dynamic processes in cells (a systems approach to biology)

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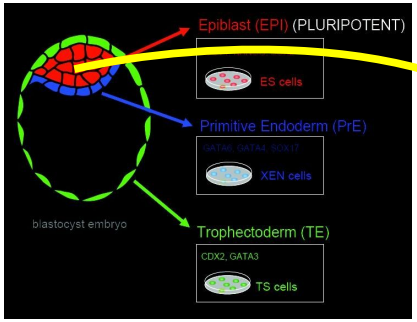
lecture 10

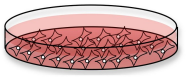
8 october 2015

pluripotency in embryonic stem (ES) cells

pre-implantation
mouse blastocyst

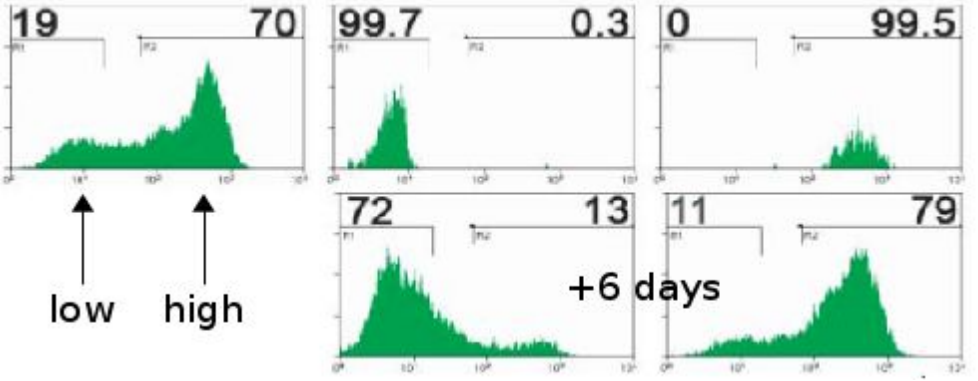


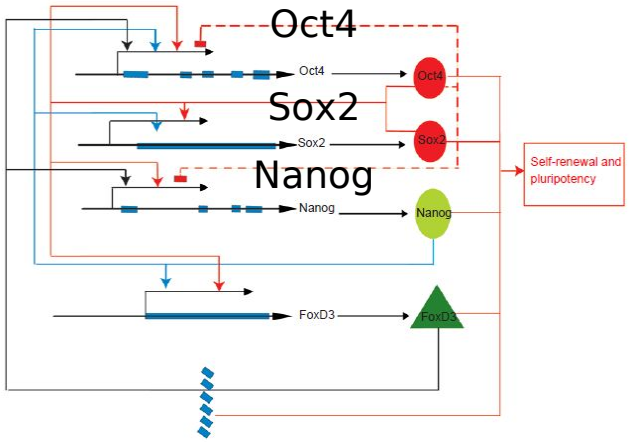
serum + LIF,
2i

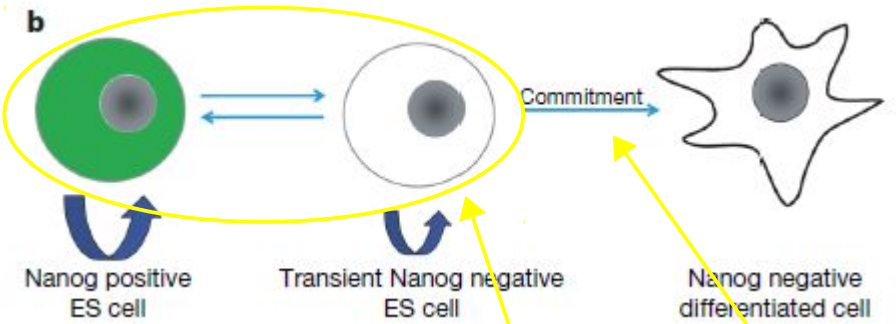


ES cells

ES cells, GFP expression from Nanog locus



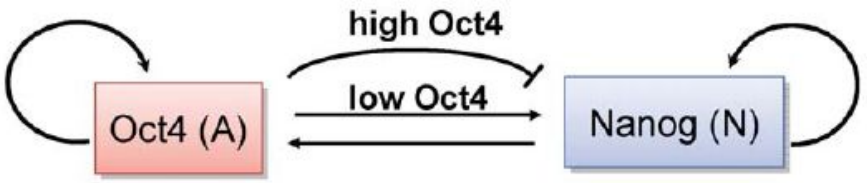




weak linkage by variation/selection (lecture 6)

Chambers et al, "*Nanog safeguards pluripotency and mediates germline development*", Nature 450:1230-4 2007; Kalmar et al *"Regulated fluctuations in Nanog expression mediate cell fate decisions in embryonic stem cells"*, PLoS Biol **7**:e1000149 2009;

excitability

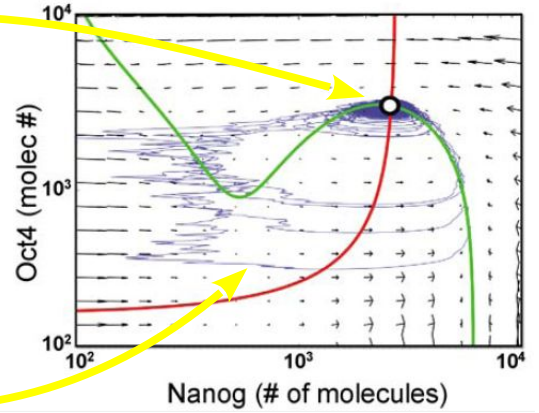


Hill-like functions n = 2, p = 1.5

$$\frac{dN}{dt} = \alpha_n + \frac{\beta_n N^n}{k_n^n + N^n} - \delta \frac{A^p}{k_x^p + A^p} N - \gamma_n N$$

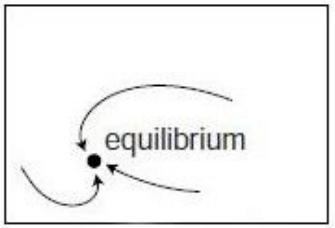
$$\frac{dA}{dt} = \alpha_a + \beta_a A N - \gamma_a A$$

there is a single, **stable steady state** with a small stability region, outside of which trajectories make **long excursions** before returning to the steady state

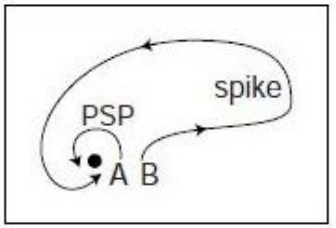


not potential dynamics!

