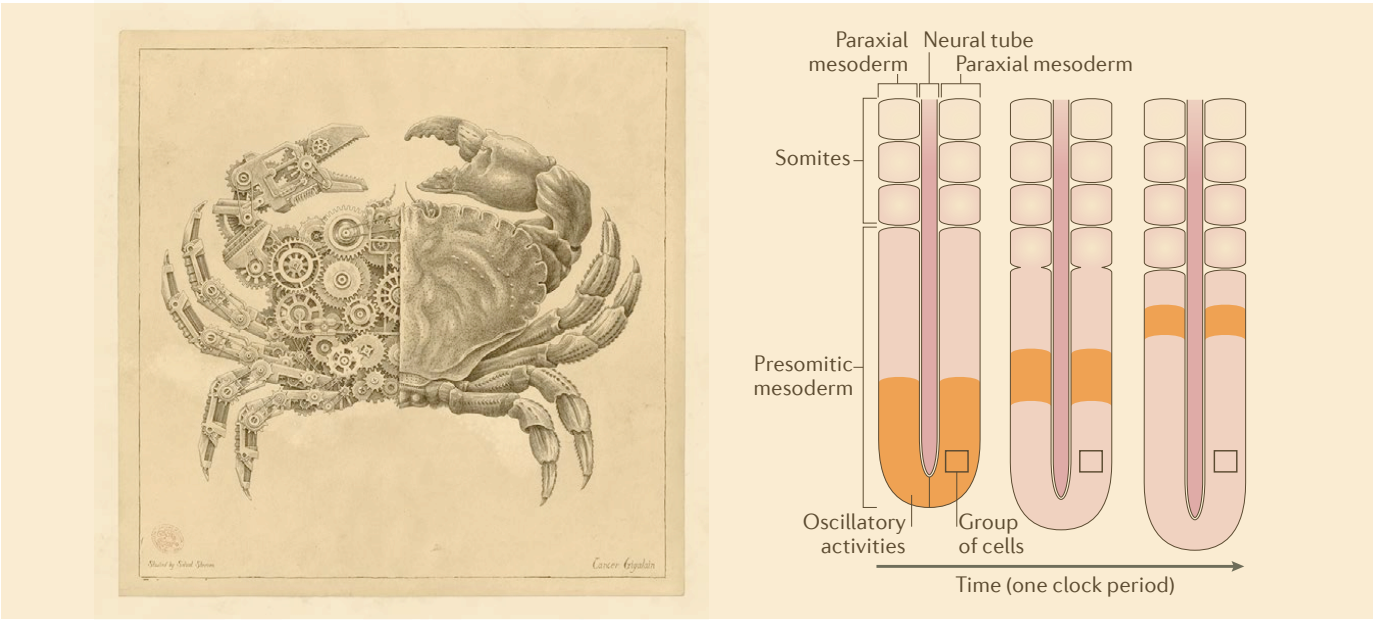
What is biological information?



Course 4: Encoding, Decoding and Representations of *Time*

Thomas Lecuit

chaire: Dynamiques du vivant

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1530

Summary of previous course

1. **Length scales are defined in chemical and mechanical systems in a variety of ways** (deterministic and self-organised models).

2. ***Shannon information theory* provides a powerful framework to:**
   - *Quantify* biological information encoded in a chemical system
   - Assess information transmission in a noisy channel, such as in any input/output system in biology.

3. ***Mutual information* provides a measurement of positional information** through the statistical structure of correlations between concentrations of molecules and spatial coordinates.

4. **In self-organised systems, exploration of other means to quantify total information: eg. positional and correlational information.**

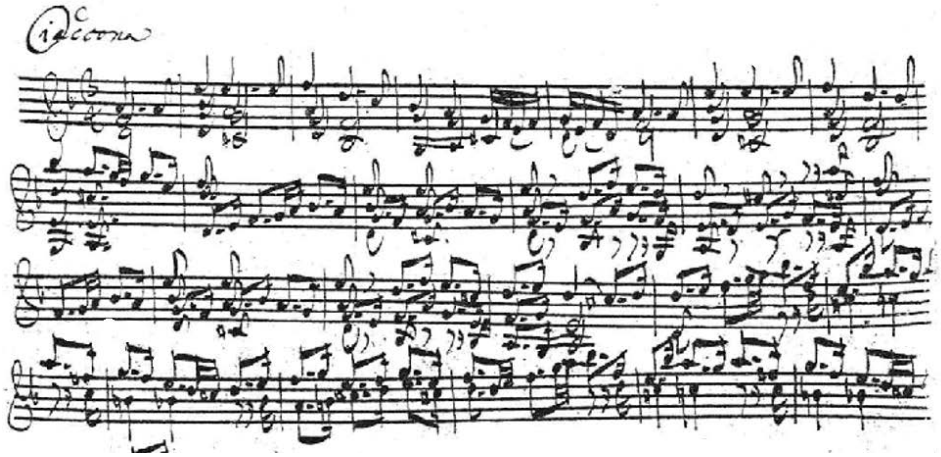
Encoding and Decoding Time

- From letters (chemical species) to « words »: sequences and combinations

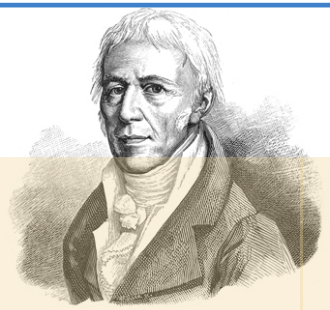
  Balance between diversity and specificity

- From « words » to patterns of words (in space and time): « sentences ».

  - *Static* chemical representation (combinatorial): « music chord ».
  - *Dynamic* chemical representation: « melody »



Lamarck - *Time* is an *inherent property* of the living



JB de Lamarck (1744-1829)

Transformism

PHILOSOPHIE

ZOOLOGIQUE.

SECONDE PARTIE.

Considérations sur les Causes physiques de la Vie, les conditions qu'elle exige pour exister, la force excitatrice de ses mouvemens, les facultés qu'elle donne aux corps qui la possèdent, et les résultats de son existence dans ces corps.

1809

Dynamics

Caractères des Corps inorganiques mis en parallèle avec ceux des Corps vivans.

Tout corps, au contraire, qui possède la vie, se trouve continuellement, ou temporairement, animé par une *force particulière* qui excite sans cesse des mouvemens dans ses parties intérieures, qui produit, sans interruption, des changemens d'état dans ces parties, mais qui y donne lieu à des réparations, des renouvellemens, des développemens, et à quantité de phénomènes qui sont exclusivement propres aux corps vivans; en sorte que, chez lui, les mouvemens excités dans ses parties intérieures altèrent et détruisent, mais réparent et renouvellent, ce qui étend la durée de l'existence de l'individu, tant que l'équilibre entre ces deux effets opposés, et qui ont chacun leur cause, n'est pas trop fortement détruit;

HISTOIRE NATURELLE

DES

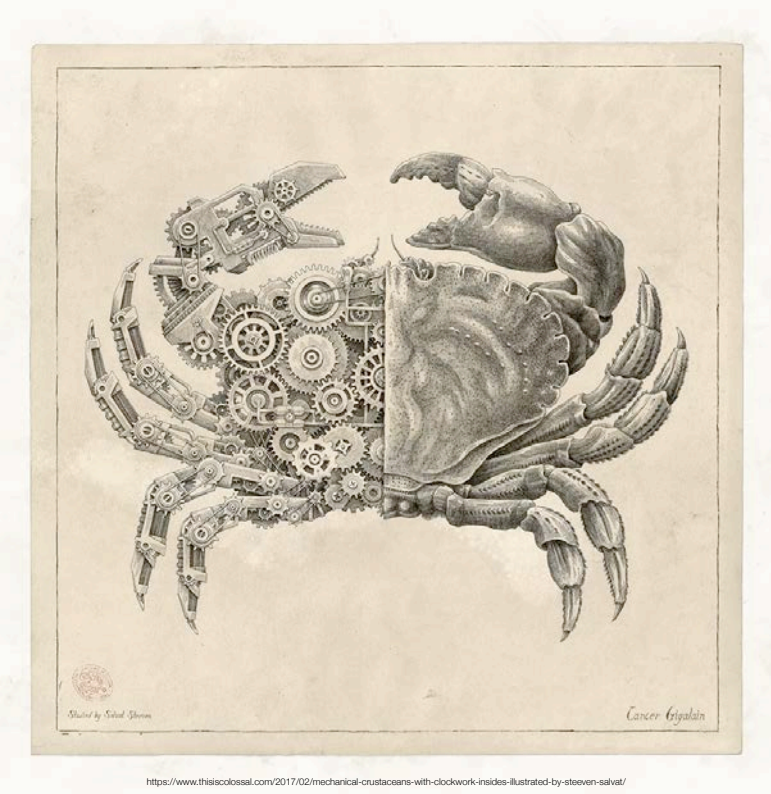
ANIMAUX SANS VERTÈBRES.

7.° La nature, dans toutes ses opérations, ne pouvant procéder que graduellement, n'a pu produire tous les animaux à-la-fois : elle n'a d'abord formé que les plus simples; et passant de ceux-ci jusques aux plus composés, elle a établi successivement en eux différens systèmes d'organes particuliers, les a multipliés, en a augmenté de plus en plus l'énergie, et, les cumulant dans les plus parfaits, elle a fait exister tous les animaux connus avec l'organisation et les facultés que nous leur observons. Or, elle n'a rien fait absolument, ou elle a fait ainsi.

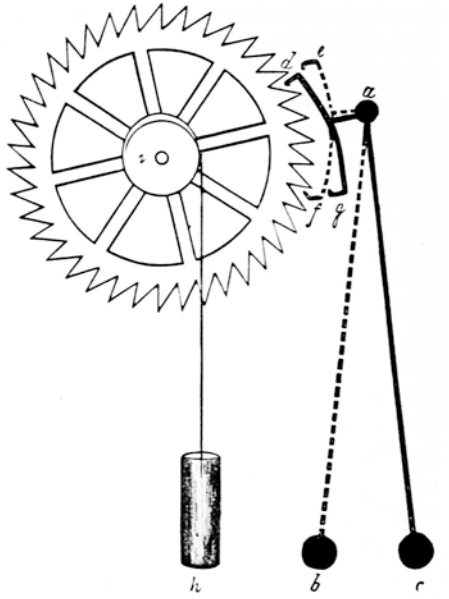
1815

Lamarck - *Time* is an *inherent property* of the living

- Time is ***constructed from within*** cells and organisms: *How*?
- Time is ***relative***: use of different time scales to organise cells and developing embryos
- **Temporal information is encoded and decoded**



https://www.thisiscolossal.com/2017/02/mechanical-crustaceans-with-clockwork-insides-illustrated-by-steeven-salvat/



http://forum.horlogerie-suisse.com/viewtopic.php?f=1&t=25216

Plan

- **Time scales in biology:**
  – Phenomenology of time and features: nested time scales (from molecules to evolution).

- **How is time encoded?**

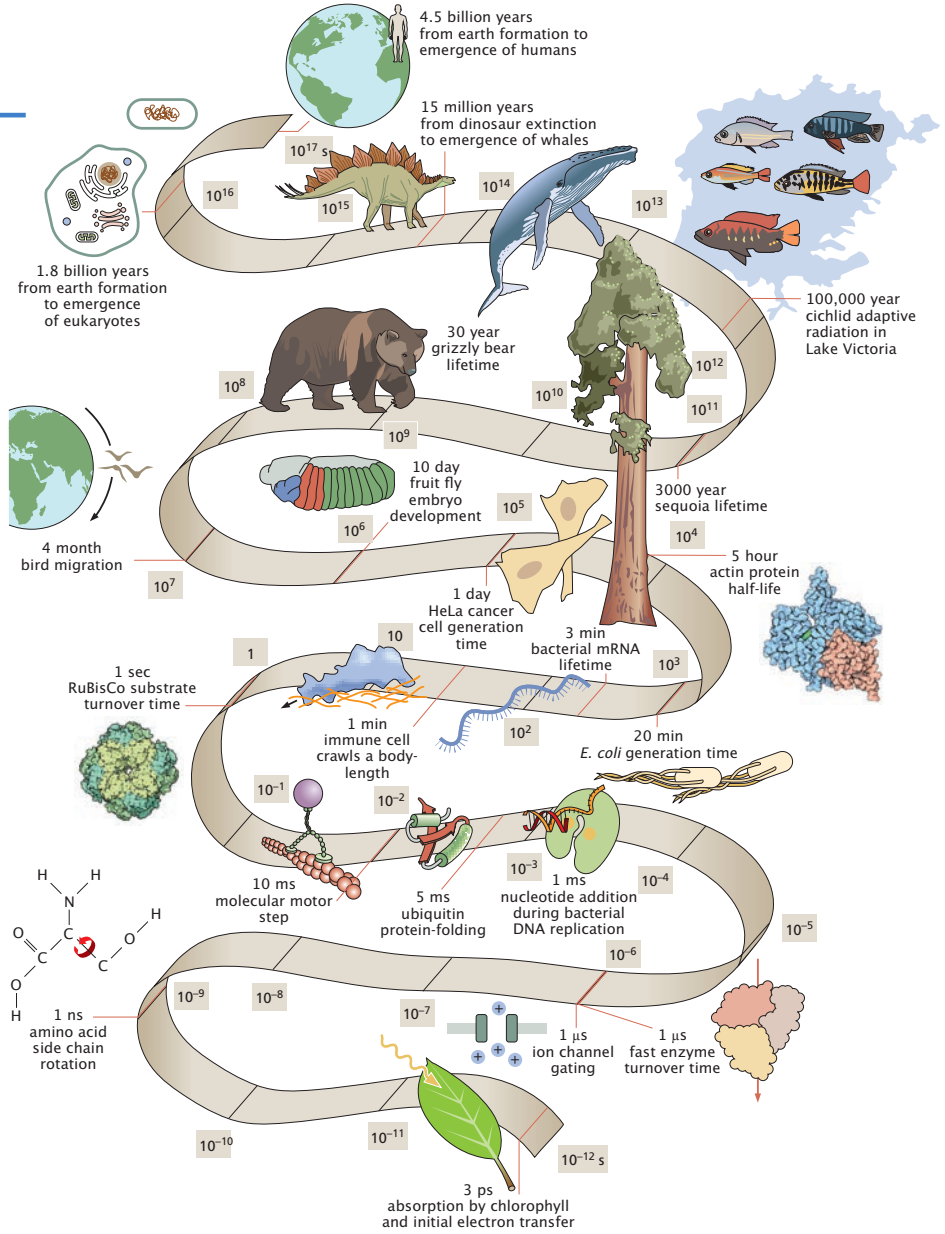
- **How is temporal information decoded?**
  – Signalling information: information encoded in dynamics.
  – Mechanical temporal information in morphogenesis.
  – Segmentation clock: decoding time to encode space

Phenomenology of time in Biology

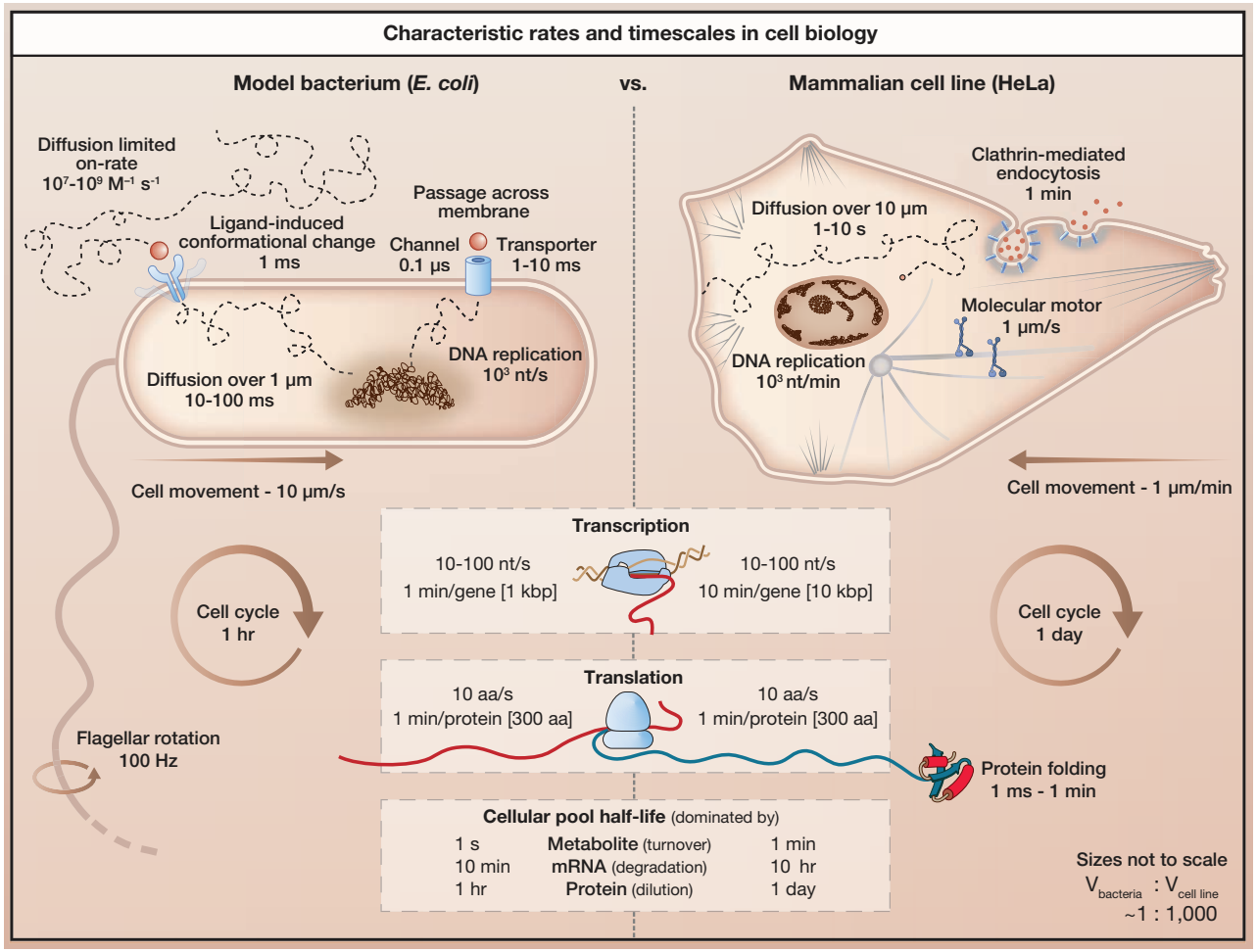
- **Life manifests over many time scales:**

(11 to 14 order of magnitude in a given organism)

- Molecular scale: 1- few ms
- Cellular scale: few minutes to hours
- Tissue scale: few 10s of min or hours
- Organismal scale: 1 day to years
- Evolutionary time:
  - –Species radiation can be « fast »
  - –Some species remain the same over longer time than major geological time.



