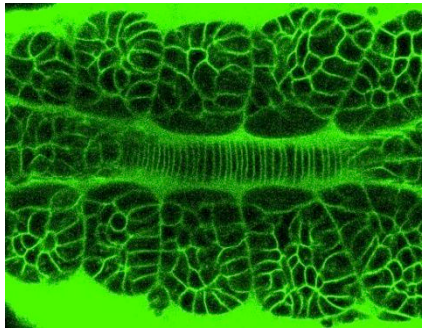
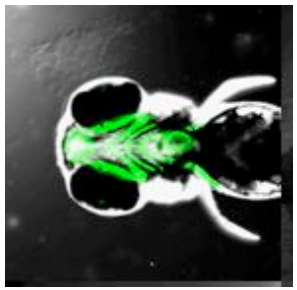
Laboratory of Embryology, Dept. Biological Sciences, University of Tokyo

Zebrafish

somitogenesis



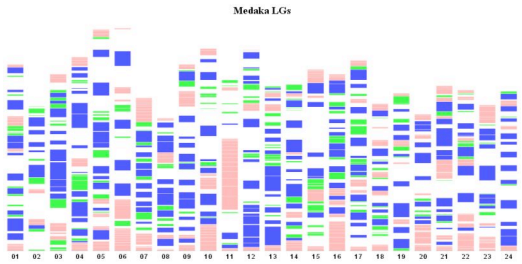
enhancer trap lines



Medaka

organogenesis mutants

medaka genome project



Why zebrafish or madaka?

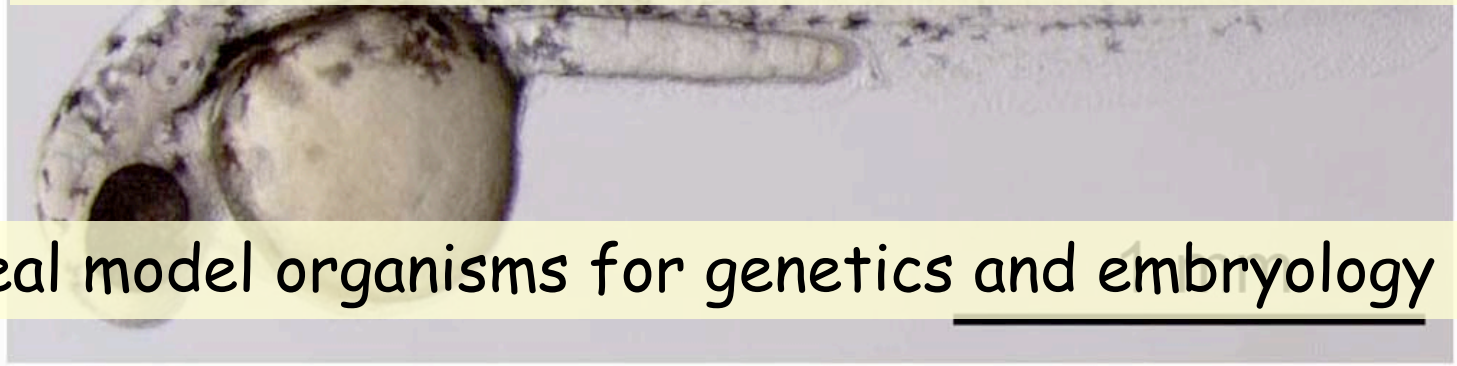
Short generation time; 2 – 3 months

They develop outside the mother

They are highly transparent

Simple body plan but comparable to higher vertebrates

80% of genes are shared by all vertebrates

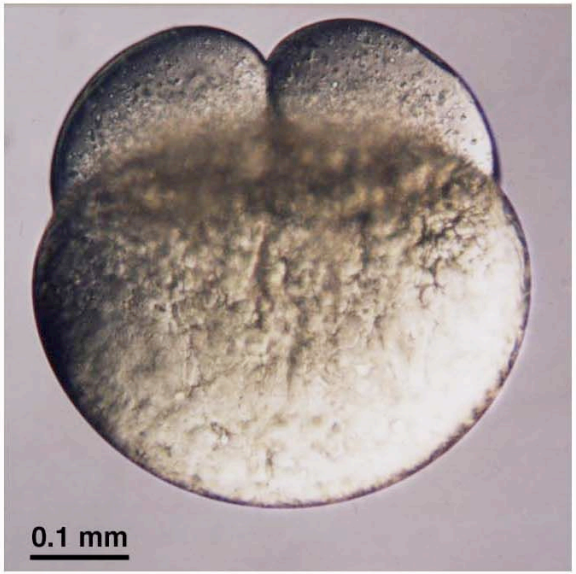


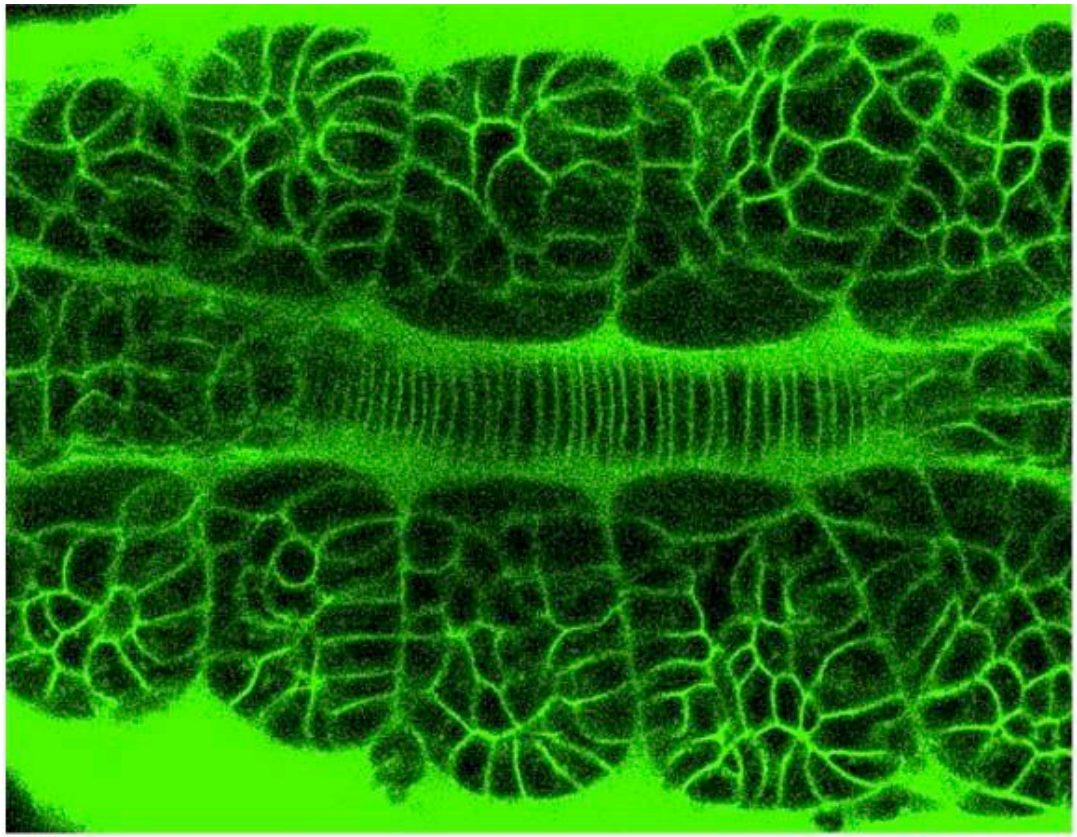
Ideal model organisms for genetics and embryology

Embryogenesis in zebrafish

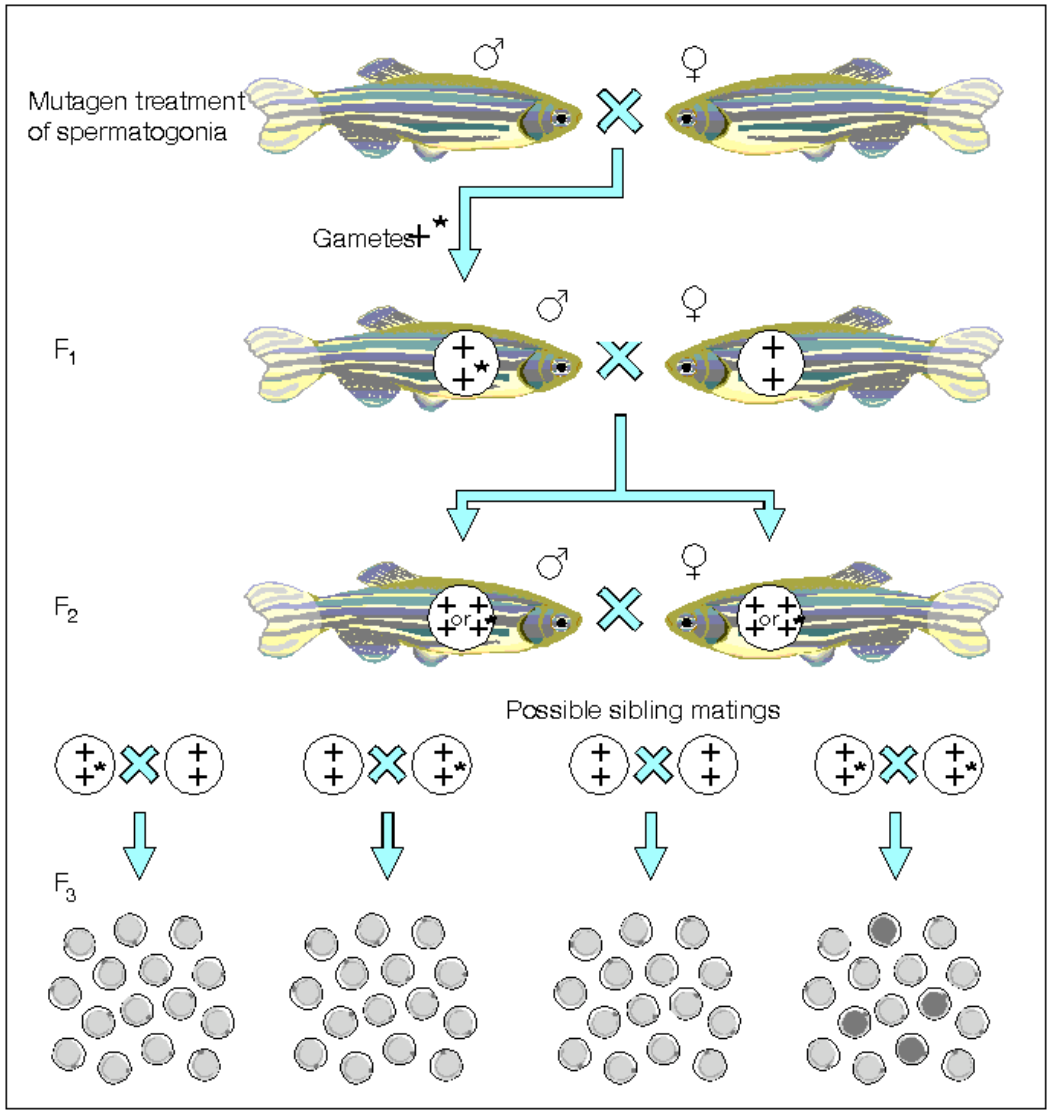
1 to 20 hours after fertilization

Embryos are transparent





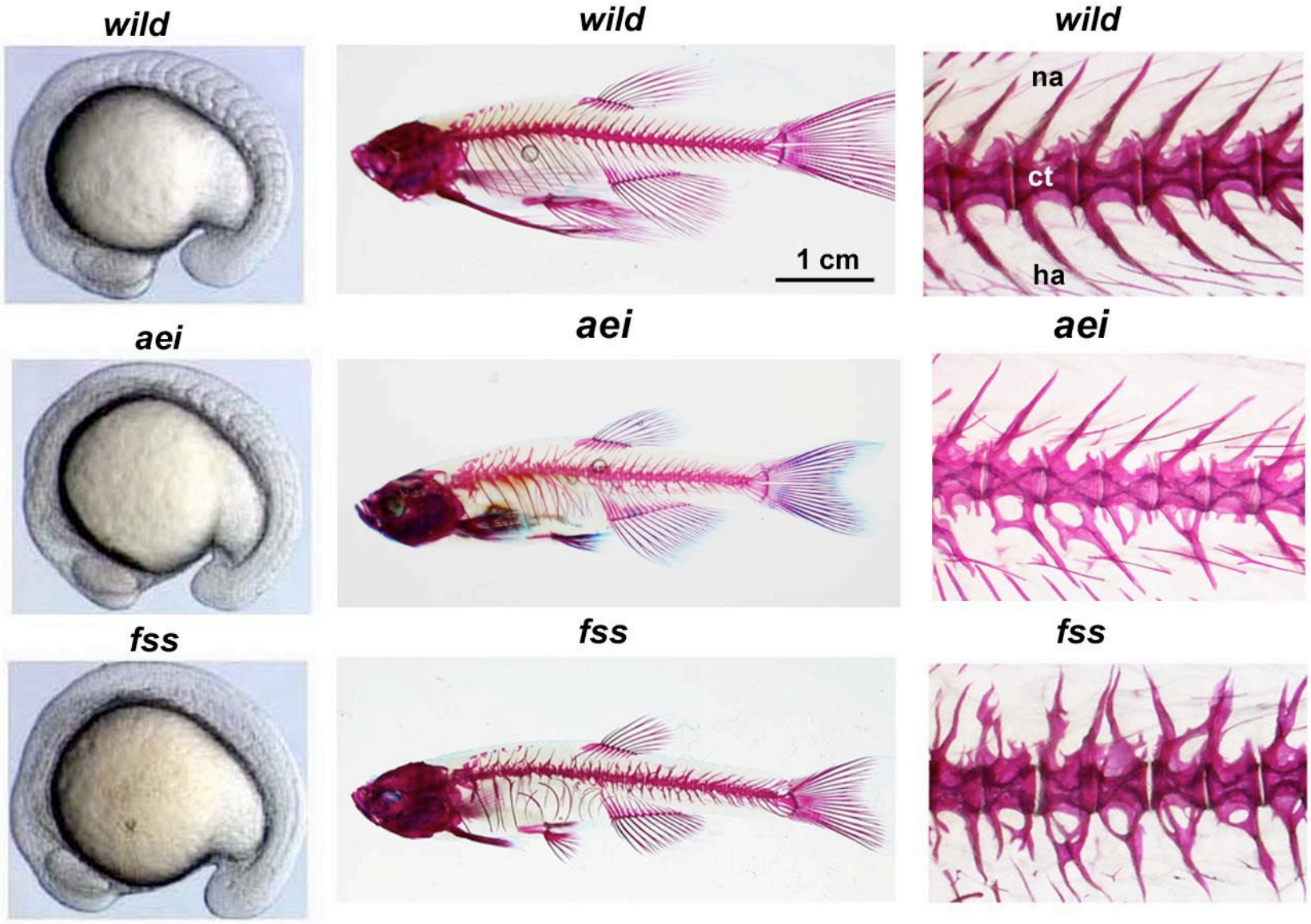
Mutagenesis screening in the laboratory



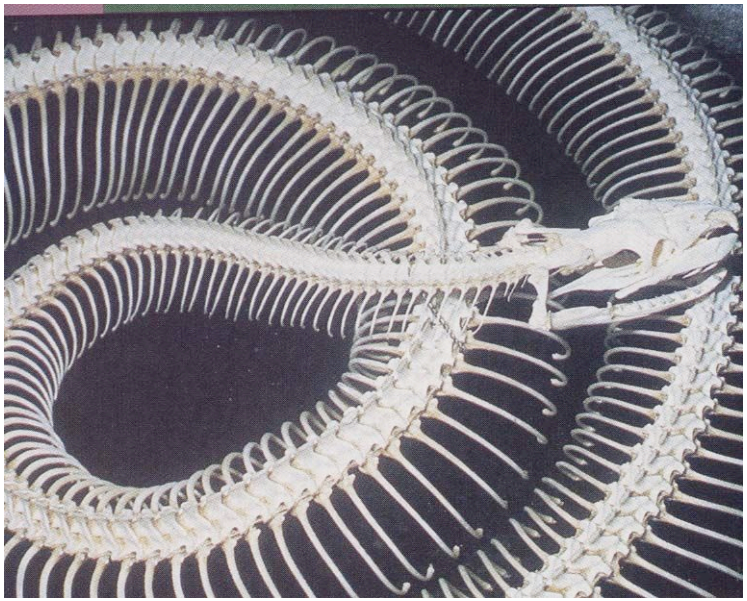
After Lewis Wolpert (2001) "Principles of Development"

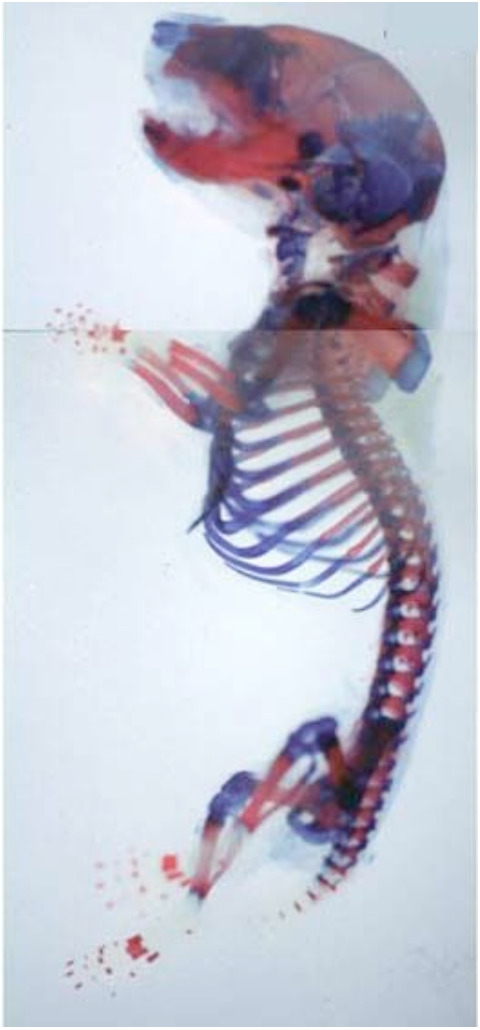
Fish facility at NIG

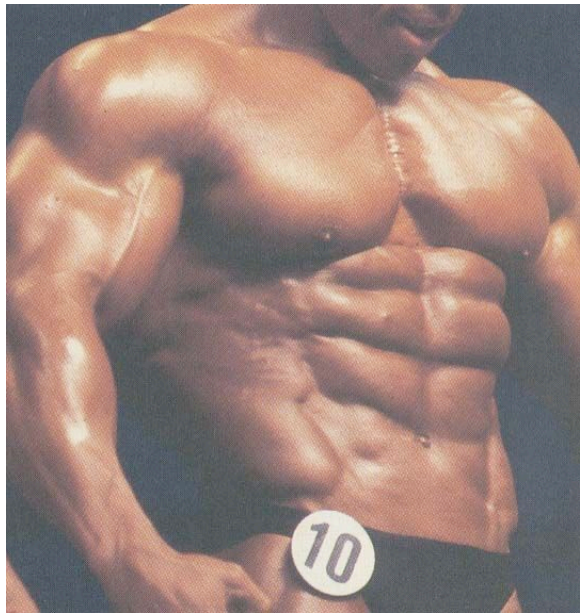
Zebrafish segmentation mutants



The segmental properties in vertebrate body

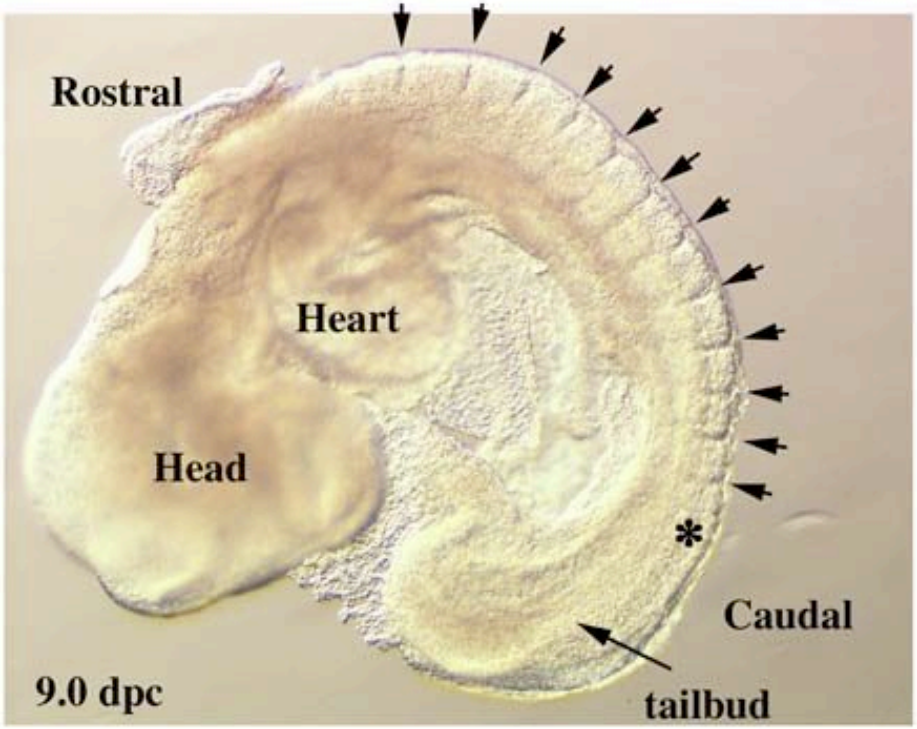




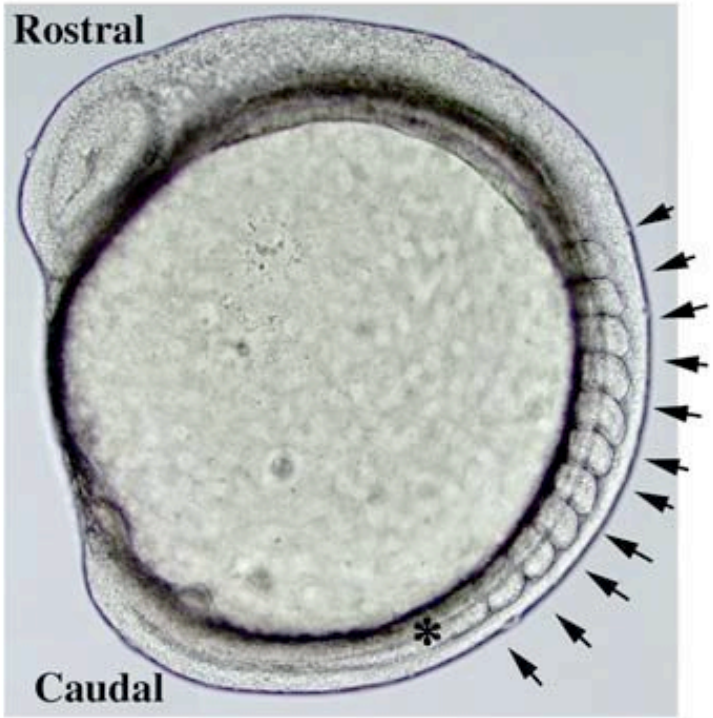


The segmental property of our trunk comes from the somites that are transiently formed during early embryogenesis

