dynamic processes in cells (a systems approach to biology)

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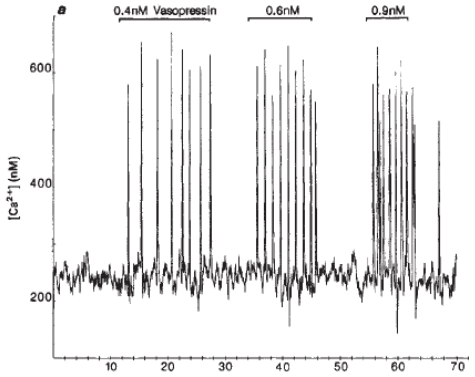
department of systems biology
harvard medical school

lecture 12
11 october 2016

Ca^{2+} oscillations

hepatocyte

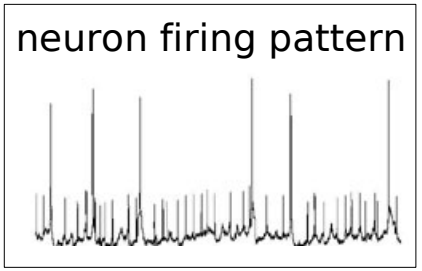


GPCR signalling

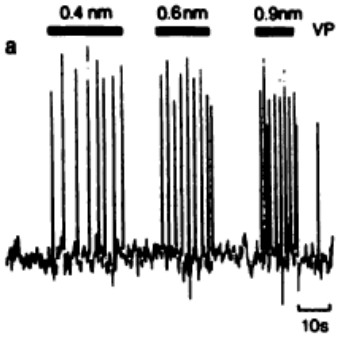
VP = vasopressin

CCh = carbachol

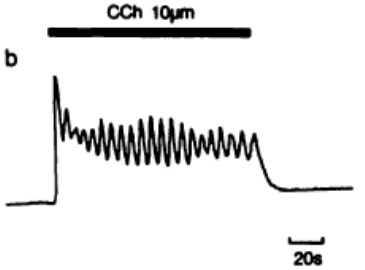
GnRH = gonadotropin releasing hormone



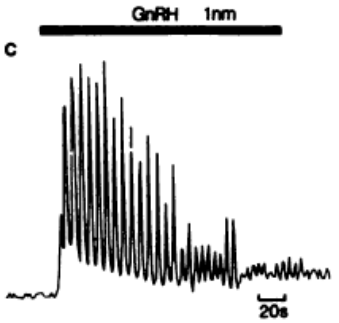
hepatocyte



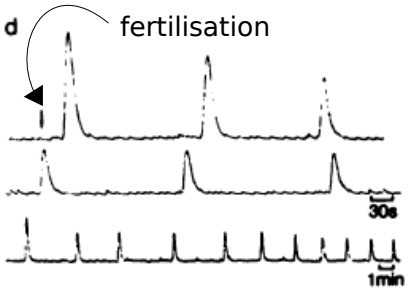
parotid gland



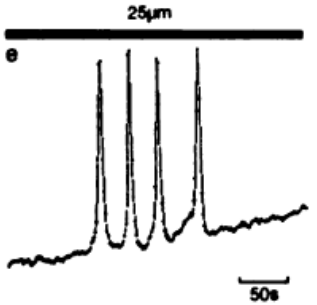
gonadotrope

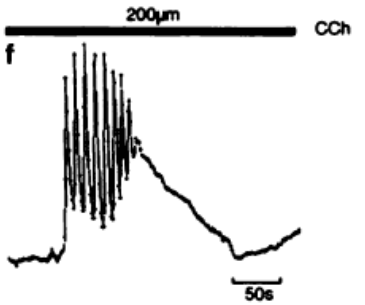


hamster egg



insulinoma cells





Woods, Cuthbertson, Cobbold, "*Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes*", Nature **319**:600-2 1986

Berridge, Galione, "*Cytosolic calcium oscillations*", FASEB J **2**:3074-92 1988

Ca^{2+} frequency encodes information

Decoding of Cytosolic Calcium Oscillations in the Mitochondria

György Hajnóczky, Lawrence D. Robb-Gaspers, Michele B. Seitz, and Andrew P. Thomas

Cell **82**:415-24 1995

Sensitivity of CaM Kinase II to the Frequency of Ca^{2+} Oscillations

Paul De Koninck and Howard Schulman*

Science **279**:227-30 1998

NFAT functions as a working memory of Ca^{2+} signals in decoding Ca^{2+} oscillation

Taichiro Tomida, Kenzo Hirose, Azusa Takizawa, Futoshi Shibasaki[1] and Masamitsu Iino[2]

EMBO J **15**:3825-32 2003

Calcium oscillations increase the efficiency and specificity of gene expression

Ricardo E. Dolmetsch*†, Keli Xu* & Richard S. Lewis

Nature **392**:933-6 1998

Cell-permeant caged $InsP_3$ ester shows that Ca^{2+} spike frequency can optimize gene expression

Wen-hong Li*†‡, Juan Llopis*, Michael Whitney§, Gregor Zlokarnik§ & Roger Y. Tsien*†¶

Nature **392**:936-41 1998

Protein Kinase C as a Molecular Machine for Decoding Calcium and Diacylglycerol Signals

Elena Oancea and Tobias Meyer* Cell **95**:307-18 1998

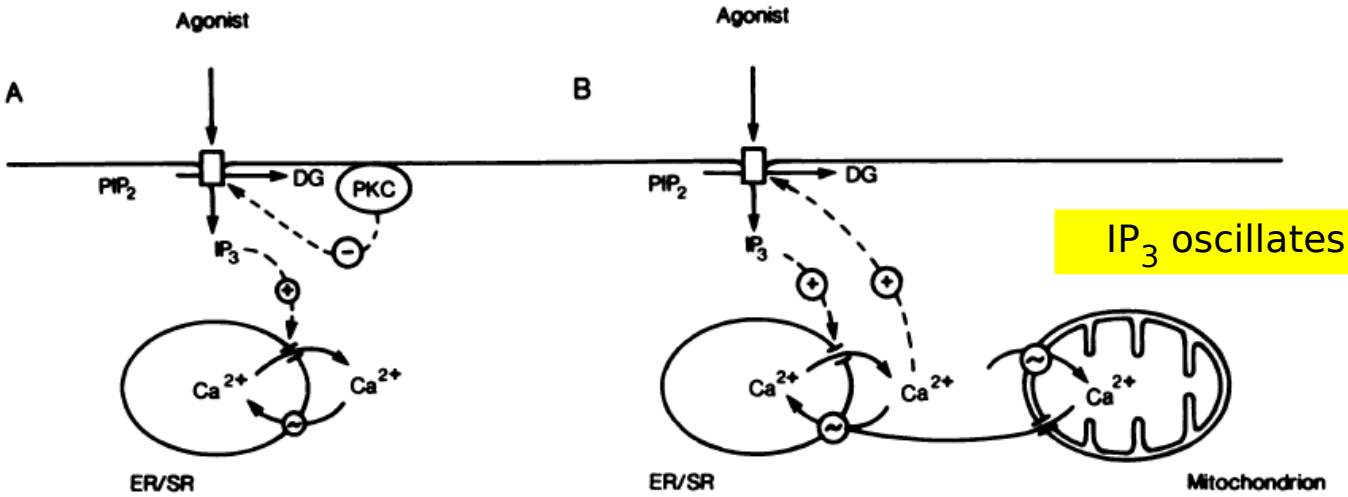
The frequencies of calcium oscillations are optimized for efficient calcium-mediated activation of Ras and the ERK/MAPK cascade

Sabine Kupzig*, Simon A. Walker†, and Peter J. Cullen*‡ PNAS **102**:7577-82 2005

two classes of oscillator architecture

receptor-controlled *

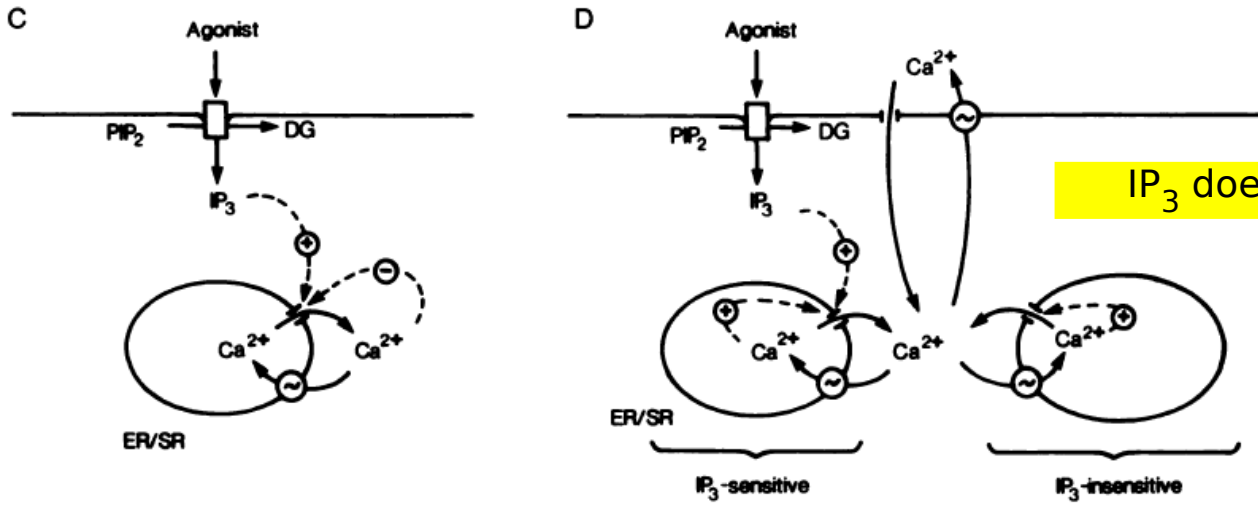
class II **



IP_3 oscillates with Ca^{2+}

2nd messenger-controlled *

class I **



IP_3 does not oscillate

* Berridge, Galione, FASEB J **2**:3074-92 1988; ** Sneyd et al, PNAS **103**:1675-80 2006

