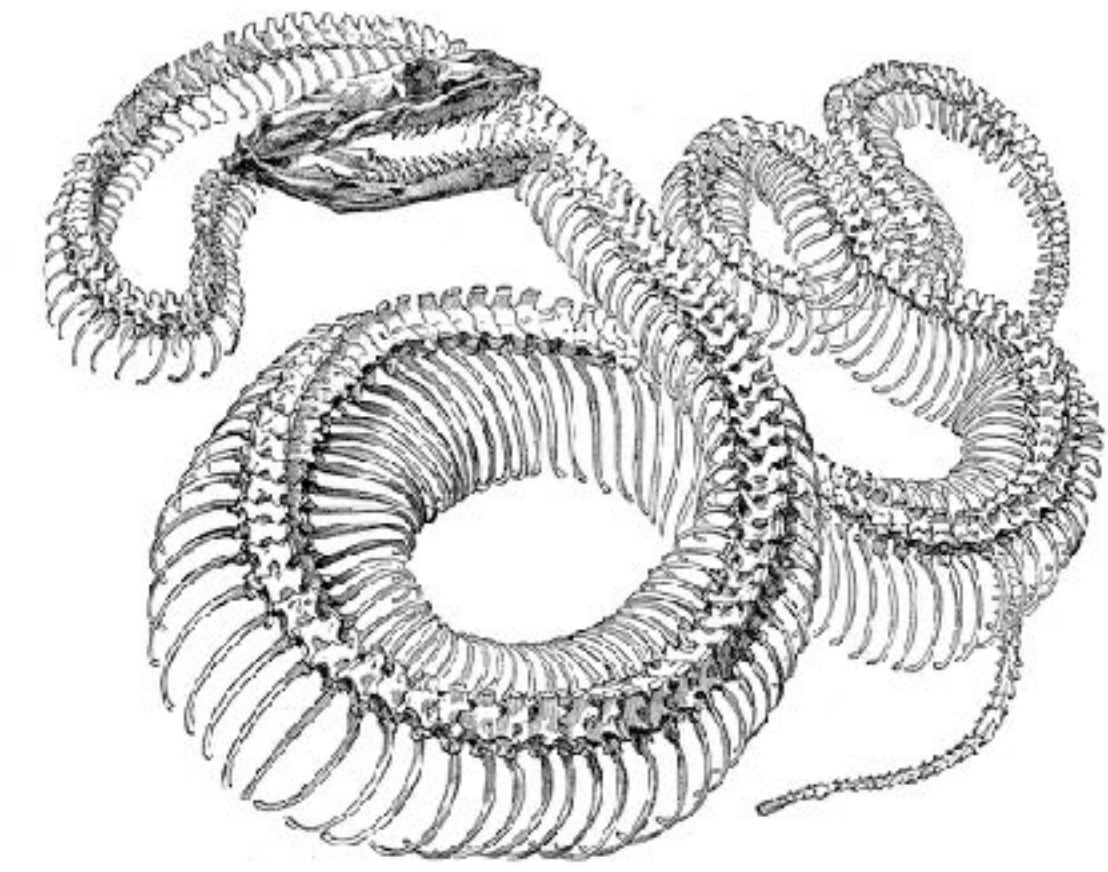
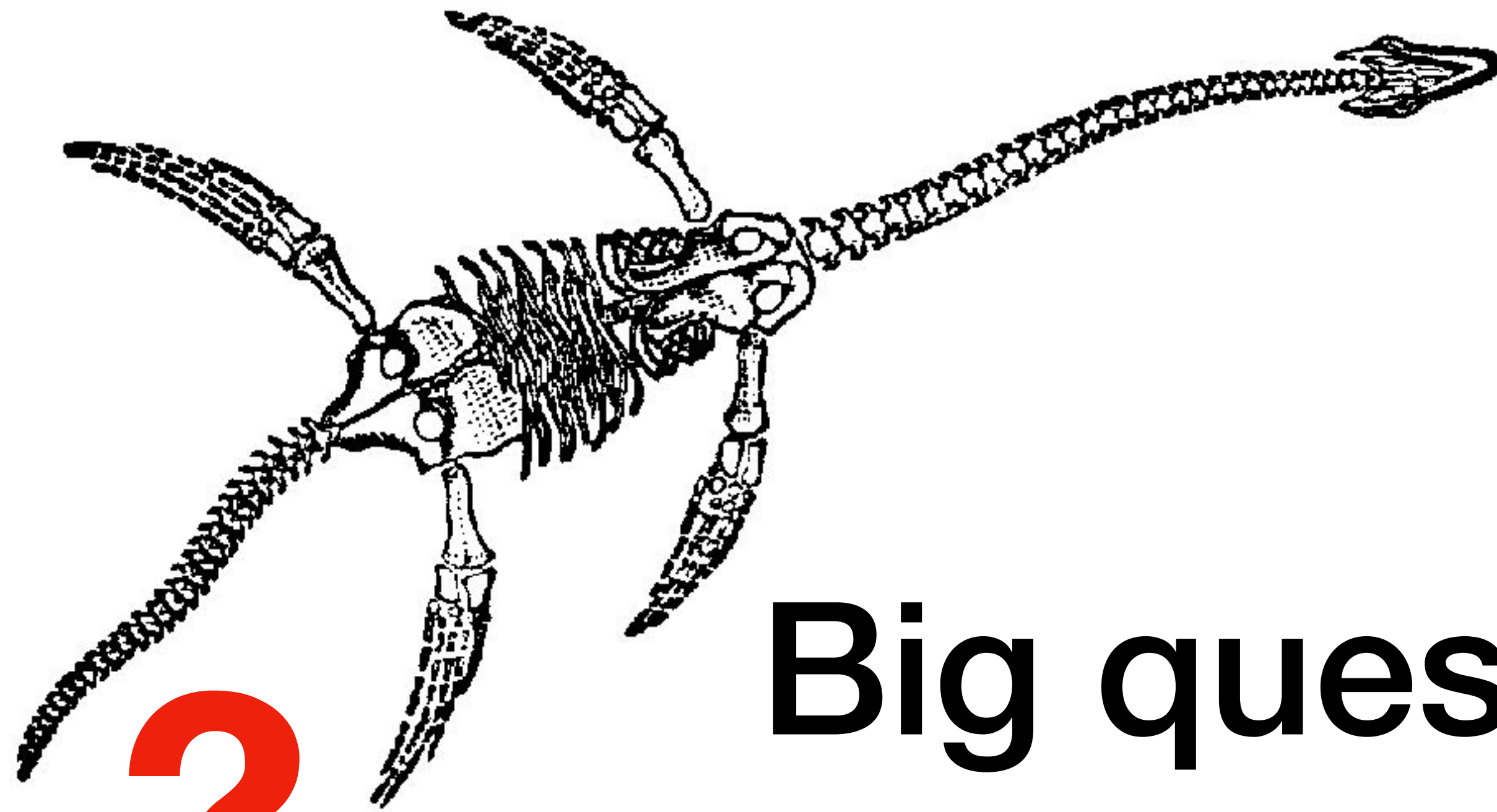
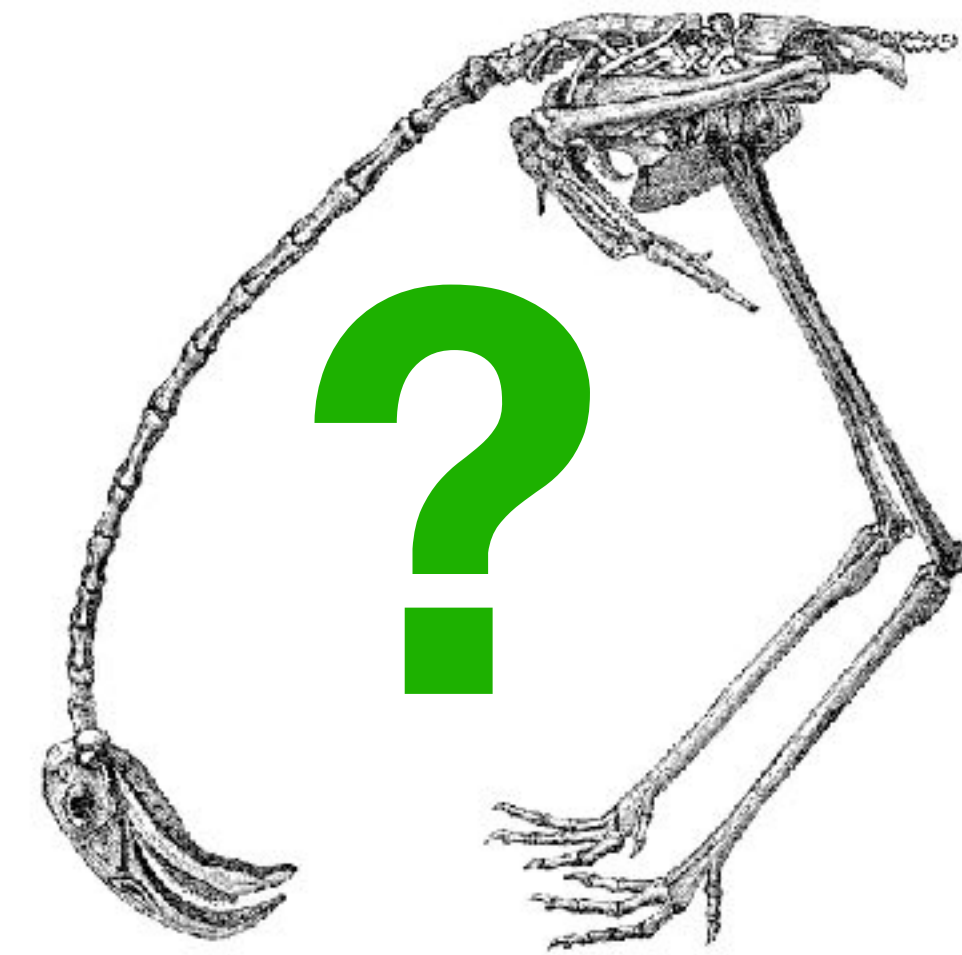
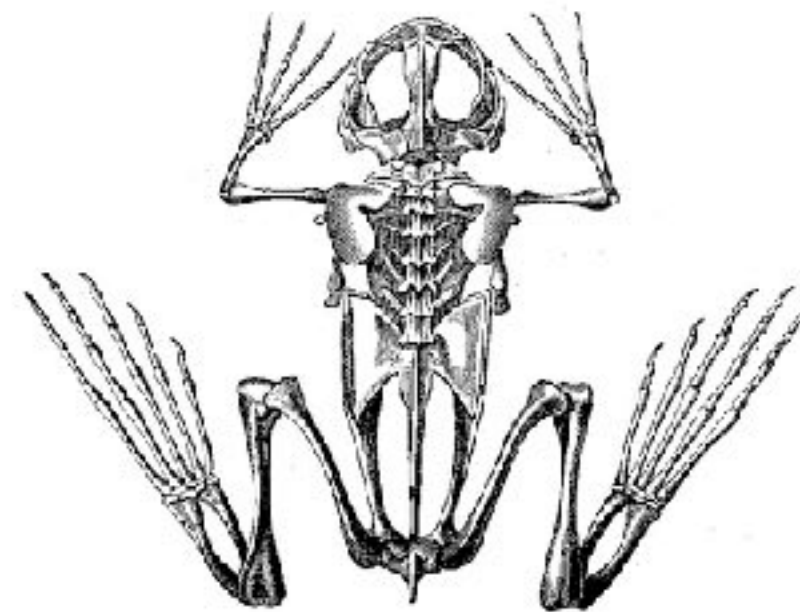
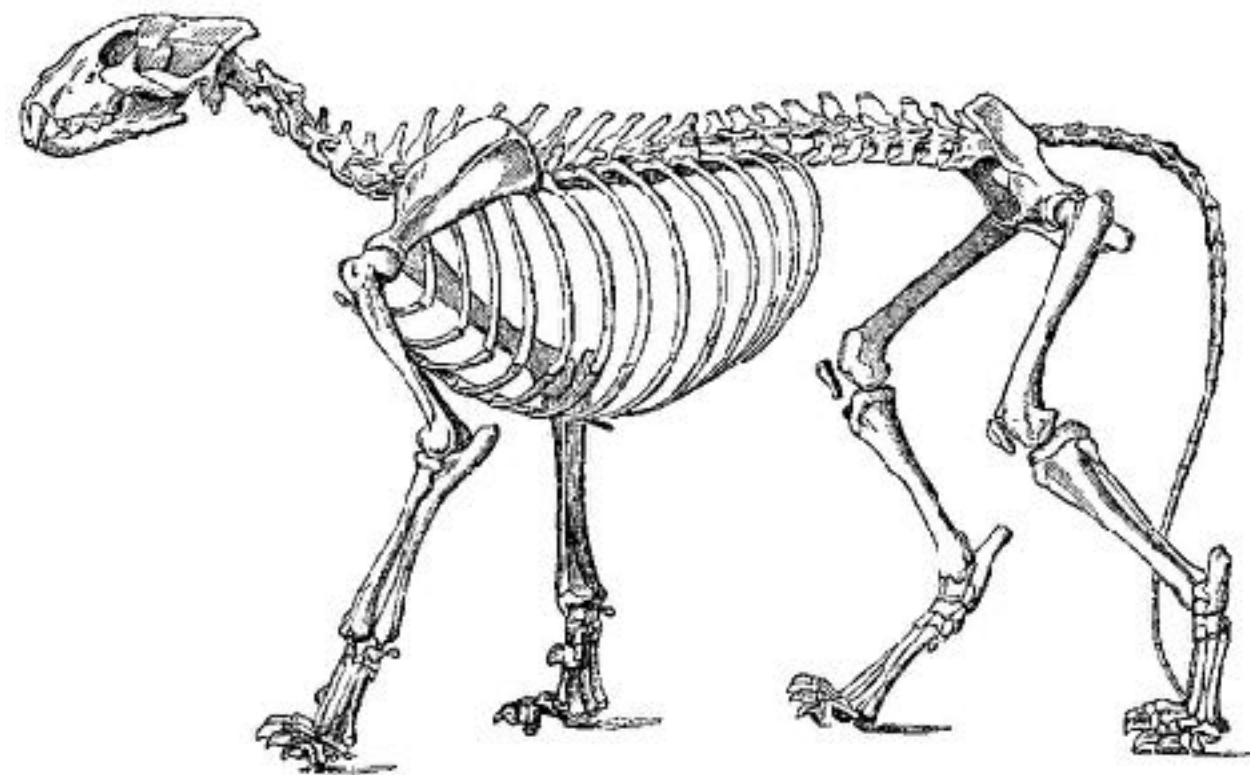
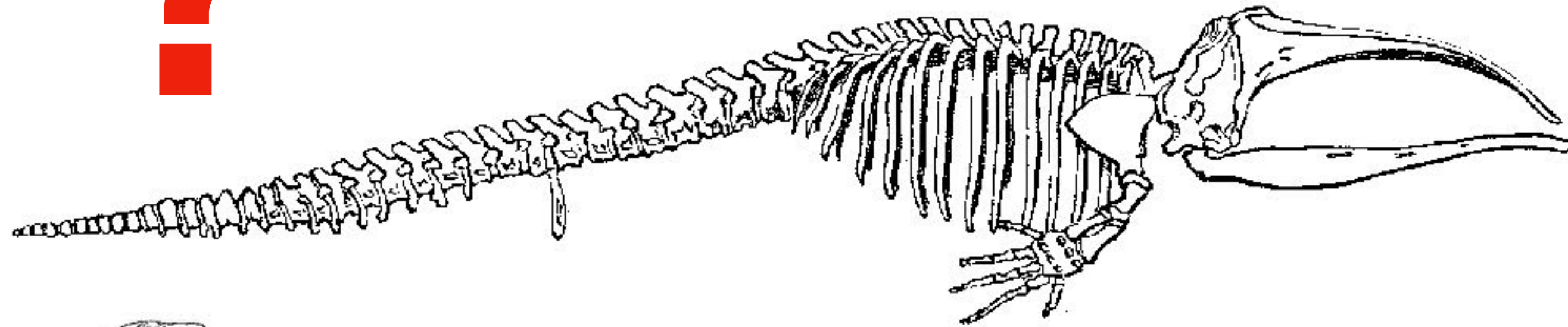


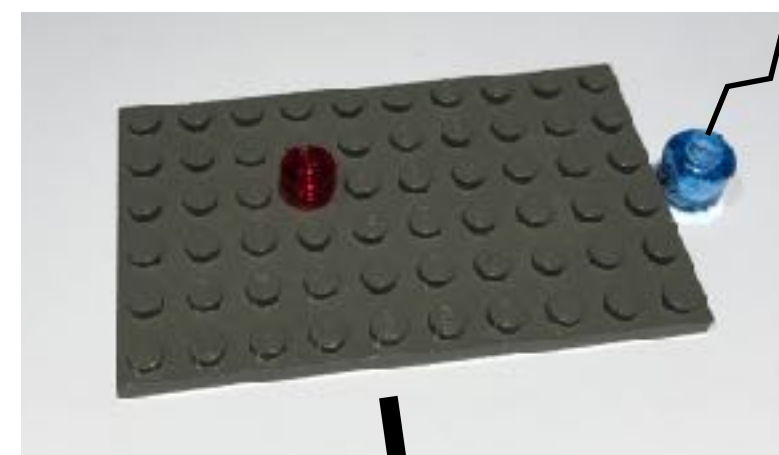


?