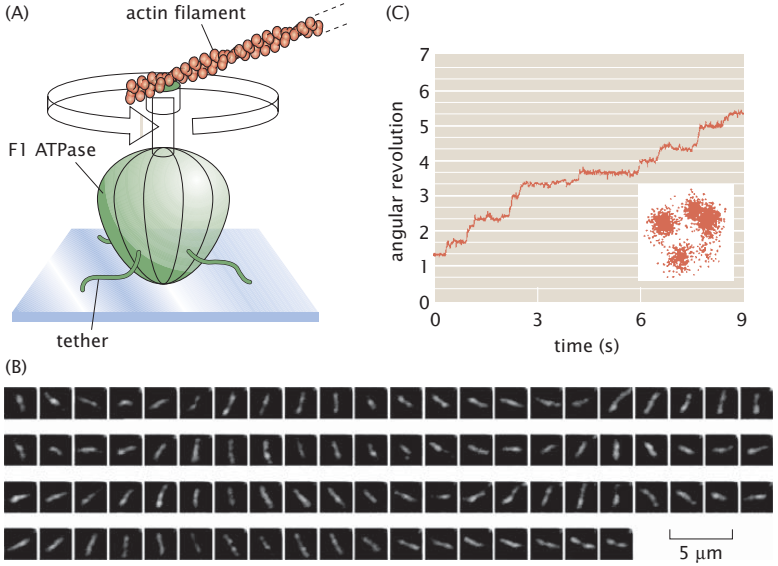
Phenomenology of time in Biology

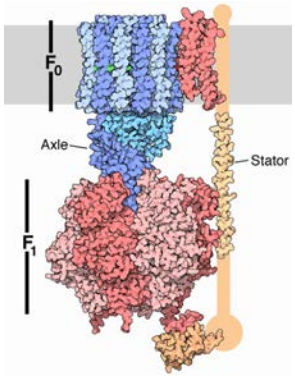


- Time scales are connected:
- **Cellular time scales emerge from molecular time scales.**
- Example: molecular oscillator such as cell cycle lasts 10 minutes to 24 hours

Phenomenology of time in Biology – Molecular cycle/oscillations

- ATP synthase Period T=10ms ~300 ATP per s

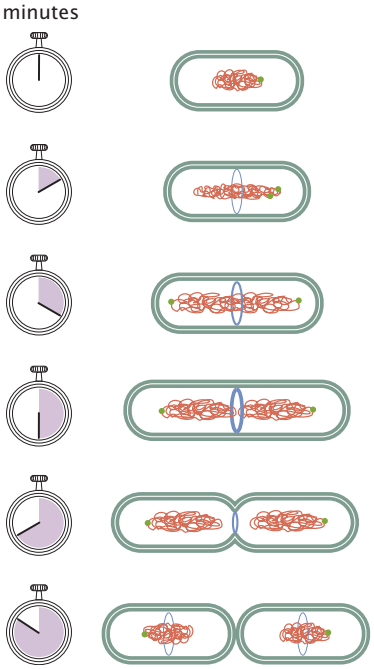




Function	Components	Period
Metabolism	Glucose, ATP, phospho-fructokinase	2 min
Signalling	Cyclic AMP, receptor, adenylate cyclase	5 min
Signalling	Ca^{2+}, $Ins(1,4,5)P_3$	> 1 s
Signalling	NF-κB, IκB, IKK	~2 h
Signalling	p53, MDM2	5 h
Signalling	Msn2, adenylate cyclase, cAMP, PKA	~10 min
Somitogenesis	Her1, Her7, Notch	30–90 min
Yeast endoreplication cycles	Cig2, Cdc10, Rum1	1–2 h
Frog egg cycles	CycB, Wee1, Cdc25, Cdc20	30 min
Circadian rhythm	PER, TIM, CLOCK, CYC	24 h

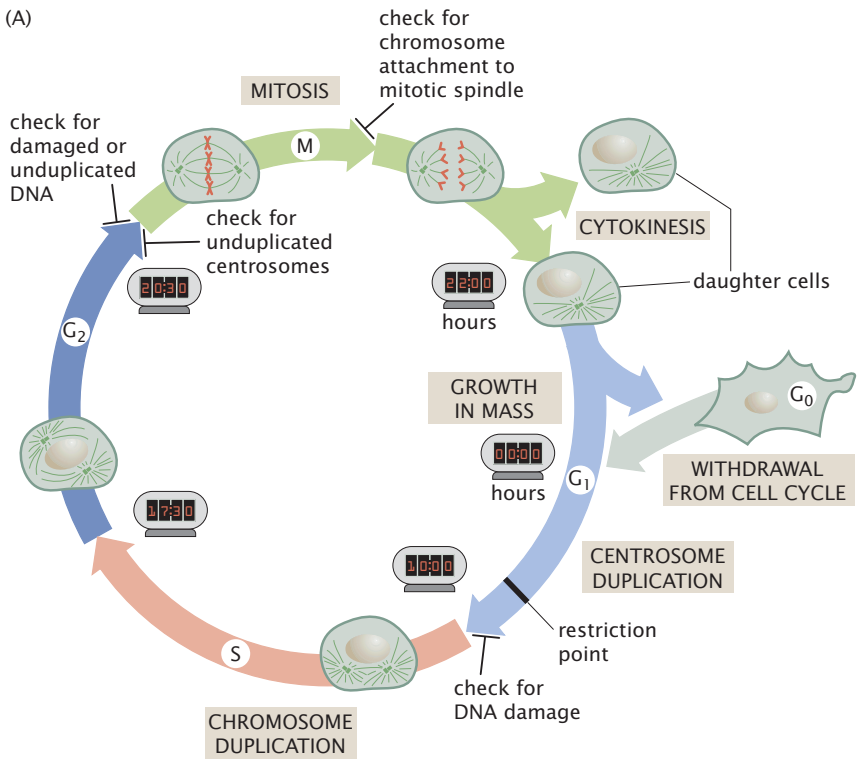
Phenomenology of time in Biology – Cell division cycle

Period T=20-60 min



Prokaryotic cell *E. coli*

Period T=500-1500 min



Eukaryotic cell

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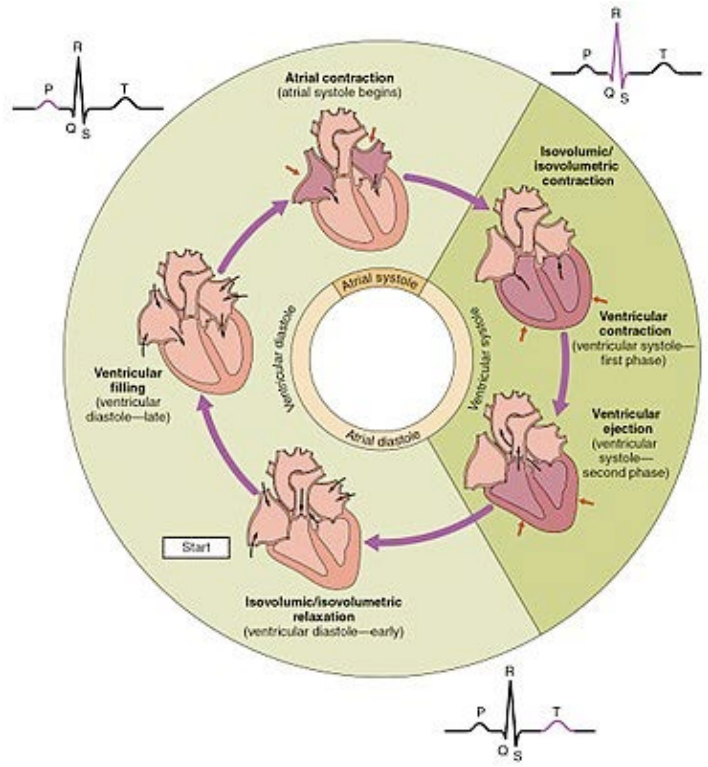
IBDM INSTITUT DE BIOLOGIE DU DÉVELOPPEMENT DE MARSEILLE

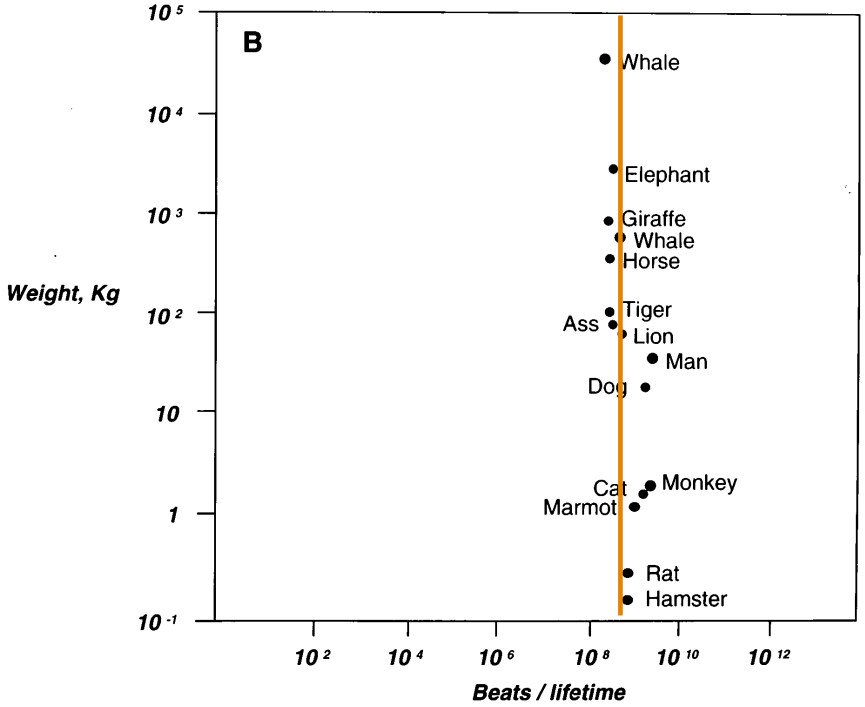
Phenomenology of time in Biology – Heart beat cycle

- Heart beat

Period T ~1s in human

ranging from 0.04s in Etruscan shrew to 10s in submerged blue whale



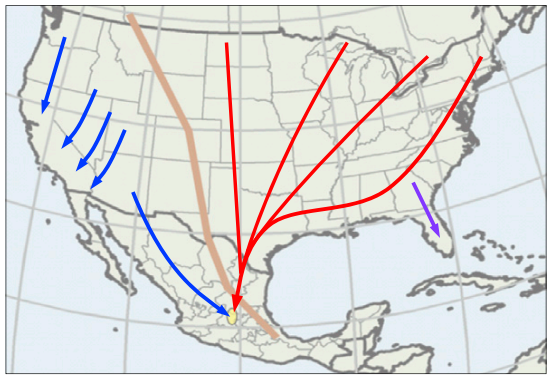


H. Levine. JACC (1997) Vol. 30, No. 4:1104–6

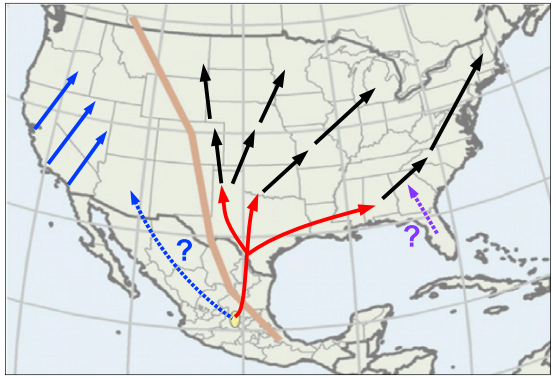
Phenomenology of time in Biology – Migration cycle

- Few hundred millions monarch butterflies, distributed in 4.5 million km^2 migrate in 2 months a 4500 km journey to a few sites 1km^2 each.
- They then migrate back, step by step, in 3-5 generations/years back to the original spots.

Migration south



Journey north



Current Biology

Period T=3-5 years

SM. Reppert and JC. De Roode *Current Biology 28*, R1009–R1022 (2018)

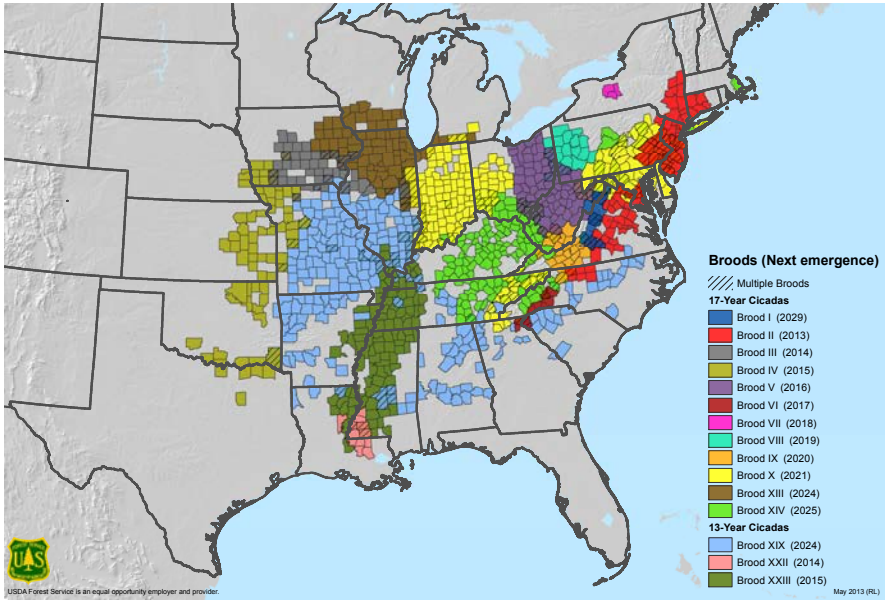
Phenomenology of time in Biology – cicada emergence cycles

Magicicada septendecim

Period T=13 or 17 years

https://www.sciencenews.org/article/mystery-synchrony
https://www.inaturalist.org/guide_taxa/370386

13-17 year broods geographical mapping (non overlapping)

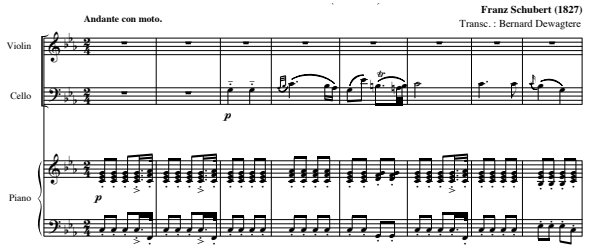


https://yardandgarden.extension.iastate.edu/article/2024/05/2024-periodical-cicada-emergence-what-should-you-expect

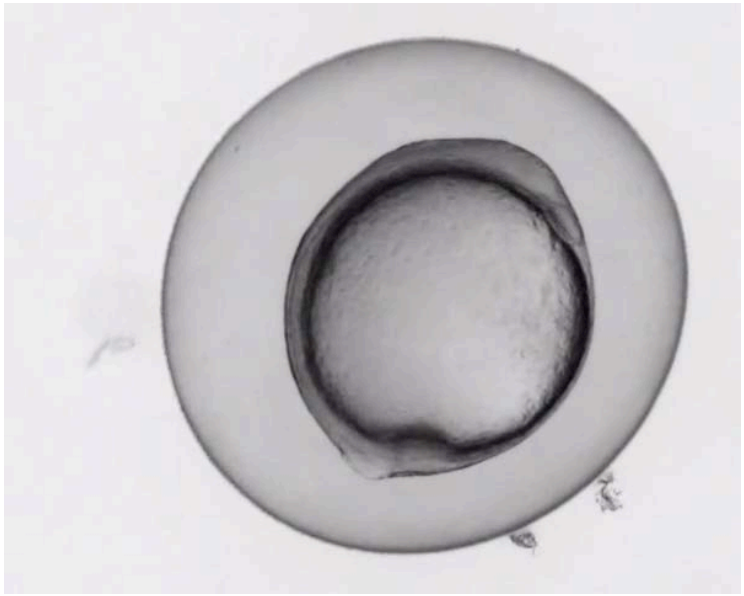
https://cicadas.uconn.edu/broods/

Embryonic development entails temporal control

Orderly temporal succession of cellular processes during embryonic development



Sea Urchin early cell division



Zebrafish embryonic development

Plan

- Time scales in biology:
  – Phenomenology of time and features: nested time scales (from molecules to evolution).

- **How is time encoded: defining time scales locally and globally.**

- How is temporal information decoded?
  – Signalling information: information encoded in dynamics.
  – Mechanical temporal information in morphogenesis.
  – Segmentation clock: decoding time to encode space

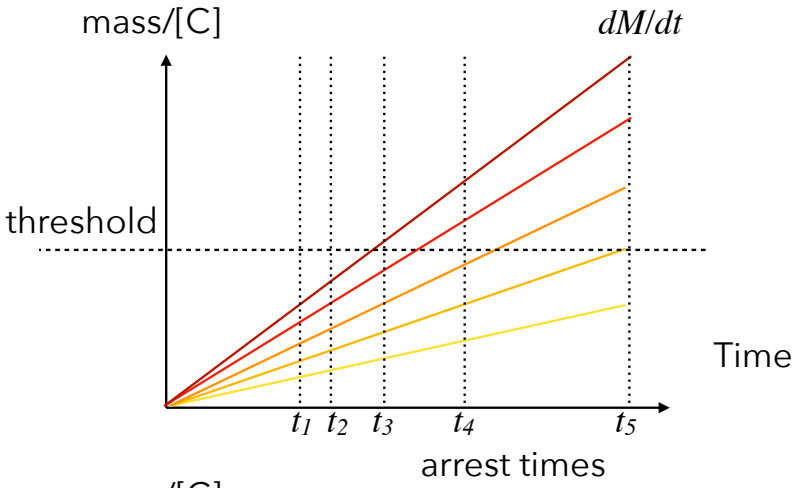
How is time generated and tuned?

Linear time: accumulation

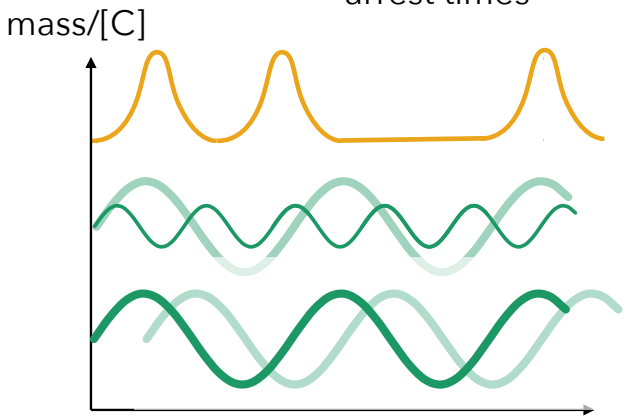
Cyclic time

How to encode temporal information?

- **Linear time:**
  - Accumulating (integration)
  - Rate



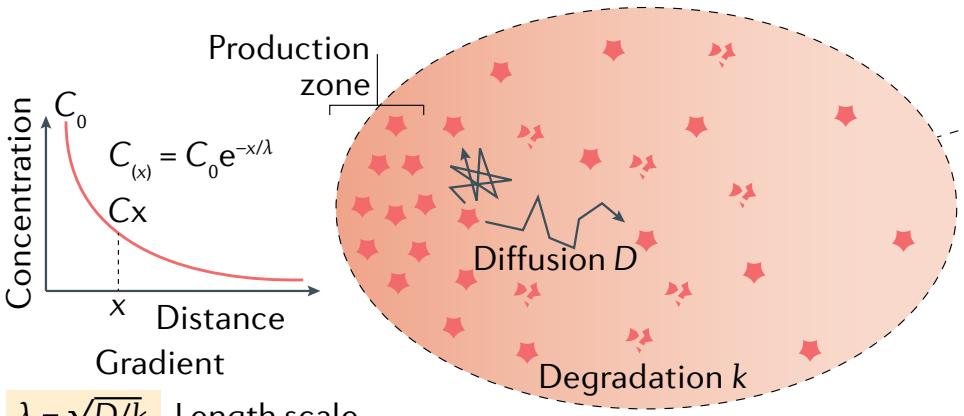
- **Cyclic or pseudo periodic dynamics**
  (eg. spikes, oscillations etc)
  - Counting
  - Frequency
  - Phase difference



Defining time scales

- Biochemical processes: diffusion, reaction waves

Diffusion



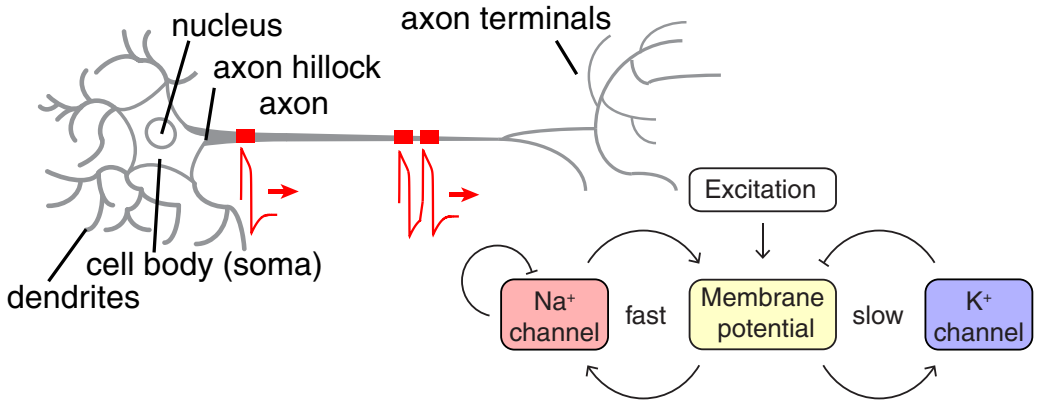
$\lambda = \sqrt{D/k}$ Length scale

$\tau = \lambda^2/D$ Timescale

$t \sim 250$s (l=50μm and D=10 μm^{2}s^{-1})

Collinet C. & Lecuit T. *Nature Rev. Mol. Cell Biol.*, 2021
doi.org/10.1038/s41580-020-00318-6

Chemical reaction wave



$t = l/v$ $t \sim 50$ms (sciatic nerve)

$v = 2(kD)^{1/2}$

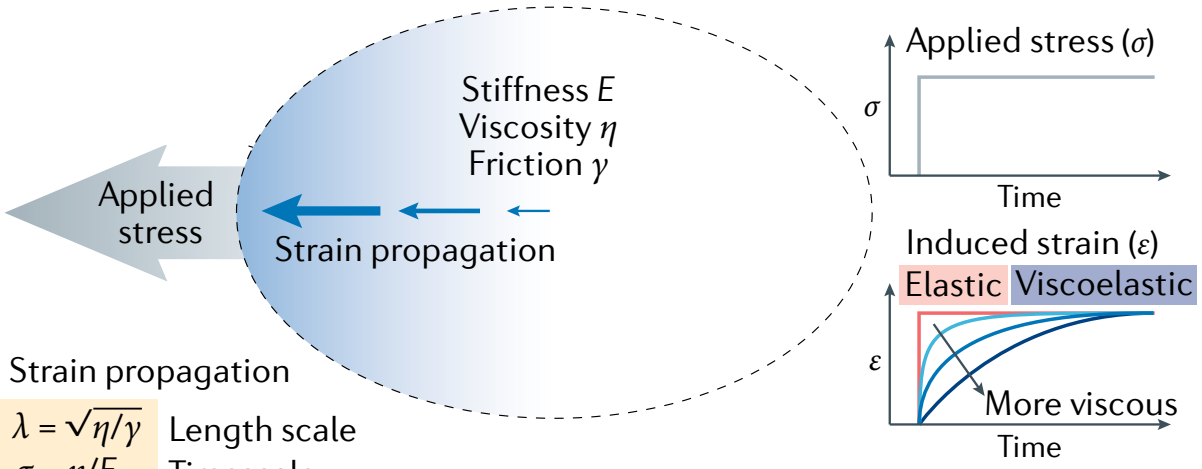
$D = 0.034\ \mathrm{m^2\ s^{-1}}$
$k = 3 \times 10^3\ \mathrm{s^{-1}}$
$v = 20\ \mathrm{m\ s^{-1}}$

reaction doubling time

L. Gelens , Anderson and J. Ferrel. MBoC, 25:3486-3493 (2014)
Showalter K, Tyson JJ *J Chem Educ* 64, 742–744 (1987)

Defining time scales

- Mechanical processes - ex: active viscoelastic flow

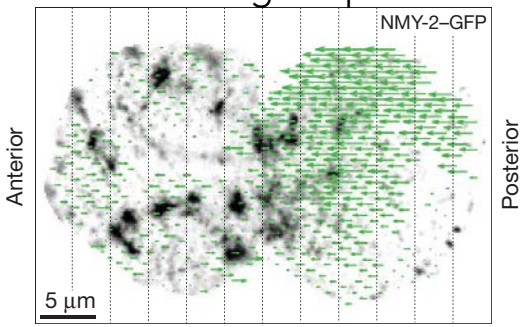


Strain propagation

$\lambda = \sqrt{\eta/\gamma}$ Length scale

$\tau = \eta/E$ Timescale

C. elegans polarization



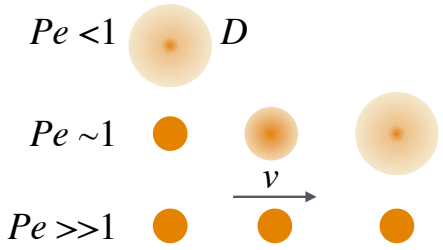
Mayer, M., Depken, M., Bois, J. S., Julicher, F. & Grill, S. W. Nature 467, 617–621 (2010).

