Dynamics, adaptation and fluctuations in bio-networks
March 24-27, Santa Barbara, California

Modelling circadian clocks and temperature-compensation

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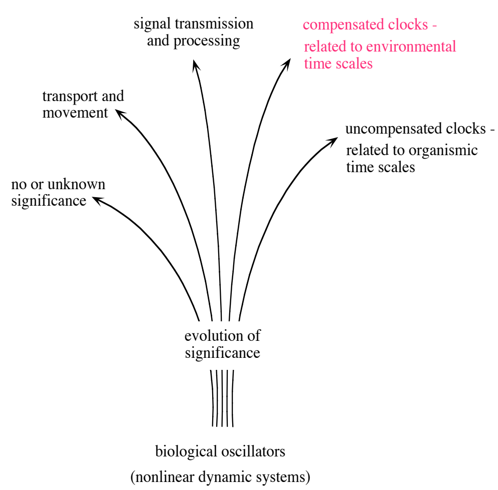
Biological Oscillators

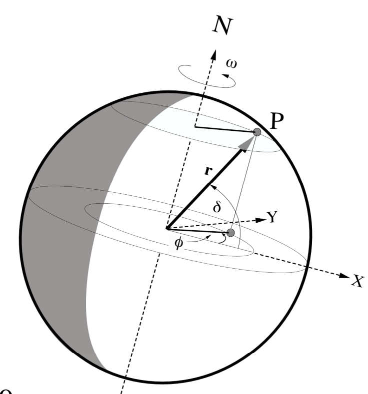


Circadian rhythms

- are important for the adaptation of organisms to their environment
  - for example to anticipate day/night changes as well as seasonal changes (important for many physiological processes).
- act as *clocks*, they have *temperature-compensation* as well as other *homeostatic regulation mechanisms* of their period against environmental influences.

Describing circadian clocks purely mathematically

The rotation/oscillation of point P along the coordinates X, Y describes an harmonic oscillator, which is used, for example, as the basis of the (harmonic) *Cosinor* or *Van der Pol* description of circadian rhythms.



$$\frac{dX}{dt} = \omega Y \qquad \frac{dY}{dt} = -\omega X \quad \Leftrightarrow \quad \ddot{X} + \omega^2 X = 0$$

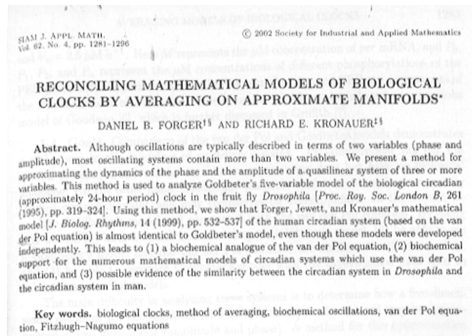
$$\omega = 1.157 \times 10^{-5} \text{ Hz}$$

Describing circadian clocks purely mathematically

Van der Pol oscillator:

$$\frac{dX}{dt} = \square[Y + \square(X \square \square X^3)]$$

$$\frac{dY}{dt} = \square\square X$$


SIAM J. APPL. MATH.
Vol. 62, No. 4, pp. 1281–1296

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RECONCILING MATHEMATICAL MODELS OF BIOLOGICAL CLOCKS BY AVERAGING ON APPROXIMATE MANIFOLDS*

DANIEL B. FORGER†§ AND RICHARD E. KRONAUER‡§

Abstract. Although oscillations are typically described in terms of two variables (phase and amplitude), most oscillating systems contain more than two variables. We present a method for approximating the dynamics of the phase and the amplitude of a quasilinear system of three or more variables. This method is used to analyze Goldbeter's five-variable model of the biological circadian (approximately 24-hour period) clock in the fruit fly *Drosophila* [*Proc. Roy. Soc. London B*, 261 (1995), pp. 319–324]. Using this method, we show that Forger, Jewett, and Kronauer's mathematical model [*J. Biolog. Rhythms*, 14 (1999), pp. 532–537] of the human circadian system (based on the van der Pol equation) is almost identical to Goldbeter's model, even though these models were developed independently. This leads to (1) a biochemical analogue of the van der Pol equation, (2) biochemical support for the numerous mathematical models of circadian systems which use the van der Pol equation, and (3) possible evidence of the similarity between the circadian system in *Drosophila* and the circadian system in man.

Key words. biological clocks, method of averaging, biochemical oscillations, van der Pol equation, Fitzhugh–Nagumo equations

Normally $\square$ is chosen to be small (0.1-0.25), which makes the van der Pol oscillator quasilinear and very similar to the harmonic oscillator.

Physical chemical approach to model circadian rhythms

”............... All living things are physico-chemical machines.....How can you make progress if you do not know physical chemistry?........”

Sinclair Lewis, *Arrowsmith*

Modelling circadian clocks in reaction kinetic terms

artistic view of an eukaryotic cell

All chemical change, no matter how complex, is believed to be due to chemical (*elementary)* processes.

If we would know all processes and their kinetics in a single cell, we would be able to give a *quantitative* description of the cell's physiology through the chemcial rate equations.

Chemical Rate Equations

Considering a set of reacting metabolites $\{S_i\}$, the reaction system is described by a set of coupled differential equations:

$$\frac{dS_i}{dt} = \sum_j n_{ij}\nu_j$$

n_{ij} denote stoichiometric coefficients

ν_j are reaction rates of individual process "*j*"

Example:

reaction 1: $A + B \xrightarrow{k_1} 2C$

reaction 2: $C + D \xrightarrow{k_2} E$

$$\Rightarrow \quad \frac{dC}{dt} = 2\nu_1 - \nu_2$$

$$\nu_1 = k_1 AB$$

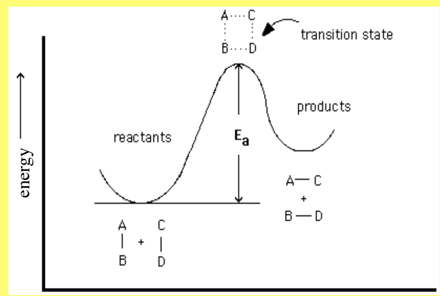
$$\nu_2 = k_2 CD$$

k_i: rate constant

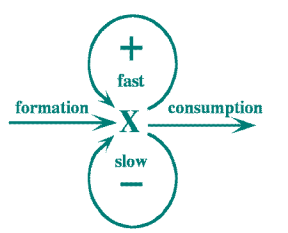
Influence of temperature on reaction rates: the Arrhenius equation

$$k = A\, e^{-\frac{E_a}{RT}}$$

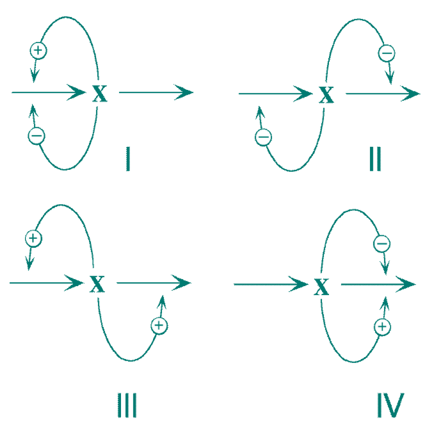
rate constant (k), activation energy (E_a), temperature (K) (T), gas constant (R), collision factor (A)



Franck's antagonistic feedback concept in physico-chemical oscillators

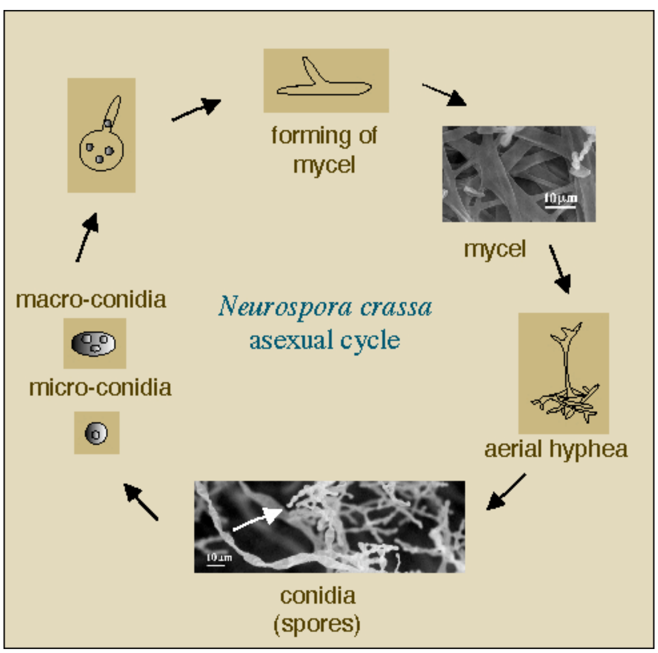


antagonistic feedback

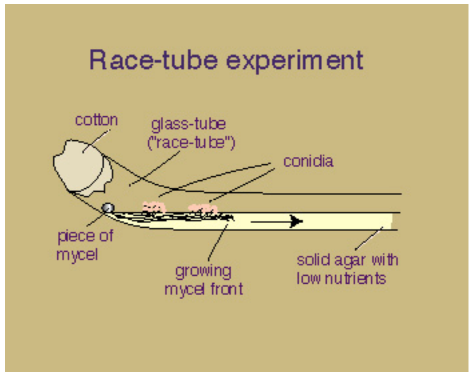


Franck, Angew. Chem. Int. Ed. Engl. 17 (1978) 1-15

Neurospora crassa: A Model Organism for the Study of Circadian Rhythms

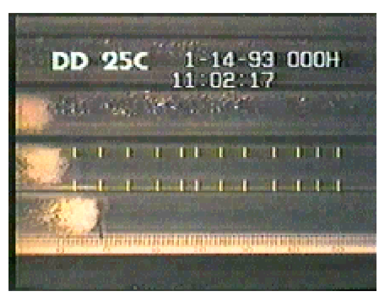


Racetube assay of clock rhythm in *Neurospora crassa*



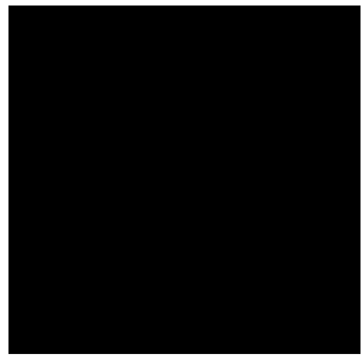
Neurospora crassa: A Model Organism for the Study of Circadian Rhythms

