Slowing down of genetic oscillations in vertebrate segmentation

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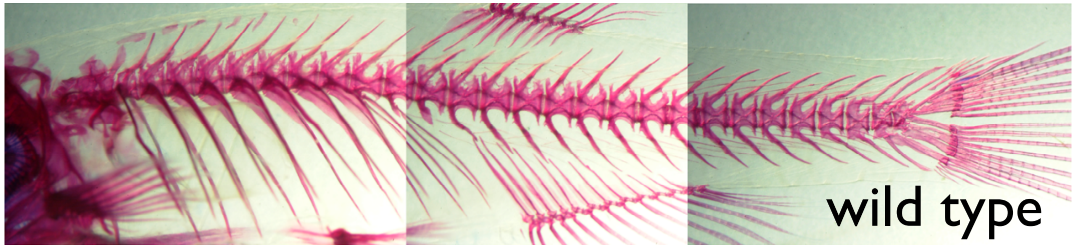
The vertebrate body axis is segmented

Introduction:
Segmentation defects: congenital malformations

Zebrafish



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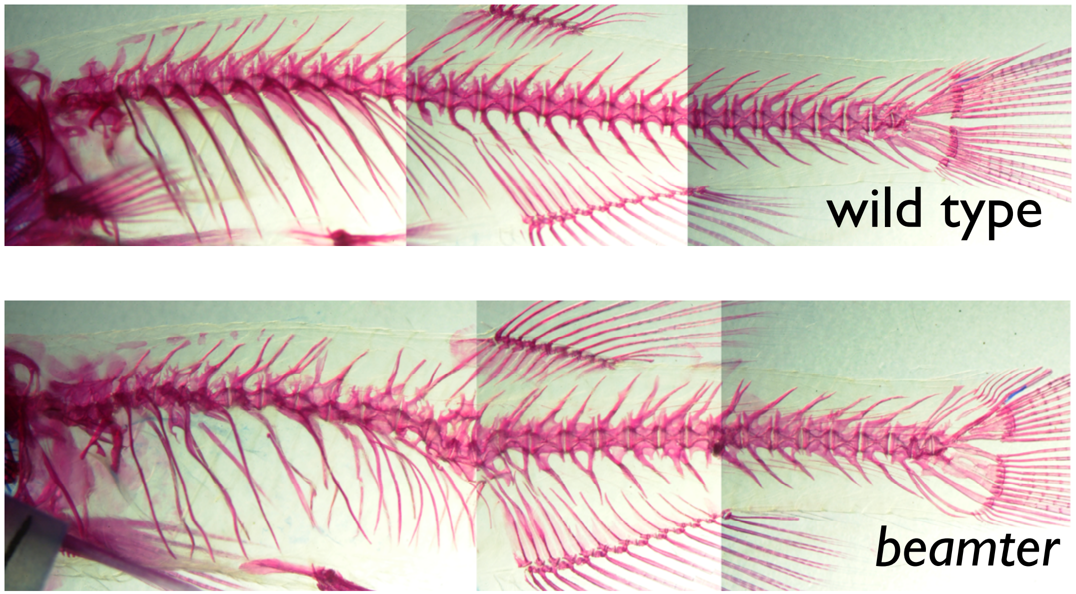

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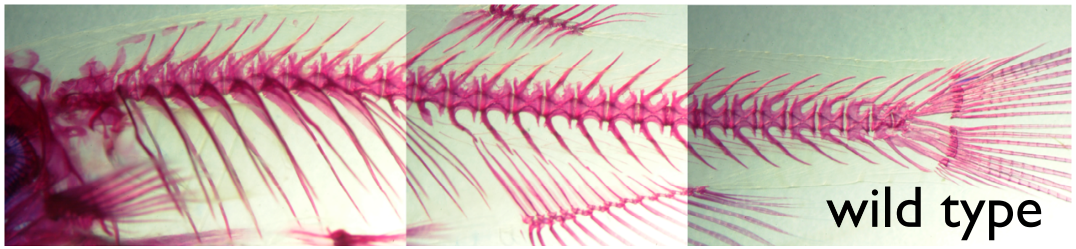
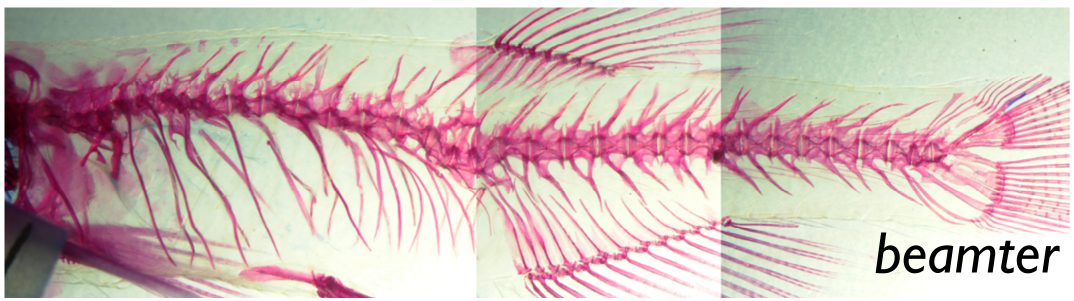
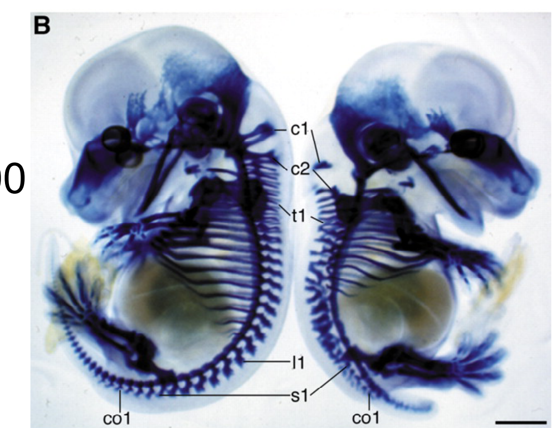




Mouse

Dunwoodie et al., 2000



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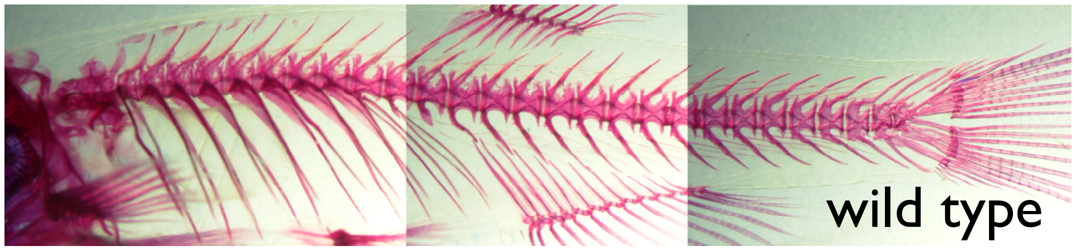
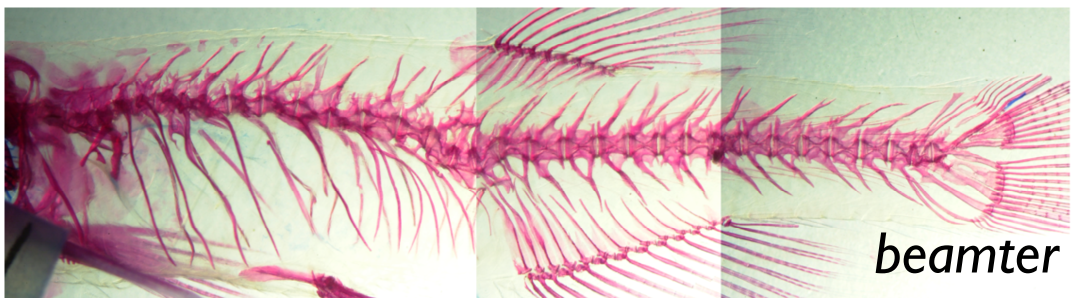
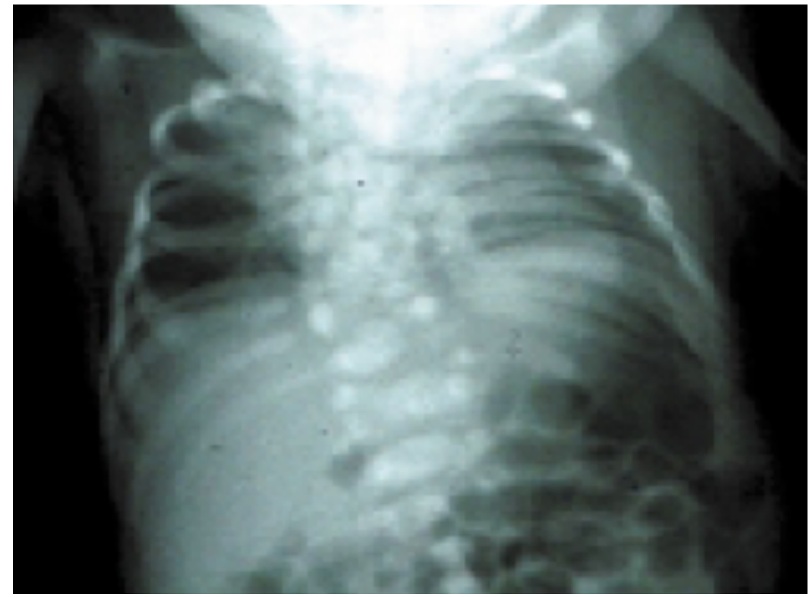