$t = l/v \sim 300\text{s}$

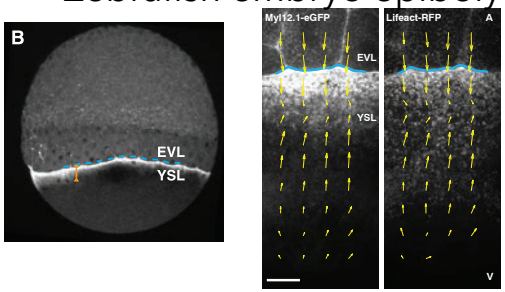
$v \sim 0.1\mu\text{m/s}$

$l \sim 30\mu\text{m}$

Pe: Peclet number measures the ratio of *advective* and *diffusive* transport rates. $Pe = L.v/D$



Zebrafish embryo epiboly



Behrndt et al, G. Sableux and CP. Heisenberg *Science*, 338:257-260 (2012)

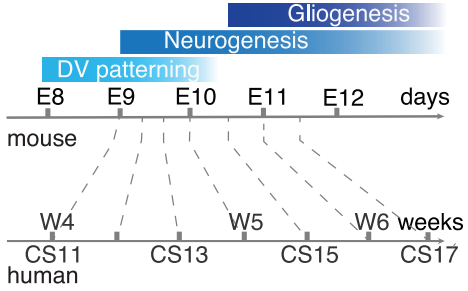
Collinet C. & Lecuit T. *Nature Rev. Mol. Cell Biol.*, 2021

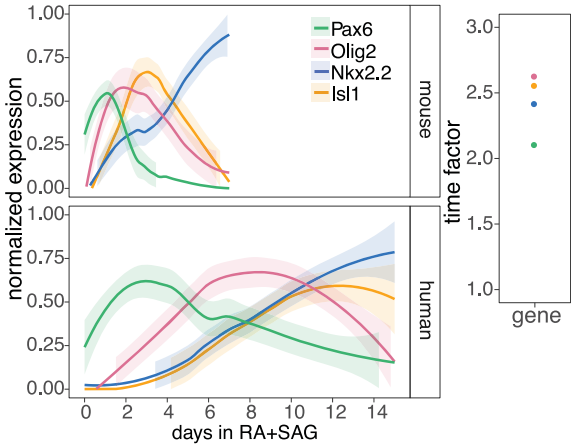
Tuning time scales *globally*

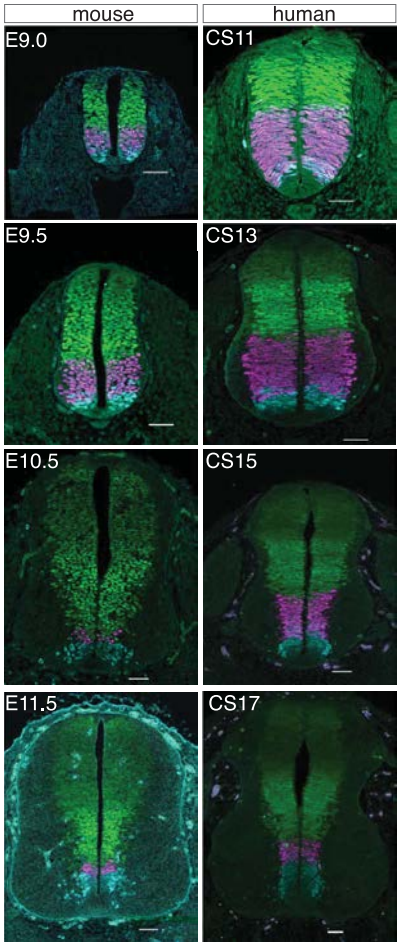
- Developmental tempo and protein stability

- Specification of motoneurons in the vertebrate neural tube depends on a Gene Regulatory Network (GRN) and growth factor signalling (Shh morphogen gradient)

- In Mouse and Human the tempo is different by a factor of ~2.5 fold (3-4 days vs 2 weeks)
- This can be recapitulated in vitro







Rayon et al. and J. Briscoe, *Science* 369, eaba7667 (2020)
Matsuda et al and M. Ebisuya, *Science* 369, eaba7668 (2020)

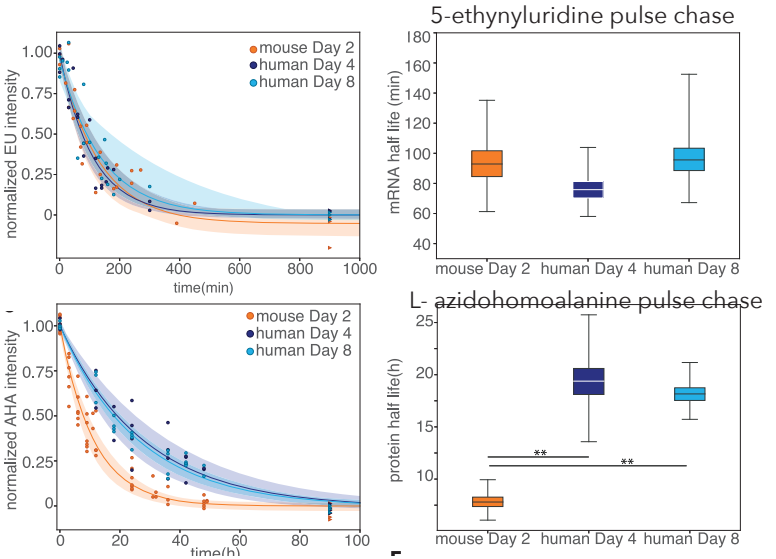
Tuning time scales *globally*

- **Developmental tempo and protein stability**

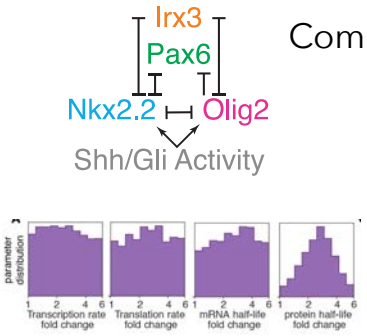
- The 2.5 fold difference in tempo is:
- *Not due to a difference sensitivity* to Shh signalling

(Similar Shh signalling dynamics is associated with different transcriptional regulation of target genes)

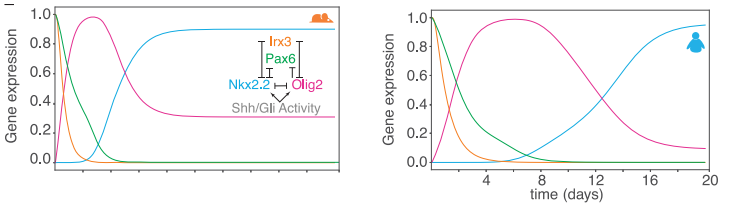
- Not associated with a difference in specie's sequence of target genes (eg. replacing Olig2 gene from human to mouse in ES cells does not change the tempo).
- **Indicates, species specific cellular environment.**
- mRNA stability (half life is not different)
- **Protein stability (half life) shows a ~2.5 fold difference**



- Computational modelling indicates higher constraints in protein stability to account for 2.5 fold change in tempo compared with other parameters.
- **A general cellular property:**
- An exogenous protein (mKate2) has different half life.
- Cell cycle duration show similar tempo difference



Computational model:

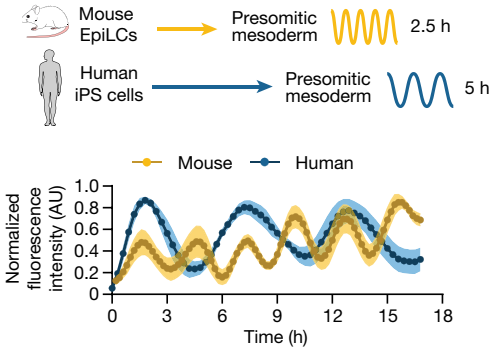


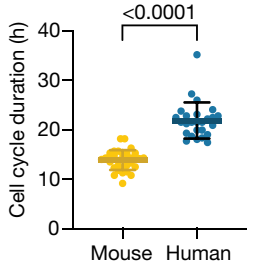
Rayon et al. and J. Briscoe, *Science* 369, eaba7667 (2020)
Matsuda et al and M. Ebisuya, *Science* 369, eaba7668 (2020)

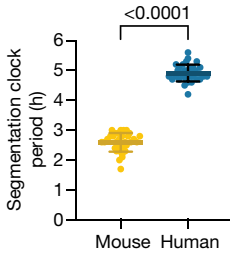
Tuning time scales *globally*

- Developmental rate and Metabolism

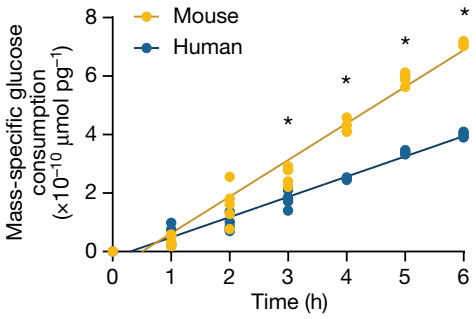
- Segmentation in vertebrates depends on sequential formation of somites based on the oscillatory dynamics of a molecular clock.
- This process can be recapitulated in vitro.
- **In humans, the clock period is 2x longer than in the mouse.**
- The cell cycle is also longer.

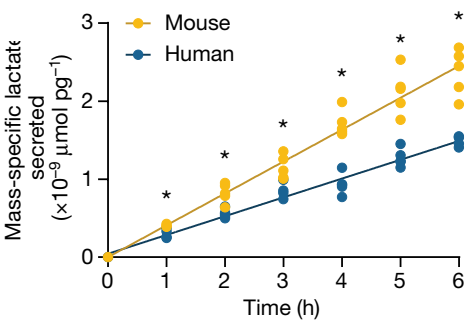


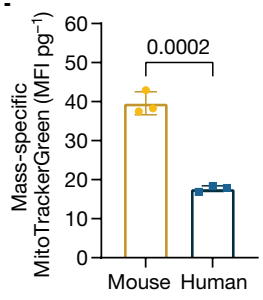




- **Metabolic rate density is higher in faster developing embryos.**
- Metabolic activity (eg. glucose consumption rate) normalised to unit mass is greater in the mouse.
  The density of mitochondria is also higher.





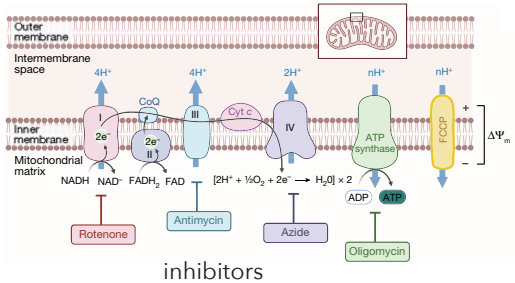


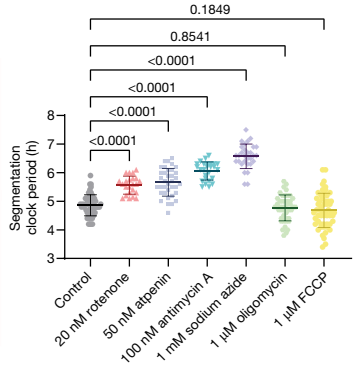
M. Diaz-Cuadros et al and O. Pourquié. *Nature* 613:550-557 (2023)

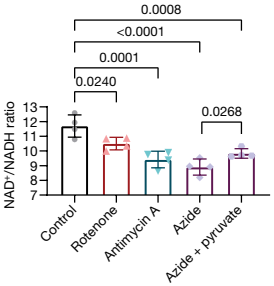
Tuning time scales *globally*

- Developmental rate and Metabolism

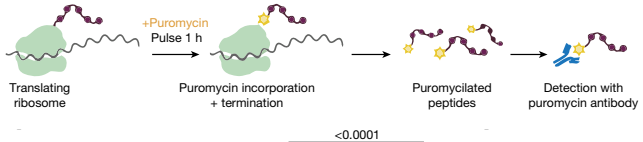
- **The Electron Transport Chain (ETC)** but not ATP synthase **affects the period of the segmentation clock.**
- Role of NAD+/NADH rather than ATP.

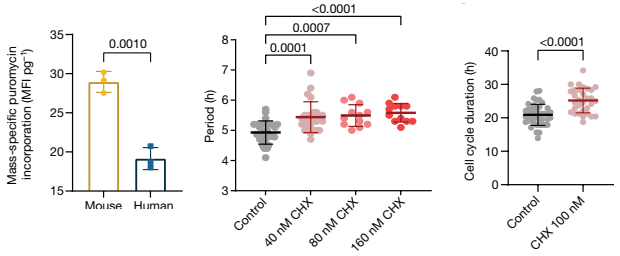


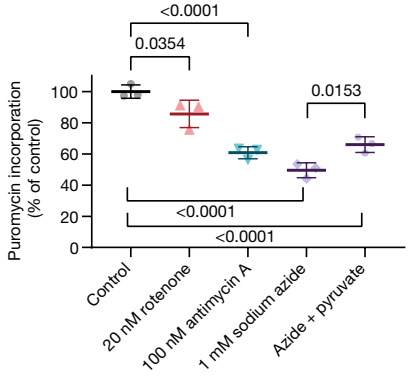




- Protein translation sets the segmentation clock period.
- The ETC and NAD+/NADH ratio affects protein translation.







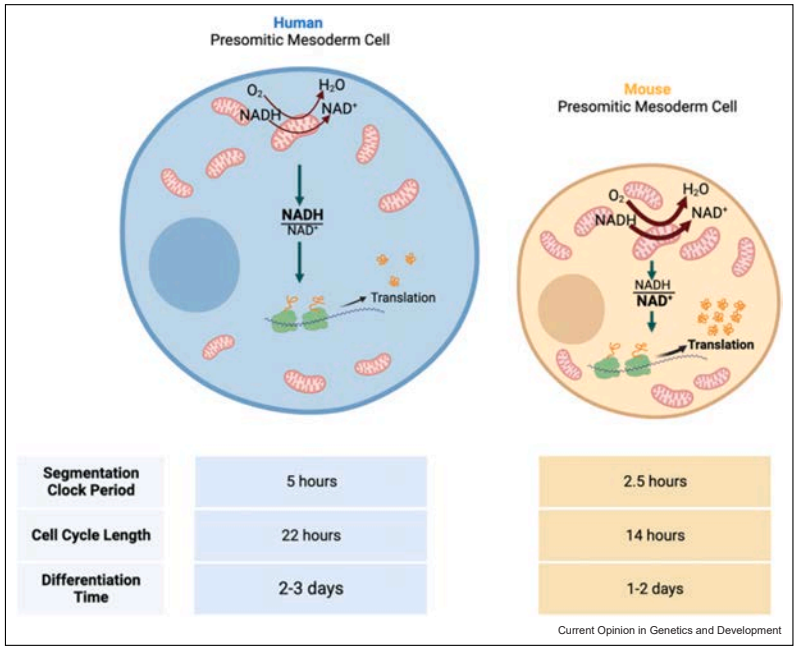
CHX: cycloheximide : protein translation inhibitor

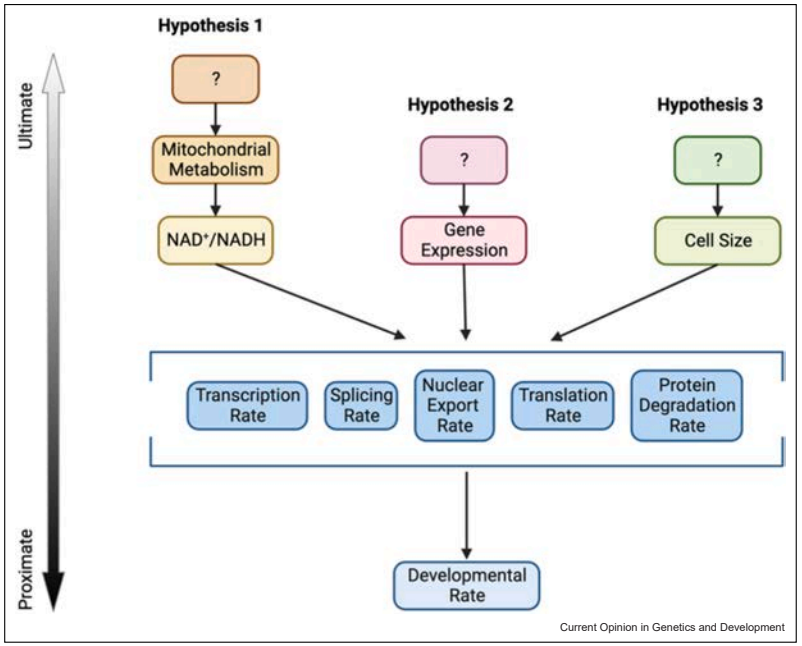
M. Diaz-Cuadros et al and O. Pourquié. *Nature* 613:550-557 (2023)

Tuning time scales *globally*

- **Developmental rate and Metabolism**

- Hypothesis: Tissue specific regulation of Electron Transport Chain and NAD+/NADH ratio could allow tissue specific developmental rate
- Mitochondria metabolism also affects the tempo of neuronal development Iwata et al., *Science* 379, 553 (2023)





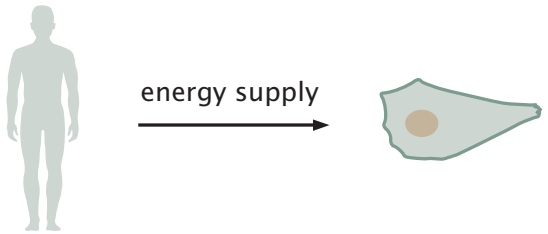
M. Diaz-Cuadros. *Current Opinion in Genetics & Development* 86:102178 (2024)

Tuning time scales *globally*

- Metabolic scaling: Kleiber law

- Sublinear scaling of metabolic power across many adult organisms
- It is not yet clear whether this also extends to embryonic development

(A)

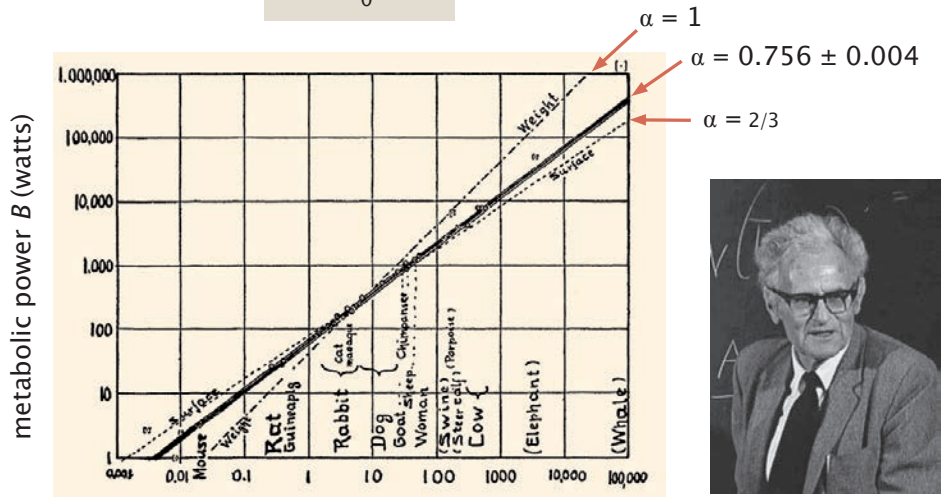


3×10^{13} cells
metabolic power B:
2000 kcal/day

1 cell
3×10^{7} ATP/cell/second

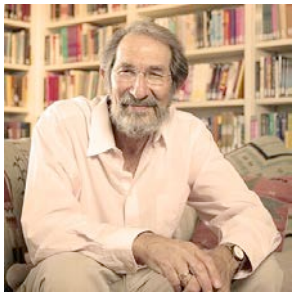
(B)

$B \propto B_0 M^{3/4}$



Max Kleiber

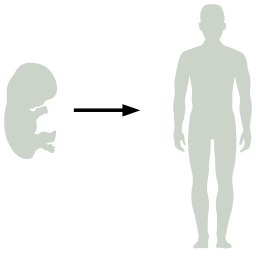
Tuning time scales globally



Geoffrey West

- **Metabolic scaling and universal growth law** (in redimensionalised time)