Influence of extracellular pH on *Neurospora's* circadian rhythm

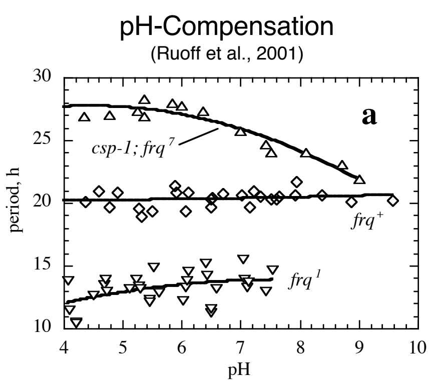


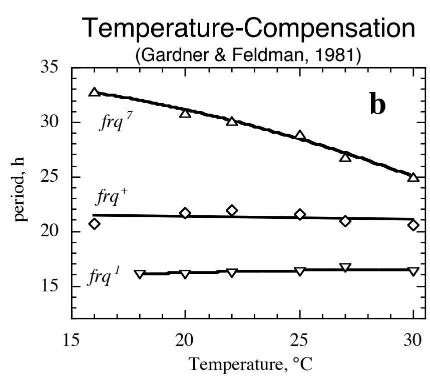
Jason C. Thoen and Van Gooch:
"Time Lapse Video Showing an Internal Circadian Clock in Mold (*Neurospora*) Growth"

Neurospora crassa growth and banding in a Petri dish



pH- and temperature-compensation in mutants of *Neurospora crassa*





Temperature-compensation in pH-oscillators

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PCCP

general scheme of a pH oscillator:

$$A^- + H^+ \rightleftharpoons AH \quad (1)$$
$$AH + H^+ + \{B\} \rightarrow 2H^+ + P^- \quad (2)$$
$$H^+ + \{C^-\} \rightarrow CH \quad (3)$$

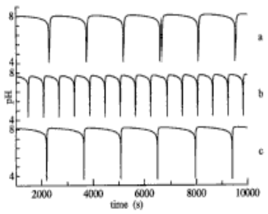


Fig. 2 Temperature compensation of the frequency in the calculated pH time series. Curve (a) was simulated with input concentrations, flow rate, and the basic set of the rate constants shown in Fig. 1(a). All the rate constants were 3 times higher than their basic values for curve (b) resulting in high frequencies. The same frequency as in curve (a) was obtained with the following constants: k_1 3.0 × 10 M s; k_{-1} 1.1 × 10 s; k_2 5.0 × 10 M s; k_3 0.40 s (curve (c)).

opposing effects on period from process (2) and (3)

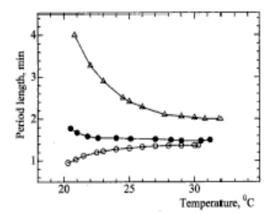
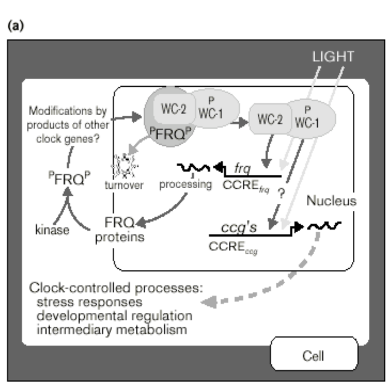


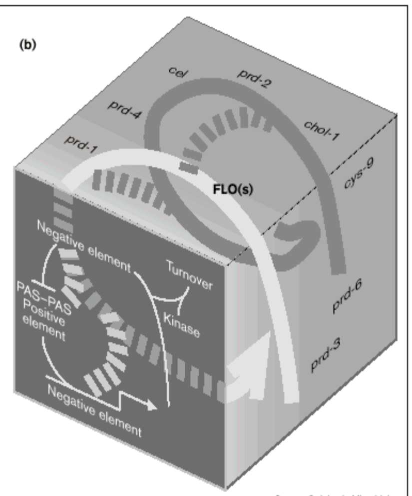
Fig. 4 Period length as a function of temperature at different input sulfite concentrations. $[H_2O_2]_0$ 0.0135, $[SO_3^{2-}]_0$ 0.0050, $[H^+]_0$ 5.0 × 10 M, k_0 3.0 × 10 s, $[SO_3^{2-}]_0$ 0.0018 (triangles), 0.0017 (full circles), 0.0015 M (open circles).

Phys. Chem. Chem. Phys., 2002, **4**, 5265–5269 **5267**

experimental finding of TempComp in the H2O2-sulfite-thiosulfate system

Negative feedback loop of *frequency* gene: Putative circadian pacemaker of the *Neurospora* clock