Meyer-Stryer model

Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5051–5055, July 1988
Biochemistry

Molecular model for receptor-stimulated calcium spiking

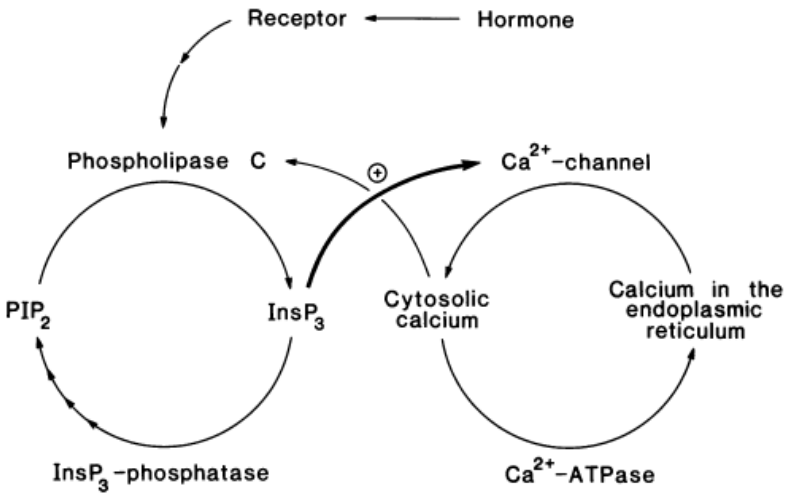
(inositol phospholipid cascade/inositol trisphosphate/calcium channels/oscillations/frequency encoding)

TOBIAS MEYER AND LUBERT STRYER

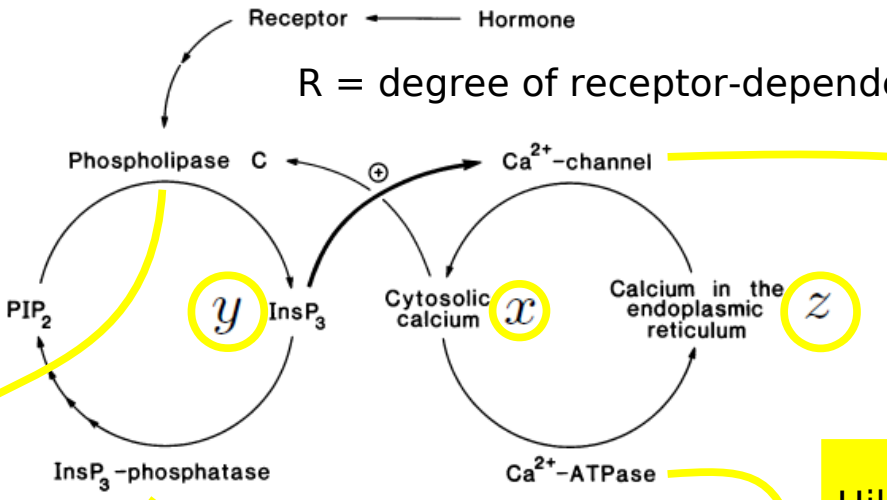
Department of Cell Biology, Sherman Fairchild Center, Stanford University School of Medicine, Stanford, CA 94305

ABSTRACT **Many cells exhibit periodic transient increases in cytosolic calcium levels rather than a sustained rise when stimulated by a hormone or growth factor. We propose here a molecular model that accounts for periodic calcium spiking induced by a constant stimulus. Four elements give rise to repetitive calcium transients: cooperativity and positive feedback between a pair of reciprocally coupled (crosscoupled) messengers, followed by deactivation and then by reactivation. The crosscoupled messengers in our model are inositol 1,4,5-trisphosphate ($InsP_3$) and cytosolic calcium ions. The opening of calcium channels in the endoplasmic reticulum by the binding of multiple molecules of $InsP_3$ provides the required cooperativity. The stimulation of receptor-activated phospholipase C by released calcium ions leads to positive feedback. $InsP_3$ is destroyed by a phosphatase, and calcium ion is pumped back into the endoplasmic reticulum. These processes generate bistability: the cytosolic calcium concentration abruptly increases from a basal level to a stimulated level at a threshold degree of activation of phospholipase C. Spiking further**

receptor controlled, class II



positive feedback



R = degree of receptor-dependent activation

NOT
Hill-like functions!

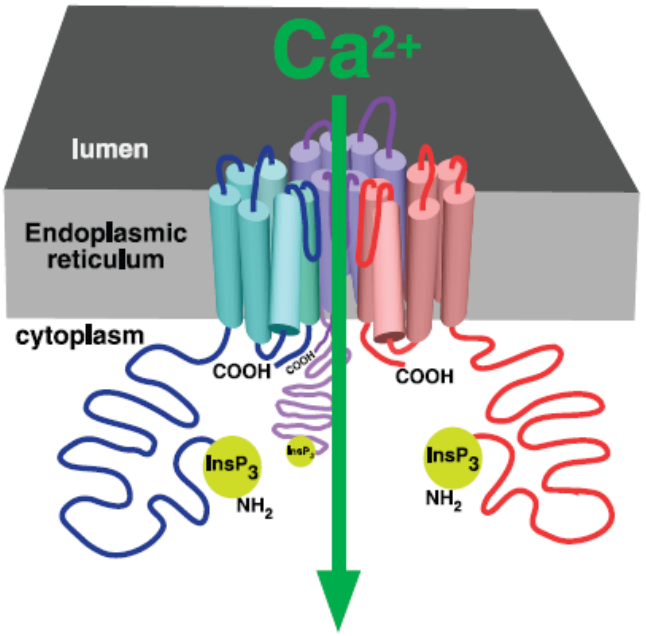
$$\frac{dy}{dt} = \frac{c_4 R x}{K_3 + x} - c_5 y \qquad \frac{dx}{dt} = -\left(\frac{c_2 x^2}{(K_2 + x)^2} - c_3 z^2\right) + \frac{c_1 y^3 z}{(K_1 + y)^3}$$

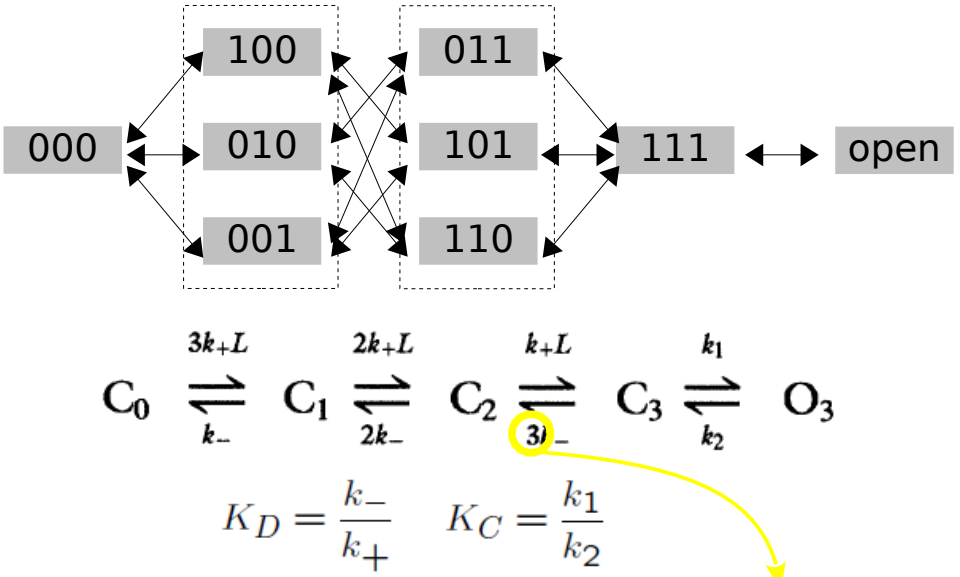
"K_1 is the $InsP_3$ concentration at which half the sites are filled" X