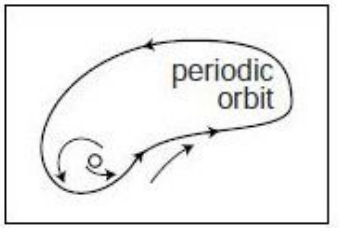
monostability



excitability

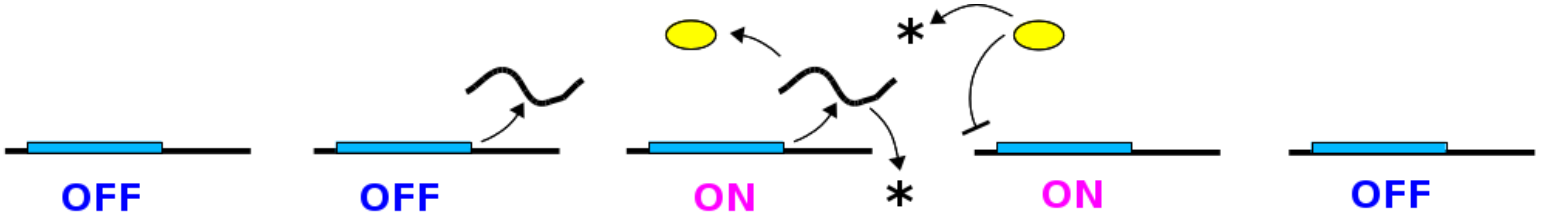


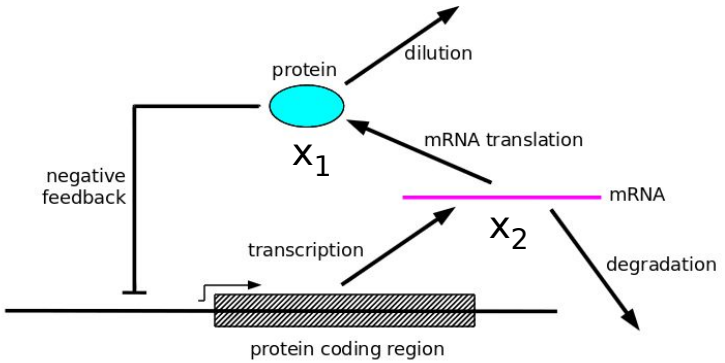
oscillation



Eugene Izhikevich, **Dynamical Systems in Neuroscience: The Geometry of Excitability and Bursting**, MIT Press, 2007

negative feedback – homeostasis or oscillation?



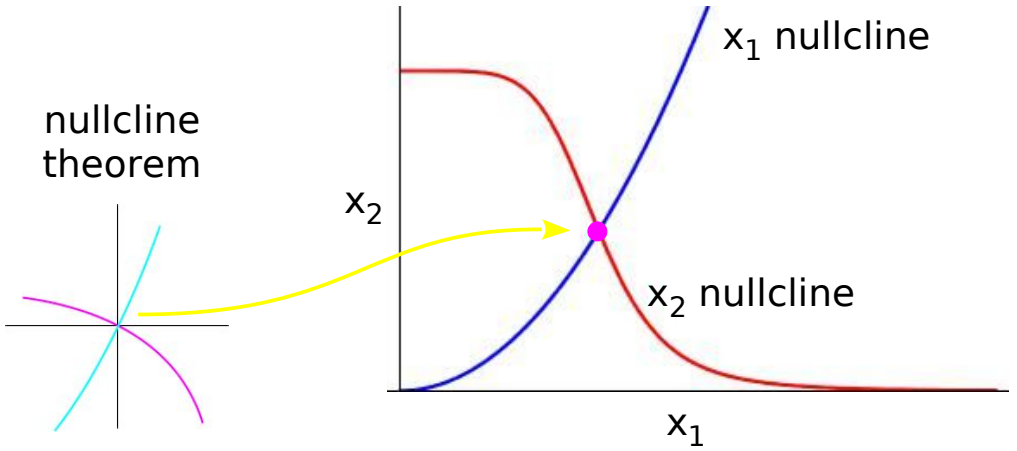


$$\frac{dx_1}{dt} = f(x_2) - ax_1$$

$$\frac{dx_2}{dt} = g(x_1) - bx_2$$

$$g(x_1) = \frac{\alpha}{K + x_1^n}$$

repressive Hill-like function

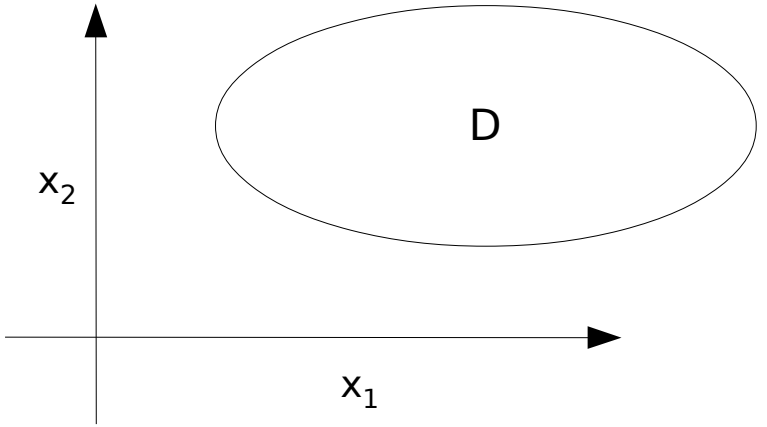


Bendixson's negative criterion

given a 2D nonlinear dynamical system

$$\frac{dx_1}{dt} = f_1(x_1, x_2)$$

$$\frac{dx_2}{dt} = f_2(x_1, x_2)$$



if D is a bounded, connected region with no holes and the trace of the Jacobian

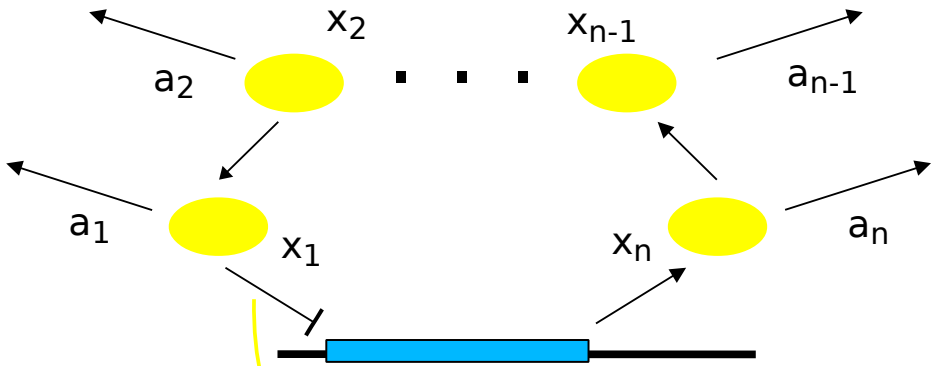
$$\mathrm{Tr}(Df) = \frac{\partial f_1}{\partial x_1} + \frac{\partial f_2}{\partial x_2}$$