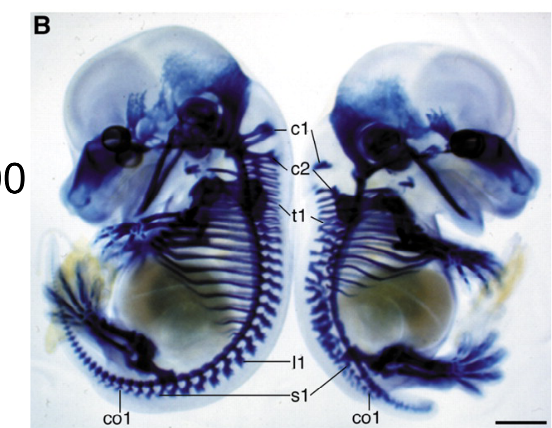
Human



Bulman et al., 2000

Mouse

Dunwoodie et al., 2000



Congenital scoliosis

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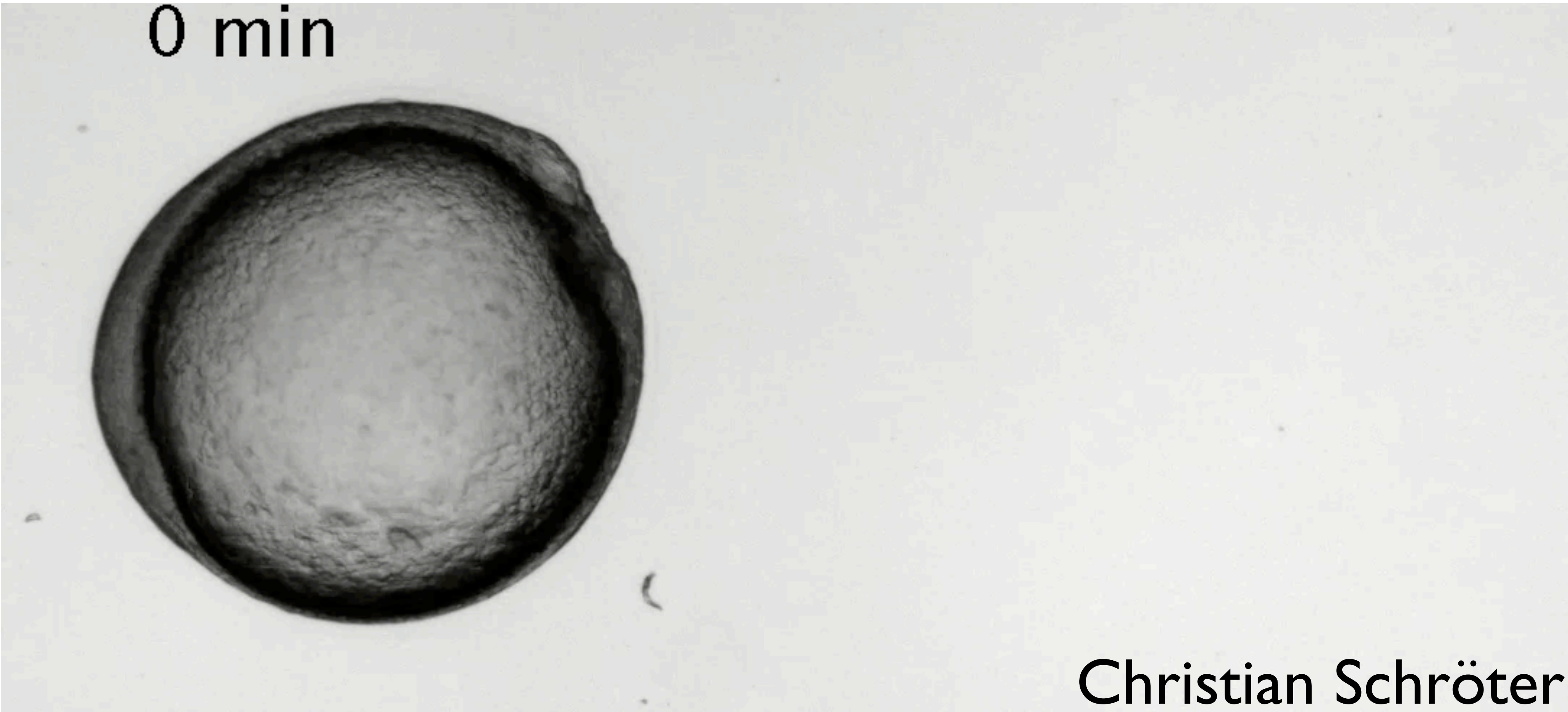
Introduction:
Somites: periodic segmentation of body axis

Somites: precursors in early embryonic development for vertebrae, ribs, skeletal muscles and dermis.



LATERAL VIEW

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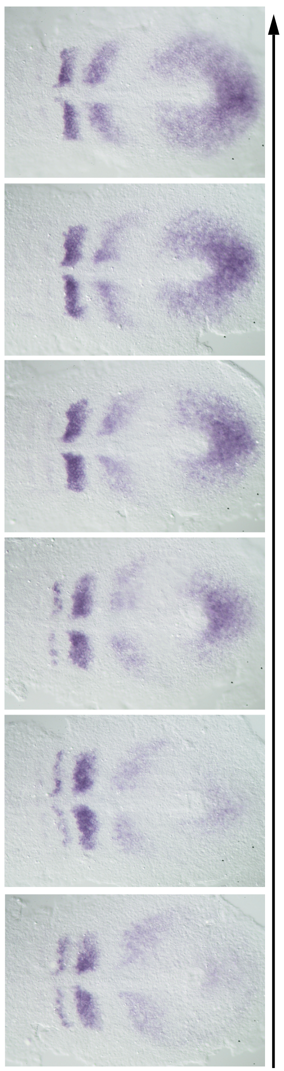
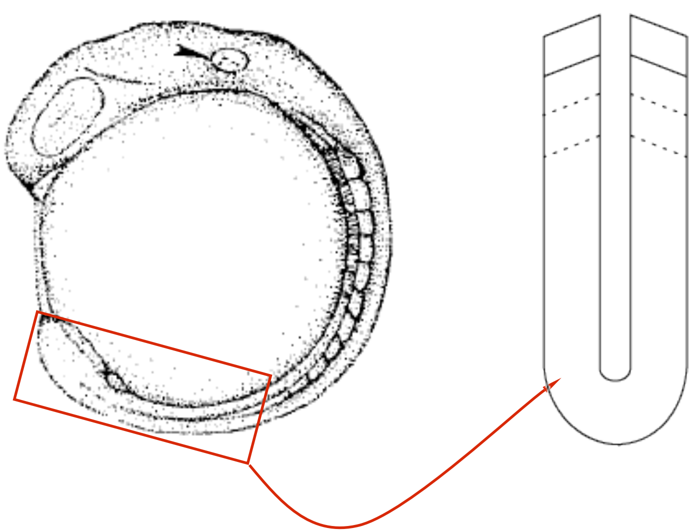
Introduction:
Genetic oscillations in the PSM



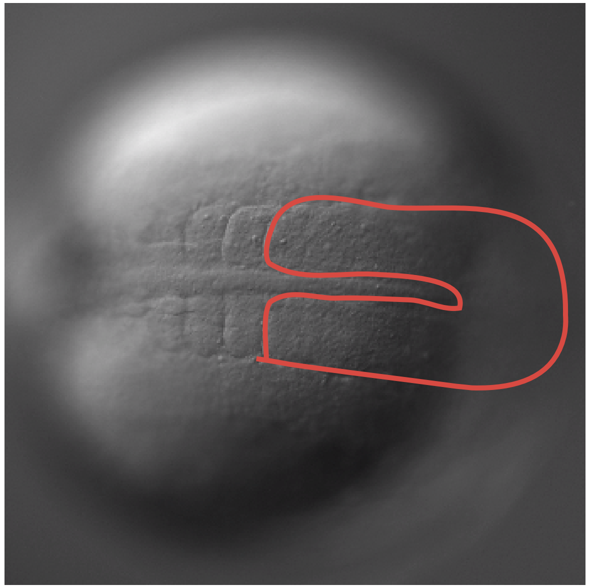
Periodically repeating wave and stripe-like expression

Periodic formation of somite boundaries

Segmentation clock



14h of development



Dorsal view: the unsegmented tissue behind the last pair of somites formed is the <u>Pre-Somitic Mesoderm</u> (PSM)