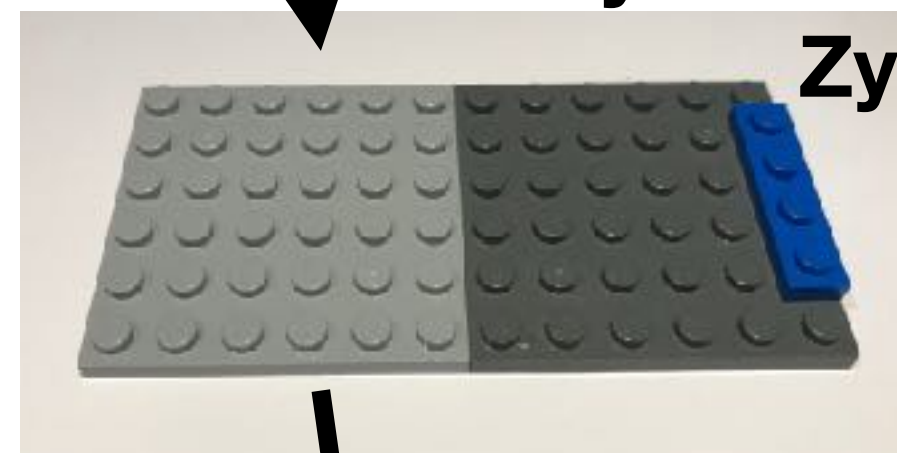
Big questions



LEGO embryo - where are we? **Pattern and growth**



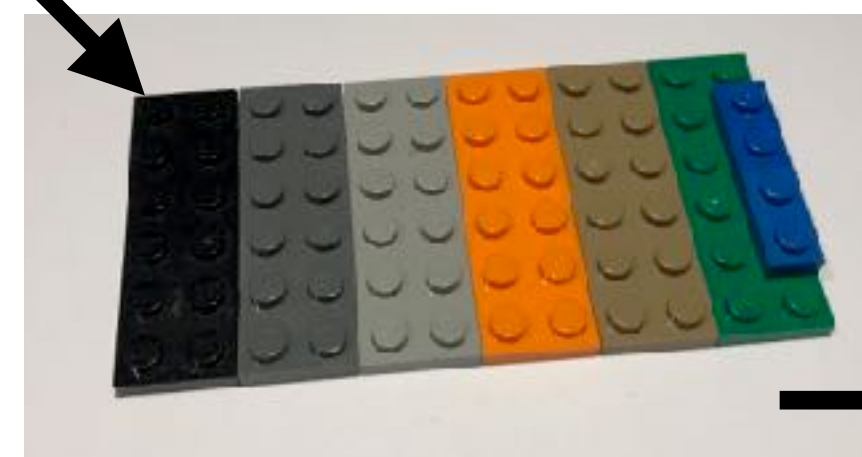
Fertilisation



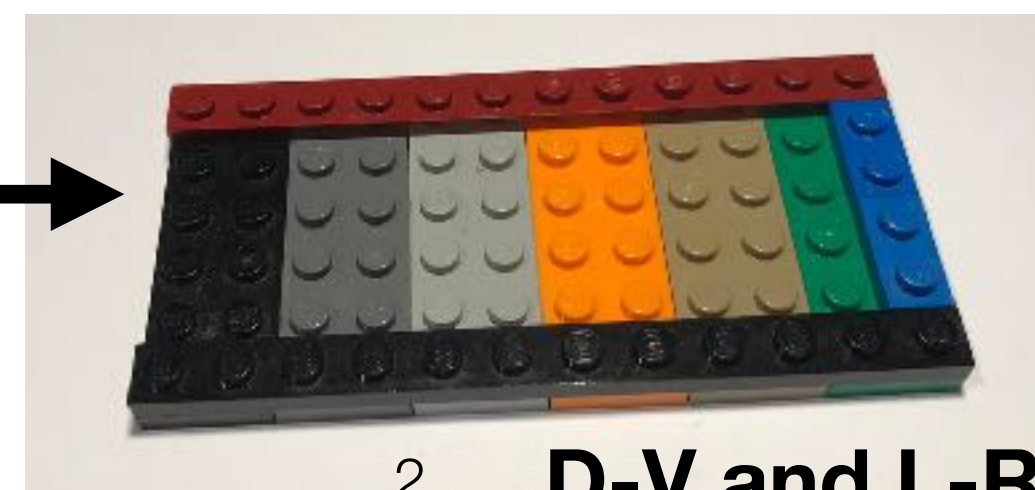
**Symmetry breaking
Zygote**



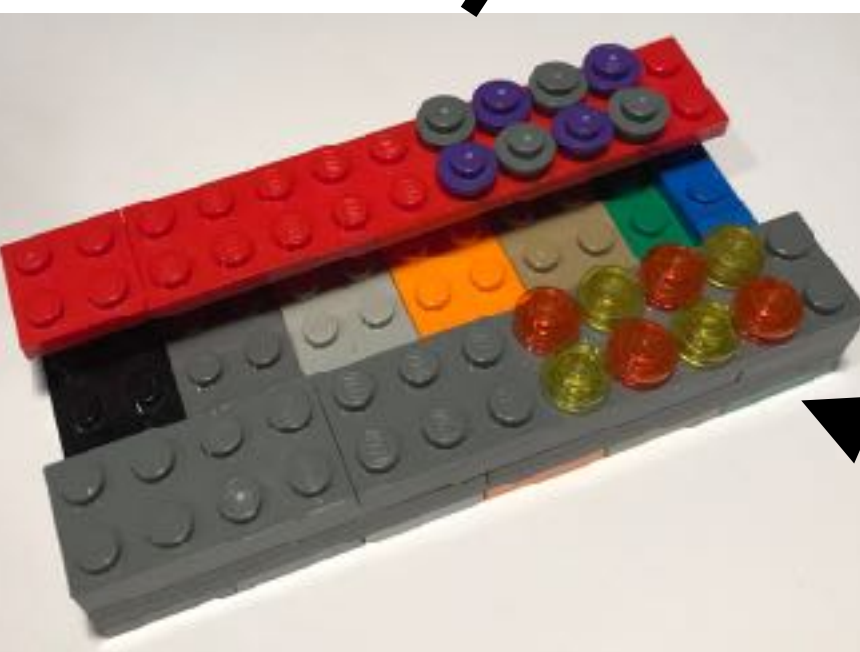
**Maternal-to-Zygotic
transition**



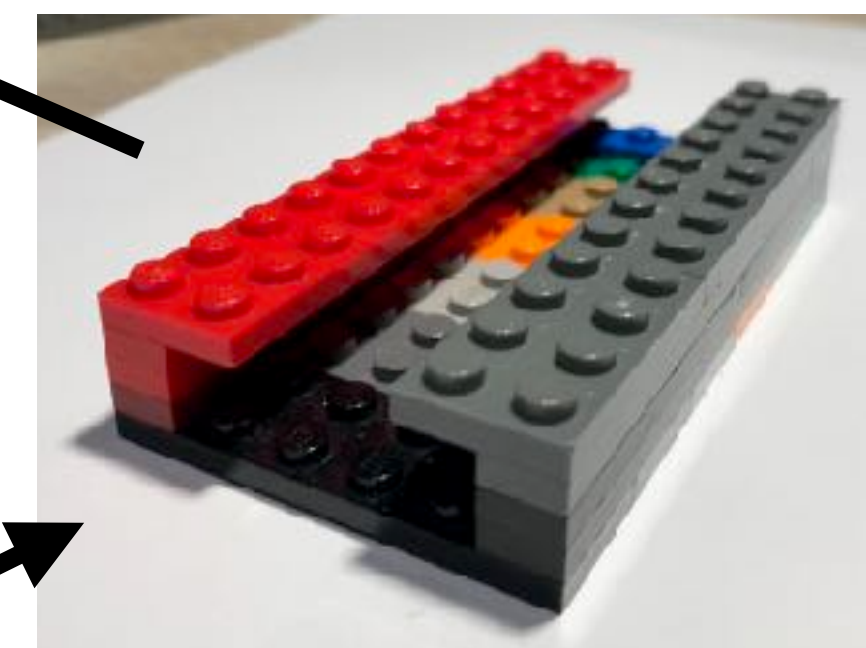
**Early patterning
A-P axis**



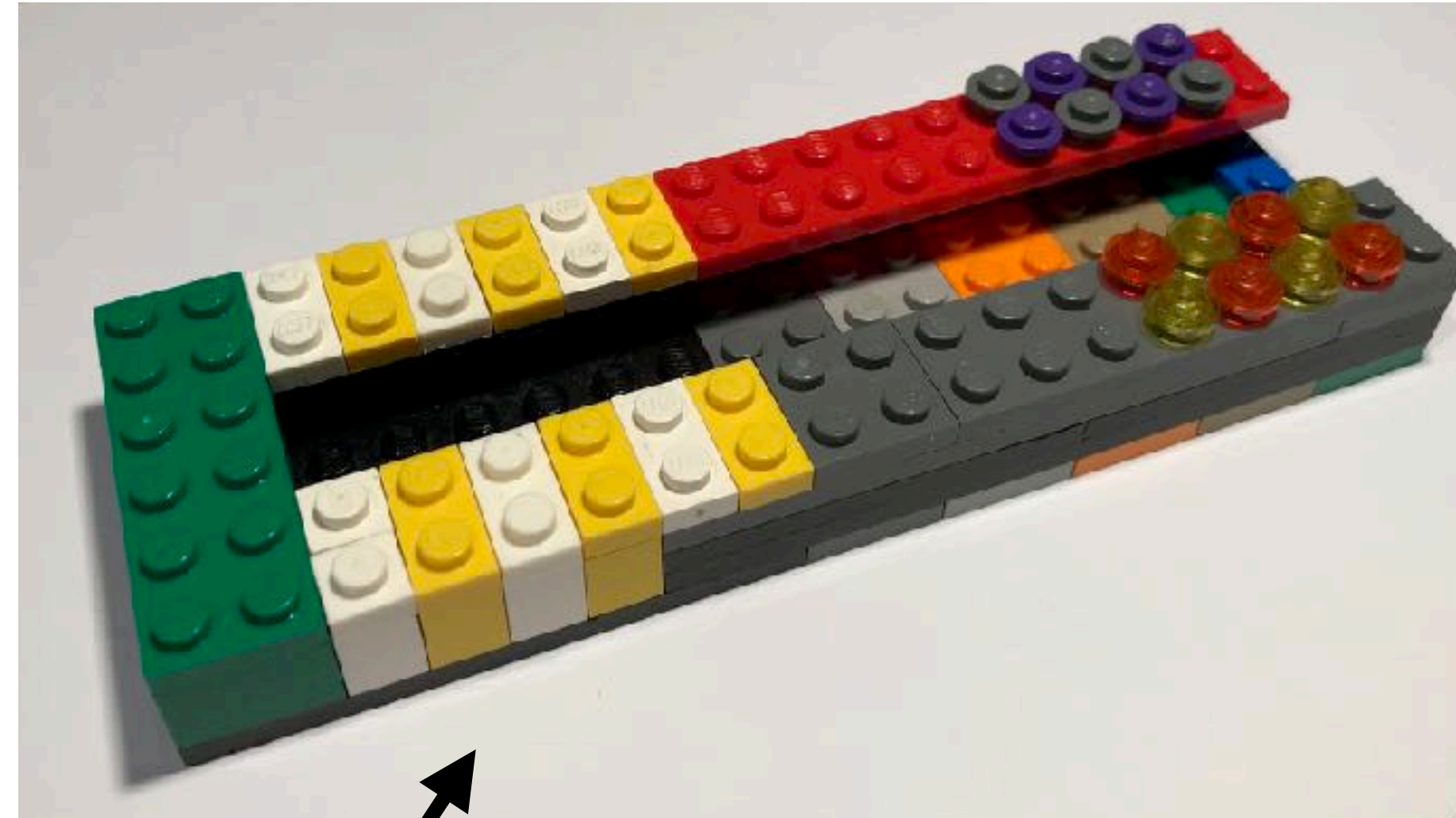
D-V and L-R axes

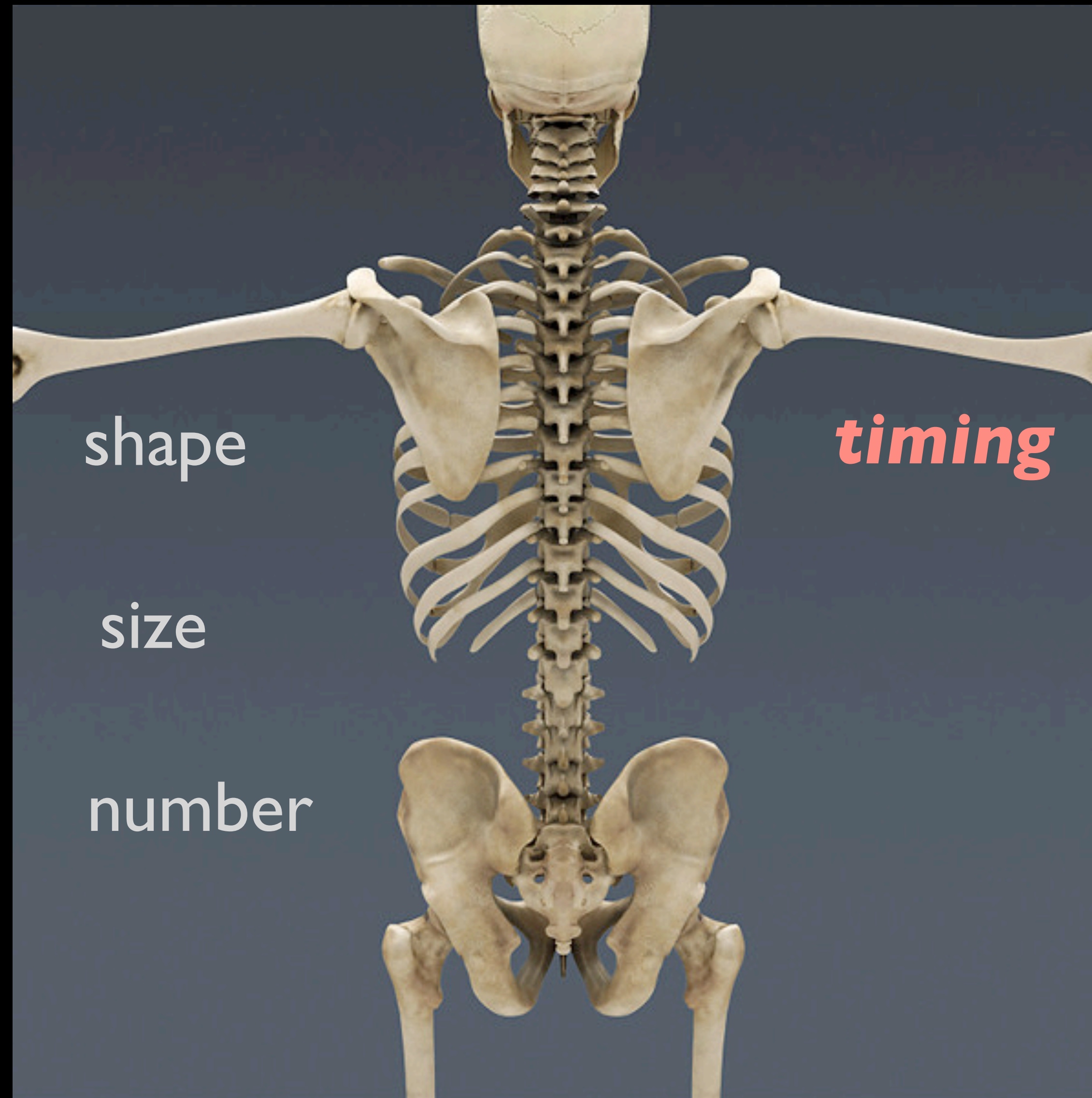


**Fine grained
Patterning**

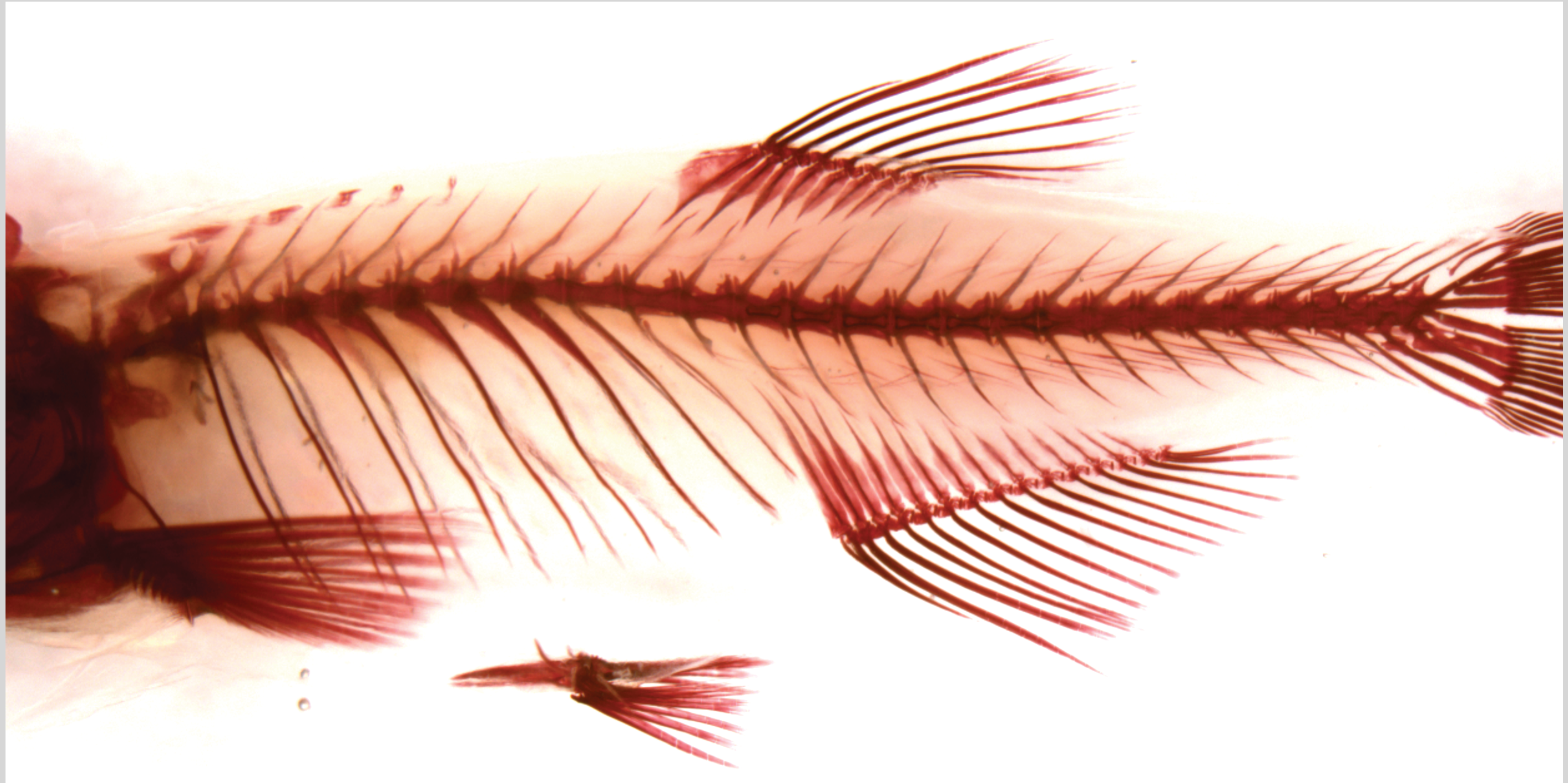


**Morphogenesis:
Gastrulation and folding**



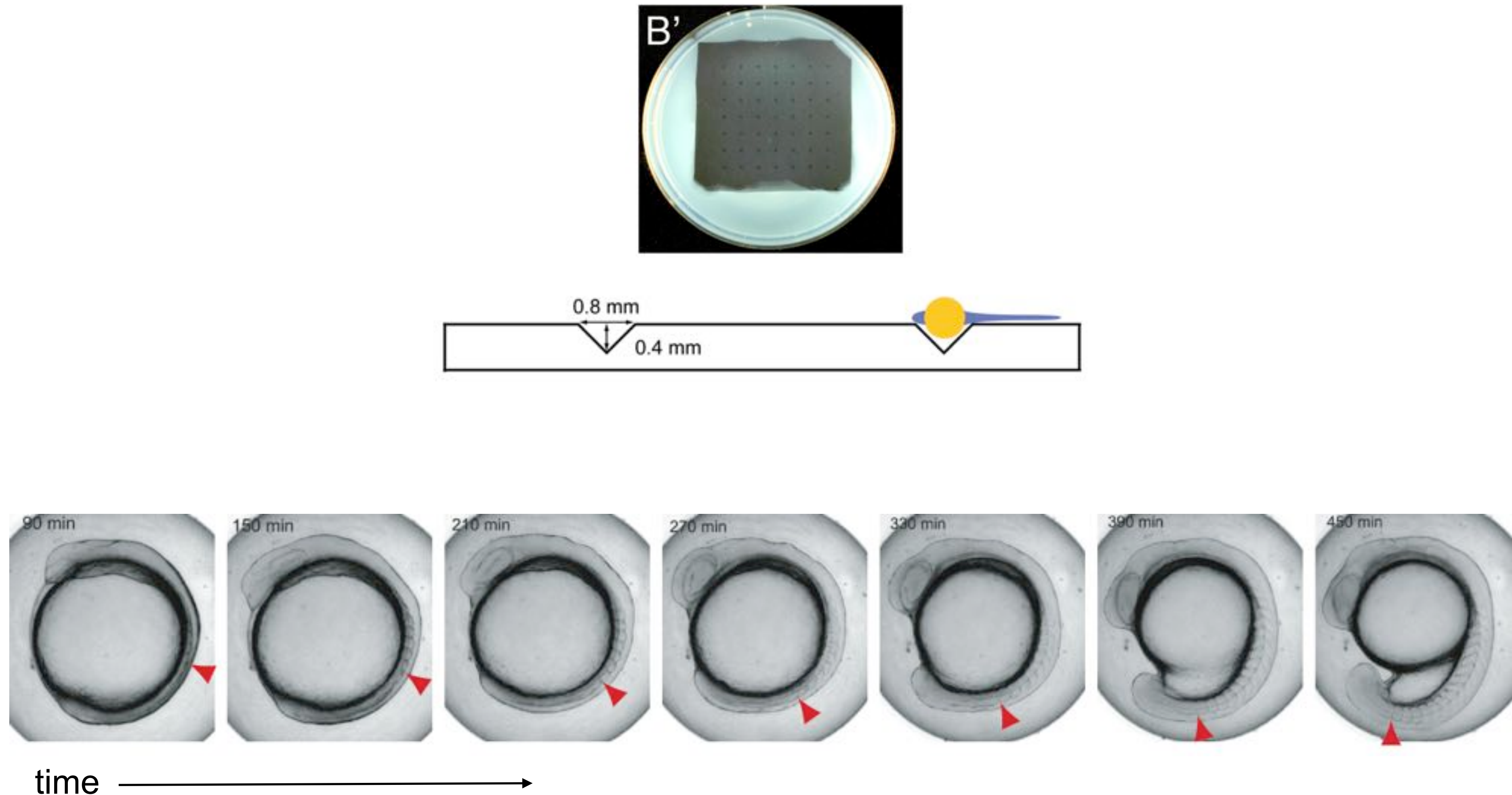


Body segments are added rhythmically and sequentially



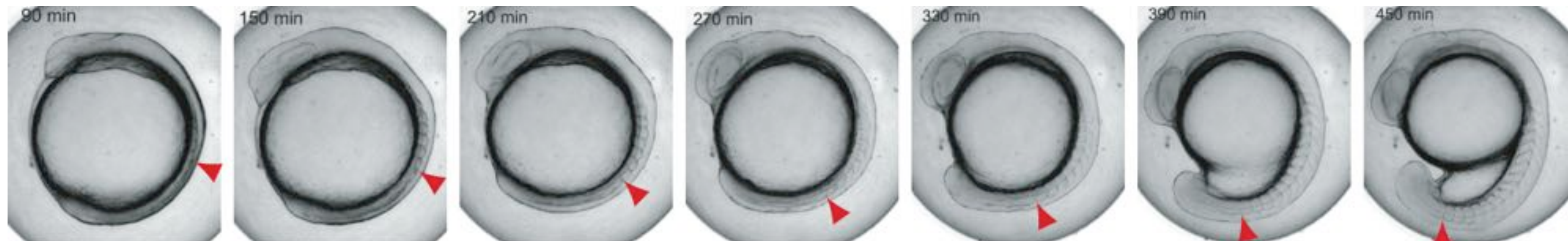
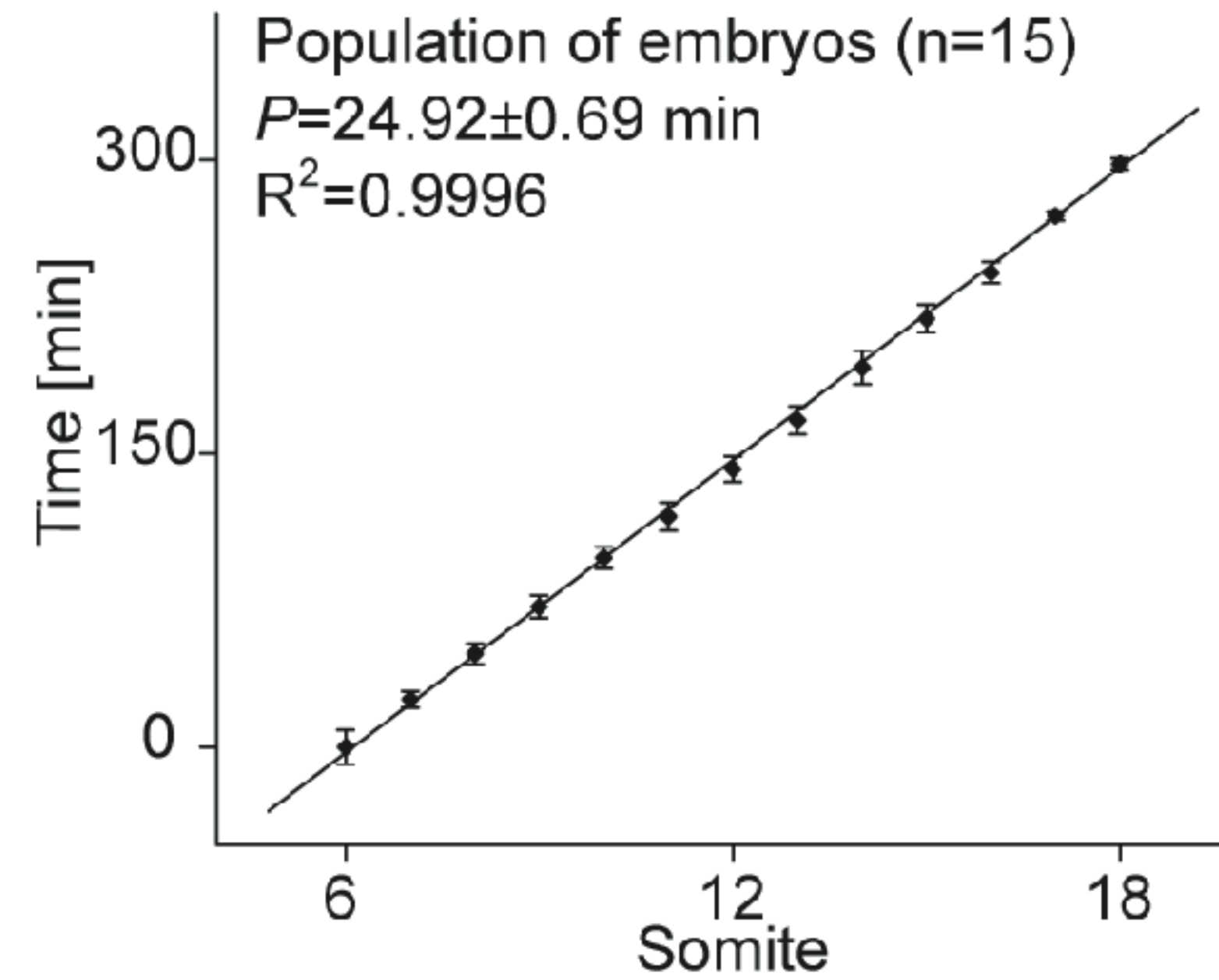
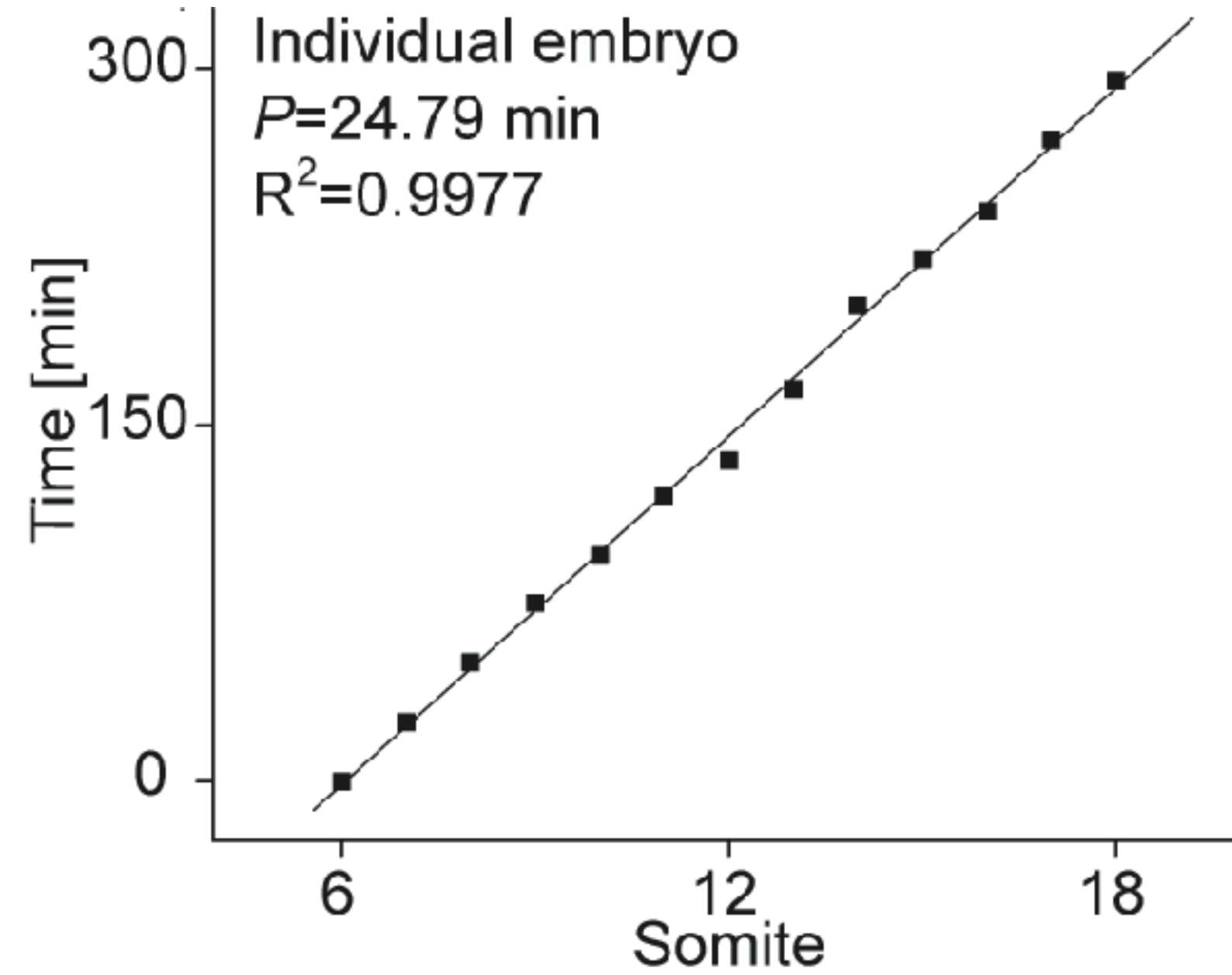
Somitogenesis rhythm can be measured throughout development

Schröter et al., 2008;
Herrgen et al., 2009



Schröter, C., Herrgen, L., Cardona, A., Brouhard, G. J., Feldman, B., & Oates, A. C. (2008). Dynamics of zebrafish somitogenesis. *Developmental Dynamics*, 237(3), 545–553. <http://doi.org/10.1002/dvdy.21458>

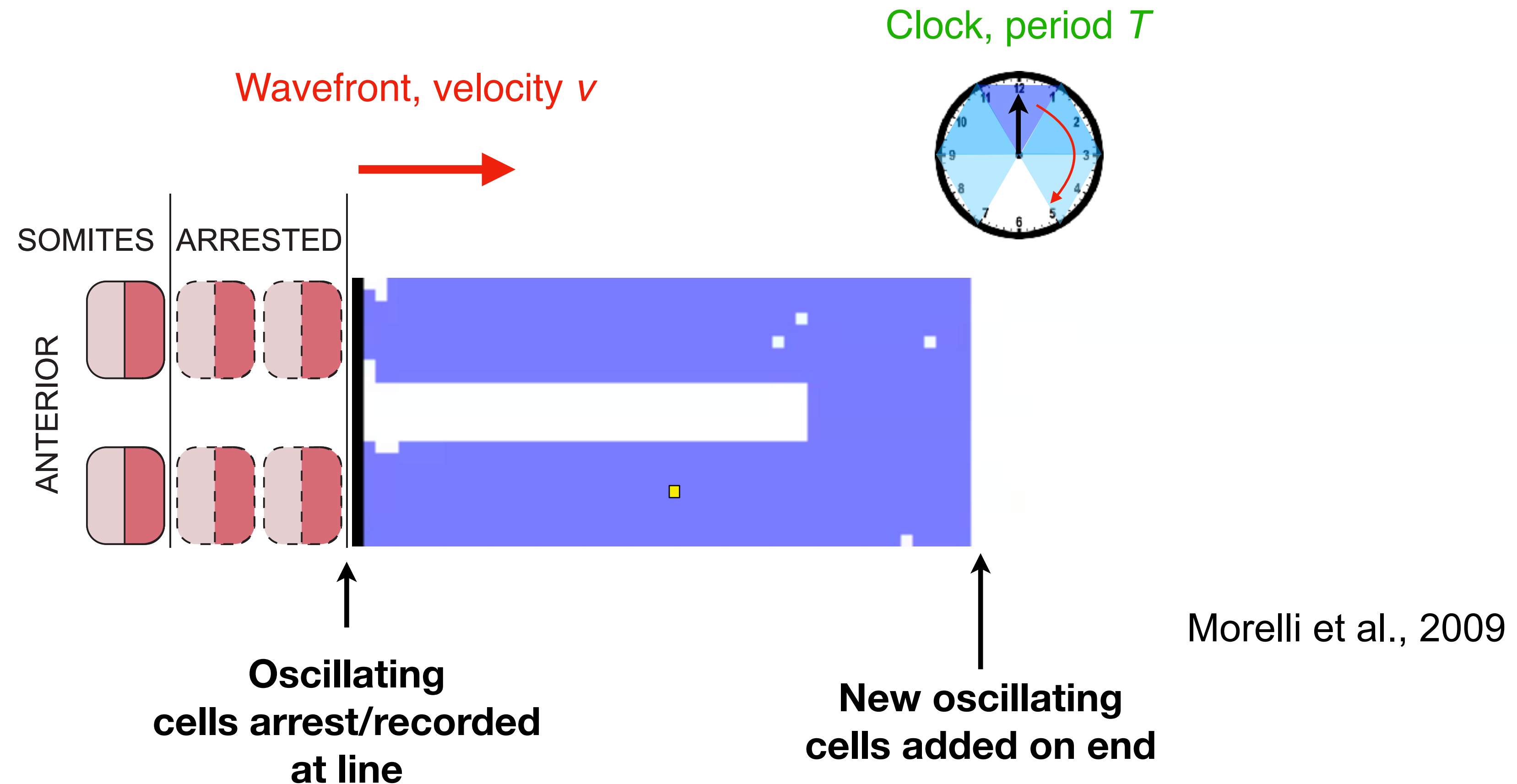
Somitogenesis period is precise



time →

A clock and wavefront mechanism can subdivide an embryo

Cooke and Zeeman, 1976



Cooke, J., & Zeeman, E. C. (1976).
A clock and wavefront model for control of the number of repeated structures
during animal morphogenesis.
Journal of Theoretical Biology, 58(2), 455–476.

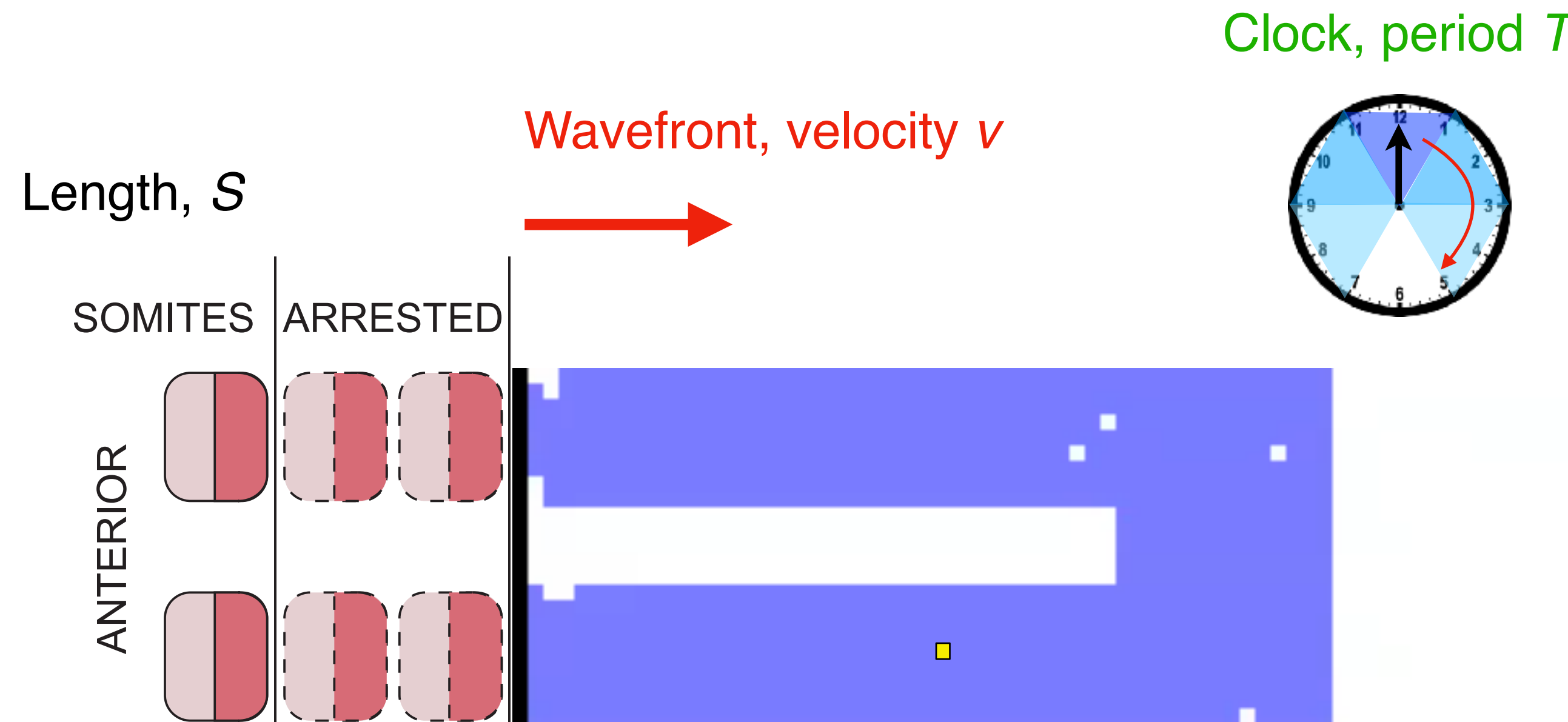
Morelli, L. G., Ares, S., Herrgen, L., Schröter, C., Jülicher, F., & Oates, A. C. (2009).
Delayed coupling theory of vertebrate segmentation.
HFSP Journal, 3(1), 55–66. <http://doi.org/10.2976/1.3027088>

Individual puzzle



A clock and wavefront mechanism can subdivide an embryo

Cooke and Zeeman, 1976

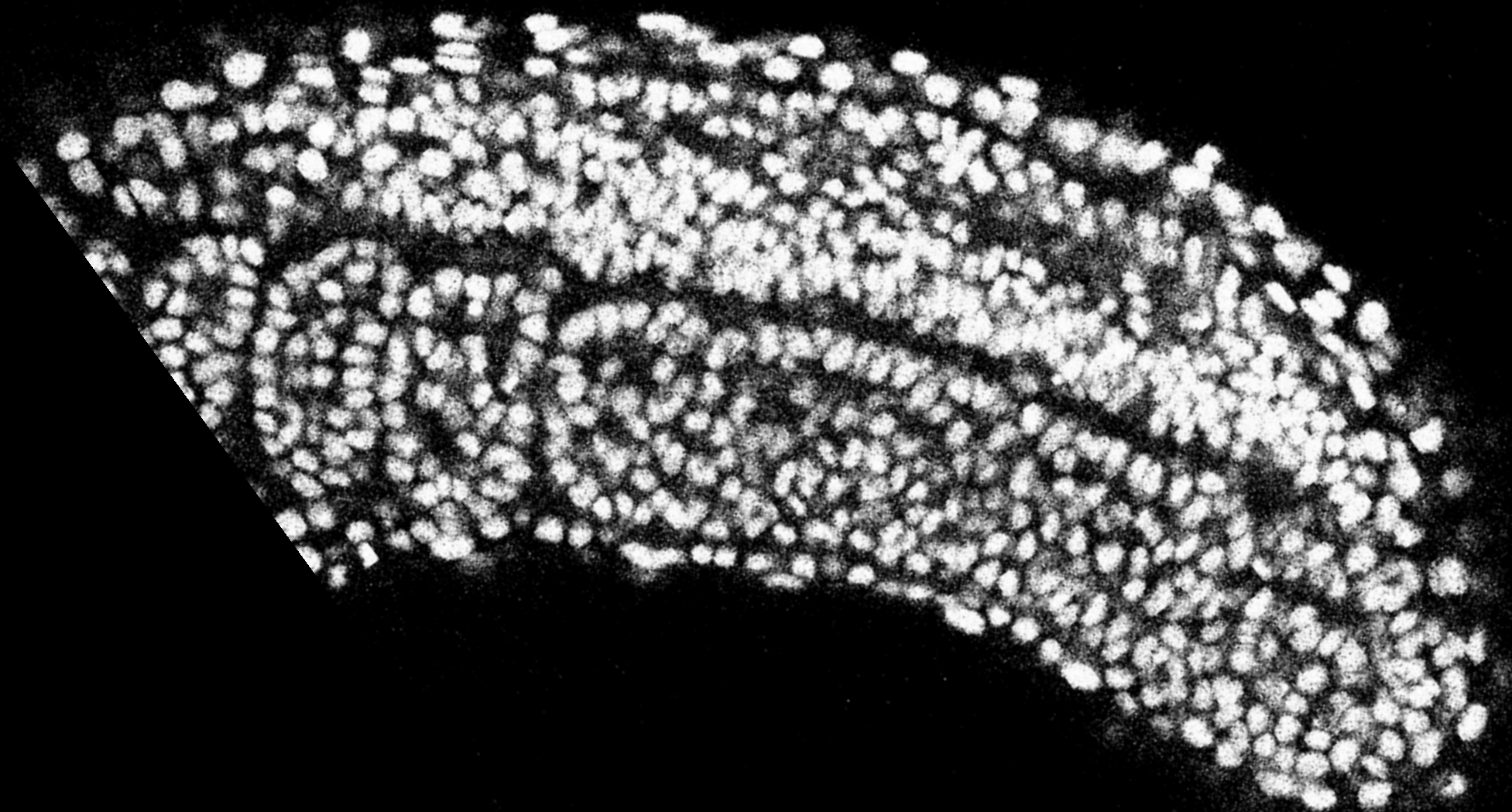


Morelli et al., 2009

What determines the segment length?