has the same sign (and is never 0) throughout D, then there are no periodic trajectories in D

for negative auto-regulation, $\mathrm{Tr}(Df) = -(a+b)$ **so no oscillation no matter how sharply the gene is turned off**

taking delays into account

there is a trade-off between **cooperativity** of repression and **time delay**, or the number of components around the feedback loop, for oscillation



$$\frac{dx_1}{dt} = x_2 - a_1 x_1$$

$$\frac{dx_2}{dt} = x_3 - a_2 x_2$$

$$\vdots \quad \vdots \quad \vdots$$

$$\frac{dx_{n-1}}{dt} = x_n - a_{n-1} x_{n-1}$$

$$\frac{dx_n}{dt} = \frac{1}{1 + x_1^p} - a_n x_n$$

repressive Hill-like function

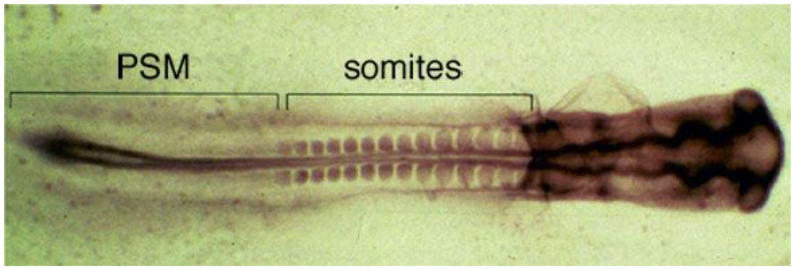
there can be no sustained oscillations if $p < \sec^n(\pi/n)$

secant conditon

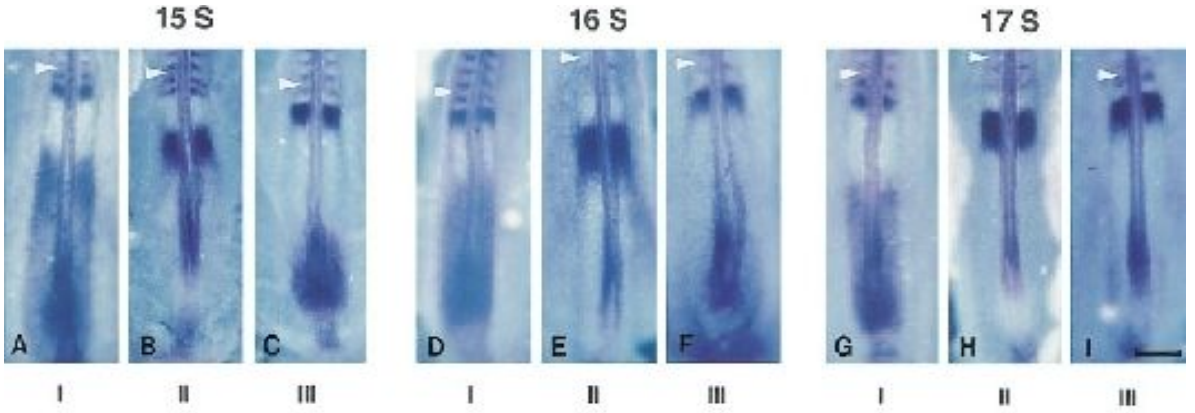
John Tyson, Hans Othmer, *"The dynamics of feedback control circuits in biochemical pathways"*, Progress in Theoretical Biology **5**:1-62 1978

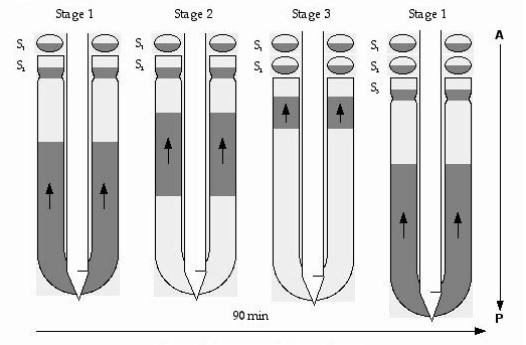
the somitogenesis clock

chick embryo



in-situ hybridisation against c-hairy1 mRNA



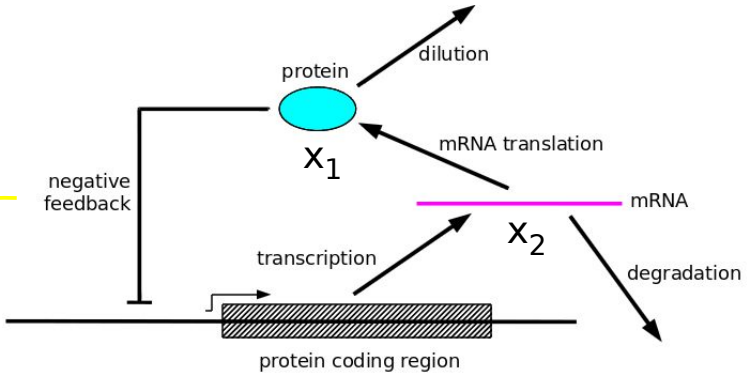


travelling wave of c-hairy1 expression is generated by a cell-autonomous oscillator with a period of 90 minutes, that does not stop when protein synthesis is blocked

Palmeirim, Henrique, Ish-Horowicz, Pourquie,*"Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis"*, Cell **91**:639-48 1997

differential-delay equations

explicit accounting for **time delay** in transcription, T_m, and translation, T_p



1946-2014

$$\frac{dx_1(t)}{dt} = ax_2(t - T_p) - bx_1(t)$$

$$\frac{dx_2(t)}{dt} = f(x_1(t - T_m)) - cx_2(t)$$

$$f(u) = \frac{k}{1 + (u/u_0)^2}$$

repressive Hill-like function

differential-delay equations are infinite-dimensional dynamical systems

Julian Lewis, *"Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator"*, Current Biol **13**:1398-408 2003; Monk, Curr Biol **13**:1409-13 2003