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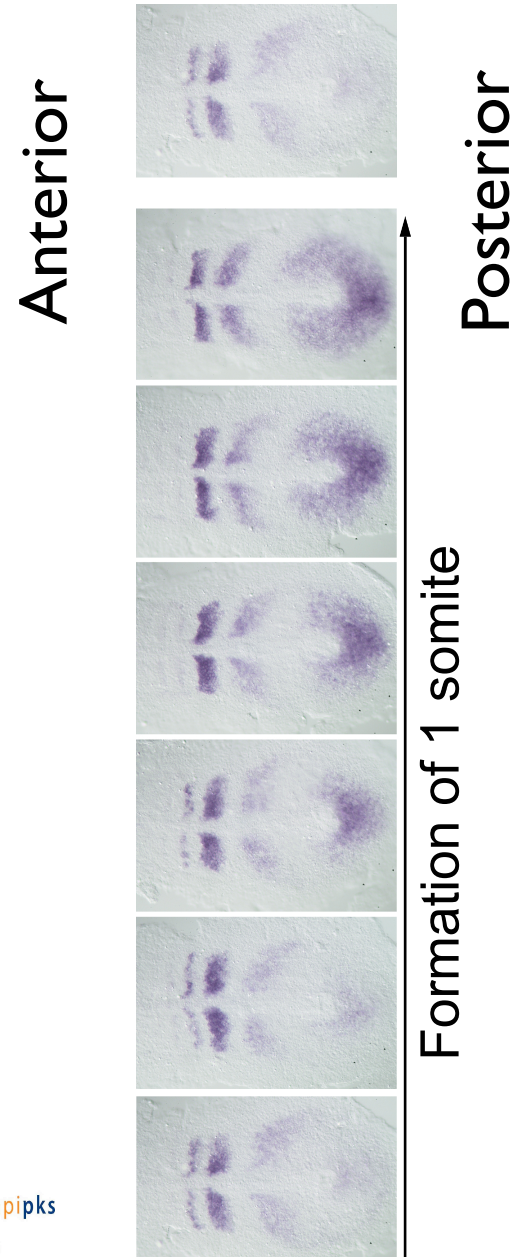
Introduction:
Genetic oscillations in the PSM



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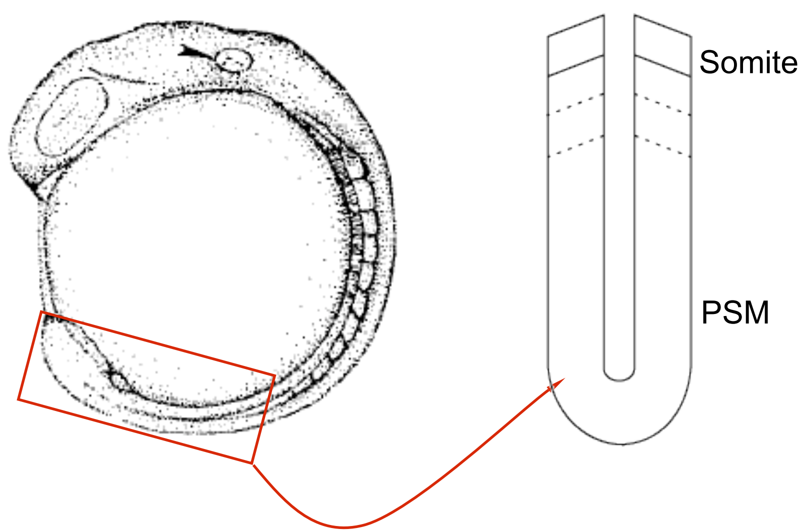

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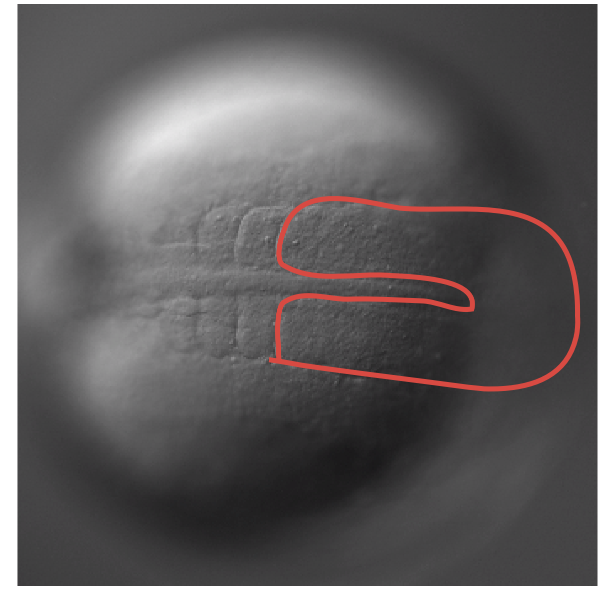

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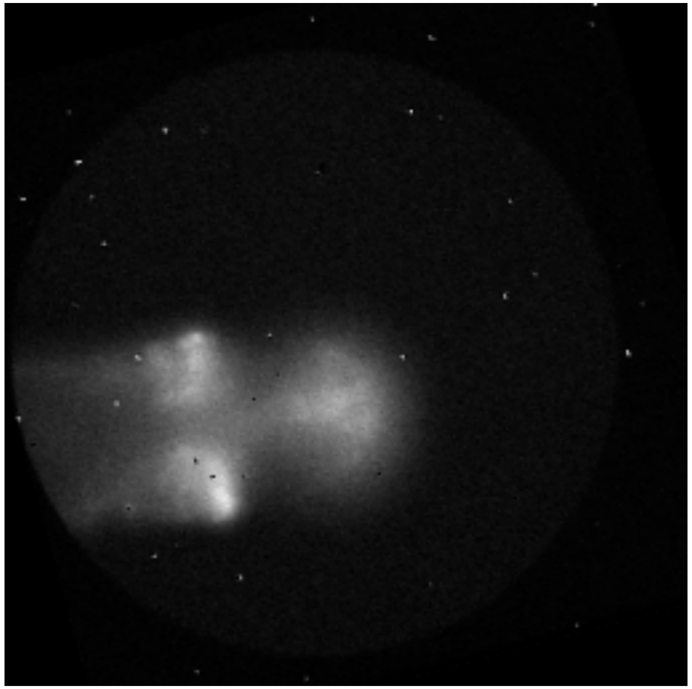
Genetic oscillations in the PSM

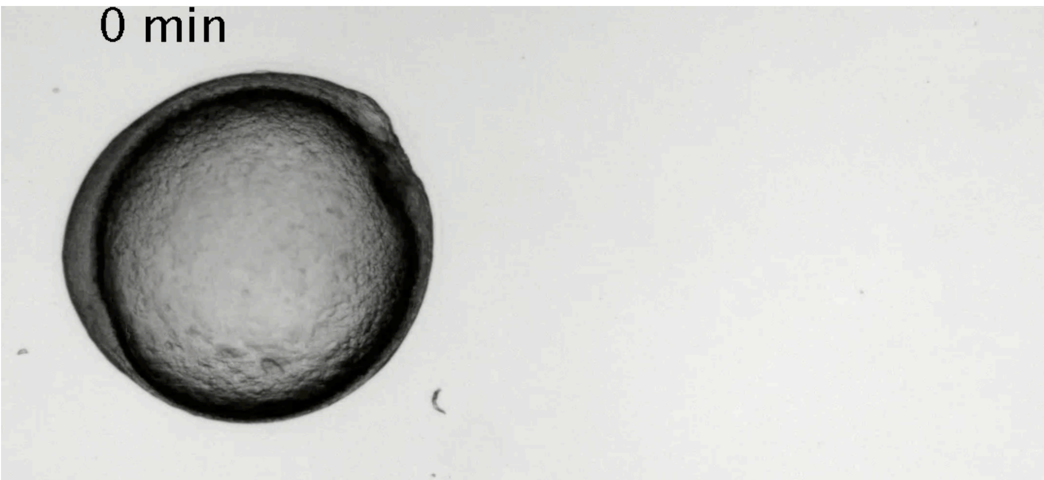


Live imaging of Hes1 expression in mouse.
Duration of the movie: 15 hours.