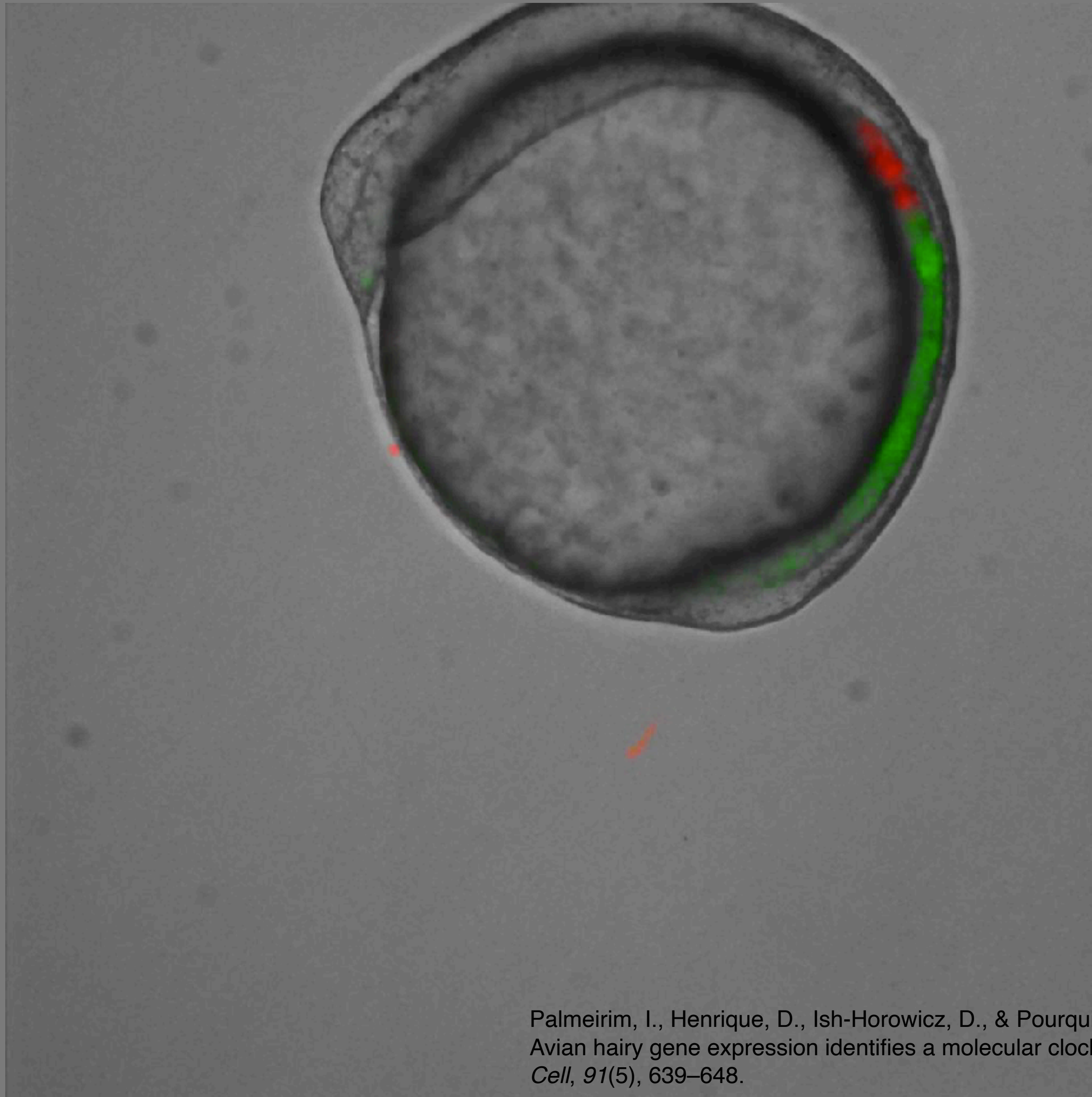
What determines the segment number?

Flow and rhythmic rearrangement of cells during somitogenesis



Histone- H2A-GFP

A “segmentation clock” ticks in the Pre-Somitic Mesoderm



Palmeirim et al., 1997

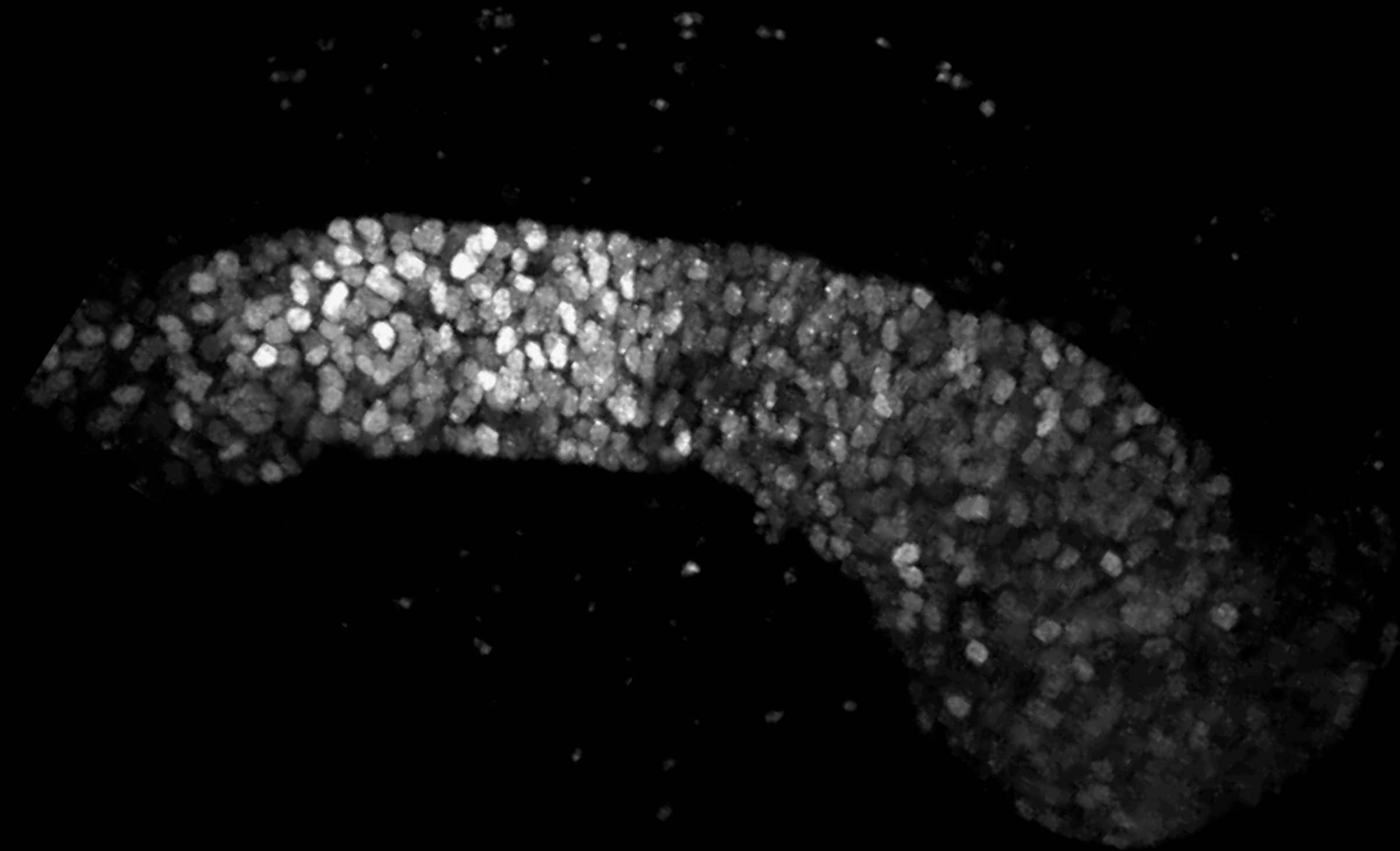
cyclic reporter gene
her1-YFP/*mespb-mKate2*

Daniele Soroldoni

Mouse: Masamizu et al., 2006;
Aulehla et al., 2008.
Zebrafish: Delaune et al., 2012;
Soroldoni et al., 2014

Palmeirim, I., Henrique, D., Ish-Horowicz, D., & Pourquie, O. (1997).
Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis.
Cell, 91(5), 639–648.

Waves of gene expression sweep across a population of genetic oscillators in the PSM



cyclic reporter gene *her1*-YFP

Zeiss 710, single photon, W 40x NA 1.0; 1 frame/~3 min;
dXY=0.35, 1024x1024; dZ= 1.0, #Z~85

David Richmond

A model for the segmentation clock...

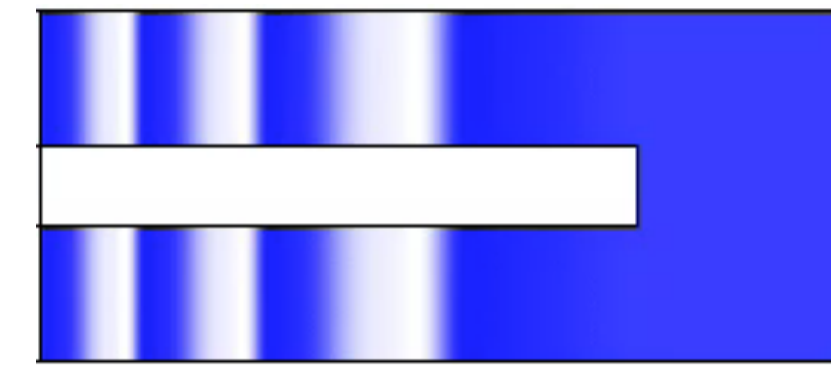


single units, local correlation, global organization

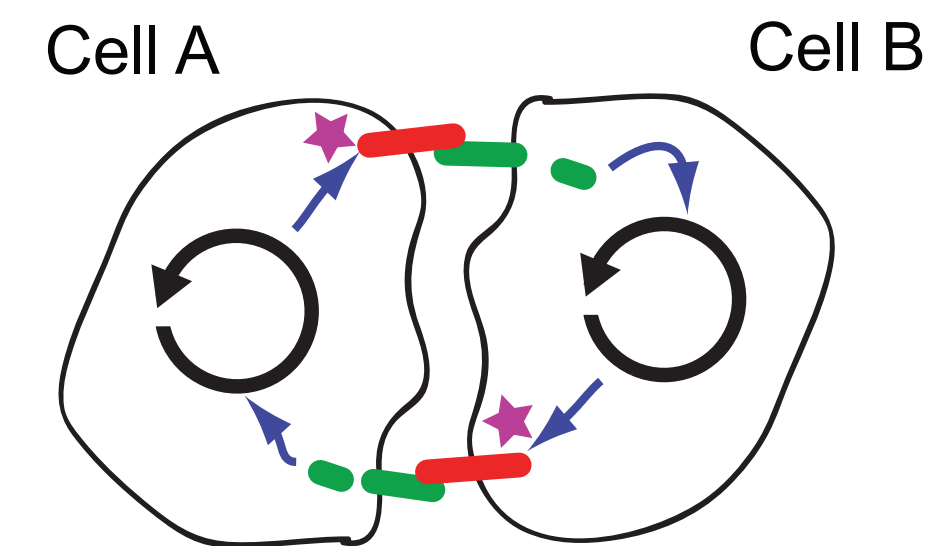
The segmentation clock is a tissue-level, rhythmic pattern generator



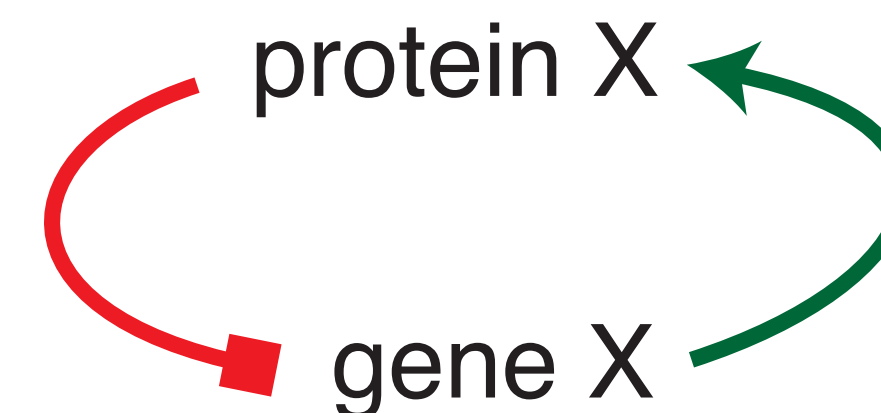
Tissue-level wave patterns



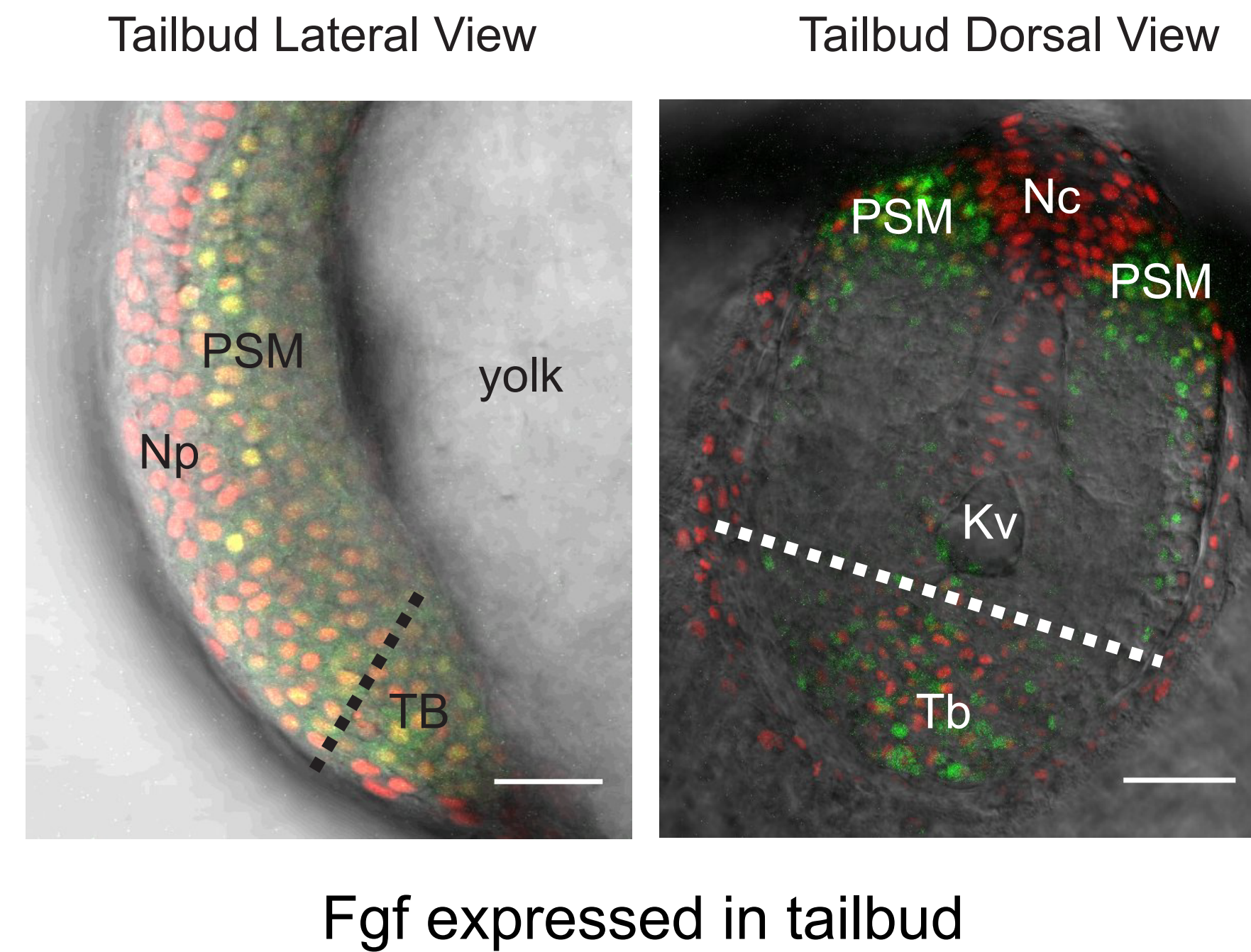
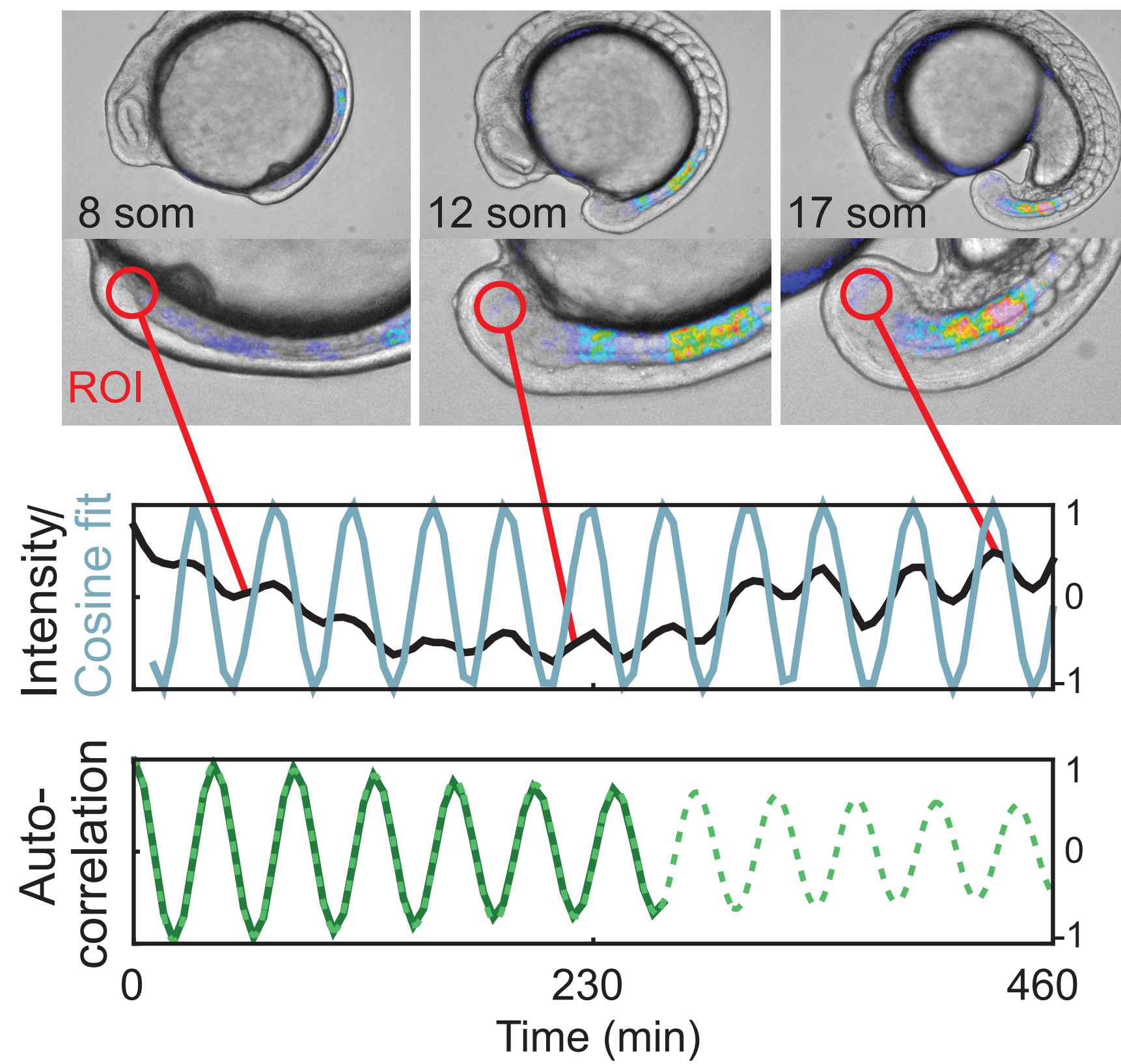
Local synchronization



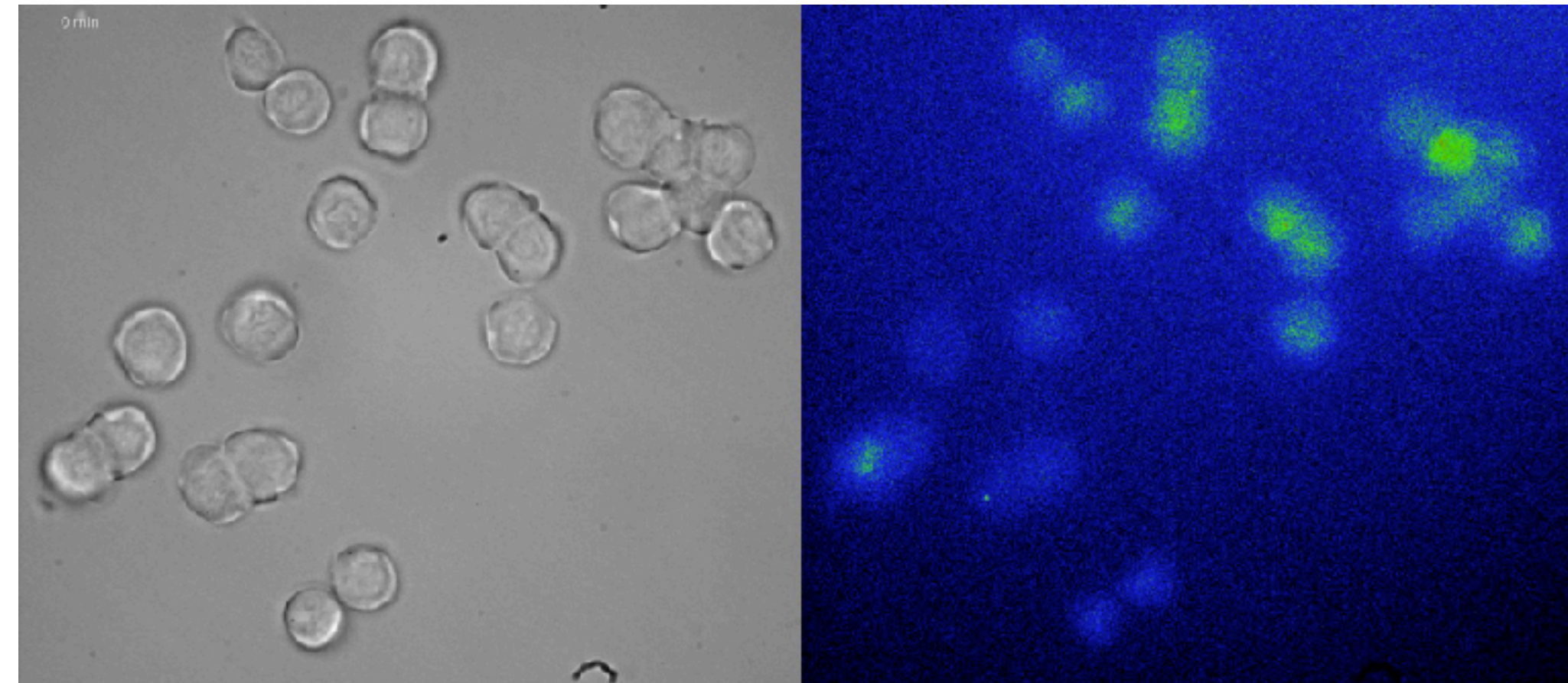
Cellular oscillators



The **tailbud** contains a persistently rhythmic progenitor population

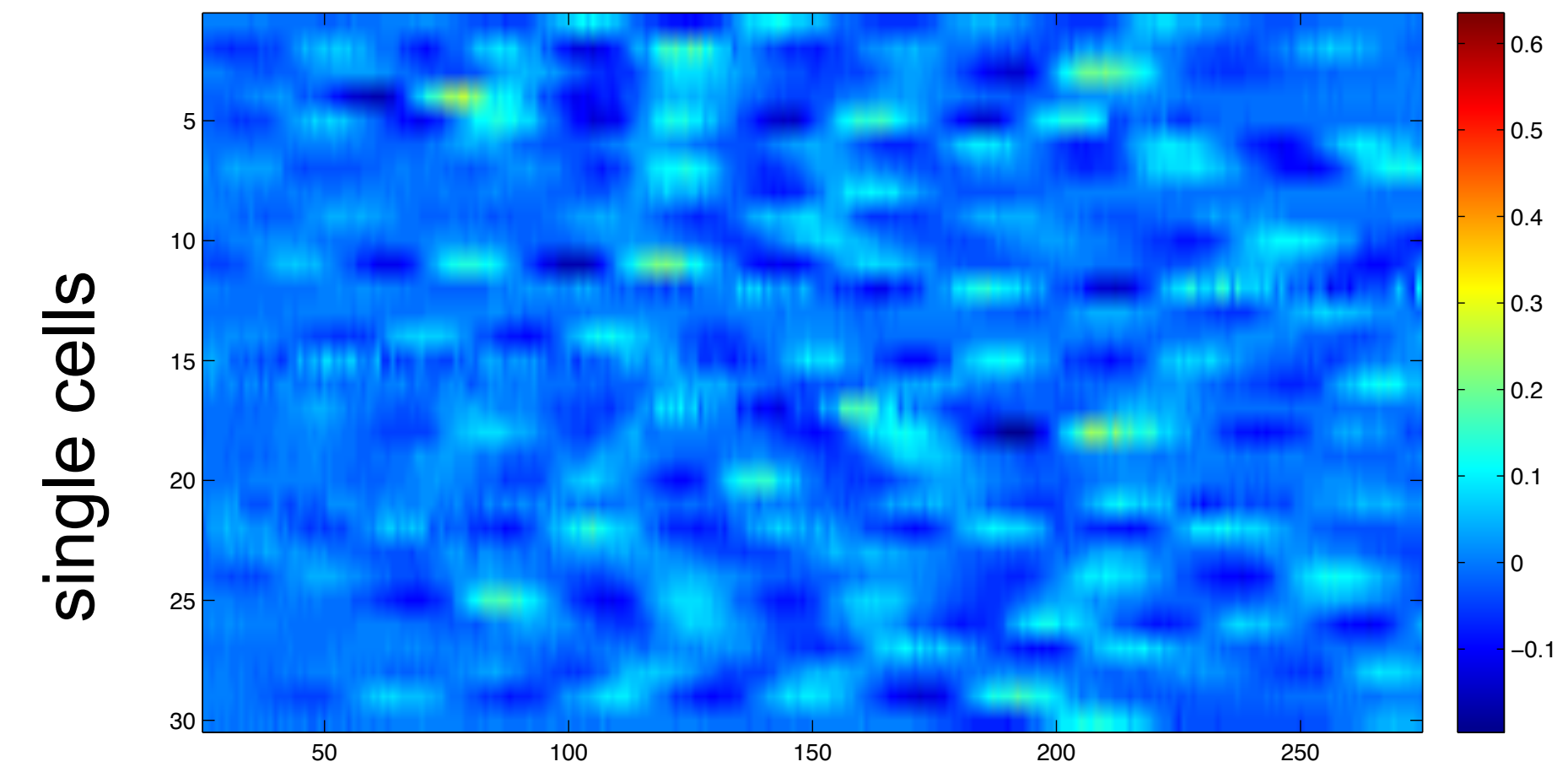
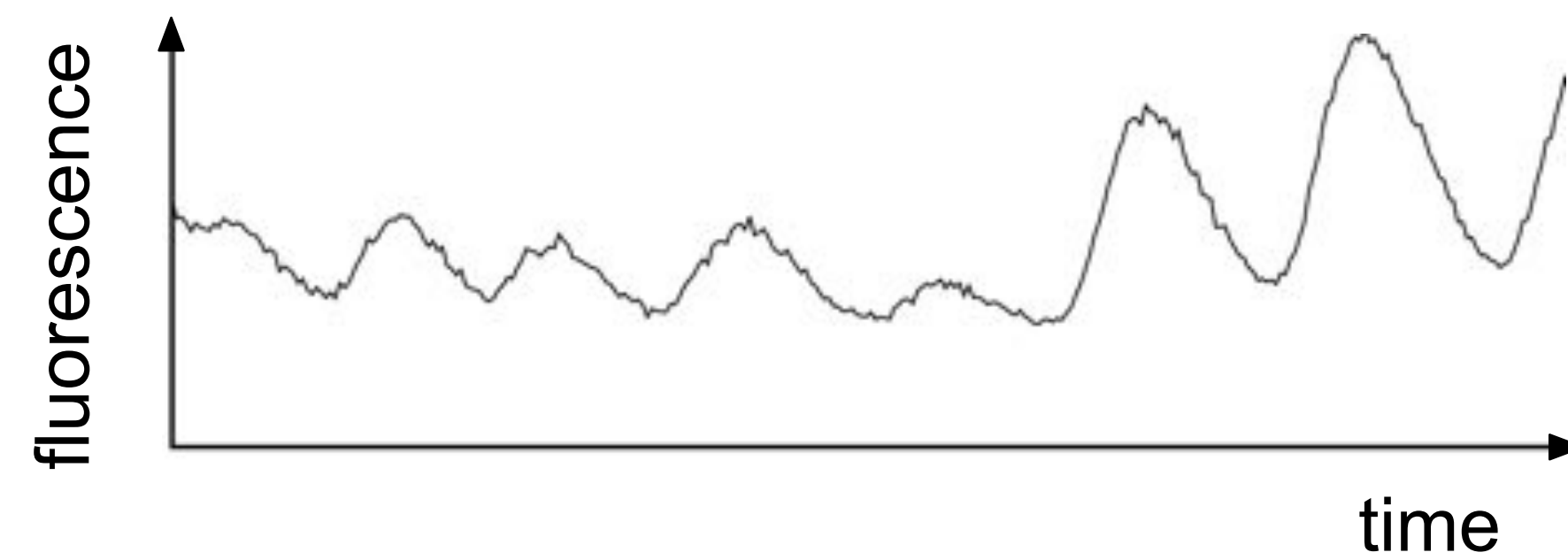


Isolated PSM cells oscillate in culture



cyclic reporter gene *her1*-YFP

Annelie Oswald

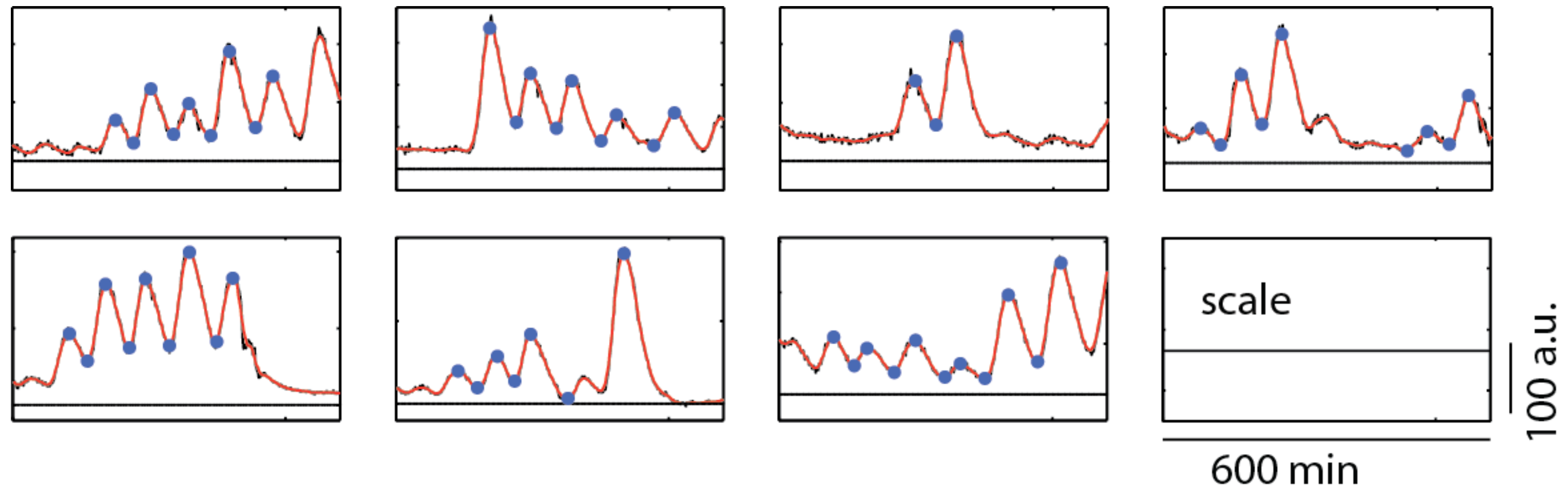


Alexis Webb

? Mouse: Masamizu et al., 2006 ?