Mouse somitogenesis



Zebrafish somitogenesis

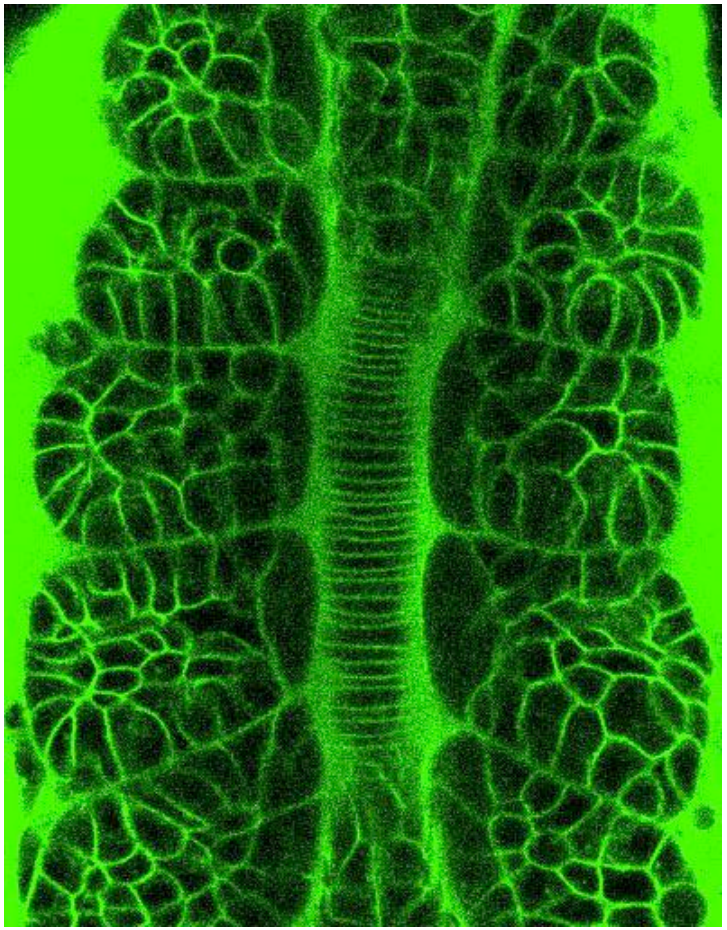


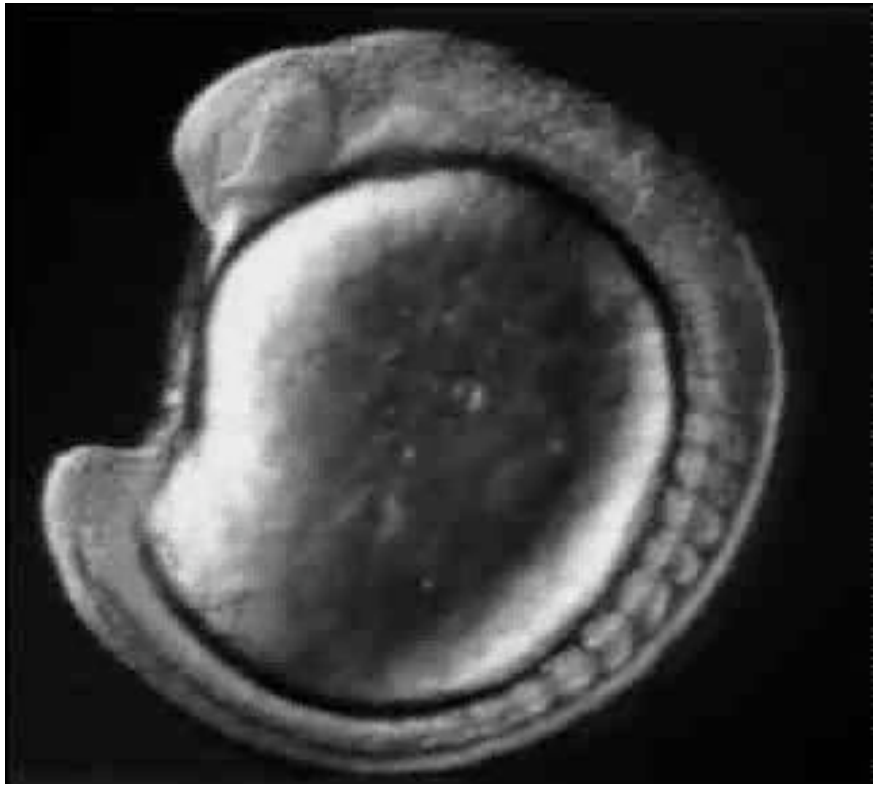
Somites → differentiate into

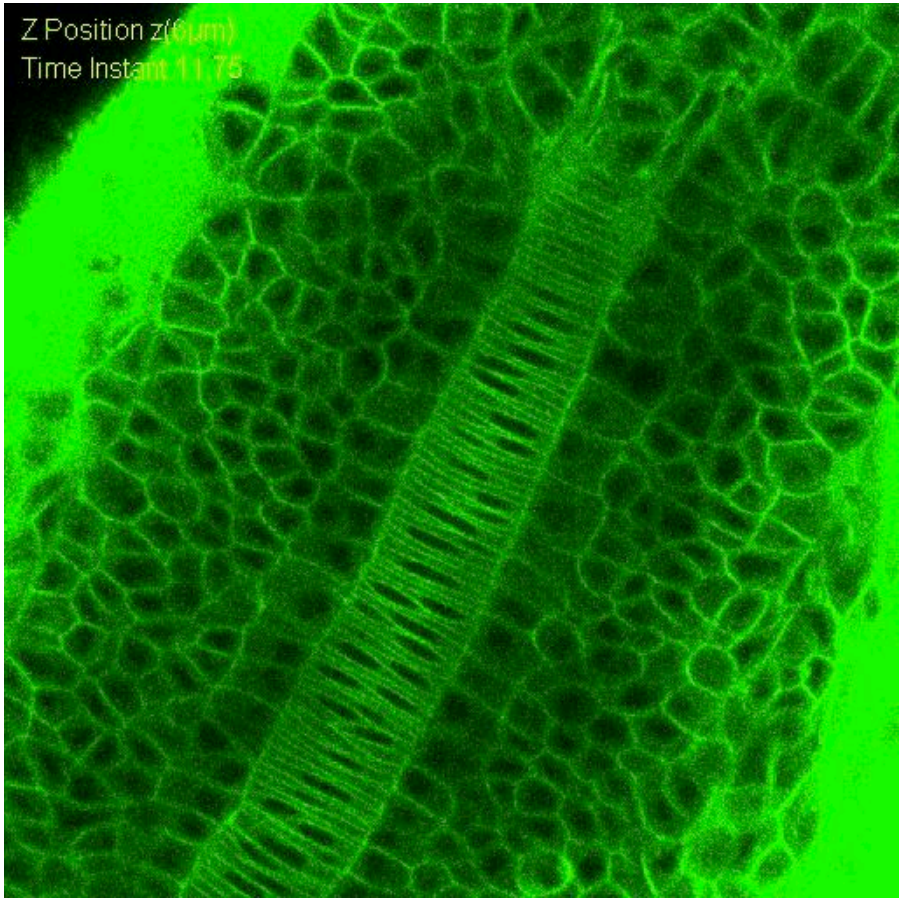
Vertebrae
Muscle
Dermis

The somite, a ball of mesenchymal cells, is formed one by one from the anterior end of the un-segmented tissue (presomitic mesoderm, PSM) in an anterior-to-posterior direction.

Presomitic mesoderm (PSM)






Z Position z(6µm)
Time Instant 11.75

Somite formation (somitogenesis) in the vertebrate trunk

Zebrafish embryos at the segmentation stage

Strict periodicity

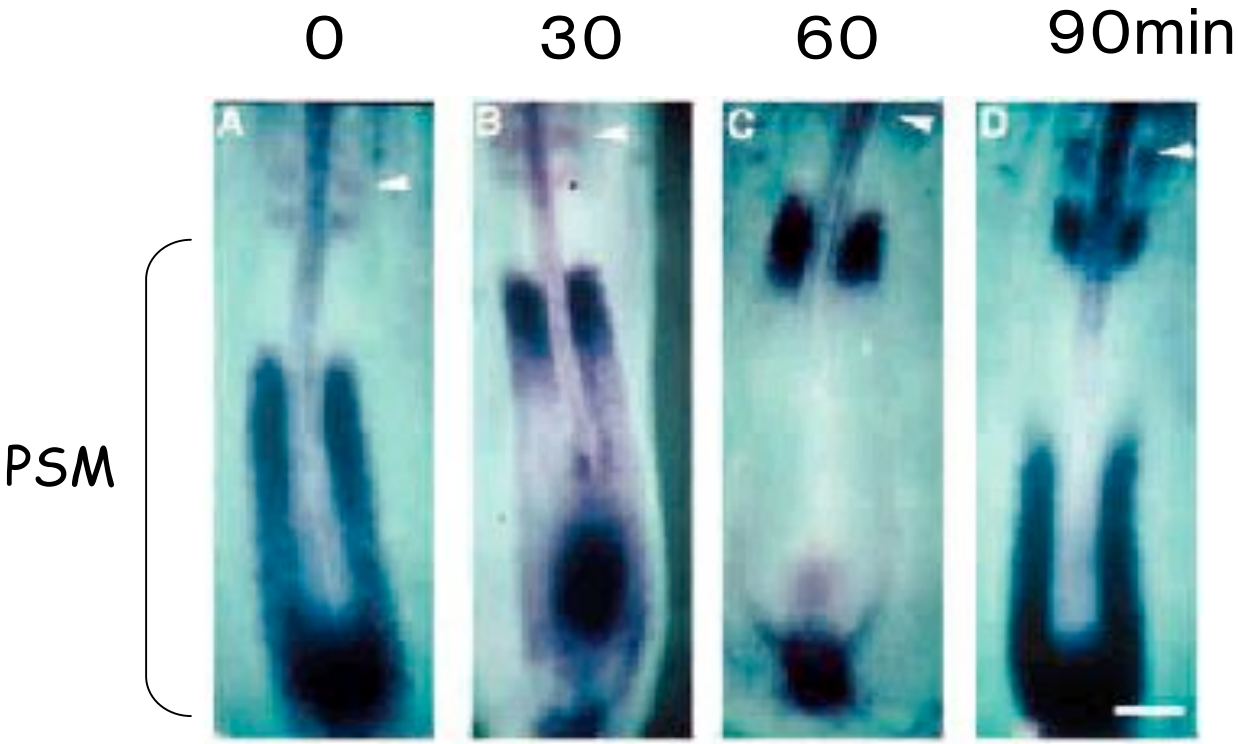
T= 30 min. for zebrafish
90 min. for chick
120 min. for mouse

In 1997, the transcription of the *hairy* genes was first reported to oscillate in the PSM (O. Pourquie).

A biological clock (segmentation clock) operates here

Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis

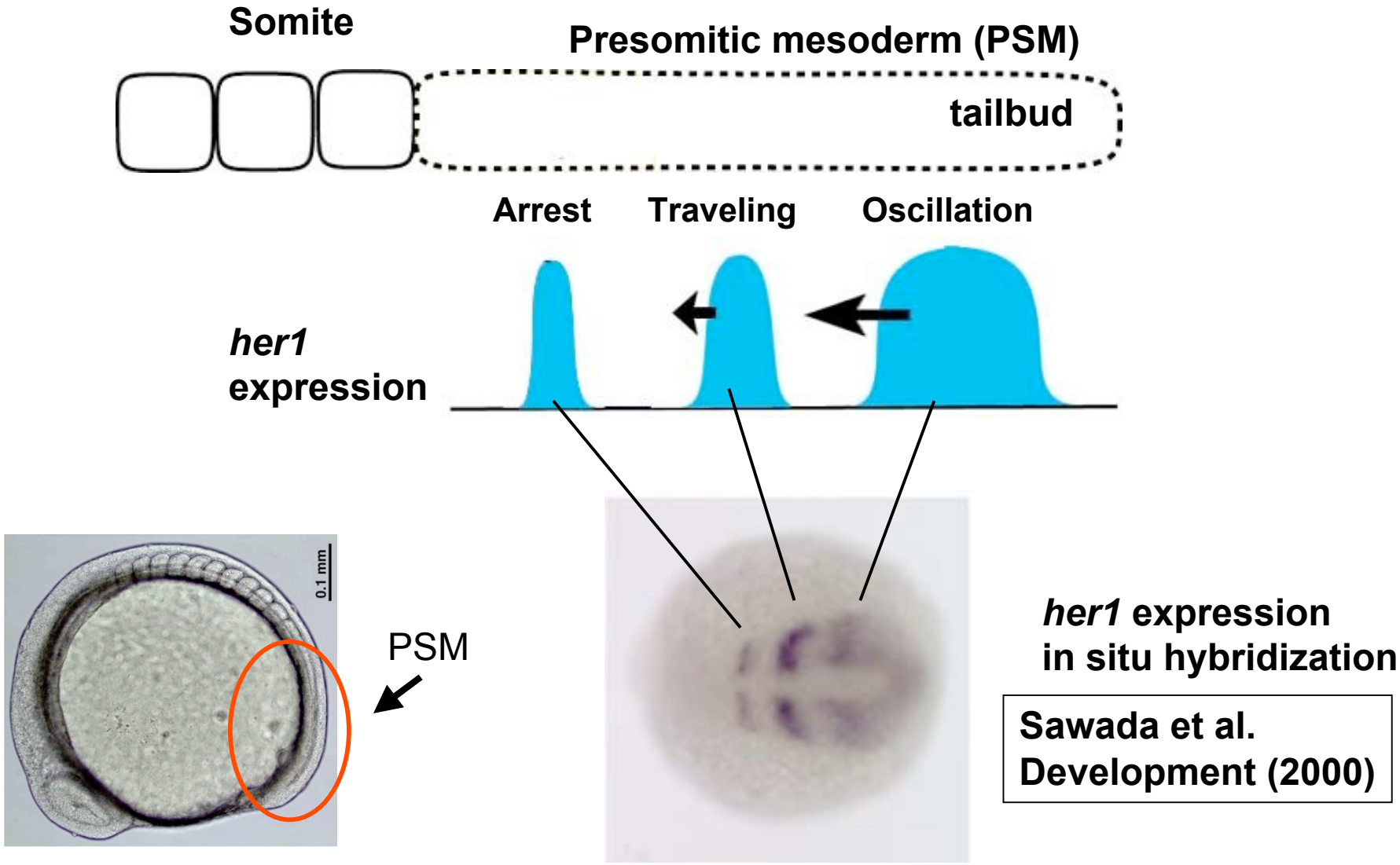
Palmeirim, Henrique, Ish-Horowicz & Pourquie (Cell 91: 639-648, 1997)



The cycle of gene expression wave corresponds to that of one-somite formation

The cyclic expression wave is created by a coordinated on-off pattern of transcription in individual cells, not by migration of PSM cells

her1 expression wave in the presomitic mesoderm



The expression domain appear in the tailbud every 30 min.

Zebrafish segmentation mutants

***fss*-type (segmentation defects)**

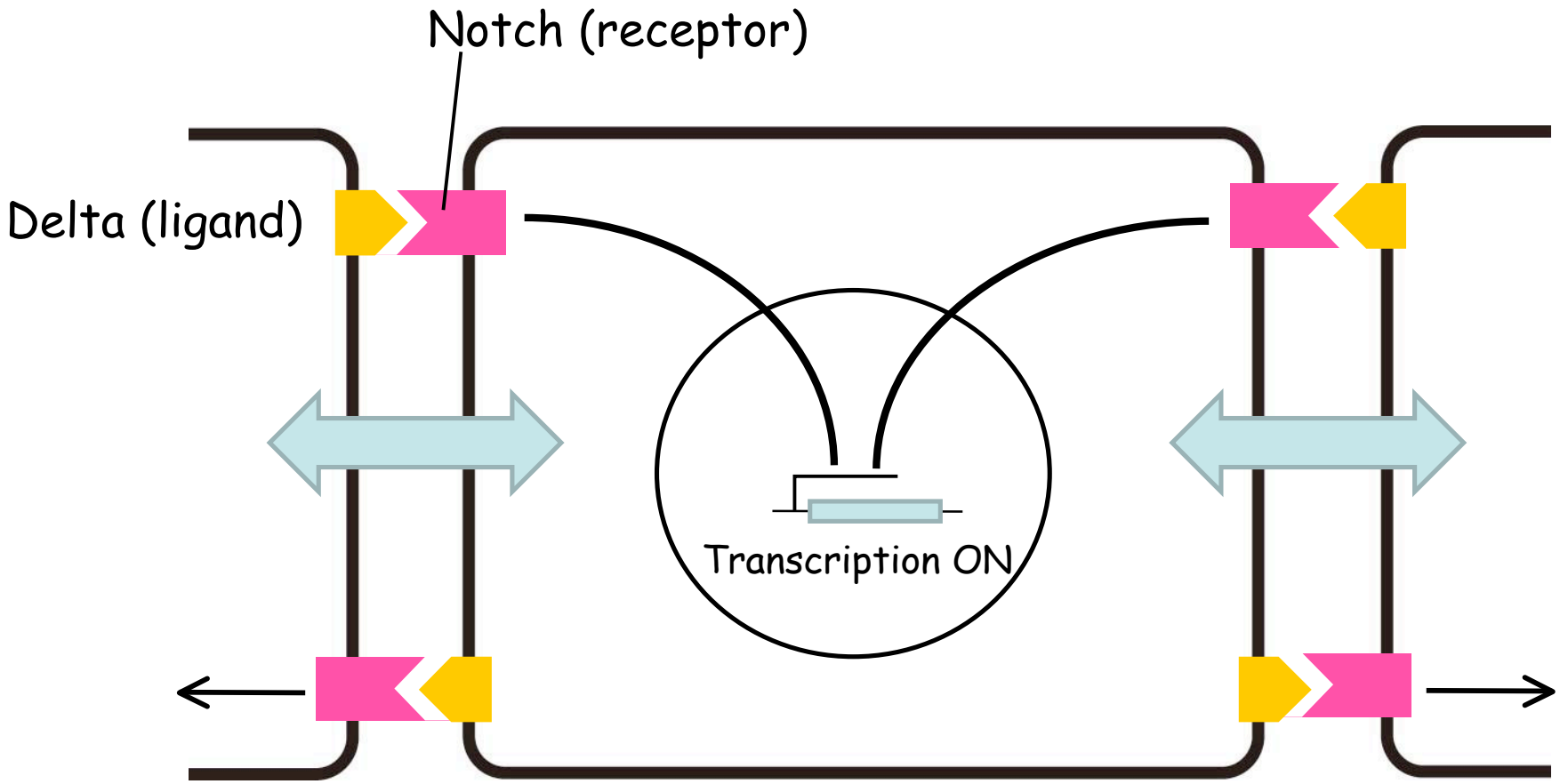
fused somites* (*fss*) = *tbx24

Nikaido et al., Nature genetics 31: 195 (2002)

beamter* (*bea*) = *deltaC
deadly seven* (*des*) = *notch1
after eight* (*aei*) = *deltaD
***white tail* (*wit*, *mib*) = a ubiquitin ligase**

After van Eden et al. Development 123: 153 (1996)
Holley et al., Genes Dev 14: 1678 (2000)
Development 129: 1175 (2002)
Ito et al., Dev. Cell 4: 67 (2003)

Notch-Delta signaling mediates intercellular communication



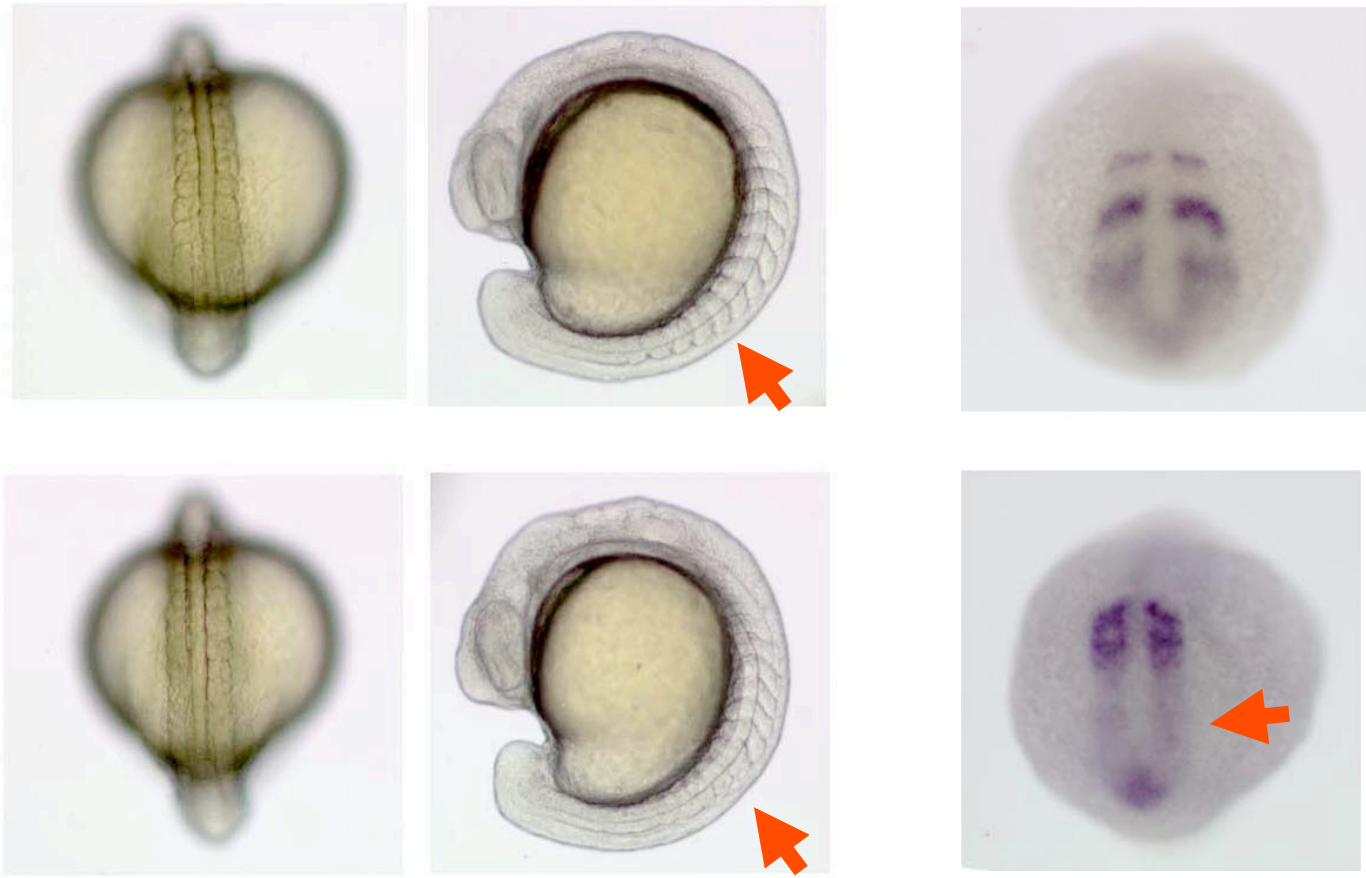
Morphology and *her1* expression in the Notch mutant

after eight* (*aei*) = *delta D

***her1* expression**

sibling

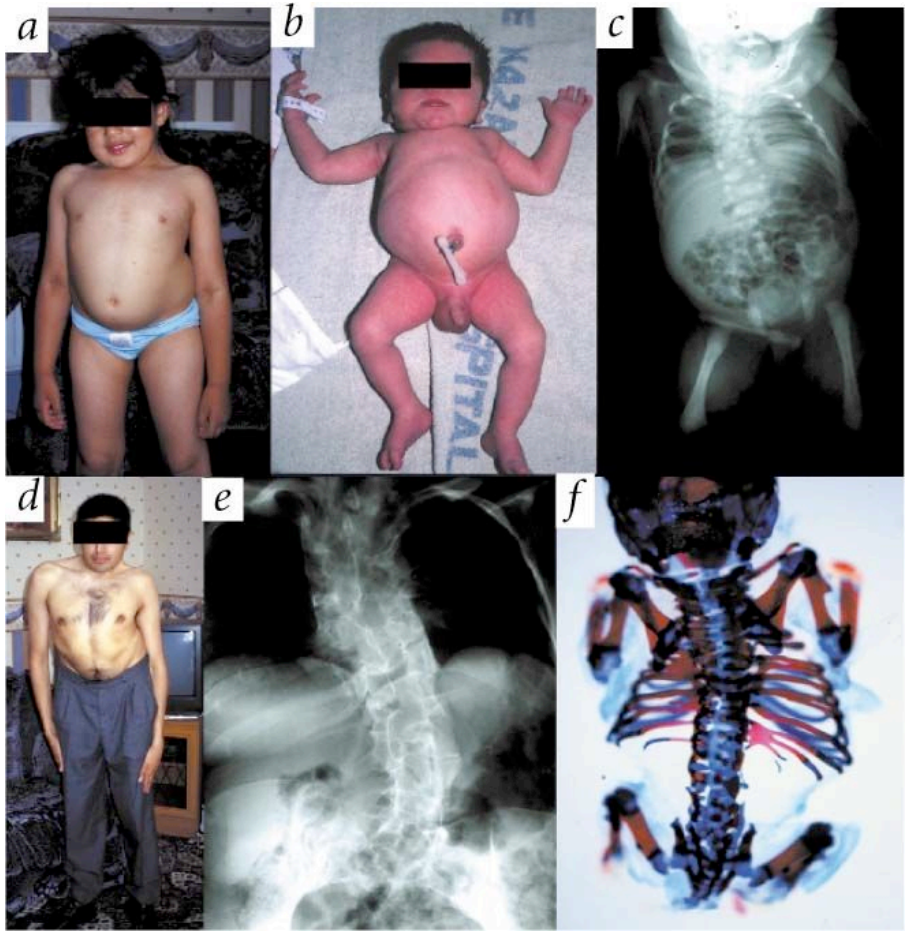
aei-/-(AR33)