Arthur Lander, "*Making sense of in biology: an appreciation of Julian Lewis*", BMC Biol **12**:57 2014

parameters chosen from the literature

a	protein synthesis rate	4.5 molecules/transcript
b	protein degradation rate	0.23 molecules/minute
c	mRNA degradation rate	0.23 molecules/minute
k	maximal mRNA synthesis rate	33 molecules/minute (1000 transcripts/hour)
u_0	feedback threshold	40 molecules (1nM in a 5 micron diameter nucleus)

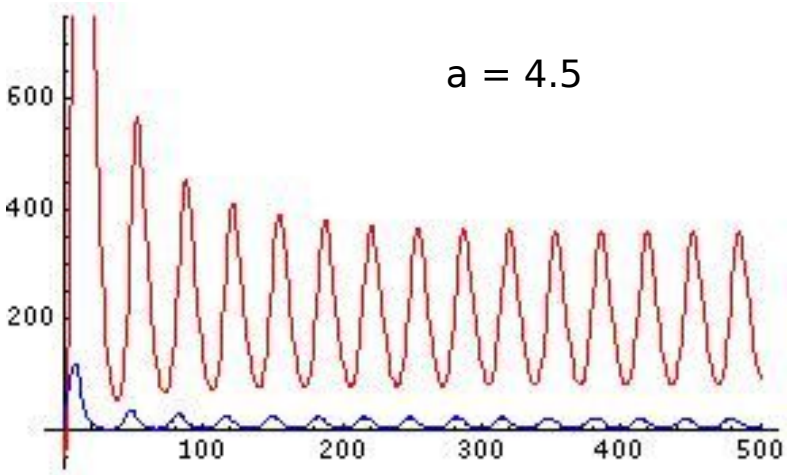
RNA Pol II speed	20 bp/sec
intron splicing	1 minute per intron
nucleo-cytoplasmic transport	4 minutes
ribosome speed	6 bp/sec

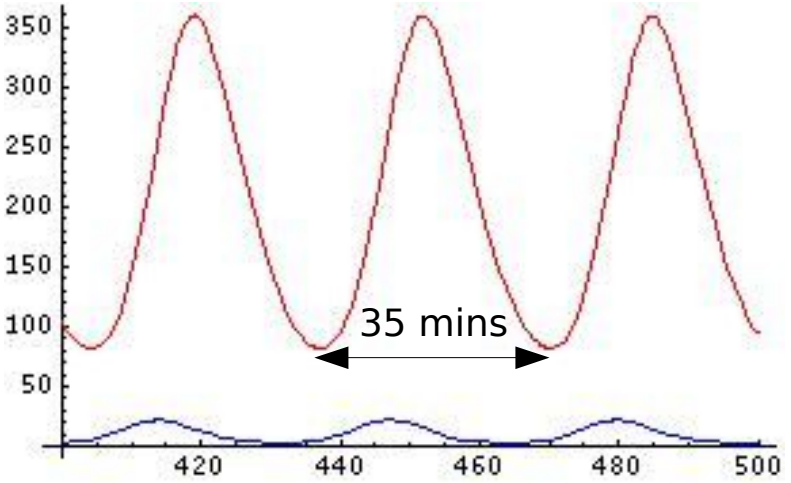
her7 primary mRNA 1280 bp, 2 introns
Her7 204 aa
expected T_m = 7.1 minutes, T_p = 1.7 minutes

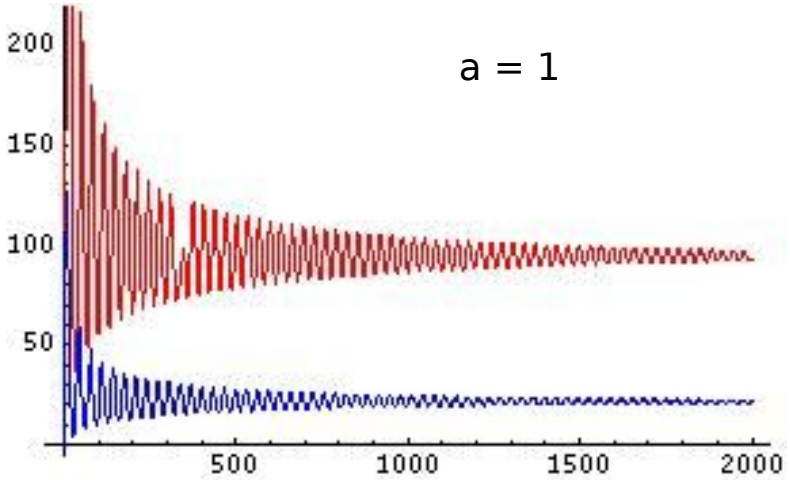
Lewis, Current Biol **13**:1398-408 2003

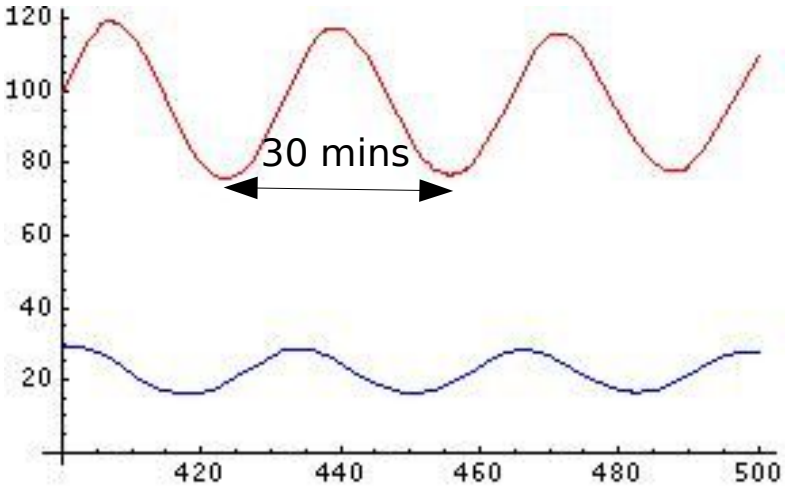
sustained oscillations robust to protein synthesis