Webb et al., *eLife* 2016

Cellular rhythms are heterogeneous

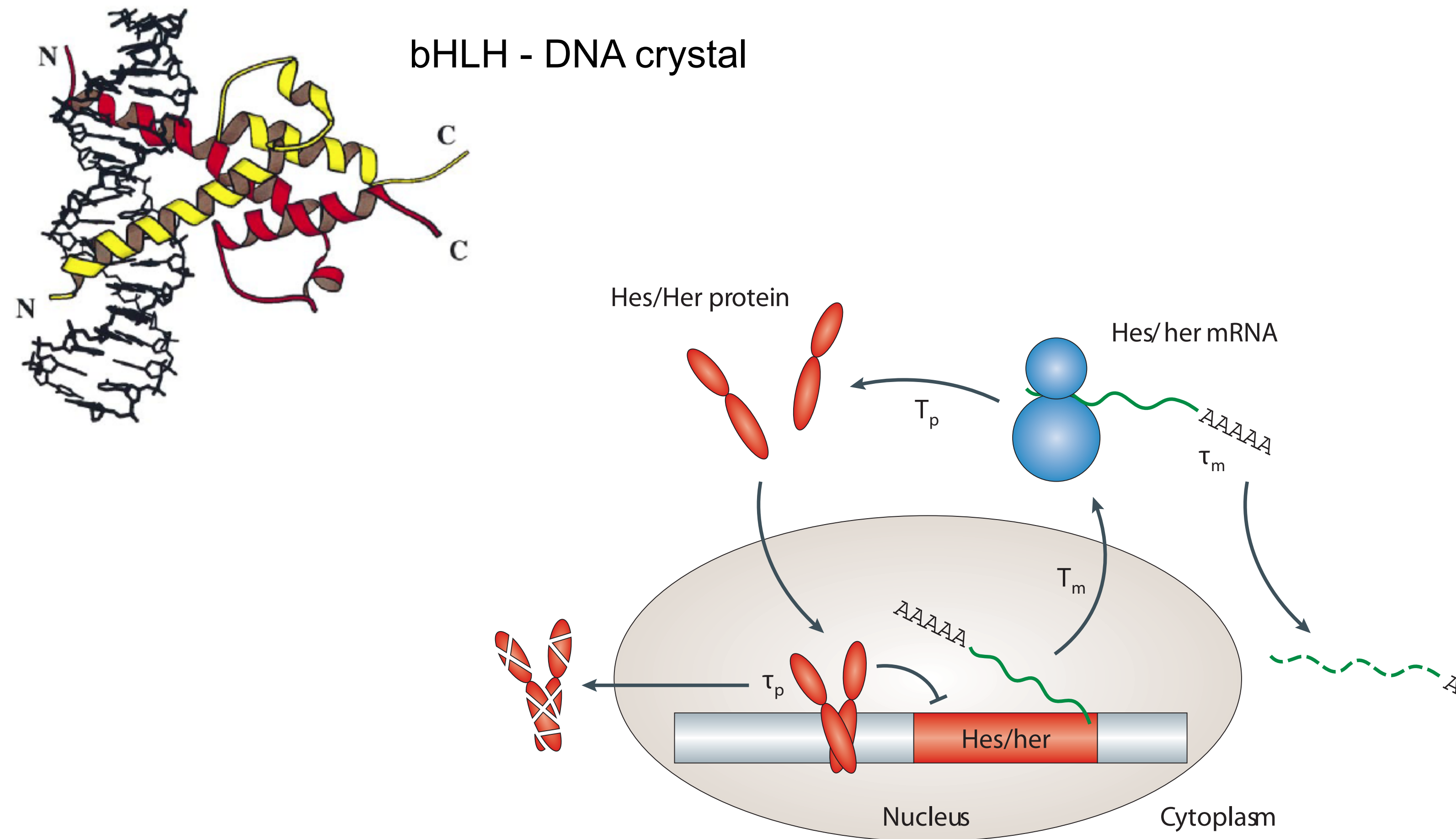


Oscillations require *negative feedback*

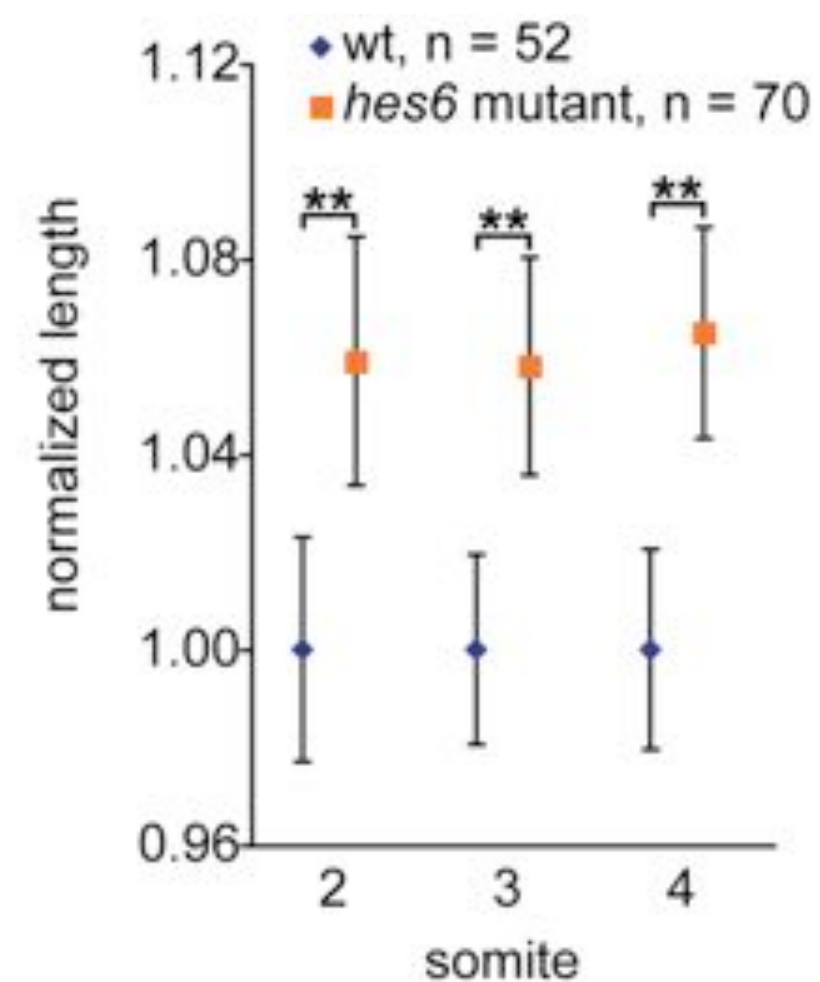
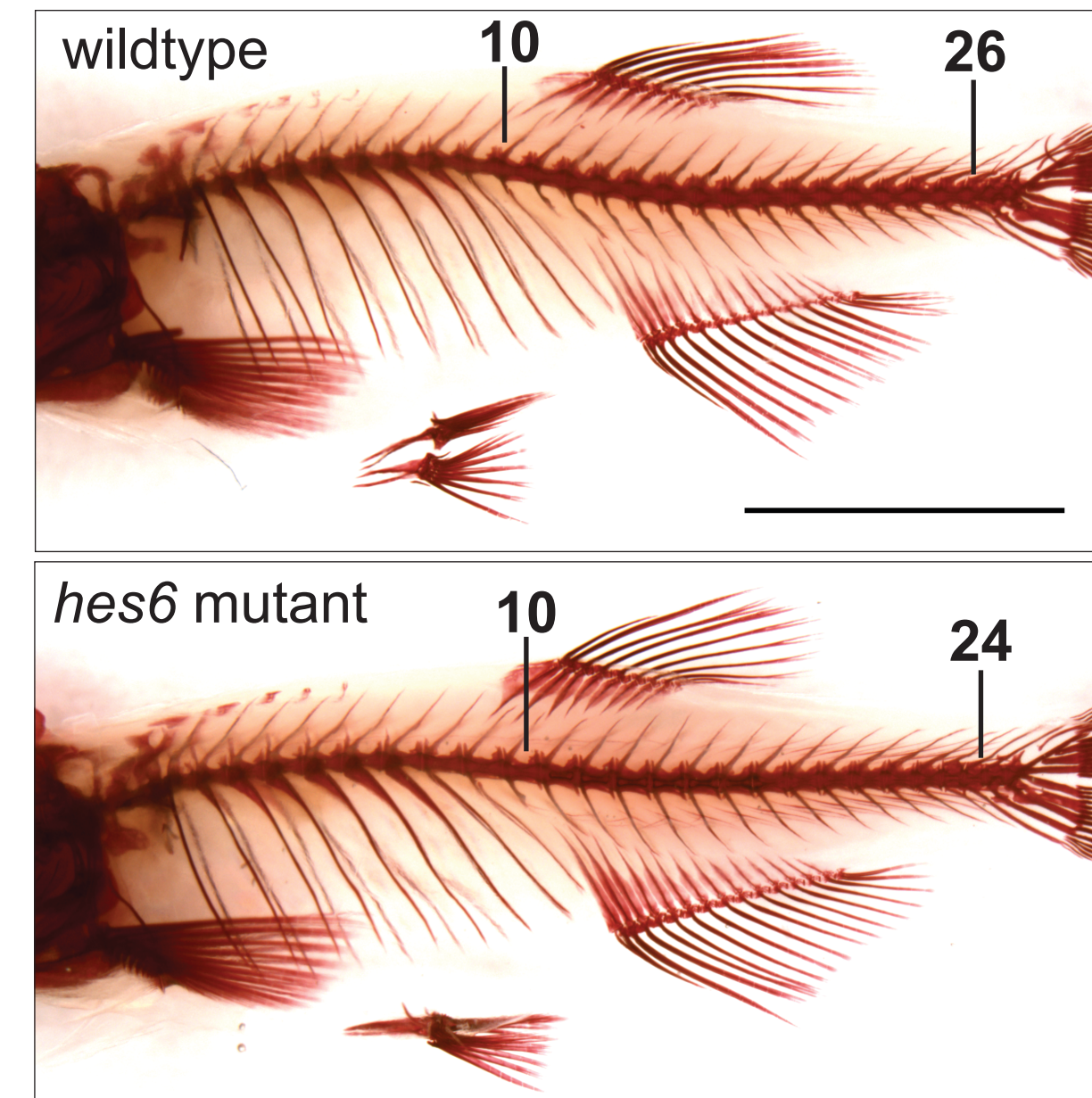
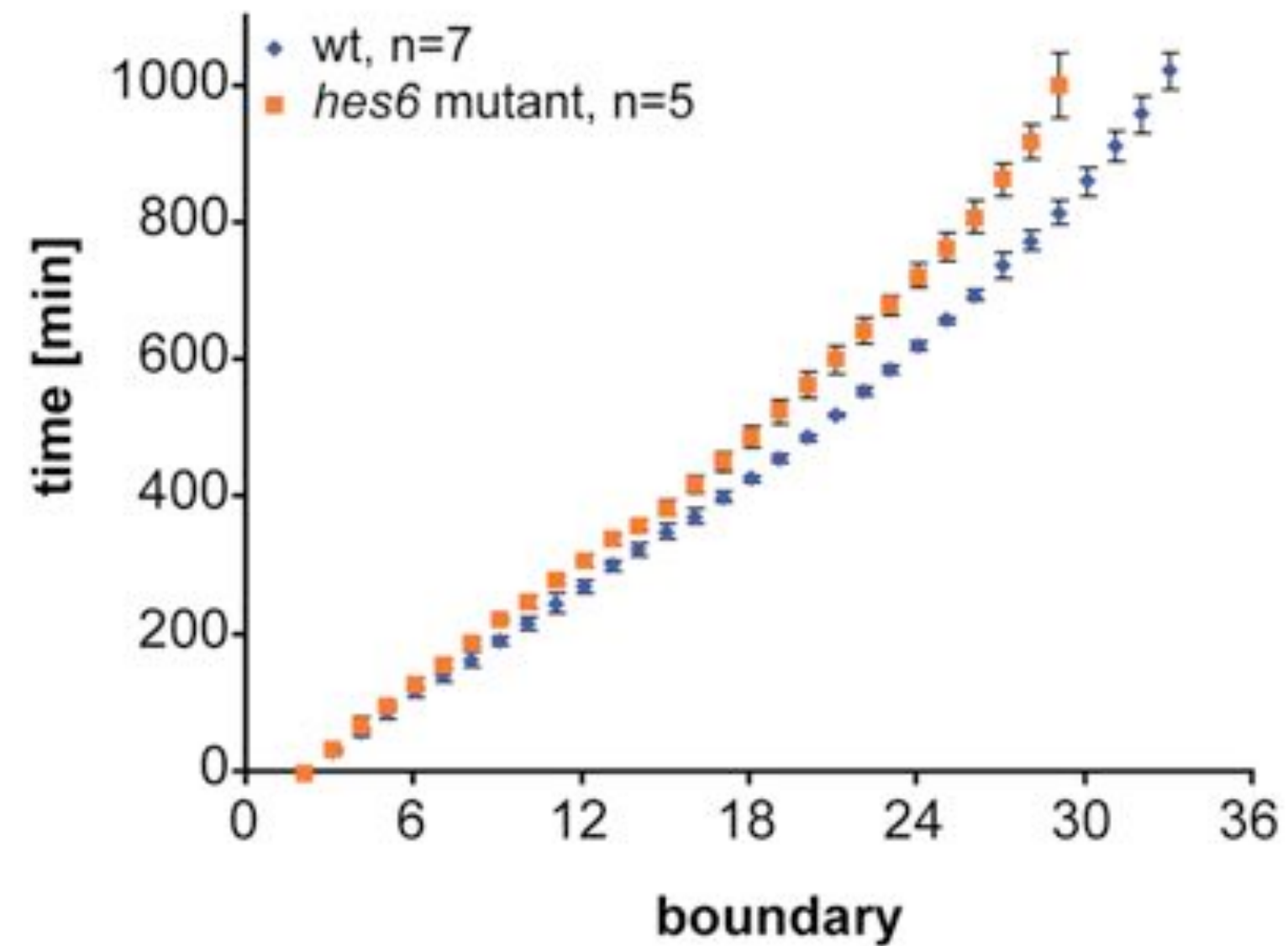


Known example: the Circadian Clock

A model for the single-cell pacemaker circuit



Period mutants: altering somitogenesis period alters segment length and number



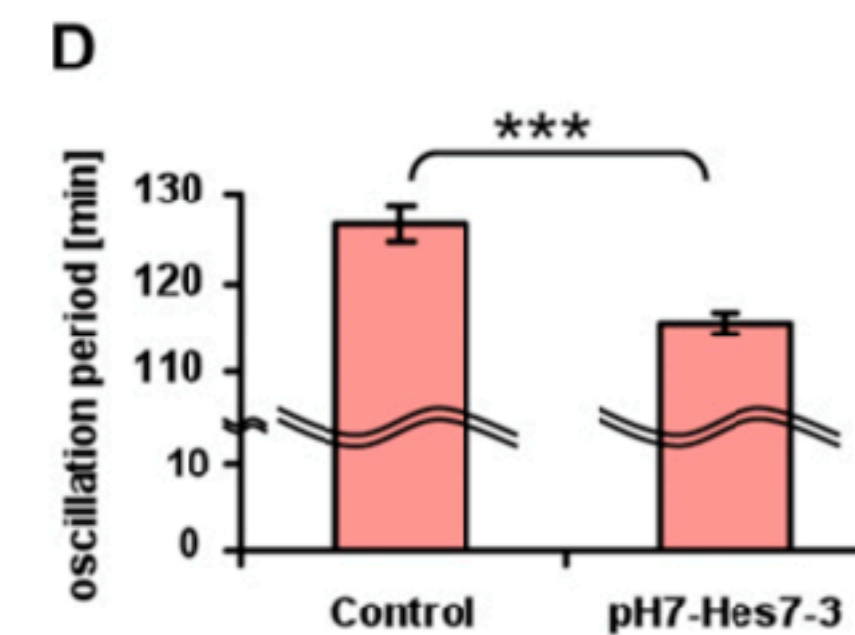
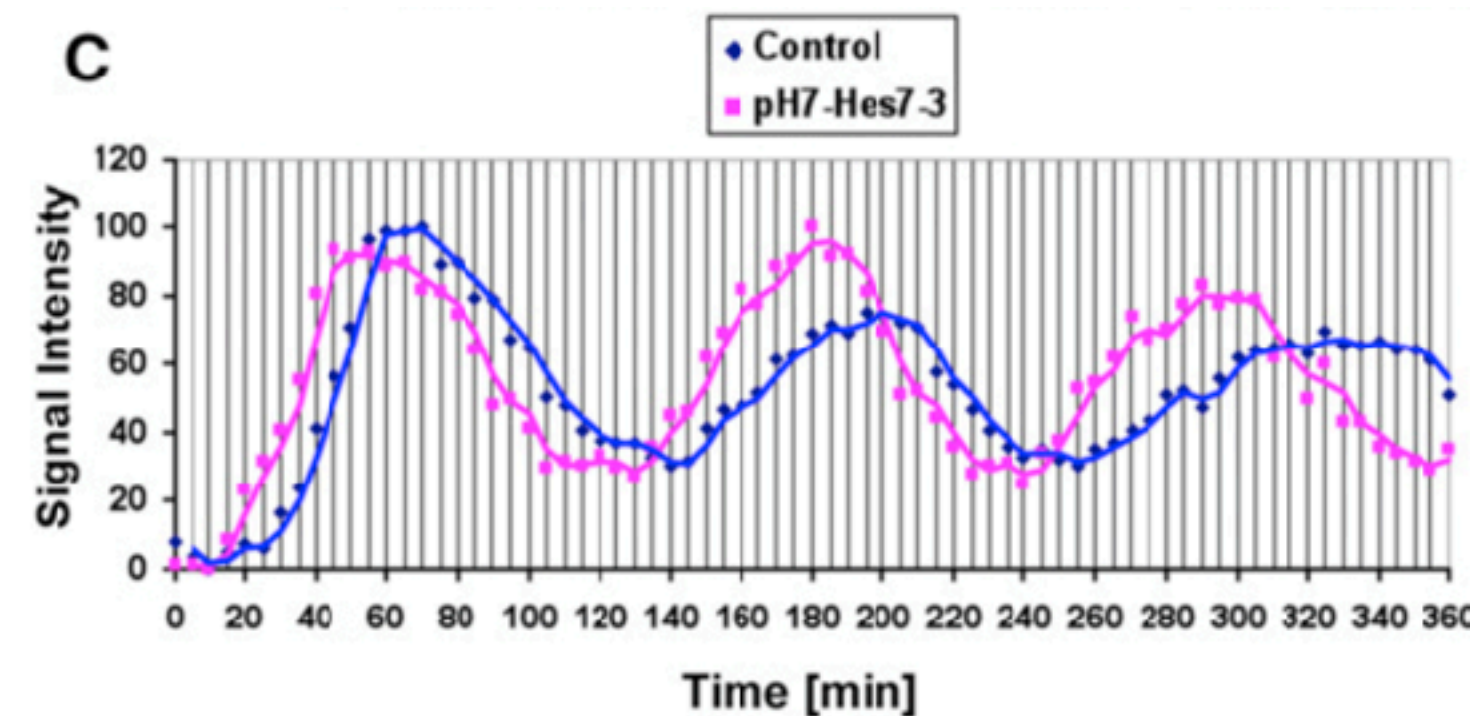
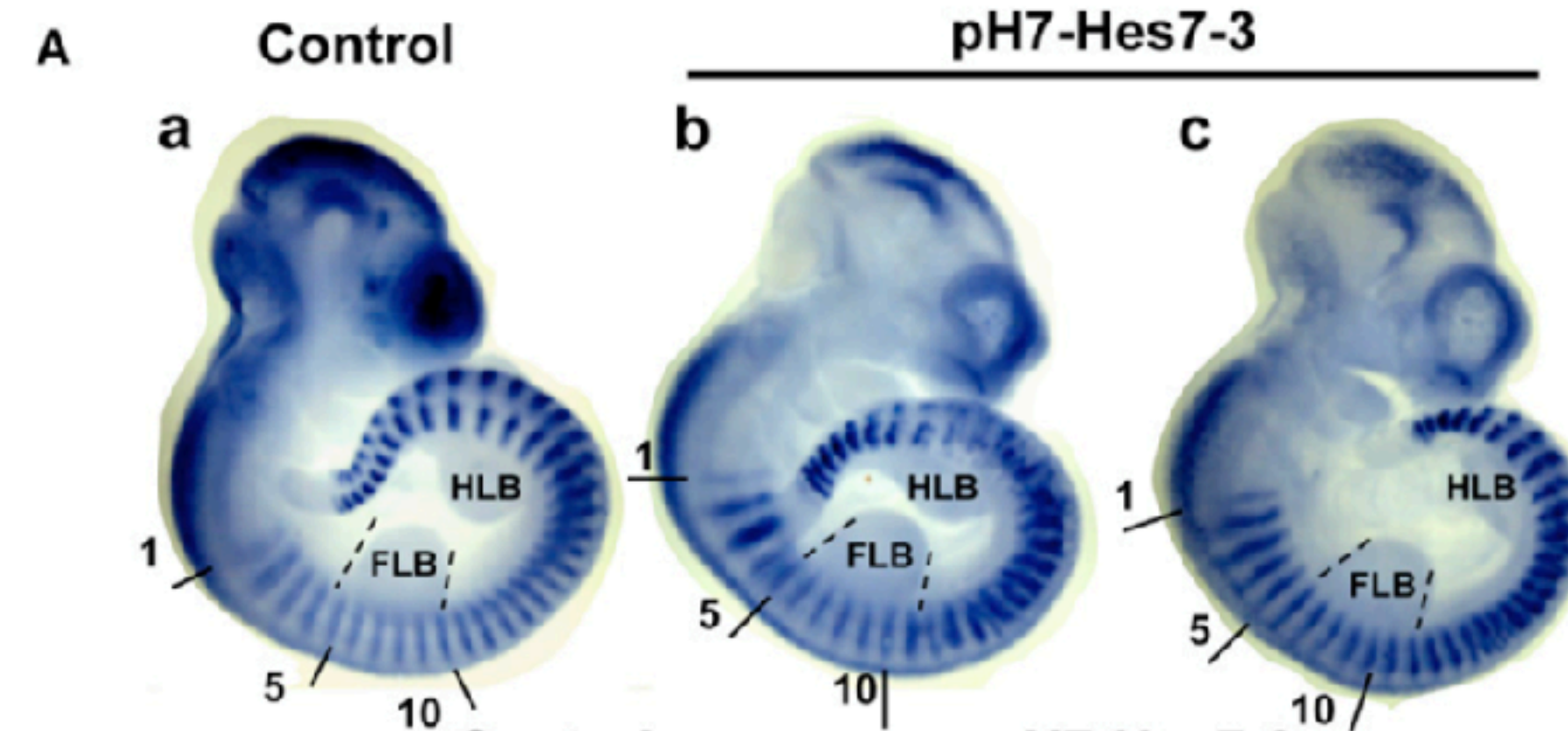
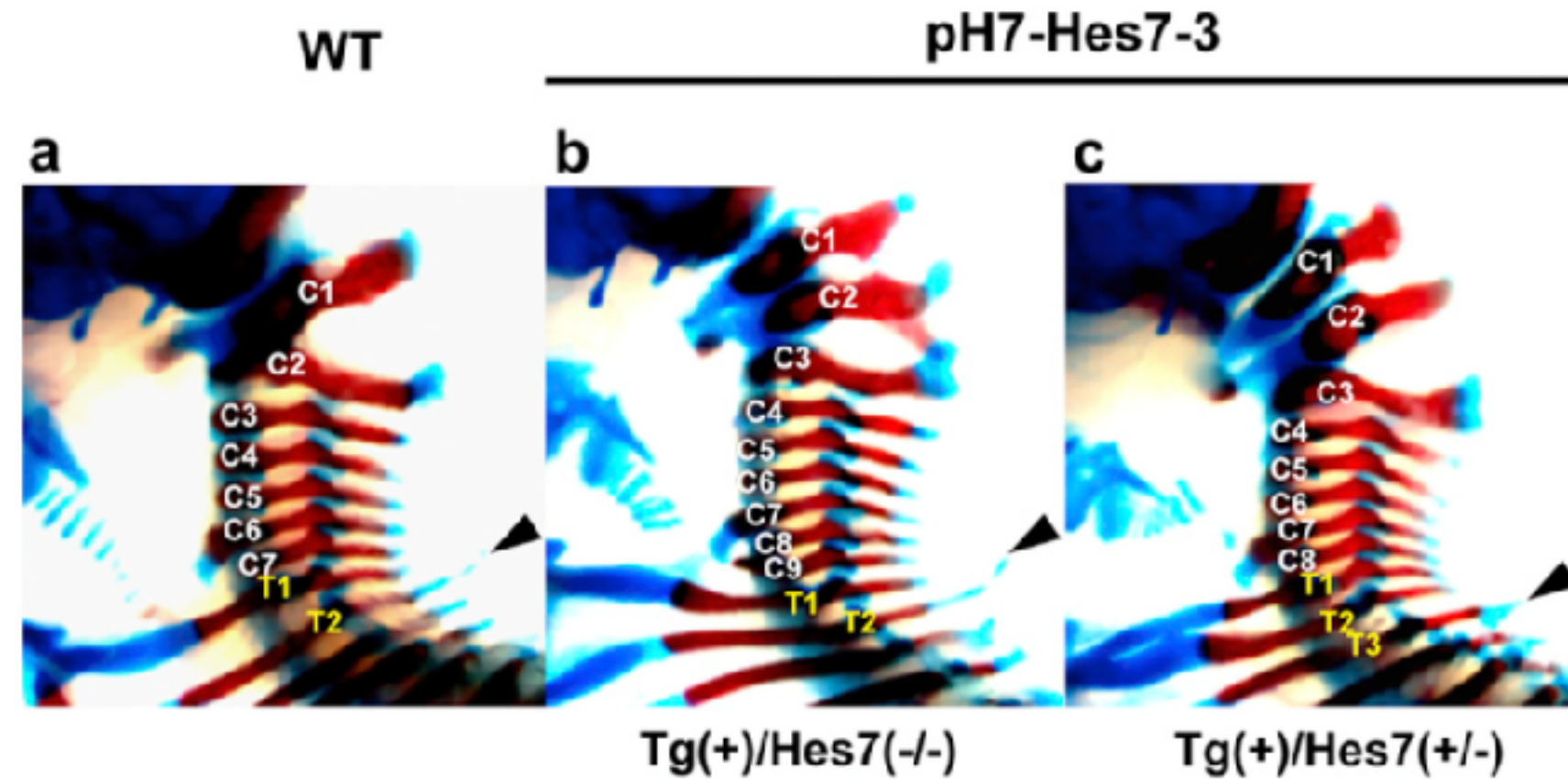
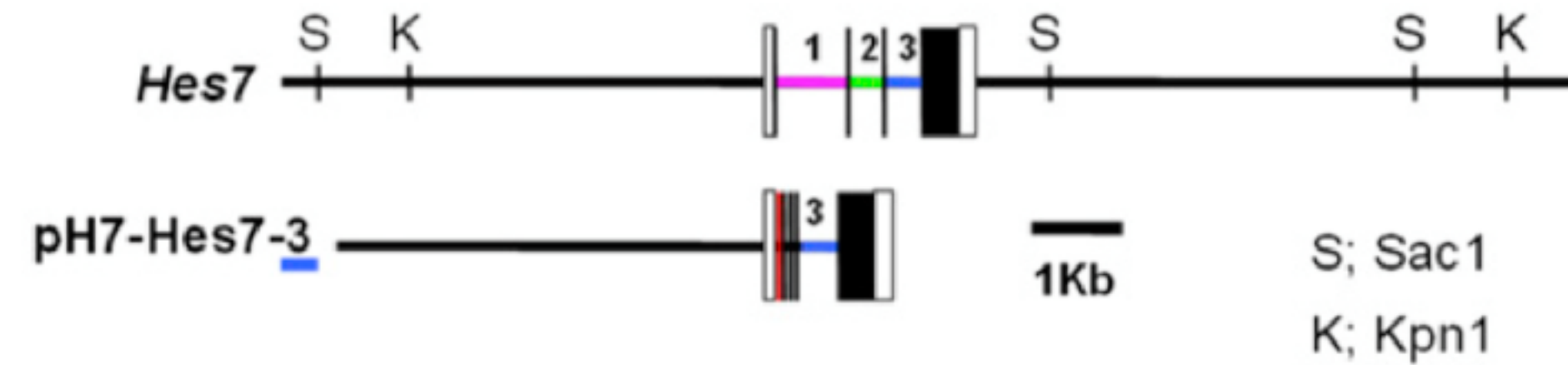
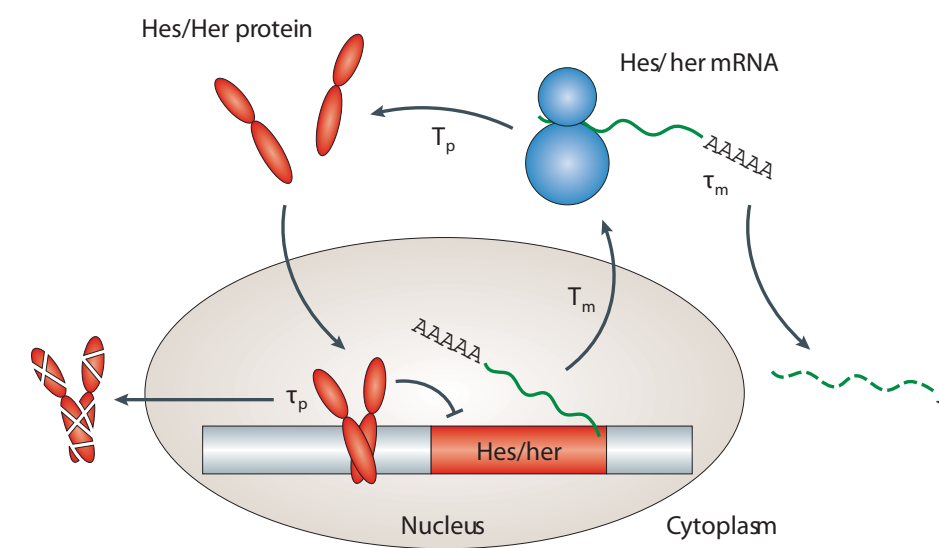
$$S = vT$$