from: Iwasaki and Dunlap
Curr. Opinion Microbiol. 2000, 3:189-196

FRQ-protein

>sp|P19970|FRQ_NEUCR FREQUENCY CLOCK PROTEIN - Neurospora crassa.

```
MADSGDKSQGMRPPPPFDSRGHPLPRRASPDKSITLENHRLARDTSSRVTS
SSALGVTESQPQLKSSPTRRNSSGESEPTNWFNQSNRNPAAAFHDESHIM
EVDPPFYQKETDSSNEESRYPPGRNPVHPPGGVQLPGFRPVAAHSTAADD   coiled-coil
YRSVIDDLTVENKRLKEELKRYKQFGSDVMRKEKLFEIKVHGLPRRKKRE
LEATLRHFAASLGDSSESTSQRRKTGRHGTAVYSSGVSLSKHDSSSSSRS
RPVDSAYNSMSTGRSSHAPHSSGPSLGRPSLTRAKSVGTQKVENYLRDTP
DGLLPHHIVMTDKEKKKLVVRRLEQLFTGKISGRNMQRNQSMPSMDAPLA
PEGTNMAPPRPPPEGLREACIQLQDGDNPRKNRSSKDNGSASNSGGDQTE
LGGTGTGSGDGSGSGGRTGNNTSPPGAIAPDQRPTRPRDLDPDRVQIPSE
NMDYIRHLGLVSPEFLQGSRTSYQDVAPDAEGWVYLNLLCNLAQLHMVNV   frq[7]:G□ D  frq[1]:G□ S
TPSFIRQAVSEKSTKFQLSADGRKIRWRGGTDGTKFSSDSSEDKSQQSPM   S513         PEST-1
TEDTEDGSDKNGRRKKRKTQQASSEIGRFGPSRSPSDTFHYKPMFVHRNS   PEST-1       NLS
SSIETSLEESMSQGSEDAVDESNMGNSKWDFSGSGTTQQRRKRRYDGAIV
YYTGAPFCTDLSGDPGDMSPTAQMTAGREVEGSGSGDEVEHVLQRTLSGS
SLPIRPLSDDRARVAEVLDFDPGNPPELVADDGSSPNDEDFVFPWCEDPA
KVRIQPIAKEVMEPSGLGGVLPDDHFVMLVTTRRVVRPILQRQLSRSTTS
EDTAEFIAERLAAIRTSSPLPPRSHRLTVAPLQVEYVSGQFRRLNPAPLP   PEST-2
PPAIFYPPFSTDSSWDDGDDLASDDEEVEEVEEDSYSEGQISRRANPHFS   PEST-2
DNNTYMRKDDLAFDTETDVRMDSDDNRLSDSGHNMRAMMPRAEAVDGDDS
PLAAVTGKEVDIVHTGSSVATAGGAESGYSSSMEDVSSS
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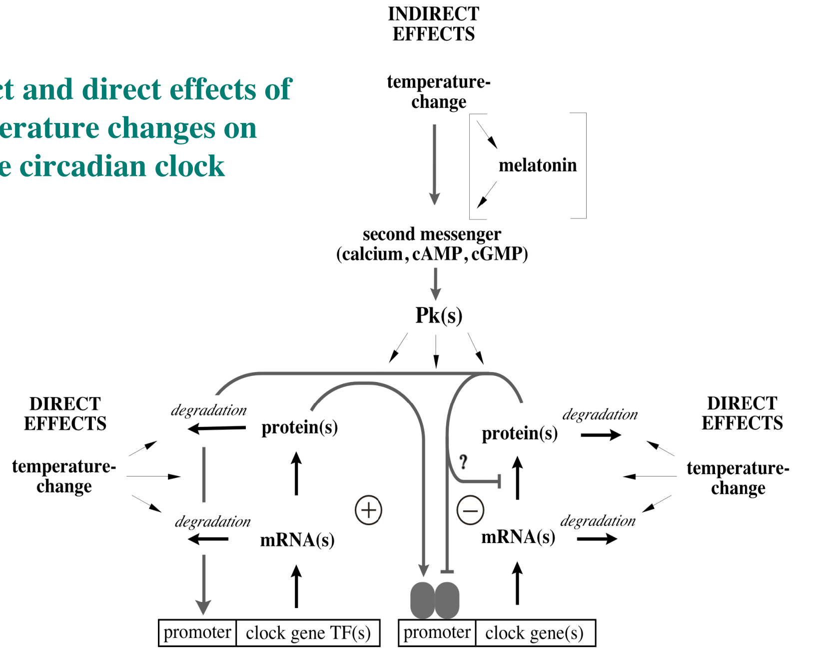
modelling temperature-compensation

Indirect and direct effects of temperature changes on the circadian clock



Rensing and Ruoff, CBI *19* (2002), 807-864

A theory of temperature-compensation

Assume that we know all (elementary) reactions $\{R_i\}$ defining a cell's physiology and its circadian rhythm.

process $R_i(T)$ $\quad k_i = A_i \exp(-E_i/RT)$

period $P = f(k_1, k_2, k_3, \ldots, k_i, \ldots)$

Antagonistic balance in temperature

$$P = f(k_1, k_2, k_3, \ldots, k_i, \ldots)$$

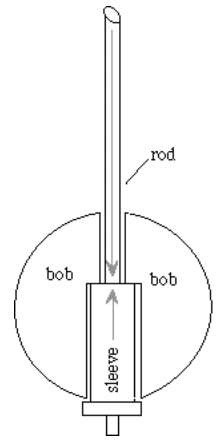
$$\frac{dP}{dT} = \sum_i \left(\frac{\partial f}{\partial k_i}\right)\left(\frac{\partial k_i}{\partial T}\right) = 0$$

$$\square \quad \sum_{i\square} \left(\frac{\partial \ln(f)}{\partial \ln(k_i)}\right) \square E_i = 0$$

Concept of opposing reactions
(antagonistic balance in temperature)

$$\square_{\square} \sum_{j}^{\square} \left(\frac{\partial \ln f}{\partial \ln k_j}\right) \square E_j = \square \sum_{i}^{\square} \left(\frac{\partial \ln f}{\partial \ln k_i}\right) \square E_i$$

(P-increasing) (P-decreasing)



Mechanical analogy:
Temp-Comp Invar pendulum

Infinite number of possibilities to realize temperature-compensation!

- For a given reaction kinetic oscillator model there is an infinite number of activation energy combinations that will lead to temperature compensation. Evolution has "realized" some of them.

$$\sum_{j} \left(\frac{\partial \ln f}{\partial \ln k_j}\right) \square E_j = \square \sum_{i} \left(\frac{\partial \ln f}{\partial \ln k_i}\right) \square E_i$$

(P-increasing) (P-decreasing)

- Analogous homeostasis conditions may be formulated for other physico-chemical properties, as for example, pH or salinitiy:

salinity: $k_i = k_{oi} \exp(\pm\square\sqrt{I})$, I = ionic strength

pH: $k_i = k_{oi} \exp(-\square_i (pH - pH_{i,opt})^2)$

Euler's summation theorem

$$P = f(k_1, k_2, k_3, \ldots, k_i, \ldots)$$

$\left(\frac{\partial \ln P}{\partial \ln k_i}\right)$ are called *control coefficients* C_i, *period elasticities*, or *sensitivity coefficients*.

The period function $P = f$ is called homogenous to degree -1 if:

$$f(tk_1, tk_2, tk_3, \ldots, tk_i, \ldots) = t^{-1} f(k_1, k_2, k_3, \ldots, k_i, \ldots) = t^{-1} P$$

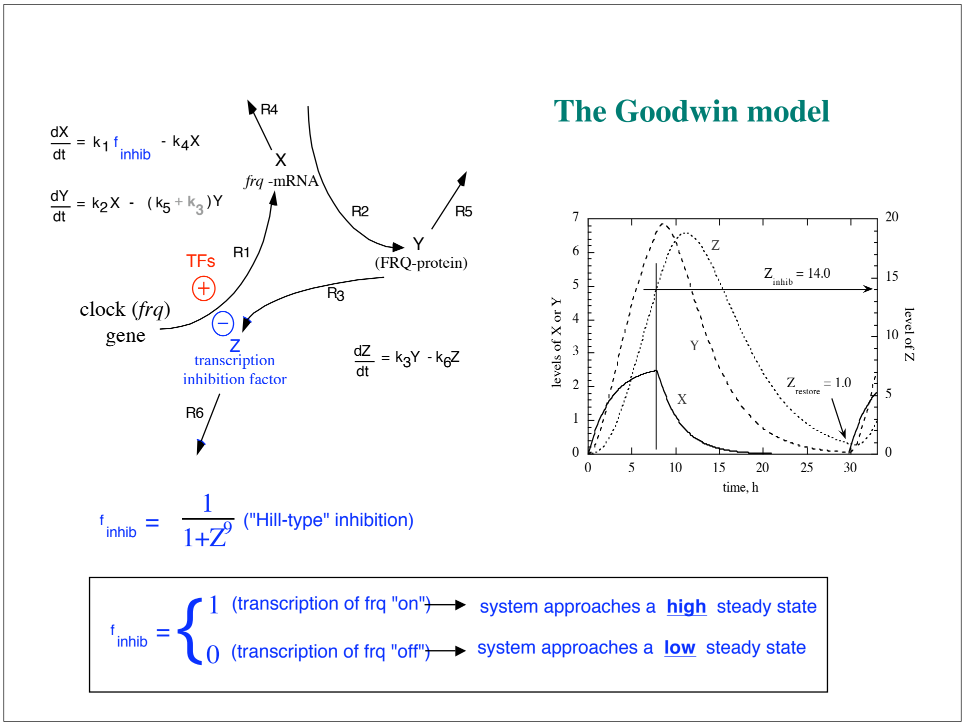
$$\Rightarrow \quad \sum_i \left(\frac{\partial \ln P}{\partial \ln k_i}\right) = -1$$

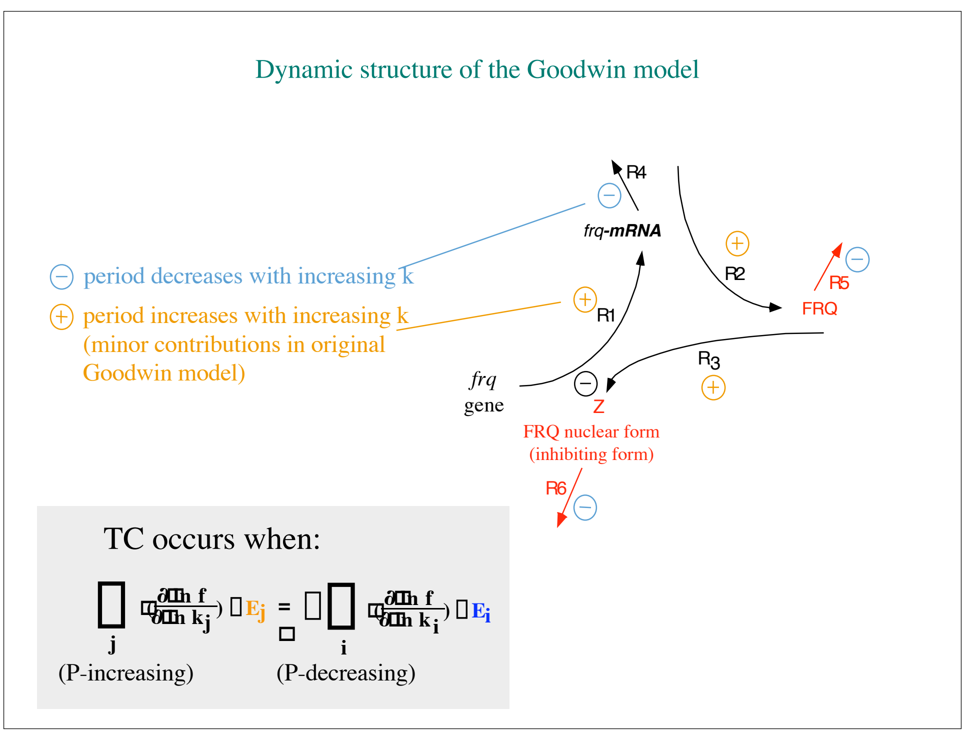
A reaction kinetic modelling strategy: working with minimal models

Ockham's razor:

"Plurality is not to be assumed without necessity"

William of Ockham, 1285-1349


The Goodwin model
dX/dt = k1 f inhib - k4X
dY/dt = k2X - (k5 + k3)Y
dZ/dt = k3Y - k6Z
R4
X
frq -mRNA
R2
R5
Y
(FRQ-protein)
R1
TFs
clock (frq)
gene
R3
Z
transcription
inhibition factor
R6
f inhib = 1/(1+Z^9) ("Hill-type" inhibition)
levels of X or Y
level of Z
time, h
Z inhib = 14.0
Z restore = 1.0
f inhib = 1 (transcription of frq "on") → system approaches a high steady state
0 (transcription of frq "off") → system approaches a low steady state

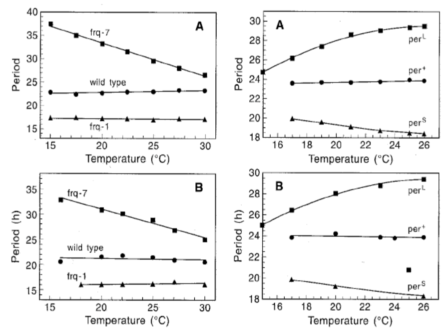

Dynamic structure of the Goodwin model
period decreases with increasing k
period increases with increasing k
(minor contributions in original
Goodwin model)
R4
frq-mRNA
R2
R5
FRQ
R1
frq
gene
R3
Z
FRQ nuclear form
(inhibiting form)
R6
TC occurs when:
(P-increasing)
(P-decreasing)

Prediction that clock protein degradation plays an important role for circadian period length and temperature-compensation

Table 1. Effect of loop rate constant values k_1, k_2, k_3 and turnover rate constant values k_4, k_5, k_6 on period length

k_1	Period[a]	k_2	Period[b]	k_3	Period[c]
0.1	23.3	0.1	23.1	0.1	23.1
1.0	23.4	1.0	23.4	1.0	23.4
10.0	23.6	10.0	23.6	10.0	23.6
100.0	23.6	100.0	24.0	100.0	24.0
1000.0	23.9	1000.0	24.6	1000.0	24.6
k_4	Period[d]	k_5	Period[e]	k_6	Period[f]
0.05	39.3	0.05	39.0	0.05	29.1
0.15	26.2	0.15	26.2	0.15	21.0
0.3	20.0	0.3	20.0	0.3	16.3
0.45	17.7	0.45	17.7	0.45	13.8
0.6	16.6	0.6	16.4	0.6	12.7

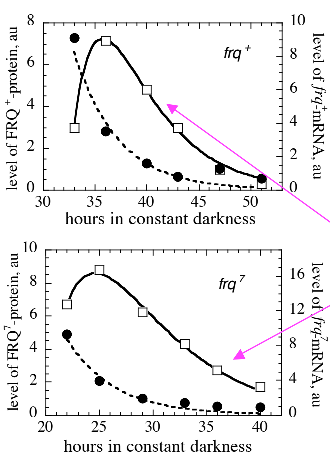
(Ruoff et al., Naturwissenschaften (1996) 83: 514-517)

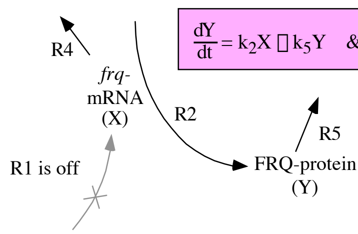


Neurospora *Drosophila*

(**A**: Goodwin model; **B**: experiment)

Estimating *frq*-mRNA and FRQ-protein half-lives in *frq[+]* and *frq[7]*





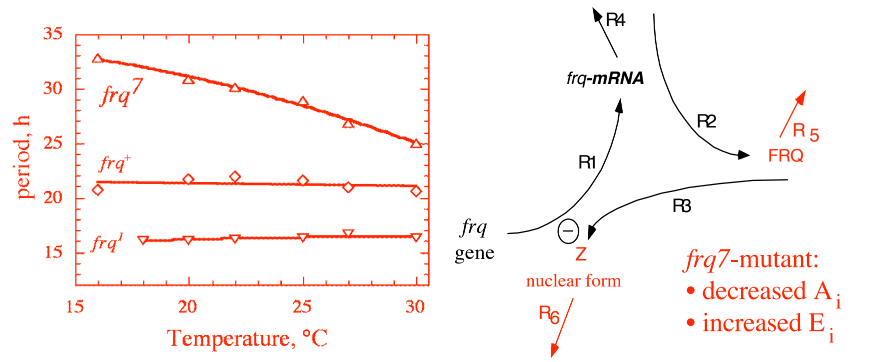
$$\frac{dY}{dt} = k_2X - k_5Y \quad \& \quad \frac{dX}{dt} = -k_4X$$

$$Y(t) = Y_{start}\exp(-k_5t) + \frac{k_2X_{start}}{k_5 - k_4}\left(\exp(-k_4t) - \exp(-k_5t)\right)$$

Species	Half-Life, h
frq[+]-mRNA	3.39 ± 0.42
frq[7]-mRNA	2.86 ± 0.31
FRQ[+]	2.9
FRQ[7]	6.2

Garceau et al., Cell 89 (1997) 469-476 (exp data)
Ruoff et al, JBR 14 (1999) 469-479 (model)

FRQ-Stability and Temperature Compensation



$$k_i = A_i \exp(-E_i/RT)$$

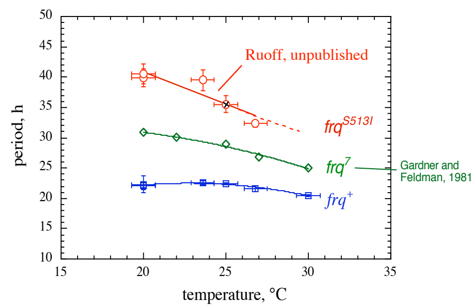
FRQ^{S513I}

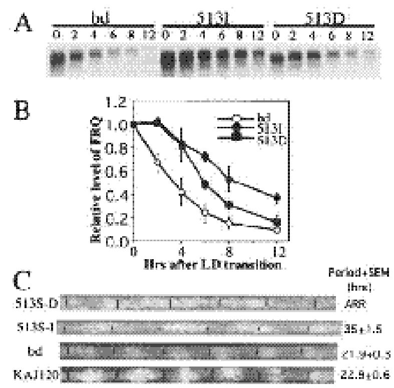
>sp|P19970|FRQ_NEUCR FREQUENCY CLOCK PROTEIN - Neurospora crassa.