METABOLIC POWER



total mass = cell number × cell mass

$$m = m_c \times N_c$$

$$B = \sum_c \left[N_c B_c + E_c \frac{dN_c}{dt} \right]$$

cell metabolic rate

energy required to produce a new cell

power allocated to sustain organism

power allocated to growth

GROWTH EQUATION

$$\frac{dm}{dt} = \left(\frac{m_c}{E_c}\right) B - \left(\frac{B_c}{E_c}\right) m$$

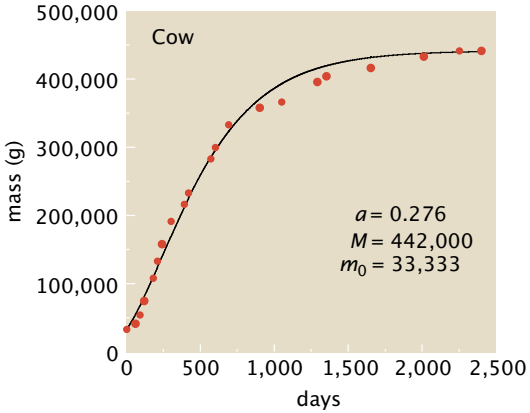
KLEIBER LAW

$$B = B_0 m^{3/4}$$

$$\frac{dm}{dt} = \underbrace{am^{3/4}}_{\propto \text{energy supply}} - \underbrace{bm}_{\propto \text{energy demand}}$$

asymptomatic mass:

$M = (a/b)^4 = (B_0 m_c / B_c)^4$

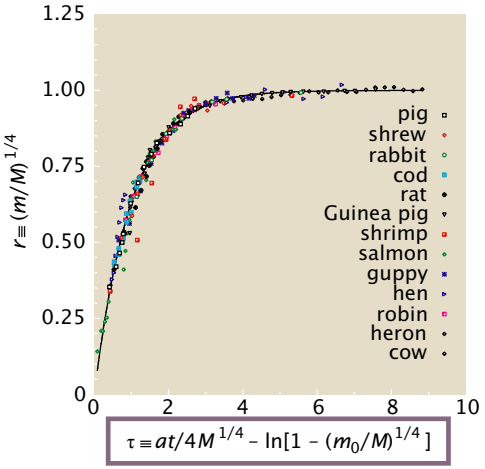


UNIVERSAL GROWTH LAW

$$r = 1 - e^{-\tau}$$

r fraction of metabolic power for sustenance

R = 1 – *r* fraction of metabolic power for growth



G. West, J. Brown and B. Endquist. *Nature* 413 (2001)

Universality of biological clock

- How to relate whole organism metabolic rate to biochemical reaction within cells?

organism metabolic rate $B = \sum_i R_i$, where R_i is the rate of energy consumption per chemical reaction i associated with metabolism

$R_i \propto$ [reactants]x(flux of reactants) x (kinetic energy of system)

allometric constraint $\propto M^{3/4}$

Boltzmann factor (temperature dependence) $e^{-E_i/kT}$

activation energy

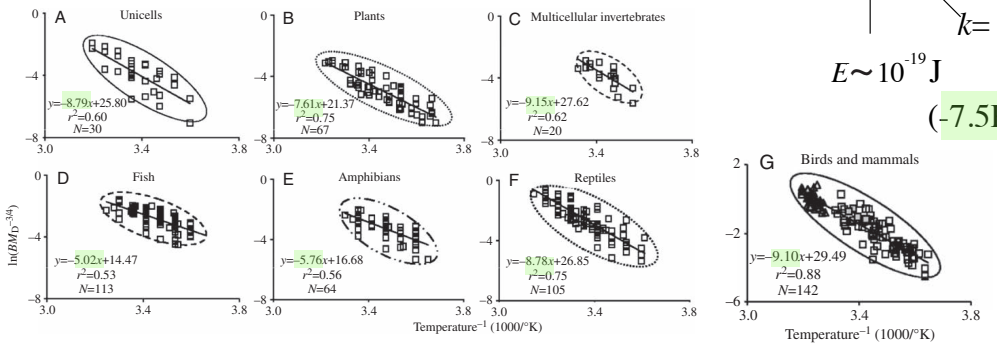
$$B \sim M^{3/4} e^{-E_i/kT}$$

prediction: $\ln(B.M^{-¾})$ should be linearly related to $1/T$ with slope $a=-E_i/k$ ~-7500 K

$k = 1.38\ 10^{-23}$ J.K^{-1}

$E \sim 10^{-19}$ J

(-7.5K with 1000/K)



Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L. (2001). *Science* 293, 2248-2251.

Universality of biological clock

- **A new definition of biological rates and times.**

average activation energy for rate limiting chemical reactions: $E \sim 10^{-19}$ J

biological rates $R \propto M_b^{-1/4} e^{-E/kT}$

biological times $t \propto M_b^{1/4} e^{E/kT}$

- **All animals run the same « clock »** adjusted for mass (internal constraint on energy delivery) and temperature (external constraint)

Plan

- Time scales in biology:
  – Phenomenology of time and features: nested time scales (from molecules to evolution).

- How is time encoded: defining time scales locally and globally.

- **How is temporal information decoded?**
  – Signalling information: information encoded in dynamics.
  – Mechanical temporal information in morphogenesis.
  – Segmentation clock: decoding time to encode space

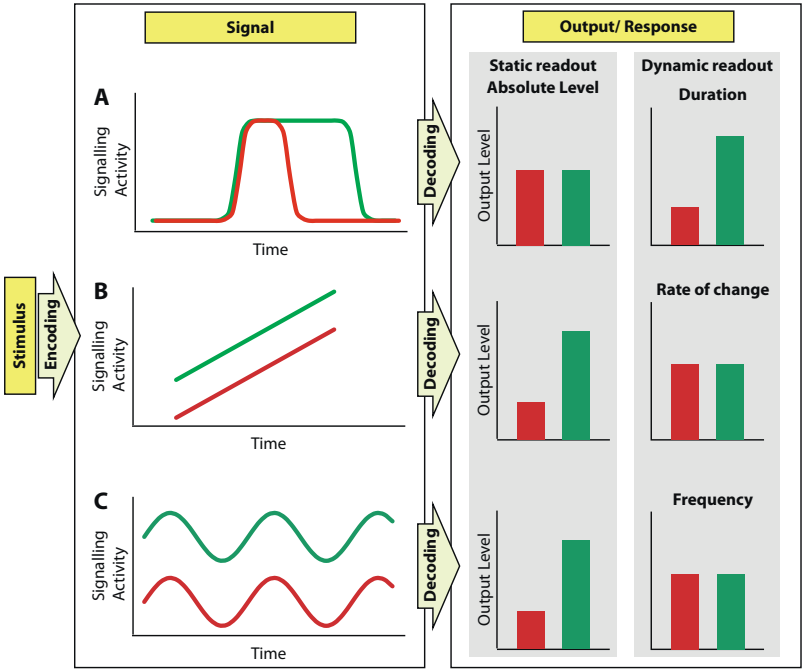
How is time information used?

Temporal information in biological signalling

- Encoding and decoding different temporal patterns of cellular signalling

The topology of signalling networks endow cells with capacity to compute various features of temporal information coming from the cell environment.

–***Duration*** of signalling instead of level: persistence detectors (coherent FFL), adaptation (incoherent FFL)
–***Frequency*** of pulsatile or oscillatory signalling
–***Number*** of pulses
–***Phase difference*** between oscillatory signals

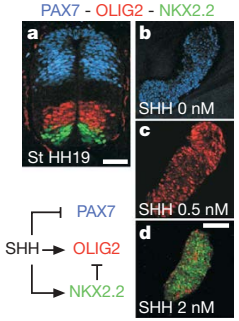


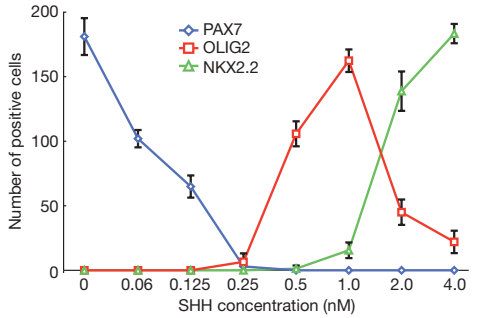
K.F. Sonnen, A. Aulehla / *Seminars in Cell & Developmental Biology* 34 (2014) 91–98

Temporal information in biological signalling

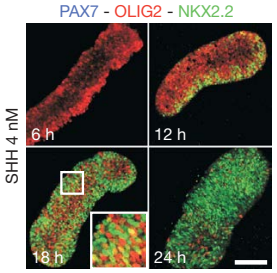
- Encoding and decoding the *duration* of signalling through *adaptation*

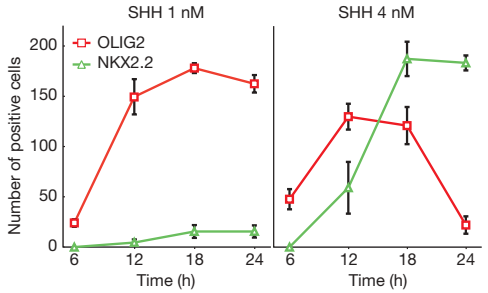
- Spatial patterning of motoneurons in the vertebrate neural tube is based on the **concentration dependent activation** of target genes by a Shh gradient.
- **The duration of signalling at constant concentration of Shh elicits dynamic changes in target gene activation.**

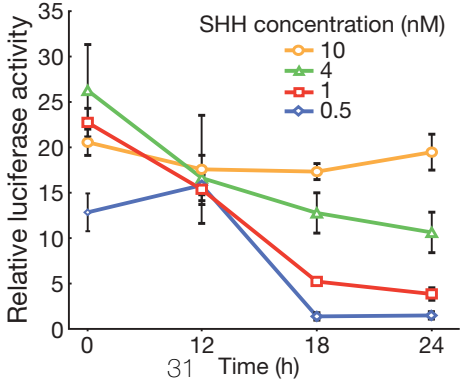




- Up to 12h, there is similar signalling activation irrespective of Shh concentration
- At 24h, Shh signalling is concentration dependent.
- **Signalling is down regulated over time**, to a greater extent as the concentration of Shh lowers.
- **Temporal adaptation of cells to Shh.**







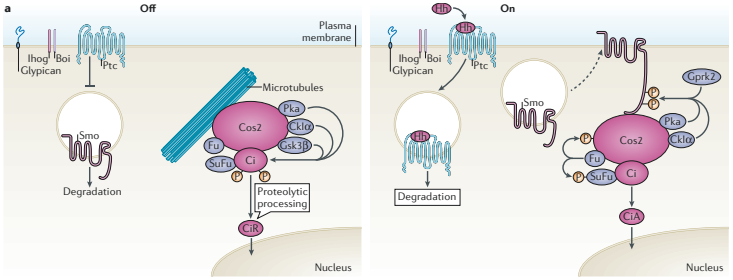
E. Dessaud et al and J. Briscoe, *Nature*, 450:717-720 (2007)

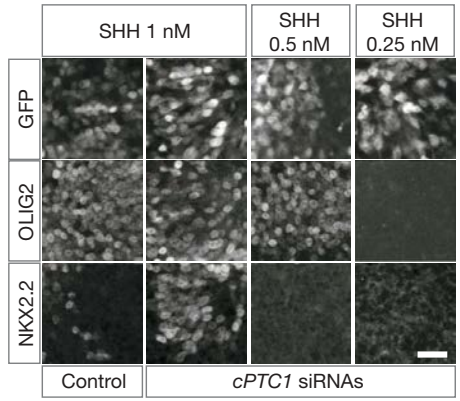
Temporal information in biological signalling

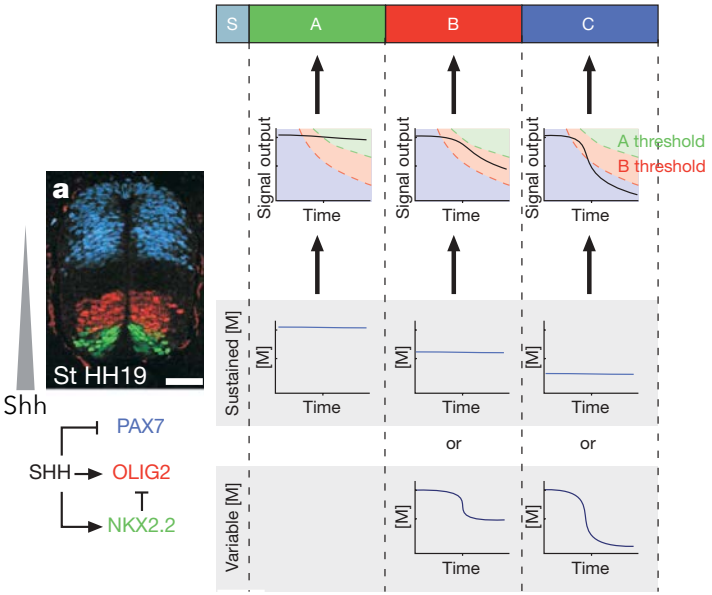
- Encoding and decoding the *duration* of signalling through *adaptation*

- Shh signalling requires inhibition of Ptc receptor, which releases inhibition of Smo receptor.
- Ptc is upregulated by Shh
- Signalling adaptation (downregulation) operates upstream of Smo receptor.
- **Ptc is required for differential activation of target genes at 24h**
- Adaptation via an *incoherent feedforward loop*.

- **Signal output declines (adapts) faster in cells exposed to lower [Shh].**
- **The progressive adaptation of cells to Shh transforms ligand exposure into periods of increased GLI activity, that are proportional to [Shh]**







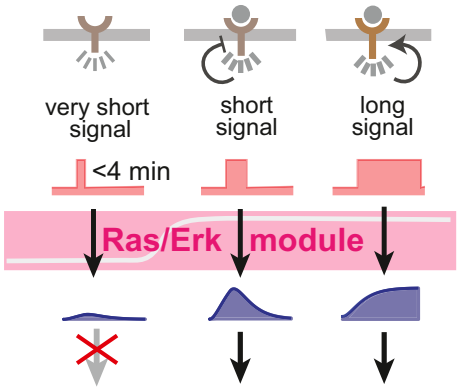
E. Dessaud et al and J. Briscoe, *Nature*, 450:717-720 (2007)

Temporal information in biological signalling

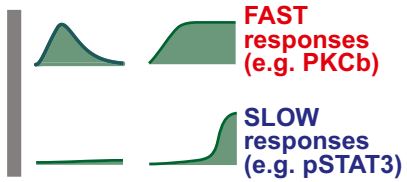
- Encoding and decoding the *duration* of signalling

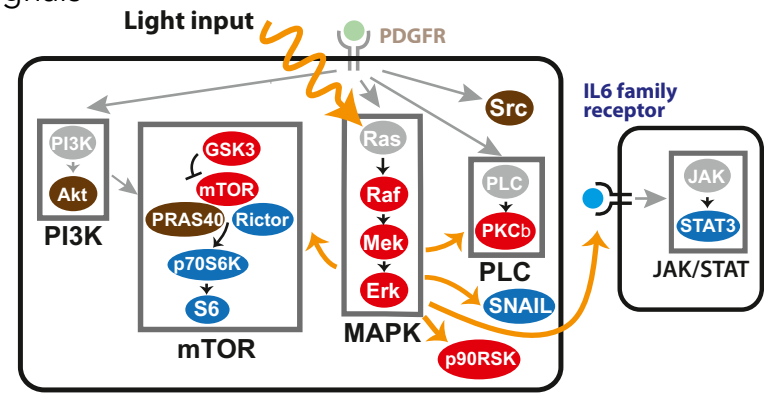
- Use optogenetics to perturb the dynamics of Ras signalling
- Precision sensing at the single cell level: Each cell is capable of singular and stable response over hours.
- ERK signalling is a **high bandwidth low pass filter**.
- **Differential modular decoding downstream of Ras/ERK:**
  - *Fast module* faithfully transmit Ras dynamics
  - *Slow module* is a persistence detector that only conveys long lasting signals

Encoding of signal *duration*



Modular decoding





Toettcher JE, Weiner OD, Lim WA. *Cell* 155:1422–34 (2013)

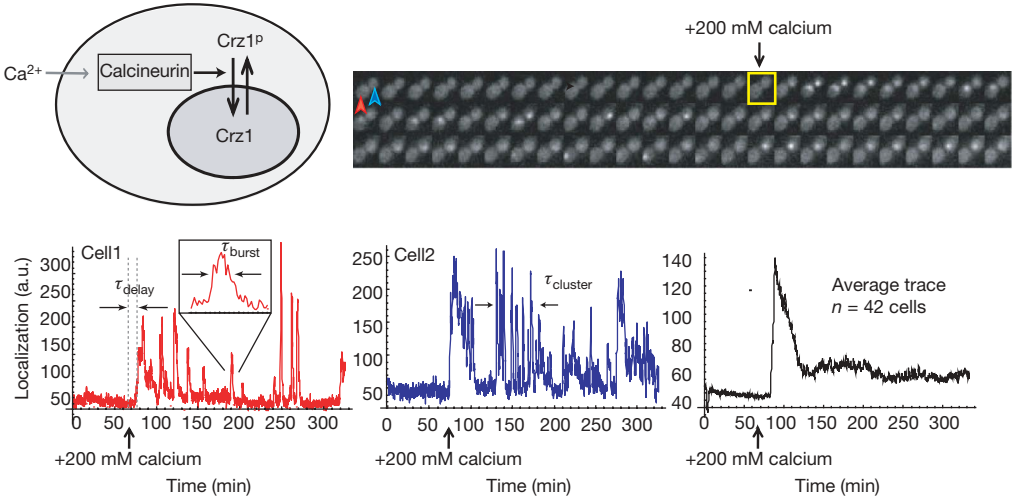
Mangan, S., and Alon, U. Structure and function of the feed-forward loop network motif. *PNAS* 100, 11980–11985 (2003)

Temporal information in biological signalling

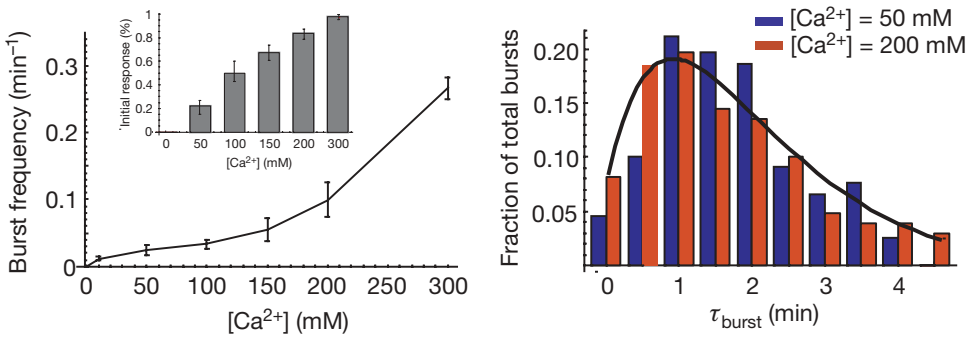
• Encoding and decoding *frequency modulated* signalling

- In budding Yeast, the transcription factor Crz1 mediates calcium stress response.
- Crz1 coordinates transcription of ~100 genes and cell response to changes in extracellular Ca^{2+}.

- Crz1-GFP translocates to nucleus in response to Ca^{2+}.
- Crz1-GFP shows **stochastic bursts of nuclear translocation** which tend to cluster.



- Ca^{2+} concentration tunes the ***burst frequency*** but **not the duration** of Crz1-GFP nuclear translocation.



L. Cai, CK Dalal and M. Elowitz *Nature*, 455:485-490 (2008)

Temporal information in biological signalling

• Encoding and decoding *frequency modulated* signalling

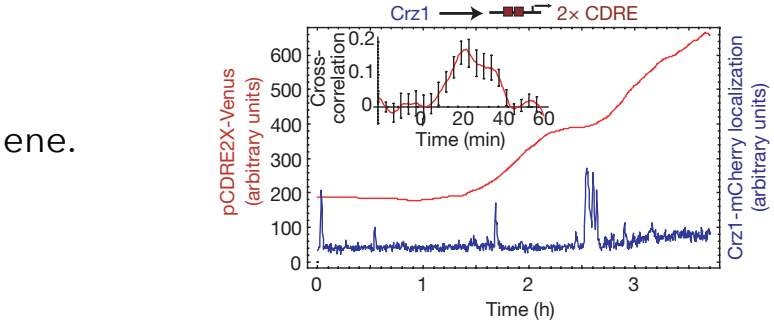
- Statistical correlation between Crz1 bursts and transcriptional activation of synthetic target gene.
- Crz1 nuclear bursts increase transcription of target gene.