$$n = d/T$$

Schröter and Oates, 2010;
Herrgen et al., 2010; Harima et al., 2013

Speeding up the clock with *fewer* introns

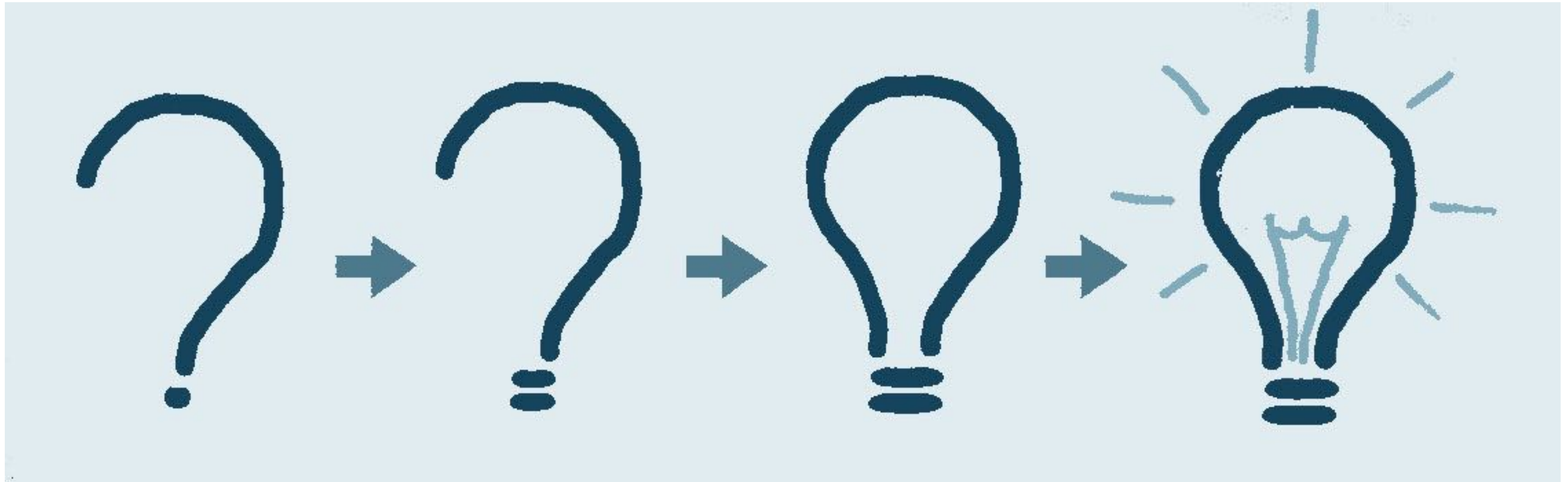
Harima et al., *Cell Reports*, 2013



1st tier: cellular oscillators

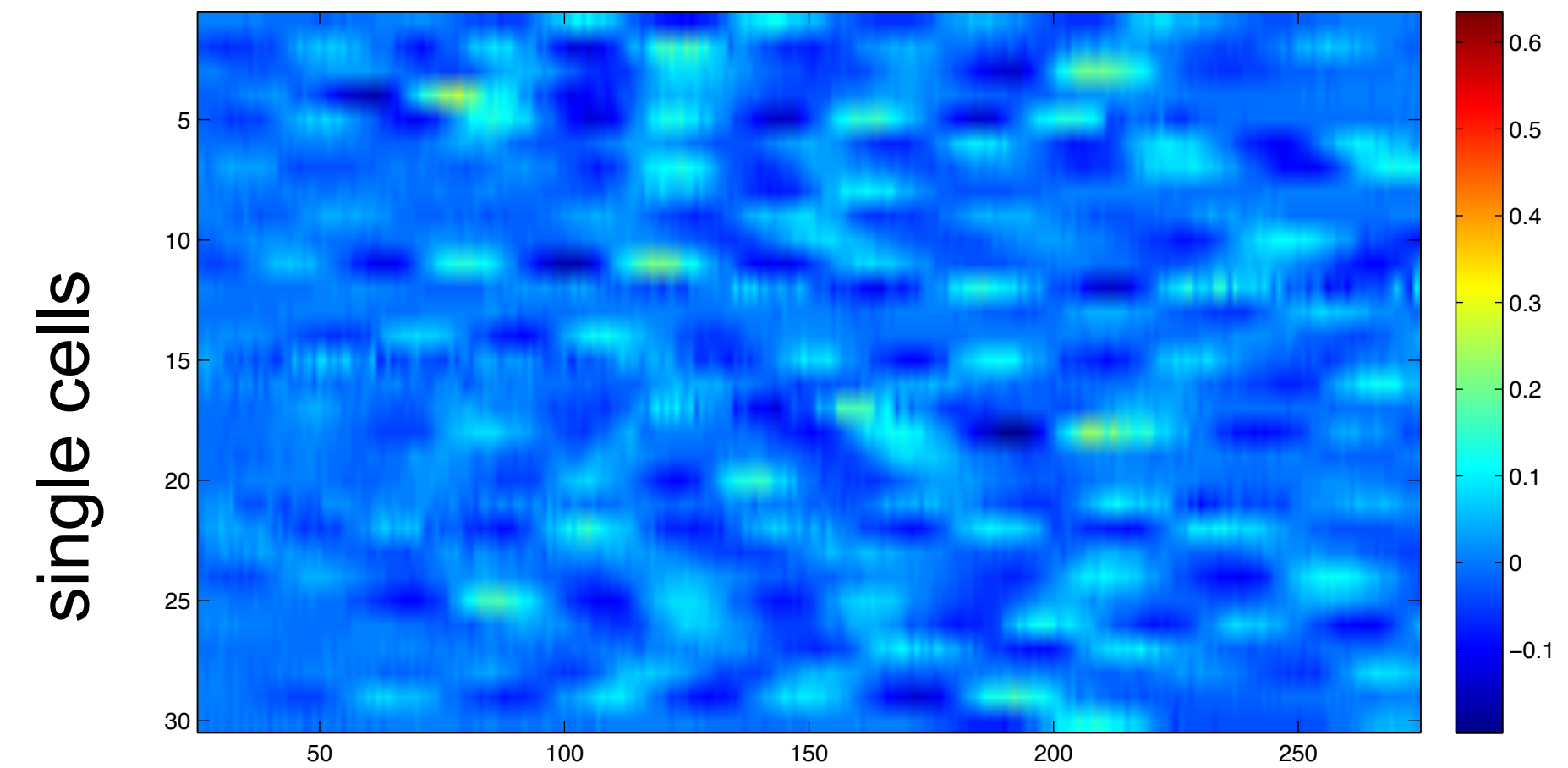
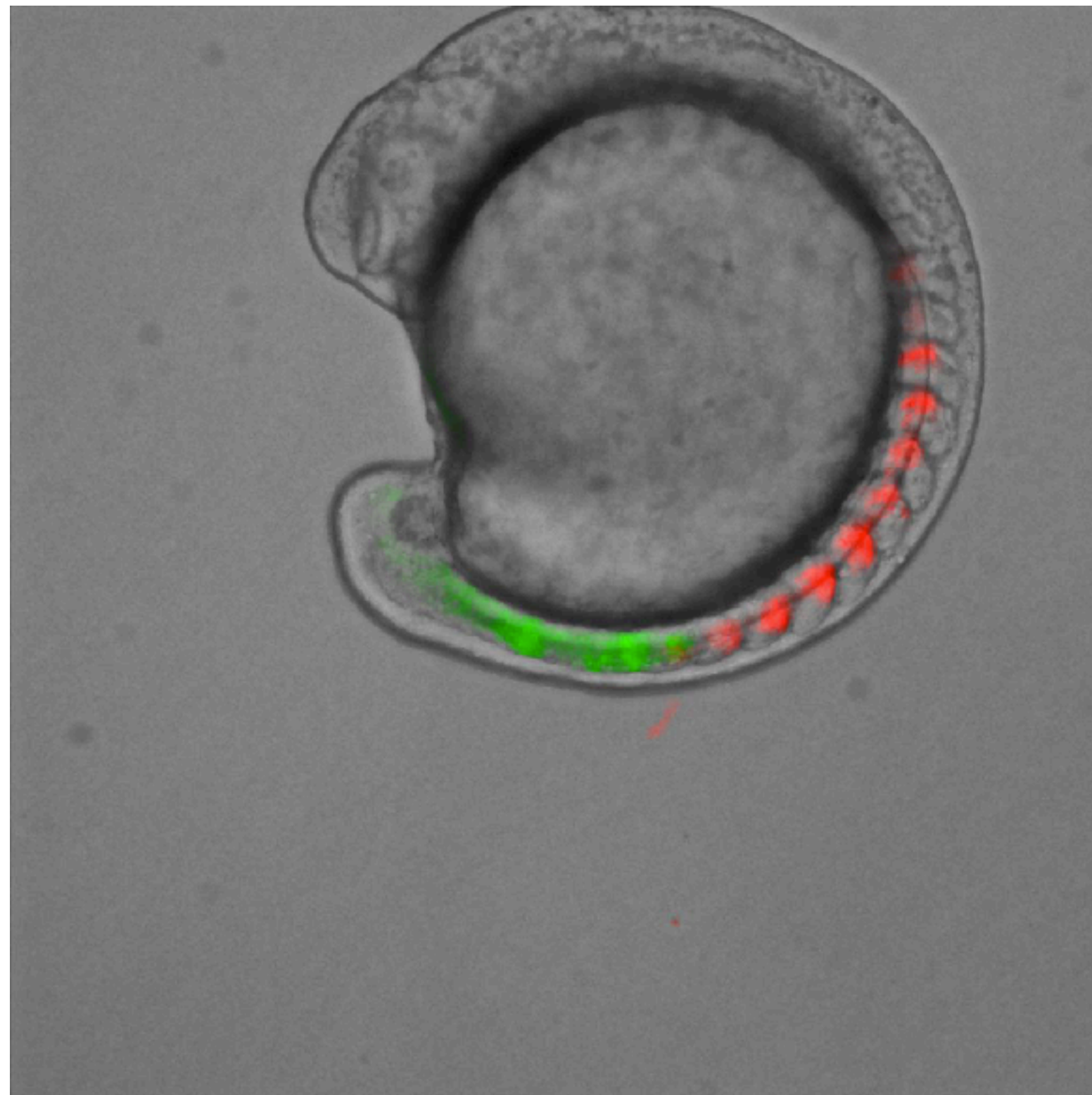
- noisy, autonomous oscillators
 - negative feedback mechanism
 - Hes/her transcriptional repressors
 - period mutants
-
- key papers:
 - Lewis, J. (2003). Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator. *Current Biology : CB*, 13(16), 1398–1408.
 - Schröter, C., & Oates, A. C. (2010). Segment number and axial identity in a segmentation clock period mutant. *Current Biology : CB*, 20(14), 1254–1258. <http://doi.org/10.1016/j.cub.2010.05.071>
 - Harima, Y., Takashima, Y., Ueda, Y., Ohtsuka, T., & Kageyama, R. (2013). Accelerating the tempo of the segmentation clock by reducing the number of introns in the Hes7 gene. *Cell Reports*, 3(1), 1–7. <http://doi.org/10.1016/j.celrep.2012.11.012>
 - Webb, A. B., Lengyel, I. M., Jörg, D. J., Valentin, G., Jülicher, F., Morelli, L. G., & Oates, A. C. (2016). Persistence, period and precision of autonomous cellular oscillators from the zebrafish segmentation clock. *eLife*, 5. <http://doi.org/10.7554/eLife.08438>

Questions?



Take a break

Noisy cellular components vs. coherent tissue-level patterns



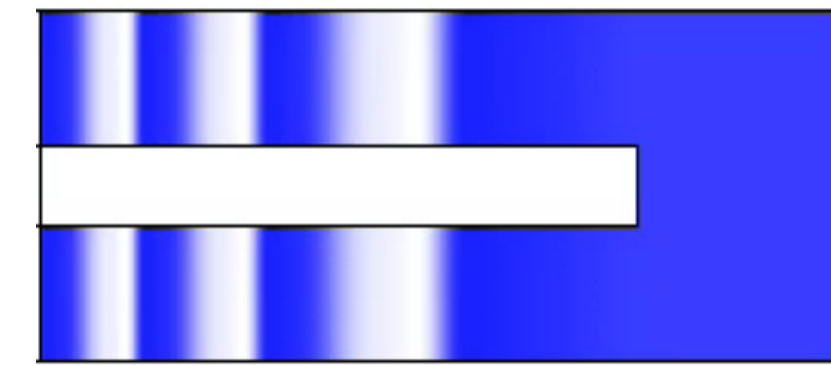
Alexis Webb

cyclic reporter gene *her1*-YFP

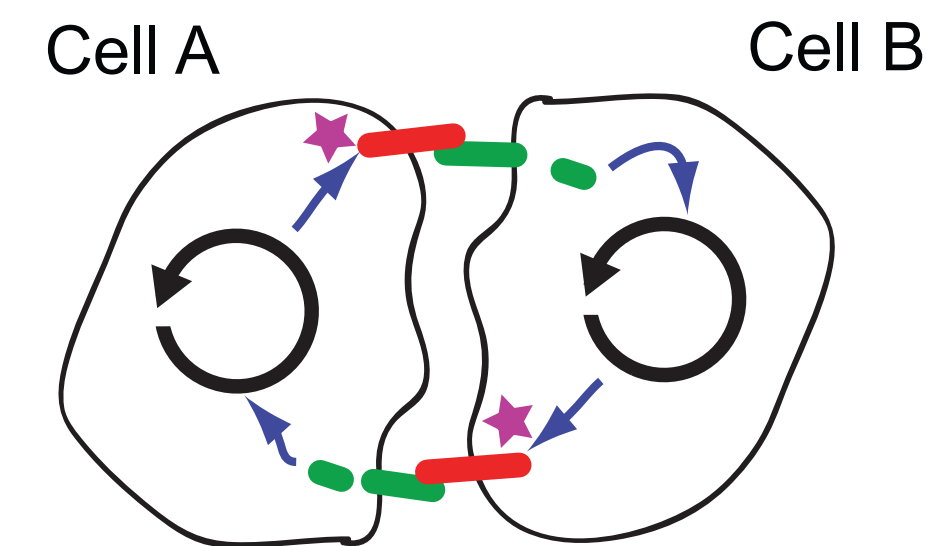
The segmentation clock is a tissue-level, rhythmic pattern generator



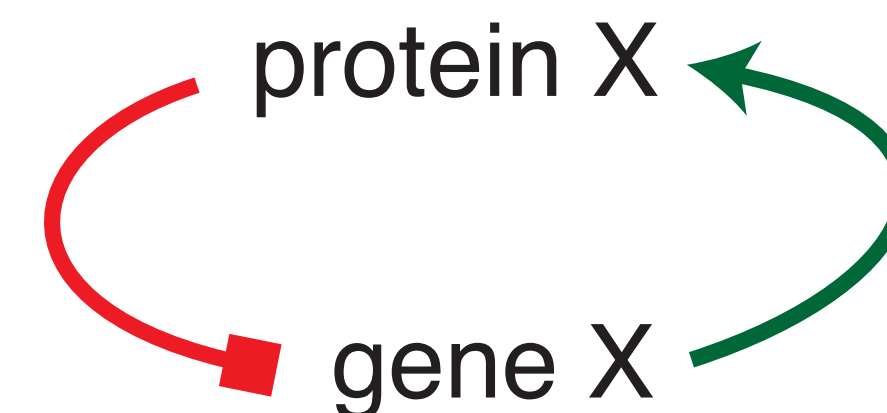
Tissue-level wave patterns



Local synchronization



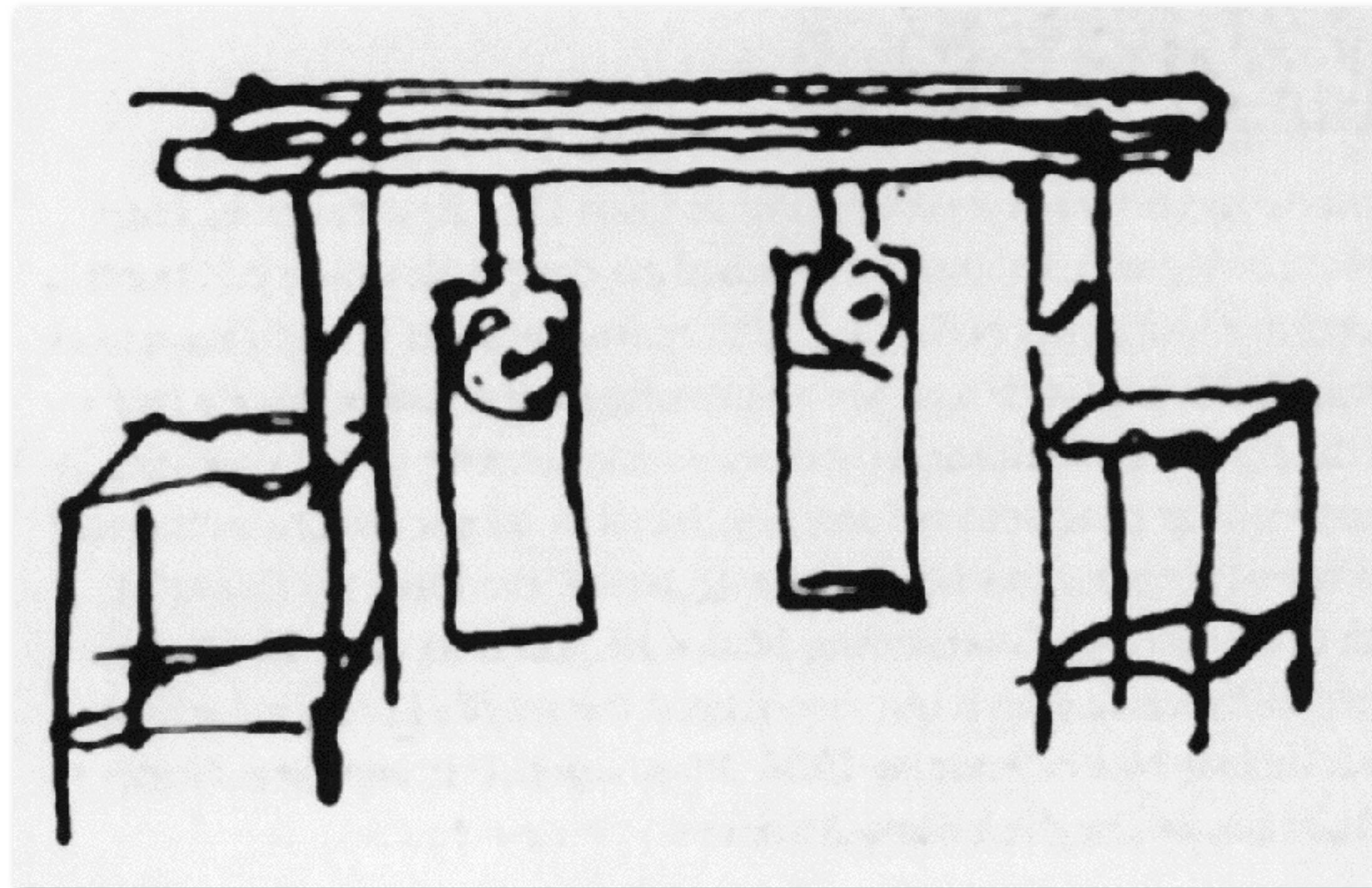
Cellular oscillators



Coupled, noisy oscillators can synchronize

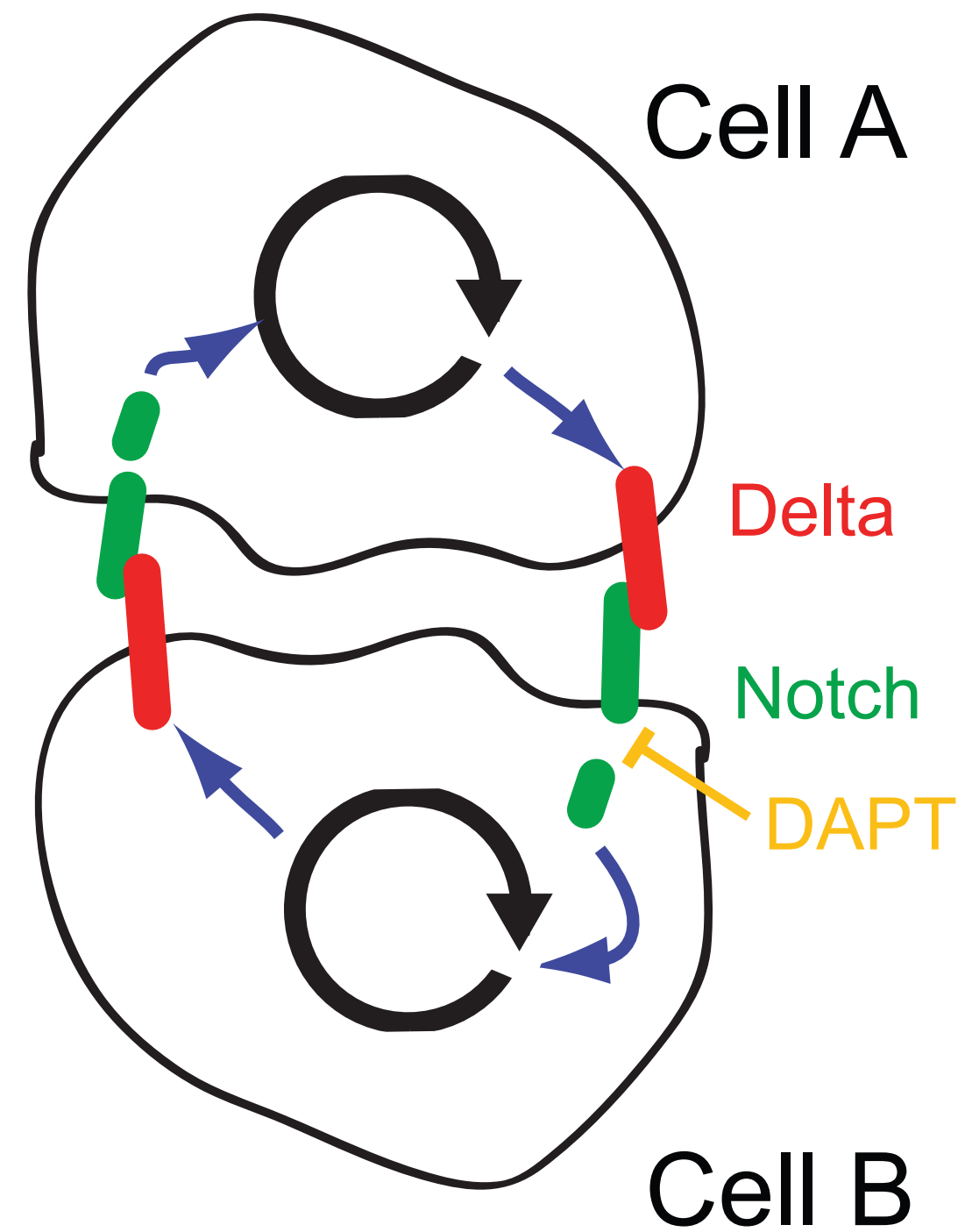


Christiaan Huygens (1629-1695)



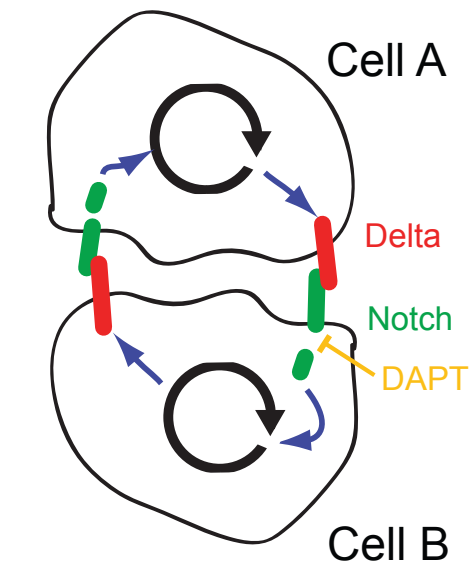
“sympathy of two clocks”

Coupling mechanism: Delta-Notch cell-cell signaling



Jiang et al., 2000
Lewis, 2003
Horikawa et al., 2006
Riedel-Kruse et al., 2007
Ozbudak et al., 2008
Delaune et al., 2012