```
MADSGDKSQGMRPPPFDSRGHPLPRRASPDKSITLENHRLARDTSSRVTS
SSALGVTESQPQLKSSPTRRNSSGESEPTNWFNQSNRNPAAAFHDESHIM
EVDPPFYQKETDSSNEESRYPPGRNPVHPPGGVQLPGFRPVAAHSTAADD
YRSVIDDLTVENKRLKEELKRYKQFGSDVMRKEKLFEIKVHGLPRRKKRE
LEATLRHFAASLGDSSESTSQRRKTGRHGTAVYSSGVSLSKHDSSSSSRS
RPVDSAYNSMSTGRSSHAPHSSGPSLGRPSLTRAKSVGTQKVENYLRDTP
DGLLPHHIVMTDKEKKKLVVRRLEQLFTGKISGRNMQRNQSMPSMDAPLA
PEGTNMAPPRPPPEGLREACIQLQDGDNPRKNRSSKDNGSASNSGGDQTE
LGGTGTGSGDGSGSGGRTGNNTSPPGAIAPDQRPTRPRDLDPDRVQIPSE
NMDYIRHLGLVSPEFLQGSRTSYQDVAPDAEGWVYLNLLCNLAQLHMVNV   frq[7]:G D  frq[1]:G S
TPSFIRQAVSEKSTKFQLSADGRKIRWRGGTDGTKFSSDSSEDKSQQSPM   S513         PEST-1
TEDTEDGSDKNGRRKKRKTQQASSEIGRFGPSRSPSDTFHYKPMFVHRNS   PEST-1       NLS
SSIETSLEESMSQGSEDAVDESNMGNSKWDFSGSGTTQQRRKRRYDGAIV
YYTGAPFCTDLSGDPGDMSPTAQMTAGREVEGSGSGDEVEHVLQRTLSGS
SLPIRPLSDDRARVAEVLDFDPGNPPELVADDGSSPNDEDFVFPWCEDPA
KVRIQPIAKEVMEPSGLGGVLPDDHFVMLVTTRRVVRPILQRQLSRSTTS
EDTAEFIAERLAAIRTSSPLPPRSHRLTVAPLQVEYVSGQFRRLNPAPLP   PEST-2
PPAIFYPPFSTDSSWDDGDDLASDDEEVEEVEEDSYSEGQISRRANPHFS   PEST-2
DNNTYMRKDDLAFDTETDVRMDSDDNRLSDSGHNMRAMMPRAEAVDGDDS
PLAAVTGKEVDIVHTGSSVATAGGAESGYSSSMEDVSSS
```

Importance of FRQ-degradation on circadian period length

Replacement of serine-513 by isoleucine (I) blocks phosphorylation at this site and results in a significantly slower FRQ-degradation and in a long circadian period length ≈ 35h as well as loss in temperature-compensation.





(Liu *et al.*, PNAS (2000) 97:234-239)

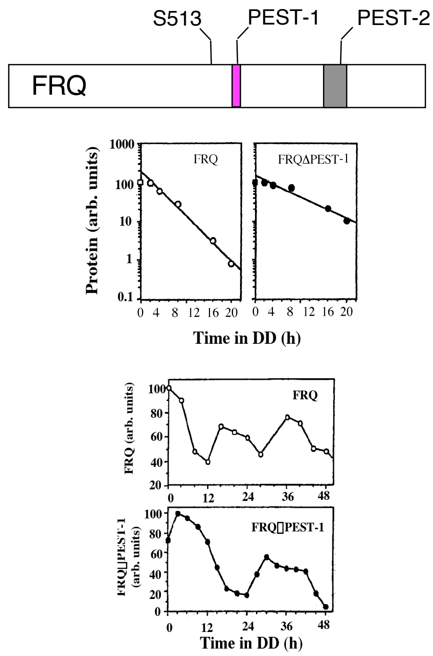
FRQ□PEST-1

>sp|P19970|FRQ_NEUCR FREQUENCY CLOCK PROTEIN - Neurospora crassa.