$$x + z = \text{constant}$$ conservation law

Meyer, Stryer, "*Molecular model of receptor stimulated calcium spiking*", PNAS **85**:5051-55 1988

the IP3 receptor is a complex beast





"statistical factors"

do not need these if you use microstates instead of aggregated states

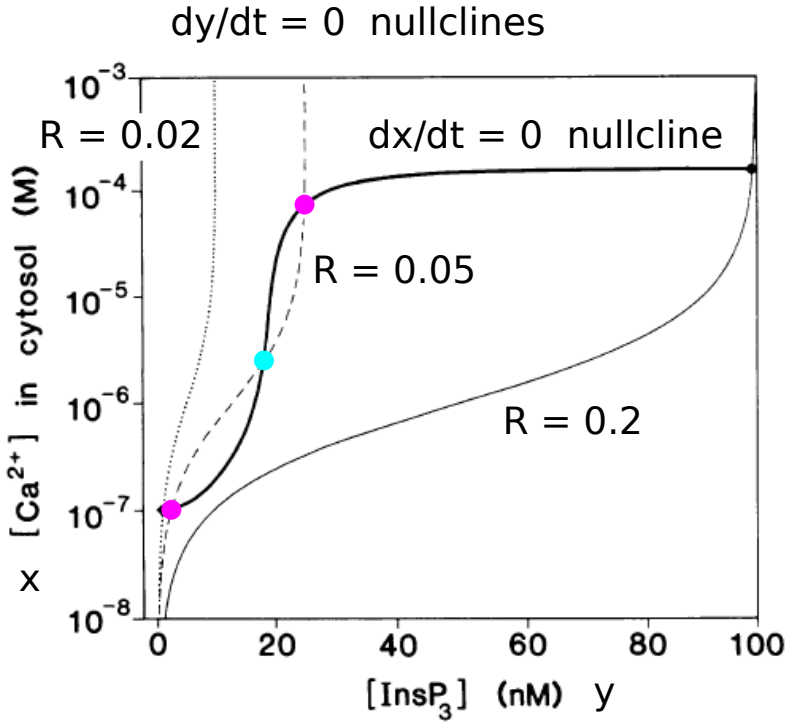
proportion of open receptors → $p(L) = \frac{(L/K_d)^3 K_c}{(1 + L/K_d)^3 + (L/K_d)K_c}$ X

Meyer, Holowka, Stryer, "*Highly cooperative opening of calcium channels by inositol 1,4,5-trisphosphate*", Science **240**:653-6 1988

Foskett, White, Cheung, Mak, "*Inositol trisphosphate Ca^{2+} release channels*", Physiol Rev **87**:593-658 2007

positive feedback – bifurcation & bistability

parameter values were determined from the literature – no fitting

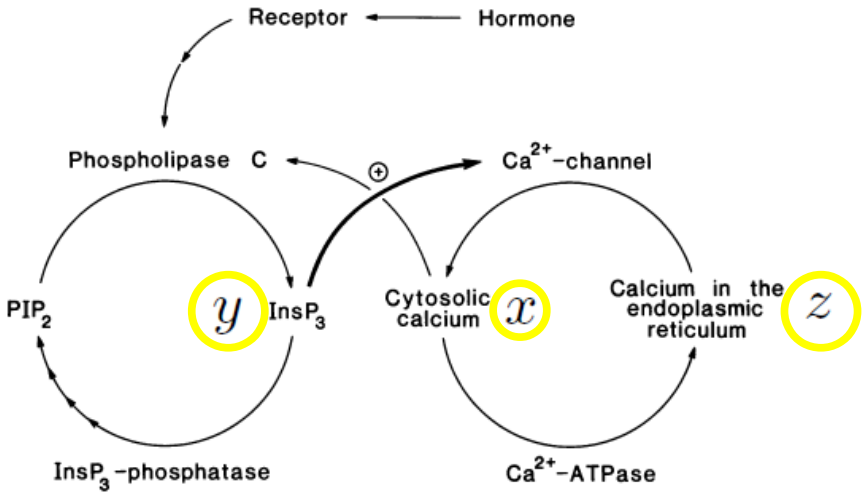


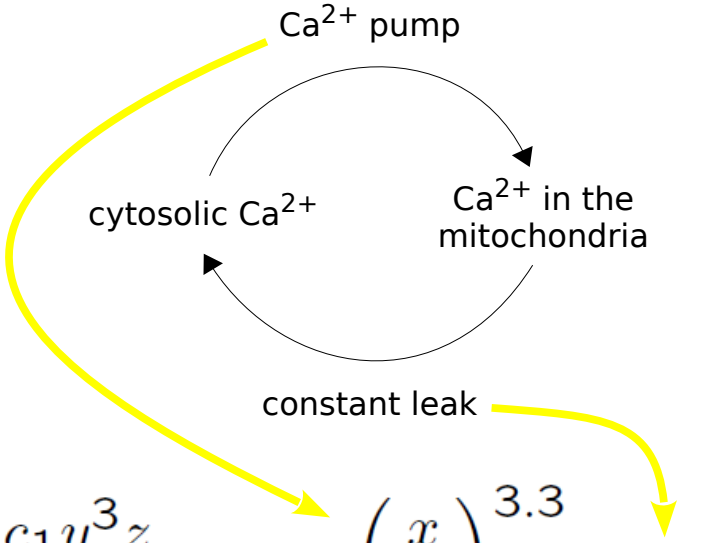
"Calculations show that cooperativity alone or positive feedback alone do not give rise to bistability."

Meyer, Stryer, "*Molecular model of receptor stimulated calcium spiking*", PNAS **85**:5051-55 1988

interlinked negative feedback

Ca2+ sequestration by the mitochondrion provides negative feedback





$$\frac{dx}{dt} = -\left(\frac{c_2 x^2}{(K_2+x)^2} - c_3 z\right) + \frac{c_1 y^3 z}{(K_1+y)^3} - c_6 \left(\frac{x}{c_7}\right)^{3.3} + c_6$$

there is no longer a conservation law

Meyer, Stryer, "*Molecular model of receptor stimulated calcium spiking*", PNAS **85**:5051-55 1988

3D dynamical system

$$\frac{dx}{dt} = -\left(\frac{c_2 x^2}{(K_2 + x)^2} - c_3 z\right) + \frac{c_1 y^3 z}{(K_1 + y)^3} - c_6 \left(\frac{x}{c_7}\right)^{3.3} + c_6$$

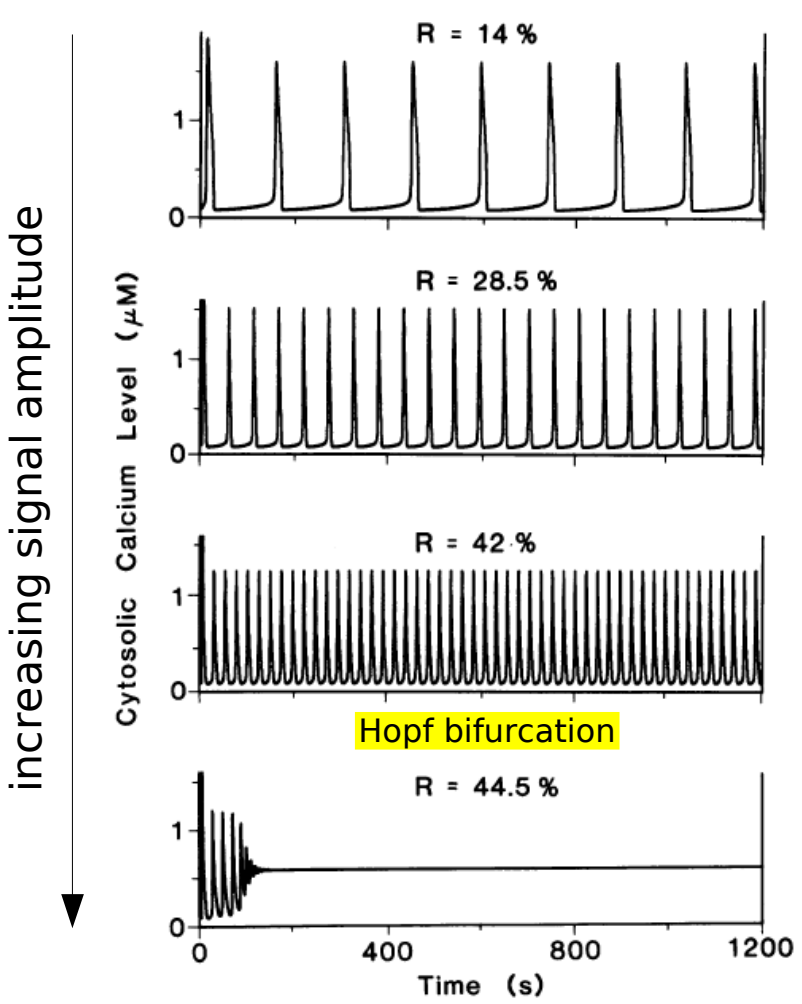
$$\frac{dy}{dt} = \frac{c_4 R x}{K_3 + x} - c_5 y$$