period in zebrafish = ~30 minutes



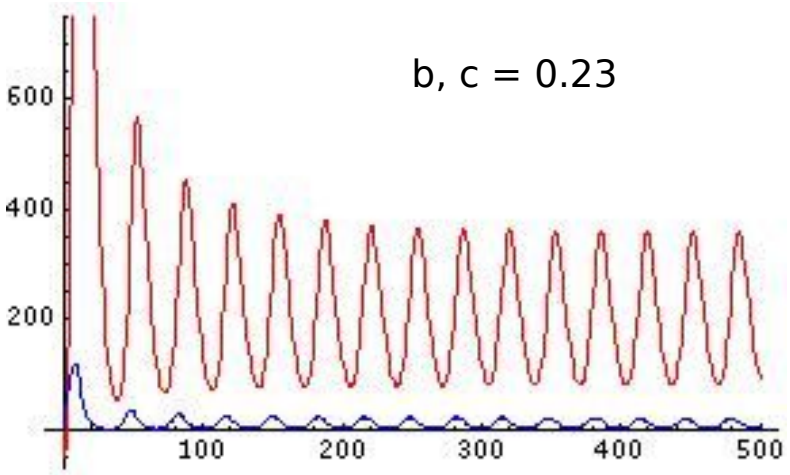


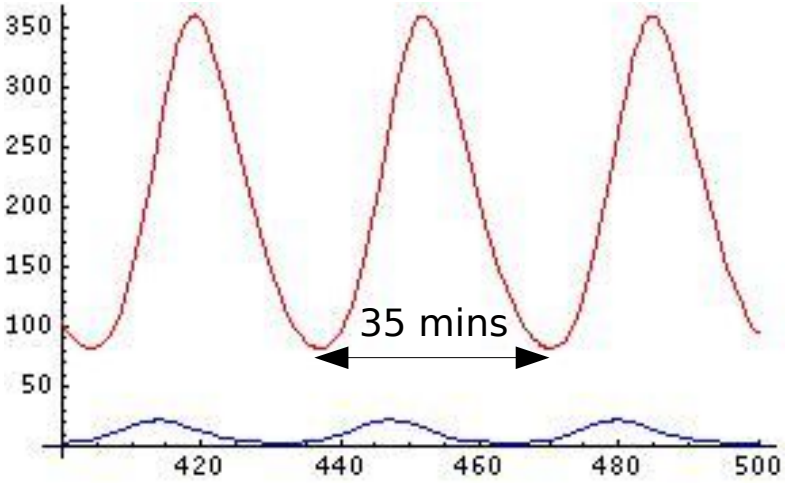


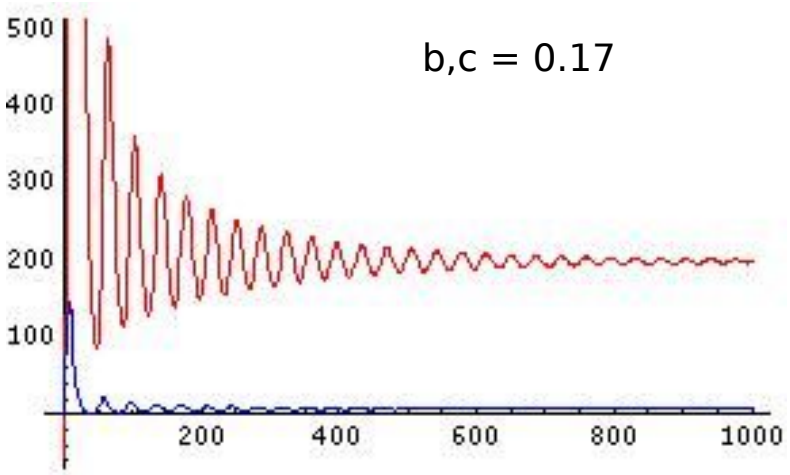


but very sensitive to protein degradation

sensitive to increase in mRNA or protein half-life

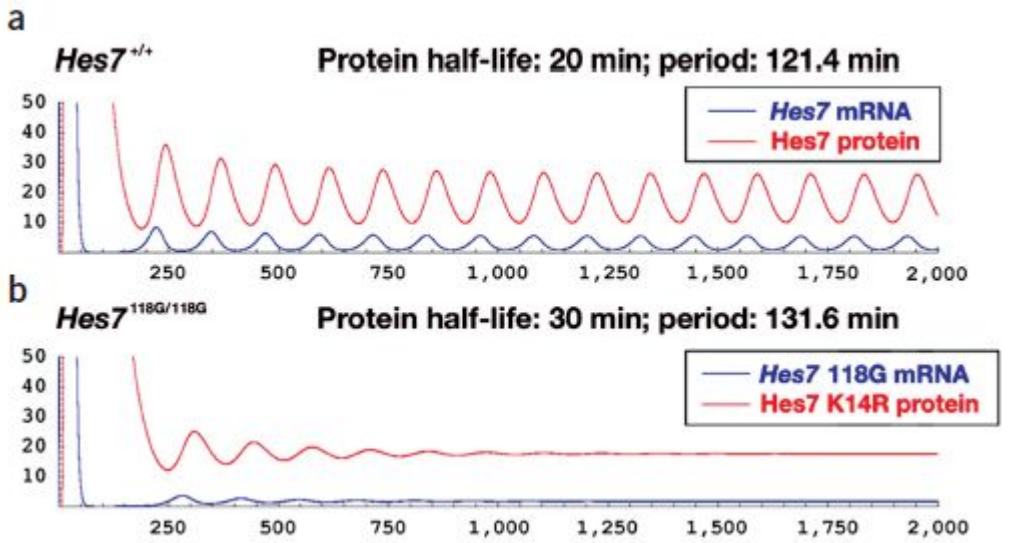


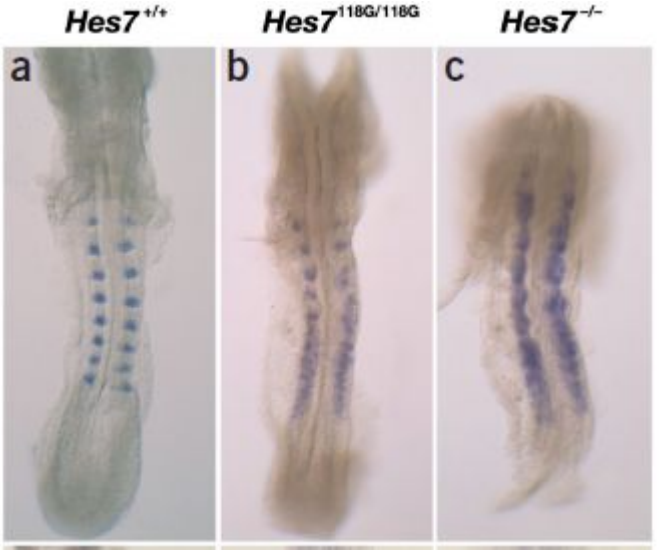




even in the mouse ...

with appropriate parameter values, the Lewis model gives a period of ~2 hours for the somitogenesis clock in the mouse