```
MADSGDKSQGMRPPPFDSRGHPLPRRASPDKSITLENHRLARDTSSRVTS
SSALGVTESQPQLKSSPTRRNSSGESEPTNWFNQSNRNPAAAFHDESHIM
EVDPPFYQKETDSSNEESRYPPGRNPVHPPGGVQLPGFRPVAAHSTAADD
YRSVIDDLTVENKRLKEELKRYKQFGSDVMRKEKLFEIKVHGLPRRKKRE
LEATLRHFAASLGDSSESTSQRRKTGRHGTAVYSSGVSLSKHDSSSSSRS
RPVDSAYNSMSTGRSSHAPHSSGPSLGRPSLTRAKSVGTQKVENYLRDTP
DGLLPHHIVMTDKEKKKLVVRRLEQLFTGKISGRNMQRNQSMPSMDAPLA
PEGTNMAPPRPPPEGLREACIQLQDGDNPRKNRSSKDNGSASNSGGDQTE
LGGTGTGSGDGSGSGGRTGNNTSPPGAIAPDQRPTRPRDLDPDRVQIPSE
NMDYIRHLGLVSPEFLQGSRTSYQDVAPDAEGWVYLNLLCNLAQLHMVNV   frq[7]:G□ D   frq[1]:G□ S
TPSFIRQAVSEKSTKFQLSADGRKIRWRGGTDGTKFSSDSSIDKSQQSPM   S513          PEST-1
TEDTEDGSDKNGRRKKRKTQQASSEIGRFGPSRSPSDTFHYKPMFVHRNS   PEST-1        NLS
SSIETSLEESMSQGSEDAVDESNMGNSKWDFSGSGTTQQRRKRRYDGAIV
YYTGAPFCTDLSGDPGDMSPTAQMTAGREVEGSGSGDEVEHVLQRTLSGS
SLPIRPLSDDRARVAEVLDFDPGNPPELVADDGSSPNDEDFVFPWCEDPA
KVRIQPIAKEVMEPSGLGGVLPDDHFVMLVTTRRVVRPILQRQLSRSTTS
EDTAEFIAERLAAIRTSSPLPPRSHRLTVAPLQVEYVSGQFRRLNPAPLP   PEST-2
PPAIFYPPFSTDSSWDDGDDLASDDEEVEEVEEDSYSEGQISRRANPHFS   PEST-2
DNNTYMRKDDLAFDTETDVRMDSDDNRLSDSGHNMRAMMPRAEAVDGDDS
PLAAVTGKEVDIVHTGSSVATAGGAESGYSSSMEDVSSS
```

Importance of FRQ-degradation on circadian period length

(Görl *et al.*, EMBO J. (2001) 20:7074-7084)

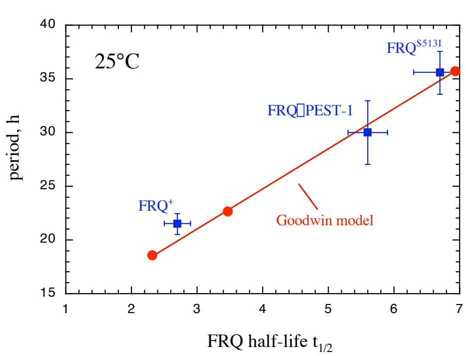


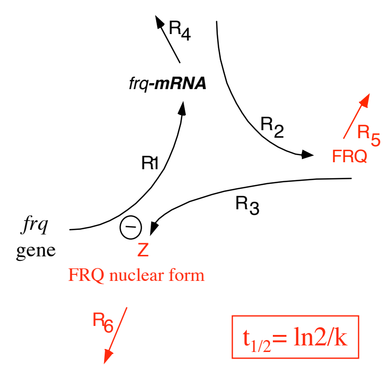
Deletion of PEST-1 leads to an increased FRQ-protein stability.

Both *frq*ΔPEST-1 RNA and FRQΔPEST-1 protein are rhythmically expressed with an increased period length of 28-30 h.

However, *frq*ΔPEST-1 has lost the conidiation rhythm.

Relationship between period length and FRQ (first-order) half-life $t_{1/2}$



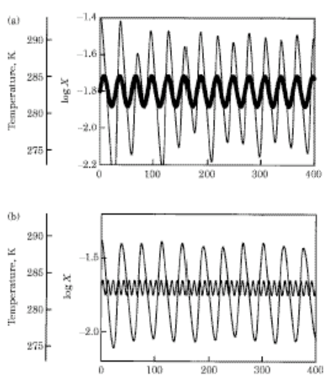


modelling entrainment by temperature cycles

&

temperature-pulse phase response curves

entrainment by temperature cycles in the Goodwin model

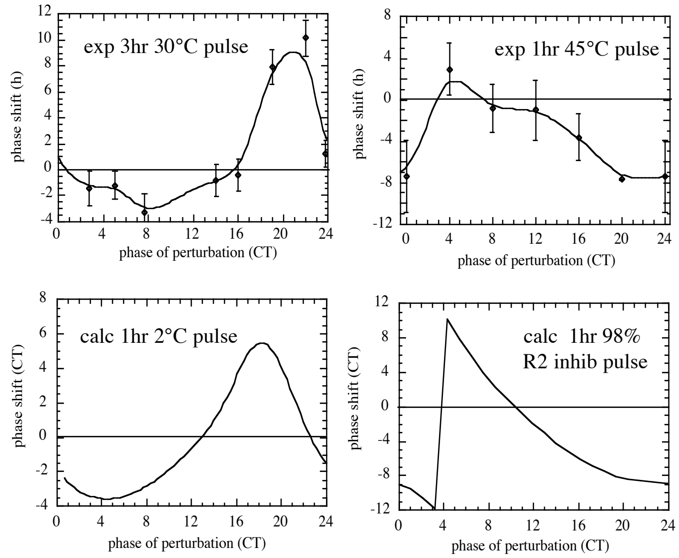


(a) Temperature entrainment using a sinusoidal temperature perturbation with period =30 and a temperature amplitude of 1°C. The inner oscillation is the entraining temperature cycle while the outer oscillation is the responding log X value.

(b) When the period is decreased to 10 the oscillator is no longer able to follow the perturbing rhythm and resets to its original unperturbed period of 38.

Ruoff & Rensing, JTB (1996) 275-285

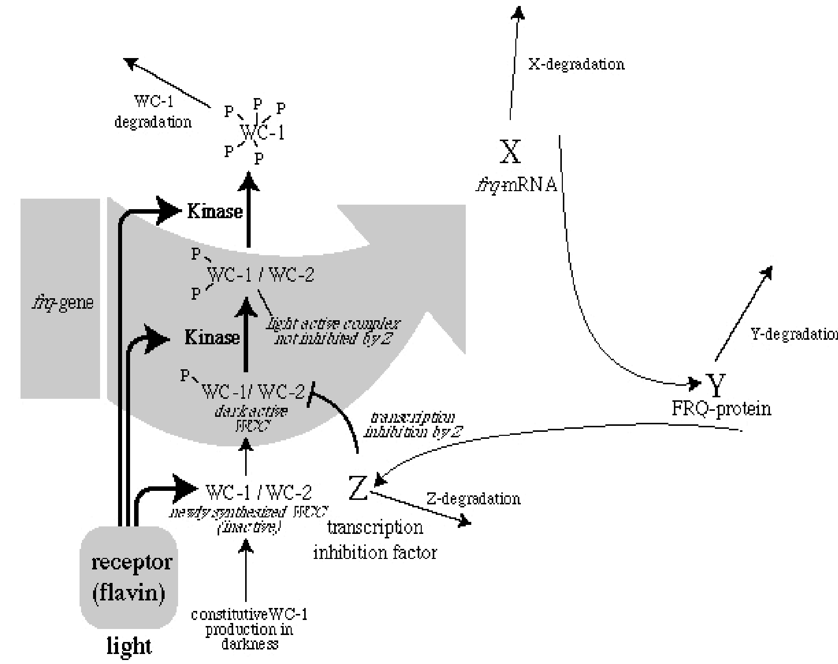
Temperature-pulse PRCs



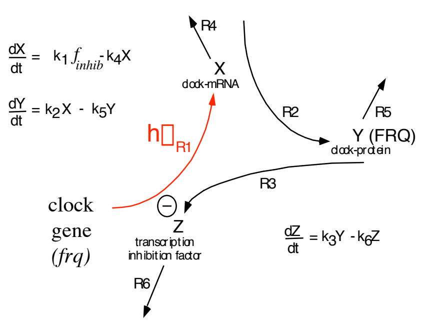
Ruoff & Rensing, JTB (1996) 275-285

Influence of light on the *Neurospora* clock:

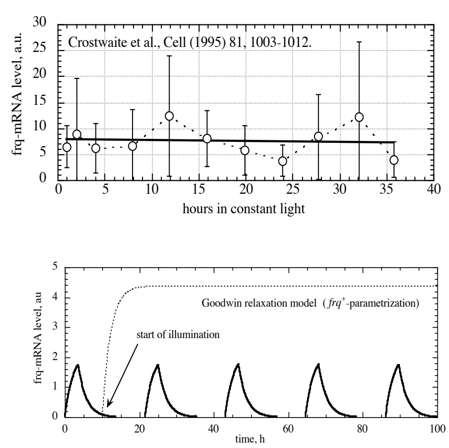
phase response curves



Constant Illumination
(Threshold Model)



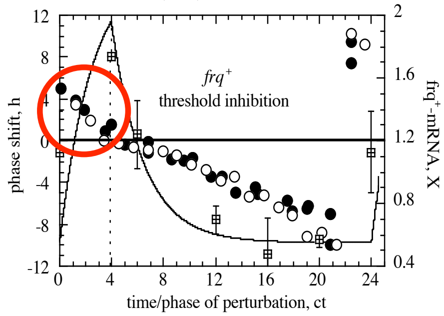
wt (DD): $k_1=k_2=k_3=1.0$ (P=21.5 h)
$k_4=k_5=k_6=0.45$



wt (LL): $k_1=2.0$

Model prediction: transcription inhibition directly after light pulse to ensure phase advances