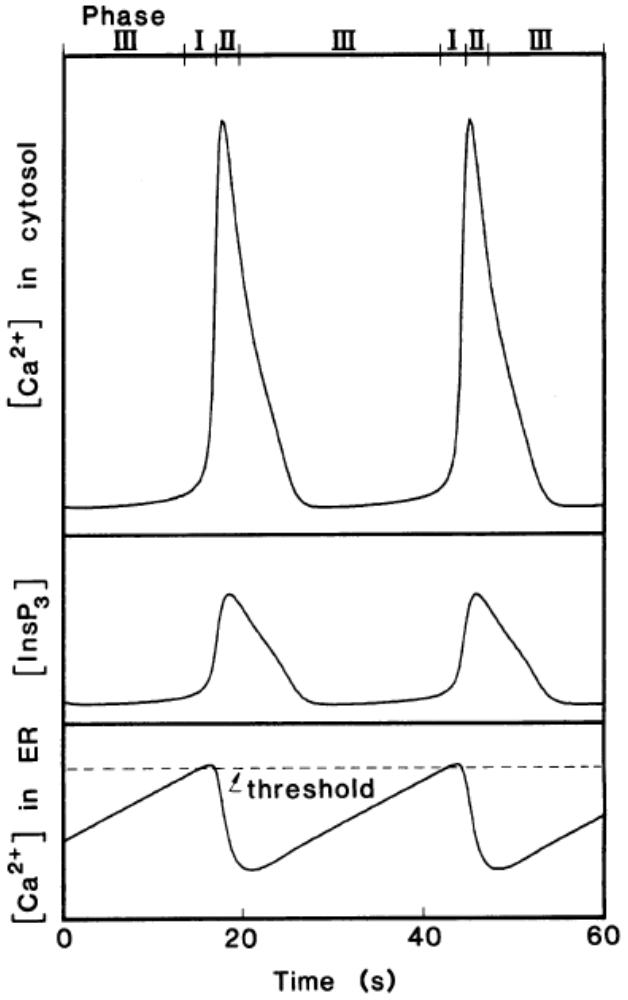
$$\frac{dz}{dt} = \left(\frac{c_2 x^2}{(K_2 + x)^2} - c_3 z\right) - \frac{c_1 y^3 z}{(K_1 + y)^3}$$

Meyer, Stryer, "*Molecular model of receptor stimulated calcium spiking*", PNAS **85**:5051-55 1988

leads to oscillation

signal amplitude to Ca^{2+} frequency conversion, without affecting Ca^{2+} peak amplitudes



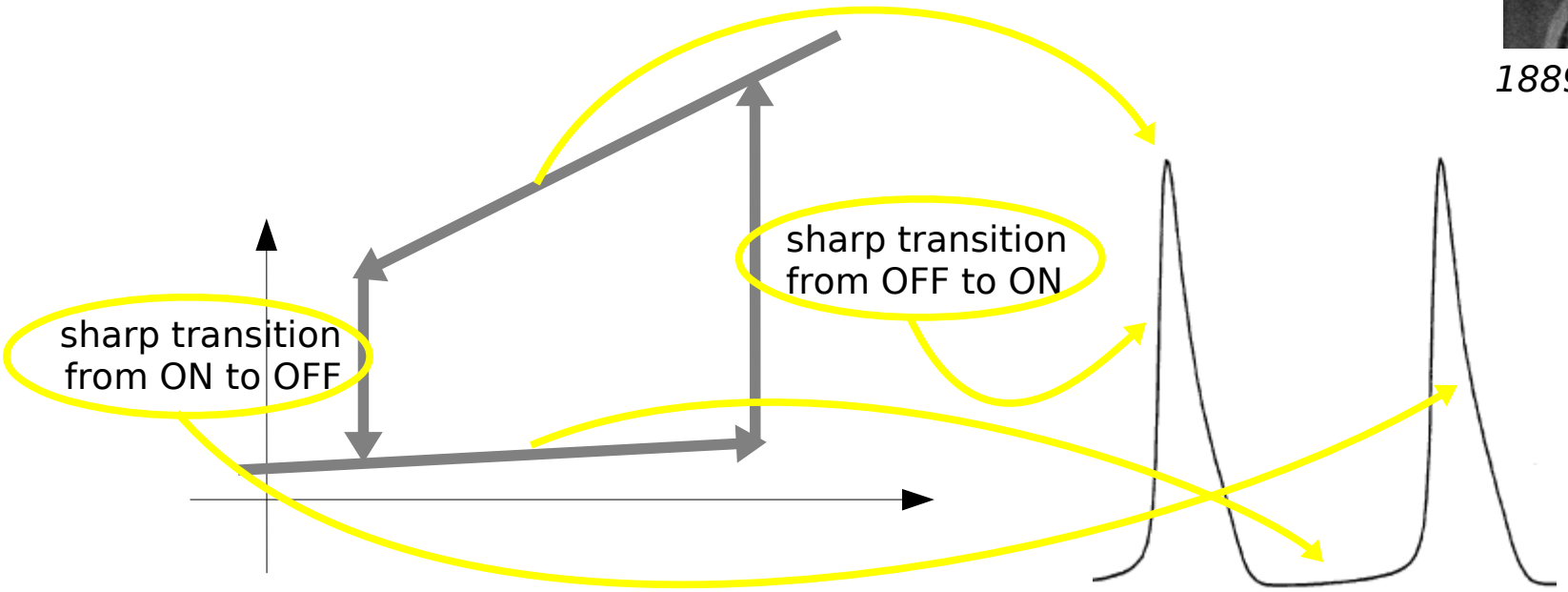


Meyer, Stryer, "*Molecular model of receptor stimulated calcium spiking*", PNAS **85**:5051-55 1988

relaxation oscillators

"Balthazar van der Pol ... was a pioneer in the field of radio ... but he also pursued the mathematical problems encountered in radio work so far that his work has formed the basis of much of the modern theory of non-linear oscillations, and he has given his name to the most typical equation of that theory" *

1889-1959

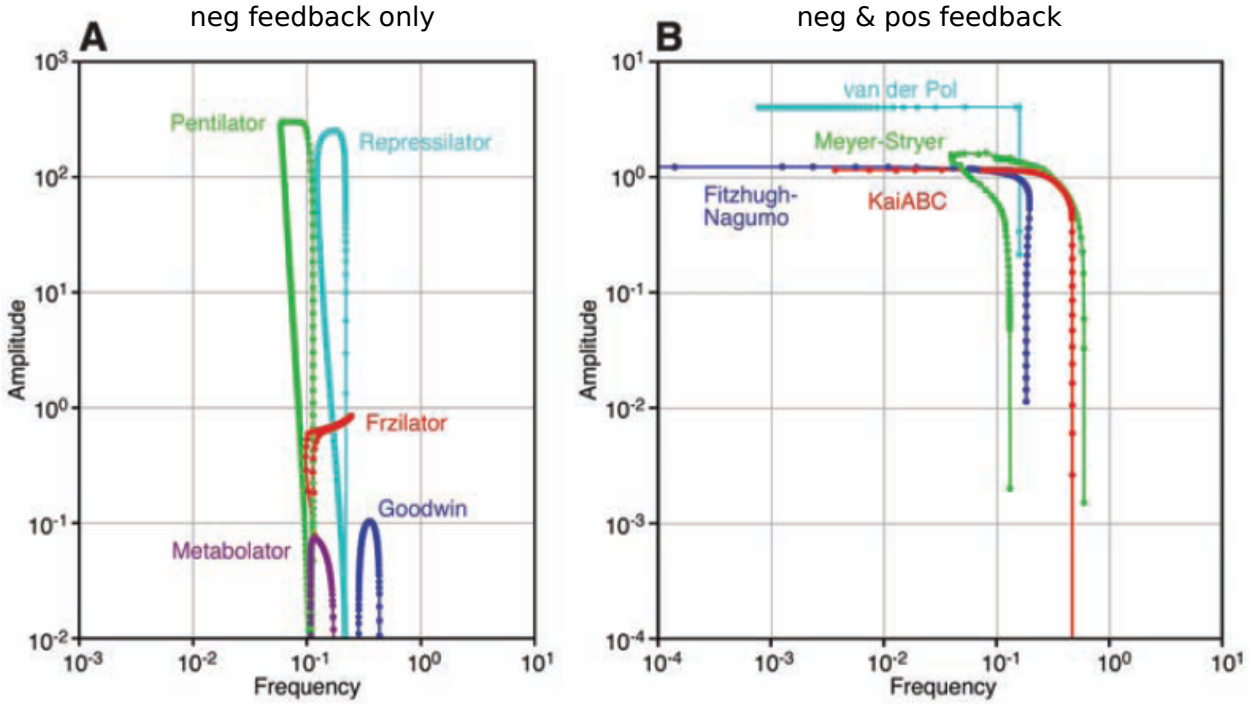


interlinked negative feedback drives hysteresis of an underlying positive feedback bistable switch

