M, Davies JA. *Organogenesis* 2011;7:83-7.

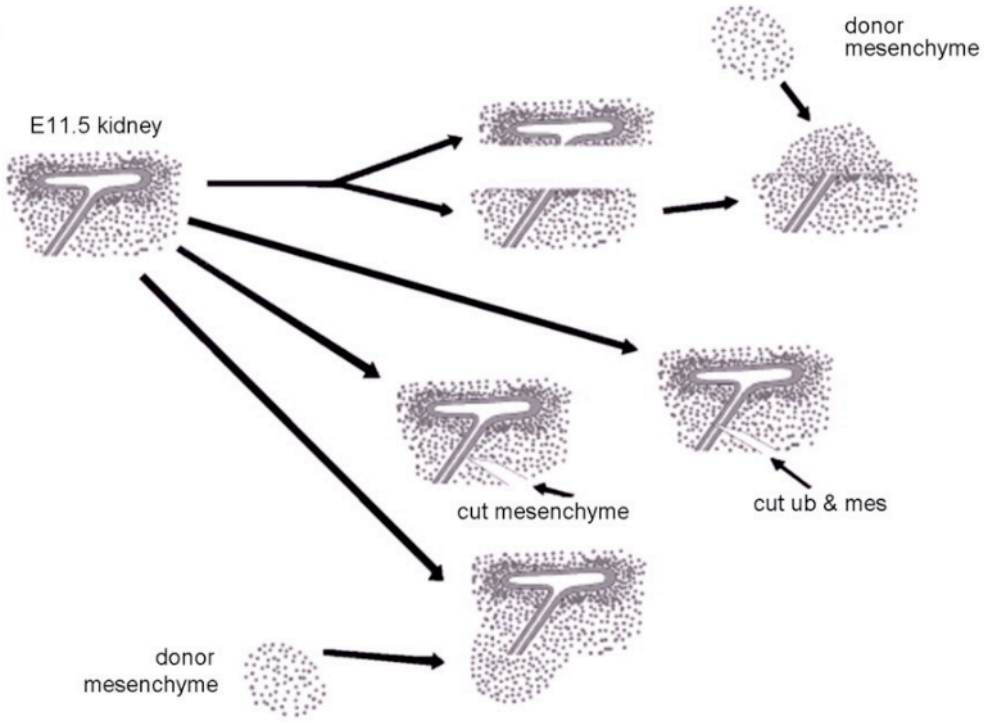
And with a few more tricks we can get more realism

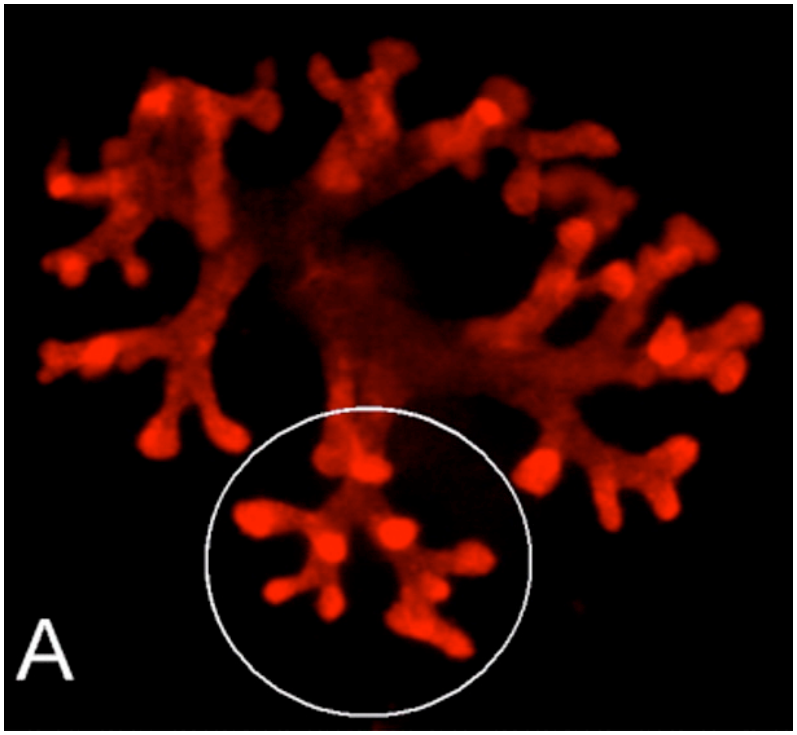
C-Hong Chang



But there is no ureter

Some years ago, we found evidence that the UB derivatives are plastic:





Development. 2008 Aug;135(15):2505-10. doi: 10.1242/dev.022145. Epub 2008 Jun 25.