Masamizu et al, 2006

Introduction:
Quantitative measurements

Period of the oscillation (time to form a somite)



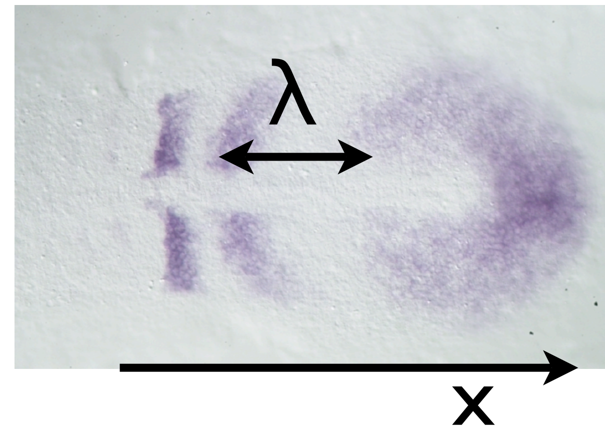
Zebrafish at 28°C → 23.5 minutes

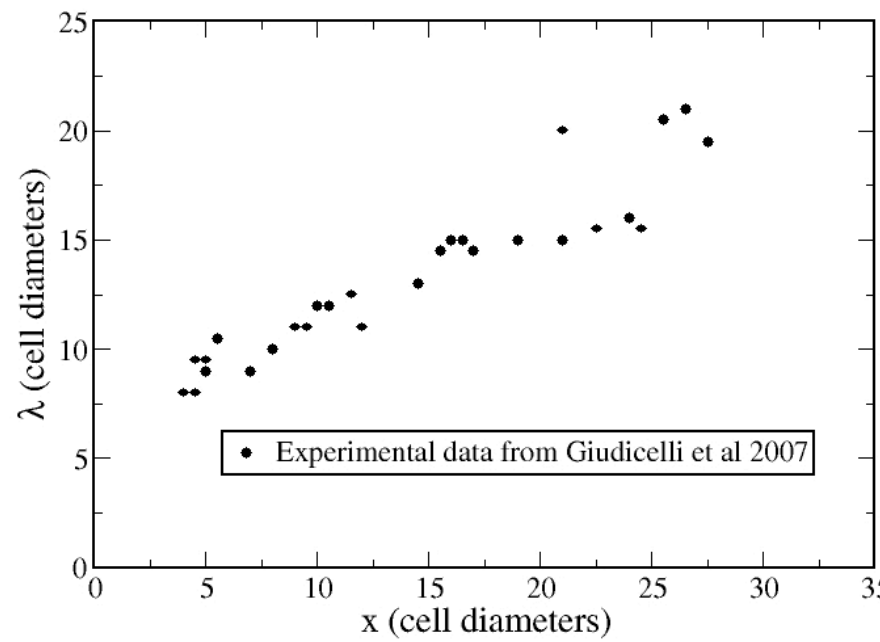
Chick ~ 90 minutes

Mouse ~ 120 minutes

In zebrafish the frequency depends linearly on temperature

Wavelength of the pattern





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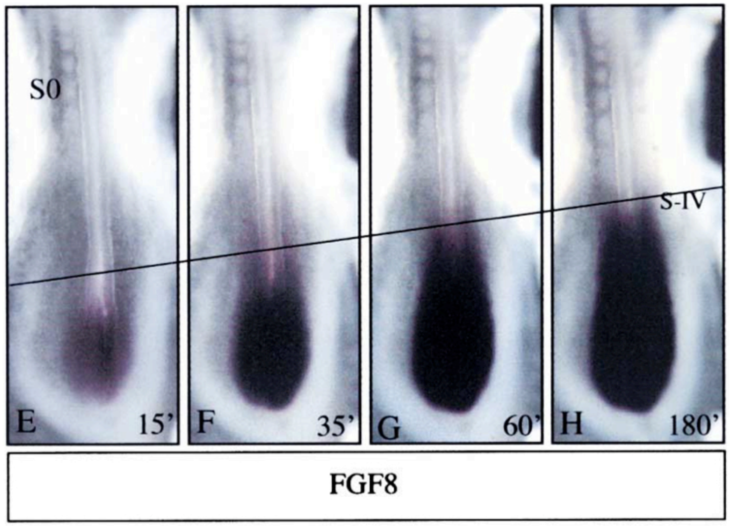
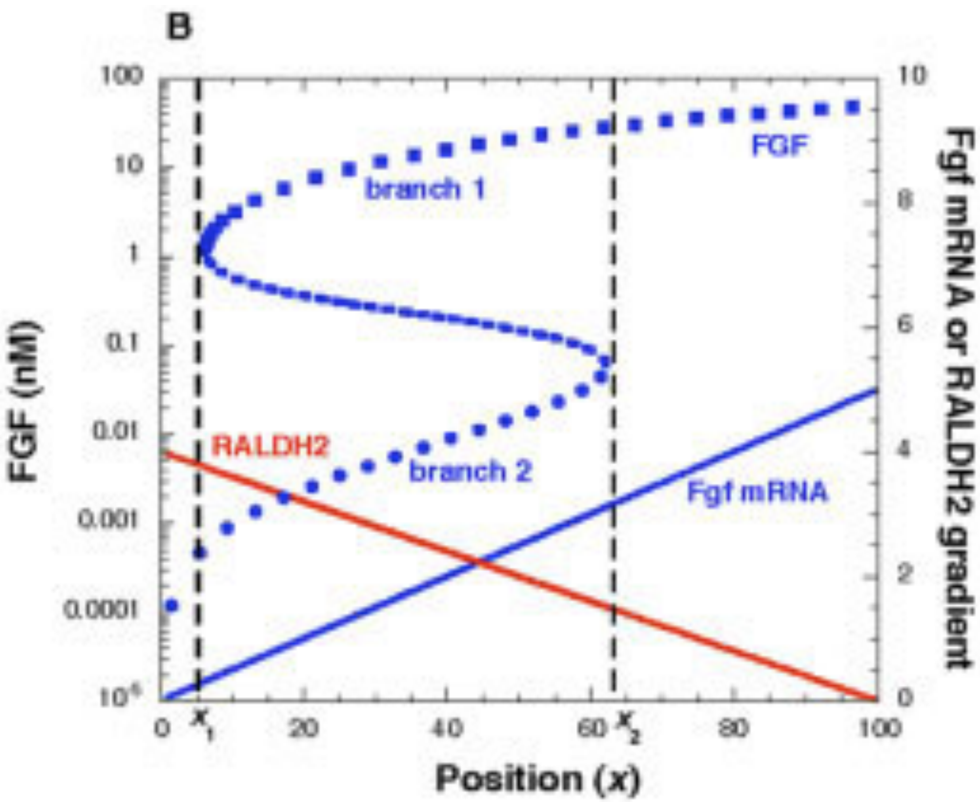
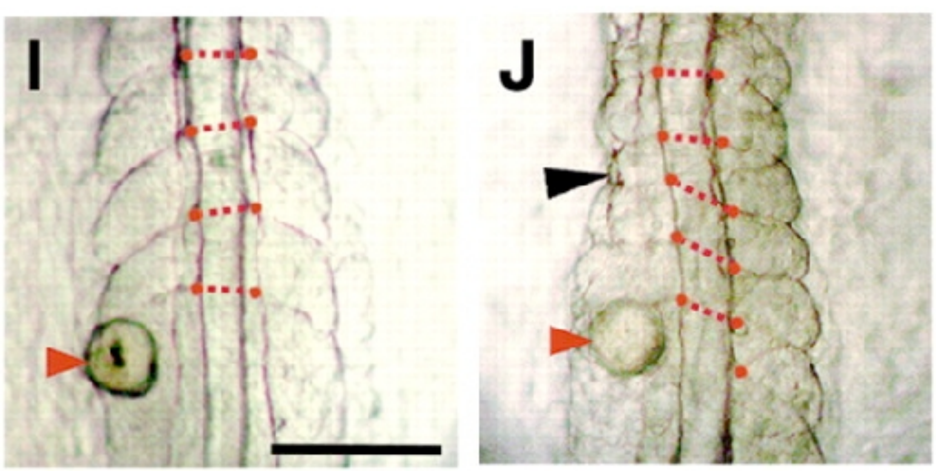
Introduction:
Signaling gradients affect somitogenesis



Dubrulle, McGrew & Pourquié 2001



Goldbeter, Gonze & Pourquié 2007



Sawada et al 2001

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Delayed Coupling theory:
Introduction of the theory

- Coupled phase oscillators
- Time delay in coupling
- Frequency profile
- Moving boundary conditions

