Chemical Oscillations and Circadian Rhythms

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Early Events

-1828 Fechner publishes the observation of an oscillating electric current in an electrochemical cell.

-1899 Ostwald observes that dissolution of chromium in acid is periodic.

-1916 Morgan discovers that CO production from formaldehyde can occur periodically.

-1921 Bray discovers periodic H_2O_2 decomposition in the presence of iodate.

-1951-1958 Belousov tries to publish his observation of what we know today is the Belousov-Zhabotinsky reaction.

- 1961 Zhabotinsky's starts to publish studies on Belousov's oscillatory reaction.

- 1968 Prague conference on chemical and biological oscillators.

- 1972 FKN mechanisms of the Belousov-Zhabotinsky reaction.

B. P. Belousov
ca. 1950
(from: *Oscillations and Traveling Waves in Chemical Systems* by Richard J. Field and Mária Burger; © 1985 by John Wiley & Sons, Inc)

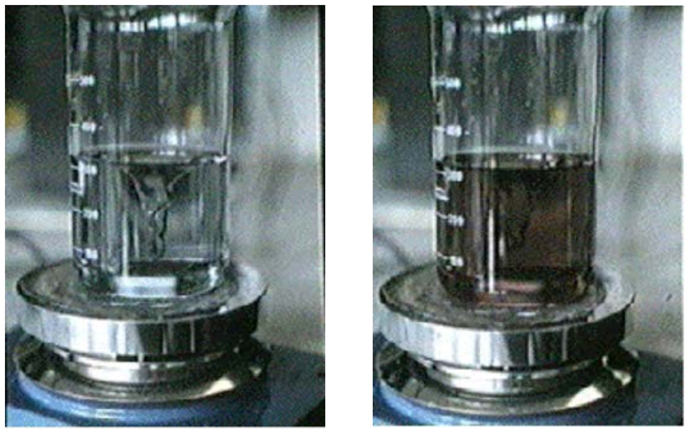
The modern history of the study of oscillating chemical reactions in the liquid phase began in Russia in 1951, when B. P. Belousov discovered temporal oscillations in the ratio [Ce(IV)]/[Ce(III)] during the cerium-ion-catalyzed oxidation of citric acid in acidic bromate.

However, Belousov was not able to get his discovery published until 1958. The first English translation of Belousov´s original manuscript appeared in *Oscillations and Traveling Waves in Chemical Systems* a book edited by Richard J. Field and Mária Burger (Wiley, 1985, ISBN 0-471-89384-6).

A. M. Zhabotinsky
Summer 1983
Photo by A. T. Winfree
(from: *Oscillations and Traveling Waves in Chemical Systems* by Richard J. Field and Mária Burger; © 1985 by John Wiley & Sons, Inc)

A. M. Zhabotinsky continued Belousov´s initial work. During the 1960´s the oscillations were characterized for a variety of different organic substrates. A review by Zhabotinsky of the early period has appeared in *Oscillations and Traveling Waves in Chemical Systems* by Richard J. Field and Mária Burger (Wiley, 1985, ISBN 0-471-89384-6).

The Belousov-Zhabotinsky Reaction (stirred system)