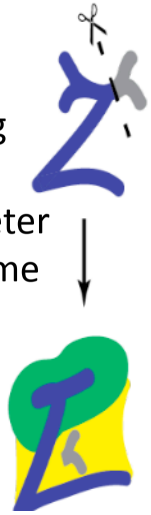
Developmental plasticity and regenerative capacity in the renal ureteric bud/collecting duct system.

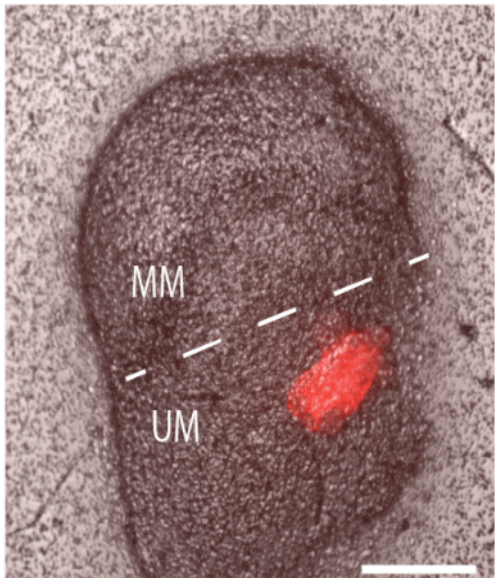
Sweeney D[1], Lindström N, Davies JA.

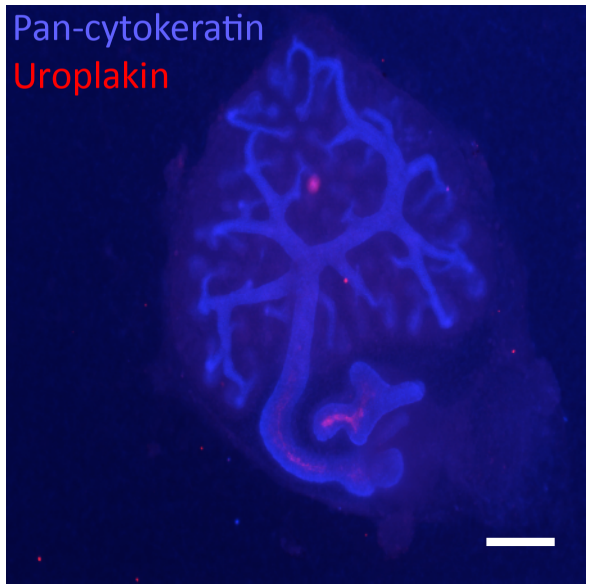
Does this work the other way round?