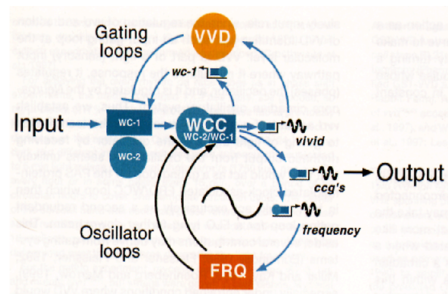
Dharmananda (1980), PhD-thesis, UC-Santa Cruz
Ruoff et al. (2001), J. theoret. Biol. **209**, 29-42.



□: calculated phase shift; □: experimental phase shift
□: experimental *frq*-mRNA; — : calculated *frq*-mRNA.

Additional inhibition of *frq*-transcription necessary directly after light pulse to get phase advances for phase of perturbations between ct0-ct4.

Heintzen et al. (2001), Cell **104**, 453-464.

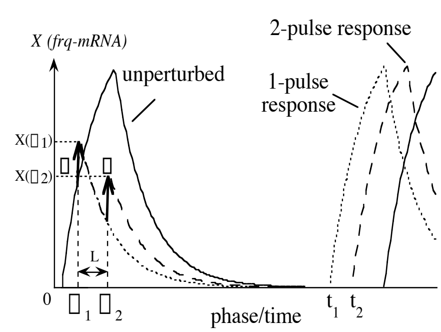


The PAS-protein VIVID (VVD) has been found to repress light input and its function appears to be related to our theoretical findings.

Phase Resetting with Two Light Pulses

Two light pulses with interval L are applied.

□□□ 12 is the additional phase shift due to the second light pulse

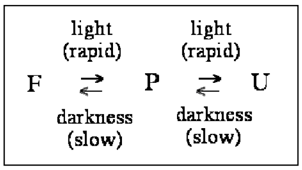


$$\square\square\square 12 = \square L + \frac{1}{k4} \ln\left(\frac{X(\square 1)}{\square\square\square X(\square 1)\exp(\square k4L)}\right)$$

Ruoff et al. (2001), JTB, **209**, 29-42.

2-Pulse Light PRC: Experimental Results and Interpretation

Kinetics of light-signal in 2-pulse light perturbations:

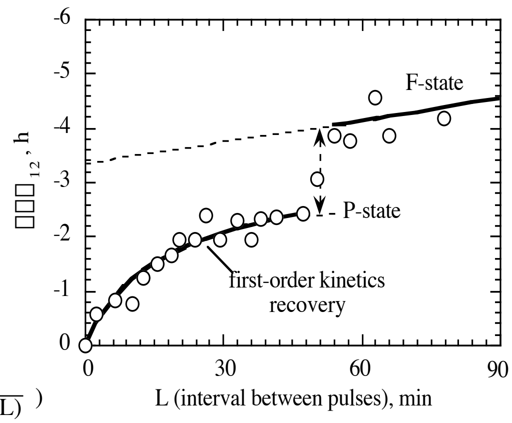


P = **P0**(1 □ exp(□t/□rp))

□ = □**P** = □**P0**(1 □ exp(□t/□rp))
= □0(1 □ exp(□t/□rp))

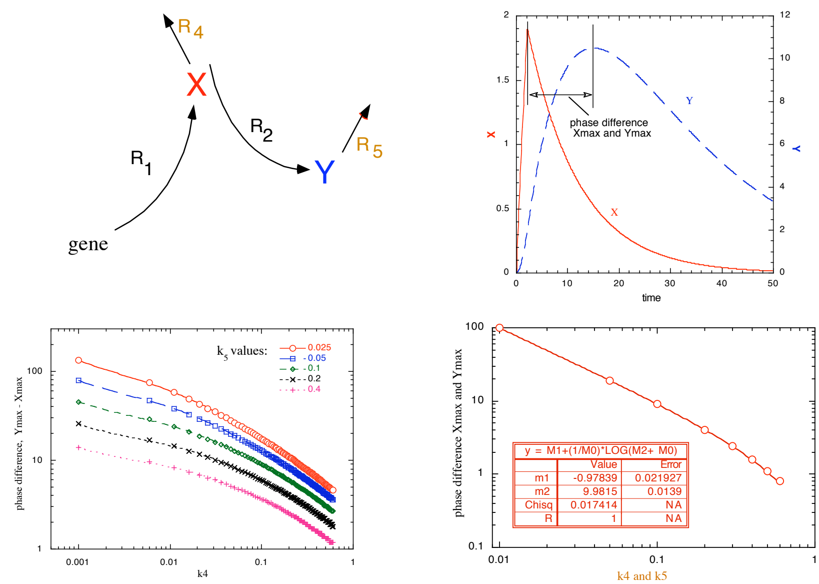
□□□ 12 = □L + 1/k4 ln (X(□ 1) / (□□+□X(□ 1)exp(□k4L)))

Exp. Result: Dharmananda, S. (1980) PhD thesis.

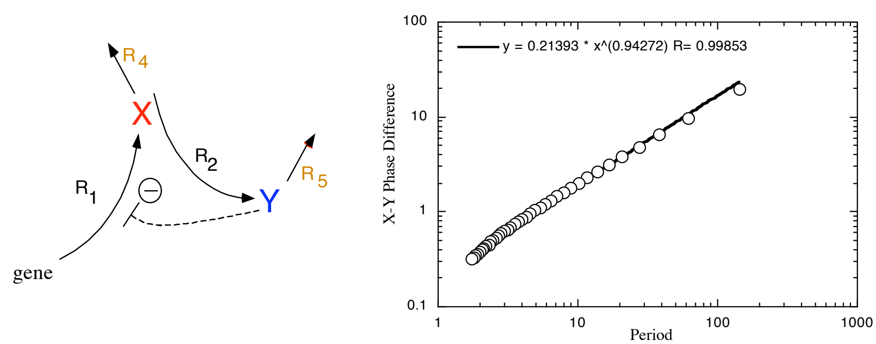


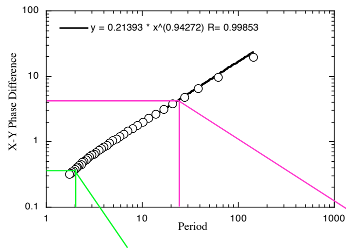
phase relationship between transcript and protein levels

phase relationship between transcript and protein levels in the transcription-translation sequence



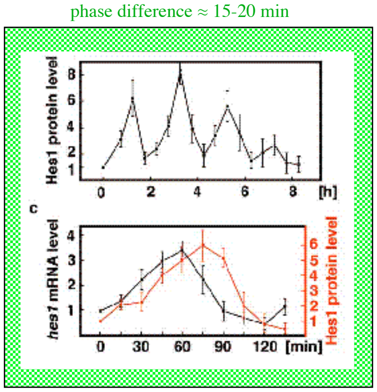
phase relationships between transcript and protein levels in the transcription-translation negative feedback loop as a function of period length





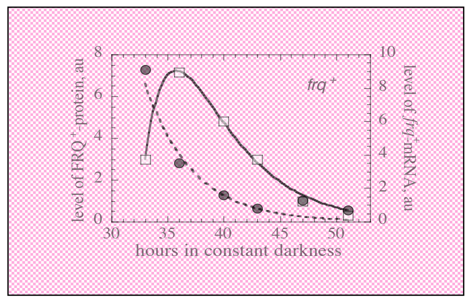
phase relationships between mRNA and expressed protein in transcription translation negative feedback oscillators

phase difference ≈ 15-20 min



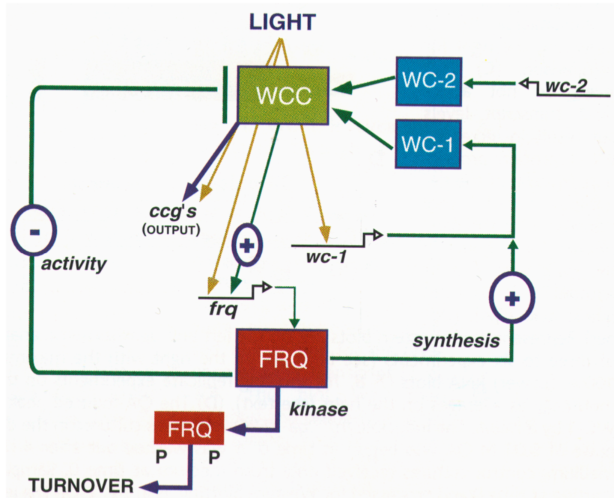
from Hirata et al., Science, 298, 2002, 840-843

phase difference ≈ 3-4 h in circadian clocks

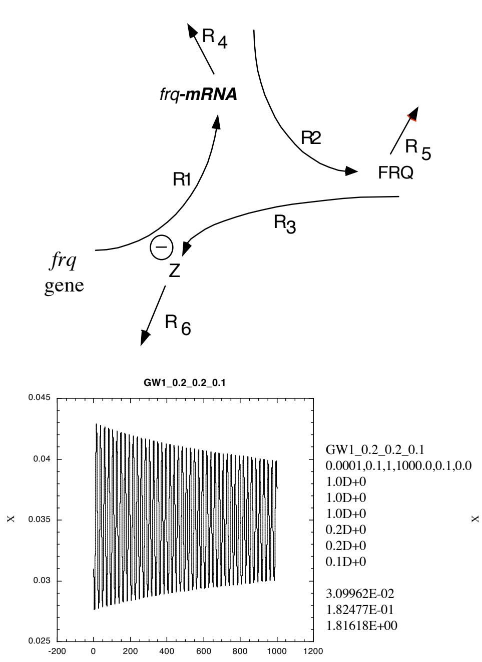


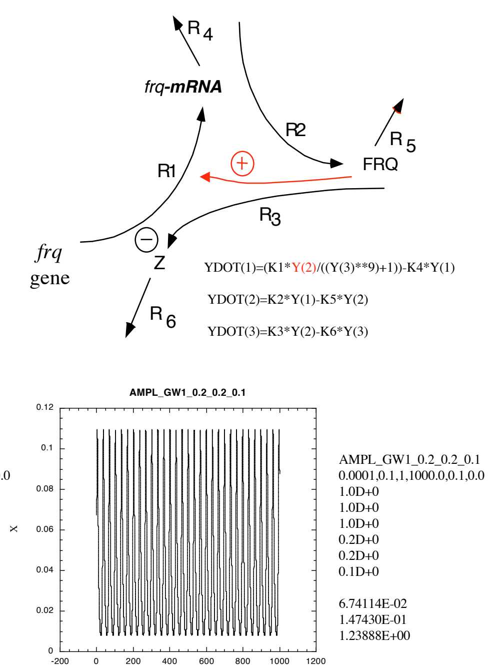
replotted from Garceau et al., Cell 89, 1997, 469-476

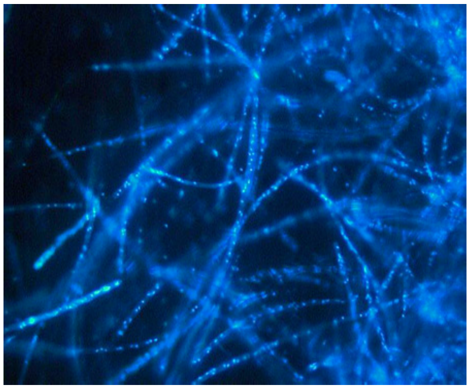
stabilizing role of positive feedback
(autocatalytic loop)



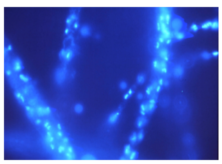
Lee *et al.*, Science *289* (2000) 107-110



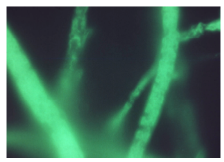




DAPI-stained mycelium to visualize nuclei (photo: P. Ruoff)



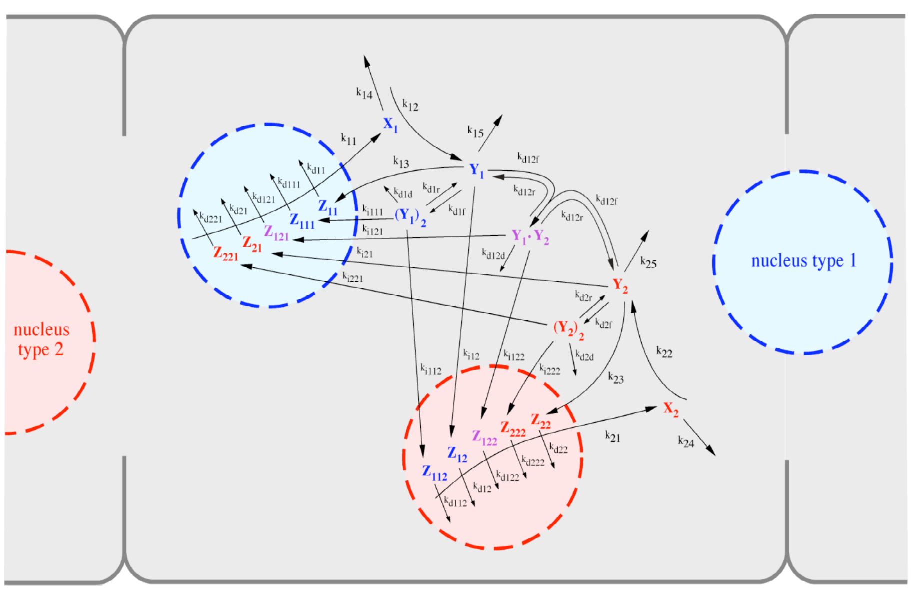
DAPI-stain



immunofluorescence of CCG-1

(photos: N. Garceau)

modelling heterokaryons
(coupled oscillators)



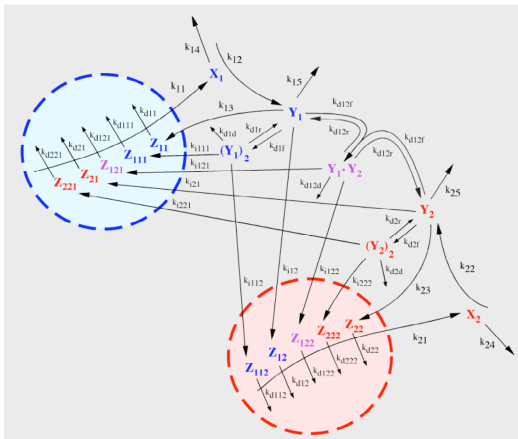