14-somite stage **6-somite stage**

Segmentation mutants in mammals

Spondylocostal dysostosis

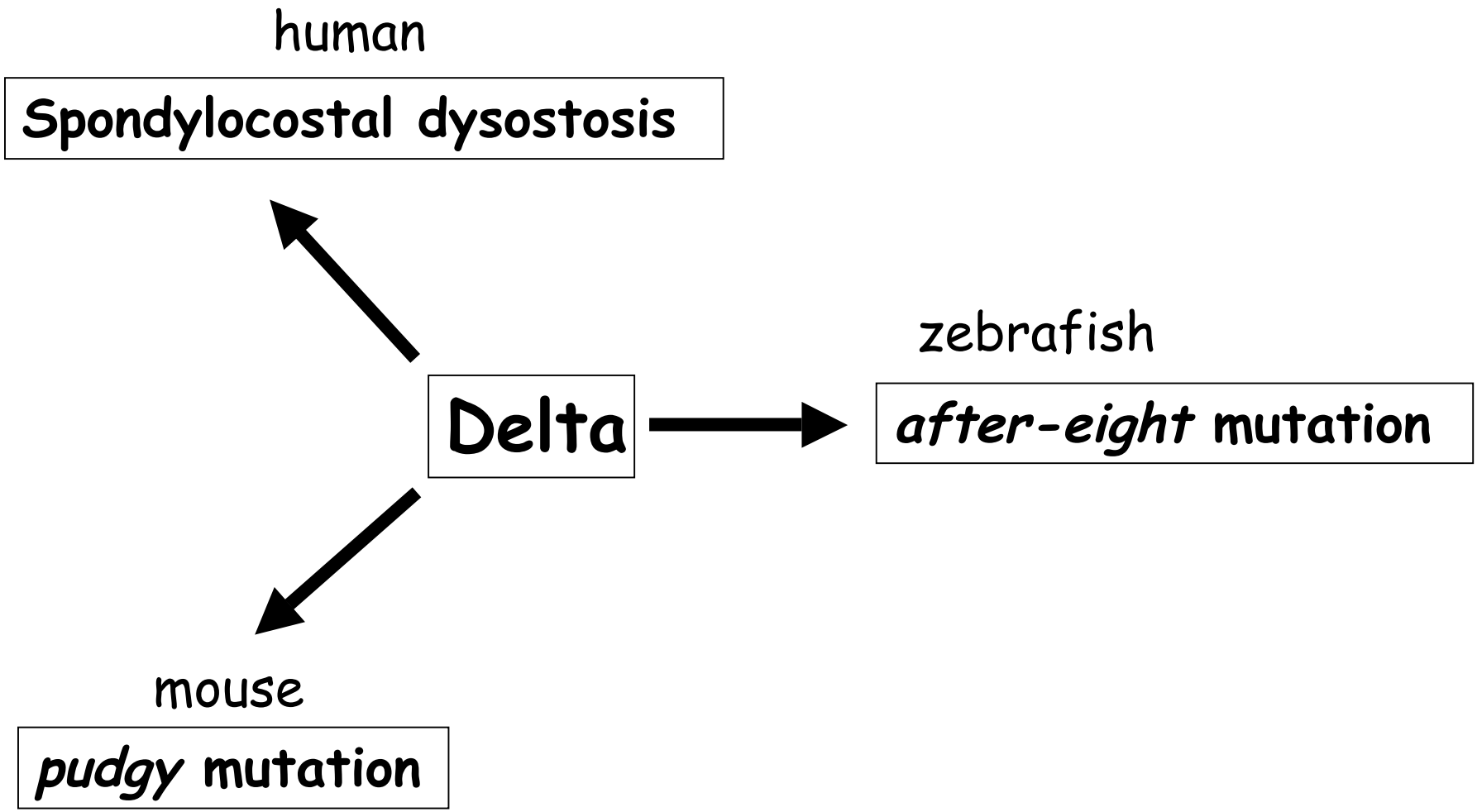


M. P. Bulman et al. (2000)

pudgy

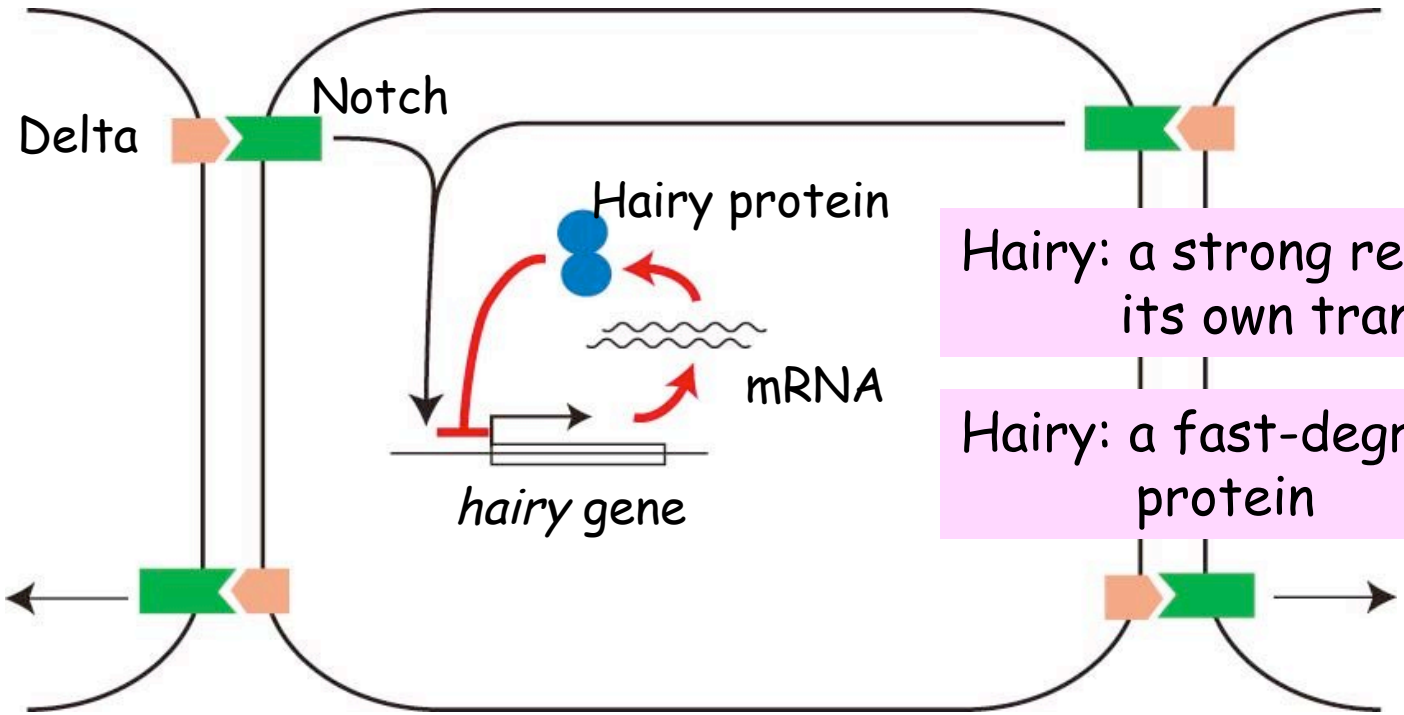
K. Kusumi et al. (1998)

Delta genes are mutated in human, mouse and zebrafish



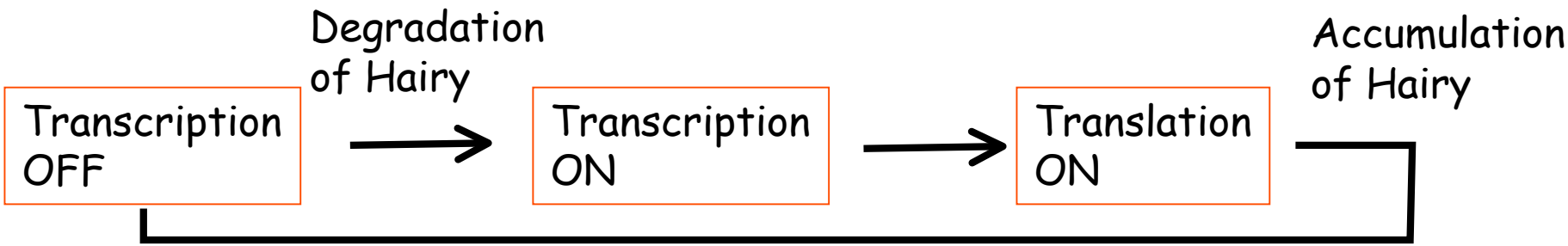
Negative-feedback loop centered by Hairy protein lines at the core of unit oscillator

Unit (cellular) oscillator

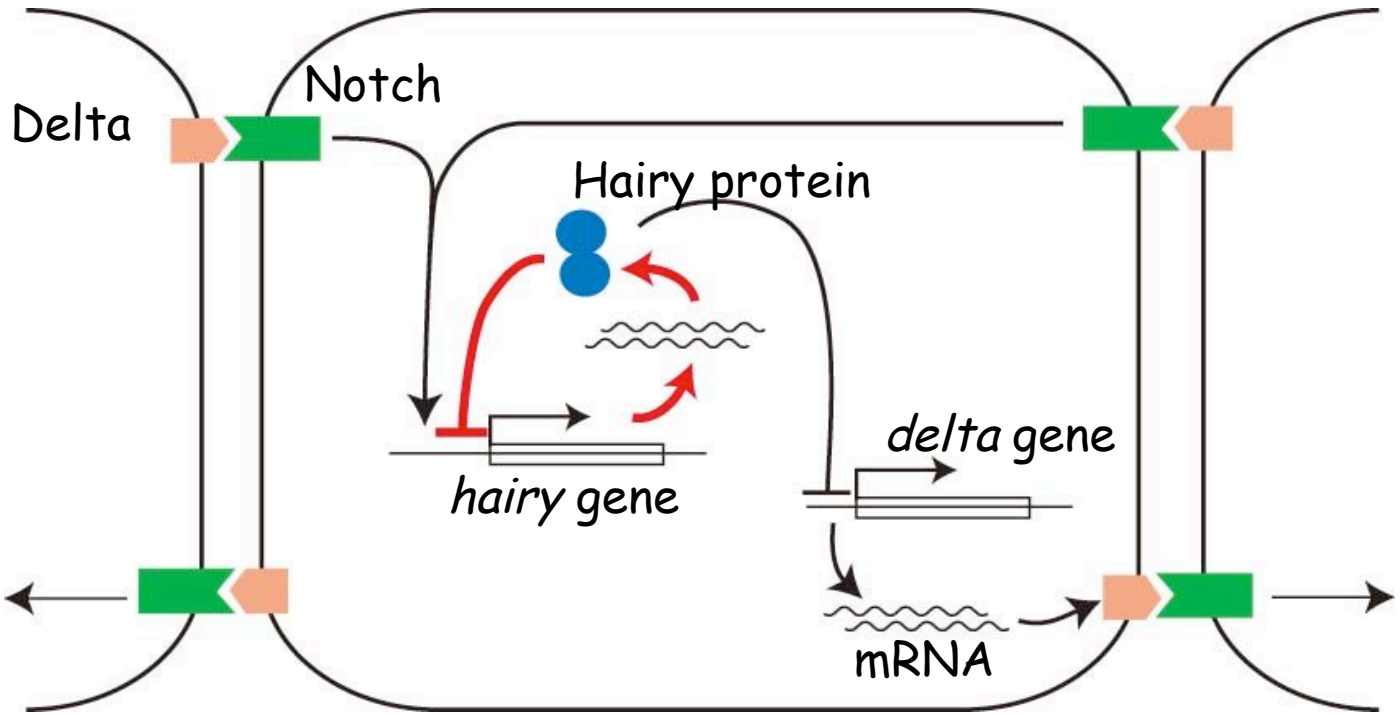


Hairy: a strong repressor to its own transcription

Hairy: a fast-degrading protein



Proposed model



Unit (cellular) oscillator

Notch-dependent intercellular communication, the activity of which is regulated by the internal hairy oscillator, couples neighbouring cells to facilitate synchronized oscillation.

Contents

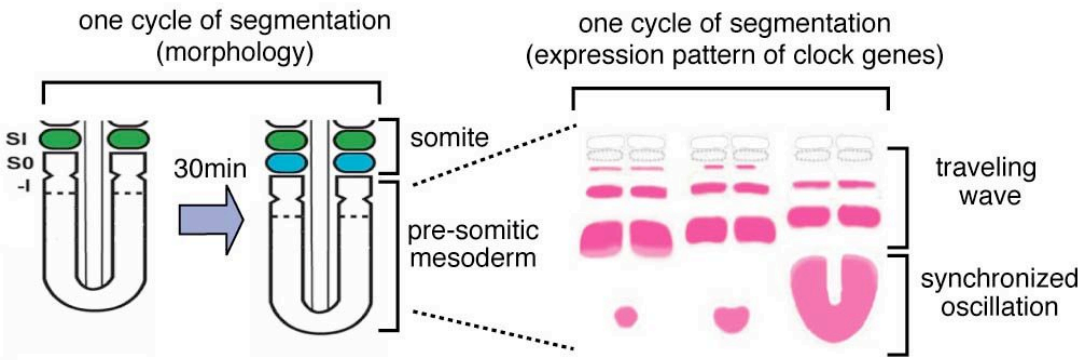
System-level property of the segmentation clock

1. Detailed *hairy* expression in the PSM
   (transcriptional dynamics of *hairy* oscillators)

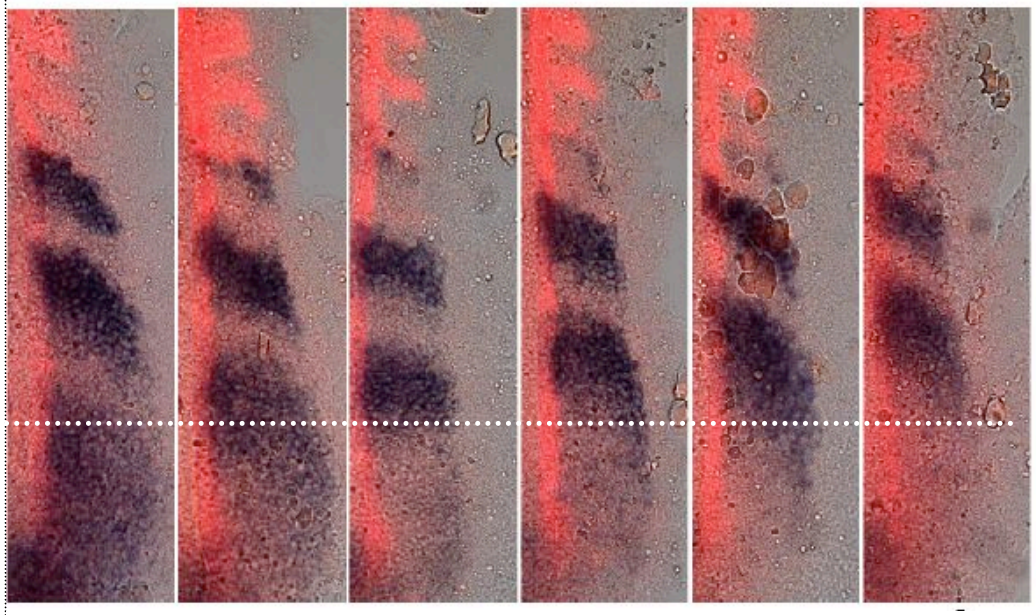
2. The segmentation clock behaves as the 'coupled oscillators' by mosaic experiments and simulation.

3. The system suffers from internal noise but is noise-resistant.

Q. What is the exact expression profile of *her1*?

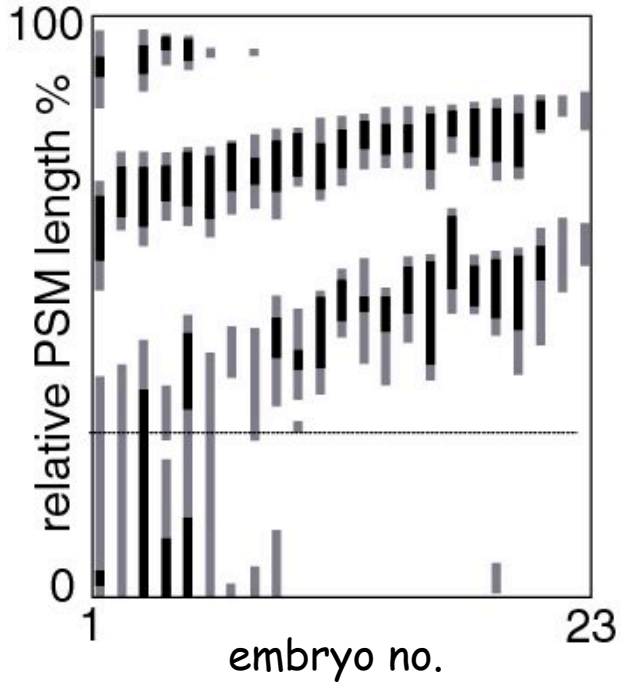


Representative pictures



0 min. → 30 min.

Time-resolution < 2min.



Traveling

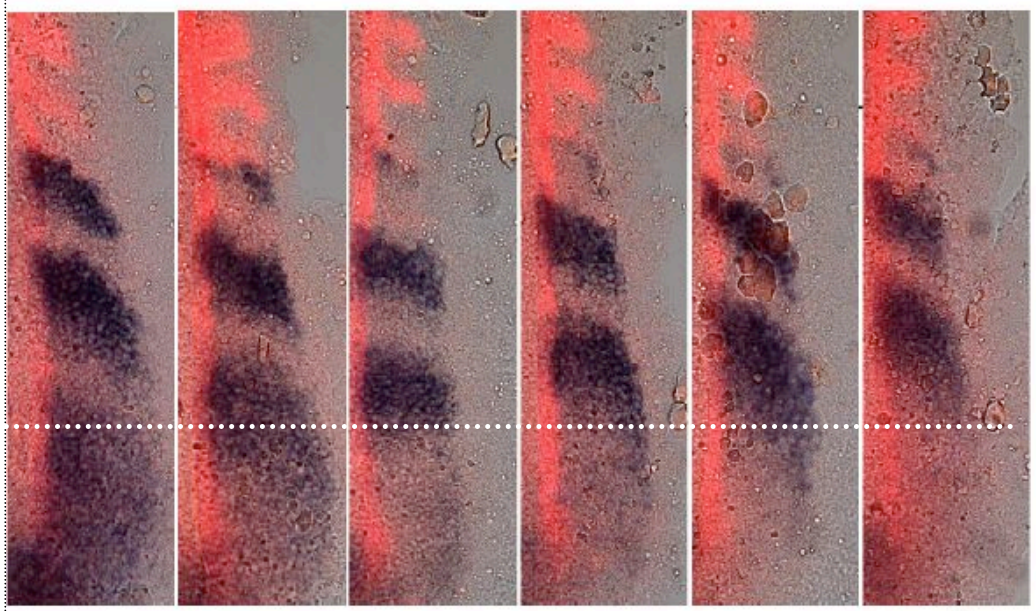
Synchronous

Q. What is the exact expression profile of *her1*?

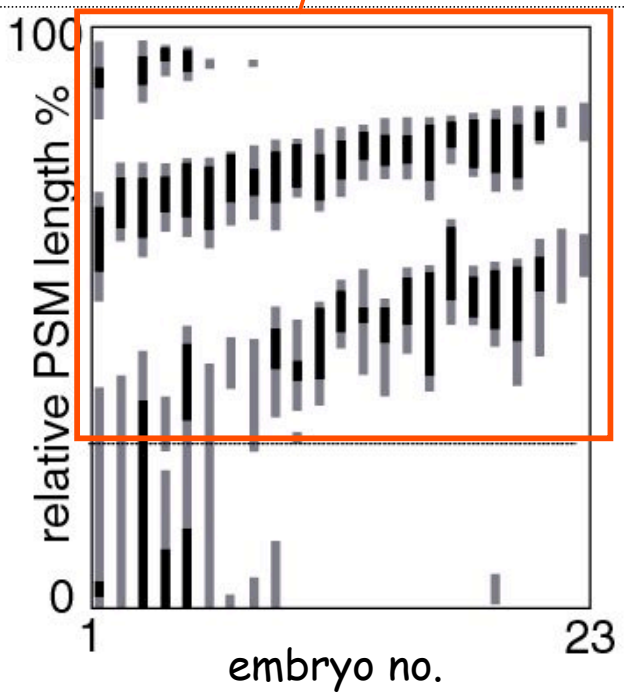
Dual mode of *her1* oscillation

Traveling wave

Representative pictures



Time-resolution < 2min.