Balthazar van der Pol, "*On relaxation oscillations*", Philosophical Magazine **2**:978-92 1926

*Mary Cartwright, "*Balthazar van der Pol*", J Lond Math Soc **35**:367-76 1960

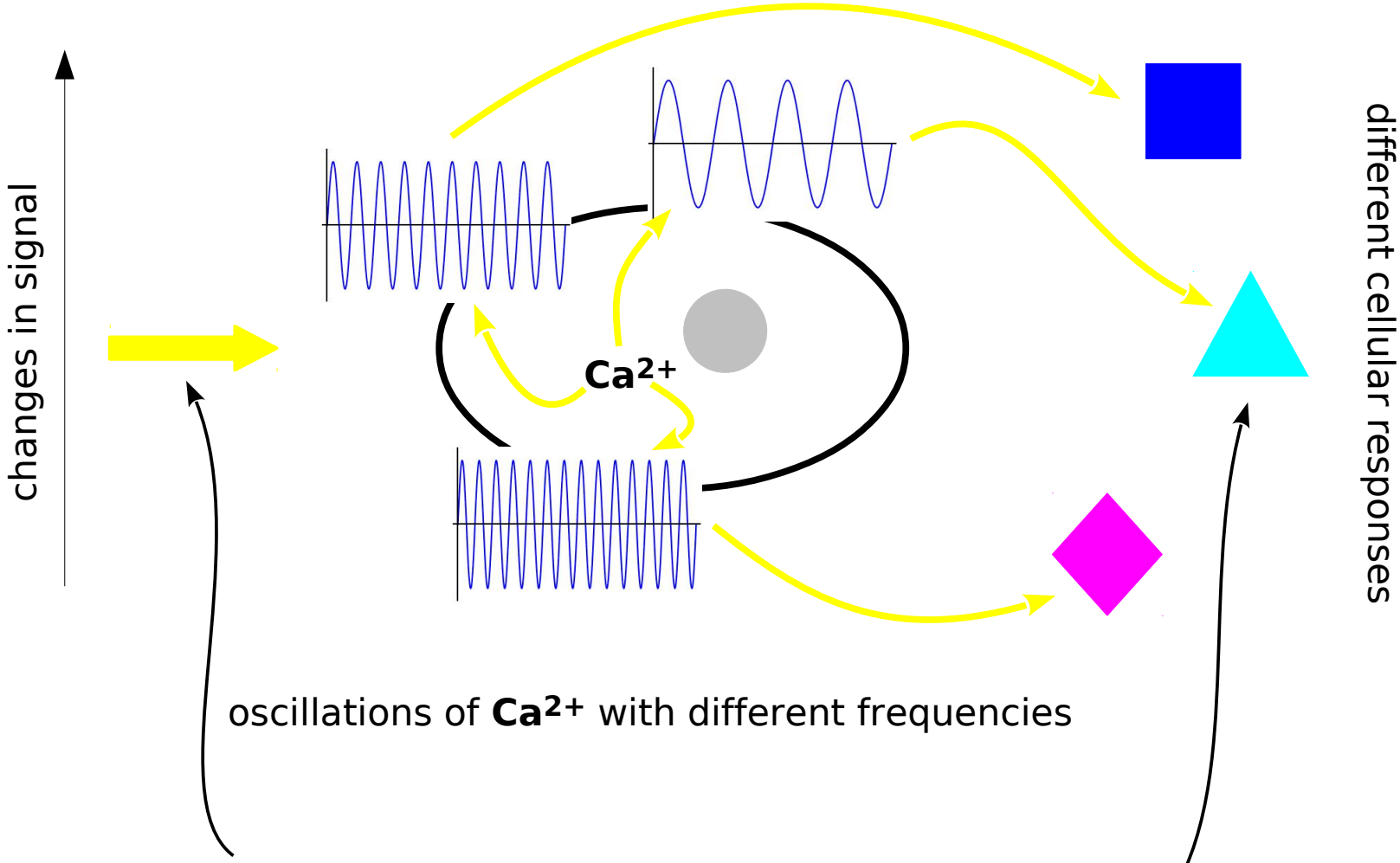
interlinked positive & negative feedback loops



"To construct amplitude vs. frequency curves, we first chose a bifurcation parameter and then identified the range of the parameter over which the system exhibited limit cycle oscillations by locating the Hopf bifurcations at which oscillations were born and extinguished. We then used an iterative algorithm to march along the chosen parameter between the two bifurcations. For each set of parameters we calculated the limit cycle solution of the system, keeping track of the amplitude and frequency of the limit cycle solution."

Tsai, Choi, Ma, Pomerning, Tang, Ferrell, "*Robust, tunable biological oscillations from interlinked positive and negative Feedback Loops*", Science **321**:126-9 2008;
Barkai, Leibler, "*Circadian clocks limited by noise*", Nature **403**:267-8, 1999

Ca^{2+} signalling – frequency modulated radio?



how are Ca^{2+} oscillations generated?

how are oscillation frequencies detected?

oscillations – Ca^{2+} is the tip of a large iceberg!

Dynamics of the p53-Mdm2 feedback loop in individual cells

Galit Lahav[1], Nitzan Rosenfeld[1], Alex Sigal[1], Naama Geva-Zatorsky[1], Arnold J Levine[2], Michael B Elowitz[3] & Uri Alon[1]

Nat Genet **36**:147-50 2004

Oscillations in NF-κB Signaling Control the Dynamics of Gene Expression

D. E. Nelson,[1] A. E. C. Ihekwaba,[2] M. Elliott,[1] J. R. Johnson,[1] C. A. Gibney,[1] B. E. Foreman,[1] G. Nelson,[1] V. See,[1] C. A. Horton,[1] D. G. Spiller,[1] S. W. Edwards,[1] H. P. McDowell,[4] J. F. Unitt,[5] E. Sullivan,[6] R. Grimley,[7] N. Benson,[7] D. Broomhead,[3] D. B. Kell,[2] M. R. H. White[1]*

Science **306**:74-8 2004

Frequency-modulated nuclear localization bursts coordinate gene regulation

Long Cai[1]*, Chiraj K. Dalal[1]* & Michael B. Elowitz[1]

Nature **455**:485-90 2008

Direct observation of frequency modulated transcription in single cells using light activation

Daniel R Larson[1]*, Christoph Fritzsch[2], Liang Sun[3,4], Xiuhau Meng[5], David S Lawrence[3,4], Robert H Singer[5,6]

ELife **2**:e00750 2015

Limits on information transduction through amplitude and frequency regulation of transcription factor activity