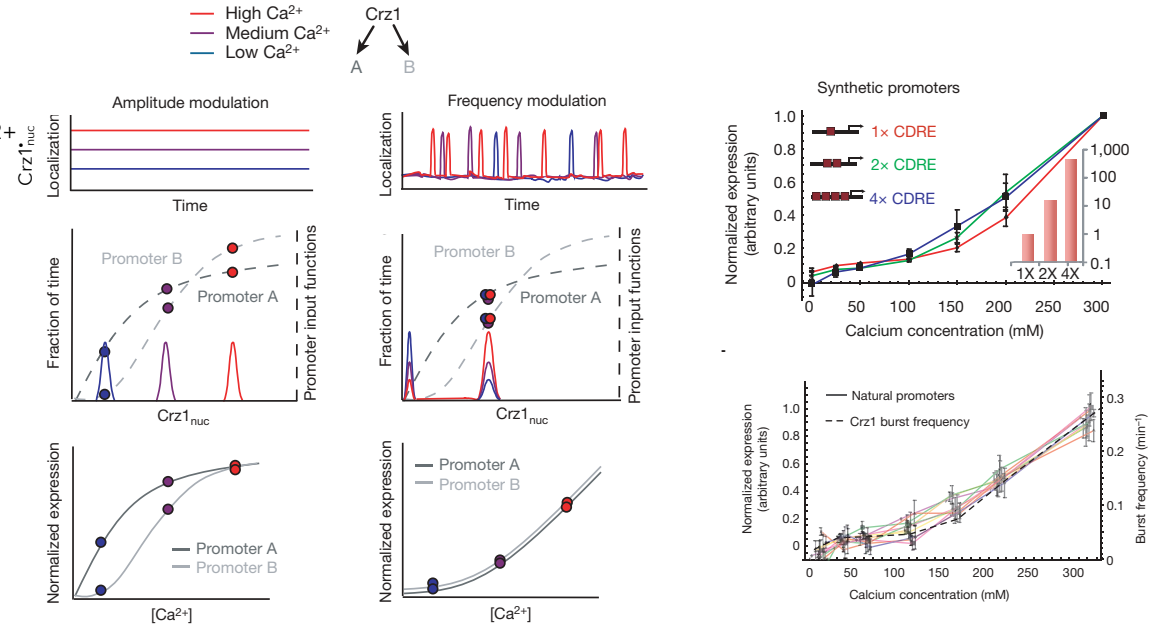
Models:

- **Amplitude modulation:**
  – Ca^{2+} controls Crz1 nuclear fraction ($Crz1_{nuc}$).
  – Different promoters, with different *input functions* (ie. transcription rate as a function of $Crz1_{nuc}$ concentration) have different normalised expression as a function of $Crz1_{nuc}$ & Ca^{2+}.

- **Frequency modulation:**
  – Ca^{2+} controls the fraction of time that Crz1 is nuclear, not the concentration.
  – Gene expression is proportional to burst frequency
  – As Ca^{2+} increases, transcription of both genes increases proportionately.
  – gene expression is coordinated.

Experimental validation



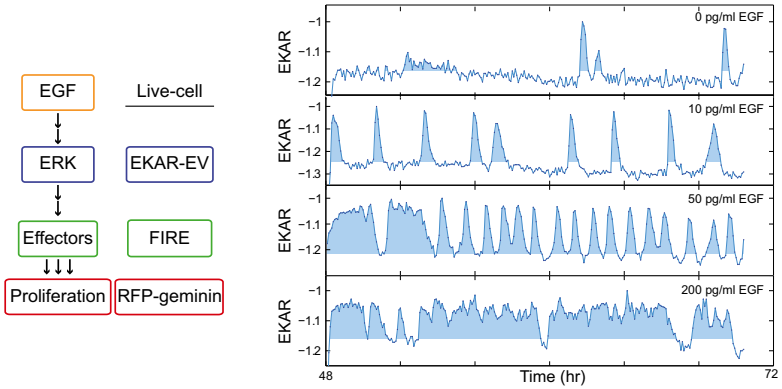


L. Cai, CK Dalal and M. Elowitz *Nature*, 455:485-490 (2008)

Temporal information in biological signalling

- Encoding and decoding *frequency modulated* signalling

- EGF induced ERK signalling pulses



Albeck JG, Mills GB, Brugge JS. *Mol Cell* 2013;49:249–61 (2013)

- NFkB signalling has also been implicated in frequency encoding.

This signalling pathway oscillates with NFkB periodically shuttling between nucleus and cytoplasm upon stimulation by the inflammatory cytokine TNFa.

The expression of late genes, like the chemokine RANTES, is only induced with high frequency pulses of NFkB, whereas early or middle genes are also expressed at lower frequencies.

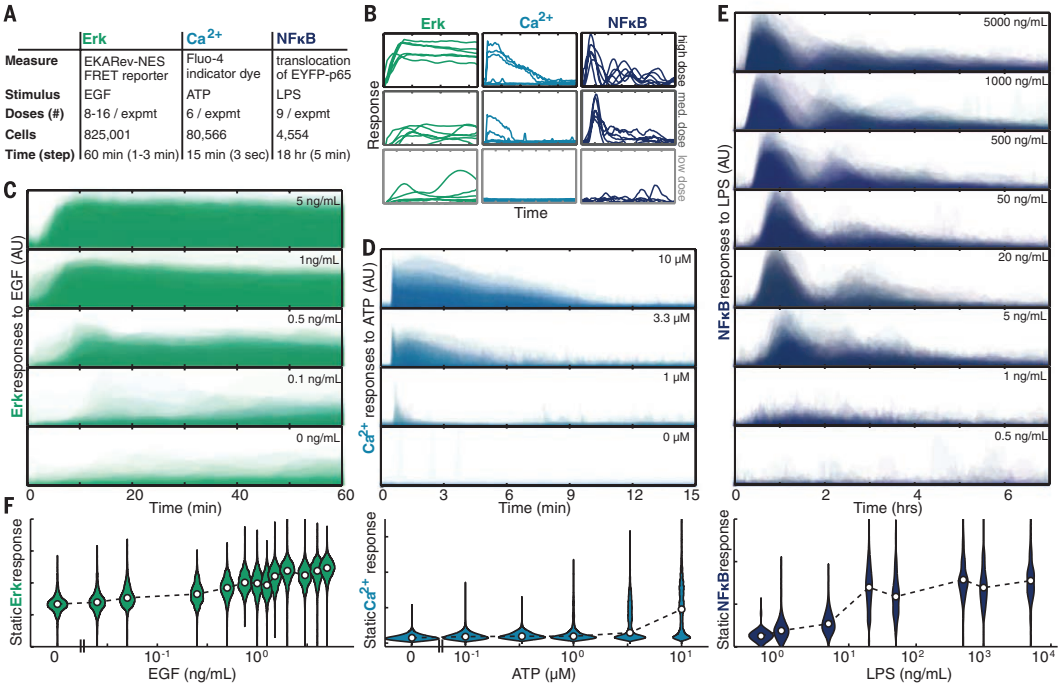
K.F. Sonnen, A. Aulehla / *Seminars in Cell & Developmental Biology* 34 (2014) 91–98

Quantifying information encoded dynamically

- Assess the information encoded dynamically versus statically
- Study the impact of extrinsic noise and intrinsic noise.

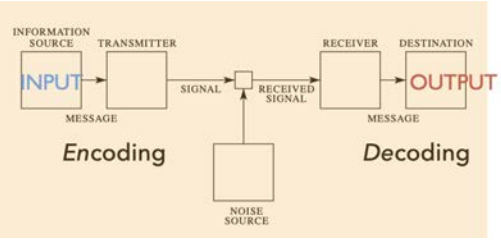
Accurate information transmission through dynamic biochemical signaling networks

Jangir Selimkhanov,[1*] Brooks Taylor,[1*] Jason Yao,[2] Anna Pilko,[2] John Albeck,[3] Alexander Hoffmann,[4,5] Lev Tsimring,[4,6] Roy Wollman[2,4,7]†



Selimkhanov et al., *Science* 346, 1371–1373 (2014)

Quantifying information encoded dynamically

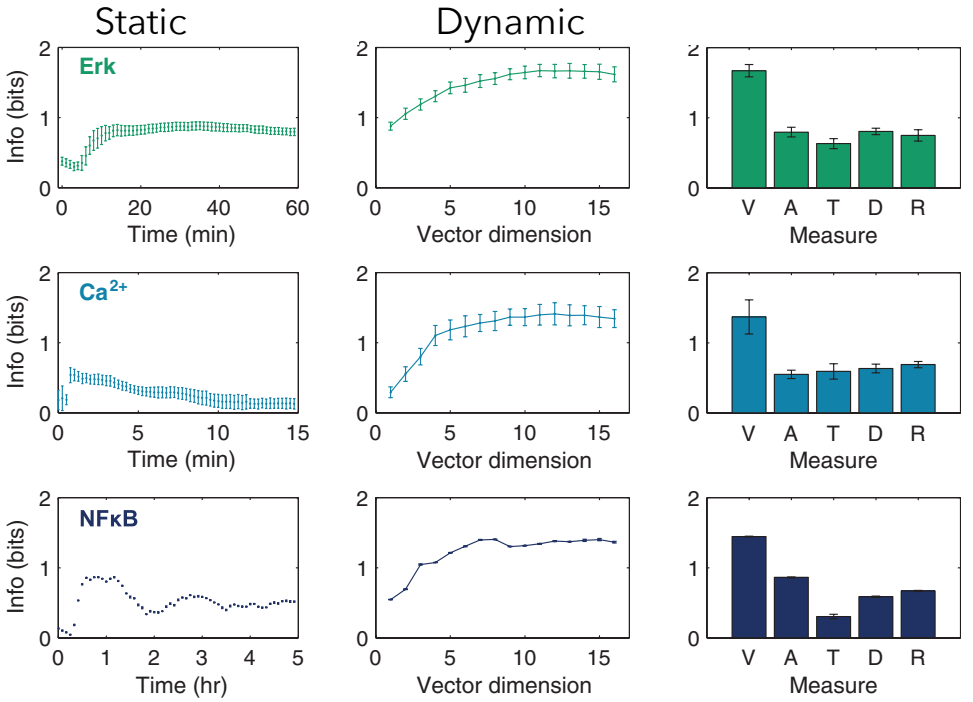


- Channel capacity is the maximum of mutual information between input and output distributions $C = \mathrm{Max}\left(H(x) - H_y(x)\right) = \mathrm{Max}\, I(x,y)$

Input X is a scalar value
Output Y is a static (scalar) or dynamic variable (multivariate vector)

- **Channel capacity (information transmission) is higher (>1 bit) using information encoded in the dynamics**
- **Impact of noise:**

For external noise, fluctuations are constrained by the internal networks that generate the dynamics such that fluctuations at different time points are deterministically correlated/interdependent.
Independent measurements can decode well the a priori internal signal of the cell.
Dynamic (but not static) information mitigates the effect of extrinsic noise.



Selimkhanov et al., *Science* 346, 1371–1373 (2014)

Quantifying information encoded dynamically

Distributed and dynamic intracellular organization of extracellular information

Alejandro A. Granados[a,b,1], Julian M. J. Pietsch[b,c,1], Sarah A. Cepeda-Humerez[d], Iseabail L. Farquhar[b,c], Gašper Tkačik[d], and Peter S. Swain[b,c,2]

Granados et al. *PNAS* 115, 6088 (2018).

Multidimensional representation of external signal changes in a set of 10 transcription factor dynamics

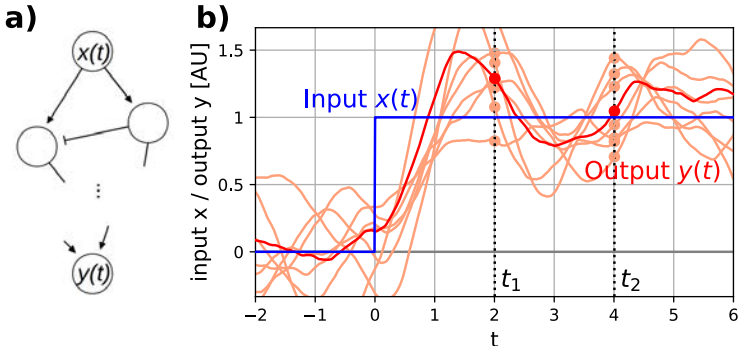
Dynamical information synergy in biochemical signaling networks

Lauritz Hahn,[1] Aleksandra M. Walczak,[1,*] and Thierry Mora[1,*]

[1] *Laboratoire de Physique de l'École normale supérieure, CNRS, PSL University, Sorbonne Université, and Université Paris Cité, Paris, France*

Hahn et al. *PRL* 131(12):128401

Analytical calculation of MI for dynamic input and output.



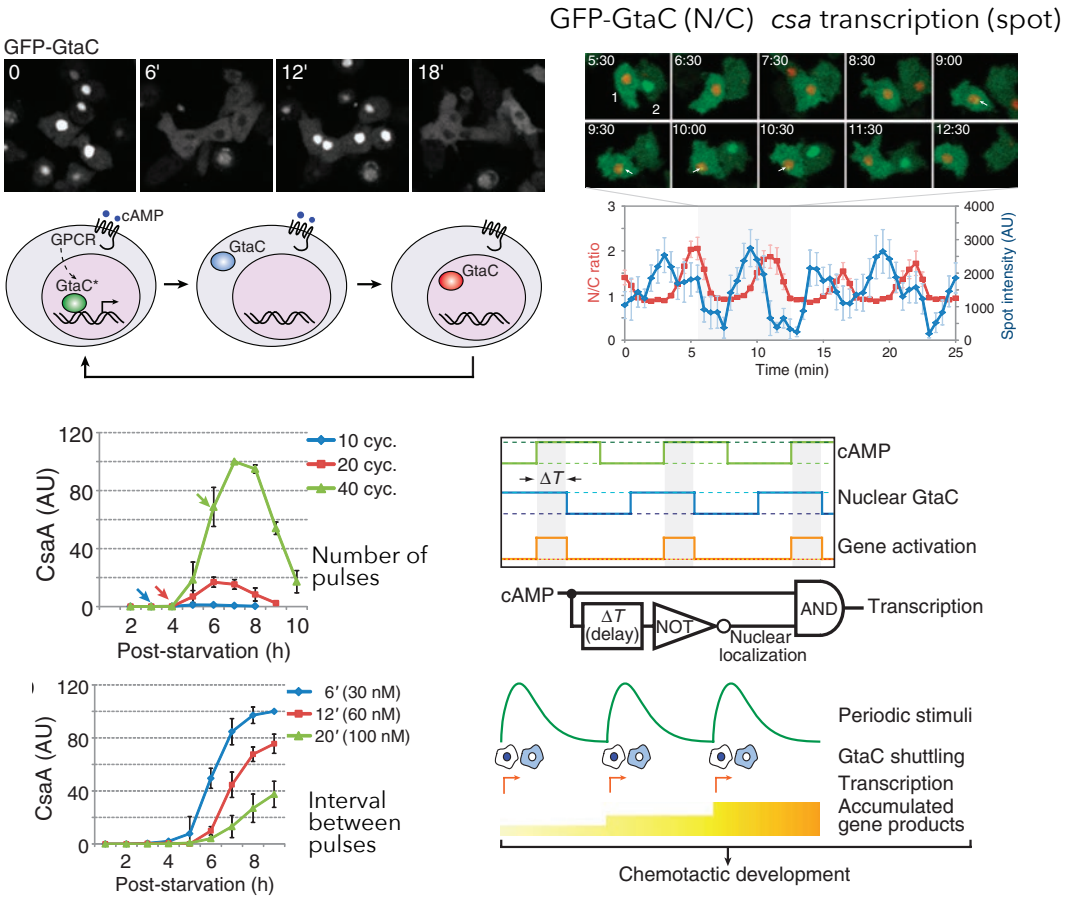
Temporal information in biological signalling

- Decoding *the number of* signalling pulses: counting mechanism

- in the social amoeba *Dictyostelium discoideum* cAMP, starvation induces social aggregation and collective motility.
- This involves waves of cAMP signalling.
- **cAMP waves and oscillatory signalling at the single cell level induce a developmental response. Continuous signalling suppresses this response.**

- ***Decoding*** oscillatory cAMP signalling requires **oscillatory nuclear shuttling of GtaC.**
- cAMP signalling induces nuclear export so persistent signalling blocks transcription.
- cAMP pulses induce burst of target gene activation.

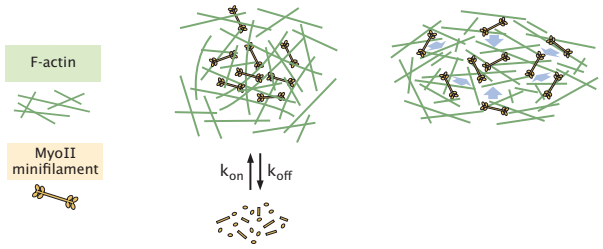
- **The *number of pulses* tunes the accumulation of target gene**

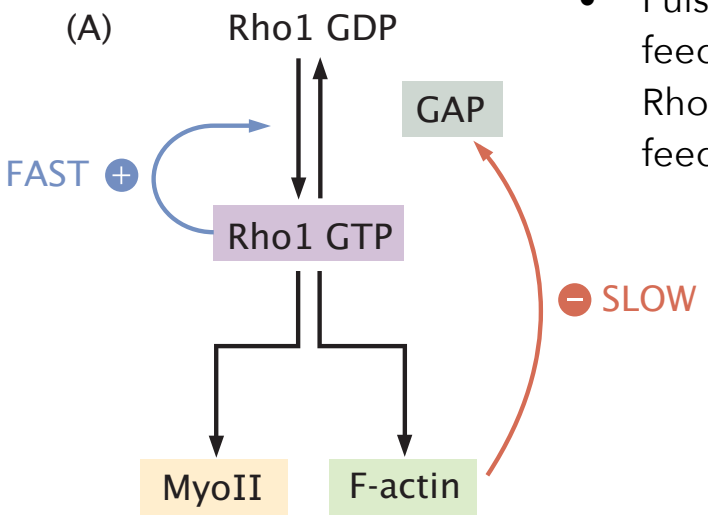


Cai et al., Science 343, 1249531 (2014). DOI: 10.1126/science.1249531

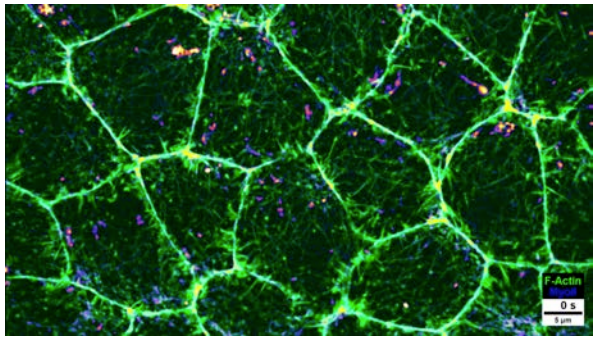
Temporal information in mechanics

- Pulsatile contractions are ubiquitous in animal morphogenesis

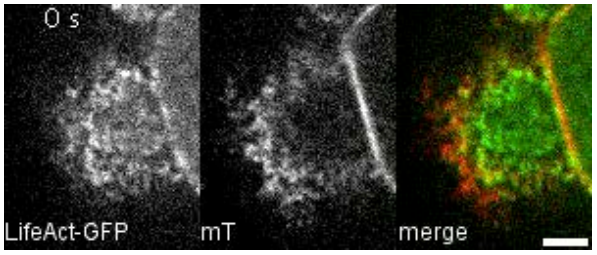




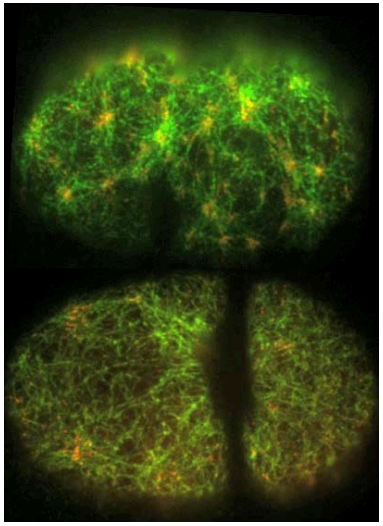
- Pulsatility arises from fast positive feedback (autocatalytic activation of Rho1) and slow or delayed negative feedback



actin and myosin in *Drosophila* embryo, (B. Dehapiot, Lecuit lab)



Mouse blastocyst (JL Maitre, Hiiragi lab)

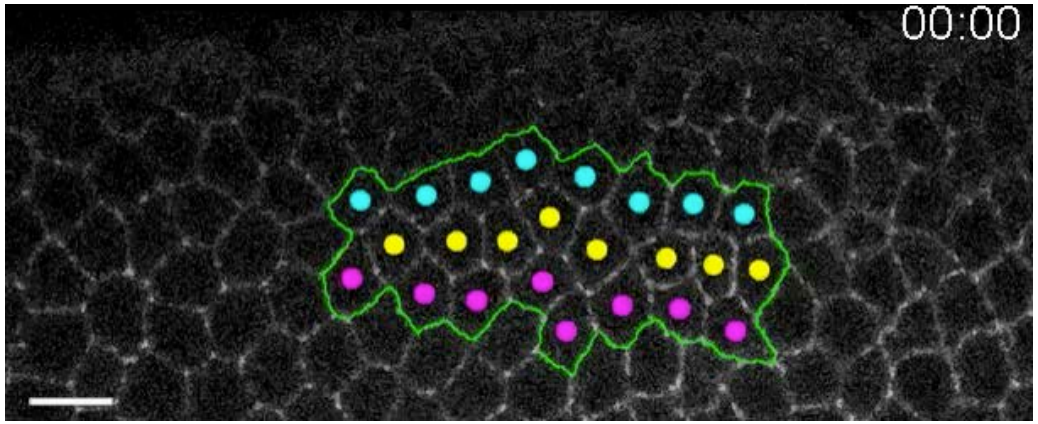


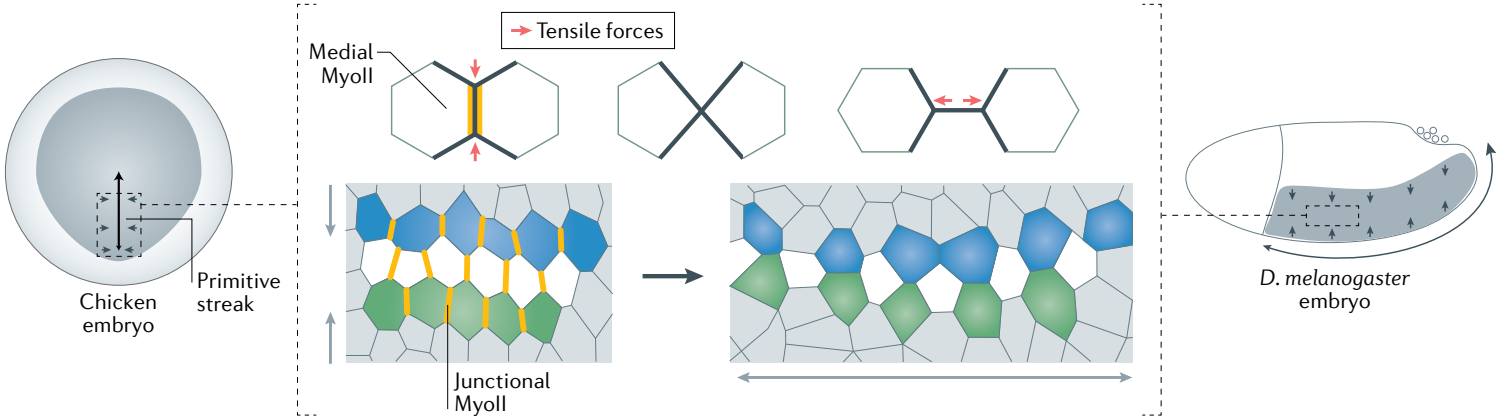
actin and myosin
C. Elegans embryo, (Munro lab)

Temporal information in mechanics

- From pulsatile cell deformations to irreversible tissue flows

- Irreversible and planar polarised changes in the topology of cell interfaces drive cell intercalation and tissue flow in vertebrate and invertebrate embryos.
- Similar to T1 transition in foams.
- This emerges from anisotropic contractile forces at cell junctions.