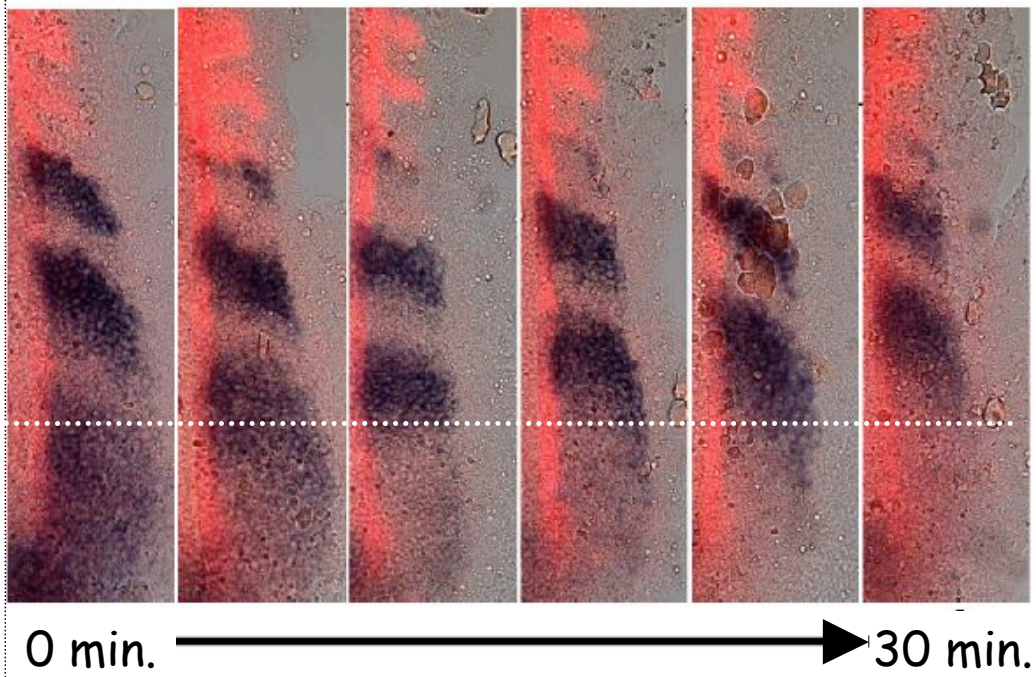


Q. What is the exact expression profile of *her1*?

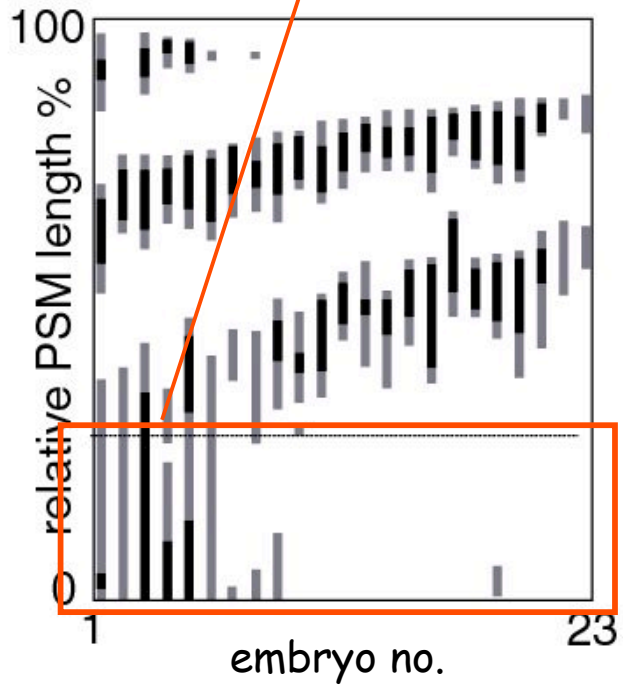
Dual mode of *her1* oscillation

Synchronized oscillation

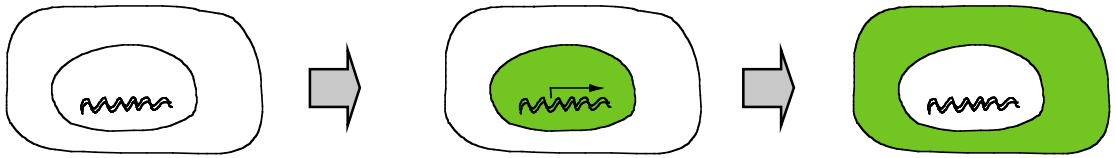
Representative pictures



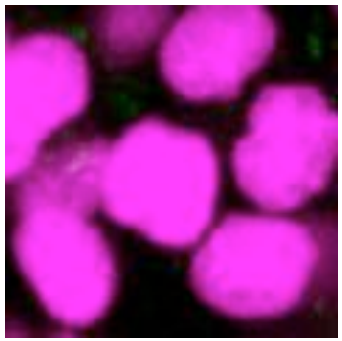
Time-resolution < 2min.

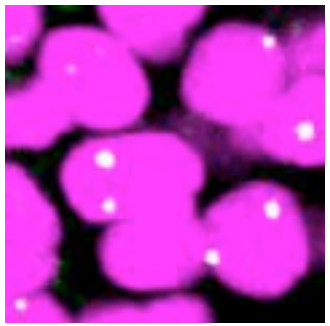


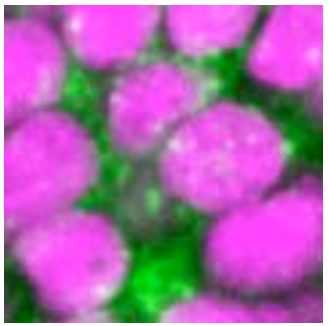
High-resolution in situ hybridization can determine the phase of individual *hairy* oscillators

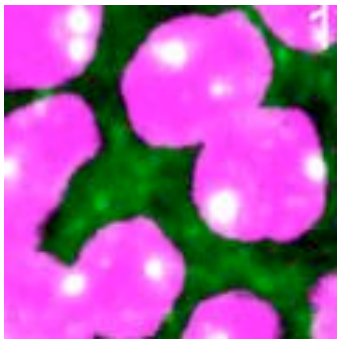


Negative → Nuclear → Cytoplasmic









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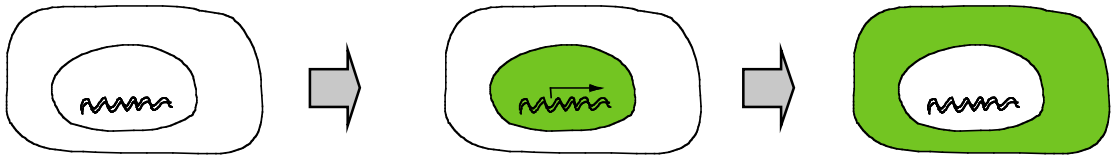

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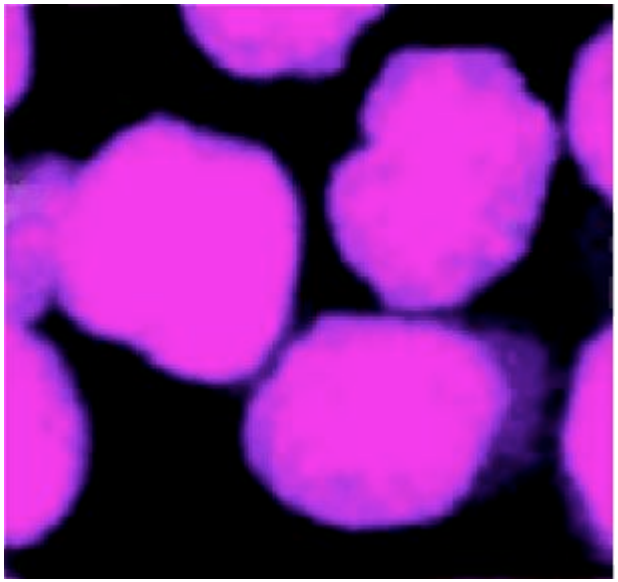
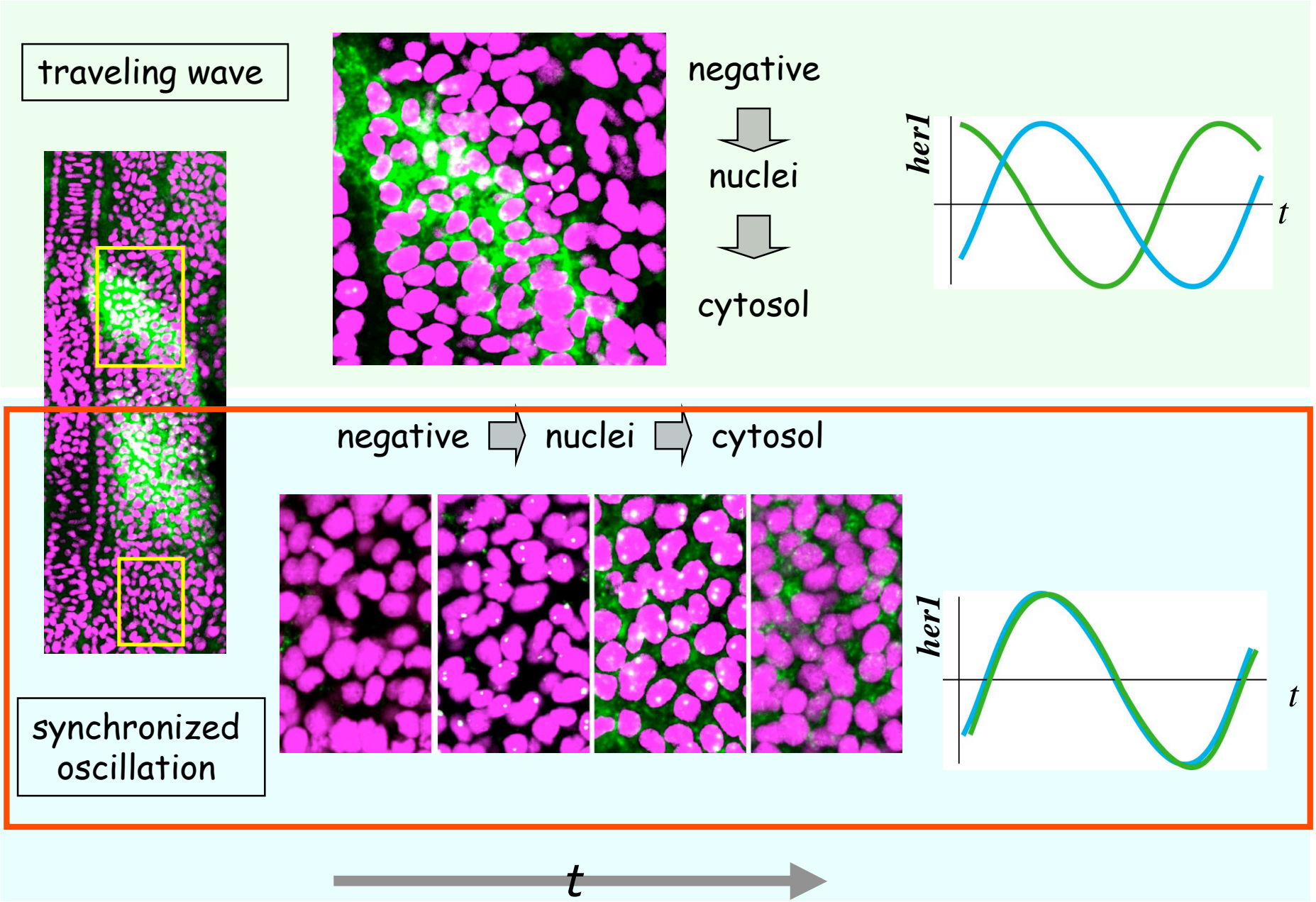
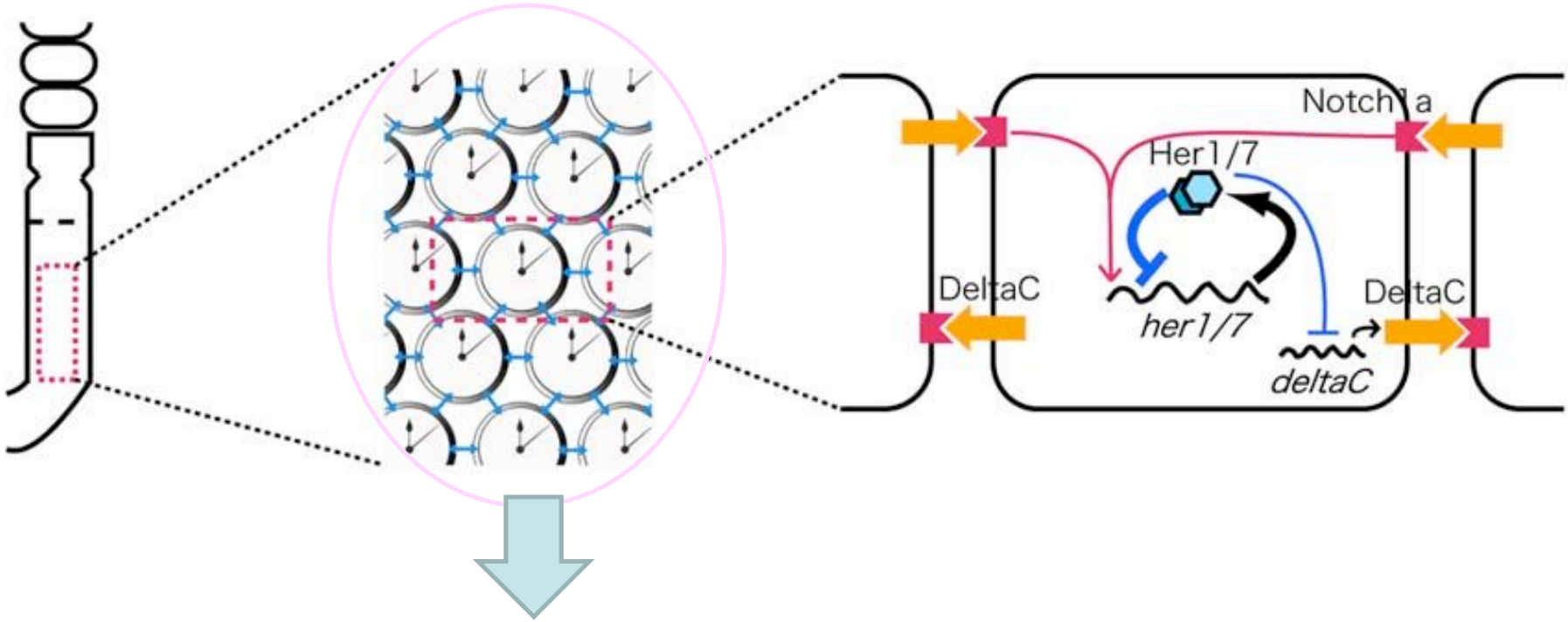


Dual mode of *her1* expression



Proposed model for the segmentation clock

1. The clock consists of numerous cellular oscillators.
2. The negative-feedback loop of Hairy proteins lies at the core of each cellular oscillator.
3. Notch-Delta dependent intercellular communication works in vivo.



Coupled oscillators

The segmentation clock behaves like 'coupled oscillators'?

In a typical 'coupled oscillators',

1. Unit oscillators cycle automatically at roughly regular time-intervals.

2. Some physical or chemical process allows unit oscillators to influence one another.

As a result of these conversations

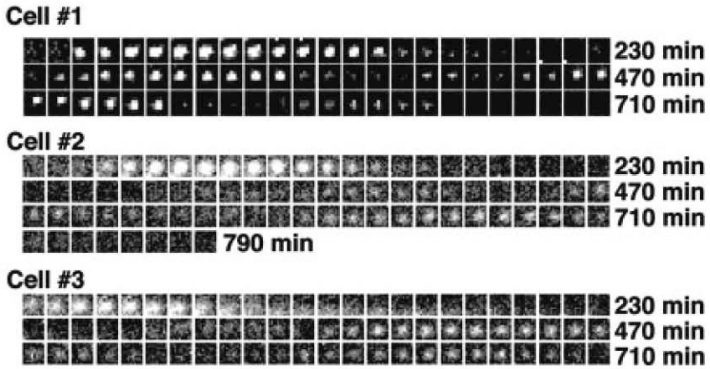
Synchronous oscillation

The segmentation clock behaves like 'coupled oscillators'?

In the segmentation clock,

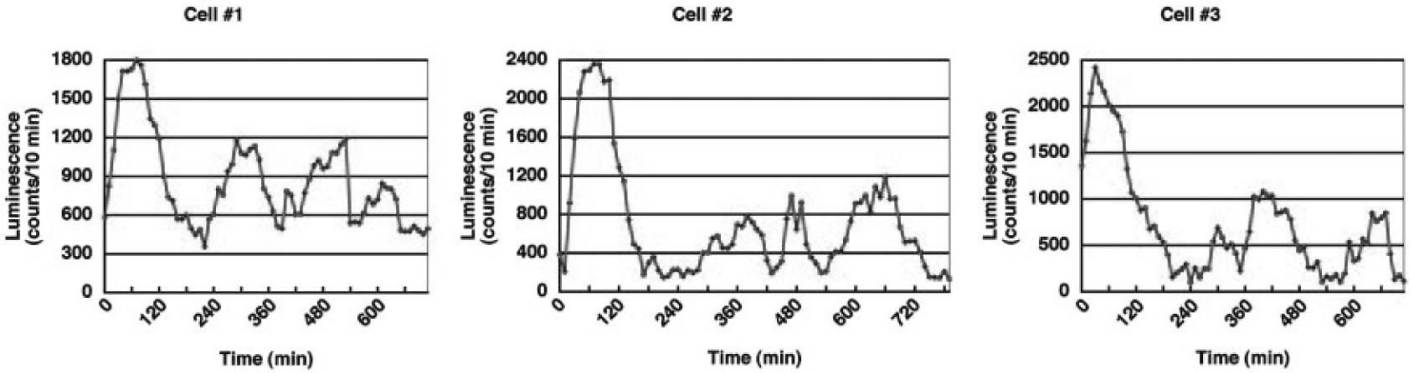
1. Unit oscillators cycle automatically at roughly regular time-intervals.

Isolated PSM cells can oscillate, though not stable (Masamizu et al., 2006)



Dissociated PSM cells of Hes1-Ub1-Luc embryos

Mazamizu et al. PNAS 103: 313–1318, 2006



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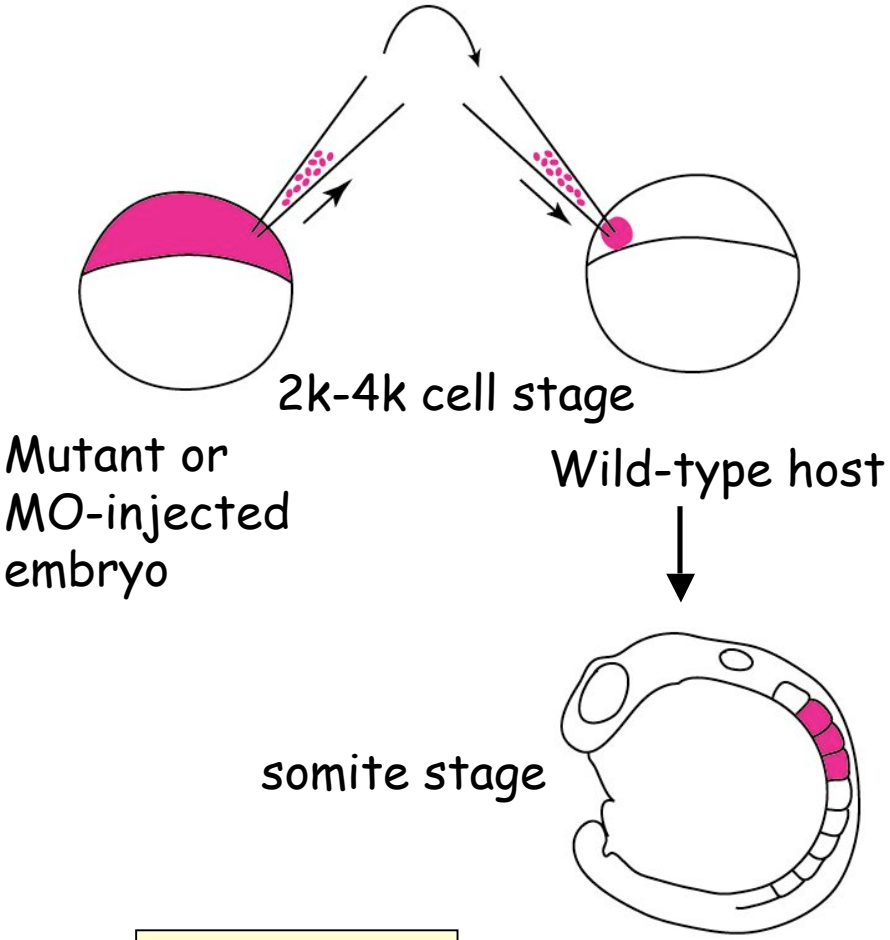
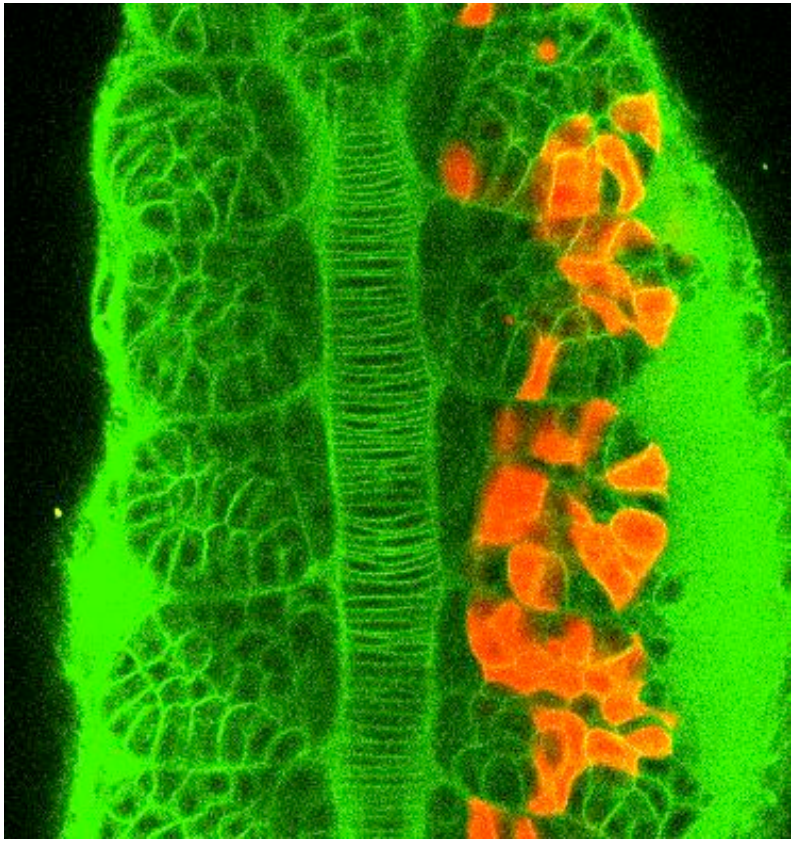
Mosaic experiments in zebrafish

Experimental scheme for the mosaic analysis



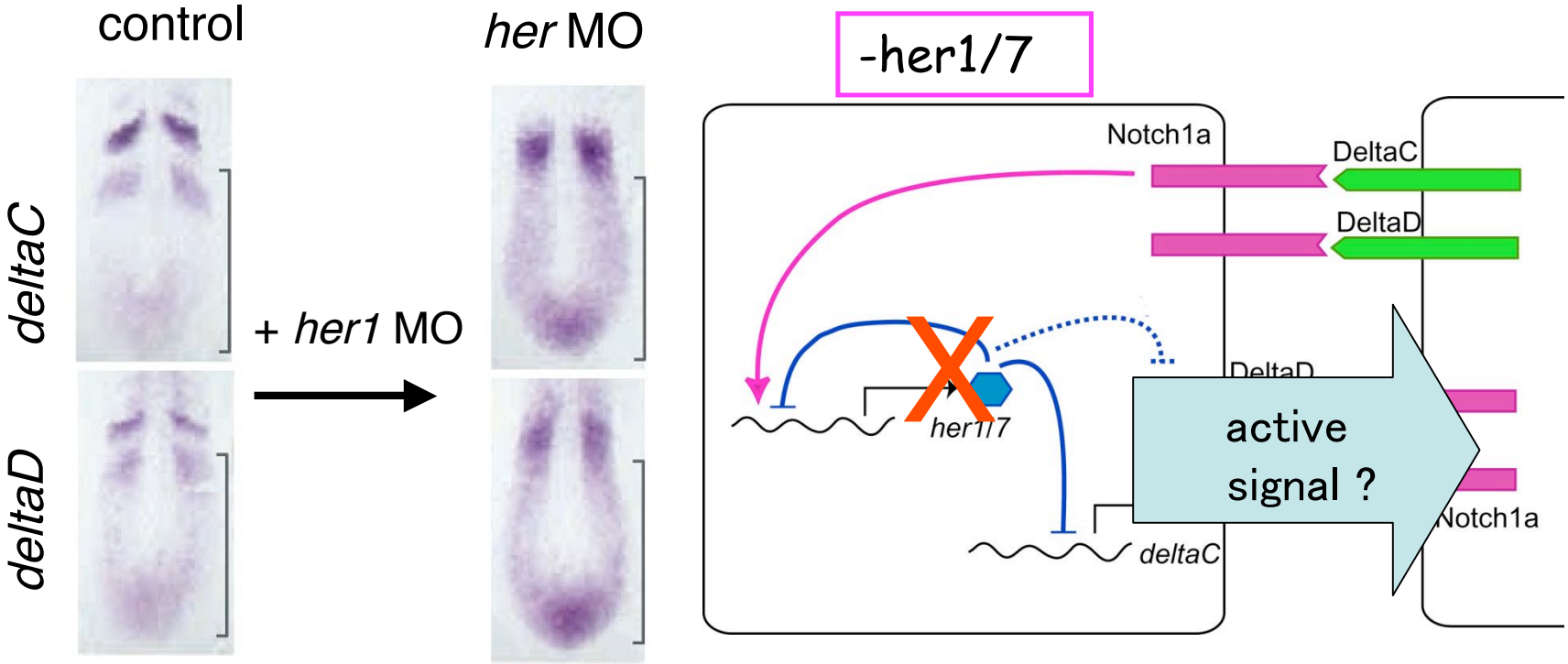


Continuous activation of Notch signaling → intact system → alterd output ?