Anders S Hansen[1,2], Erin K O'Shea[1,2,3]*

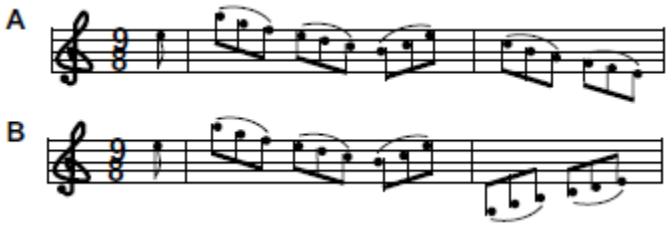
ELife **4**:e06559 2015

oscillations – the tip of the iceberg

TNF-induced gene expression oscillates in time

Li Sun, Guozhe Yang, Mone Zaidi, Jameel Iqbal *

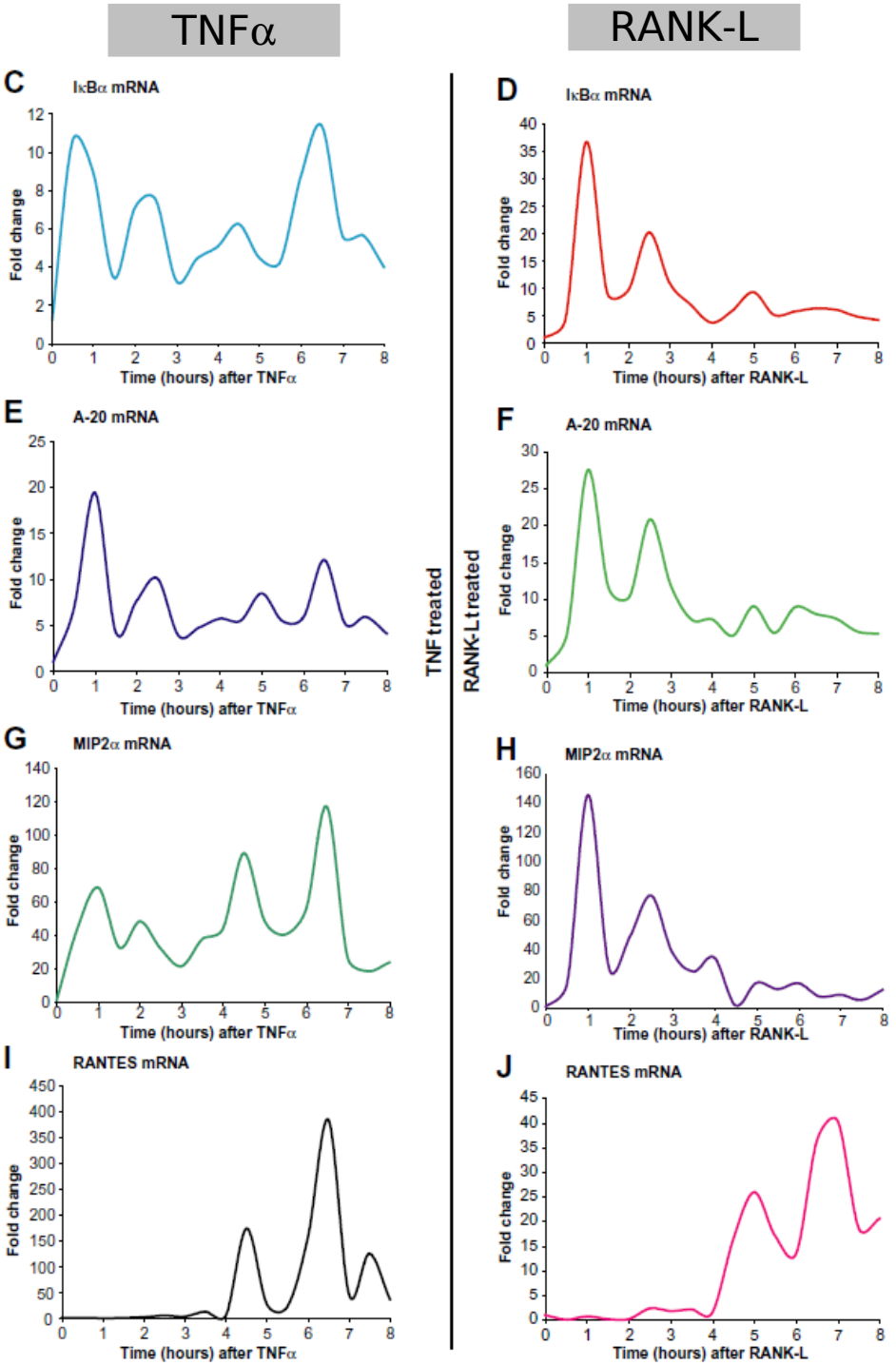
Biochem Biophys Res Comm **371**:900-5 2008



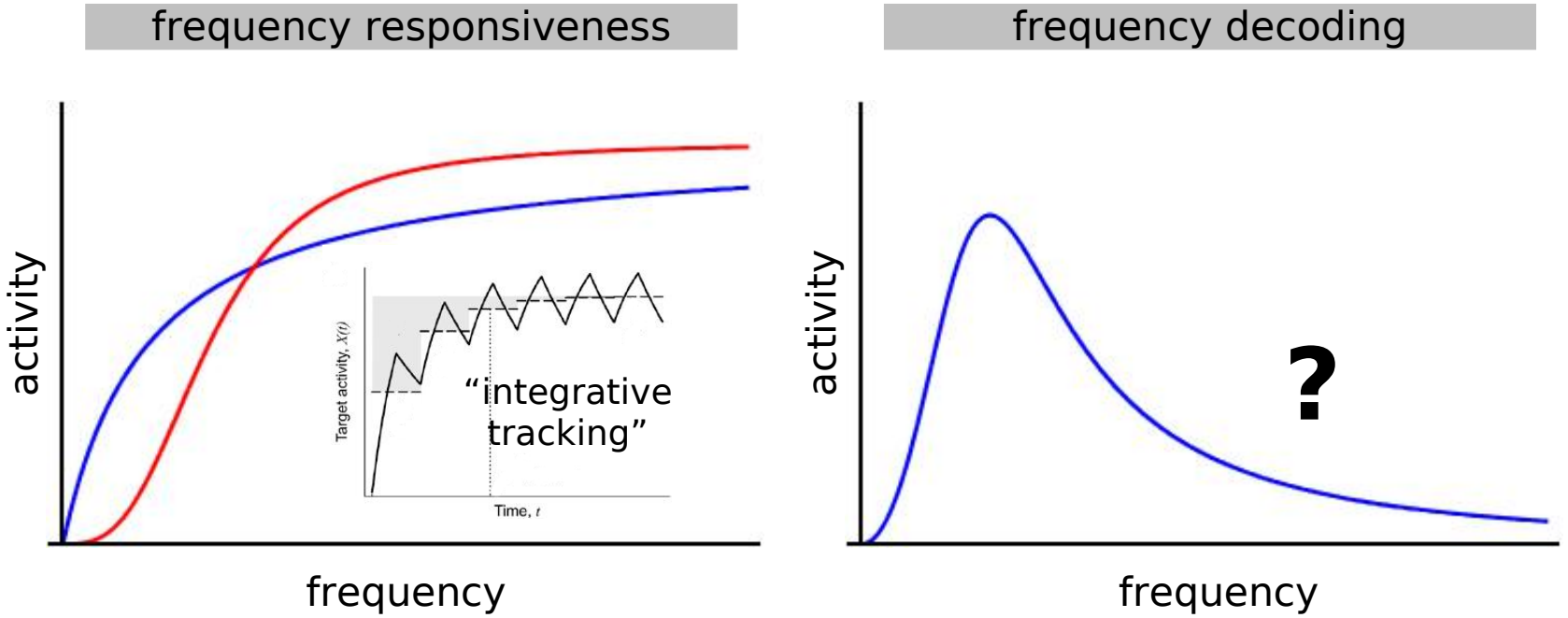
reviews:

Paszek, Jackson, White, "*Oscillatory control of signalling molecules*", Curr Op Gen Dev **20**:670-6 2010

Behar, Hoffmann, "*Undestanding the temporal codes of intra-cellular signals*", Curr Op Gen Dev **20**:684-93 2010



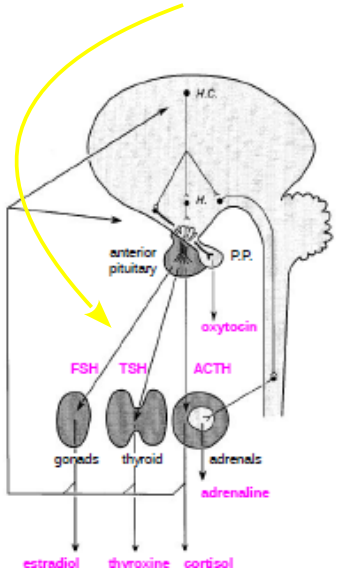
how are oscillation frequencies detected?



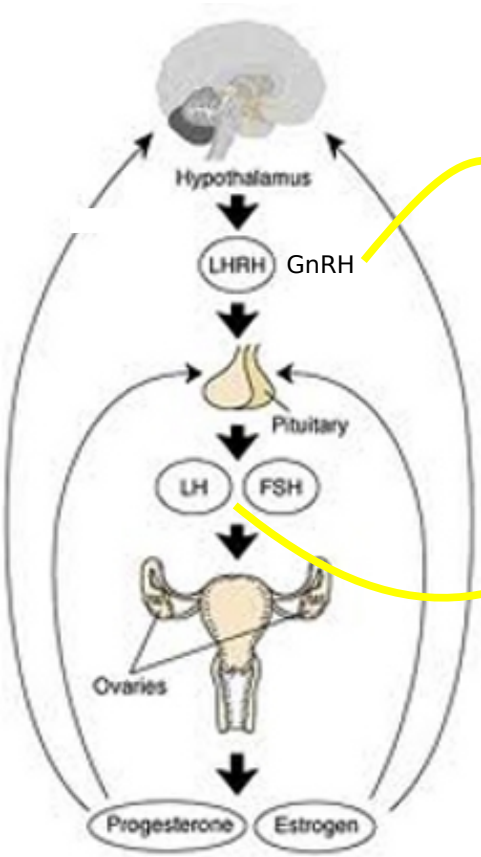
Salazar, Politi, Hofer, "*Decoding of calcium oscillations by phosphorylation cycles: analytic results*", Biophys J **94**:1203-15 2008