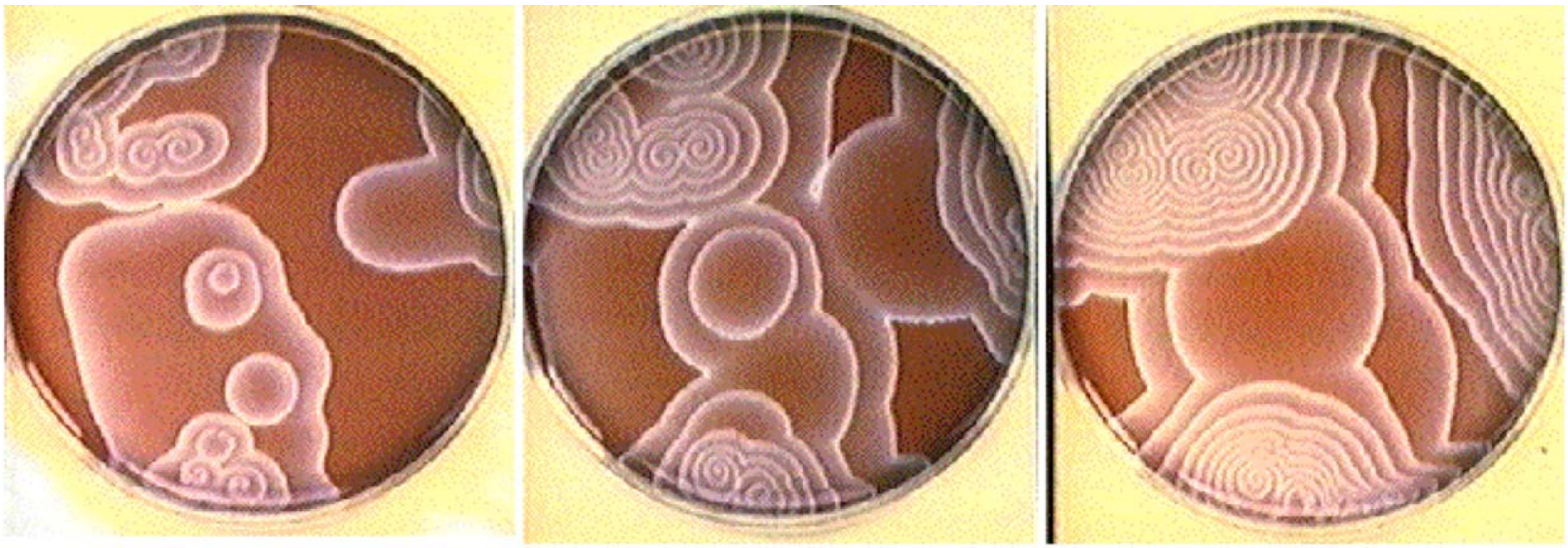


- 1M H_2SO_4
- organic subsrate
- $KBrO_3$ or $NaBrO_3$
- Ce^{3+}, Ce^{4+} (Mn^{2+}, $Fe(phen)_3^{2+}$ or $Ru(bpy)_3^{2+}$)

Fe-catalyzed system

Oscillatory fluorescence in the Ru-catalyzed system (which is also light-sensitive)

The Belousov-Zhabotinsky Reaction (unstirred thin layer in a Petri-dish)



Traveling oxidation waves

The FKN mechanism
(1972)

Dick **F**ield   Endre **K**örös   Dick **N**oyes

University of Stavanger

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Oscillations in Chemical Systems. II. Thorough Analysis of Temporal Oscillation in the Bromate–Cerium–Malonic Acid System

Richard J. Field, Endre Körös, and Richard M. Noyes*

Contribution from the Department of Chemistry, University of Oregon, Eugene, Oregon 97403, the Institute of Inorganic and Analytical Chemistry, L. Eötvös University, Budapest, Hungary, and the Physical Chemistry Laboratory, Oxford University, Oxford, England. *Received April 3, 1972*

3 overall reactions in the BZ reaction

$$BrO_3^- + 2Br^- + 3CH_2(COOH)_2 + 3H^+ \longrightarrow 3BrCH(COOH)_2 + 3H_2O \quad (A)$$

$$BrO_3^- + 4Ce^{3+} + CH_2(COOH)_2 + 5H^+ \longrightarrow BrCH(COOH)_2 + 4Ce^{4+} + 3H_2O \quad (B)$$

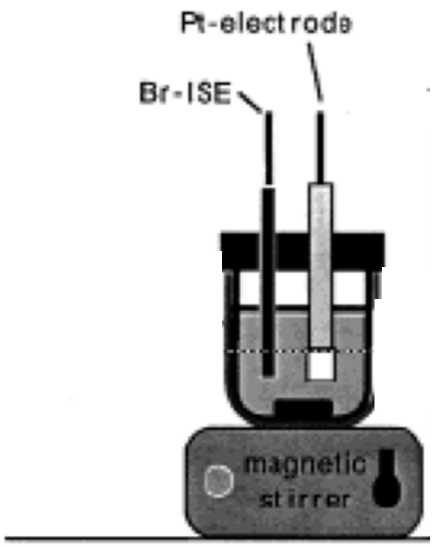
$$4Ce^{4+} + BrCH(COOH)_2 + 2H_2O \longrightarrow Br^- + 4Ce^{3+} + HCOOH + 2CO_2 + 5H^+ \quad (R10)$$