Cells that constitutively express Delta were obtained by her-MO injection

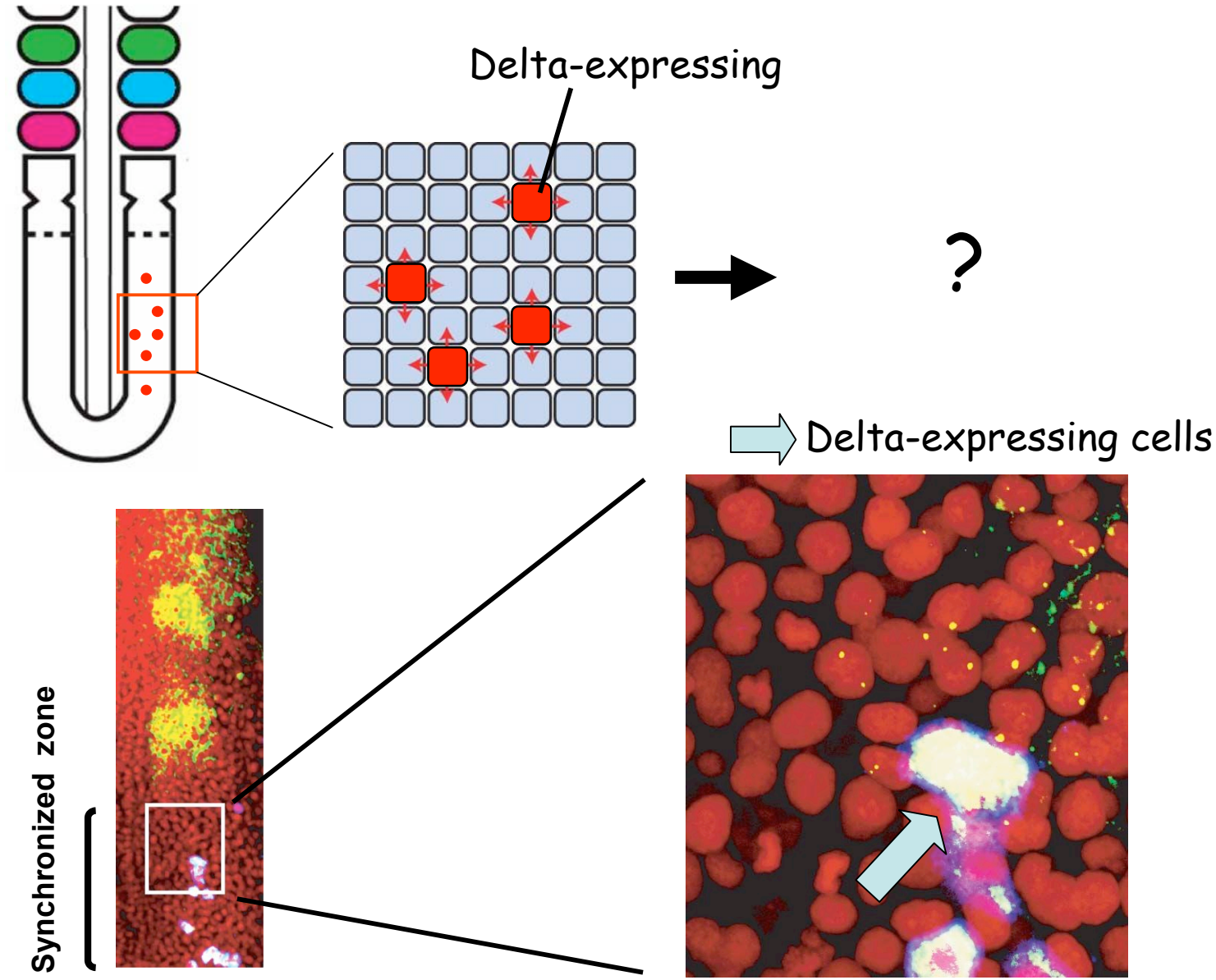
MO: morpholino anti-sense oligonucleotide

Up-regulation of *deltaC* &*D* expression in *her*-MO embryos

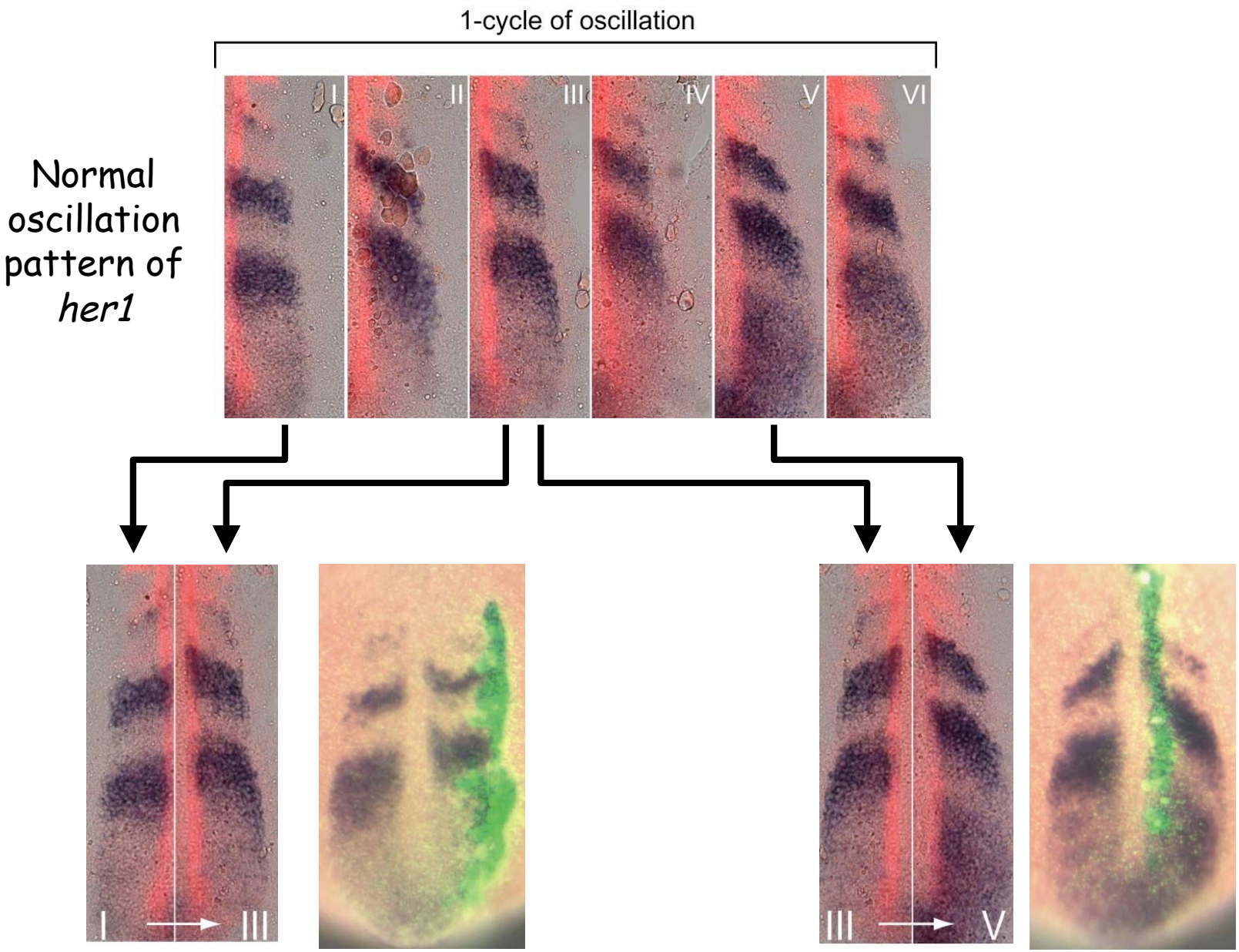


Oates & Ho (2002), Development 129: 2929
Holley et al., (2002), Development 129: 1175

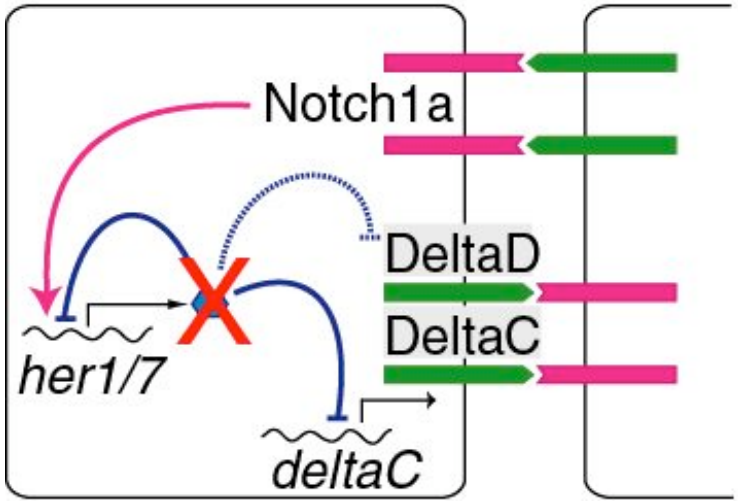
Acceleration by Delta-expressing cells in the synchronized zone

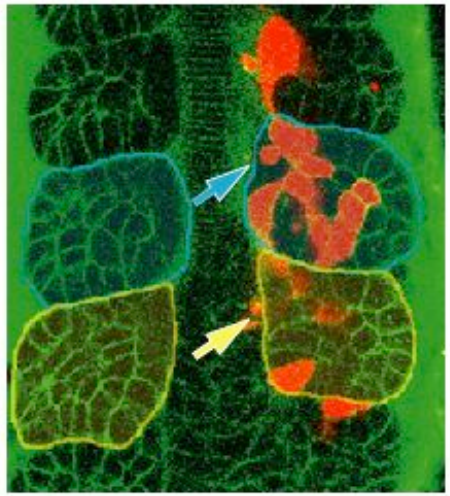


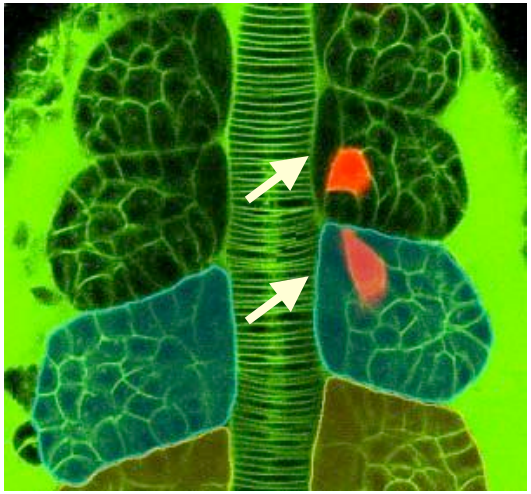
Accelerated wave on the transplanted side



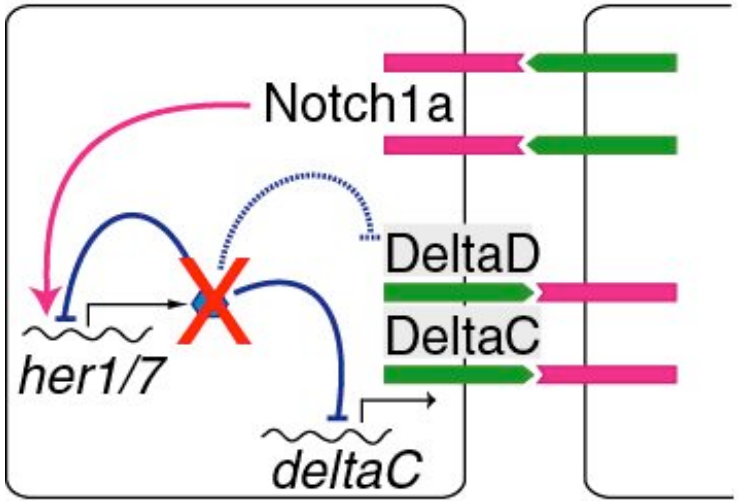
Delta-expressing cells anteriolry shift segment positions in a delta-dependent fashion

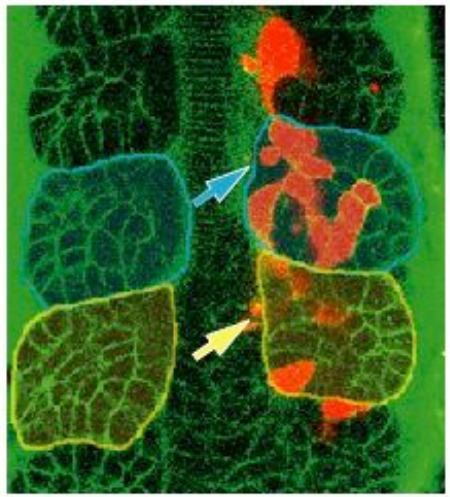


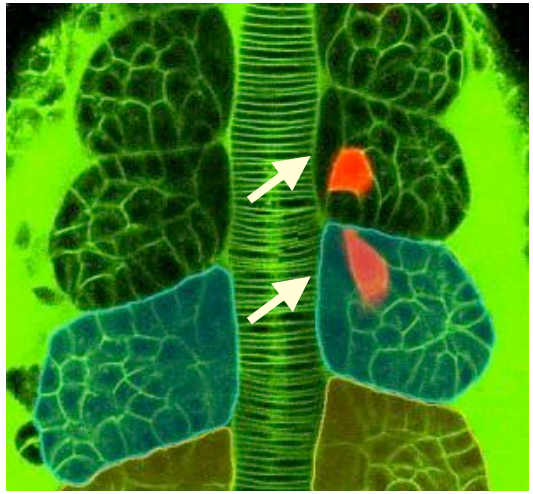


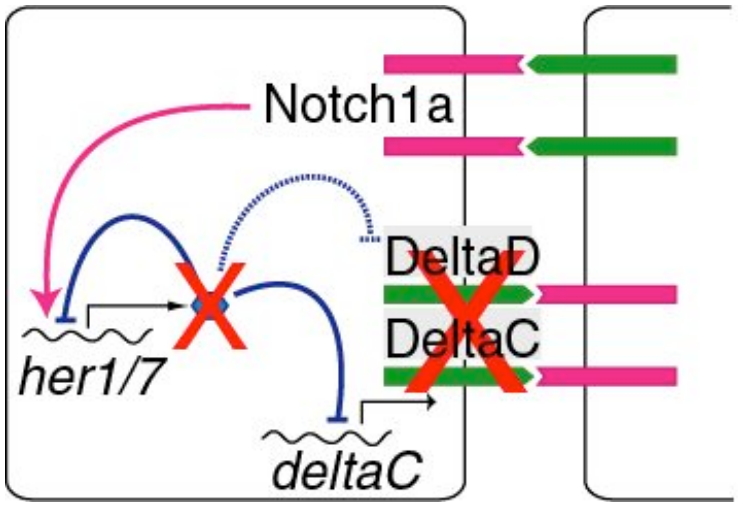


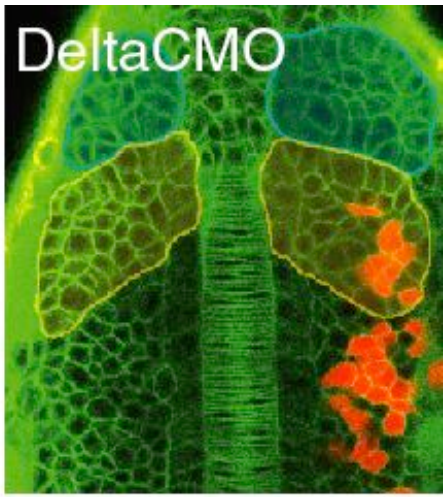
herMO-cells anteriolry shift segment positions in a delta-dependent fashion

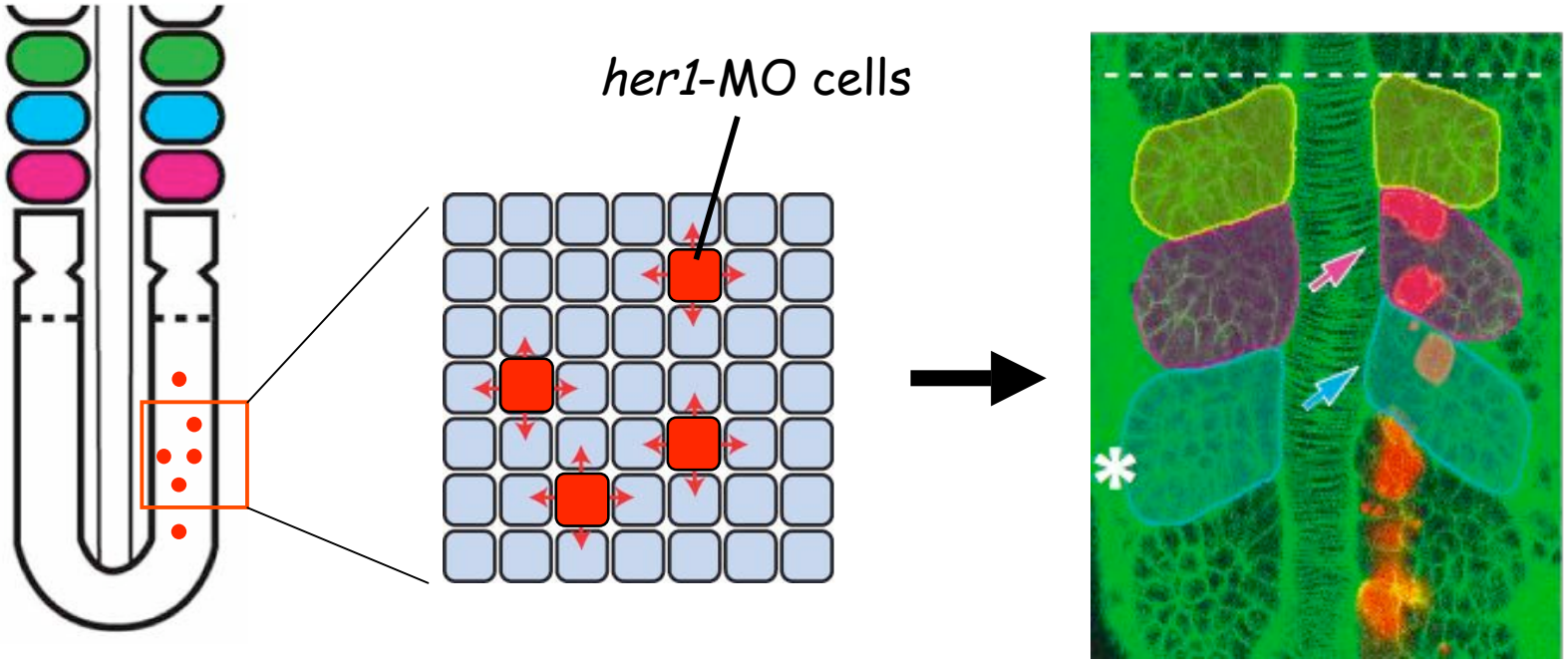












Delta-expressing donor cells locally accelerate *hairy* oscillation in the posterior PSM