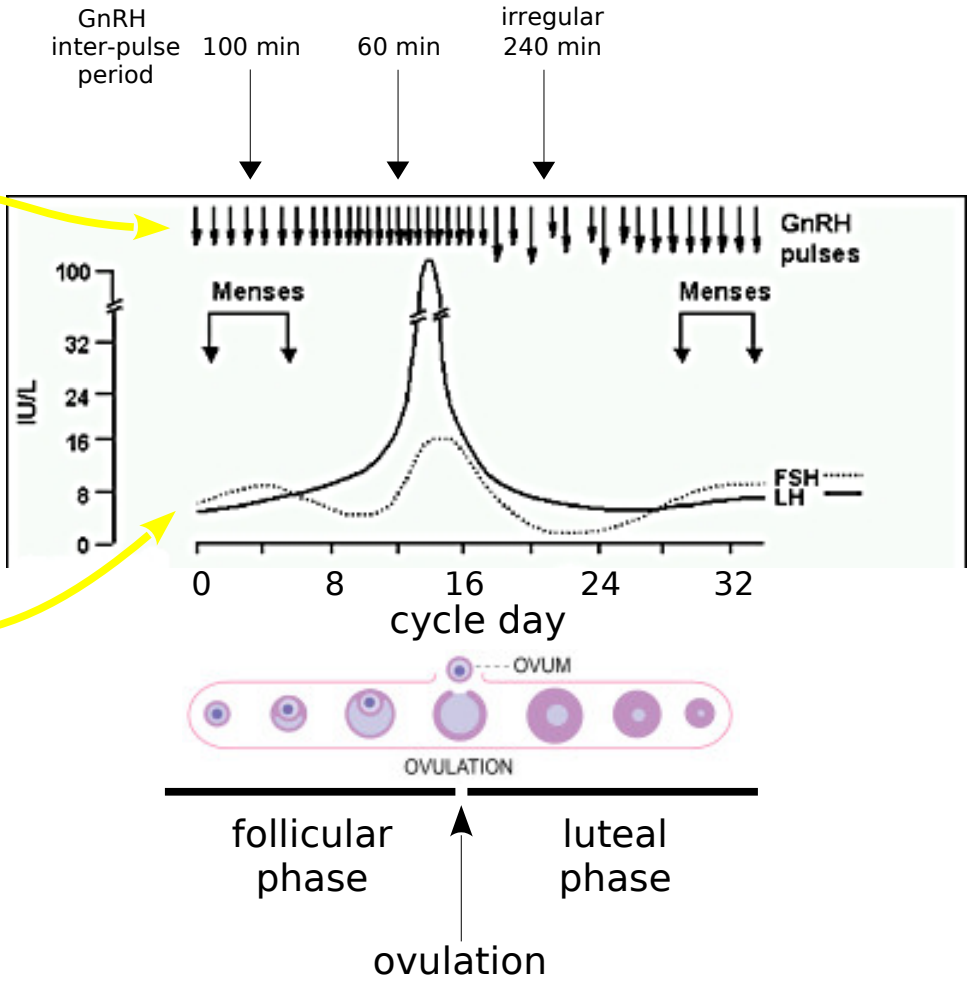
repetitive pulses in the endocrine system

hypothalamic-pituitary-gonadal (HPG) axis



endocrine axes
(HPG, HPT, HPA)

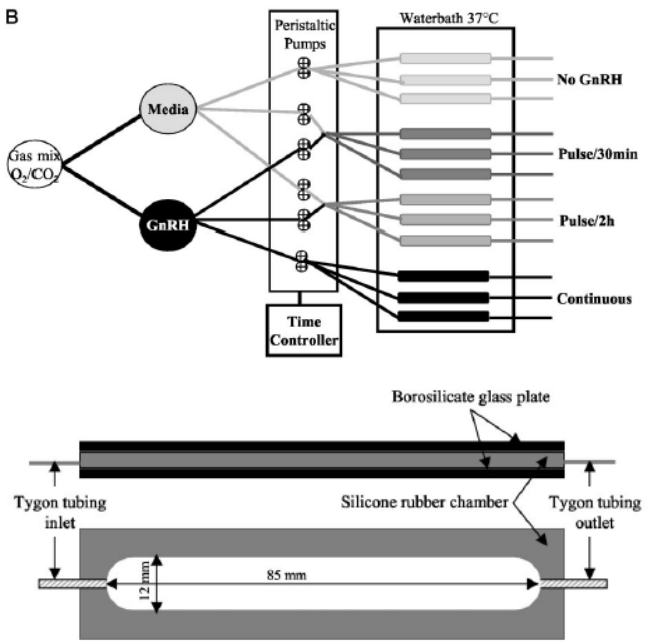


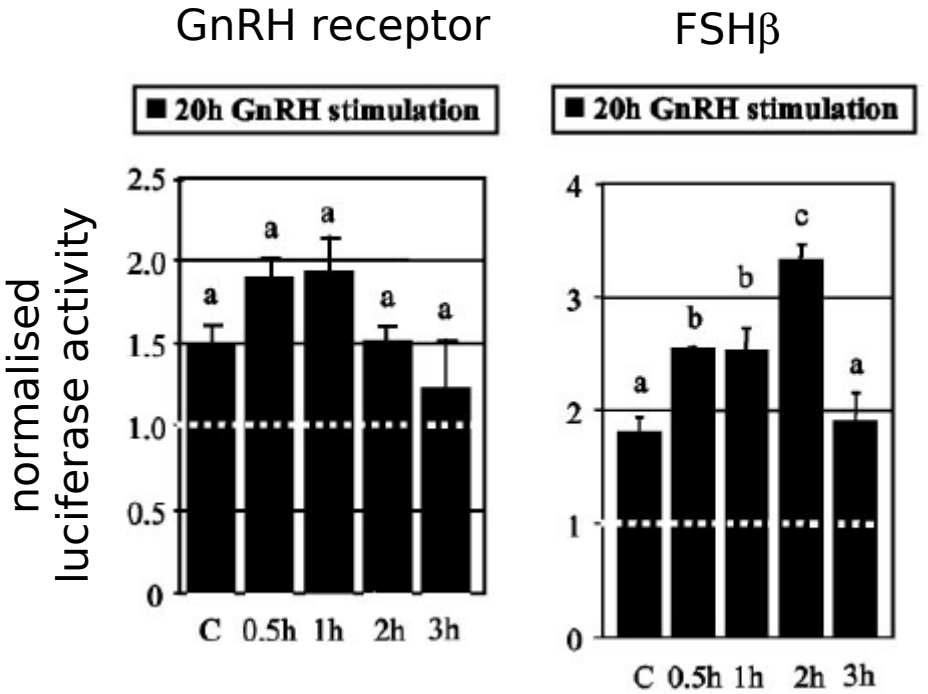


Marshall, Griffin, "*The role of changing pulse frequency in the regulation of ovulation*", Human Reproduction **8**(suppl 2): 57-61 1993

decoding of GnRH pulses

LβT2 gonadotrope cells transfected with luciferase reporters for GnRHR and FSHβ



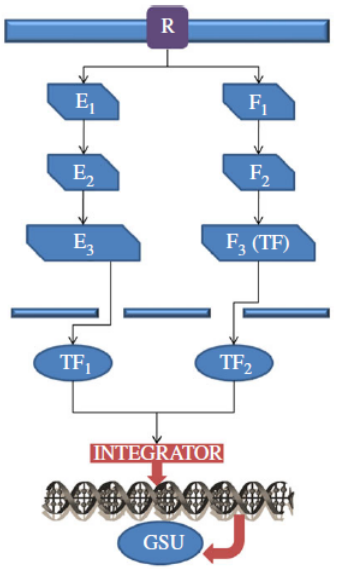


10nM GnRH pulses, 5 min pulse width, 1 pulse every X hours

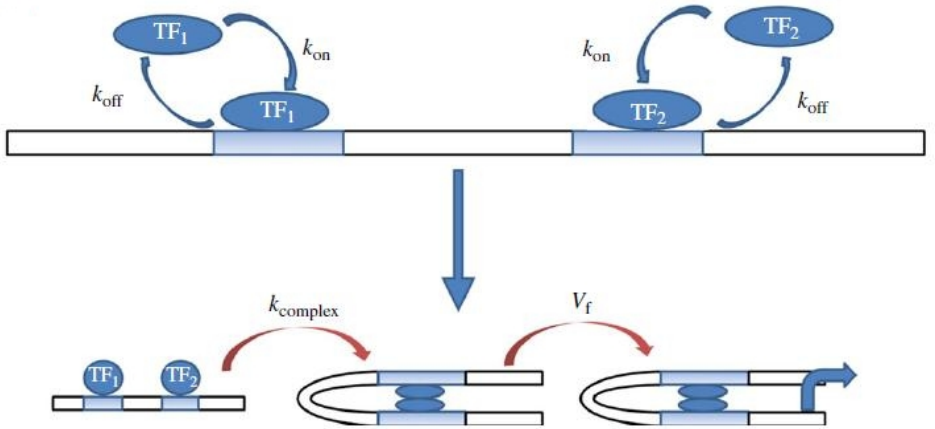
Bedecarrats, Kaiser, "*Differential regulation of gonadotropin subunit gene promoter activity by pulsatile gonadotropin-releasing hormone (GnRH) in perifused LT2 Cells: role of GnRH receptor concentration*", Endocrinol **144**:1802-11 2003