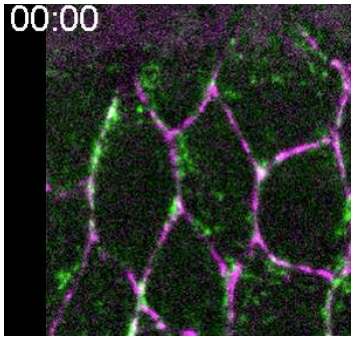


Collinet C. & Lecuit T. *Nature Rev. Mol. Cell Biol.*, (2021)
C. Bertet, L. Sulak and T. Lecuit. *Nature* 429(6992):667-71. (2004)

Temporal information in mechanics

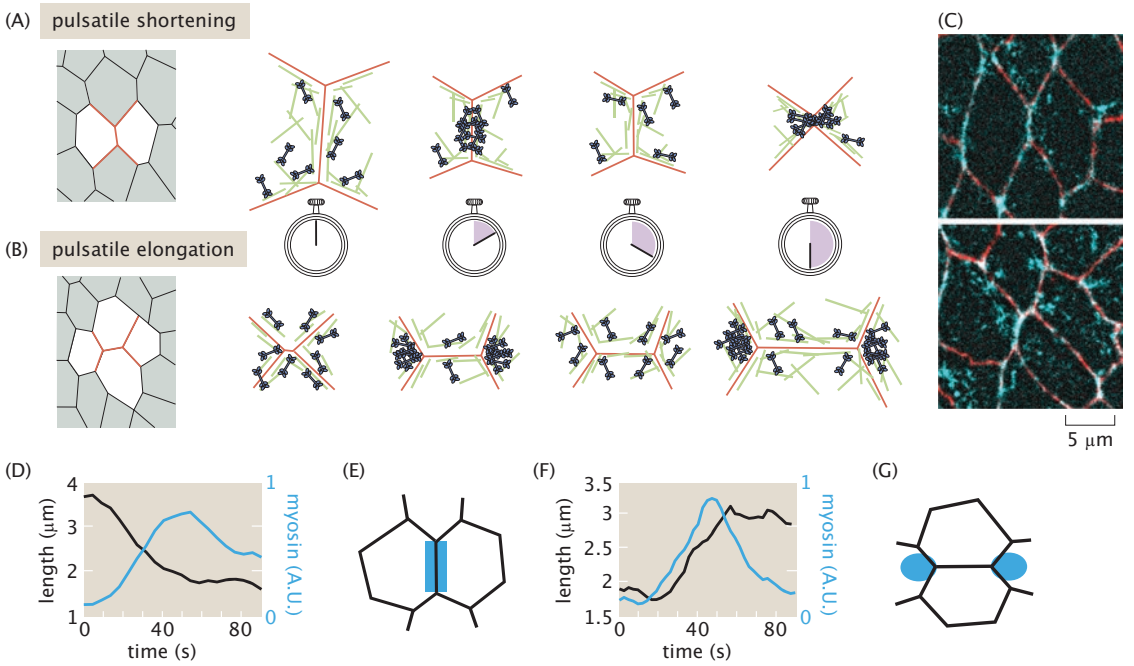
- From pulsatile cell deformations to irreversible tissue extension

- Polarized junction remodelling is sped up by pulsatile and flow of actomyosin contractile networks.



Myosin2
E-cadherin

- How do pulses of actomyosin contraction drive irreversible deformations? (ie. instead of pulsatile and reversible deformations)



M. Rauzi et al. *Nature*. 468(7327):1110-4 (2010)

Collinet C, Rauzi M, Lenne PF, Lecuit T. *NCB* 17(10):1247-58 (2015)

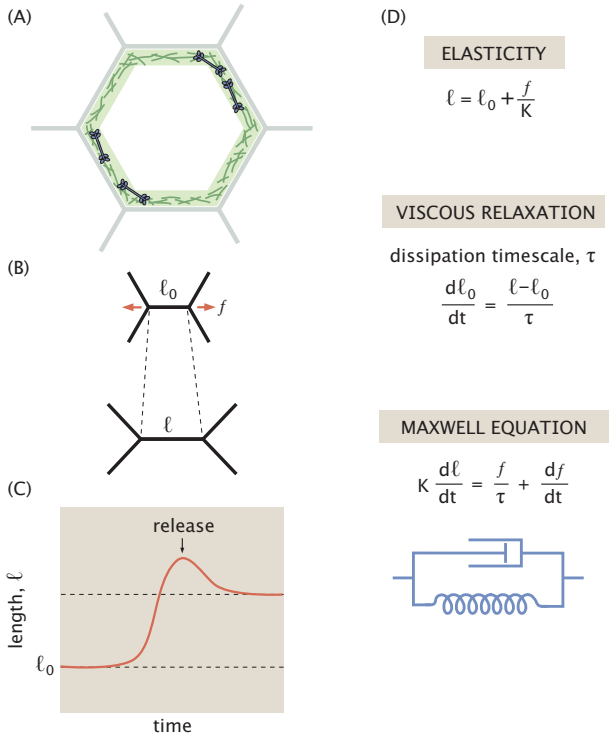
The Restless Cell: Continuum Theories of Living Matter. C. Hueschen and R. Phillips. (2024). *PUP.*

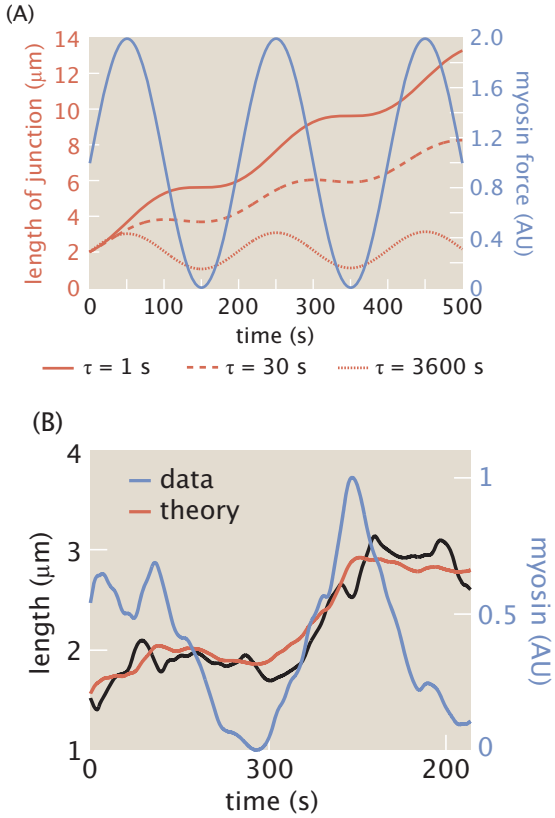
Temporal information in mechanics

- From pulsatile cell deformations to irreversible tissue extension

Computing different mechanical time scales determines the reversibility of deformation

- Time scale of deformation: period of actomyosin pulses ~120s.
- Dissipation time scale (emerging from turnover rate of actin, cross linkers, myosin2, E-cadherin complex binding kinetics etc) dictates junctions dynamics.
- **If time scale of deformation shorter than dissipation time scale, deformation is reversible (~elastic behaviour).**
  If longer, then deformations are irreversible.





Clément, R, Dehapiot, B, Collinet, C, Lecuit, T, Lenne, PF (2017), *Curr Biol* 27 3132-3142 e4.

The Restless Cell: Continuum Theories of Living Matter. C. Hueschen and R. Phillips. (2024). *PUP*

Plan

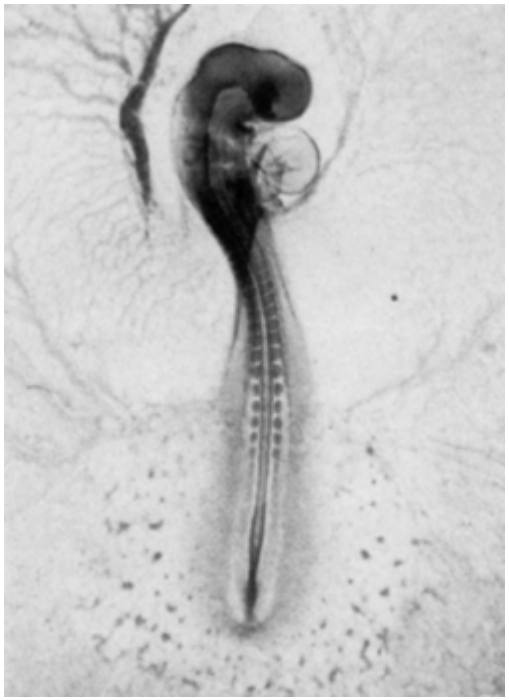
- Time scales in biology:
  – Phenomenology of time and features: nested time scales (from molecules to evolution).
  – Cycles and linear time (counting versus accumulating).

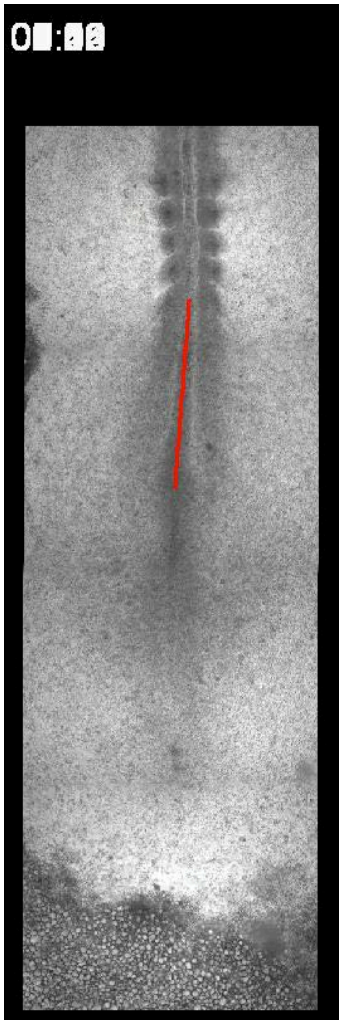
- How is time encoded: defining time scales locally and globally.

- **How is temporal information decoded?**
  – Signalling information: information encoded in dynamics.
  – Mechanical temporal information in morphogenesis.
  – Segmentation clock: decoding time to encode space

Temporal information in morphogenesis

Case study: the segmentation clock





Olivier Pourquié
(Harvard Medical School)

When theory precedes experiments

« L'essence de la théorie des catastrophes c'est de ramener les discontinuités apparentes à la manifestation d'une évolution lente sous-jacente » René Thom.

A Clock and Wavefront Model for Control of the Number of Repeated Structures during Animal Morphogenesis

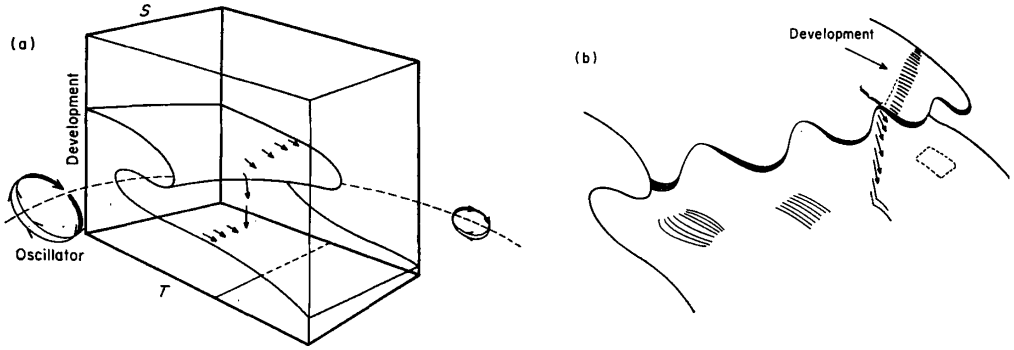
J. COOKE†
National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, England
AND
E. C. ZEEMAN
Institute of Mathematics, University of Warwick, Coventry, Warwick, England

J. theor. Biol. (1976) **58,** 455–476

The model involves an interacting "clock" and "wavefront". The clock is is a smooth cellular oscillator, for which cells throughout the embryo are assumed to be phase-linked. The wavefront is a front of rapid cell change moving slowly down the long axis of the embryo; cells enter a phase of rapid alteration in locomotory and/or adhesive properties at successively later times according to anterior–posterior body position. In the model, the smooth intracellular oscillator itself interacts with the possibility of the rapid primary change or its transmission within cells, thereby gating rhythmically the slow progress of the wavefront. Cells thus enter their rapid change of properties in a succession of separate populations, creating the pattern.

- A model inspired by the « catastrophe theory » (R. Thom)
- A model for scaling of patterns (Turing model: wavelength is not dependent on system size)
- « Implausibility » of positional information based model: too many discrete values to respond to…

- Key features of Clock and Wavefront model:
  - **Wave front** of *sudden* cell changes (discontinuity)
  - **Clock: smooth oscillation** of phase-linked cells
  - **Slow posterior movement** of the wave front



The segmentation clock -discovery

- **Formation of somites is associated with cyclic gene expression**

- The mouse segmentation gene *hairy* shows very dynamic expression patterns even within the 90 min required to produce a new somite.

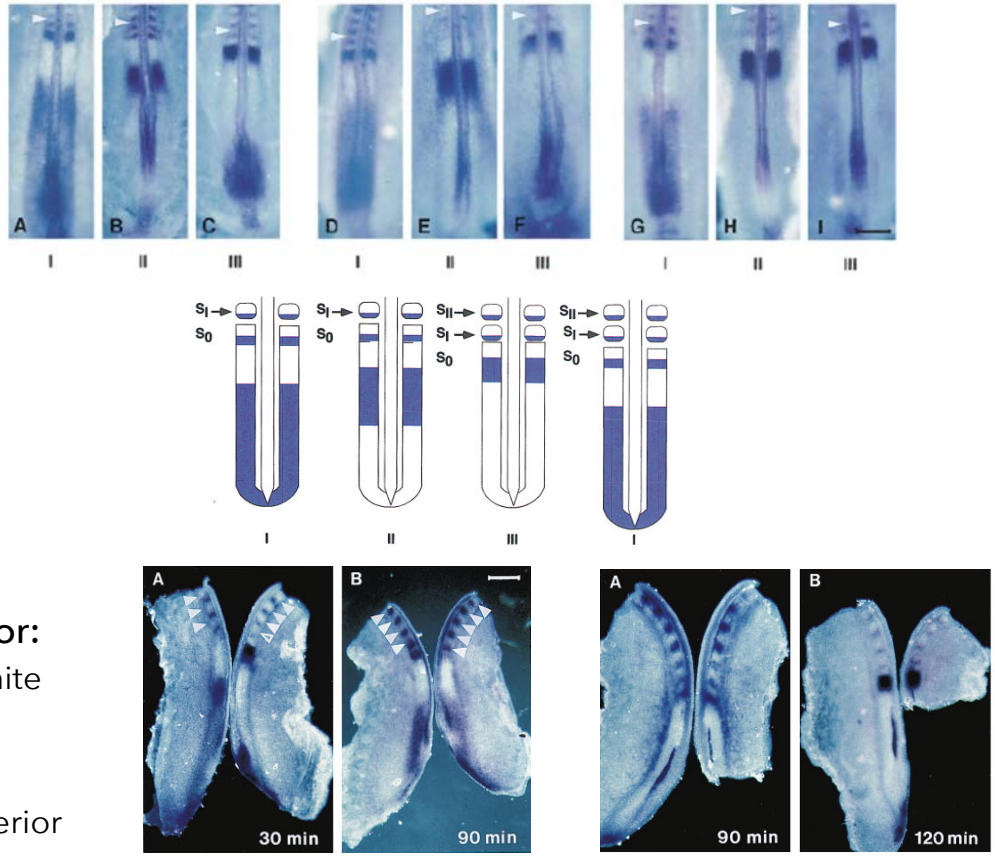
- **Evidence of cyclic gene expression** (T=90min):
  –split embryo in 2 halves, fix the left part immediately and let the right part develop for increasing amount of time, then fix.
  –After 30 min the hairy stripe has shifted to a more anterior region
  –After 90 min, the expression pattern becomes symmetric but a new segmented somite formed.

- **Associated with a kinematic wave towards the anterior:**

The wave stops and is associated with the formation of a new somite

- **The cyclic gene expression is autonomous:**

it does not depend on a signal propagating from posterior to anterior

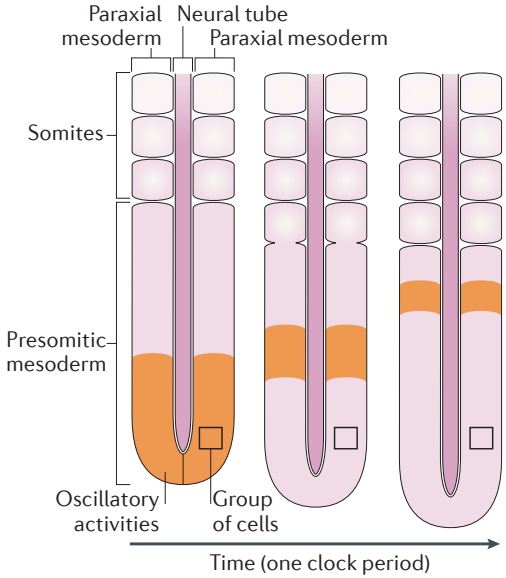


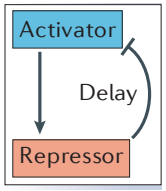
Palmeirim, I., Henrique, D., Ish-Horowicz, D., and Pourquié , O. *Cell* 91, 639–648 (1997)

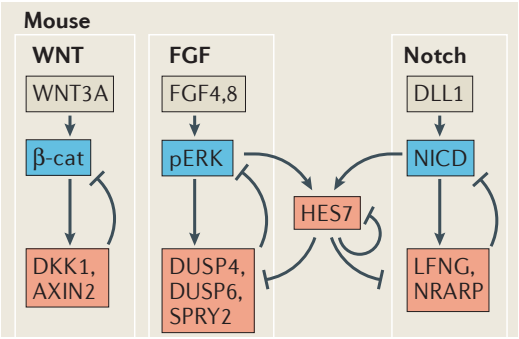
The segmentation clock -cycling genes

- 3 signalling pathways show oscillatory signalling dynamics - mouse/chicken

- The Notch, Wnt and FGF signalling pathways show cyclic expression
- The Notch and FGF pathways are coupled
- **Oscillatory dynamics** of these pathways is associated with negative feedback regulation with a time delay







- Direct visualisation of Lfng-Venus oscillations in living mouse embryos confirms the existence of cyclic gene expression and the emergence of a kinematic wave across the PSM associated with somite formation

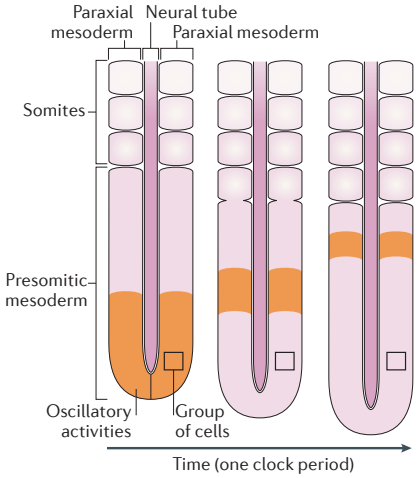
Hubaud and Pourquié. *Nature Rev Mol. Cell Biol*. 15: 709-721 (2014)

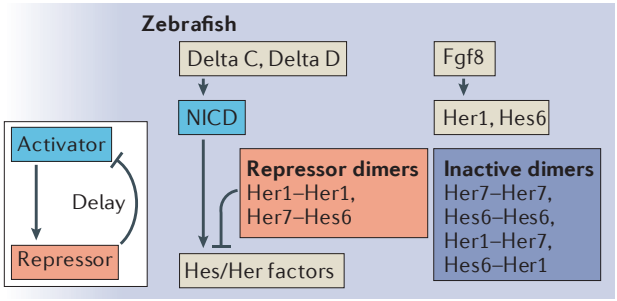
Lauschke, V. M., Tsiairis, C. D., Francois, P. & Aulehla, A. *Nature* 493, 101–105 (2012).