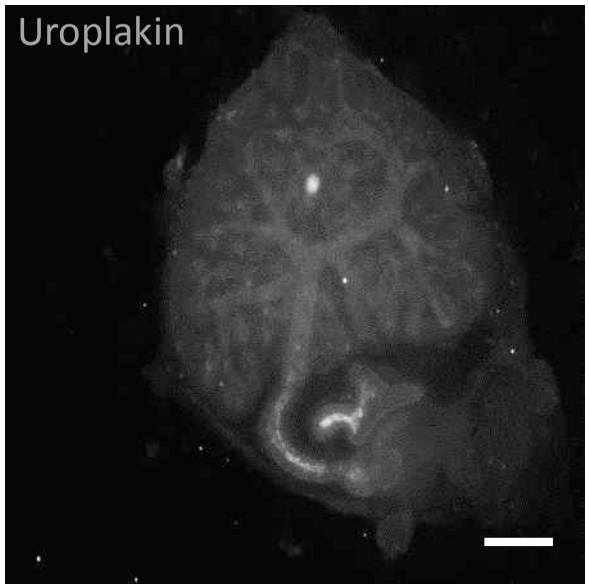
Chris Mills

Developing collecting duct to ureter mesenchyme

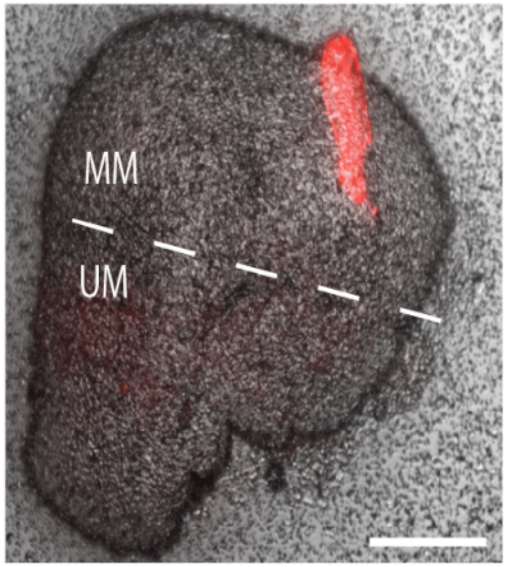


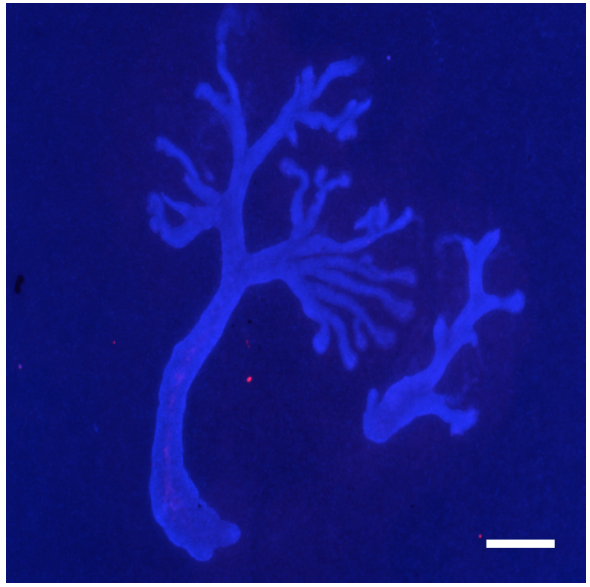