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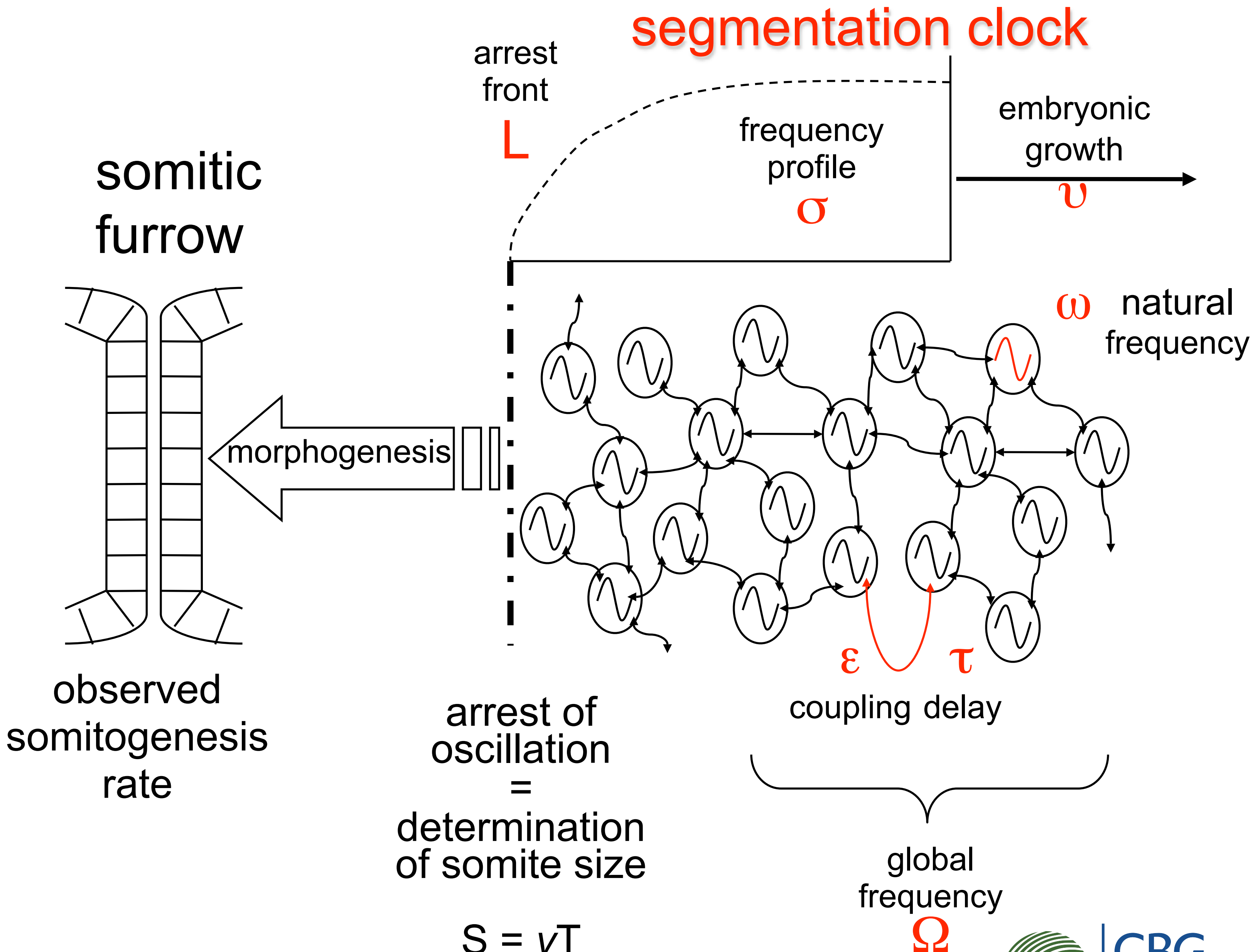

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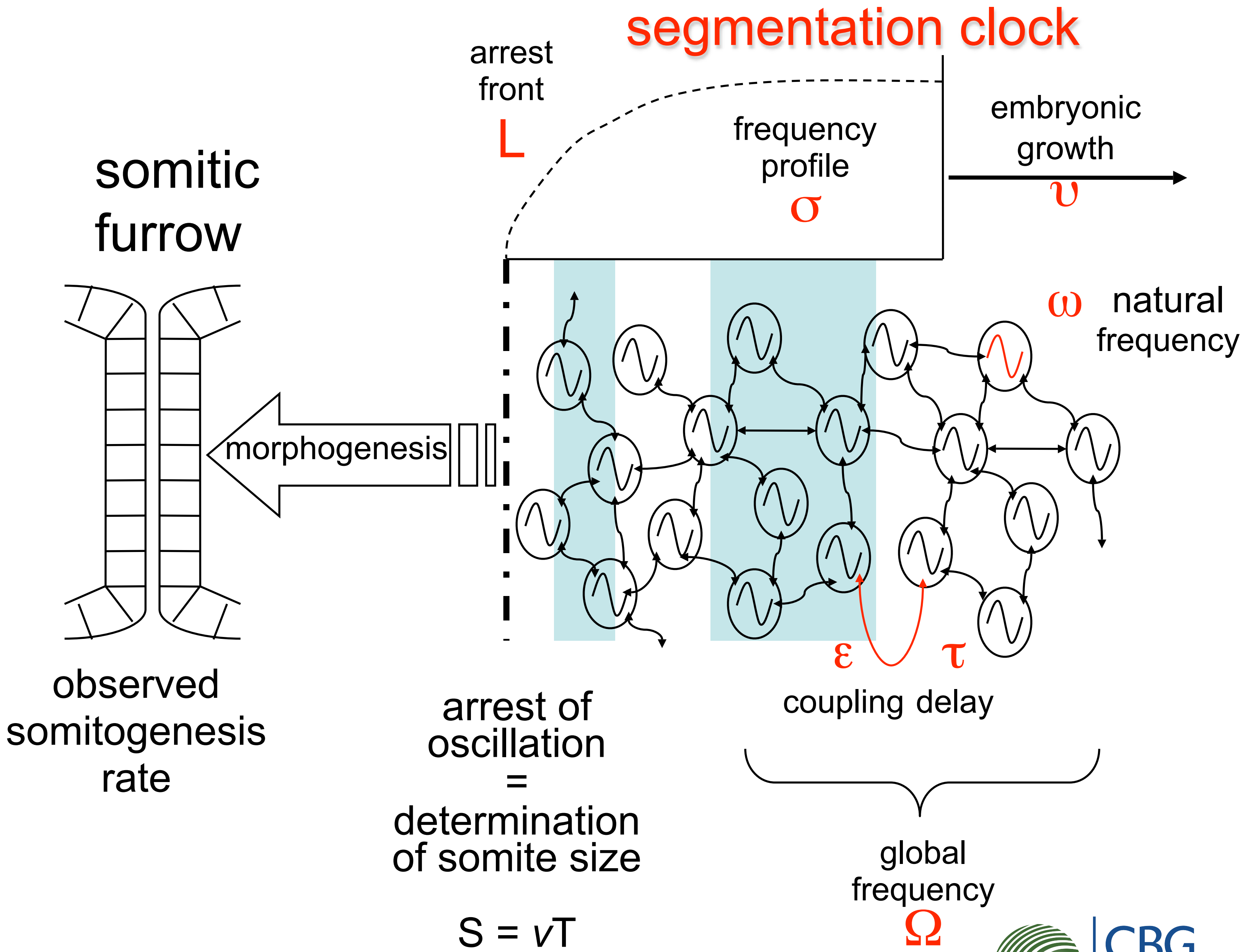

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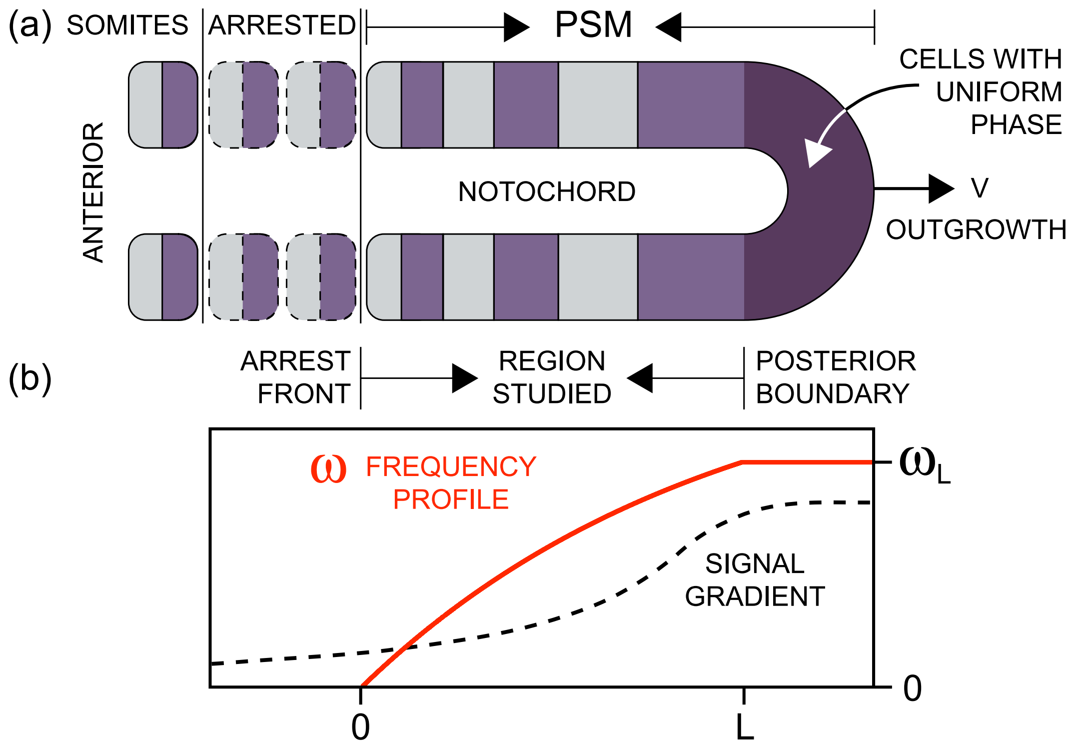
Delayed Coupling theory: Definition of the system



θ_i = phase of oscillator i
ω_i = intrinsic frequency of oscillator i
ϵ = coupling strength
τ = time delay in the coupling
ζ = noise terms
σ = width of the frequency profile

$$\boxed{\frac{\partial \theta_i(t)}{\partial t} = \omega_i(t) + \frac{\varepsilon}{n} \sum_{\langle k \rangle} \sin\left[\theta_k(t-\tau) - \theta_i(t)\right] + \zeta_i(t)}$$