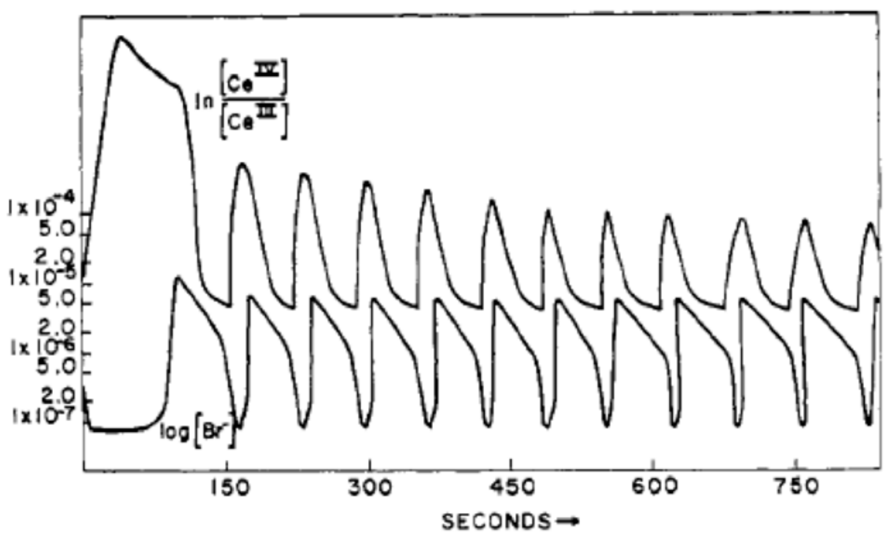
Each one of these reactions is virtually irreversible.

Process B occurs when the concentration of Br-ion has moved below a critical concentration.

Relaxation type of oscillations (oxidation spikes)

- long bromide ion consuming period
- short autocatalytic period and bromide ion regeneration





The Oregonator
(Field & Noyes 1974)

$$A + Y \rightleftharpoons X$$
$$X + Y \rightleftharpoons P$$
$$B + X \rightleftharpoons 2X + Z$$
$$2X \rightleftharpoons Q$$
$$Z \rightleftharpoons fY.$$

Species:

$X = HBrO_2$

$Y = Br^-$

$Z = Ce(IV)$

$A, B = BrO_3^-$

$P = HOBr$

$$dX/dt = k_{M1}AY - k_{M2}XY + k_{M3}BX - 2k_{M4}X^2, \quad \text{(Ia)}$$
$$dY/dt = -k_{M1}AY - k_{M2}XY + fk_{M5}Z, \quad \text{(Ib)}$$
$$dZ/dt = k_{M3}BX - k_{M5}Z. \quad \text{(Ic)}$$

The Field-Noyes equations show close analogy to the Huxley-Hodgkin equations.