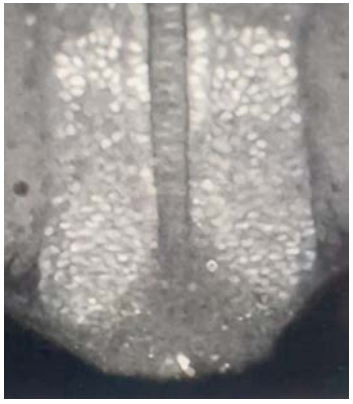
The segmentation clock -cycling genes

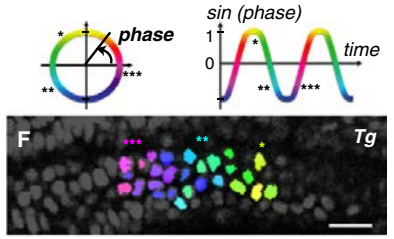
- 3 signalling pathways show oscillatory signalling dynamics - zebrafish

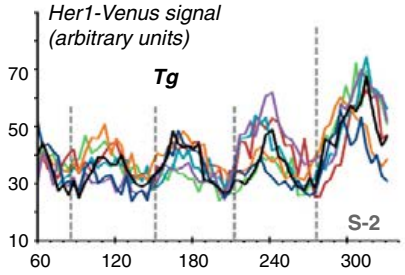
- A segmentation clock is also associated with segmentation of the mesoderm in zebrafish embryos.
- Notch and Fgf signalling are oscillatory
- Oscillations are believed to require a Her1 transcriptional core network.
- Direct observation of Her1YFP expression dynamics reveals synchronous oscillations









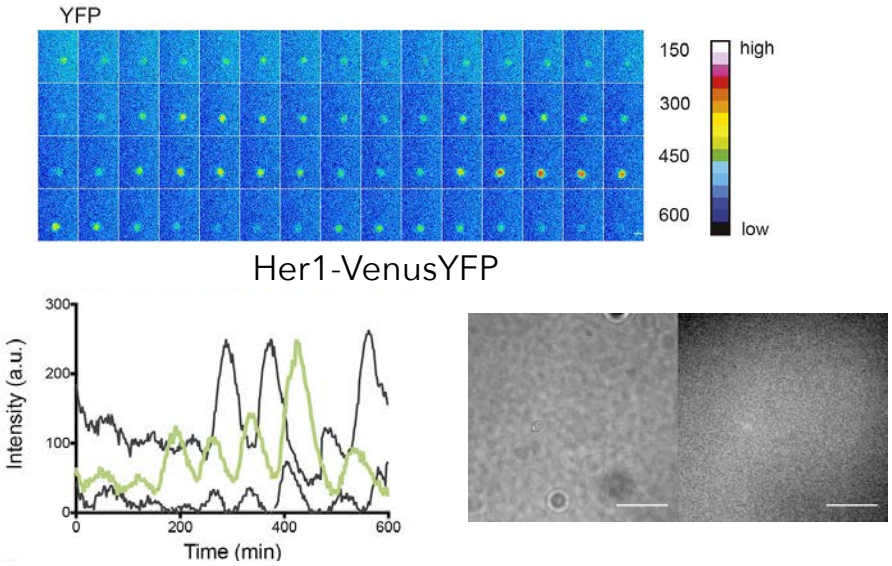


E. Delaune et al, and S. Amacher. *Developmental Cell* 23, 995–1005 (2012)

Oscillations in isolated cells in vitro

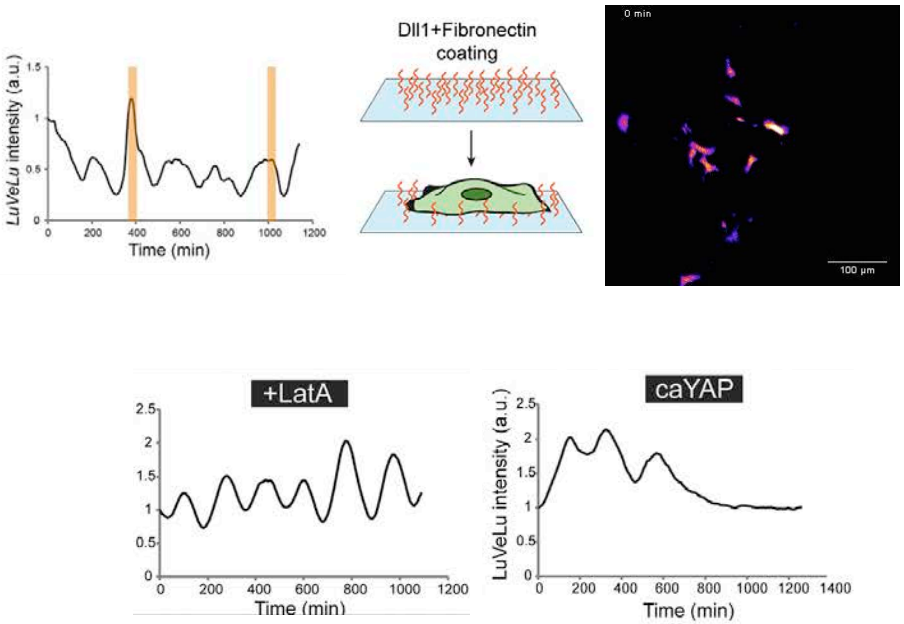
- Dissociated cells from the PSM are oscillatory in vitro

- Oscillations are believed to require a Her1 transcriptional core network.
- Oscillations in dissociated cells are not regular in period and amplitude.



A.B. Webb et al. and A.C. Oates *eLife* 2016;5:e08438.

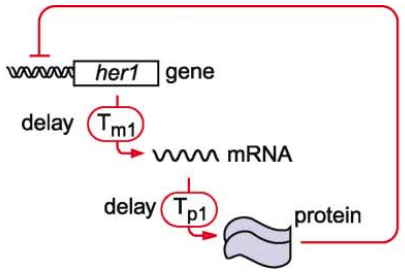
Yap-dependent mechanical signal acts as a control parameter for single cell oscillations



Hubaud et al, Mahadevan, Pourquié. *Cell* 2017 171, 668–682

Single cell oscillator: Autoinhibition with a delay

- Facts:
- Oscillations are independent of Notch signalling.
- Oscillations are observed in isolated cells.
- Oscillations are believed to require a Her1 transcriptional core network.

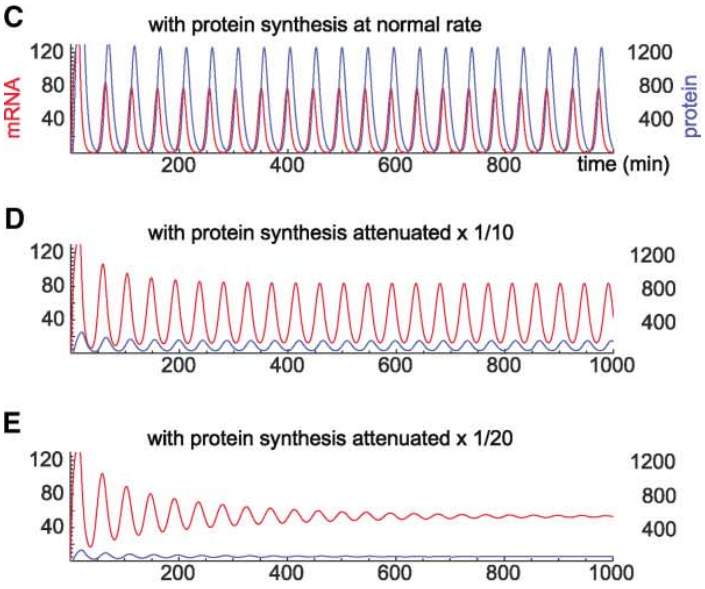


- **Model of single cell oscillator based on delayed auto inhibition (negative feedback). J. Lewis.**
- The delay may emerge from production of the Her1 mRNA and protein.
- The dynamics of mRNA m(t) depends on protein concentration at t-delay.

$$\frac{dp(t)}{dt} = am(t - T_p) - bp(t)$$

$$\frac{dm(t)}{dt} = f(p(t - T_m)) - cm(t)$$

$$f(p) = \frac{k}{1 + p^2/p_0^2},$$



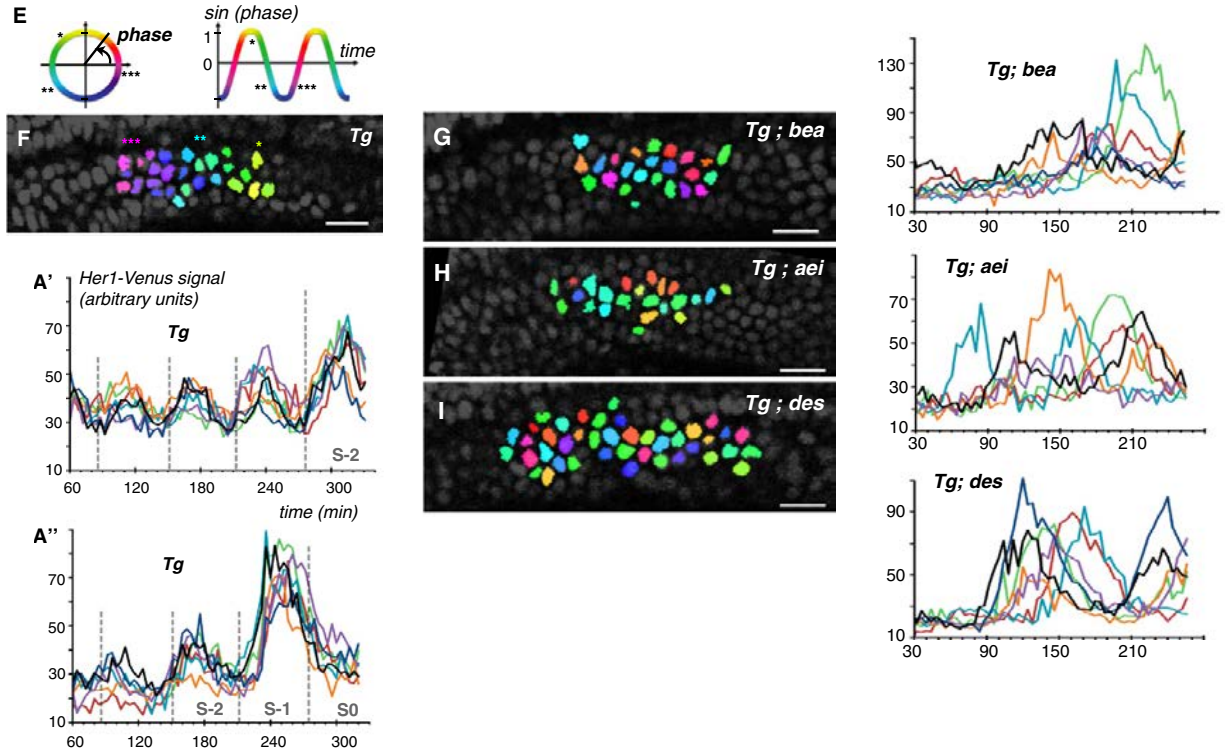
- **Sustained oscillations require production rate of protein beyond a critical value p_0** (for negative FB to manifest). Or else, damped oscillations due to degradation.

Lewis, J. *Curr. Biol*. 13, 1398–1408 (2003).

Oscillator coupling during segmentation

- Single cell oscillations are synchronous in the presomitic mesoderm.
- **Emergence of a phase gradient** along the PSM.
- In conditions that reduce Notch signalling, cells still oscillate in the PSM, but cells are out of phase.
- **Notch is not required for single cell oscillations but for coupling of individual oscillators.**

Jiang, Y. J. et al. and J. Lewis. Notch signalling and the synchronization of the somite segmentation clock. *Nature* 408, 475–479 (2000).



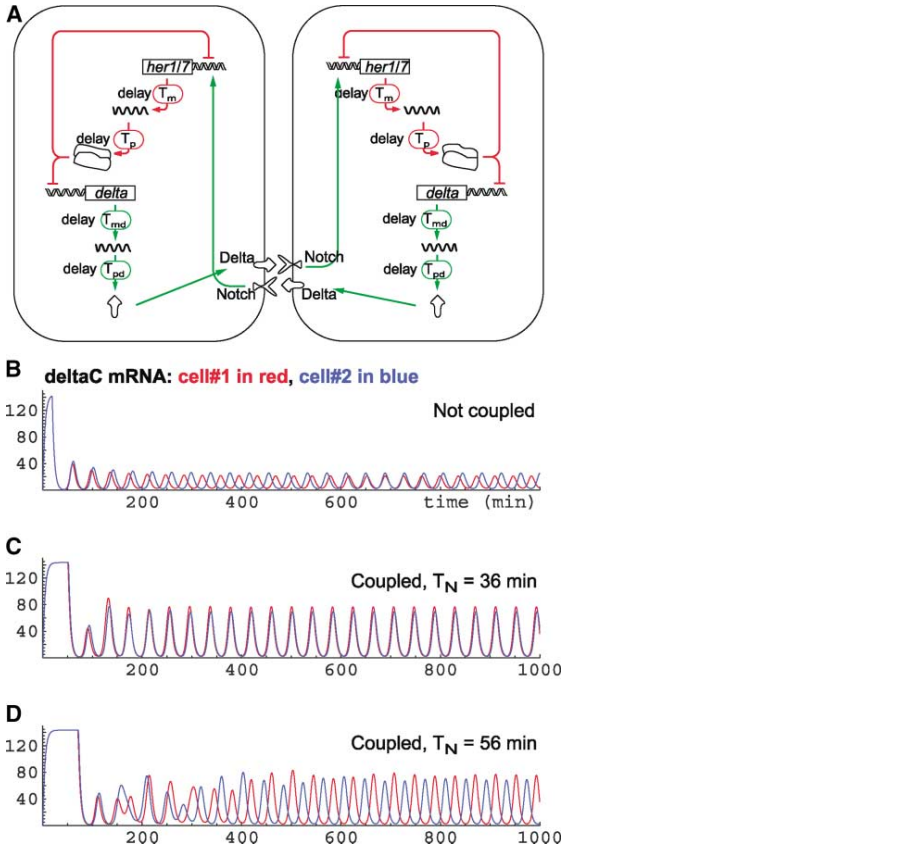
E. Delaune et al, and S. Amacher. *Developmental Cell* 23, 995–1005 (2012)

Oscillator coupling during segmentation

- Notch is not required for single cell oscillations but for coupling of individual oscillators.

- Model of coupled oscillators: J. Lewis
- The synchronisation of 2 neighbouring cells require **a specific time delay.** This delay is associated with production of eg. Delta ligand mRNA, protein, export to the cell surface, activation of Notch in neighbouring cell.

- **Activation of Her1 depends on delayed negative FB (intrinsic oscillator) and on Notch positive input via coupling.** Depending on the length of the delayed coupling T_N, synchronisation may or may not be possible.



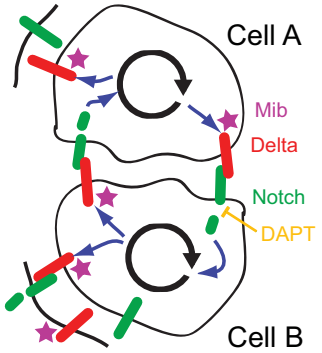
Lewis, J. *Curr. Biol*. 13, 1398–1408 (2003)

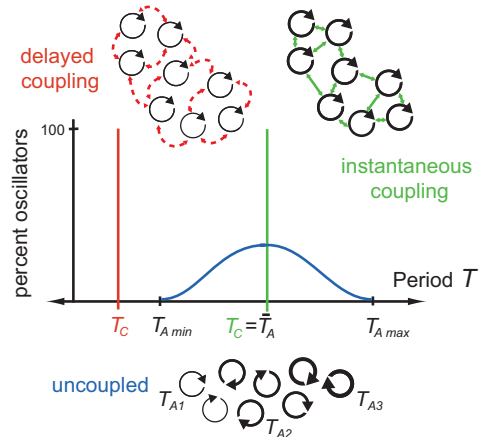
Jiang, Y. J. et al. and J. Lewis. *Nature* 408, 475–479 (2000)

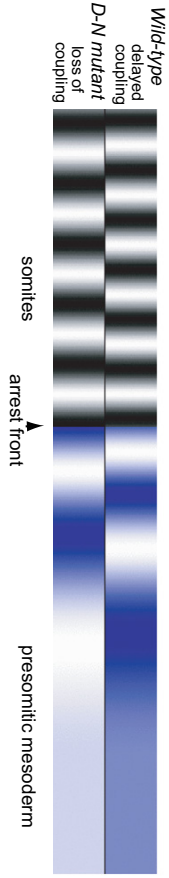
See earlier also: Winfree AT. *J Theor Biol* ;16:15–42 (1967)

Oscillator coupling during segmentation

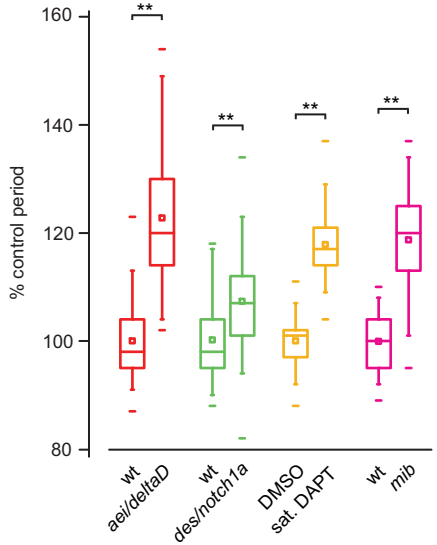
- Model of coupled oscillators: F. Jülicher.
- Delayed coupling is expected to modify the collective period of oscillation.







- Experimental observation of increased period in conditions where Notch signalling is reduced consistent with the model.



L. Herrgen et al., F. Jülicher and A. Oates *Current Biology* 20, 1244–1253 (2010)

From *time* encoding to *space* decoding

A Clock and Wavefront Model for Control of the Number of Repeated Structures during Animal Morphogenesis

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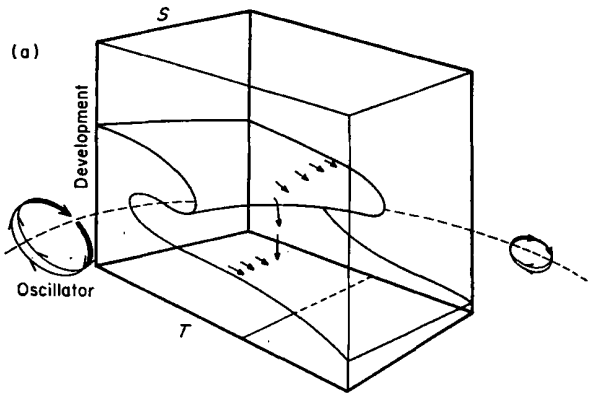
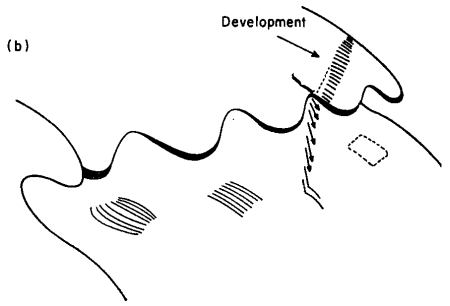
J. theor. Biol. (1976) **58**, 455–476

The model involves an interacting "clock" and "wavefront". The clock is is a smooth cellular oscillator, for which cells throughout the embryo are assumed to be phase-linked. The wavefront is a front of rapid cell change moving slowly down the long axis of the embryo; cells enter a phase of rapid alteration in locomotory and/or adhesive properties at successively later times according to anterior–posterior body position. In the model, the smooth intracellular oscillator itself interacts with the possibility of the rapid primary change or its transmission within cells, thereby gating rhythmically the slow progress of the wavefront. Cells thus enter their rapid change of properties in a succession of separate populations, creating the pattern.