Blocking Notch disrupts segmentation and wave pattern

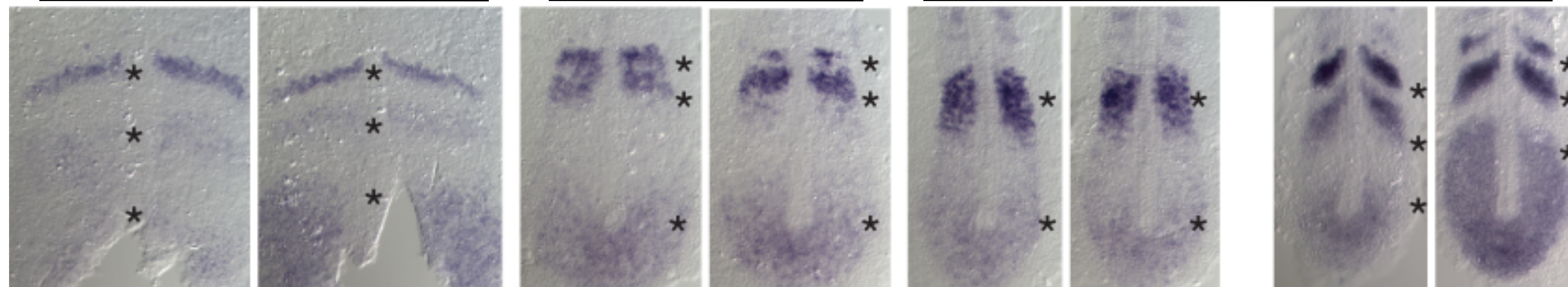


deltaC

1 som

7 som

12 som

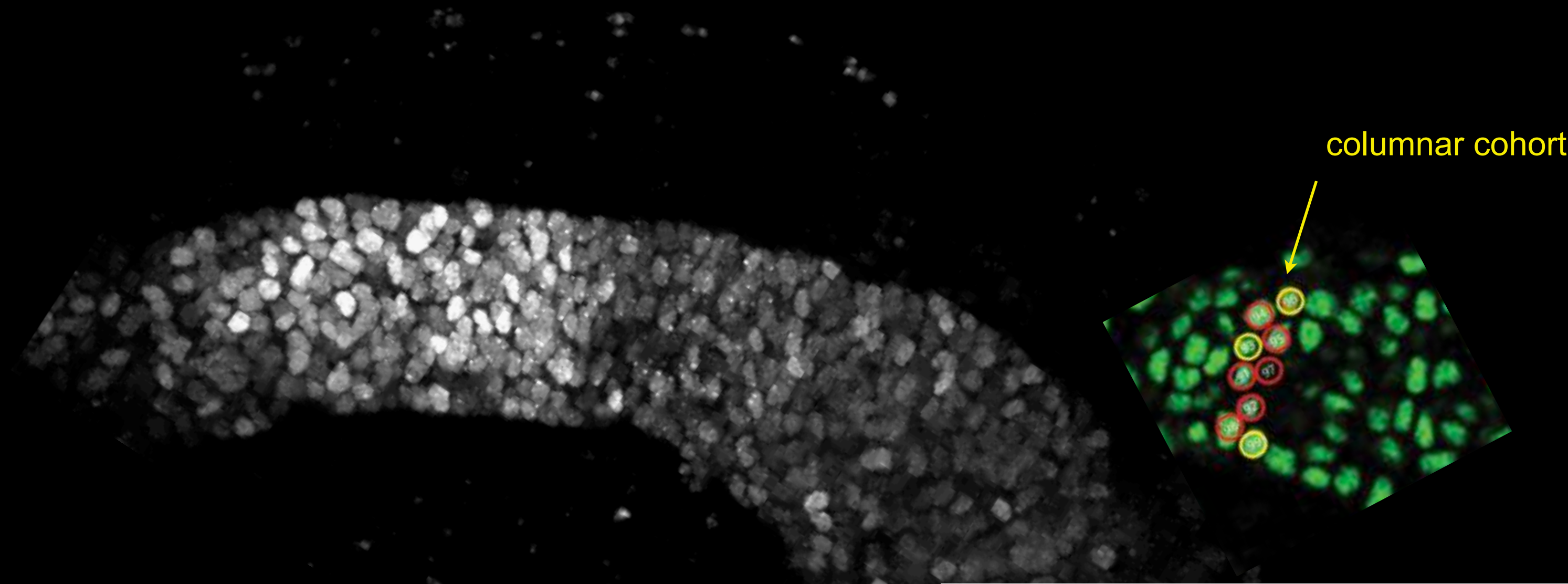


Early

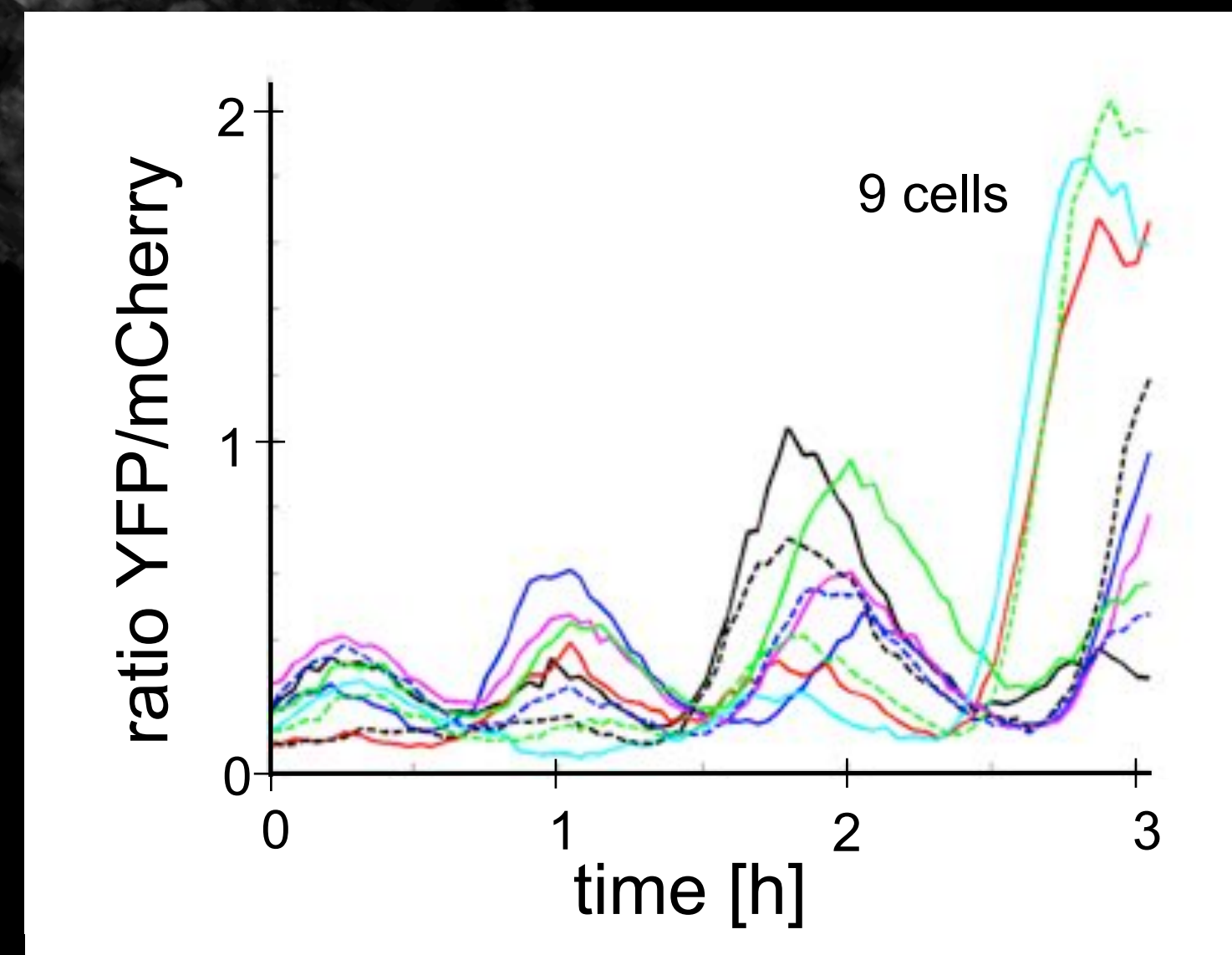
Late

WT

Neighboring cells oscillate together



cyclic reporter gene *her1*-YFP nuclear
marker H2A -mCherry

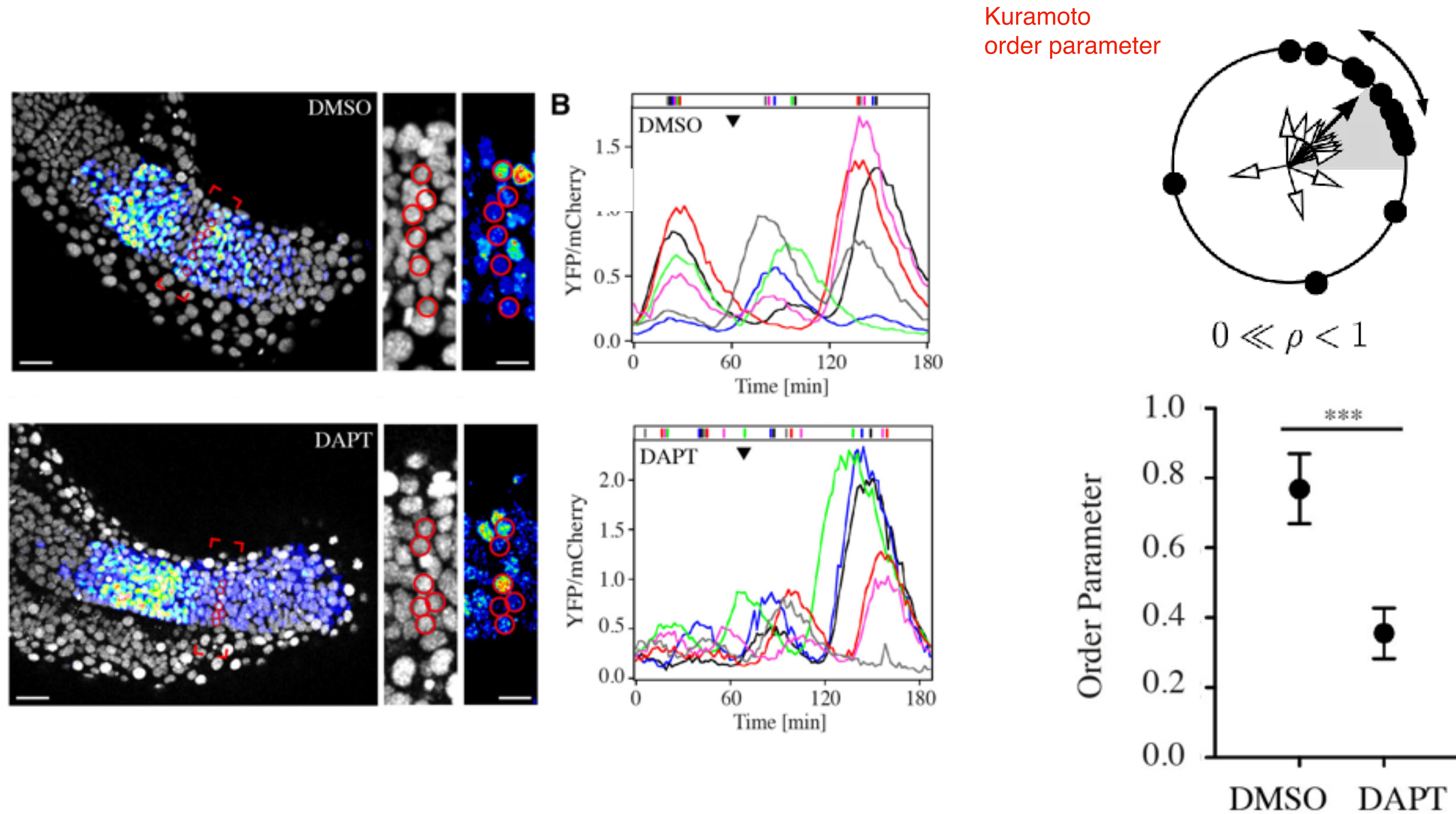


Zeiss 710, single photon, W 40x NA 1.0; 1 frame/~3 min;
dXY=0.35, 1024x1024; dZ= 1.0, #Z~85

David Richmond

Without Notch, cells keep oscillating, but are no longer synchronized

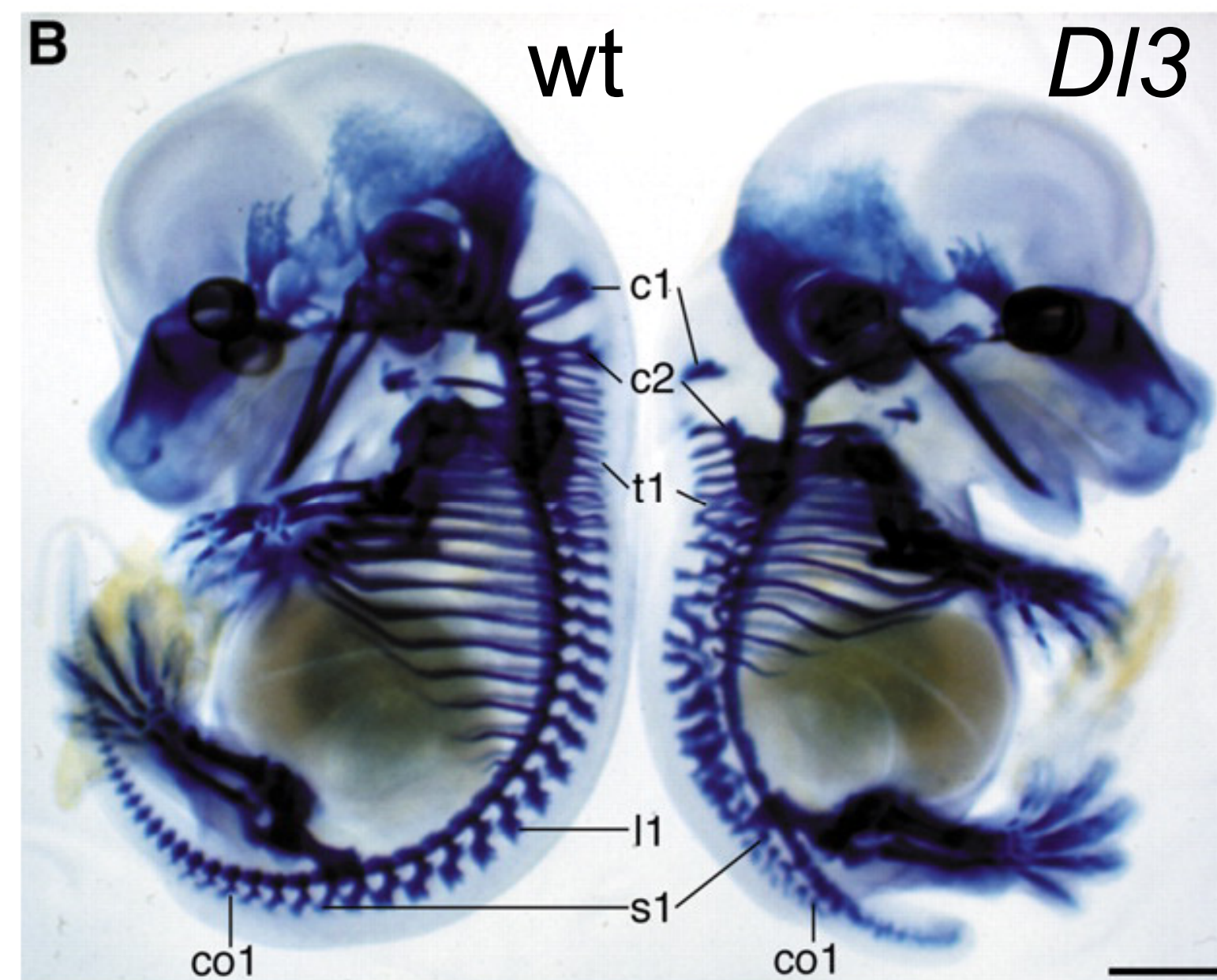
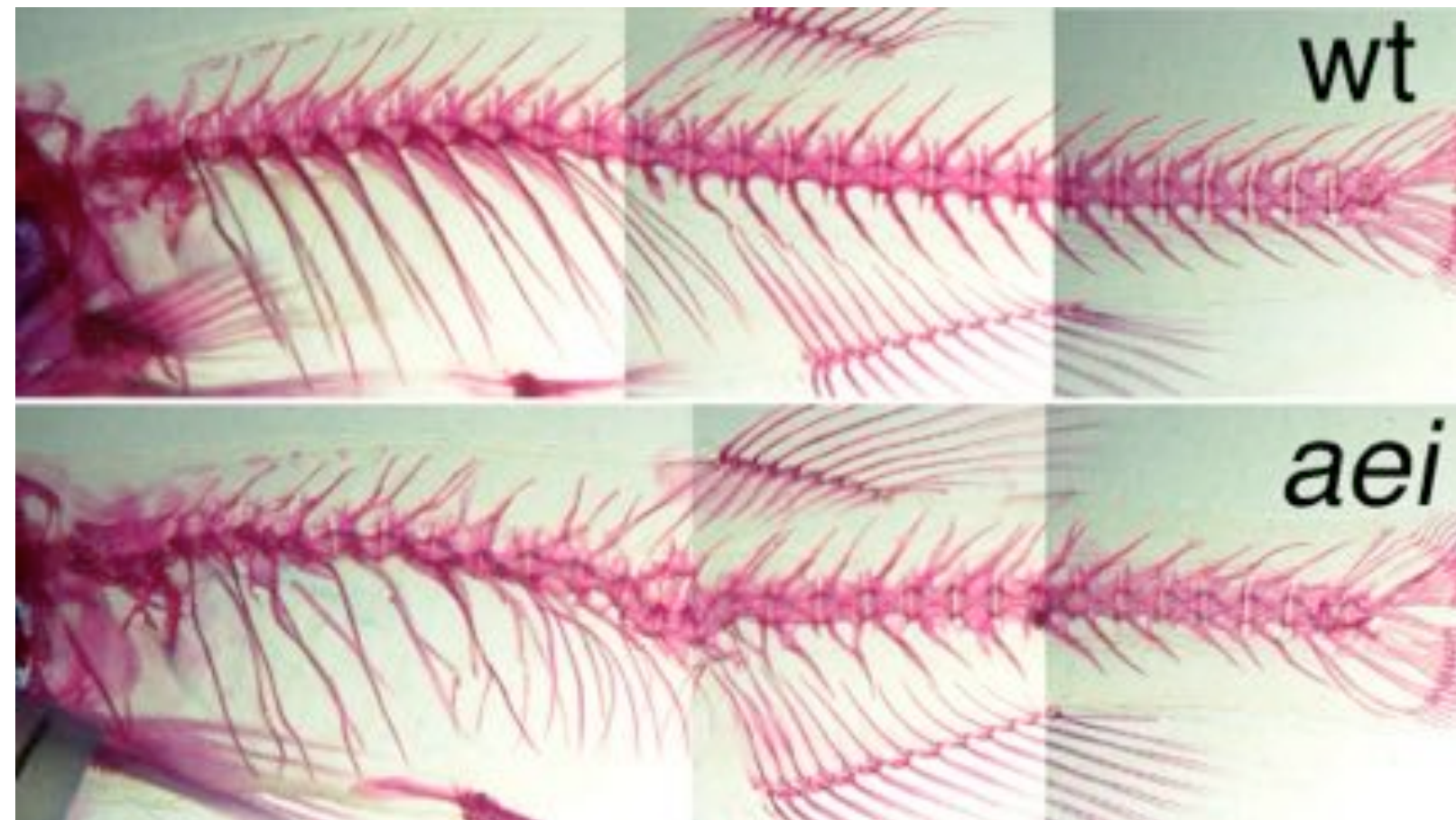
Delaune et al., 2012



n = 8 experiments; 85 cells; approx. 80K pair-wise comparisons

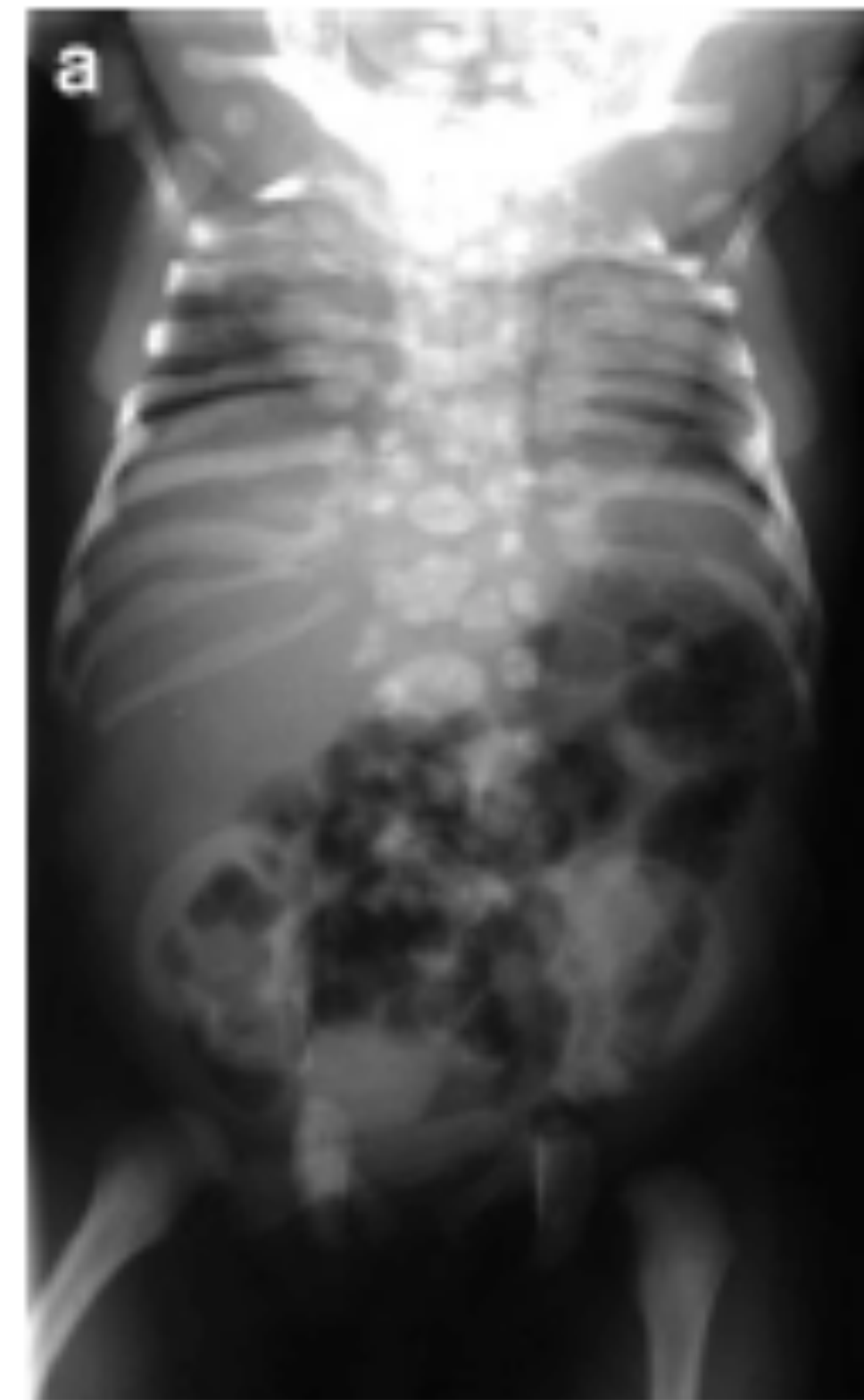
David Richmond

Synchronization defects cause congenital scoliosis



Dunwoodie et al., 2002

DLL3



Turnpenny et al., 2007

2nd tier: local synchronization

- neighboring oscillators communicate
- Delta-Notch signaling is involved
- synchronization overcomes noise
- required for correct segmentation

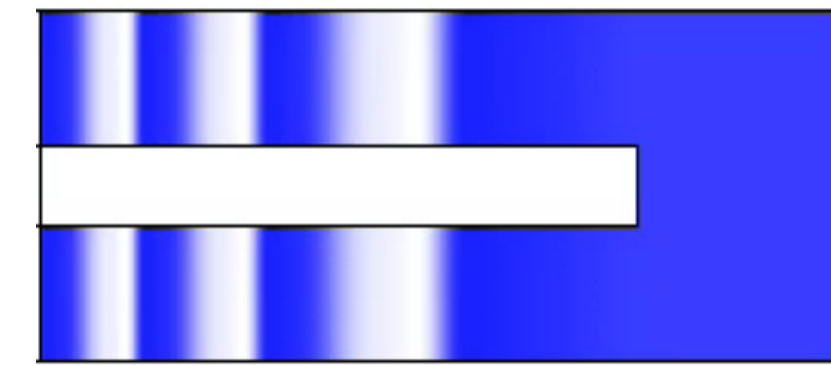
- key papers:

- M P Bulman, K Kusumi, T M Frayling, C McKeown, C Garrett, E S Lander, R Krumlauf, A T Hattersley, S Ellard, and P D Turnpenny (2000). Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. *Nature Genetics*, 24(4), 438–441. <http://doi.org/10.1038/74307>
- Dunwoodie, S. L., Clements, M., Sparrow, D. B., Sa, X., Conlon, R. A., & Beddington, R. S. P. (2002). Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene Dll3 are associated with disruption of the segmentation clock within the presomitic mesoderm. *Development (Cambridge, England)*, 129(7), 1795–1806.
- Riedel-Kruse, I. H., Müller, C., & Oates, A. C. (2007). Synchrony dynamics during initiation, failure, and rescue of the segmentation clock. *Science (New York, NY)*, 317(5846), 1911–1915. <http://doi.org/10.1126/science.1142538>
- Herrgen, L., Ares, S., Morelli, L. G., Schröter, C., Jülicher, F., & Oates, A. C. (2010). Intercellular coupling regulates the period of the segmentation clock. *Current Biology : CB*, 20(14), 1244–1253. <http://doi.org/10.1016/j.cub.2010.06.034>
- Delaune, E. A., François, P., Shih, N. P., & Amacher, S. L. (2012). Single-cell-resolution imaging of the impact of Notch signaling and mitosis on segmentation clock dynamics. *Developmental Cell*, 23(5), 995–1005. <http://doi.org/10.1016/j.devcel.2012.09.009>

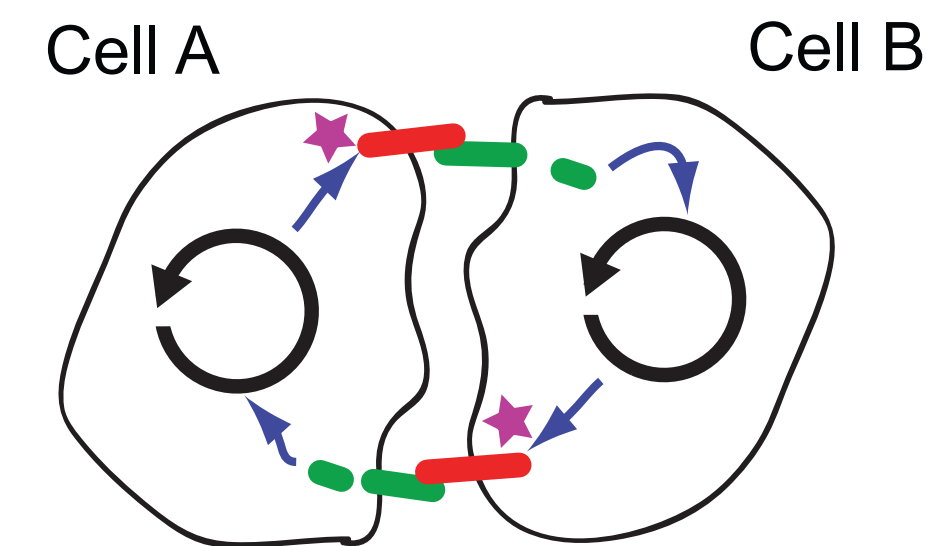
The segmentation clock is a tissue-level, rhythmic pattern generator



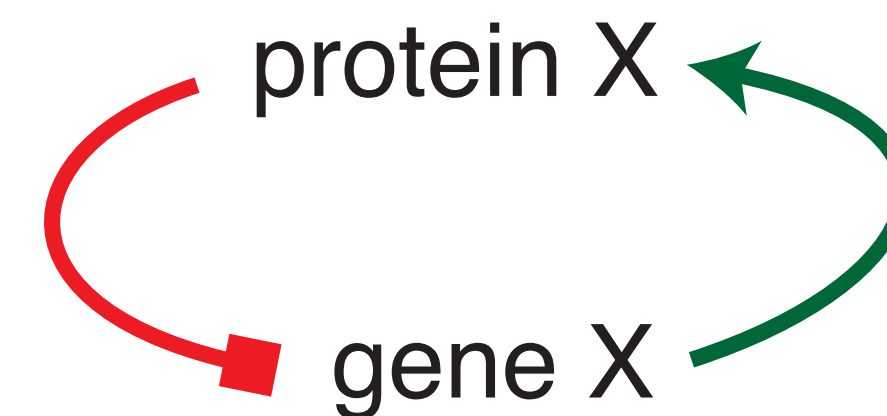
Tissue-level wave patterns



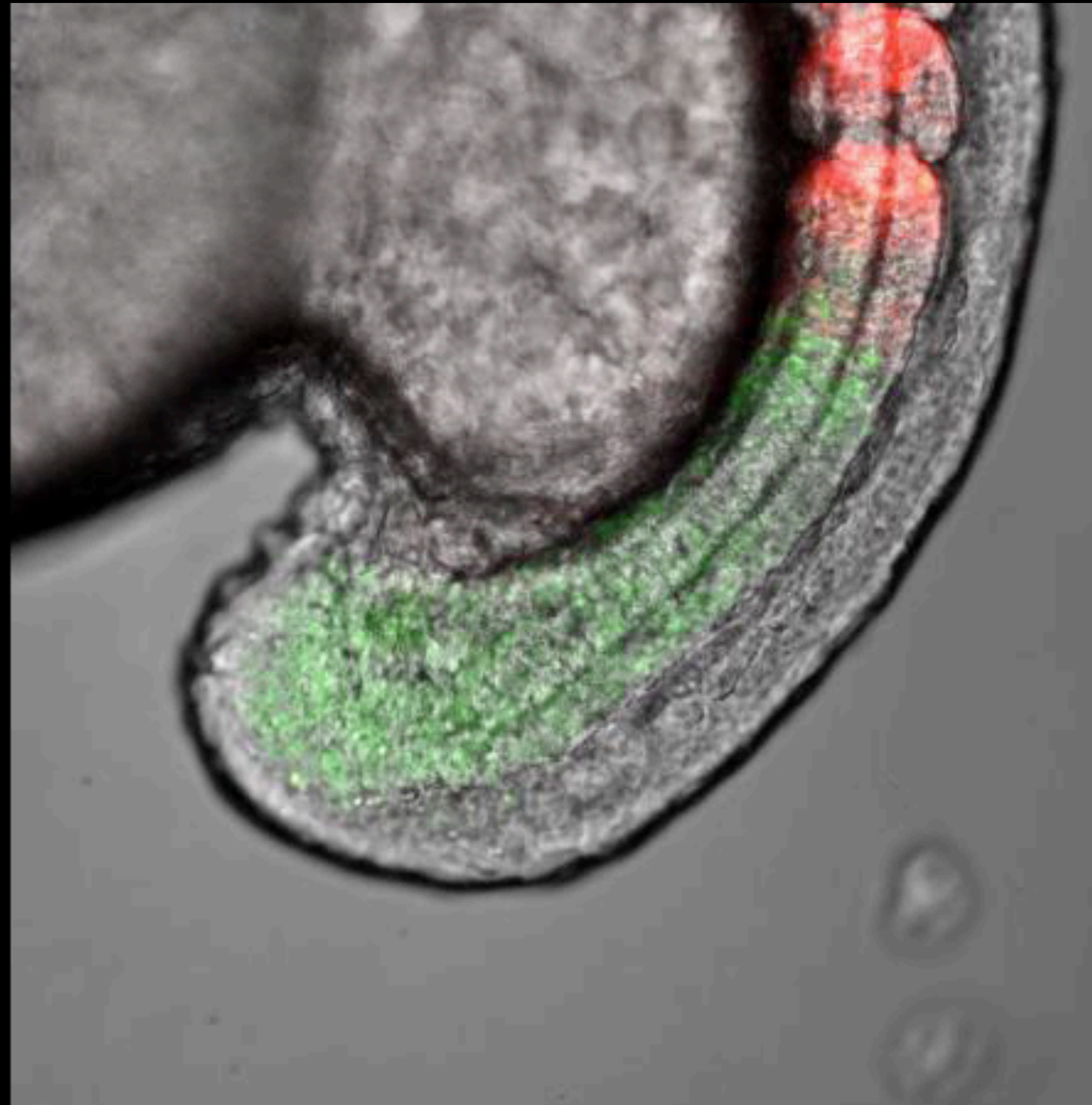
Local synchronization



Cellular oscillators



What generates these patterns?