The Oregonator

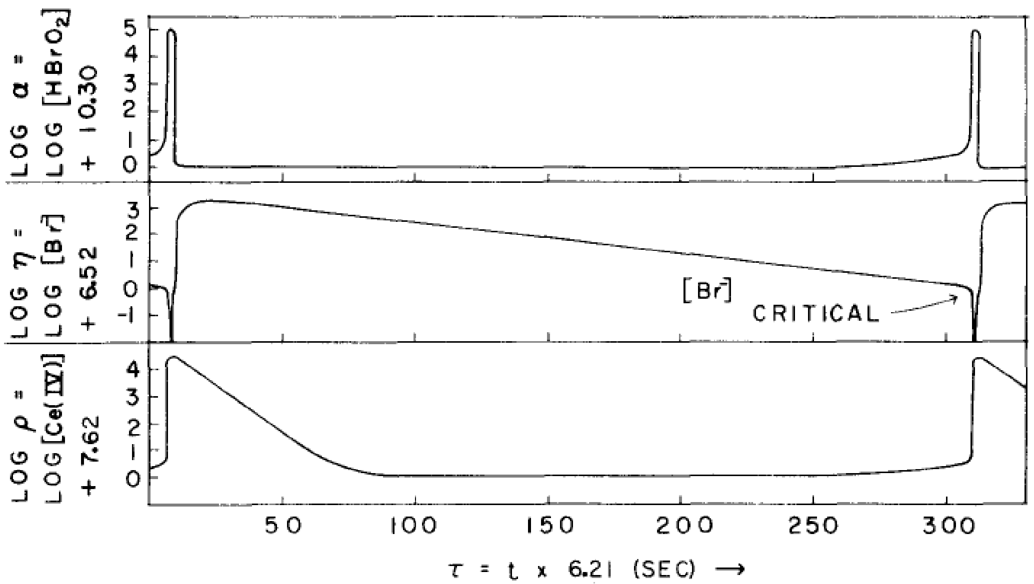
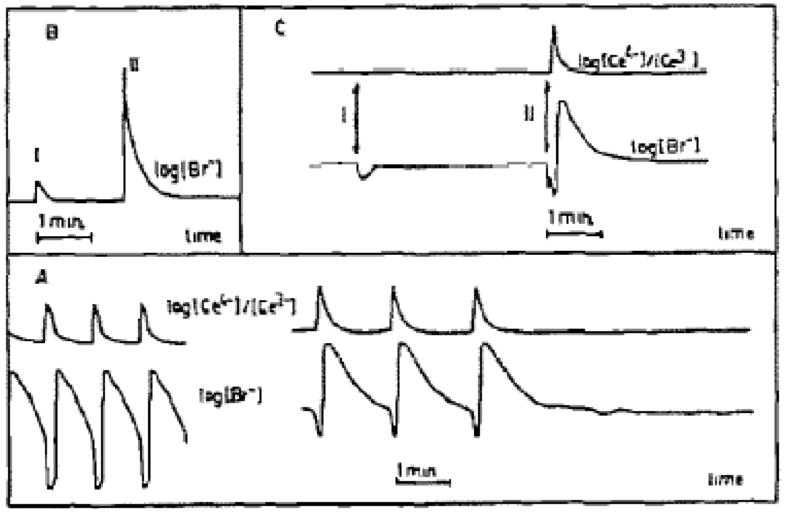
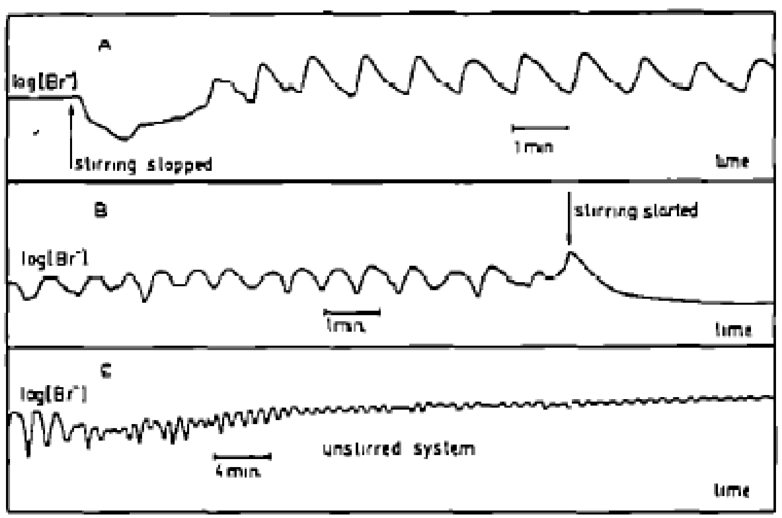