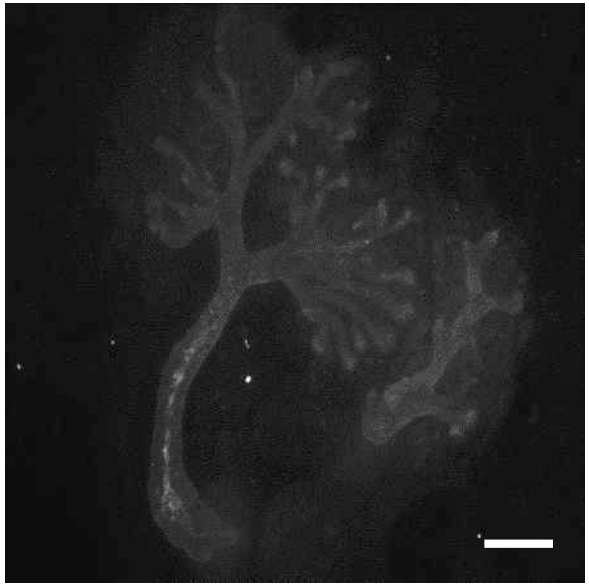




Developing ureter to metanephric mesenchyme

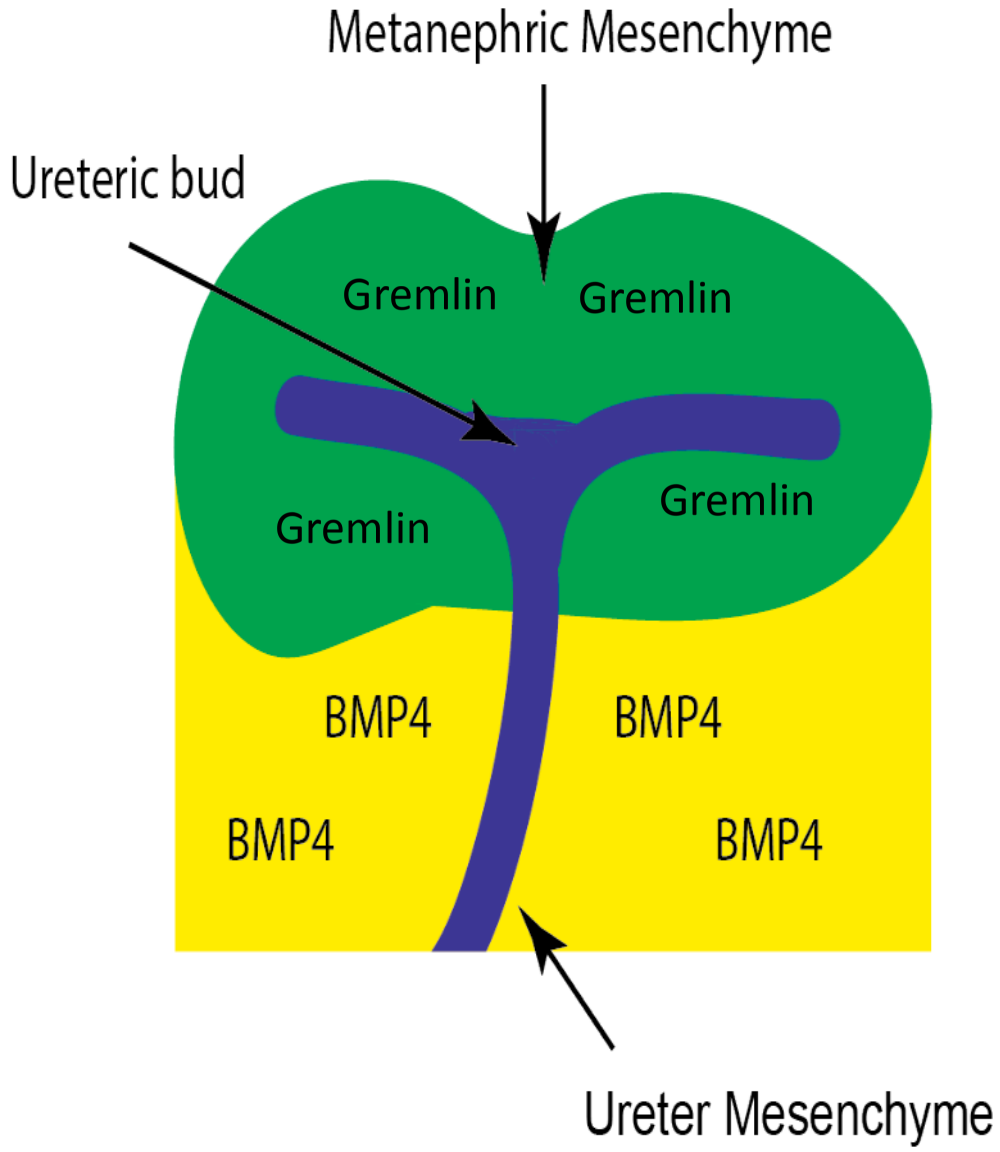




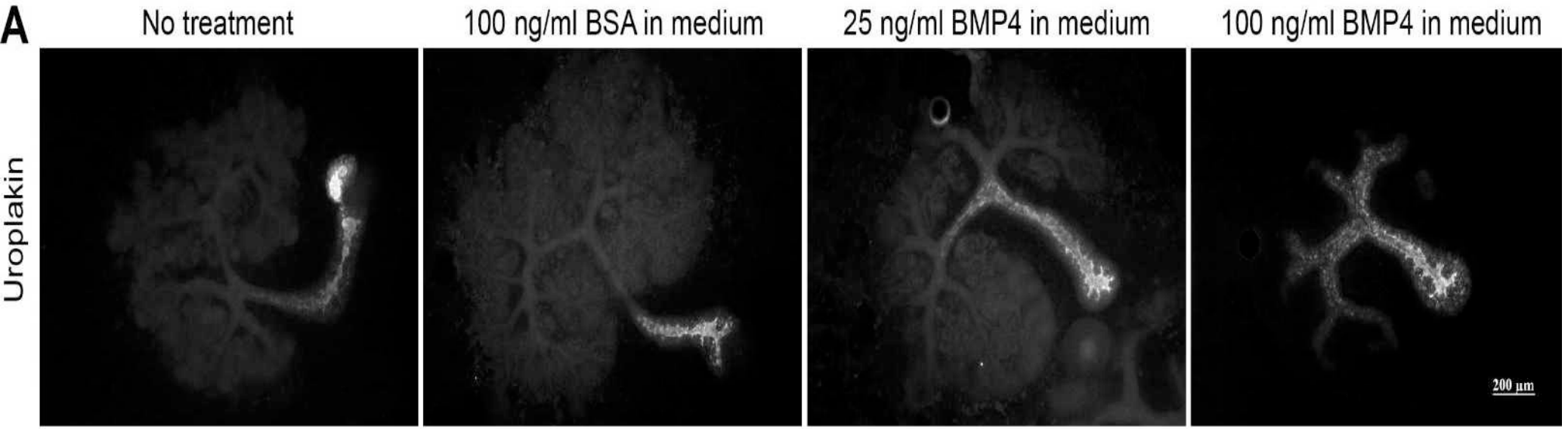


Scale bar = 200µm

Is BMP4 the signalling molecule?



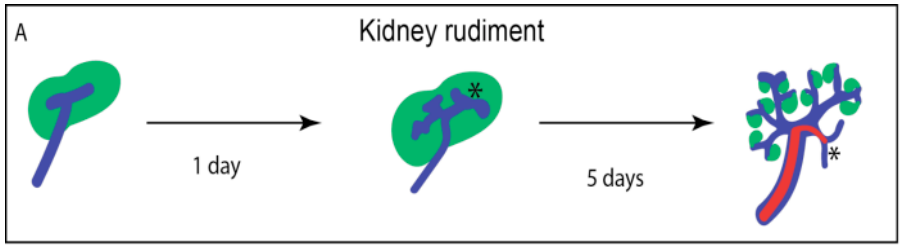
BMP4 treatment of kidneys leads to an expansion of uroplakin expression