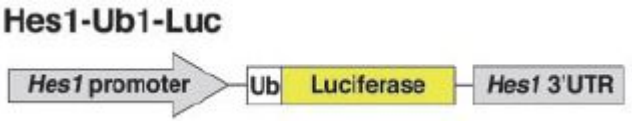
in-situ hybridisation with Uncx4.1

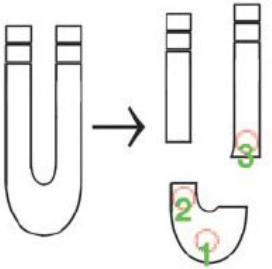
Hirata, Bessho, Kokubu, Masamizu, Yamada, Lewis, Kageyama, *"Instability of Hes7 protein is crucial for somite segmentation clock"*, Nature Genetics **36**:750-4 2004

Gunawardena, "*Models in biology: 'accurate descriptions of our pathetic thinking'*", BMC Biol **12**:29 2014

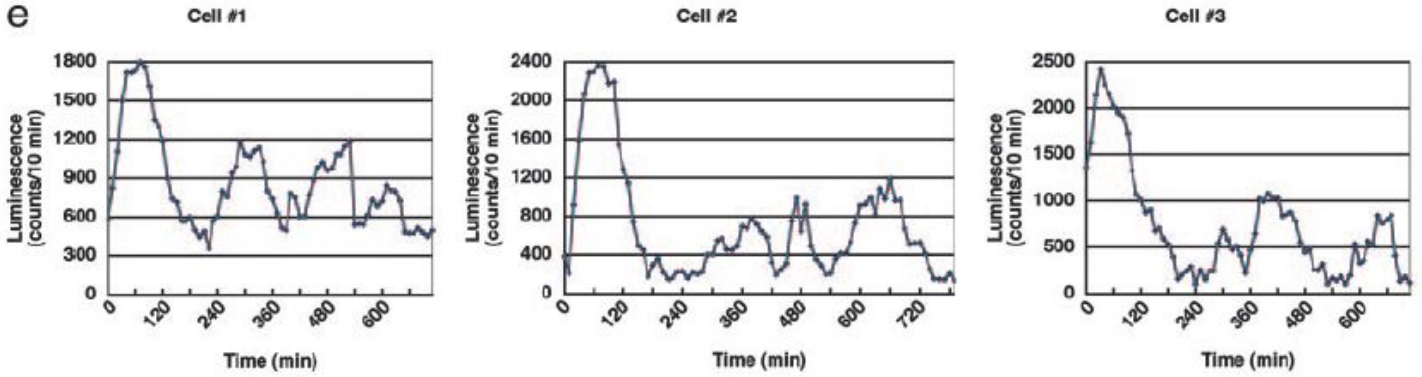
simplicity emerging from complexity?

Masamizu et al PNAS **103**:1313-8 2006





pre-somitic mesoderm cells were disaggregated in cell culture



individual cells oscillate very poorly

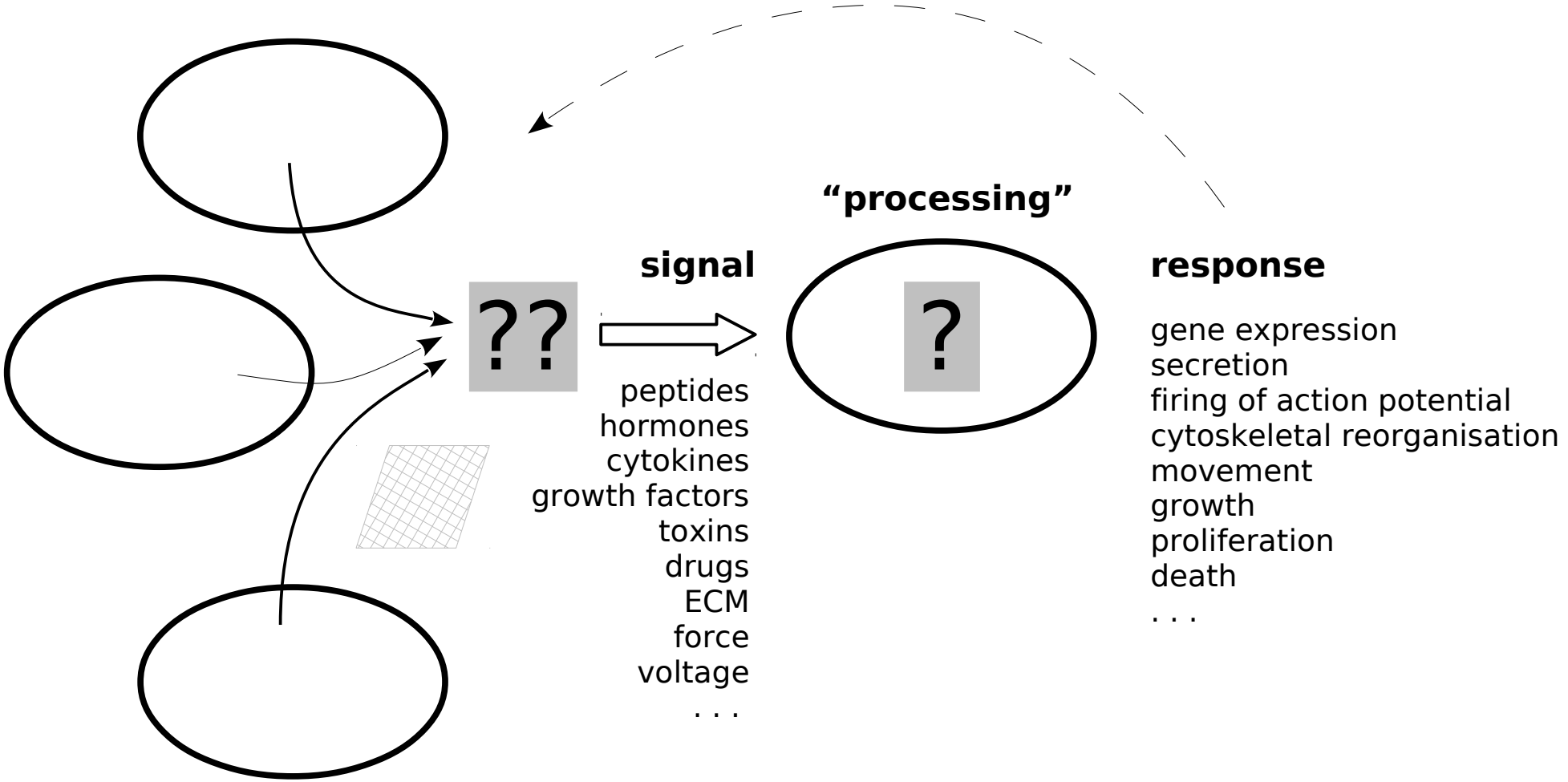
there must be signals and synchronisation in the tissue for regular somitogenesis but none of these mechanisms are present in the Lewis model

so why is the model so accurate?