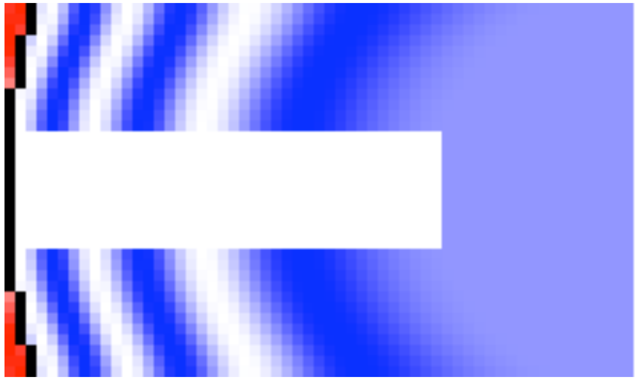
$$\omega_i(t) = \begin{cases} \omega_\infty(1 - e^{-\frac{L}{\sigma}}) \equiv \omega_L & \text{if } i \geq (L + vt) \\ \omega_\infty(1 - e^{-\frac{i - vt}{\sigma}}) & \text{if } vt < i < (L + vt) \\ 0 & \text{if } i \leq vt \end{cases}$$

Nearest neighbor Kuramoto model with delayed coupling, frequency profile and moving boundary conditions.

Delayed Coupling theory:
How does it look like?

Simulation in a 2-dimensional geometry

Arrested segments



Oscillations in the PSM

Red: pattern left behind the arrest front (out of the scope of our theory).

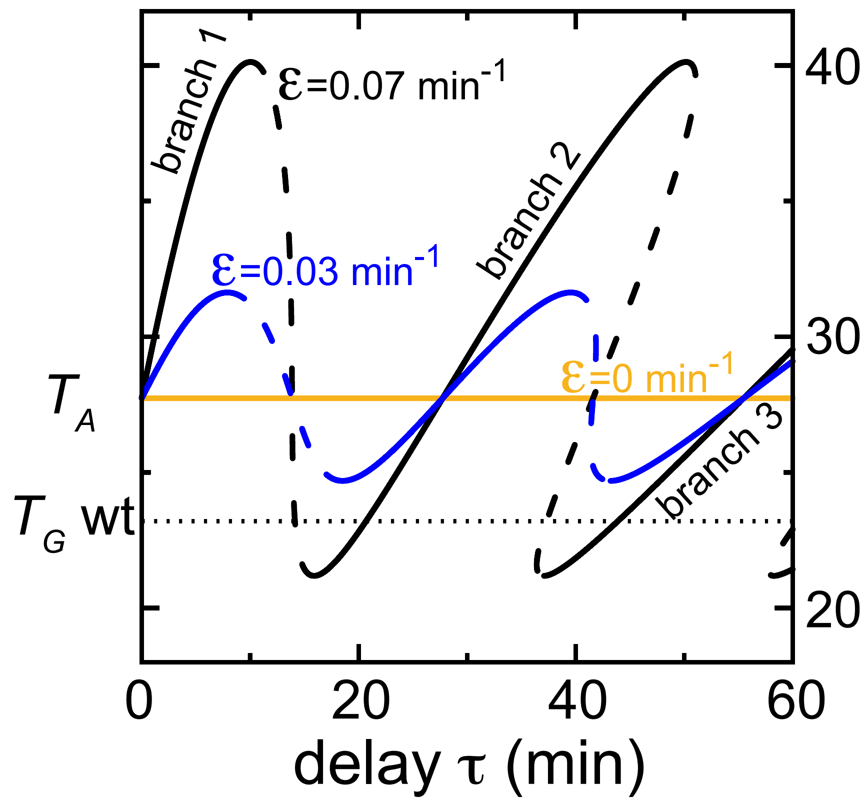
Blue: it is the dynamic gene expression in this tissue what our equations describe.

Delayed Coupling theory:
Solution: global frequency Ω (Ω=2π/T)

Steady state ansatz:

$$\varphi_j(t) = \Omega t + \phi_j$$

$$\left(\Omega = \frac{2\pi}{T_G}\right)$$

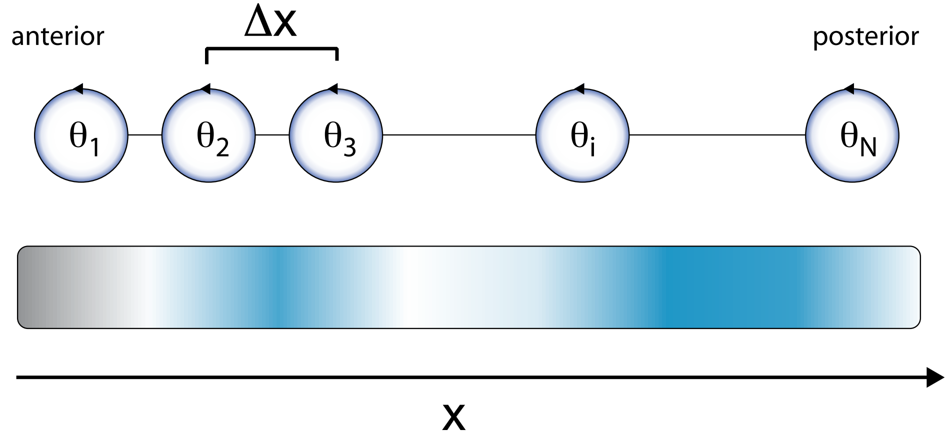


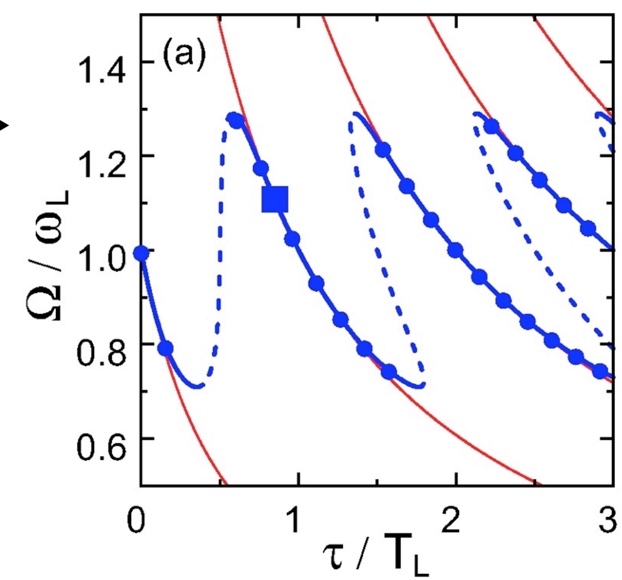
$$\boxed{\Omega = \omega_L - \varepsilon \sin(\Omega\tau)}$$

Stability: $\cos(\Omega\tau) > 0$

Delayed Coupling theory: Continuum limit: pattern wavelength

To make the equations more tractable, a **continuum limit** (in fact, an infinite number of them depending on the value of the delay) can be defined.





$$\boxed{\dot{\varphi}(x,t) = \bar{\omega}(x) + v\nabla\varphi(x,t) + \frac{\bar{\epsilon}(x)}{2}\nabla^2\varphi(x,t)}$$

$$\bar{\omega}(x) = \omega(x)\frac{\left[1+\frac{2\pi m}{\omega_l}\epsilon(x)\right]}{1+\epsilon(x)\tau} \qquad \bar{\epsilon}(x) = \epsilon(x)\frac{\left[1+\frac{2\pi m}{\omega_l}\epsilon(x)\right]}{1+\epsilon(x)\tau}$$

$m \equiv \left[\frac{\tau}{T}\right]$, [ ] stands for integer part

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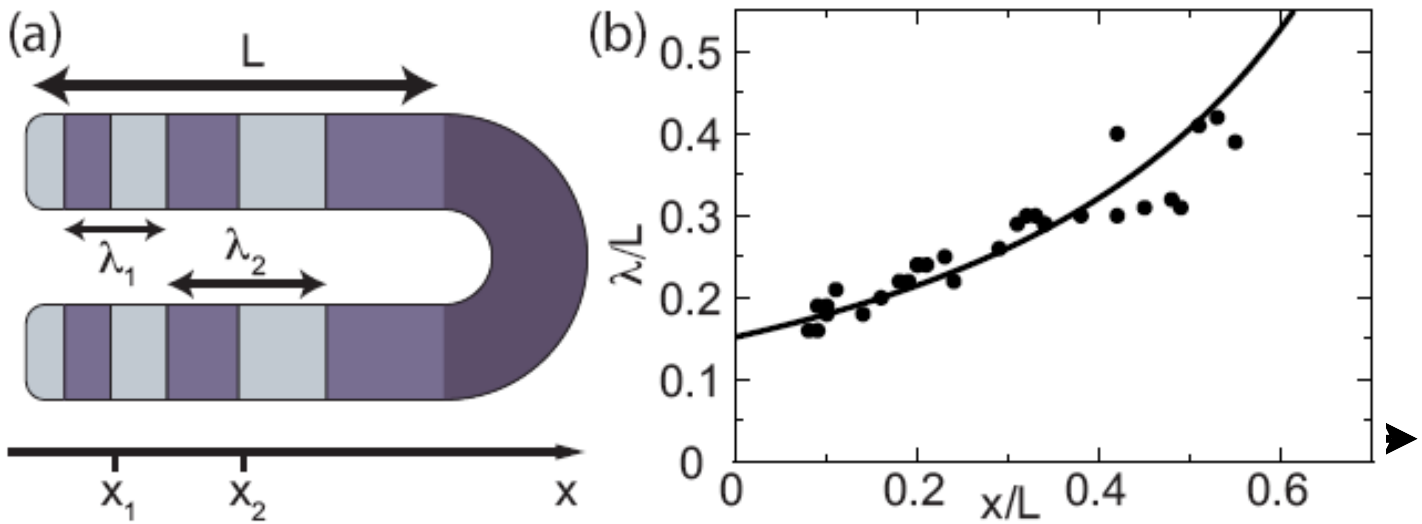
Delayed Coupling theory: Continuum limit: pattern wavelength



$$x \approx \sigma \log \left[\frac{\sinh(\lambda/2\sigma)}{\pi\nu^{-1}(1+\eta)^{-1} + (\lambda/\sigma)e^{-L/\sigma}} \right]$$