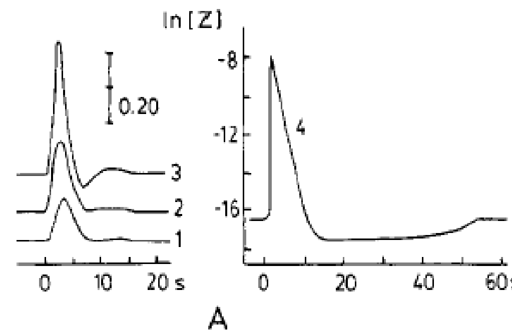
FIG. 1. Traces of log[Ce(IV)] (ρ), log[Br^-] (η) and log[$HBrO_2$] (α) vs time (τ) obtained by numerical integration of Eqs. III, which result from the Field, Körös, and Noyes mechanism for the Belousov reaction. The integration used $f=1$. Process A is occurring during the long stretches when $HBrO_2$ (α) is low and Process B is occurring when the sharp spikes of $HBrO_2$ (α) appear.

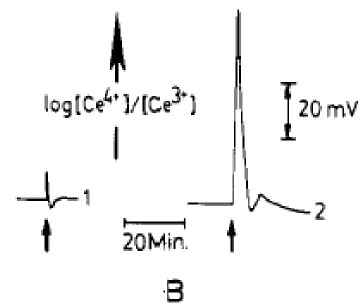
Excitability in the BZ reaction





(Oregonator)





(experiment)

Ruoff, CPL, 1982

Silver Ion Induced Oscillations

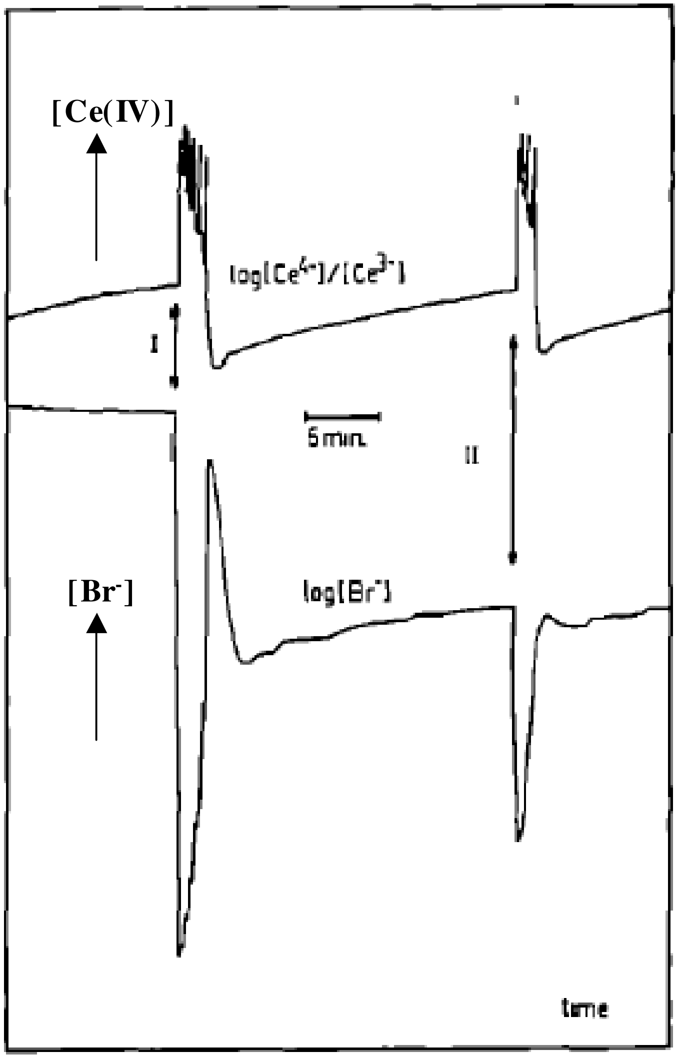


Fig 4 When the excitable state is treated with an excess of $AgNO_3$, Ce^{4+} oscillations appear. I = 5 0 x 10^{-3} mol/dm³, II = 1.0 x 10^{-4} mol/dm³.