Delta-dependent conversations function in vivo in such a way that they accelerate the clock oscillation in adjacent cells

$$\frac{p(t)}{dt} = \text{protein synthesis rate - protein degradation rate}$$

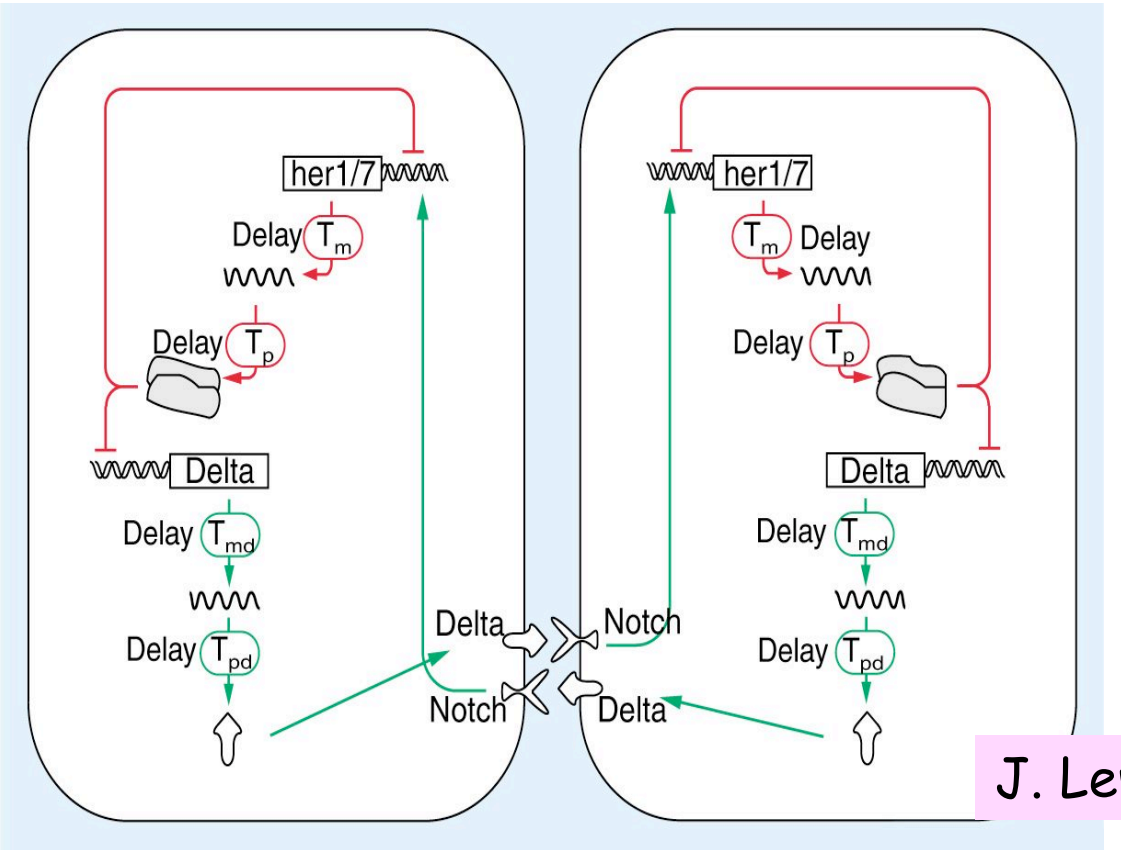
$$= am(t-Tp) - bp(t)$$

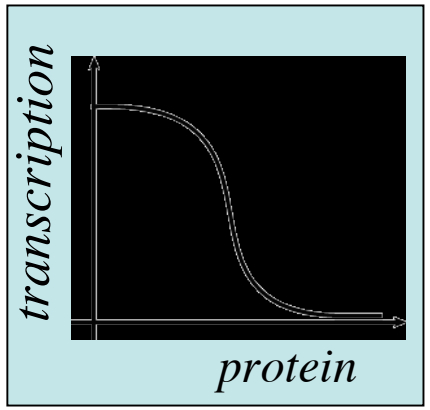
$$\frac{m(t)}{dt} = \text{mRNA synthesis rate - mRNA degradation rate}$$

$$= f(p(t-Tm)) - cm(t)$$

$f(p)$ = Her dependent inhibition

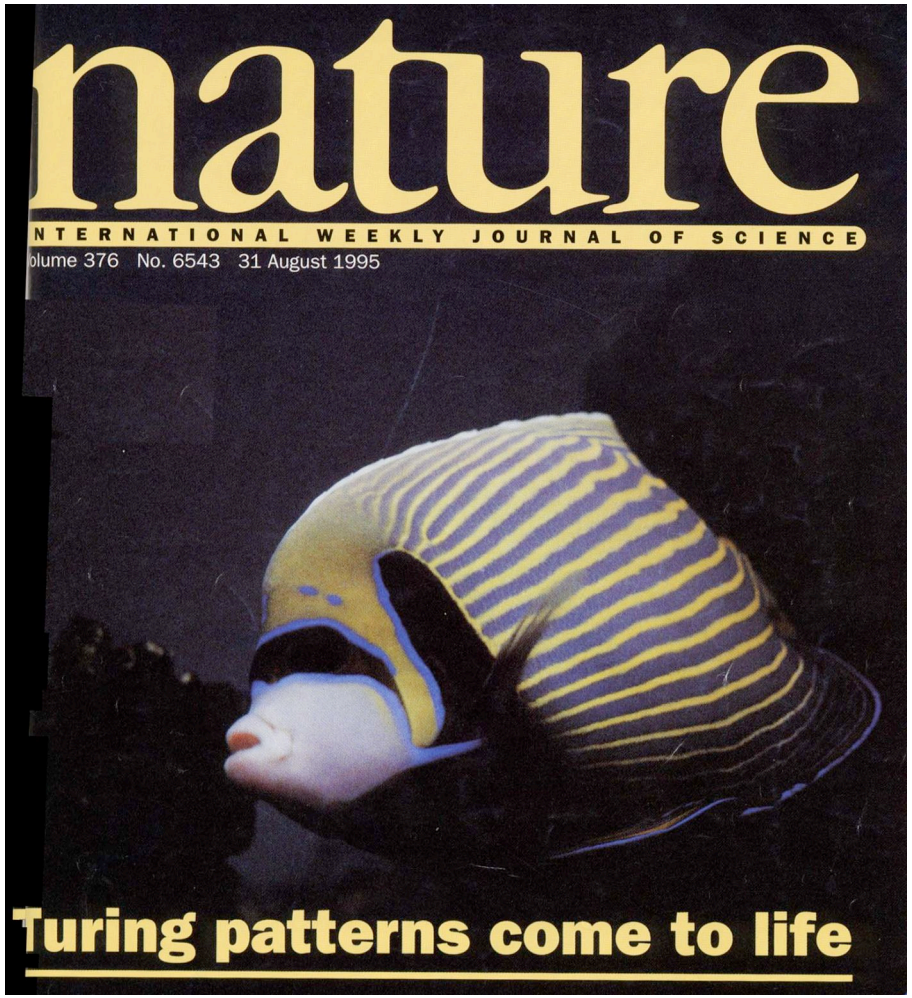
$$= \frac{k}{1+p^2}$$



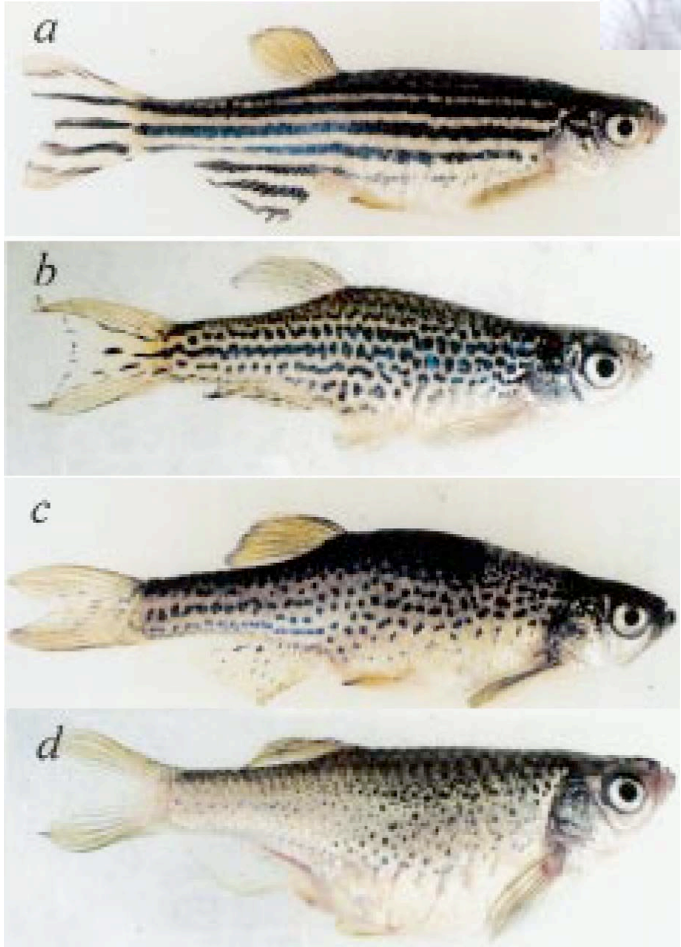


J. Lewis (2003) Curr. Biol. 13: 1398

A reaction-diffusion wave on the skin of fish (Shigeru Kondo, Nagoya Univ.)

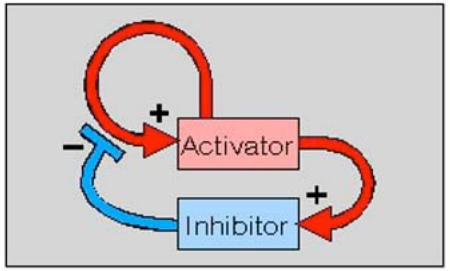


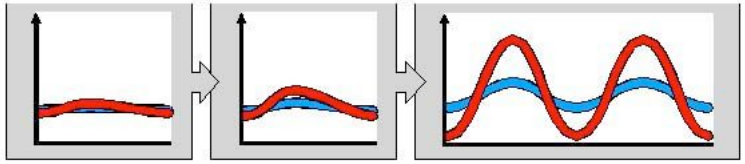
S. Kondo, Nature, 1995 & 1996



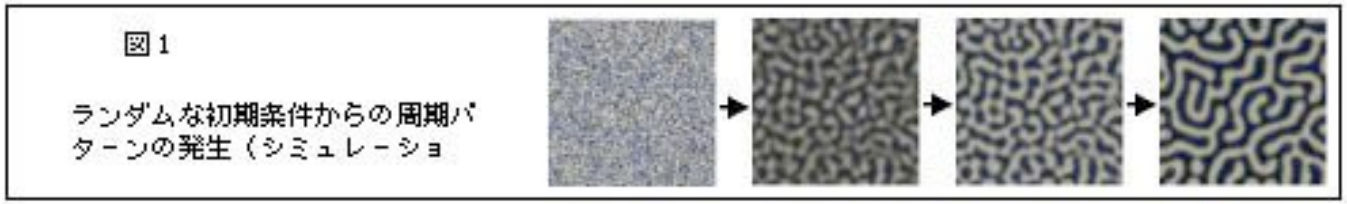
S. Kondo, Mech. Dev. 1998

Reaction-Diffusion Turing System

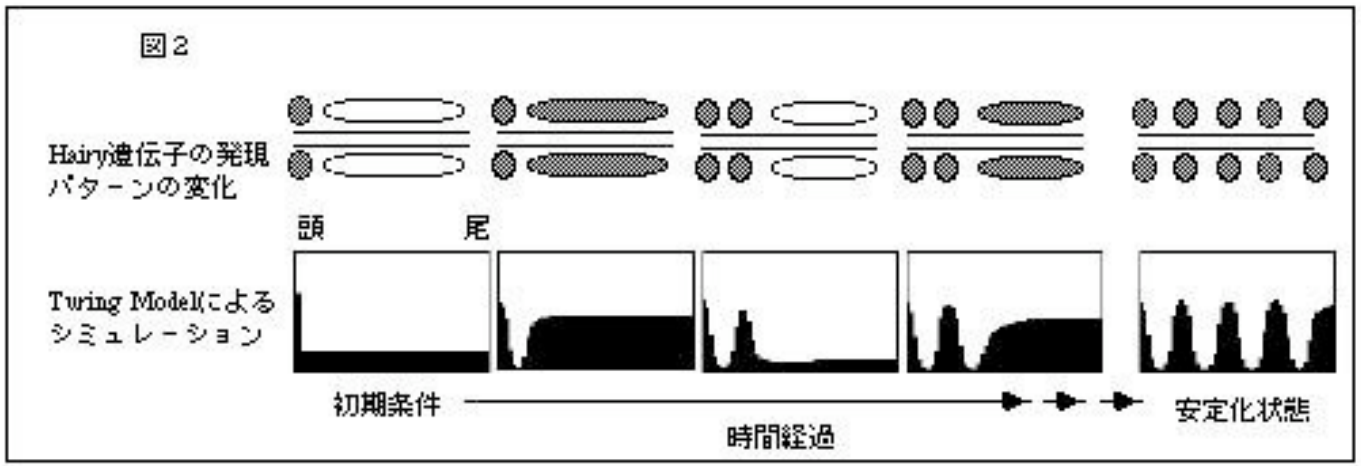




Pigment pattern

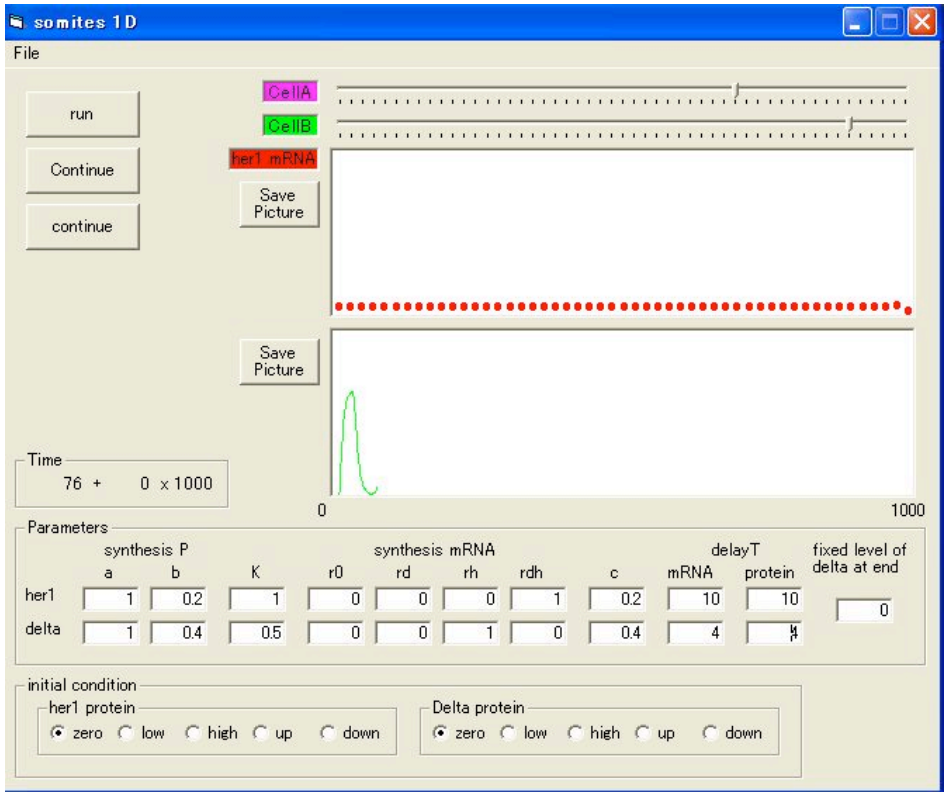


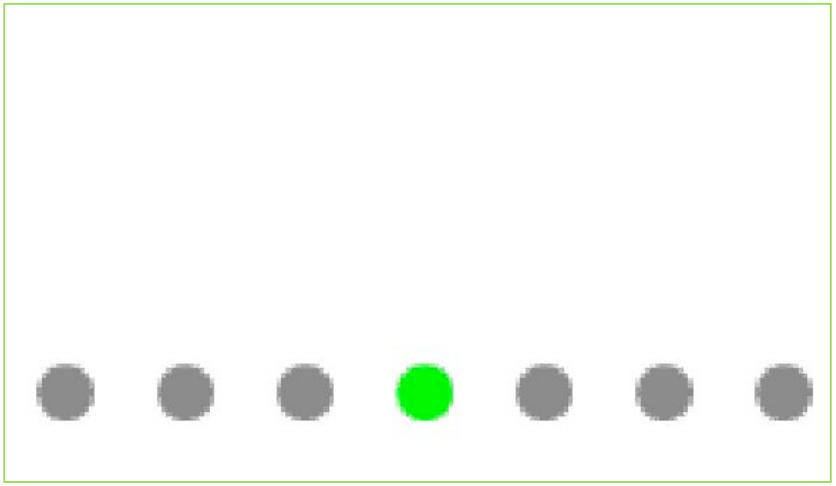
Somite Formation



From Dr. Shigeru Kondo

Simulator constructed by Dr. Shigeru Kondo (Nagoya Univ.)





Vol 441|8 June 2006|doi:10.1038/nature04861 nature

ARTICLES

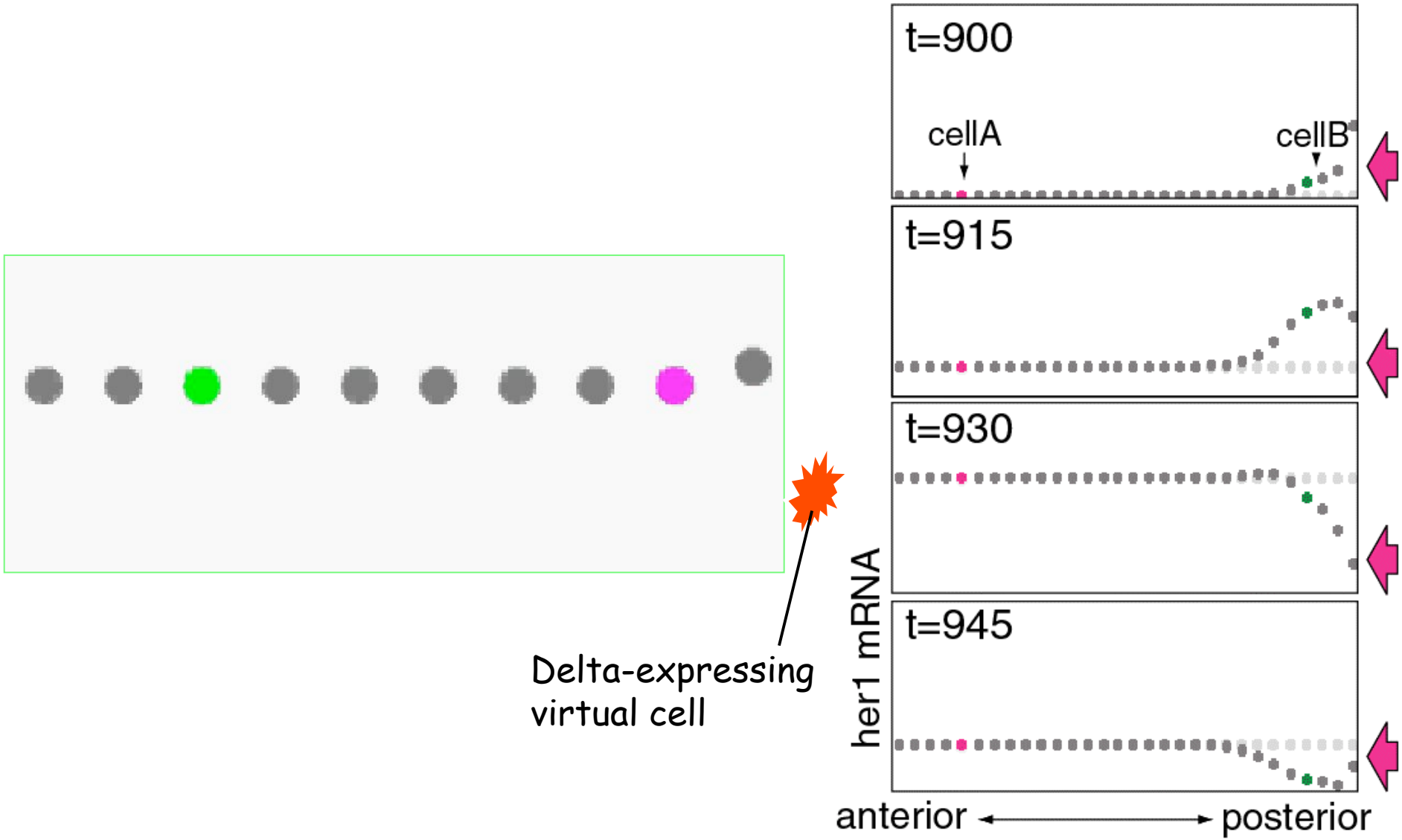
Noise-resistant and synchronized oscillation of the segmentation clock

Kazuki Horikawa[1]*, Kana Ishimatsu[1]*, Eiichi Yoshimoto[2]*, Shigeru Kondo[2] & Hiroyuki Takeda[1]

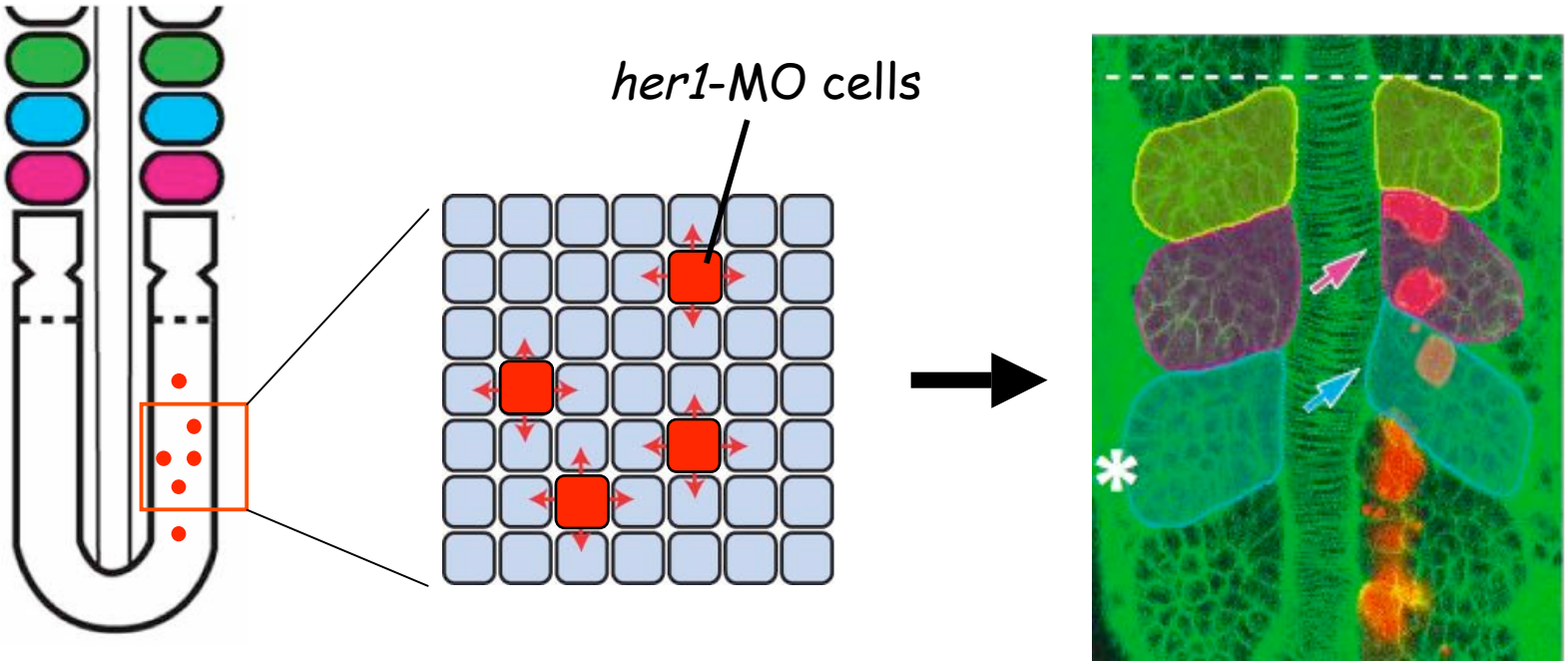
Periodic somite segmentation in vertebrate embryos is controlled by the 'segmentation clock', which consists of numerous cellular oscillators. Although the properties of a single oscillator, driven by a *hairy* negative-feedback loop, have been investigated, the system-level properties of the segmentation clock remain largely unknown. To explore these characteristics, we have examined the response of a normally oscillating clock in zebrafish to experimental stimuli using *in vivo* mosaic experiments and mathematical simulation. We demonstrate that the segmentation clock behaves as a coupled oscillator, by showing that Notch-dependent intercellular communication, the activity of which is regulated by the internal *hairy* oscillator, couples neighbouring cells to facilitate synchronized oscillation. Furthermore, the oscillation phase of individual oscillators fluctuates due to developmental noise such as stochastic gene expression and active cell proliferation. The intercellular coupling was found to have a crucial role in minimizing the effects of this noise to maintain coherent oscillation.