Combining information from the literature, observations at the Oates lab and the fit to the experimental wavelengths, we are able to determine a **complete set of parameters** for zebrafish at 28°C and at the 10 somite stage.

T	Period of somite formation[a]	23.5 min
Ω	Collective frequency, $2\pi/T$	0.267 min^{-1}
S	Somite size (our own experimental estimation)	6 cd
v	Velocity of the arrest front, $v=S/T$	0.255 cd min^{-1}
ε	Coupling strength[b]	0.07 cd^2 min^{-1}
L	PSM length	39 cd
σ	Decay length of the frequency profile	36 cd
τ	Time delay	Not determined
ω_L	Intrinsic frequency in the posterior PSM	

[a] Schröter *et al.* (2008).
[b] Riedel-Kruse *et al.* (2007).

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L.G. Morelli, S. Ares, L. Herrgen, C. Schröter, F. Jülicher and A.C. Oates, *The HFSP Journal, (2009)*

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Delayed Coupling theory:
zebrafish vs. mouse mode

Arrested segments

Oscillations in the PSM

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Delayed Coupling theory: zebrafish vs. mouse mode

Arrested segments

Oscillations in the PSM

$$N \approx s^{-1}\left(p - \frac{1}{(e^{-p} - 1)}\right)$$

N: number of stripes

s=S/L=vT/L normalized somite length

p: normalized characteristic width of the frequency profile

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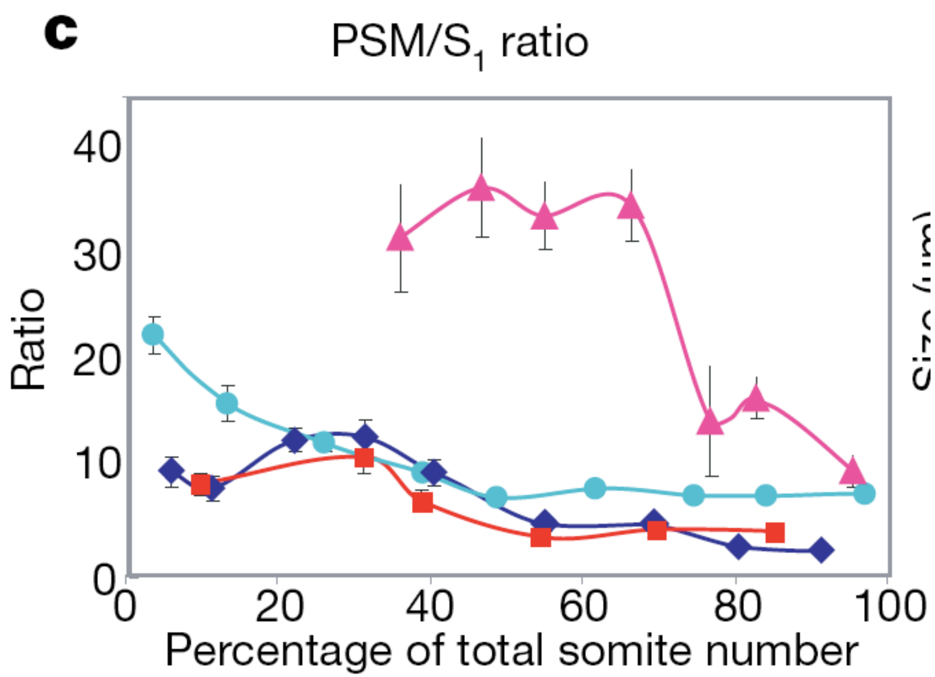
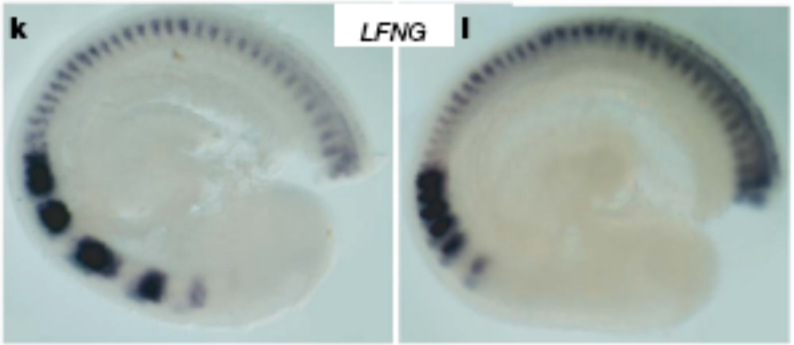
Slowing down of genetic oscillations





30% somites formed

Mouse and chicken: N=1 stripe

Zebrafish: N=2.7 stripes

Snake: N up to 9 stripes

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Gomez et al 2008

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Conclusions

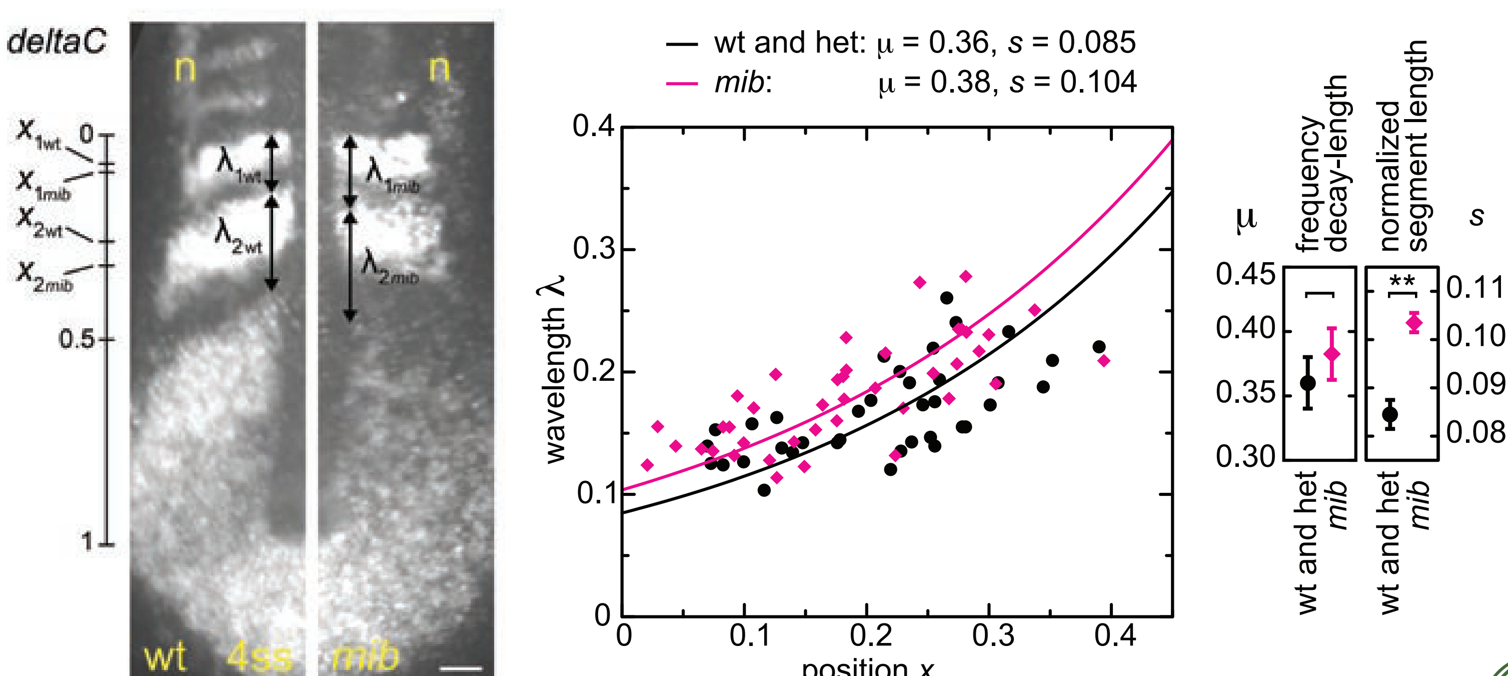
We have developed the Delayed Coupling Theory (DCT) as a tool to analyze quantitative spatiotemporal data from genetic oscillations in vertebrate segmentation.