Gunawardena, "*Models in biology: 'accurate descriptions of our pathetic thinking'*", BMC Biol **12**:29 2014

5. signal transduction & information processing

from the outside to the inside



processing = computing, not plumbing

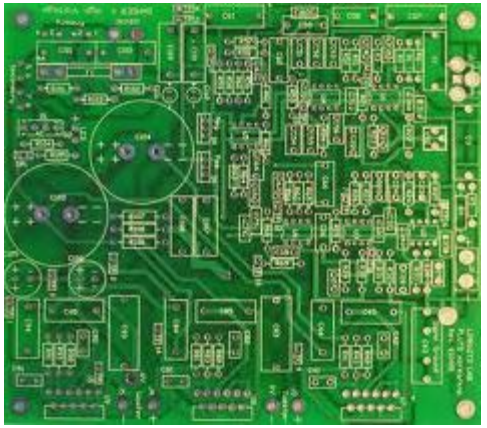
signal

cytoplasm

nucleus

X

signal



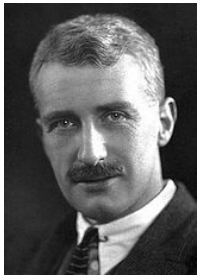
???

✓

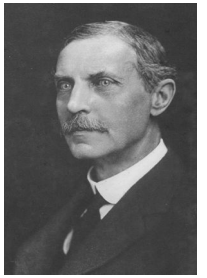
receptor theory of drug action

THE MODE OF ACTION OF NICOTINE AND CURARI, DETERMINED BY THE FORM OF THE CONTRACTION CURVE AND THE METHOD OF TEMPERATURE COEFFICIENTS. By A. V. HILL, B.A., *Scholar of Trinity College, Cambridge.*

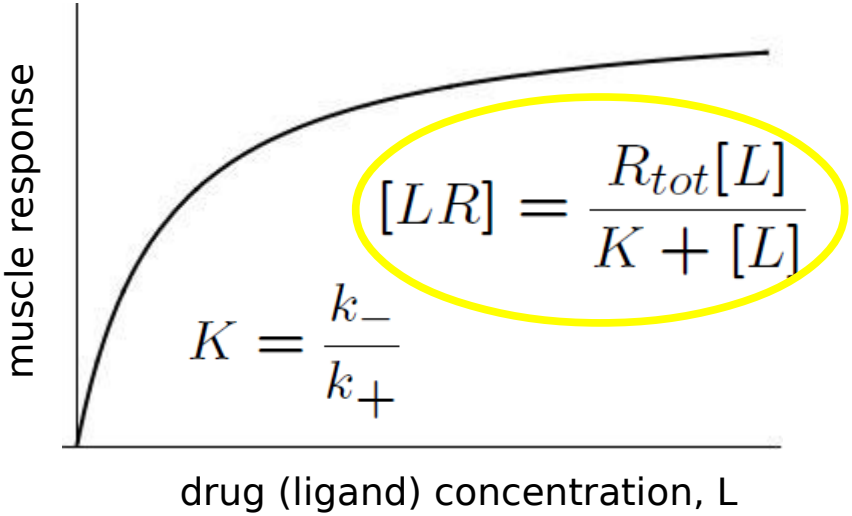
A V Hill, J Physiol **39**:361-73 1909



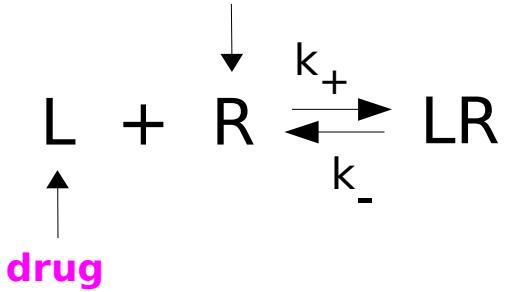
1886-1977



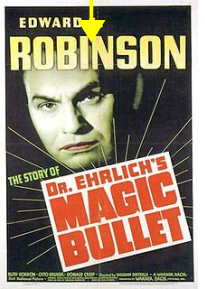
1852-1925



"receptive substance"



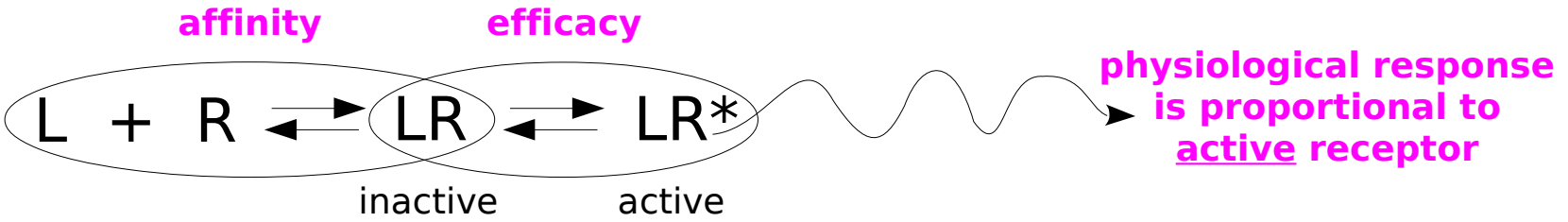
1854-1914

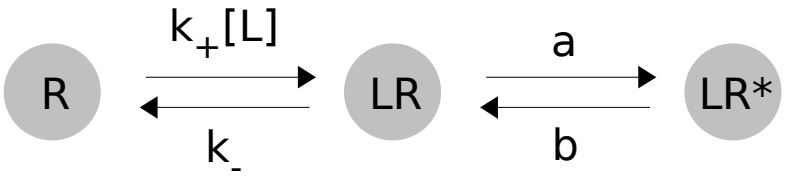


physiological response is assumed proportional to the amount of bound receptor

explained many aspects of pharmacology

"affinity" and "efficacy" are dependent but different concepts





$$[LR] = \left(\frac{k_+}{k_-}\right)[L][R] \qquad [LR*] = \left(\frac{a}{b}\right)[LR]$$

$$[LR*] = \frac{\left(\frac{a}{a+b}\right) R_{tot}[L]}{K\left(\frac{b}{a+b}\right) + [L]}$$

cannot determine affinity by fitting

D Colquhoun, *"The quantitative analysis of drug receptor interactions: a short history"*, Trends Pharmacol Sci **27**:149-57 2006