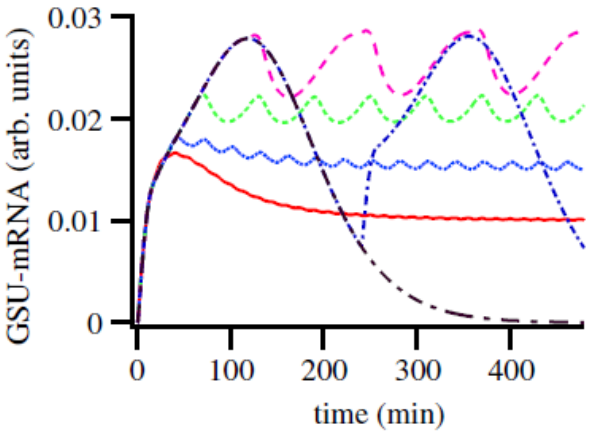
implementation by “parallel cooperation”

“parallel activation”



“cooperative gate”



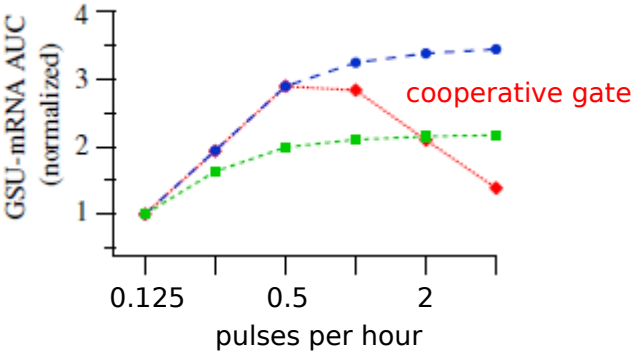


100nM 5min pulse

pulses/hour

black dash-dotted	= 0.125
blue dash-dotted	= 0.25
pink dashed	= 0.5
green dashed	= 1
blue dotted	= 2
red solid	= 4

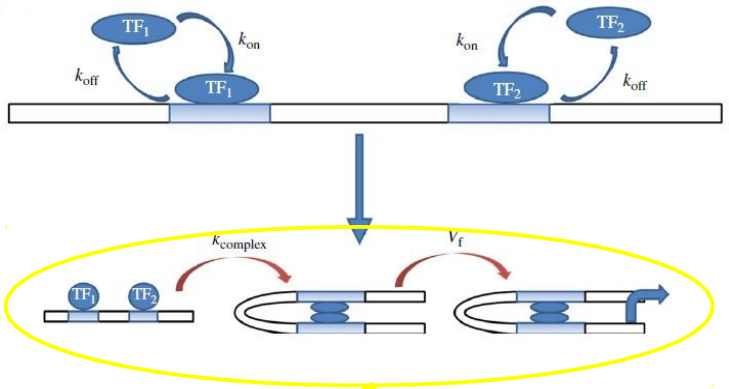
frequency decoding



Tsaneva-Atanasova, Mina, Caunt, Armstrong, McArdle, “*Decoding GnRH neurohormone pulse frequency by convergent signalling modules*”, J R Soc Interface **9**:170-82 2012

but how does this work at the promoter?

"In the CO-OPERATIVE GATE scenario, the transcription factors bind at independent promoter sites. After initial binding of the transcription factors to DNA the transcription factors interact to bring promoter sites in close proximity."



$$\frac{\mathrm{d}[\mathrm{GSU}]}{\mathrm{dt}} = K_{\mathrm{complex}} \times \left(\frac{[\mathrm{TF_1}][\mathrm{TF_2}][\mathrm{DNA_{TOT}}]^2 / K_{\mathrm{d_{TF1}}} K_{\mathrm{d_{TF2}}}}{(1 + [\mathrm{TF_1}]/K_{\mathrm{d_{TF1}}} + [\mathrm{TF_2}]/K_{\mathrm{d_{TF2}}})^2} \right) - d_{\mathrm{GSU}}[\mathrm{GSU}],$$

?

X

$$\begin{aligned} [\mathrm{TF_1}] + [DNA] &\rightleftharpoons [\mathrm{TF_1} : DNA], \\ [\mathrm{TF_2}] + [DNA] &\rightleftharpoons [\mathrm{TF_2} : DNA], \\ [\mathrm{TF_1} : DNA] + [\mathrm{TF_2} : DNA] &\rightleftharpoons [\mathrm{TF_1} : \mathrm{TF_2} : DNA]. \end{aligned}$$

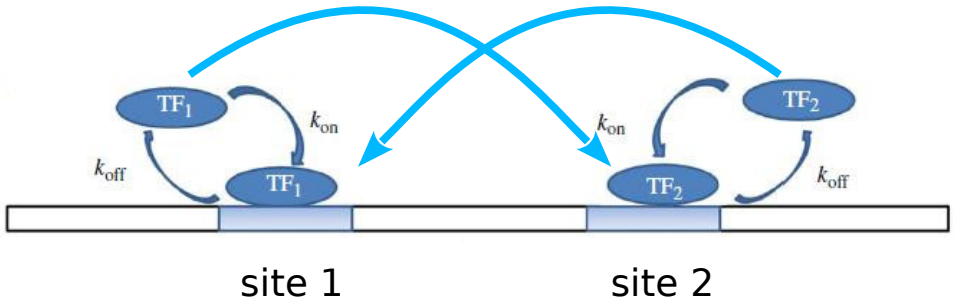
!!

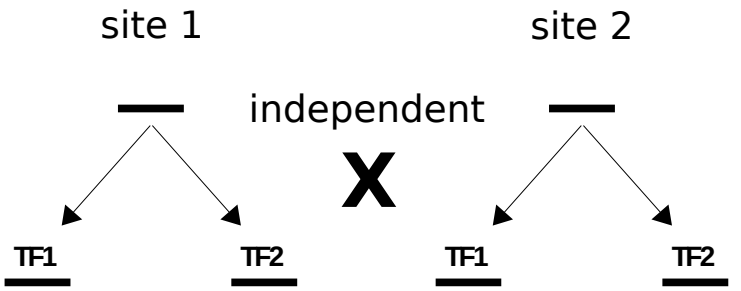
Tsaneva-Atanasova, Mina, Caunt, Armstrong, McArdle, "*Decoding GnRH neurohormone pulse frequency by convergent signalling modules*", J R Soc Interface **9**:170-82 2012 and SI

oops ... but, happily, all is not lost

if both transcription factors bind to each site with the same affinity but in a **mutually exclusive way**

and the two bindings are **independent** of each other (no cooperativity)





that gives the partition function $(1 + [TF_1]/K_{d_{TF1}} + [TF_2]/K_{d_{TF2}})^2$ in the denominator

the numerator term $[TF_1][TF_2]/(K_{d_{TF1}} K_{d_{TF2}})$ arises if both TFs must be bound for transcription to occur – “transcriptional synergy”

taking stock

systems biology = from dead molecules to living organisms

different views of the organism

machine	entity that evolves through descent with modification	entity that develops through epigenetic self-organisation

the evolution of complexity reveals the complexity of evolution

weak linkage learning

time-scale separation can get around the complexity – linear framework

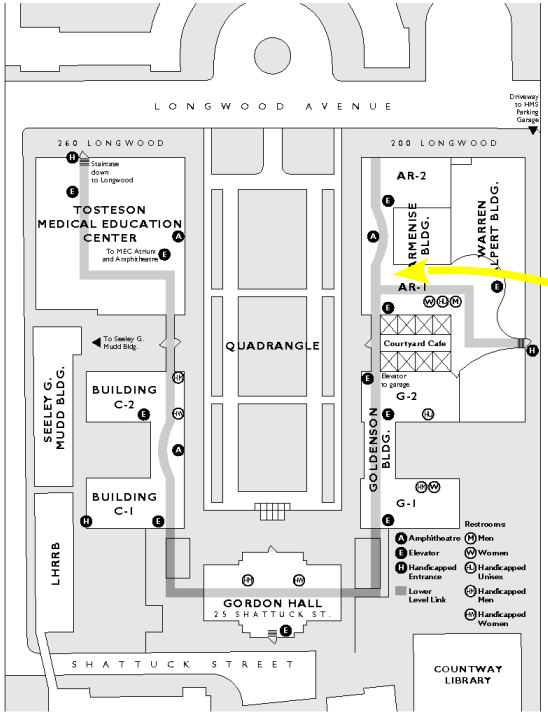
dynamical systems and landscapes – a metaphor for cellular identity

monostability, bistability, excitability, oscillation

interlinked feedbacks orchestrate homeostasis and information processing

the end of the road

thank you for participating in the course. i hope you enjoyed it. please remember to fill out the course evaluations or stop by and tell me in person what you thought about the course.



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ENJOY THE REST OF THE COURSE !