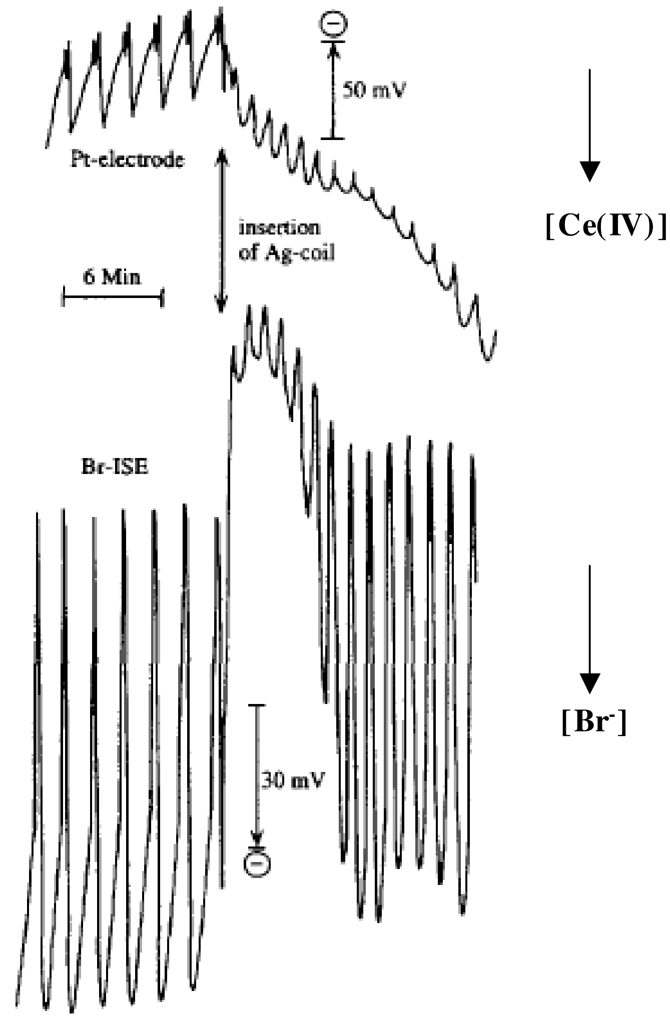


Fig. 1. Generation of high frequency oscillations when inserting the Ag-coil. Initial concentrations: $[H_2SO_4]_0 = 1.5$ M, $[Ce(SO_4)_2]_0 = 1 \times 10^{-3}$ M, $[NaBrO_3]_0 = 4 \times 10^{-3}$ M, [malonic acid]$_0 = 0.1$ M.

Oxidation pulse propagation

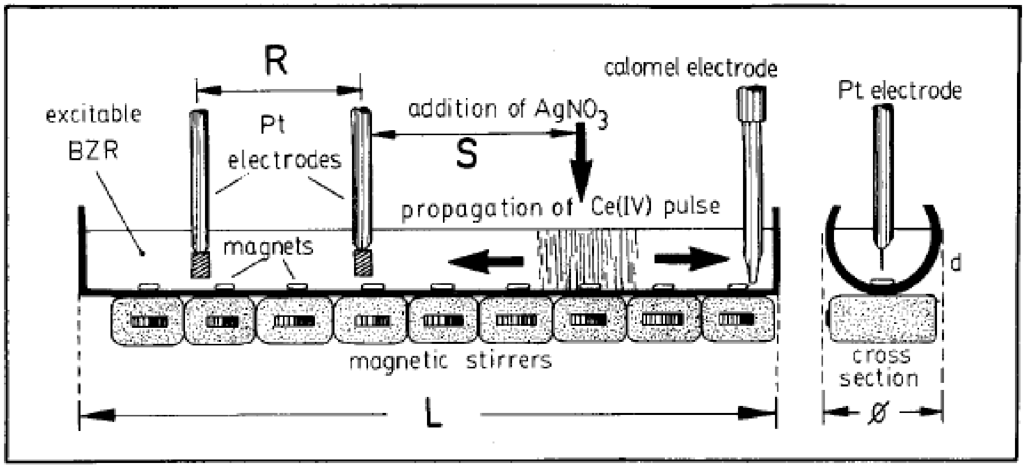


Fig. 1. Propagation experiment in the excitable BZR. Dimensions of tube: $L = 60$ cm, $\varnothing = 7$ cm, and d (thickness of wall) = 0.5 cm. R and S are variable distances. The total reaction volume is 1 l