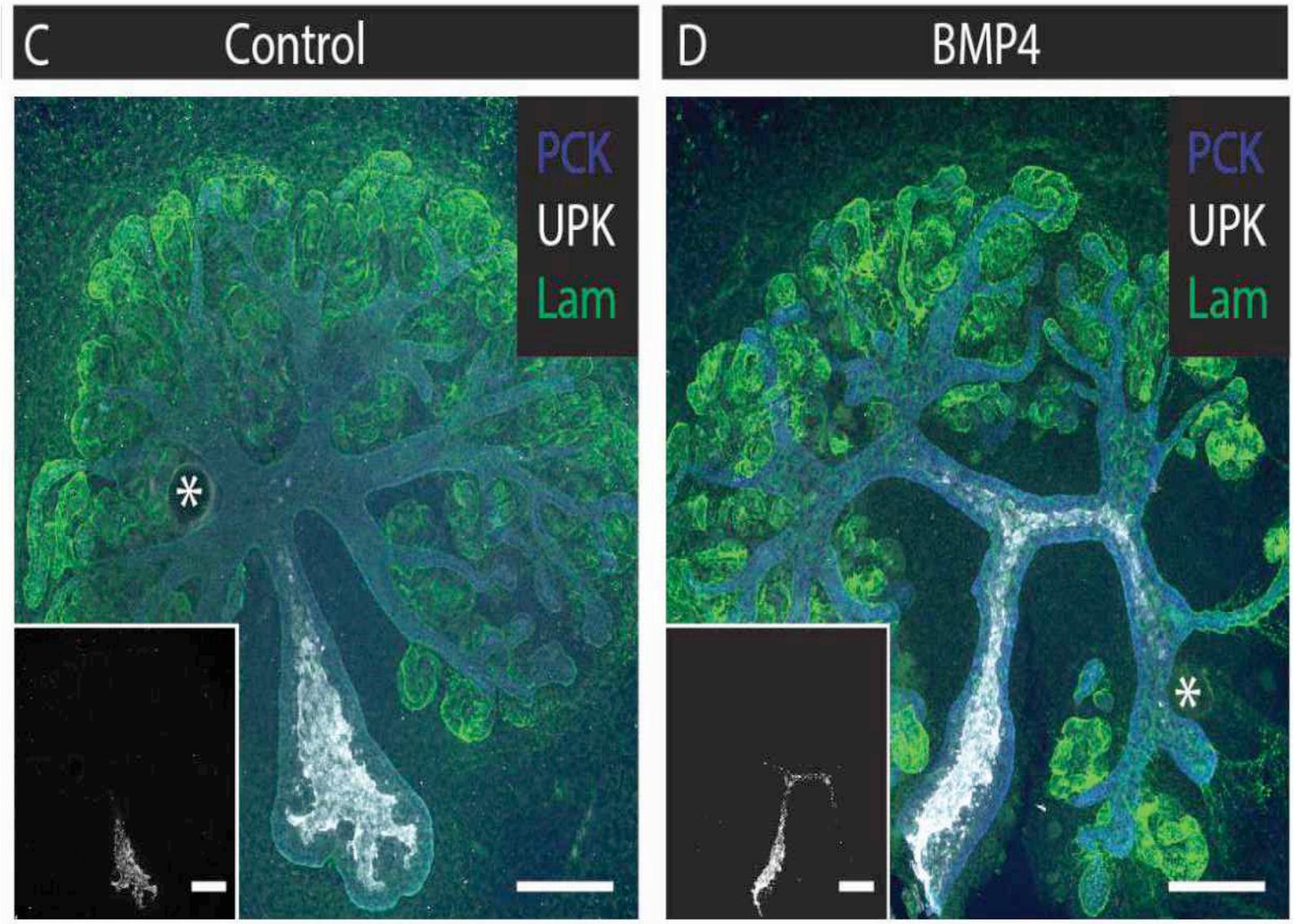


Experiment first published in:

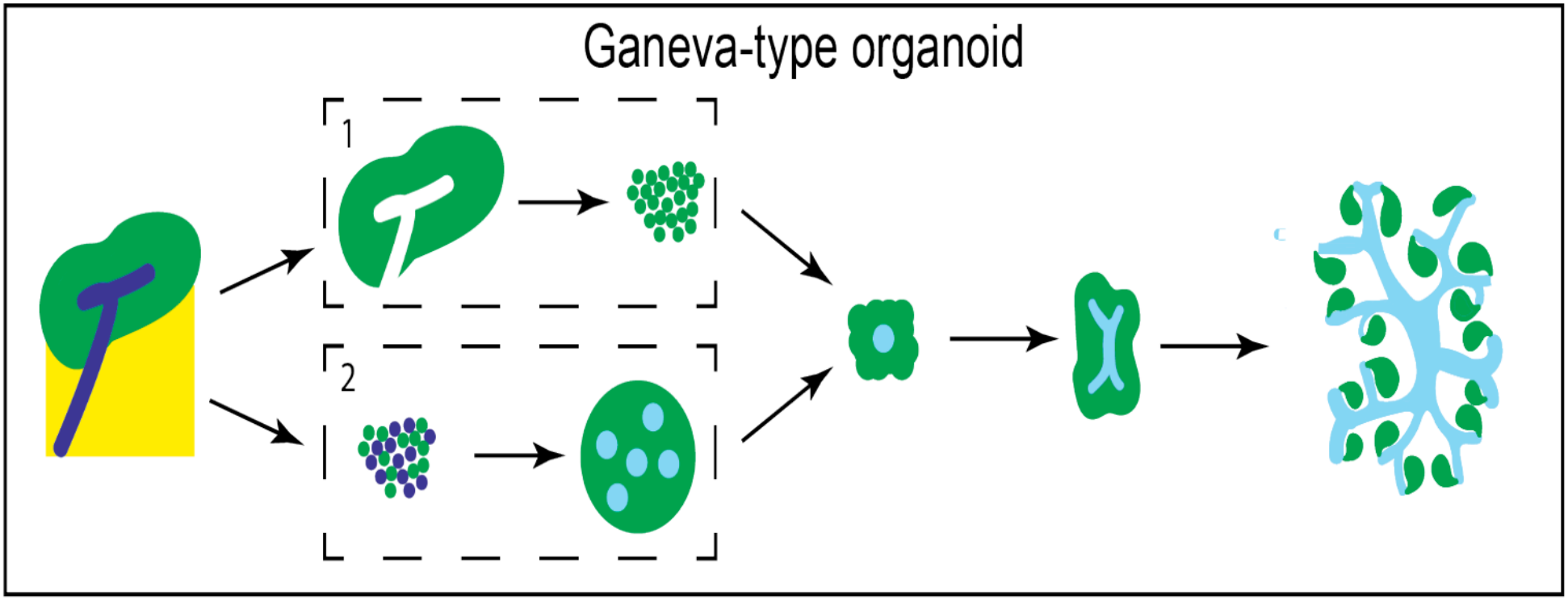
Brenner-Anantharam, A., Cebrian, C., Guillaume, R., Hurtado, R., Sun, T.T., and Herzlinger, D. Tailbud-derived mesenchyme promotes urinary tract segmentation via BMP4 signaling. *Development*, **134**, 1967–1975 (2007).