- Key features
  - **Wave front** of *sudden* cell changes (discontinuity)
  - **Clock: smooth oscillation** of phase-linked cells
  - **Slow posterior movement** of the wave front



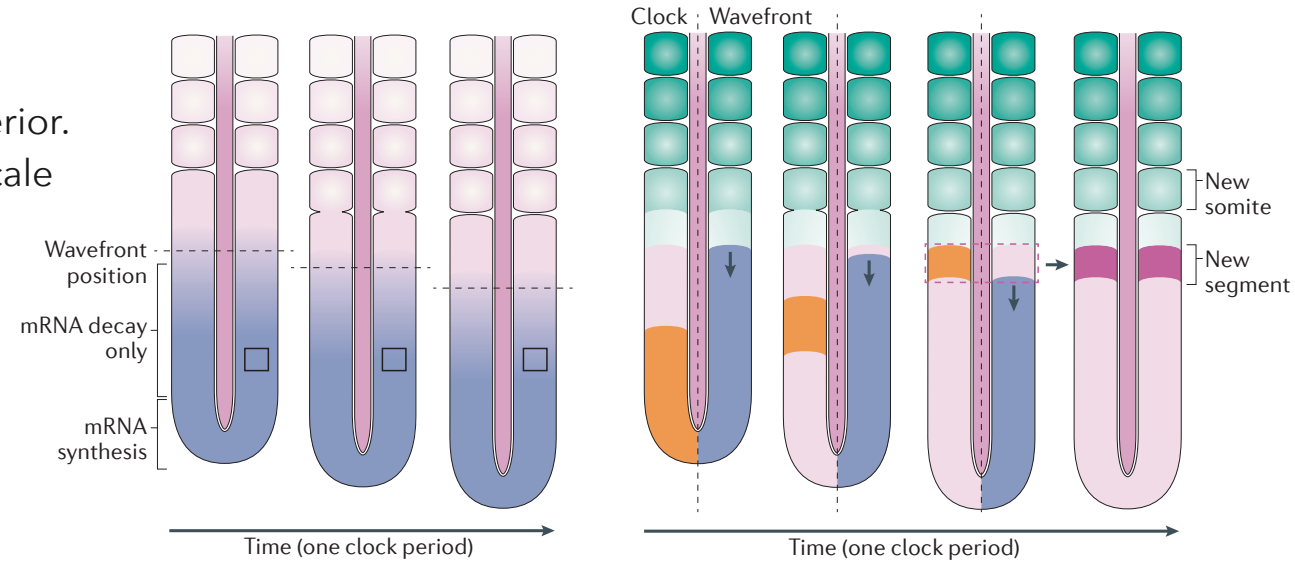


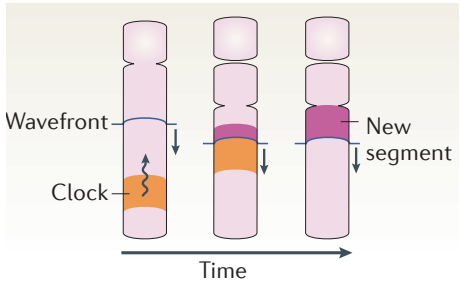
From *time* encoding to *space* decoding

- The clock and wave front model - the principle

- Antero-posterior gradient of FGF protein.
- As the PSM grows, it shifts towards the posterior.
- This leads to the posterior shift of the FGF scale invariant gradient.

- **The clock (orange) and wavefront (blue line) are independent entities** that determine the segments.
- Only one phase of the clock (orange) triggers segment determination (pink).
- The position of the wavefront determines the position of the posterior boundary of a newly determined segment.

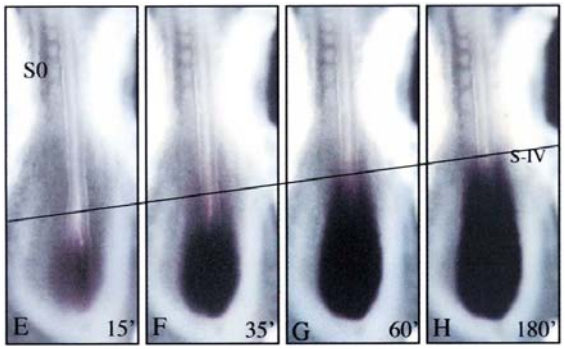




Hubaud and Pourquié. *Nature Rev Mol. Cell Biol*. 15: 709-721 (2014)

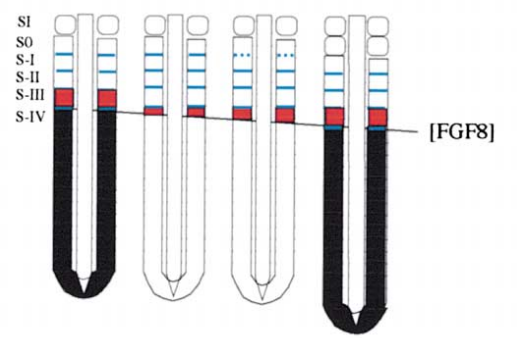
From *time* encoding to *space* decoding

- The clock and wave front model - the evidence



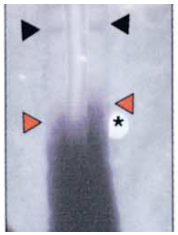
FGF8 gradient front

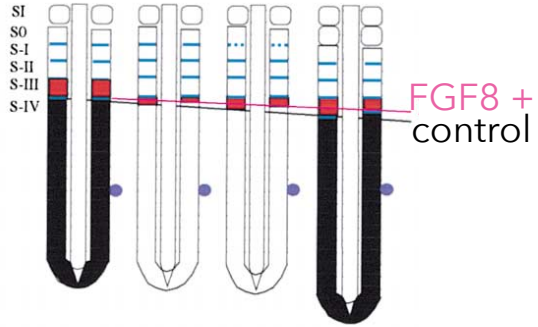
exposure time



- Posterior regression of an FGF8 gradient determines the position of somites

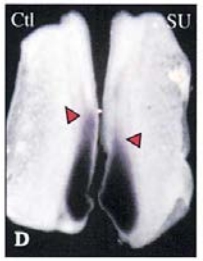
FGF8 bead (*)

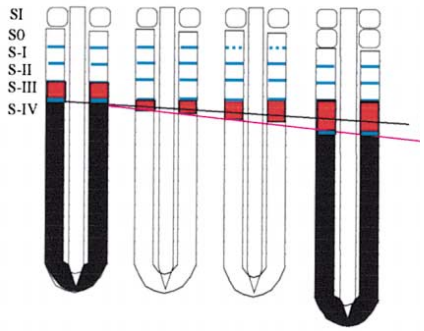




- Anterior shift in FGF8 front
- Smaller, anterior shifted somite

FGF8 inhibition (drug)





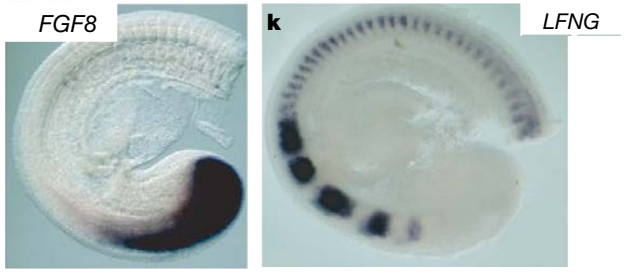
- Posterior shift in FGF8 front
- Larger, posterior shifted somite

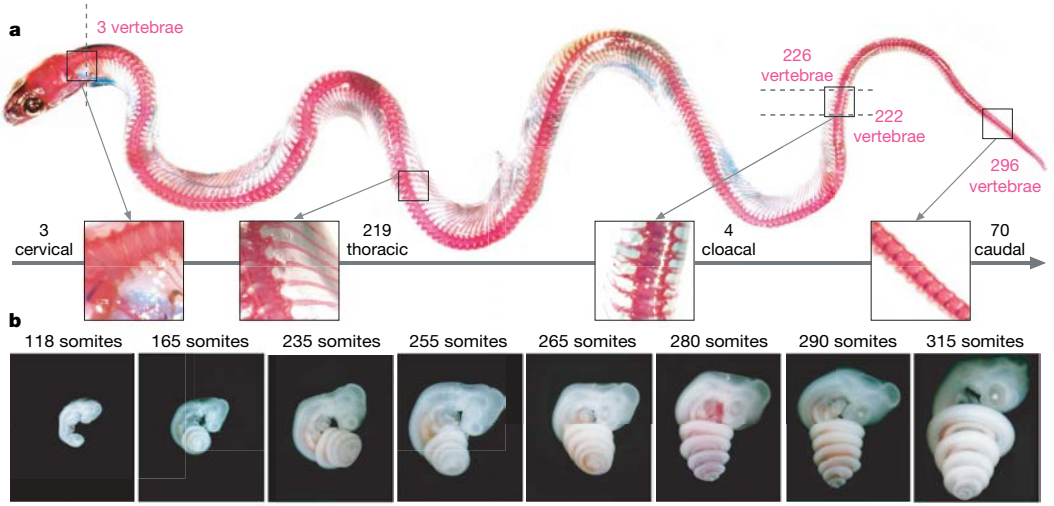
J. Dubrulle, M.J. McGrew and O. Pourquié. *Cell*, 106, 219–232 (2001)

From *time* encoding to *space* decoding

- Variations in segment number : snakes

- Snakes have a large number of vertebrae (315 in corn snakes).
- The segmentation clock in snakes is presumably the same as in chick embryos…
  – clock genes: Notch, LFng.
  – wave front: FGF8, Wnt3A that re



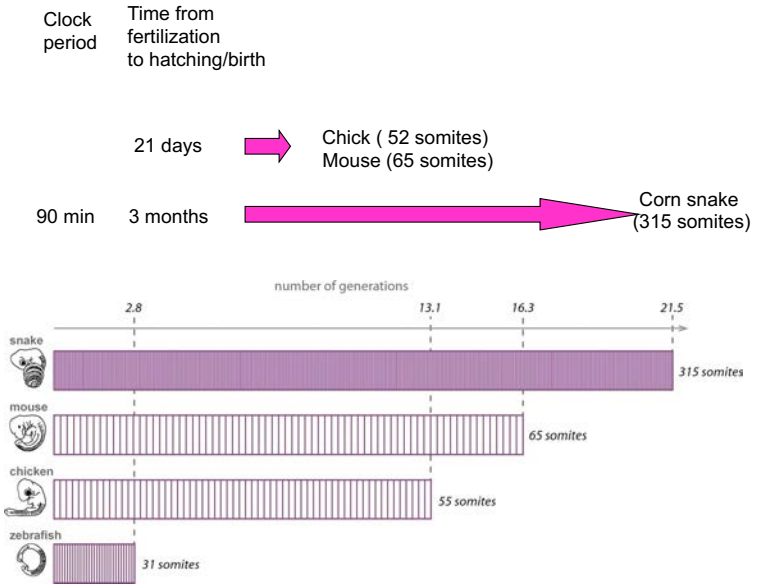


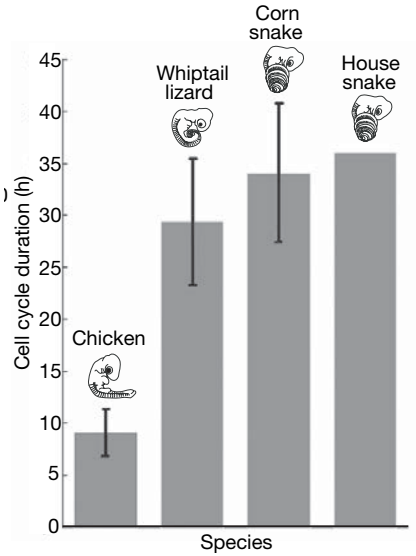
Gomez C, et al. J. Lewis and O. Pourquié, *Nature*, 454(7202):335-9 (2008).

From *time* encoding to *space* decoding

- **Variations in segment number : computing the difference between clock and developmental time scales.**

- The clock period in corn snake embryos is 90 min, similar to chick and mouse embryos.
- The growth of the PSM is slower in snakes (3-3.5x) but lasts longer.
- Thus, the posterior movement of the wavefront is presumably slower than in chick.
- **The relative time constant of the clock with respect to cell/tissue growth accounts for smaller size of somites in snakes** over the same embryonic time window.
- **Since development lasts longer, the number of segments is much larger.**



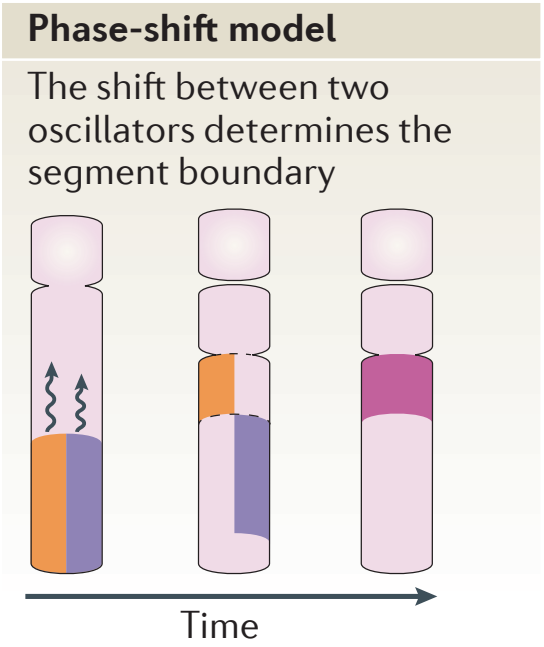


Gomez and Pourquié https://doi.org/10.1002/jez.b.21305

Gomez C, et al. J. Lewis and O. Pourquié, *Nature*, 454(7202):335-9 (2008).

From *time* encoding to *space* decoding

Modulation of Phase Shift between Wnt and Notch signaling oscillations controls segmentation



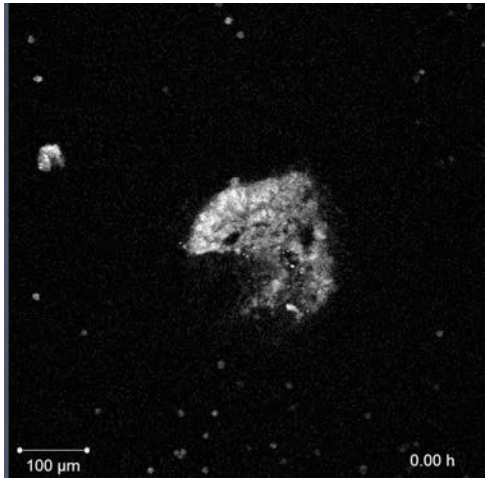
From *time* encoding to *space* decoding

Phase-shift model of segmentation

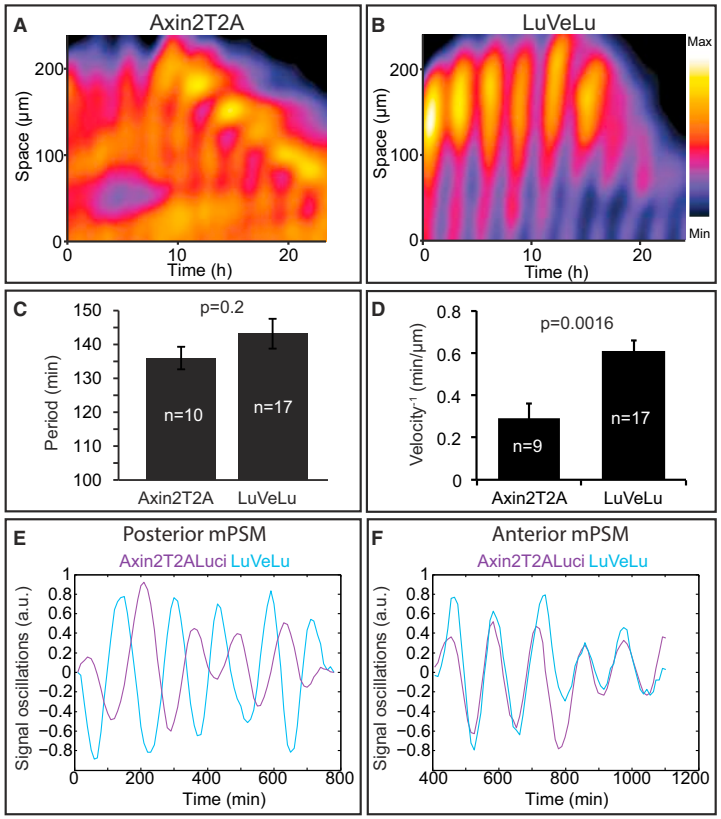
- Ex vivo cultured mouse PSM cells produce **kinematic waves and segments.**
- **Both Notch and Wnt oscillate and produce kinematic waves.**

- **Relative phase-shift between Wnt and Notch signaling oscillations is changing along the PSM length.**

Lfng-VenusYFP



Wnt signalling Notch signalling



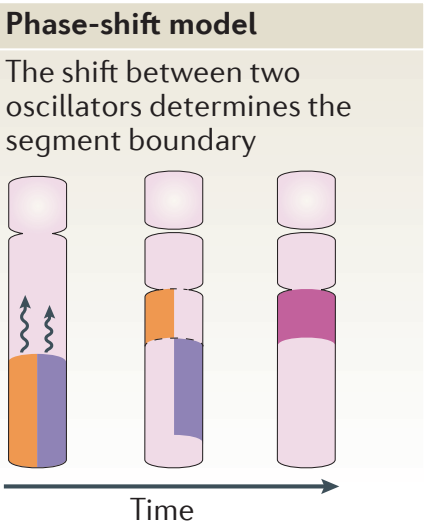
Sonnen et al. and A. Aulehla, *Cell* 172, 1079–1090 (2018)

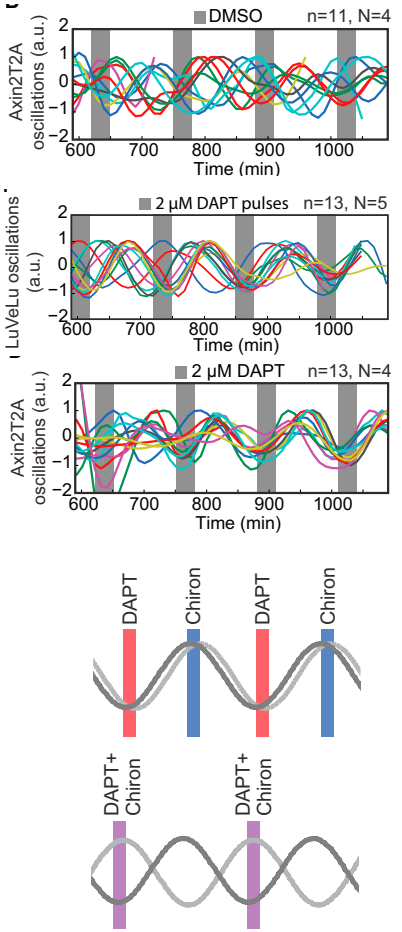
From *time* encoding to *space* decoding

Phase-shift model of segmentation

- Using microfluidic, entrainment of Wnt and Notch oscillations by drugs that activate Wnt (Chiron) or inhibit Notch (DAPT).
- Cross-entrainment between two oscillators.

- Entraining the endogenous rhythms with simultaneous pulses of Chiron and DAPT, resulted in anti-phase Wnt and Notch signalling oscillations in anterior PSM, and led to segmentation defects.
- **Relative timing of Wnt and Notch signaling oscillations is critical for segmentation.**





Sonnen et al. and A. Aulehla, *Cell* 172, 1079–1090 (2018)

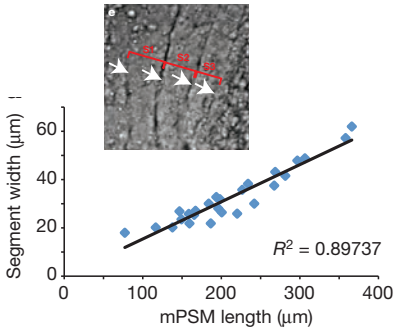
From *time* encoding to *space* decoding

- Scaling of segmentation based on phase-gradient encoding

- Ex vivo cultured mouse PSM cells produce **kinematic waves and segments.**
- **There is a phase shift between neighbouring cells and a phase gradient** across the PSM.

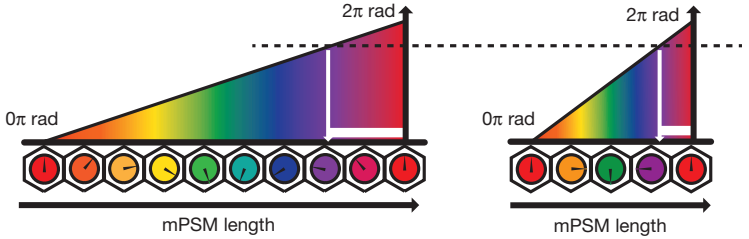
$$v = \partial\varphi/\partial t \,\big/\, \partial\varphi/\partial x$$

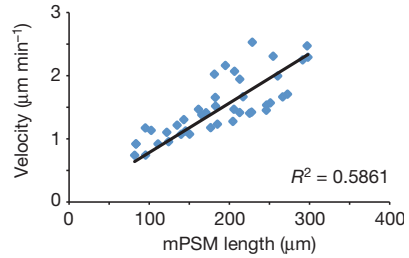
velocity of wave | clock frequency | phase gradient

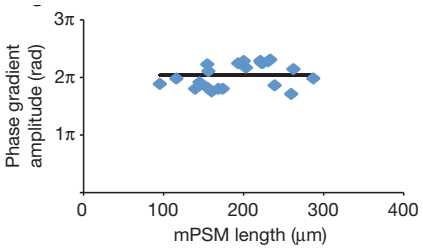


Scaling mechanism:

- As the PSM length shortens, segments become smaller.
- This indicates **scaling of segments to tissue size**
- The velocity of the wave also scales with PSM length.







- **The amplitude of the phase gradient is 2π irrespective of tissue size.**
- **Therefore, the phase gradient scales with tissue size.**

Lauschke, V. M., Tsiairis, C. D., Francois, P. & Aulehla, A. *Nature* 493, 101–105 (2012).

Conclusions

- Time can be *encoded* locally and globally in variety of ways:
  - Chemical systems (diffusion, trigger wave), mechanical systems (advection, material properties)
  - Importance of energetics/metabolism.

- How is temporal information *decoded*?
  - **Signalling: information decoded in *dynamics* of signal.**
    - signal duration
    - signal frequency
    - signal burst counts etc
  - **Mechanical deformation** in morphogenesis: **information decoded through *comparison of different time scales*.**
    - eg. deformation and viscous relaxation, or growth and relaxation.
  - **Developmental patterning**
    - The segmentation clock: **decoding time to encode space**
    - Neuronal identity: **temporal encoding of transcription factor series.**