Nature 441: 719 – 723, 2006

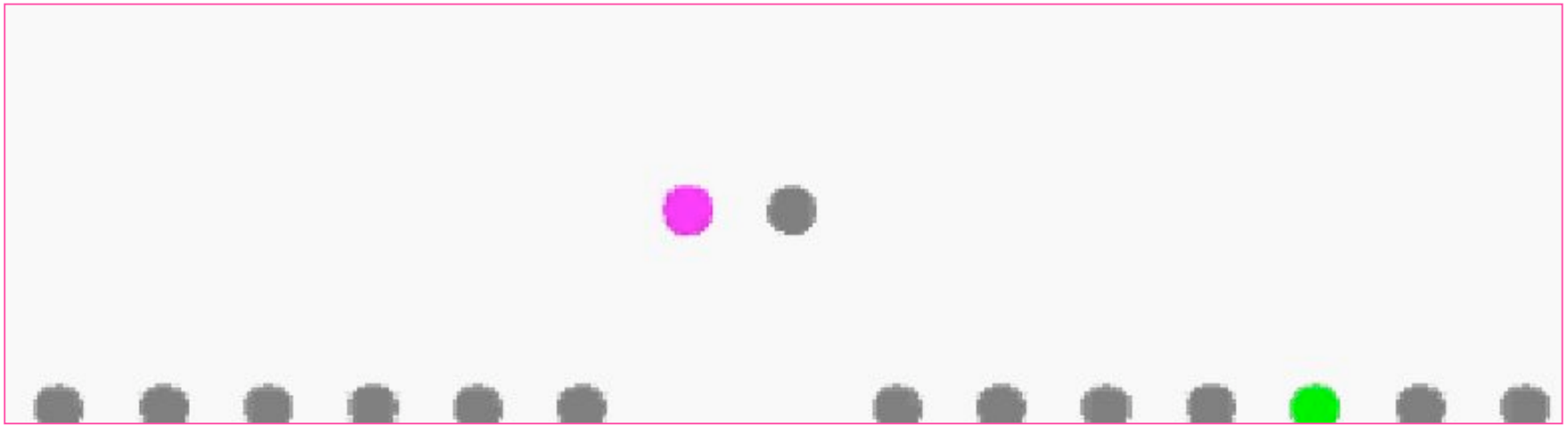
Simulator constructed by Dr. Shigeru Kondo (Nagoya Univ.)



In vivo response to exogenous Notch stimuli can be predicted by the simulator

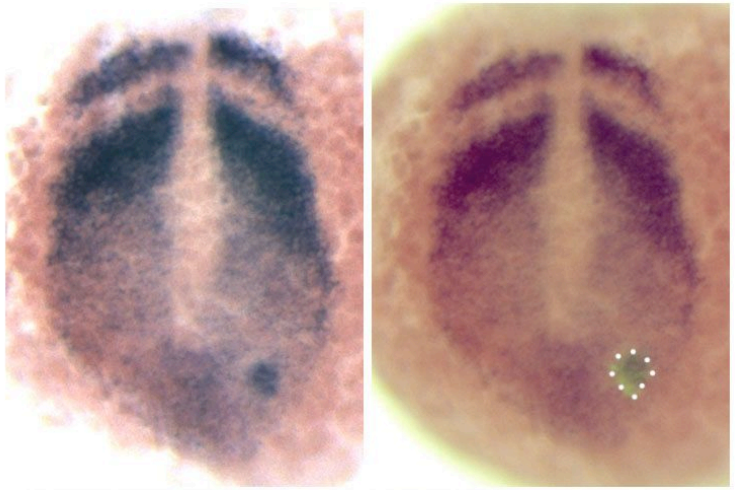


What will happen when normally oscillating cells with a different phase are placed in silico?

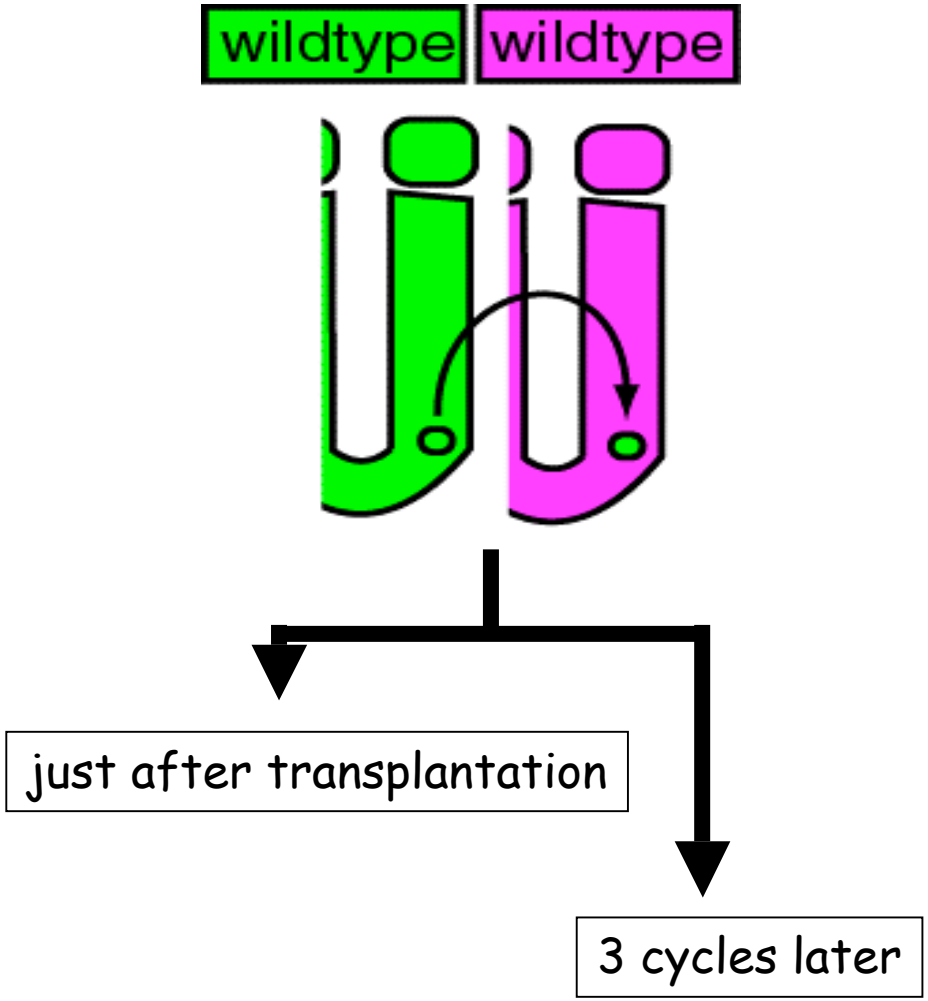


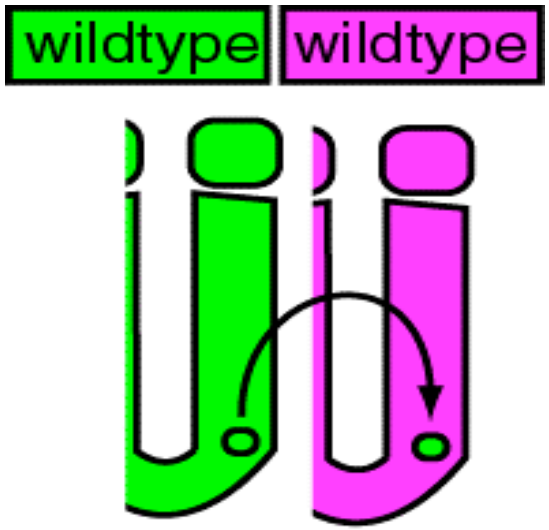
What will happen when normally oscillating cells with a different phase are placed in vivo?

just after transplantation



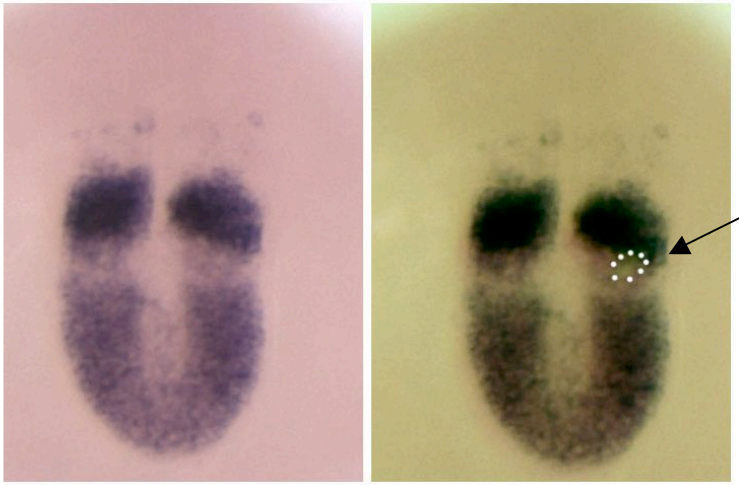
9/22 showed out of phase





3 cycles later

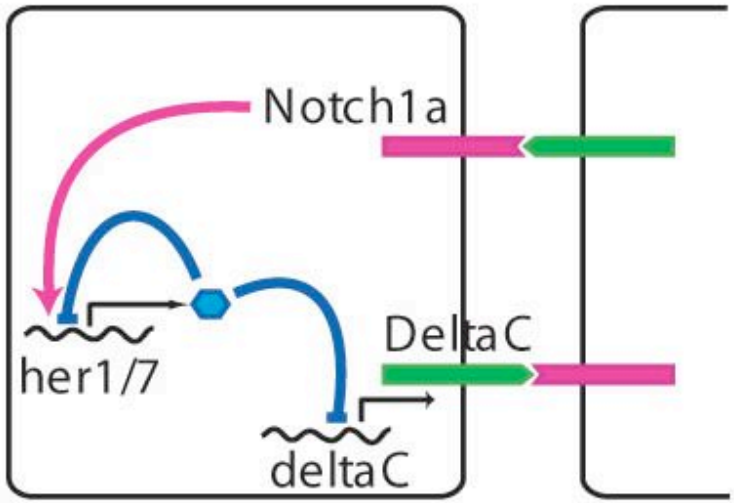
Fluorescent view



24/26 were adjusted in phase

The segmentation clock has the strong ability to couple individual oscillators

This is one of the most characteristic features of 'coupled-oscillators', designated as 'phase synchronization.



$$\frac{d(\text{protein})}{dt} = \text{synthesis of protein - degradation}$$
$$= ap(t-Tp) - bp(t)$$

$$\frac{d(\text{mRNA})}{dt} = \text{synthesis of mRNA - degradation}$$
$$= f\{p(t-Tm)\} - cm(t)$$

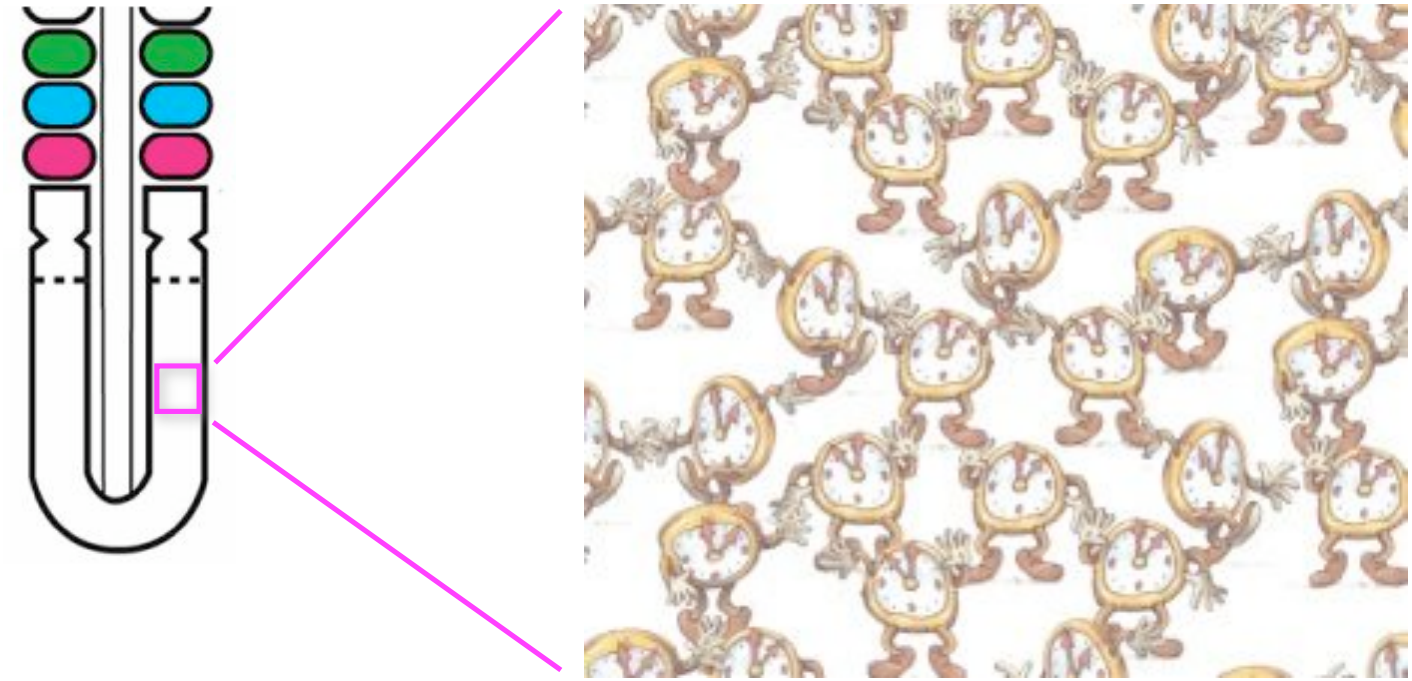
f(p)=basal activation+HER-dependent repression
+Delta dependent activation+combinatorial regulation

The proposed molecular circuit works in vivo

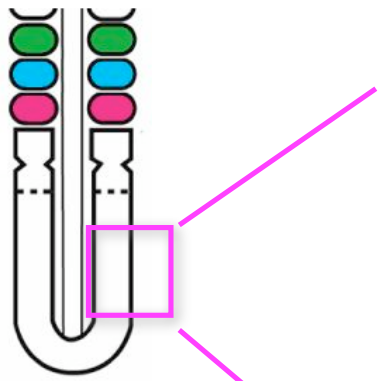
↓

The segmentation clock behaves as 'coupled oscillators'

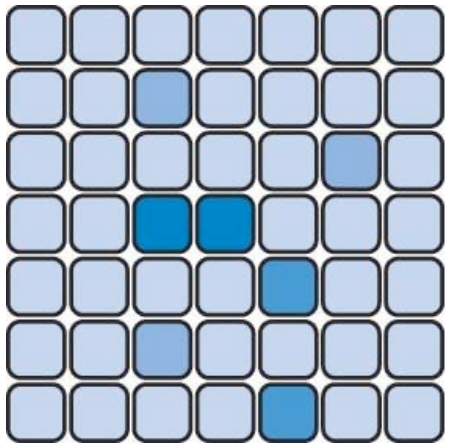
The 'coupled oscillators' is known to be a robust system to internal and external noise

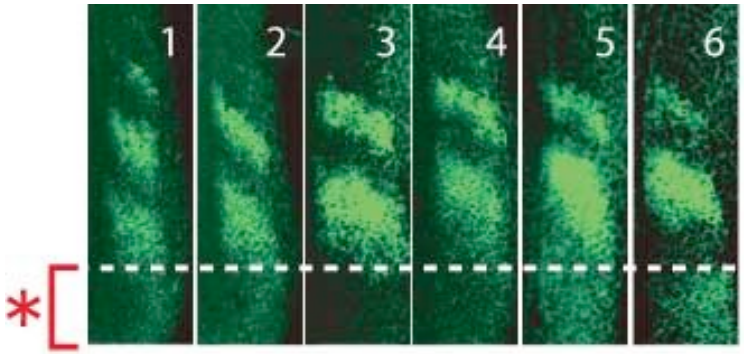


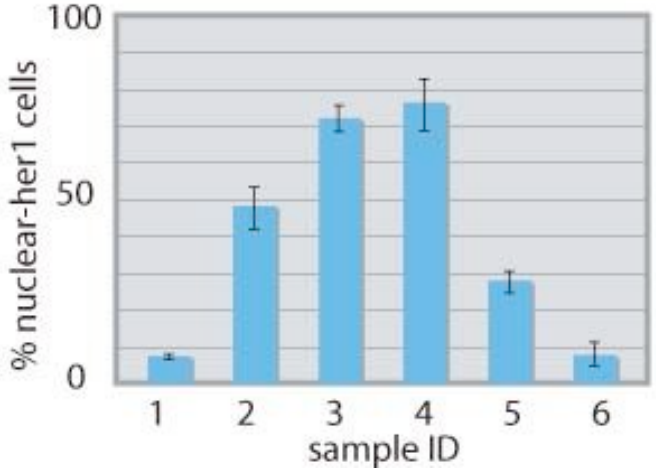
Fluctuations of oscillation phase in the synchronized zone



A population of cellular oscilators

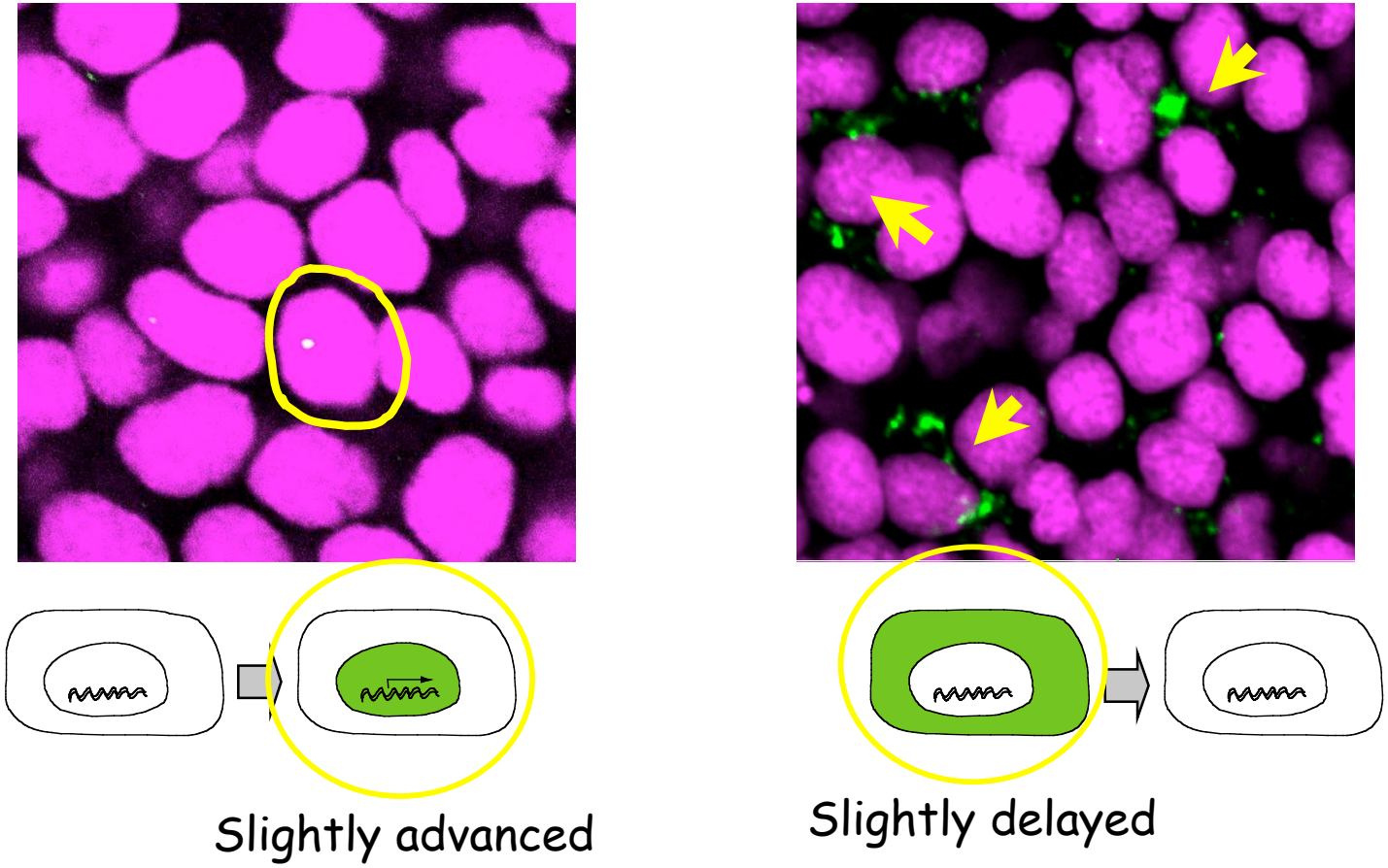






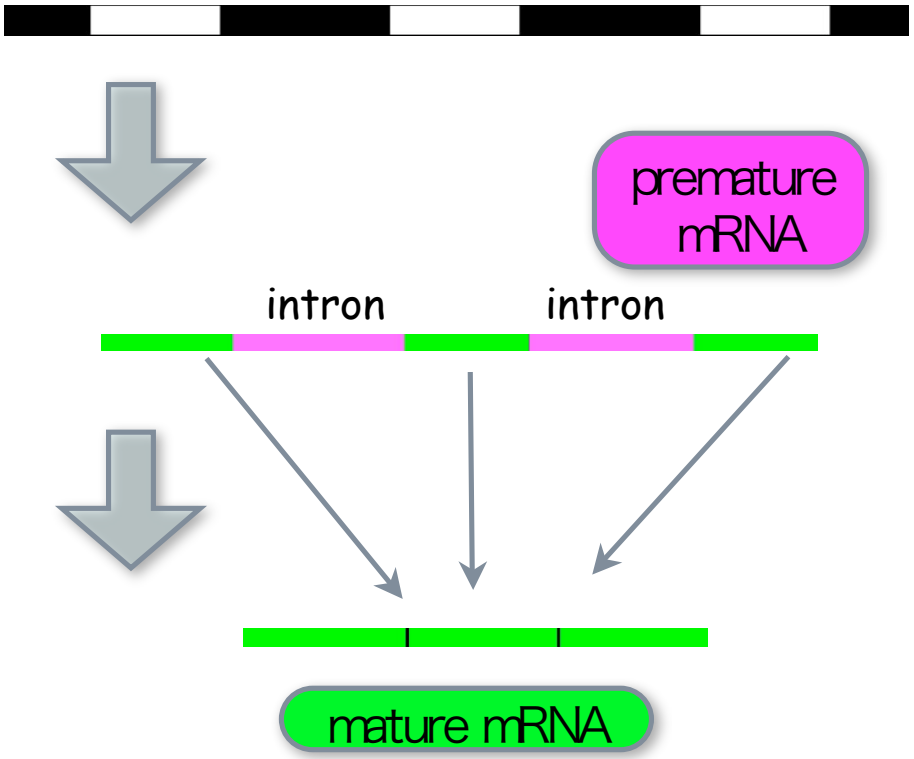
10-20% of PSM cells are always out of phase in the synchronized oscillation zone