Collecting ducts treated locally with BMP4 express uroplakin





Applying asymmetric signals to Ganeva organoids



Ganeva-type kidneys respond to BMP4 treatment by expressing uroplakin

Chris Mills

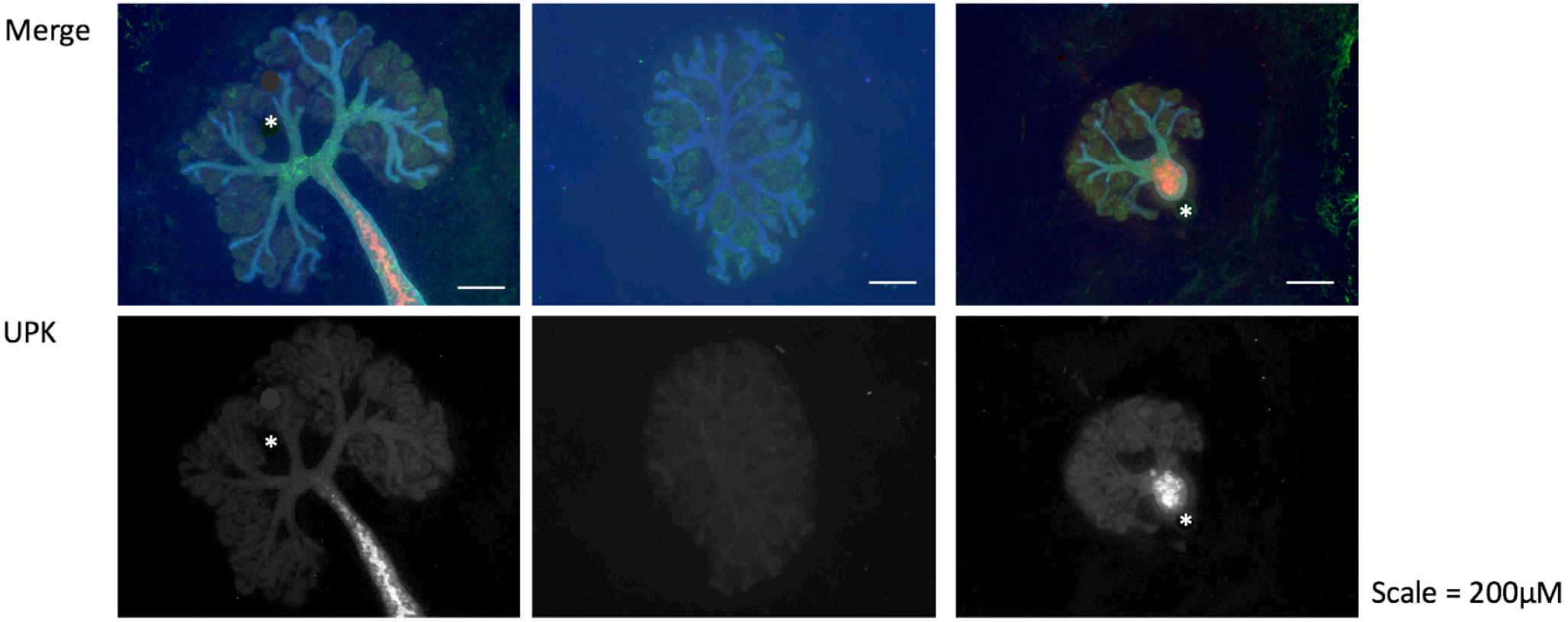
Normal kidney

Engineered

control bead

Engineered

signalling bead



Orange = uroplakin, a marker of ureter maturation

Going up a level...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“The possession of knowledge does not kill the sense of wonder and mystery. There is always more mystery”.