anterior

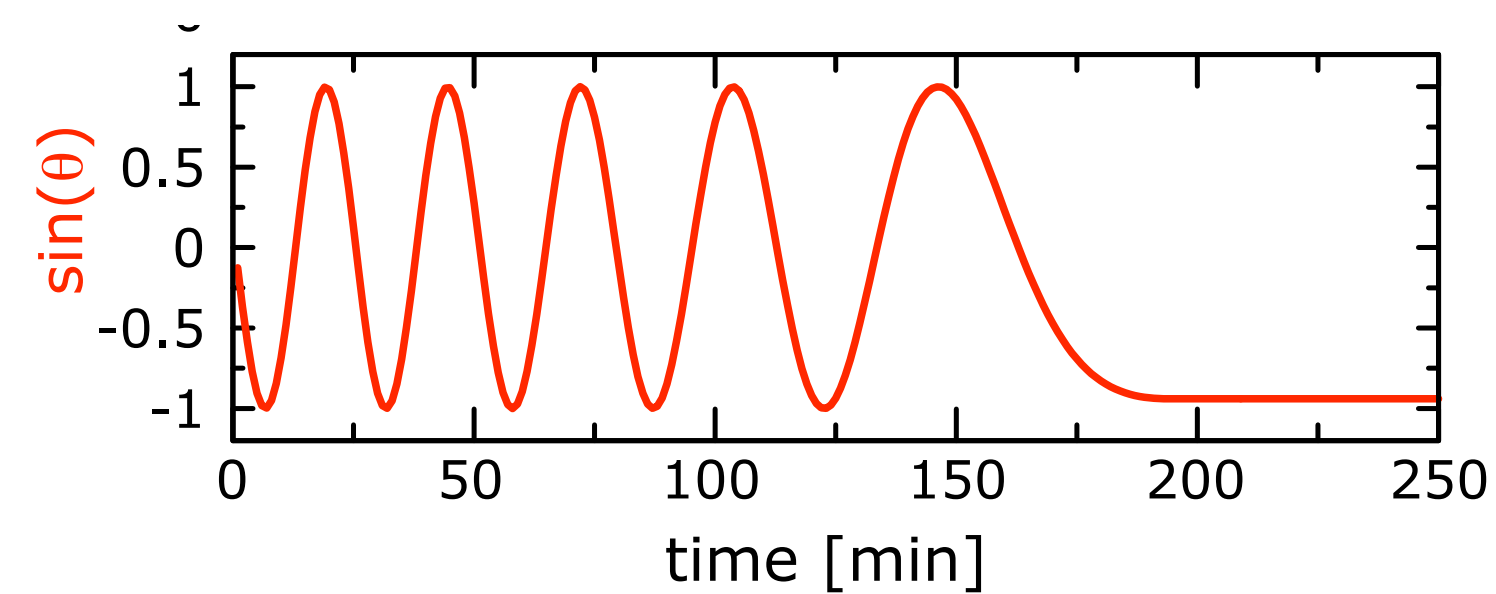
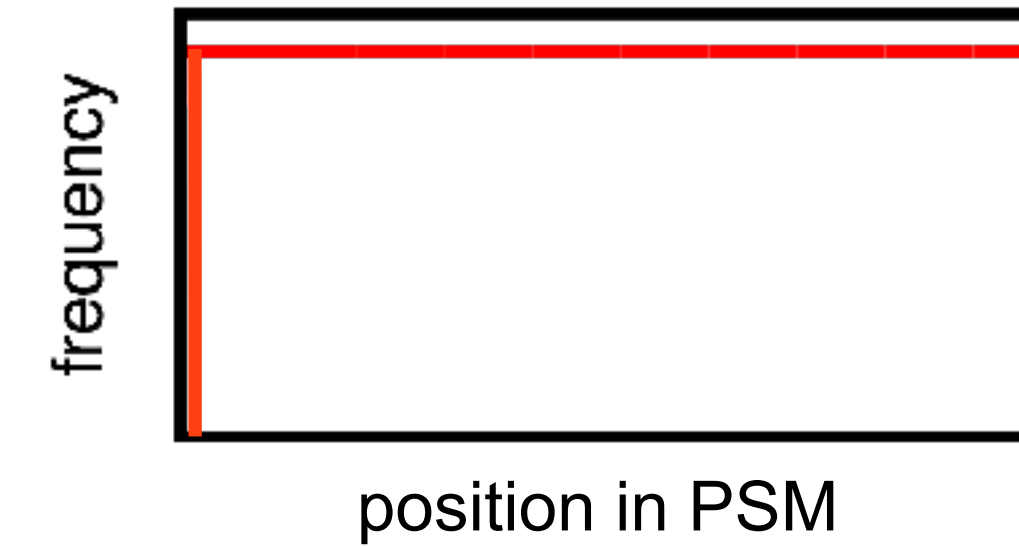
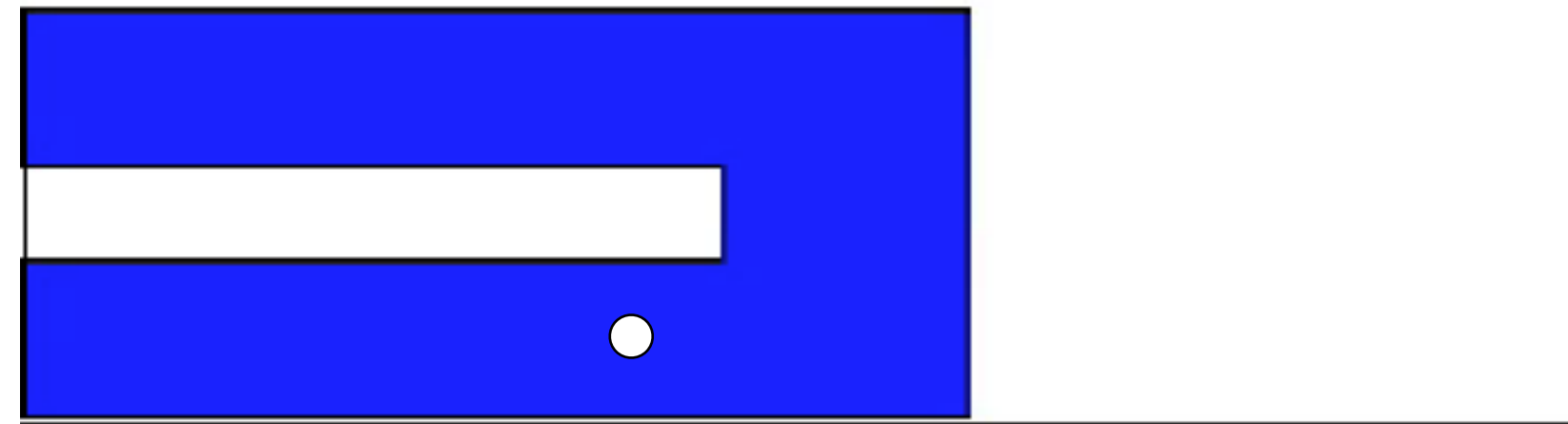
differentiation

posterior

waves

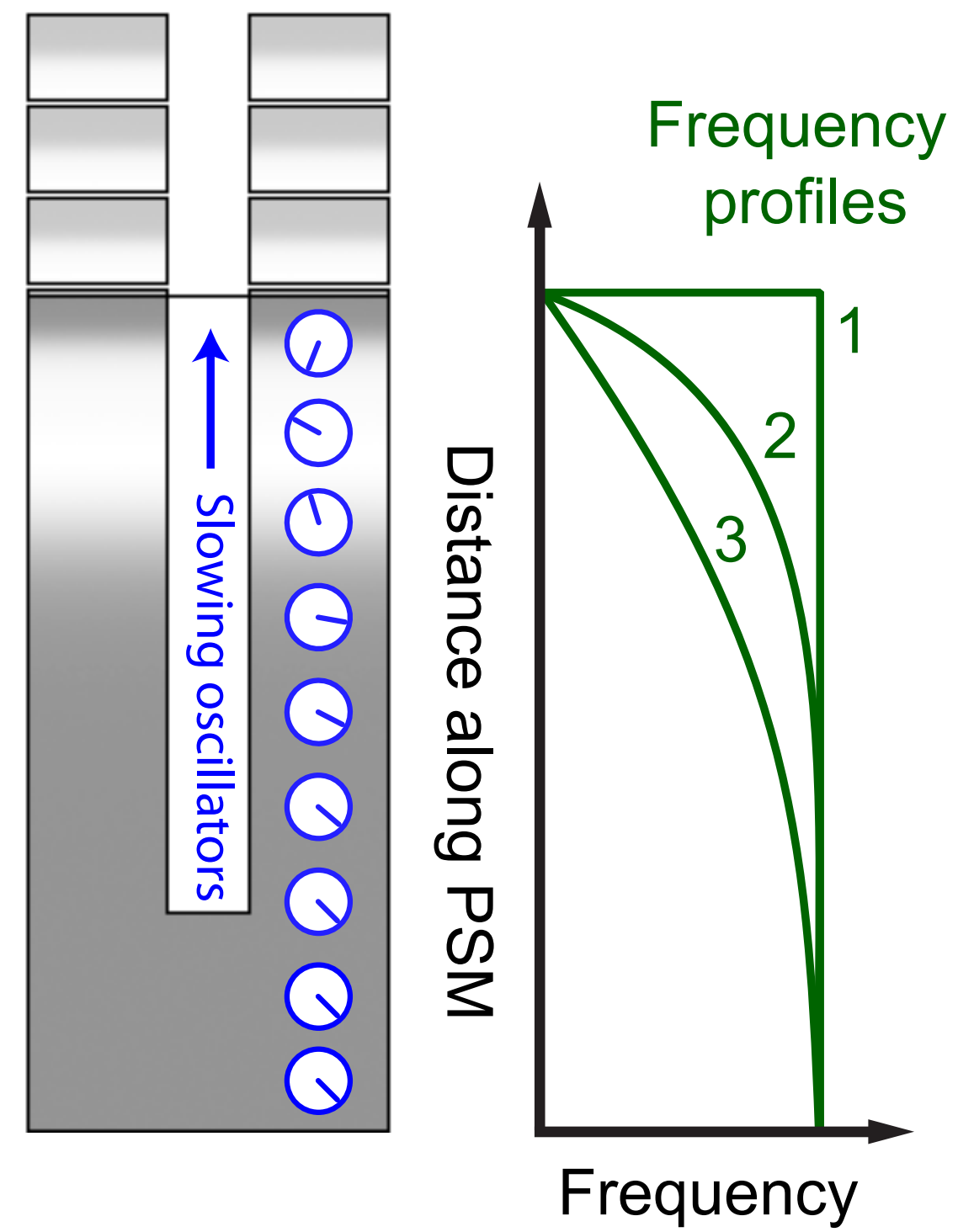
Gene expression wave patterns suggest cells are gradually slowing frequency before arresting

abrupt arrest
=
uniform pattern

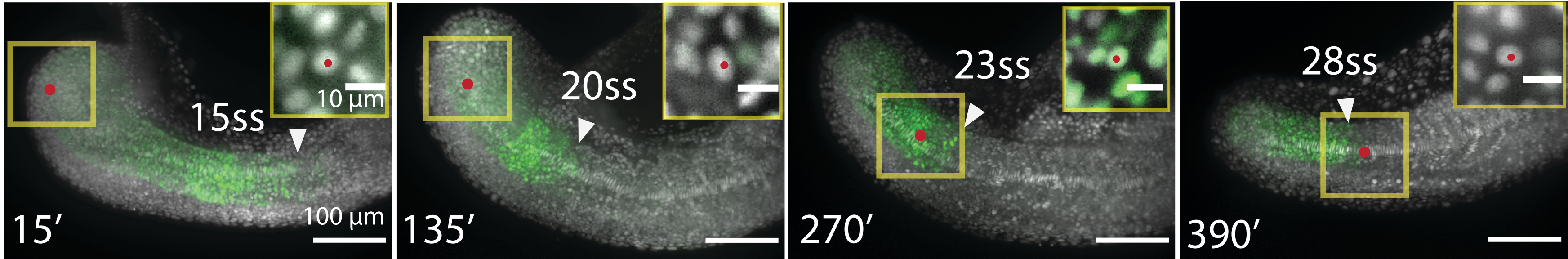


Changing the *rate* of slowing changes the pattern

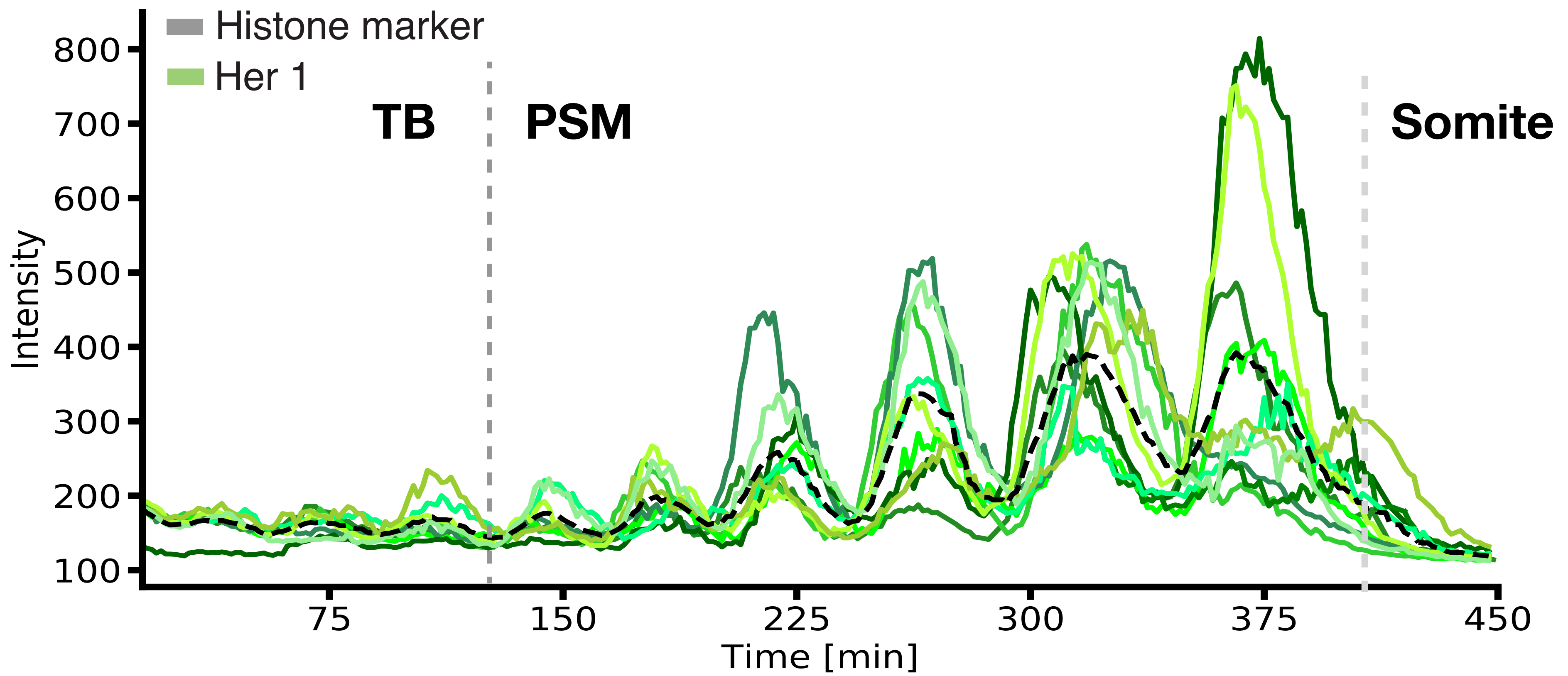
The spatial frequency profile



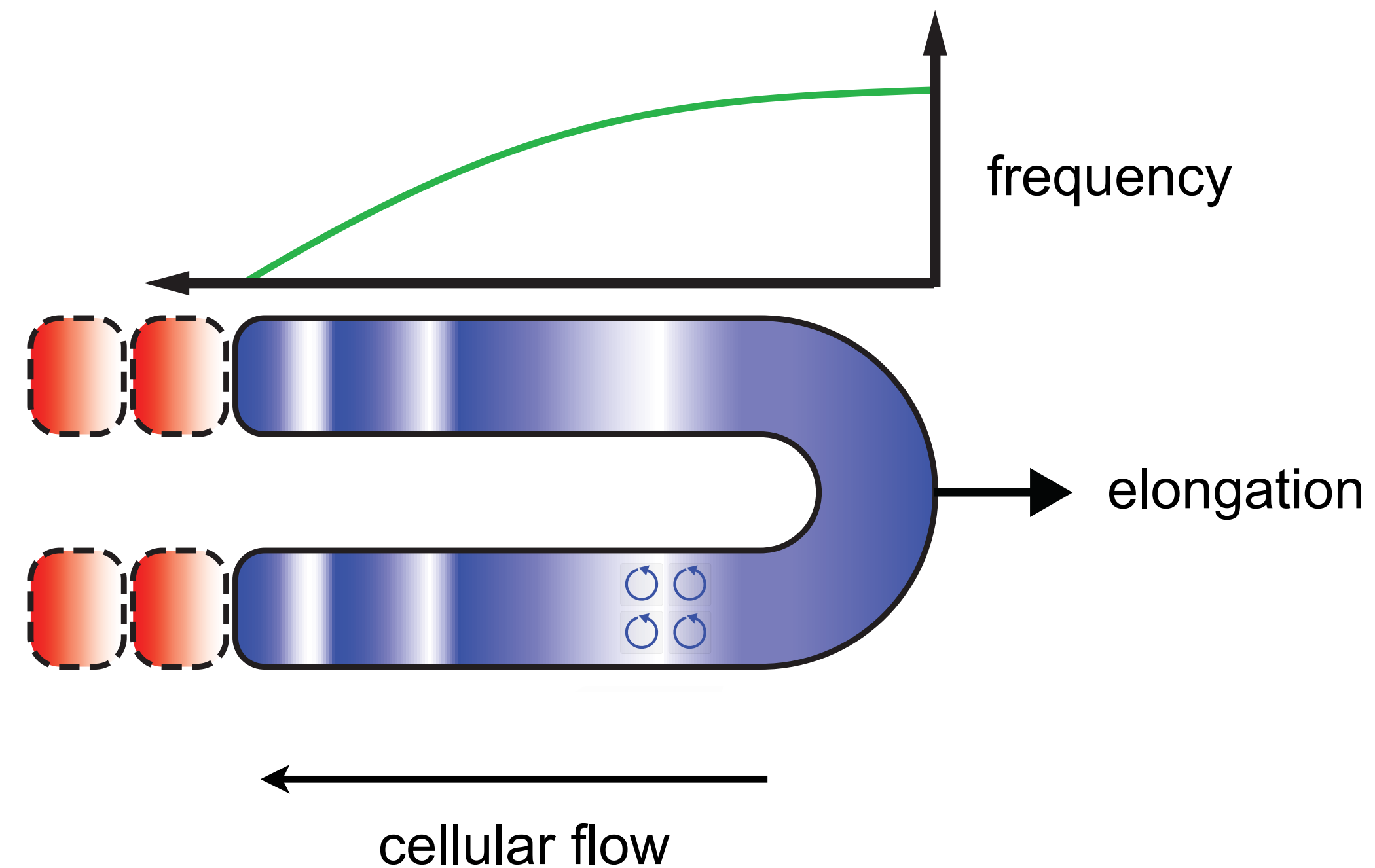
Cells slow period, increase amplitude, and arrest before somite formation



Viventis
Microscopy

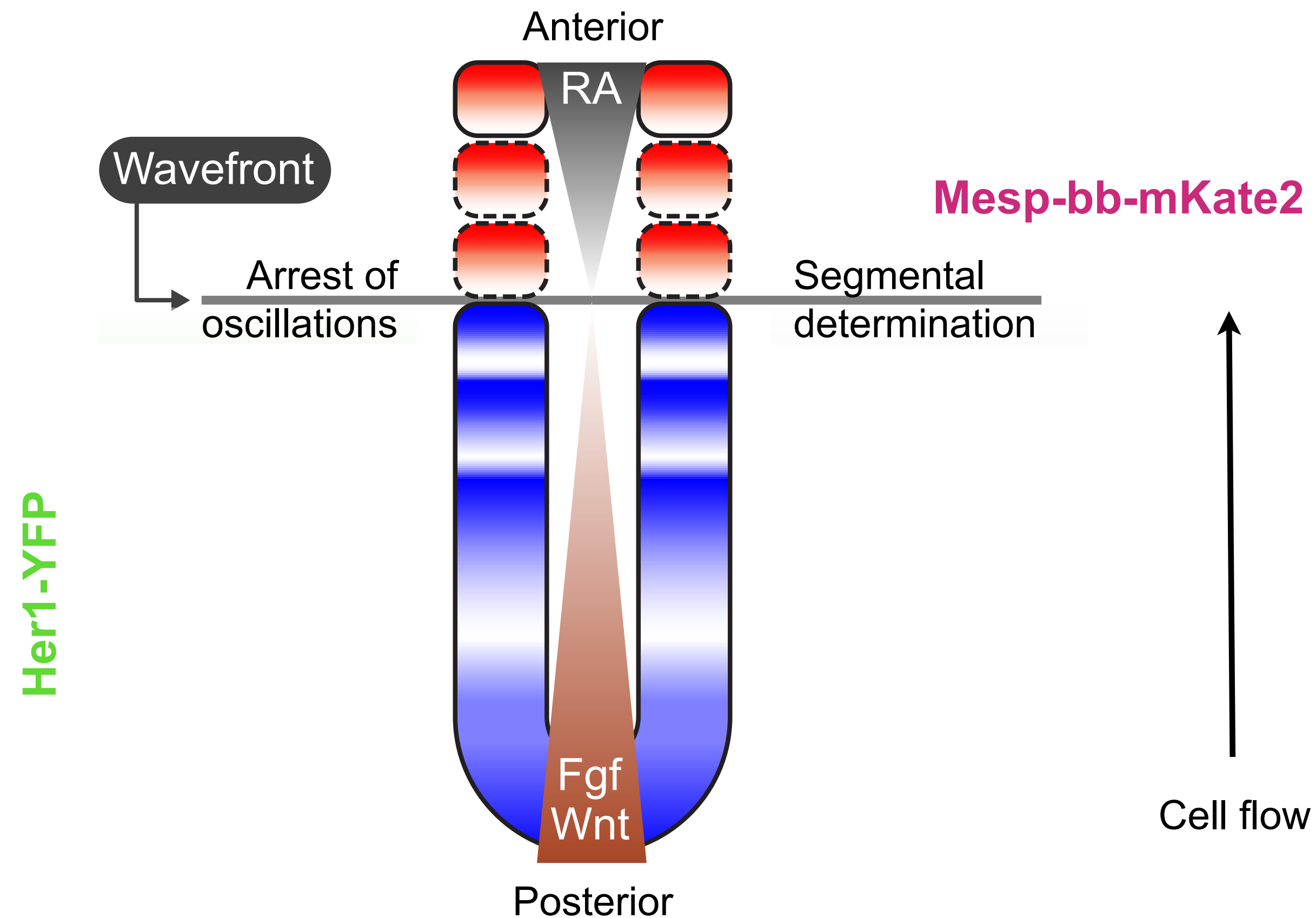


Wave patterns arise from slowing of cellular oscillators in transit across the PSM



Waves: How are oscillators slowed and stopped?

Whence cometh the positional information?



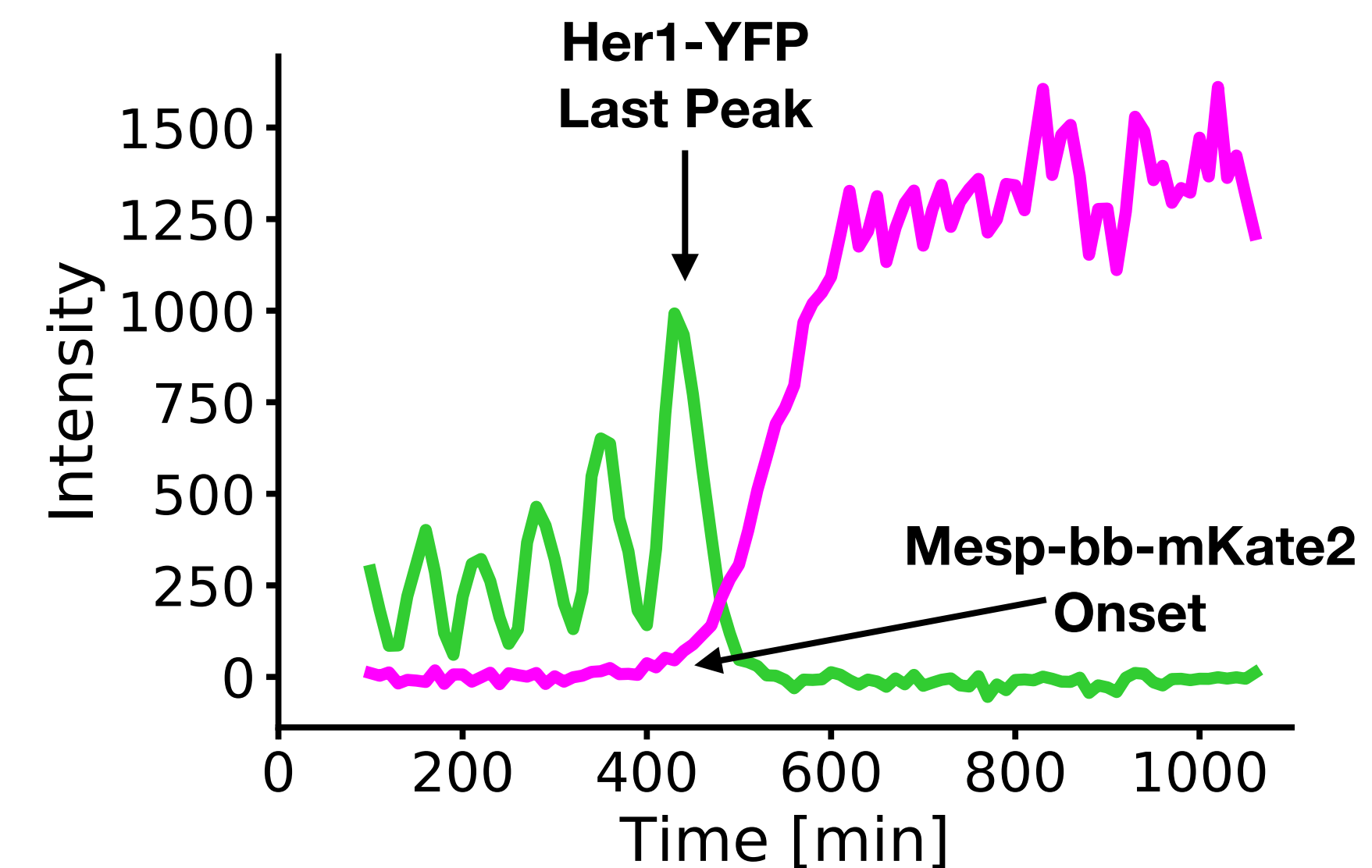
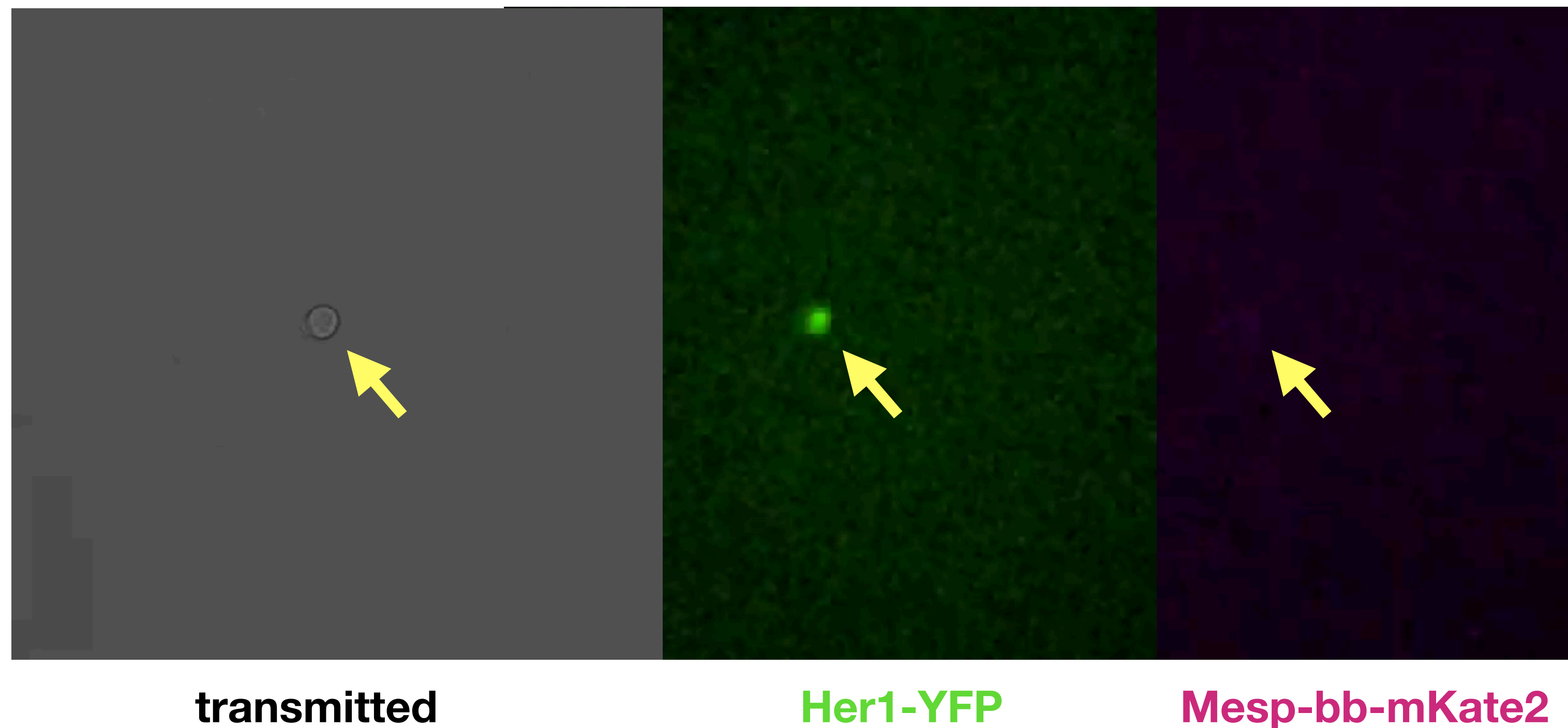
Dubrulle et al., 2001
 Sawada et al., 2001
 Aulehla et al., 2003
 Diez del Corral et al., 2003
 Dubrulle and Pourquie, 2003
 Moreno and Kintner, 2004
 Naiche et al., 2011
 Bajard et al., 2014
 Sari et al., 2018
 Simsek et al., 2018