$k_{11}=k_{12}=k_{13}=k_{21}=k_{22}=k_{23}=k_{i12}=k_{i21}=1.0$ (transcription, translation, nuclear import)

$k_{14}=k_{24}=0.28$ (mRNA degradation)

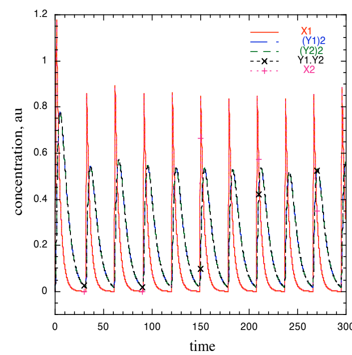
$k_{d11}=k_{d22}=k_{15}=k_{25}=k_{d1d}=k_{d2d}=0.18$ (FRQ, $(FRQ)_2$ degradation)

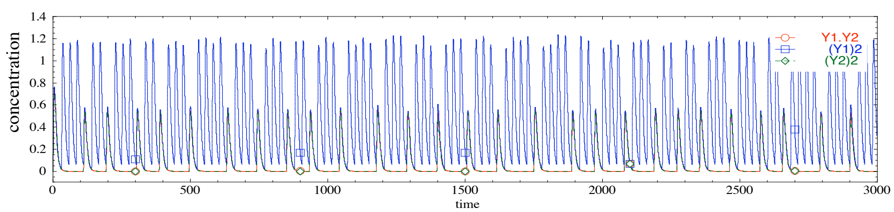
$k_{d1f}=k_{d2f}=k_{d12f}=1$ □ 10^6; $k_{d1r}=k_{d2r}=k_{d12r}=0.01$(reversible $(FRQ)_2$ formation)

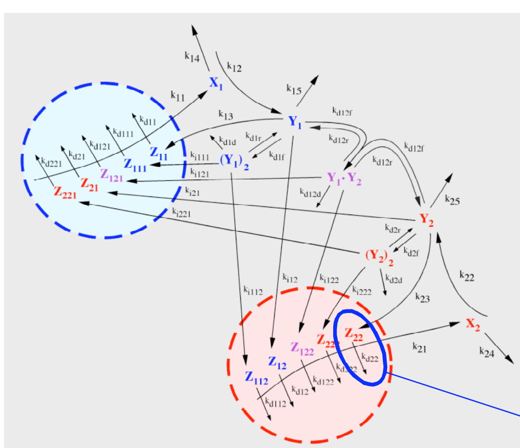
remaining rate constants = 0 (no FRQ-dimer import into nucleus)



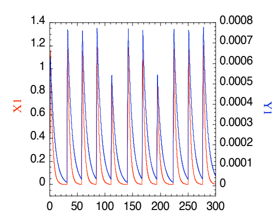
homokaryon example

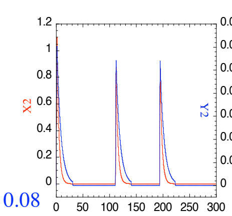




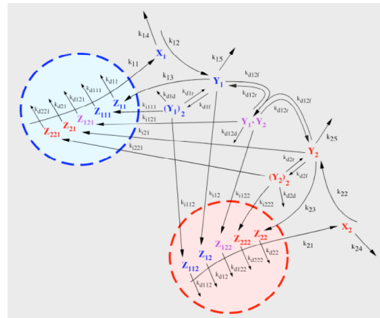


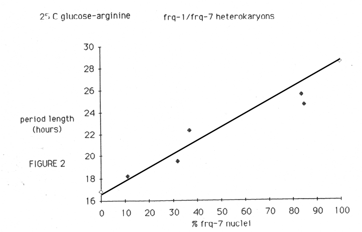
k_{d22}: 0.18 □ 0.08



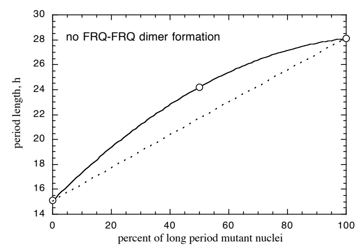


heterokaryon periods





from J. J. Loros: "Studies on FRQ-9, a Recessive Circadian Clock Mutant of *Neurospora crassa*" University of California Santa Cruz, 1984.



calculated from above model with no FRQ dimer formation

Summary

- Importance of *Ockham's razor* to remove redundancy in models, i.e., create minimal models.
- Minimal models can be further developed in accordance with experimental findings.
- More concerted interactions between experimentalist and theoreticians/modellers are needed in order to do more systematic experimental studies to get data for building models.

Baker Library, Dartmouth College

Lysefjord, near Stavanger

Thanks to

- Prof. Jay Dunlap for support during my stay at the Genetics Department, Dartmouth Medical School.
- Stavanger University College for giving me the possibility for a sabbatical leave.

Appendix

Euler's summation theorem

$$P = f(k_1, k_2, k_3, \ldots, k_i, \ldots)$$

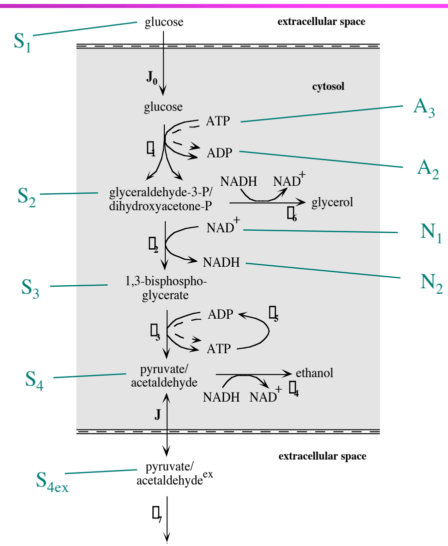
$\left(\frac{\partial \ln P}{\partial \ln k_i}\right)$ are called *control coefficients* C_i, *period elasticities*, or *sensitivity coefficients*.

The period function $P = f$ is called homogenous to degree -1 if:

$$f(tk_1, tk_2, tk_3, \ldots, tk_i, \ldots) = t^{-1} f(k_1, k_2, k_3, \ldots, k_i, \ldots) = t^{-1} P$$

$$\Rightarrow \quad \sum_i \left(\frac{\partial \ln P}{\partial \ln k_i}\right) = -1$$

The temperature-compensated glycolytic oscillator: testing Euler's summation theorem and antagonistic balance condition for temperature-compensation



$$\frac{dS_1}{dt} = J_0 - v_1 \qquad v_1 = k_1 S_1 A_3 \left[1 + \left(\frac{A_3}{K_I}\right)^q\right]^{-1}$$

$$\frac{dS_2}{dt} = 2v_1 - v_2 - v_6 \qquad v_2 = k_2 S_2 N_1$$

$$\frac{dS_3}{dt} = v_2 - v_3 \qquad v_3 = k_3 S_3 A_2$$

$$\frac{dS_4}{dt} = v_3 - v_4 - J \qquad v_4 = k_4 S_4 N_2$$

$$\frac{dN_2}{dt} = v_2 - v_4 - v_6 \qquad v_5 = k_5 A_3$$

$$\frac{dA_3}{dt} = -2v_1 + 2v_3 - v_5 \qquad v_6 = k_6 S_2 N_2$$

$$\frac{dS_4^{ex}}{dt} = \varphi J - v_7 \qquad v_7 = k S_4^{ex}$$