Anaïs Nin

John Conway's Game of Life

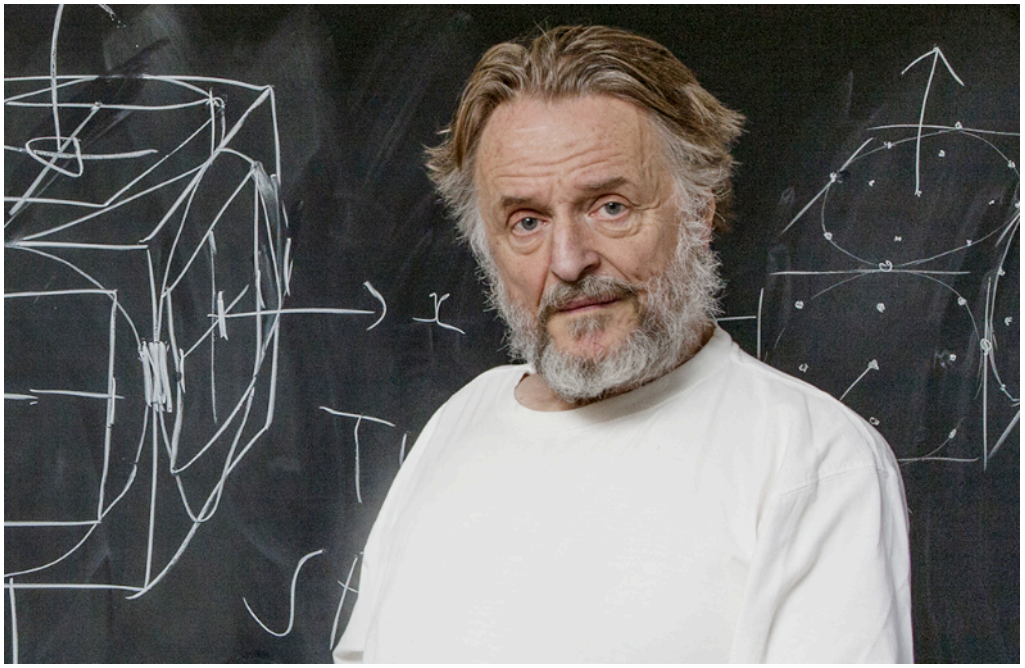
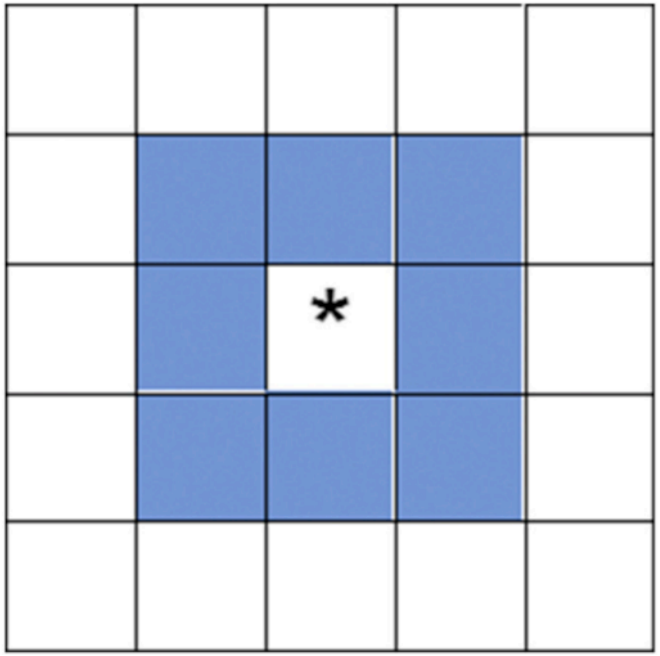


Photo credit: Princeton University, Office of Communications, Denise Applewhite



1. A cell that has fewer than two neighbours dies from lack of trophic support.
2. A cell that has four or more neighbours dies from overcrowding (for example, through build up of toxins).
3. If exactly three cells are neighbours of an empty location, one of them divides so that one daughter stays where the mother was and the other occupies the previously-empty location (since mothers and daughters are instantly equivalent, it makes no difference which of the cells is considered to have divided).