Using the DCT, we have shown for the first time that the slowing down of the genetic oscillations along the PSM is qualitatively different in different vertebrate species.


Thank you!

Delayed Coupling theory:
Applications and testing in somitogenesis

Applying the theory to analyze data from **mutants** or embryos subject to certain **treatments**, comparison with wild type allows to **determine** which **parameters** have been **affected** by the mutation or the treatment: confirm or reject **roles for genes**.



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Delayed Coupling theory:
How does it look like?

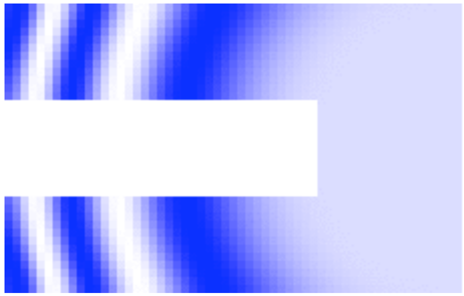
$$\tau = 0 \text{ min}$$

Arrested segments

Oscillations in the PSM

Delayed Coupling theory:
How does it look like?

$\tau = 0 \text{ min}$



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Oscillations in the PSM

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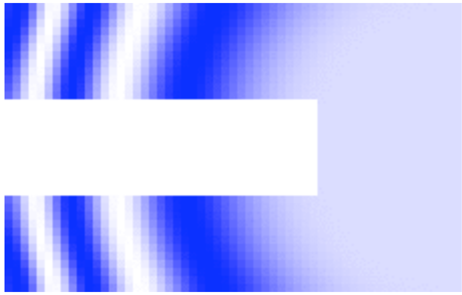


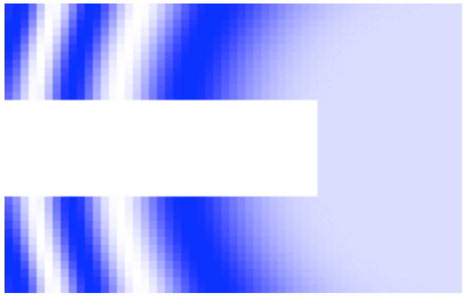
$T = 28 \text{ min}$

Arrested segments

Oscillations in the PSM

Delayed Coupling theory: How does it look like?

$\tau = 0 \text{ min}$



$T = 28 \text{ min}$

$\tau = 7 \text{ min}$

Arrested segments

Oscillations in the PSM

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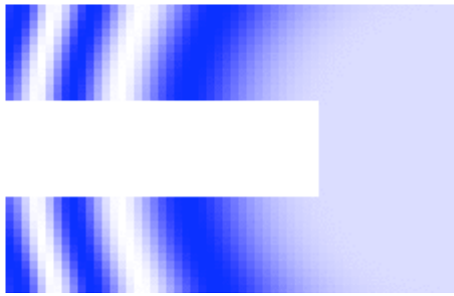
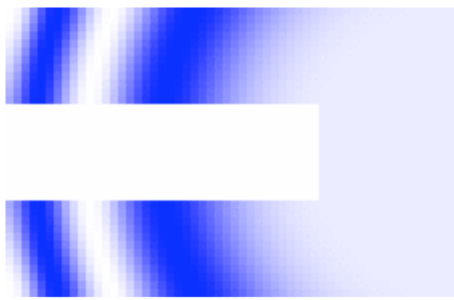
$\tau = 0 \text{ min}$



$T = 28 \text{ min}$

$\tau = 7 \text{ min}$



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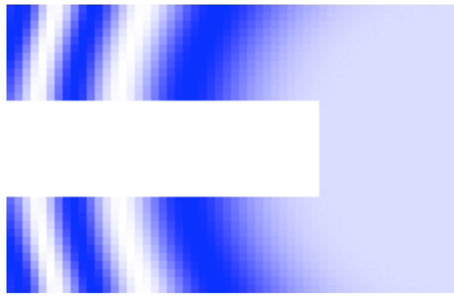

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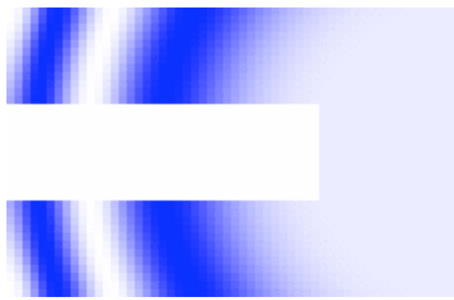

$T = 39 \text{ min}$

Arrested segments

Oscillations in the PSM

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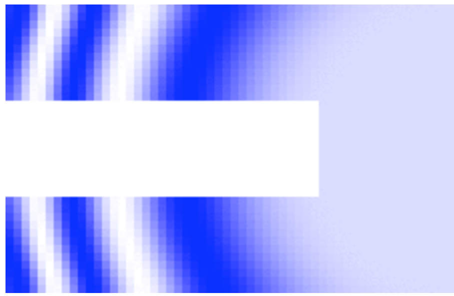
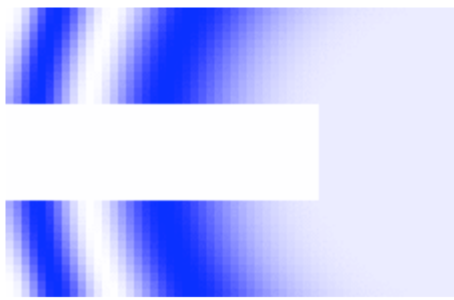
Delayed Coupling theory: How does it look like?

$\tau = 0$ min



$T = 28$ min

$\tau = 7$ min



$T = 39$ min

$\tau = 21$ min

Arrested segments

Oscillations in the PSM

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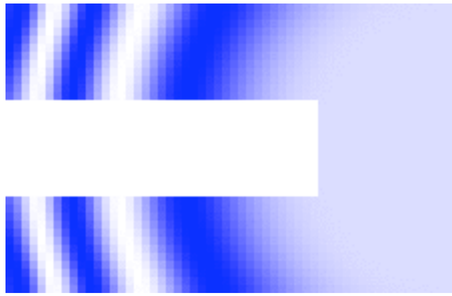
Delayed Coupling theory: How does it look like?

$\tau = 0$ min



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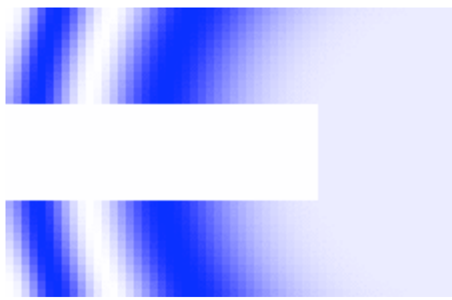

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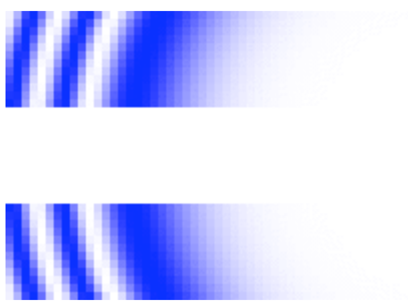

Arrested segments

Oscillations in the PSM

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Delayed Coupling theory:
How does it look like?

$\tau = 0$ min $\quad T = 28$ min

$\tau = 7$ min $\quad T = 39$ min

$\tau = 21$ min $\quad T = 24$ min

Arrested segments

Oscillations in the PSM

Delayed Coupling theory:
How does it look like?

$\tau = 0$ min $\qquad T = 28$ min

$\tau = 7$ min $\qquad T = 39$ min

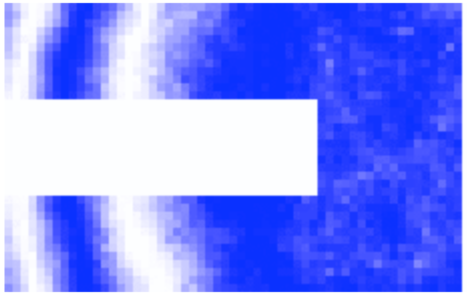
$\tau = 21$ min $\qquad T = 24$ min

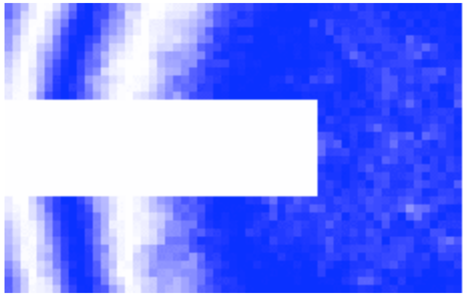
Arrested segments

???

Oscillations in the PSM

Delayed Coupling theory: Robustness and coupling disruption





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