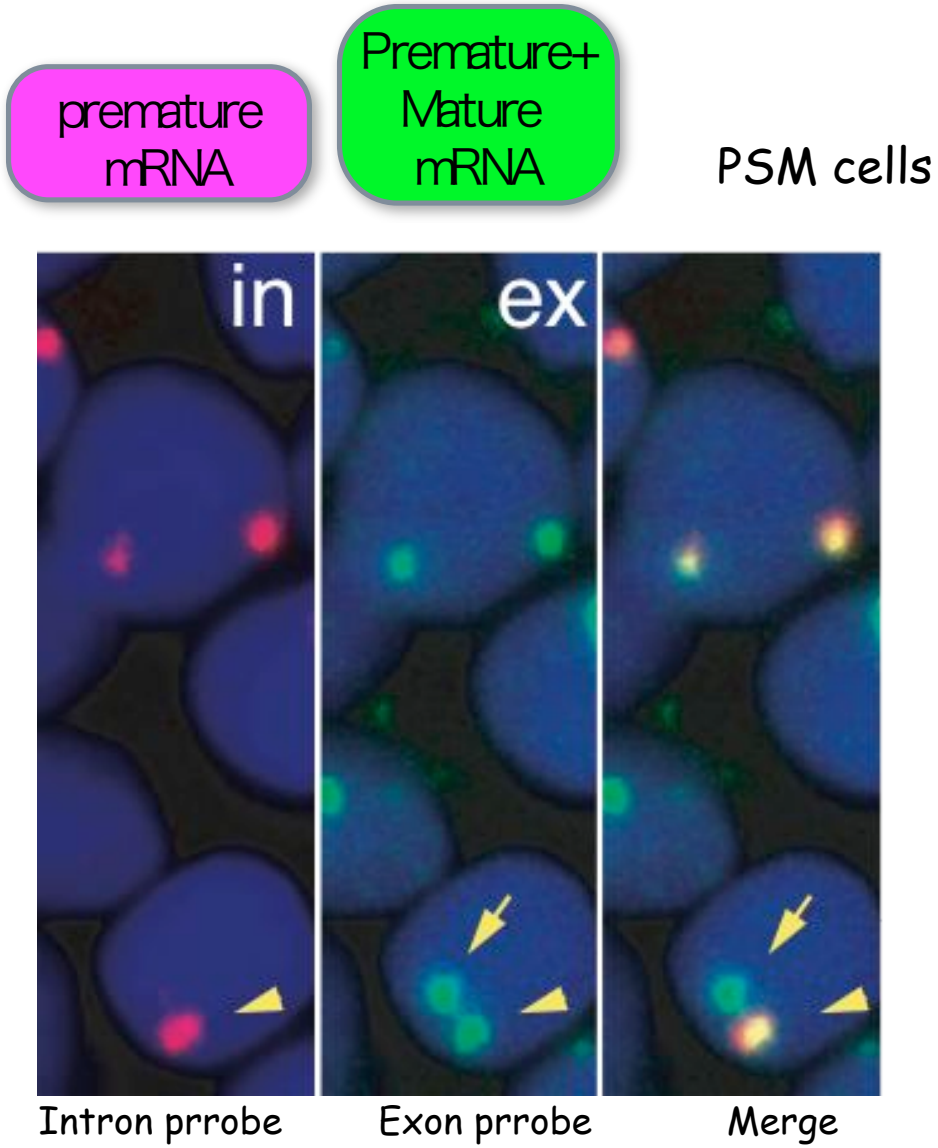
Fluctuations in cellular oscillators



The stochastic nature of rhythm-generating reactions: transcription, translation and translocation are by themselves stochastic processes at the molecular level.

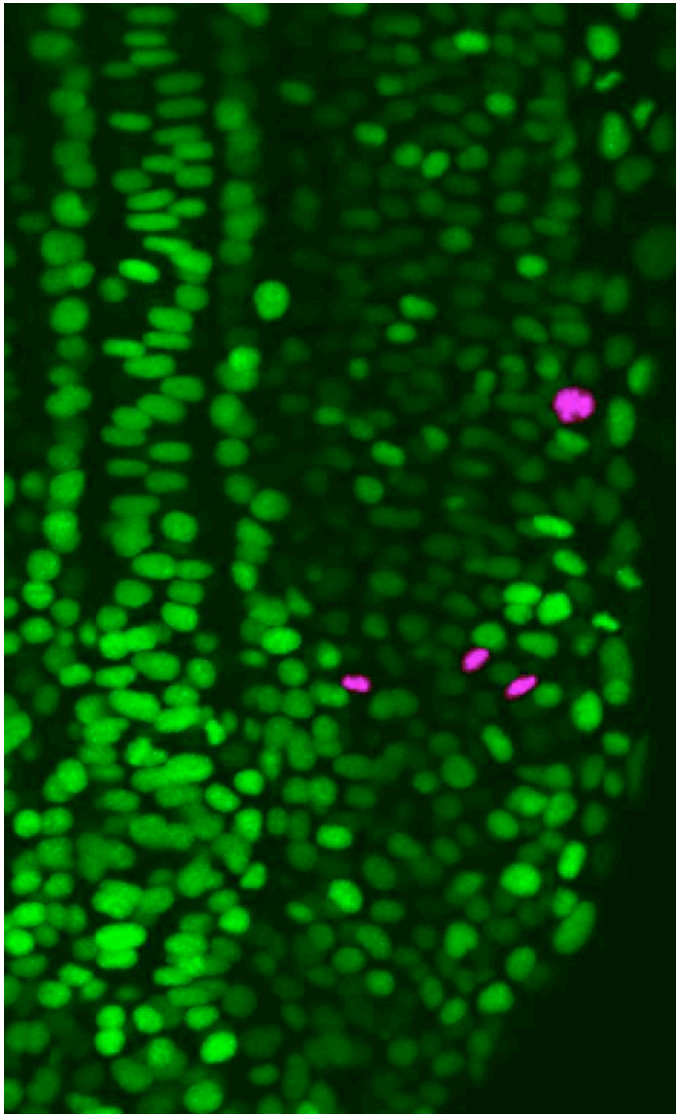
Different timing in transcription between the two alleles



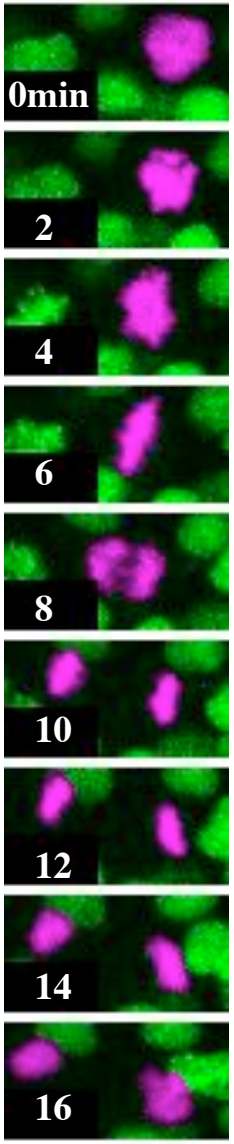


Distribution pattern of mitotic-active cells

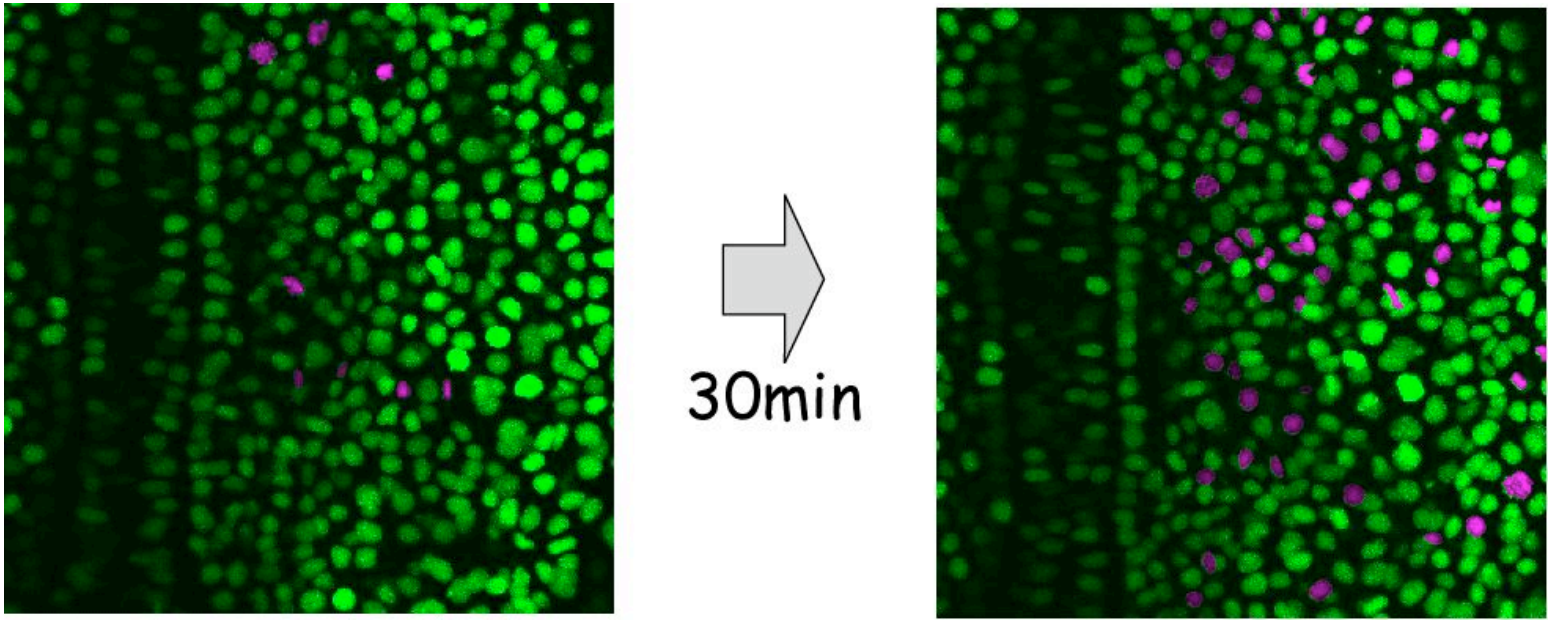
Histon 2B mRNA-injected live embryos



Distribution pattern of mitotic-active cells



Histon 2B mRNA-injected live embryos

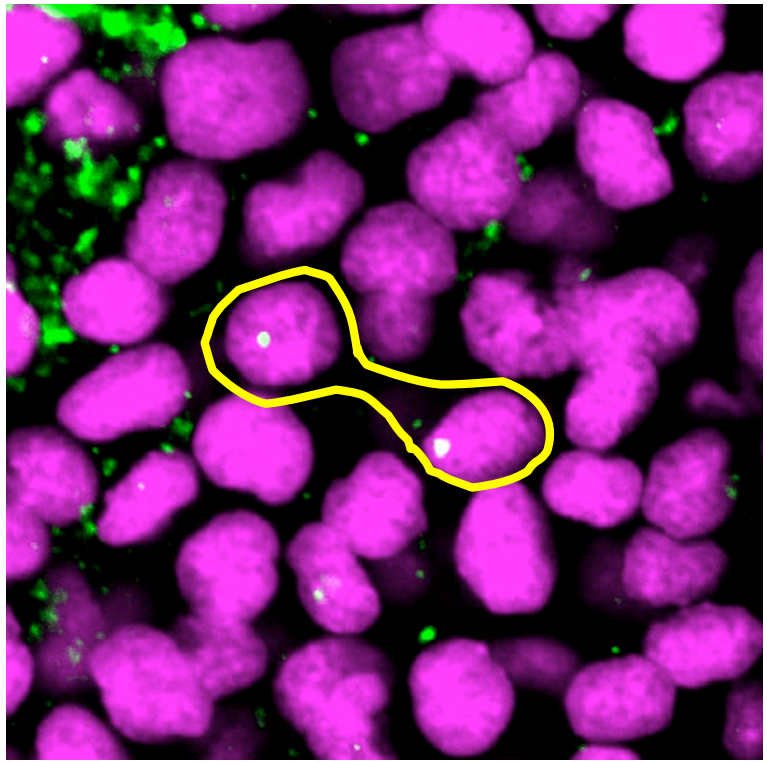


- estimated period of mitotic-phase is approx.15 min.

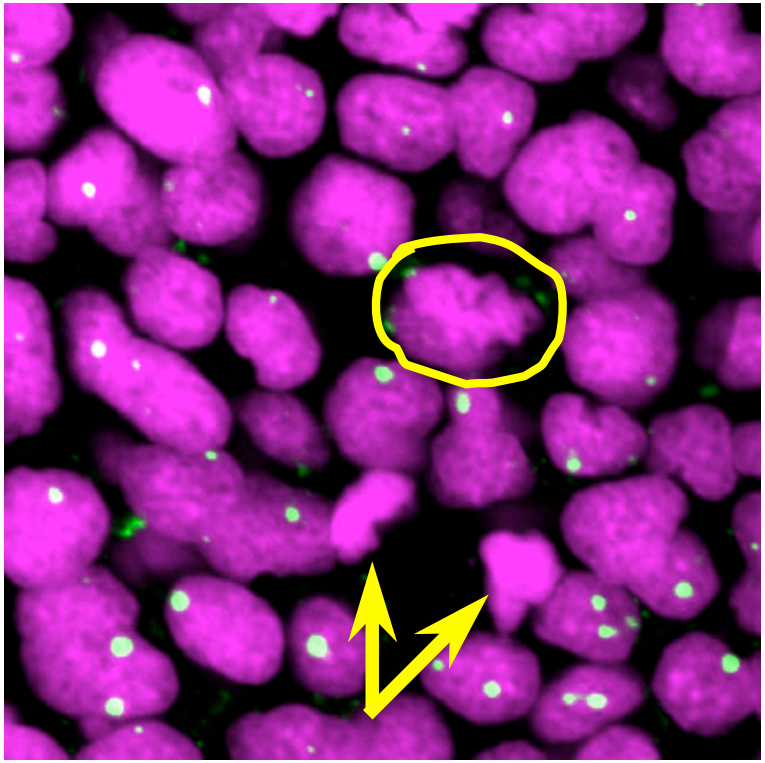
- 10-15% of cells in the posterior PSM enters mitotic phase during one round of oscillation.

cell-division affects *her1* oscillation

Much delayed or advanced cells often found as a pair

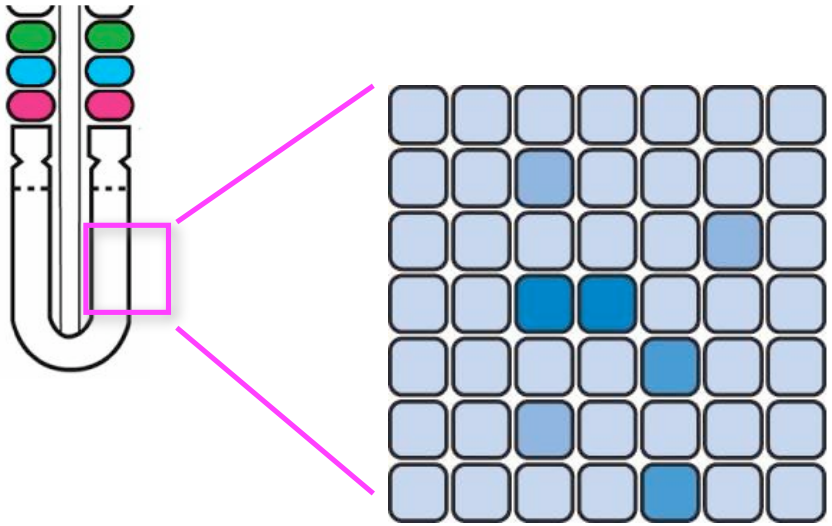


Transcription is arrested during mitosis



-> cell divisions would delay *her1* oscillation period

The segmentation clock works under noisy environment



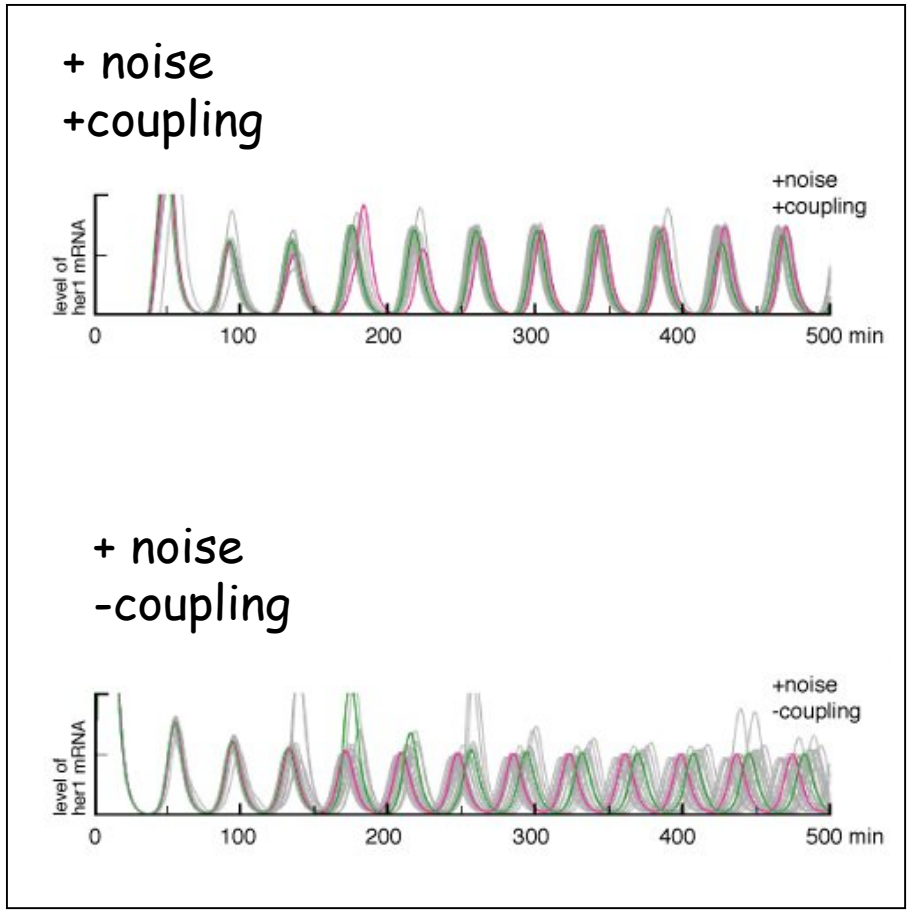
10 -20% of oscillators are out of phase due to internal noise

1.Stochastic nature of biochemical reactions
2.Active cell-proliferation

The presence of noise-reduction mechanism?

Notch-mediated cell communication plays a crucial role?

The synchrony is disrupted in the absence of celluar coupling when biological noise is introduced



By Kondo's lab.

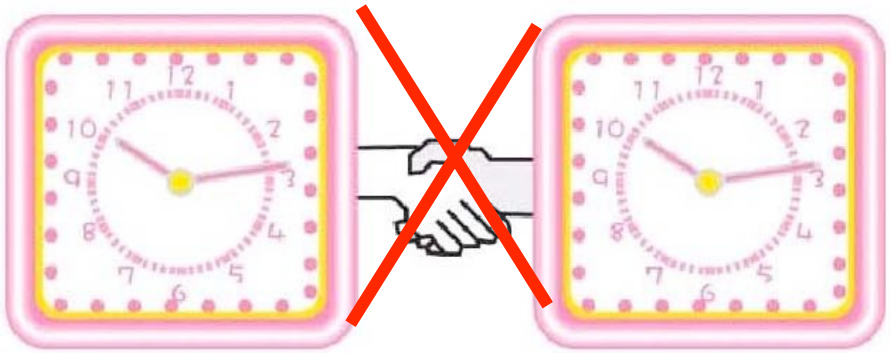
In silico experiment

The oscillation frequency and phase are differentially affected by noise as follows:

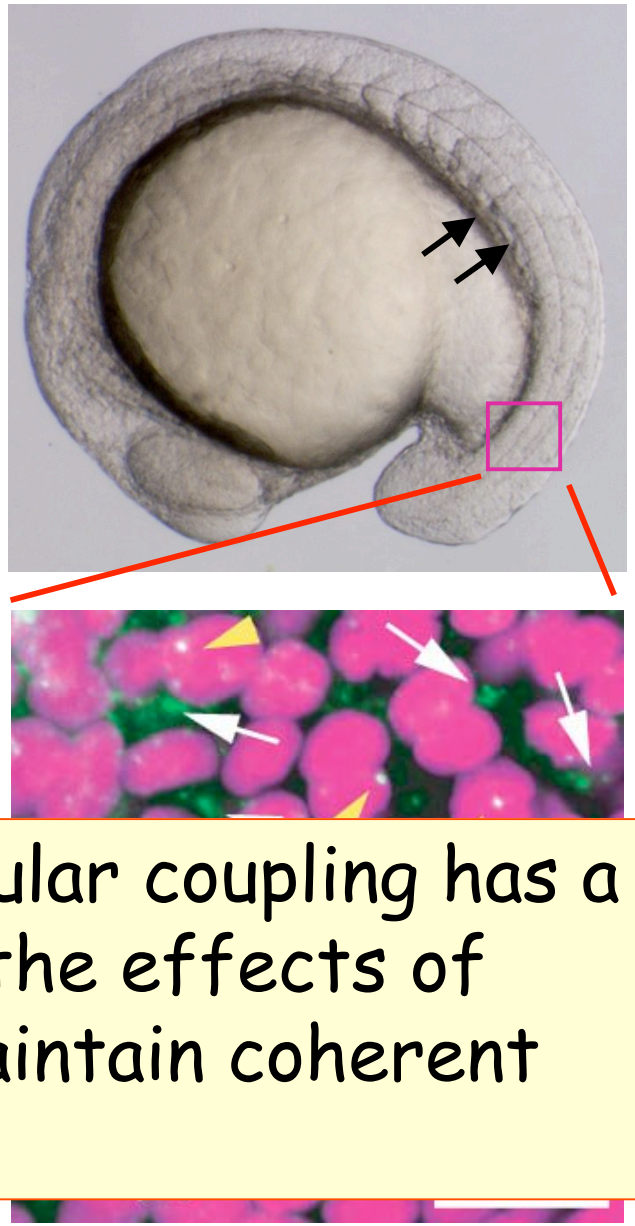
(1) cells display different free-running frequencies reflecting variations in cellular conditions as is seen in the stochastic gene expression.

(2) the oscillation phase in a certain proportion of cells is delayed by mitosis

The synchrony is disrupted when the Notch activity is reduced



Treatment with DAPT



DAPT: a chemical inhibitor to Notch signaling

Notch-mediated intercellular coupling has a crucial role in minimizing the effects of developmental noise to maintain coherent oscillation

Conclusions

The structure and system-level property of the segmentation oscillator

The segmentation clock consists of multiple cell-level oscillators connected through intercellular communication (Delta-Notch) and behaves as 'coupled oscillators'. It behaves like 'coupled oscillators'

The system is robust to the developmental noise
(stochastic gene expression, cell proliferation, genetic variation, etc.).

Noise reduction – essential for pattern formation?

Two conflicting requirements in developing embryos:
1.Rapid growth --- active cell-proliferation
2.Precise pattern formation --- strict transcriptional regulation

Collaborators

Prof. Shigeru Kondo (Nagoya Univ.)

Mr. Eiichi Yoshimoto (Nagoya Univ.)

Main contributors