but “receptive substances” remained a theory

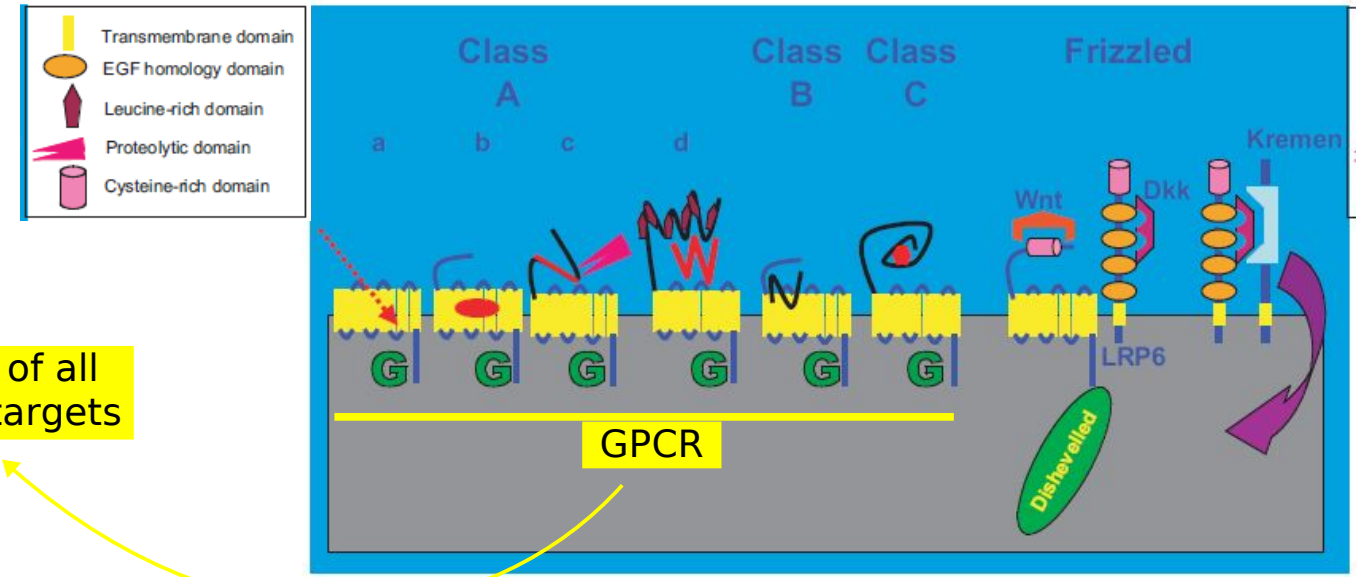
“The adrenotropic receptors are those hypothetical structures or systems located in, on or near the muscle or gland cells affected by epinephrine. ... Although little can be said at the present time as to the fundamental nature of the adrenotropic receptor ... this concept should be useful when studying the various actions of epinephrine.”

R Ahlquist, *“A study of the adrenotropic receptors”*, Am J Physiol **153**:586-600 1948

until the first receptor (the nicotinic acetylcholine receptor – an ion channel) was biochemically isolated

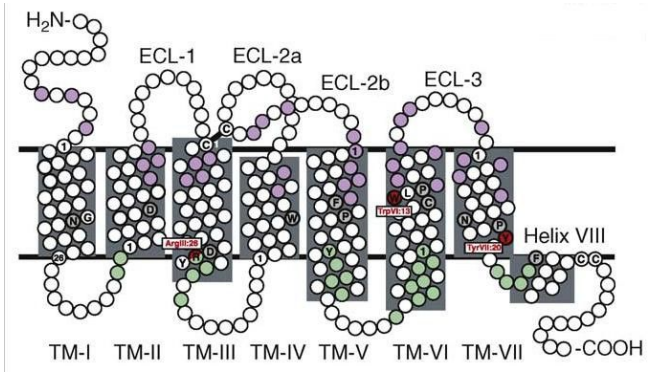
Changeux, Kasai, Lee, *“Use of a snake venom toxin to characterize the cholinergic receptor protein”*, PNAS **67**:1241-7 1970; Changeux & Edelstein, “*Allosteric mechanisms of signal transduction*”, Science **308**:1424-8 2005.

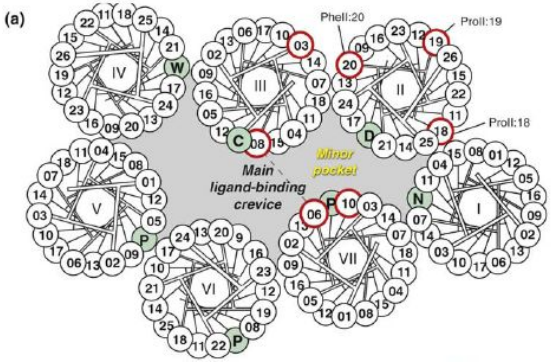
seven transmembrane (7TM) receptors



30% of all drug targets

~800 7TM-enconding genes in the human genome





Nygaard et al, "*Ligand binding and micro-switches in 7TM receptor structures*", Trends Pharmacol Sci, **30**:249-59 2009; Rosenkilde et al, "*The minor-binding pocket: a major player in 7TM receptor activation*", Trends Pharmacol Sci, **31**:567-74 2010; Pierce, Premont, Lefkowitz, Nature Rev Mol Cell Biol **3**:639-50 2002

the long road to molecular understanding

P&M 1994

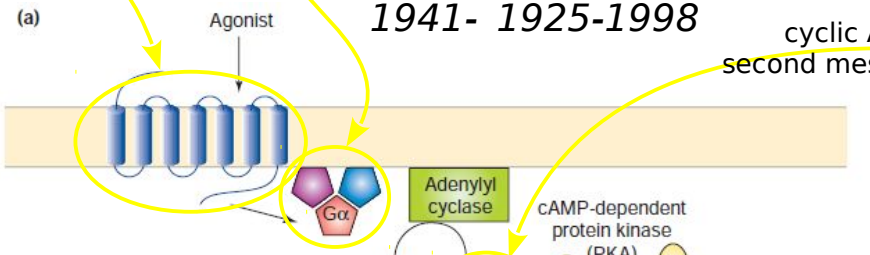
1941- *1925-1998*

Chem 2012

1943- *1955-*

G-proteins

GPCRs



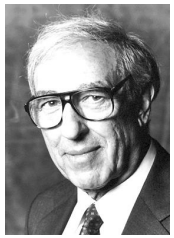
cyclic AMP second messengers

P&M 1971

1915-1971

student

P&M 1992



1920- *1918-2009*

reversible protein phosphorylation

glycogen metabolism

P&M 1947

1896-1957 *1896-1984*

student