J. Wolf and R. Heinrich, Biochem. J. *345* (2000) 321-334.

Parameter Values of the Reference State[a]

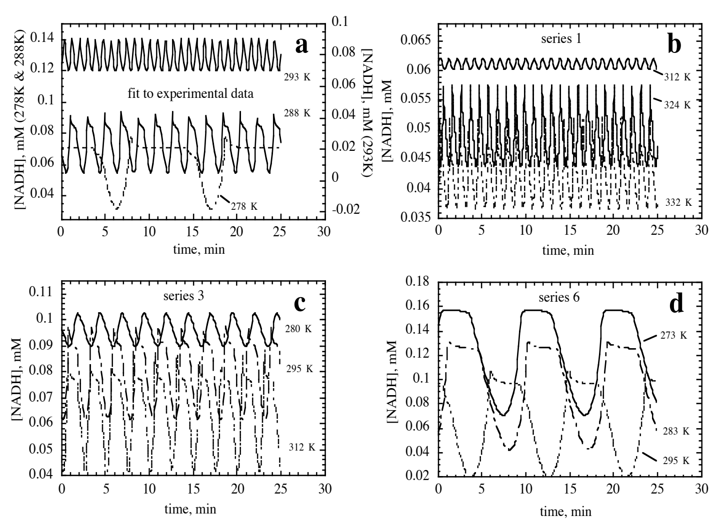
Parameter	Value
J_0	2.5 mMmin^{-1}
k_1	100.0 mM^{-1} min^{-1}
k_2	6.0 mM^{-1} min^{-1}
k_3	16.0 mM^{-1} min^{-1}
k_4	100.0 mM^{-1} min^{-1}
k_5	1.28 min^{-1}
k_6	12.0 mM^{-1} min^{-1}
k	1.8 min^{-1}
//	13.0 min^{-1}
q	4.0
K_1	0.52 mM
N	1.0 mM
A	4.0 mM
□	0.1

[a] resulting in a period length of 1.17 min.

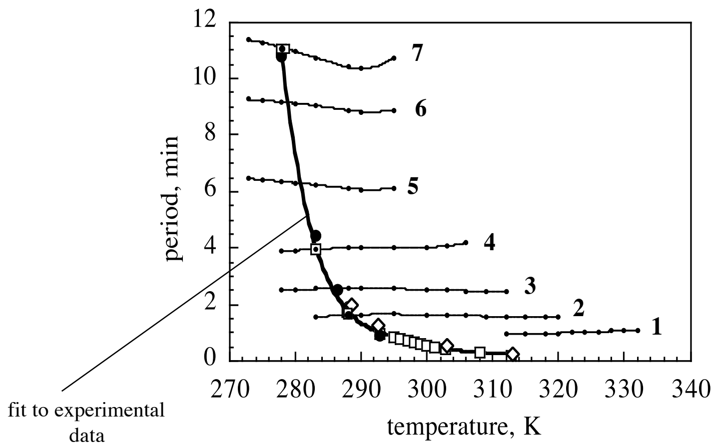
Initial Concentrations for Reference State Oscillations

Compound	Concentration
glucose	1.187 mM
glyceraldehyde-3-P/ dihydroxyacetone-P	0.193 mM
1,3-bisphosphoglycerate	0.050 mM
pyruvate/acetaldehyde	0.115 mM
external pyruvate/acetaldehyde	0.077 mM
ADP	1.525 mM
ATP	2.475 mM
NAD^+	0.923 mM
NADH	0.077 mM

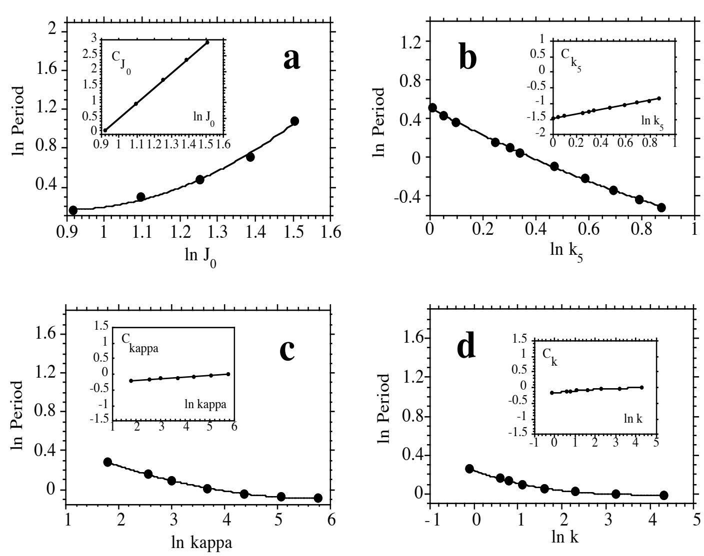
Temperature-compensated and non-temperature-compensated oscillations in the Wolf-Heinrich model



Temperature-compensated oscillations and experimental periods



Calculating Control Coefficients C_i



Testing Euler's summation theorem

$$\frac{dS_1}{dt} = J_0 - v_1 \qquad v_1 = k_1 S_1 A_3 \left[1 + \left(\frac{A_3}{K_1}\right)^q\right]^{-1}$$

$$\frac{dS_2}{dt} = 2v_1 - v_2 - v_6 \qquad v_2 = k_2 S_2 N_1$$

$$\frac{dS_3}{dt} = v_2 - v_3 \qquad v_3 = k_3 S_3 A_2$$

$$\frac{dS_4}{dt} = v_3 - v_4 - J \qquad v_4 = k_4 S_4 N_2$$

$$\frac{dN_2}{dt} = v_2 - v_4 - v_6 \qquad v_5 = k_5 A_3$$

$$\frac{dA_3}{dt} = -2v_1 + 2v_3 - v_5 \qquad v_6 = k_6 S_2 N_2$$

$$\frac{dS_4^{ex}}{dt} = J - v_7 \qquad v_7 = k S_4^{ex}$$

Control coefficients C_i for the oscillation period in the reference state

control coefficient	value (± 1% variation)	value (± 10% variation)
C_{J_0}	+0.69	+0.68
C_{k_1}	−0.21	−0.23
C_{k_2}	+0.04	+0.06
C_{k_3}	−0.01	−0.01
C_{k_4}	+0.26	+0.27
C_{k_5}	−1.24	−1.27
C_{k_6}	−0.26	−0.28
C_k	−0.13	−0.12
C_{κ}	−0.17	−0.15
C_{K_1}	−0.86	−0.84
$\sum_i C_i$ (without K_1)	−1.02	−1.05
$\sum_i C_i$ (with K_1)	−1.88	−1.89

Testing the antagonistic balance equation

For calculating the balance sums (last row) values for control coefficients have been used corresponding to a 10% change of parameters (c.f. Table 3)

Parameter	E_i, kJ/mol (experimental)[a]	E_i, kJ/mol (series 1)	E_i, kJ/mol (series 3)	E_i, kJ/mol (series 6)	E_i, kJ/mol (series 7)
J_0	16.2	43.9	46.1	41.4	51.0
k_3	44.9	31.7	48.7	30.4	39.2
k_4	58.7	44.2	38.0	48.1	42.4
k_1	13.8	16.0	19.1	14.0	23.3
k_2	60.7	10.4	11.8	10.8	11.9
k_5	41.2	13.3	17.9	15.1	19.8
k_6	31.4	23.7	16.2	24.0	15.7
κ (kappa)	15.9	22.3	19.0	18.7	19.7
k	24.3	30.0	32.2	34.1	31.5
K_I[b]	47.0	12.2	11.4	8.6	10.0
$\sum_i C_{k_i} E_i$	-79.0	-2.3	-6.1	-1.8	-3.6

[a] fit to Hemker et al. data, Fig. 3 (large solid dots)
[b] in case of K_1, E_i is interpreted as $\Delta H^0_{K_1}$