- **Extrinsic?**
 - radio receiver
 - neighboring cells
- **Intrinsic?**
 - gradient by inheritance
- **Mix?**

Aulehla and Pourquie, 2010, *CSH Perspect. Biol.*;
 Oates et al., 2012, *Development*;
 Mallo 2015, *FEBS J.*

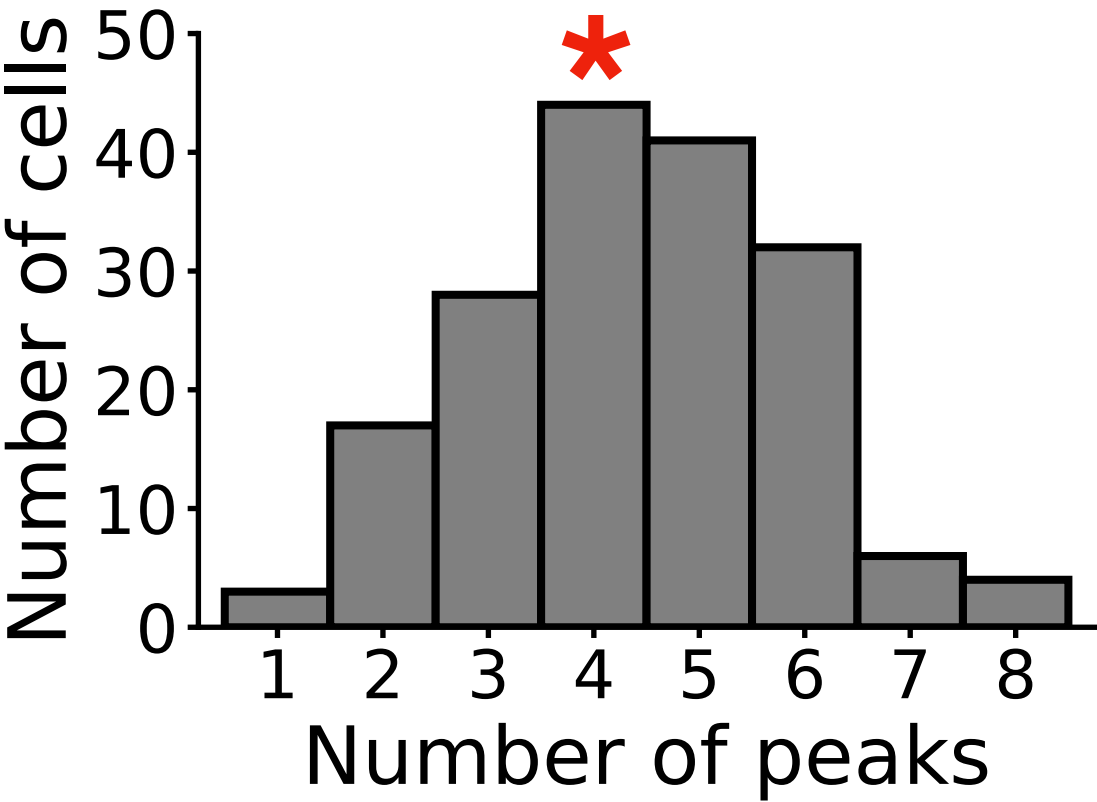
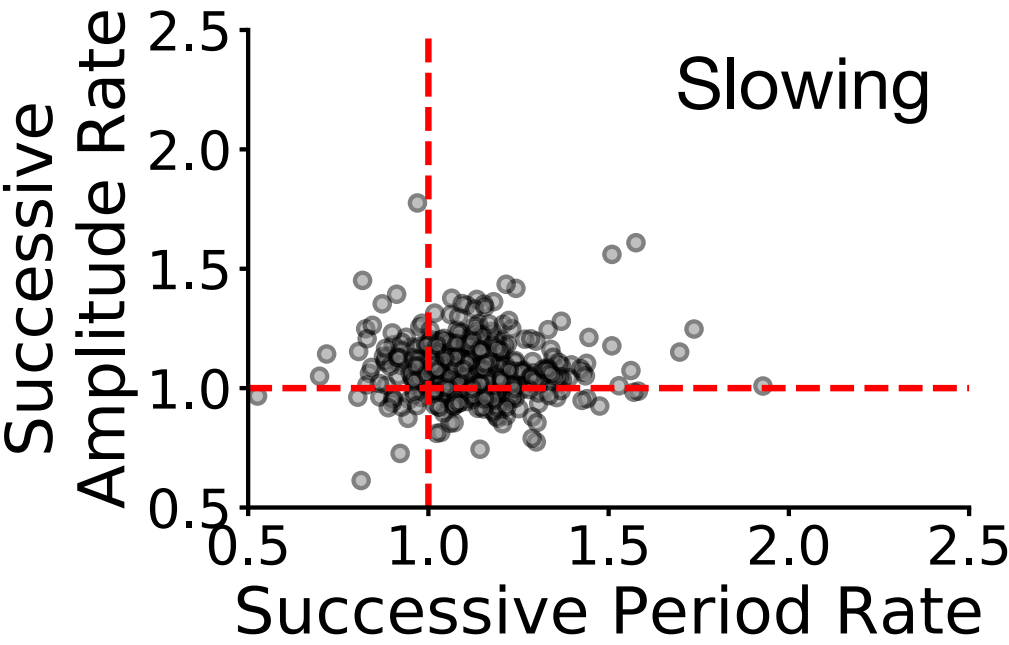
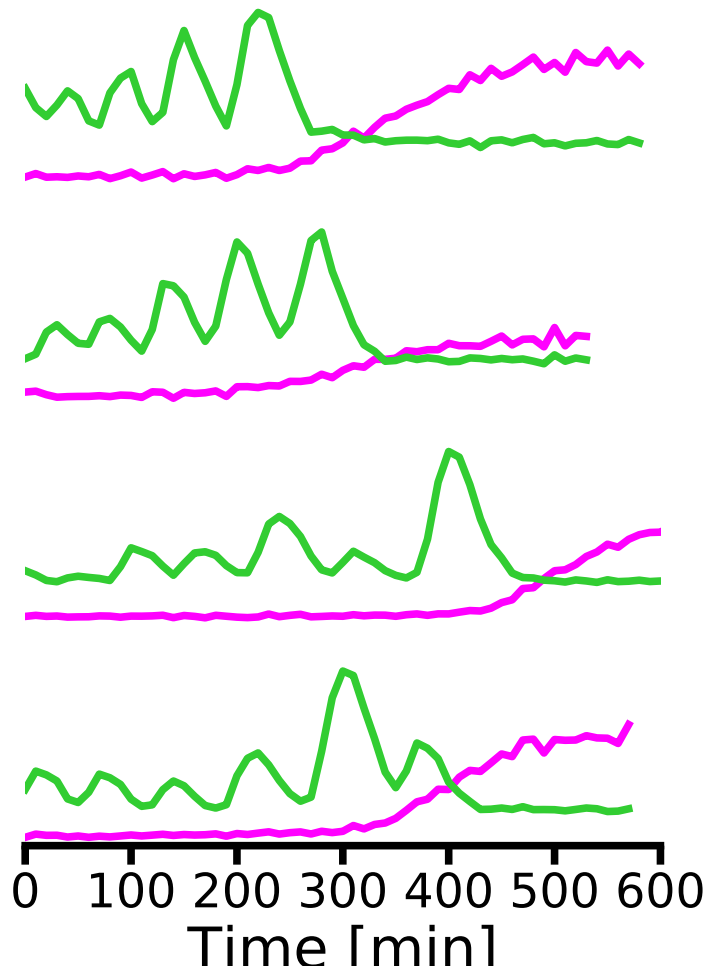
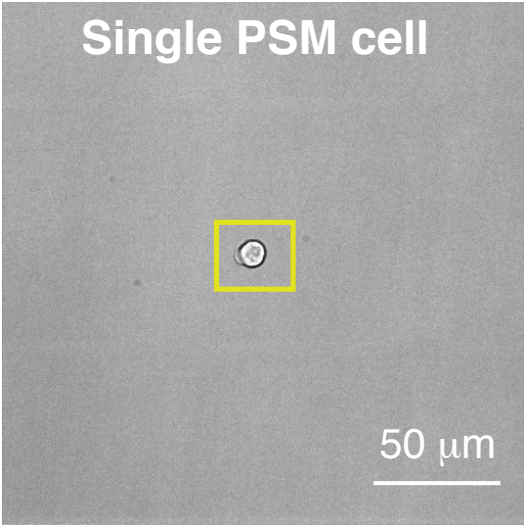


Cells **oscillate**, then stop and **differentiate** in culture

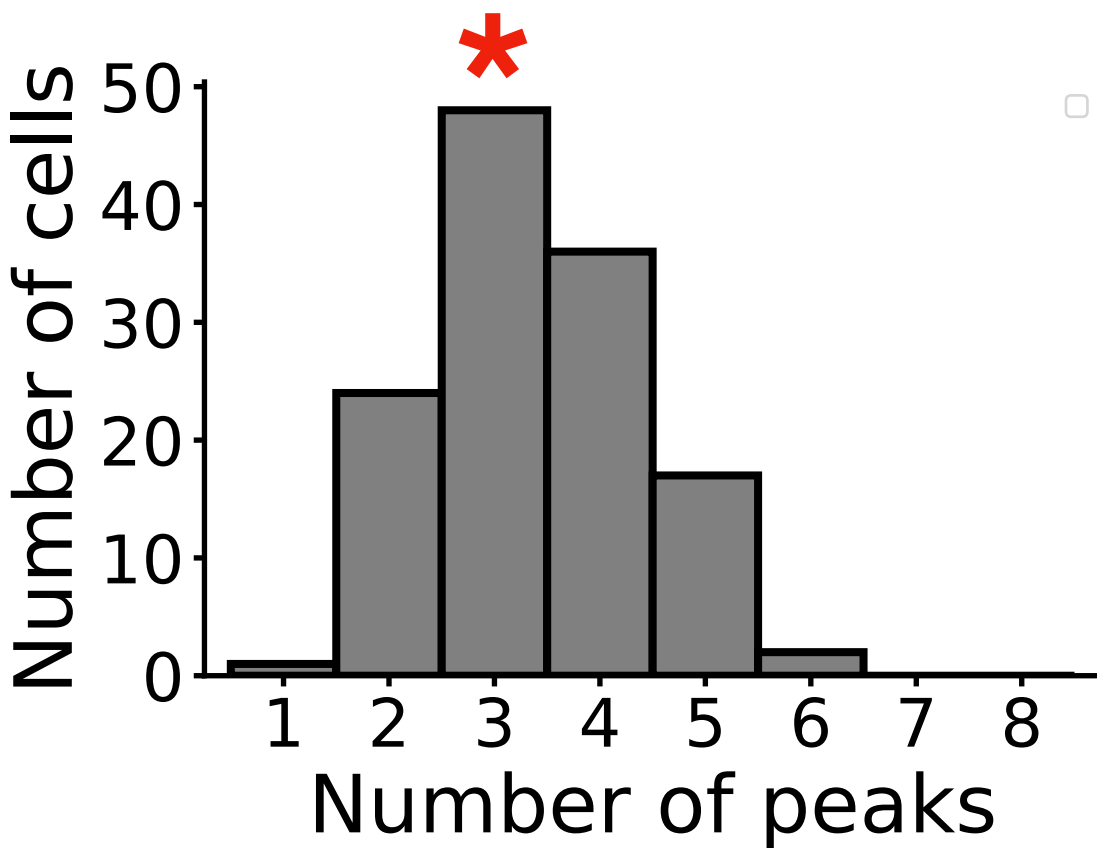
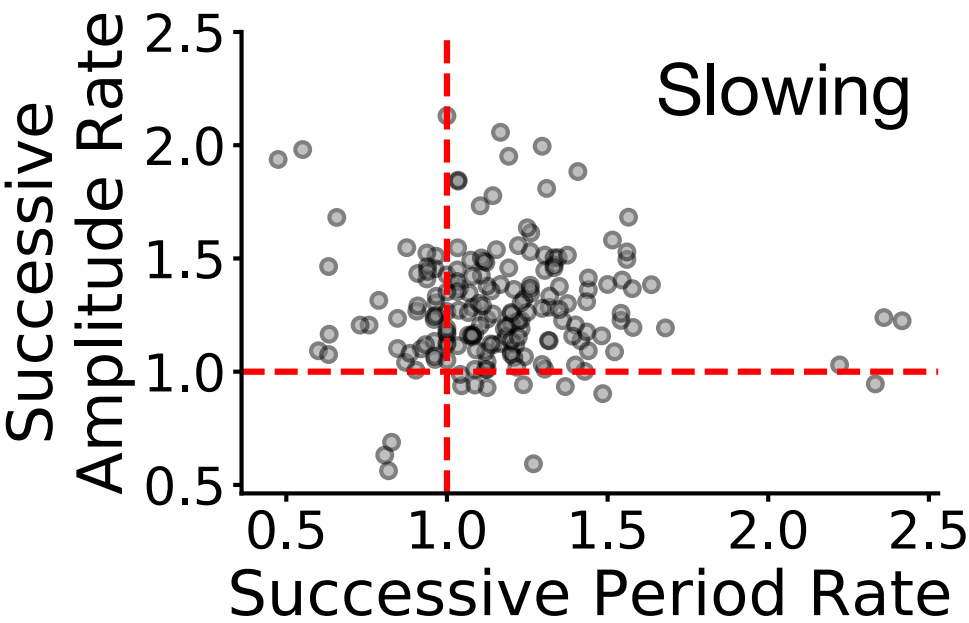
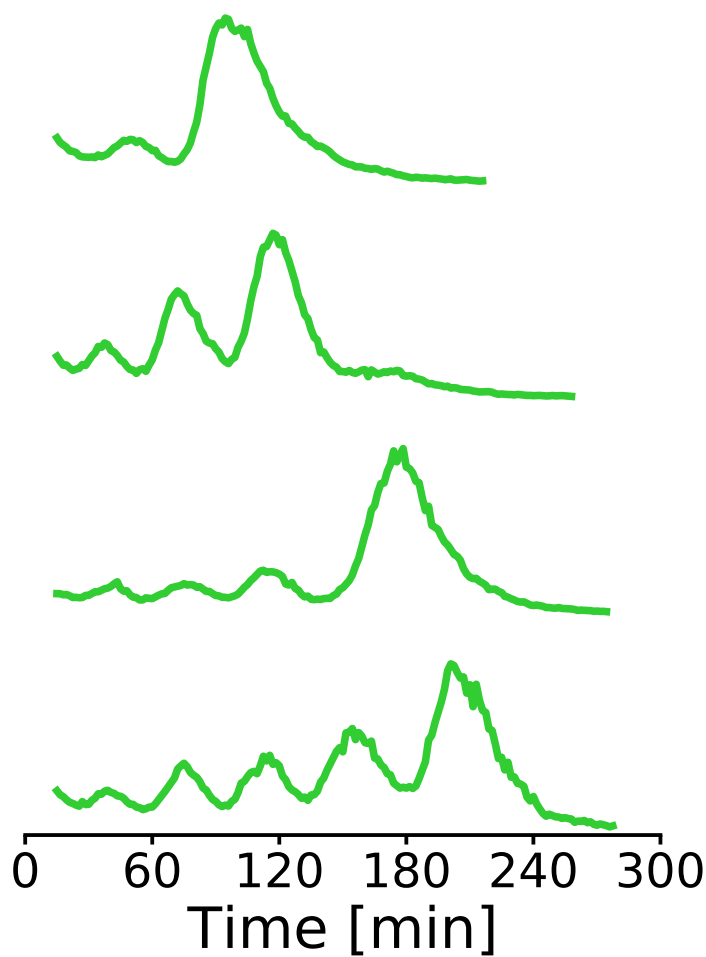


Cultured cells recapitulate embryo dynamics, with higher variability

Culture - PSM⁴



Embryo - PSM⁴

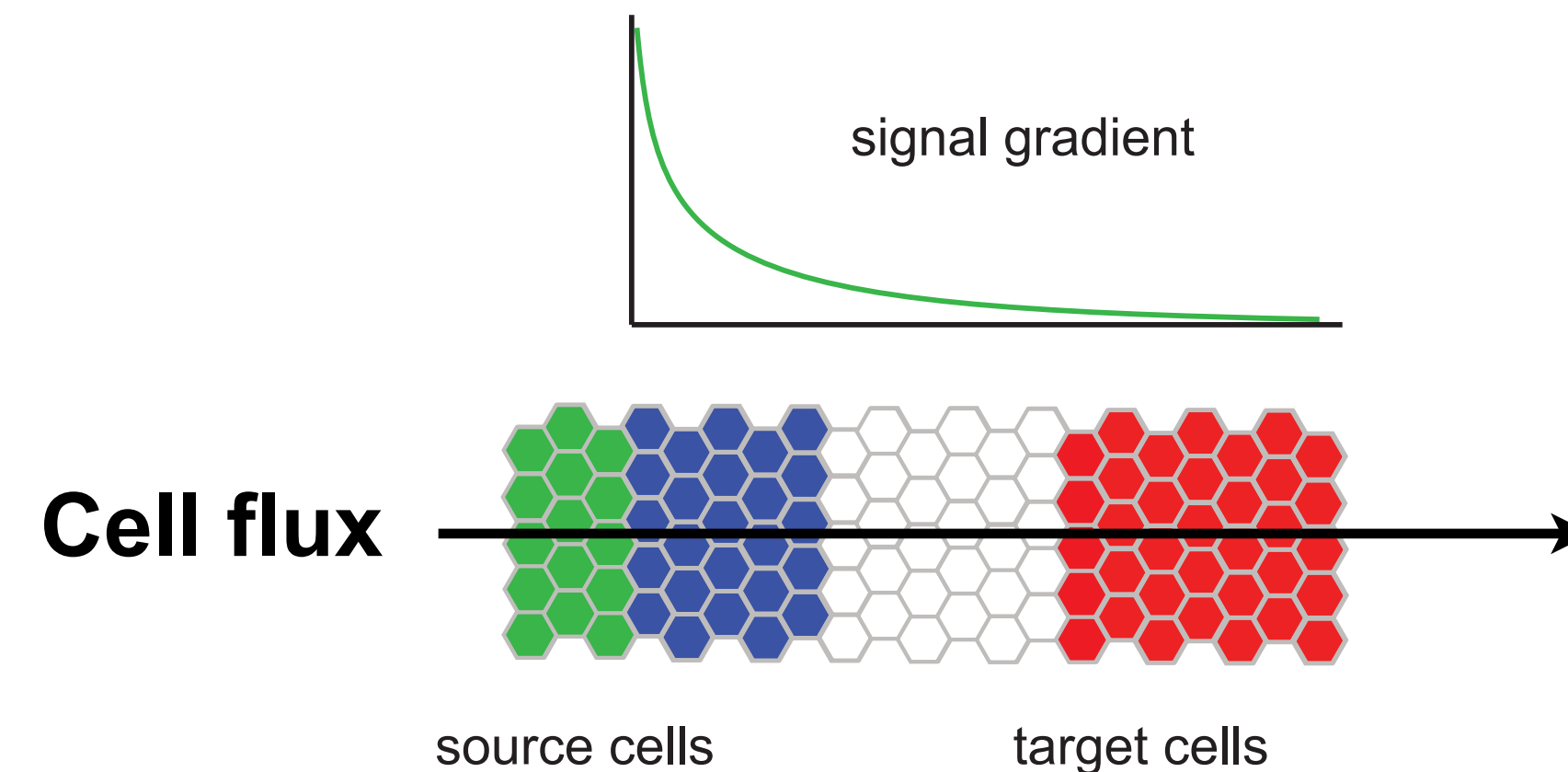


Cell dynamics underlying wave patterns are generated intrinsically, not extrinsically

Active signaling from neighboring cells or spatial gradients not required

Supports gradient by inheritance

➡ **internal timer**



Positional information in PSM is a temporal axis

“Wavefront” in a single cell



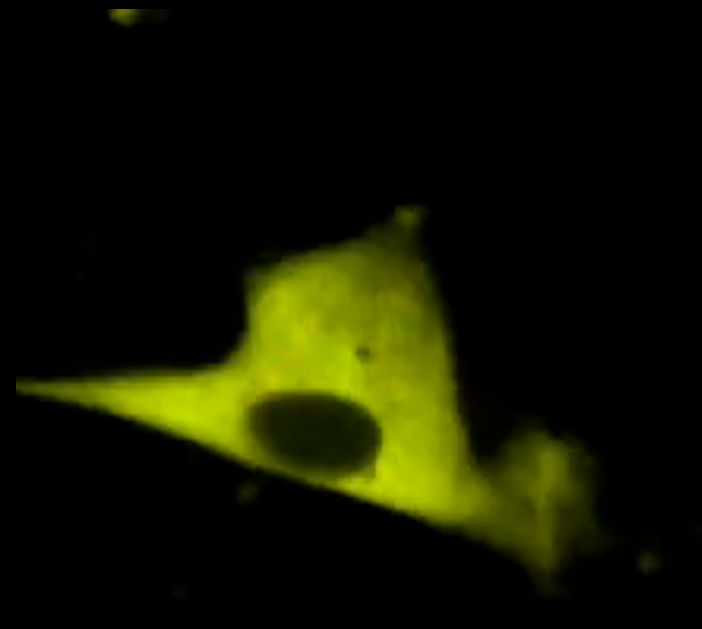
3rd tier: tissue-level waves

- cells slow down before stopping
- Slowing generates phase differences
- gradients of signals involved
- **but**, cells have autonomous program
- gradient by inheritance
- key papers:
 - Dubrulle, J., McGrew, M. J., & Pourquié, O. (2001). FGF signaling controls somite boundary position and regulates segmentation clock control of spatiotemporal Hox gene activation. *Cell*, 106(2), 219–232.
 - Sawada, A., Shinya, M., Jiang, Y. J., Kawakami, A., Kuroiwa, A., & Takeda, H. (2001). Fgf/MAPK signalling is a crucial positional cue in somite boundary formation. *Development (Cambridge, England)*, 128(23), 4873–4880.
 - Aulehla, A., Wiegraebe, W., Baubet, V., Wahl, M. B., Deng, C., Taketo, M., et al. (2008). A beta-catenin gradient links the clock and wavefront systems in mouse embryo segmentation. *Nature Cell Biology*, 10(2), 186–193. <http://doi.org/10.1038/ncb1679>
 - Bajard, L., Morelli, L. G., Ares, S., Pecreaux, J., Jülicher, F., & Oates, A. C. (2014). Wnt-regulated dynamics of positional information in zebrafish somitogenesis. *Development (Cambridge, England)*, 141(6), 1381–1391. <http://doi.org/10.1242/dev.093435>
 - Soroldoni, D., Jörg, D. J., Morelli, L. G., Richmond, D. L., Schindelin, J., Jülicher, F., & Oates, A. C. (2014). Genetic oscillations. A Doppler effect in embryonic pattern formation. *Science (New York, NY)*, 345(6193), 222–225. <http://doi.org/10.1126/science.1253089>
 - <https://elifesciences.org/reviewed-preprints/93764>

Emerging roles for biological rhythms:

Inflammation

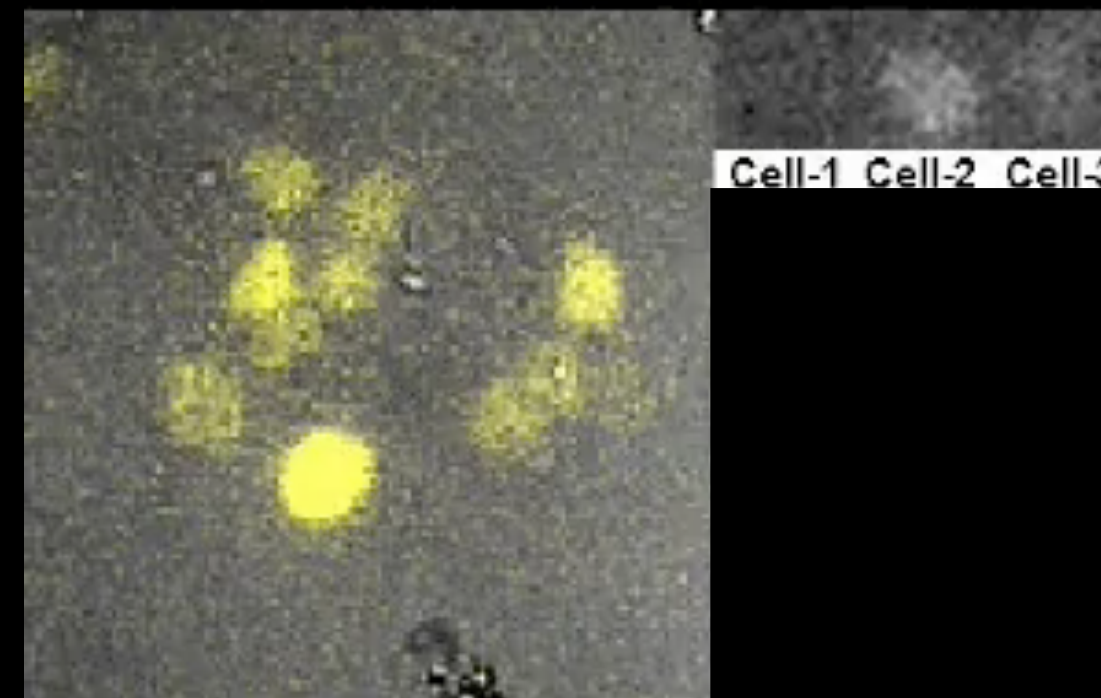
NF- κ B



Spiller et al., 2010

Stem cells

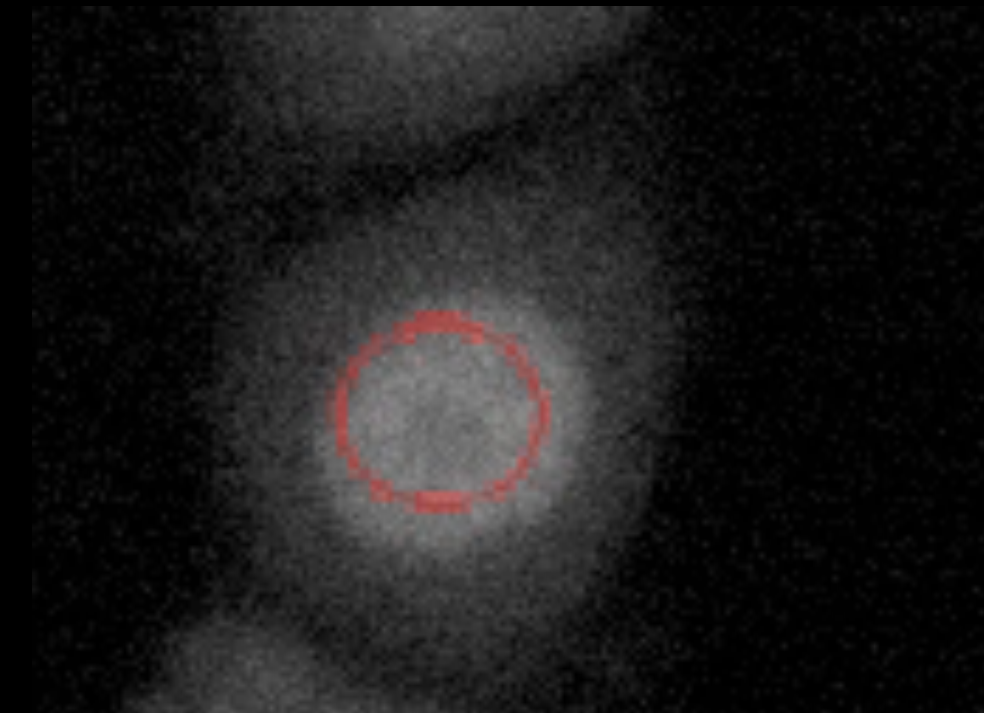
Hes-1



Imayoshi et al., 2013

DNA damage

p53-Mdm2



Loewer et al., 2010

in vivo? ...temporal therapy?

Questions?