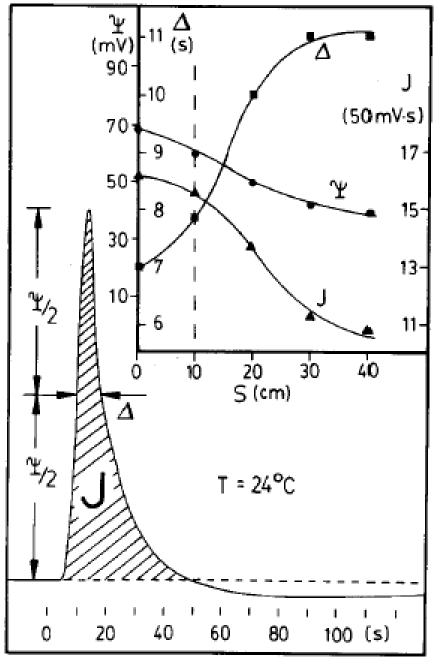


Fig. 3. Propagating wave initiated 10 cm from detecting electrode. Insert: amplitude Ψ, half-width Δ and time integral J as a function of S. Same amount of $AgNO_3$ used as in Fig. 2

Facilitation phenomena

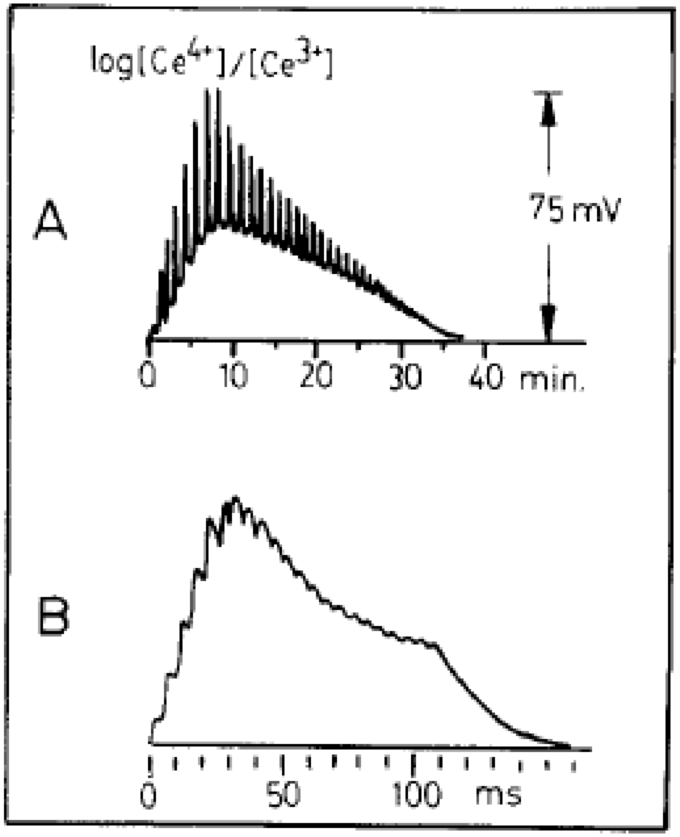


Fig. 4. A) Dynamic behaviour of an aged excitable BZR. 370 min after the reagents have been mixed, 0.3 ml of an 0.025 *M* $AgNO_3$ solution was added ($t=0$). Initial concentrations: malonic acid 0.28 *M*, Ce(IV) $2.1\cdot10^{-2}$ *M*, $KBrO_3$ 0.1 *M* and H_2SO_4 1 *M*. B) Facilitation phenomena in curarized frog muscles (see [10])

10. Katz, B.: Nerve, Muscle and Synapse, p. 140. New York: McGraw-Hill 1966

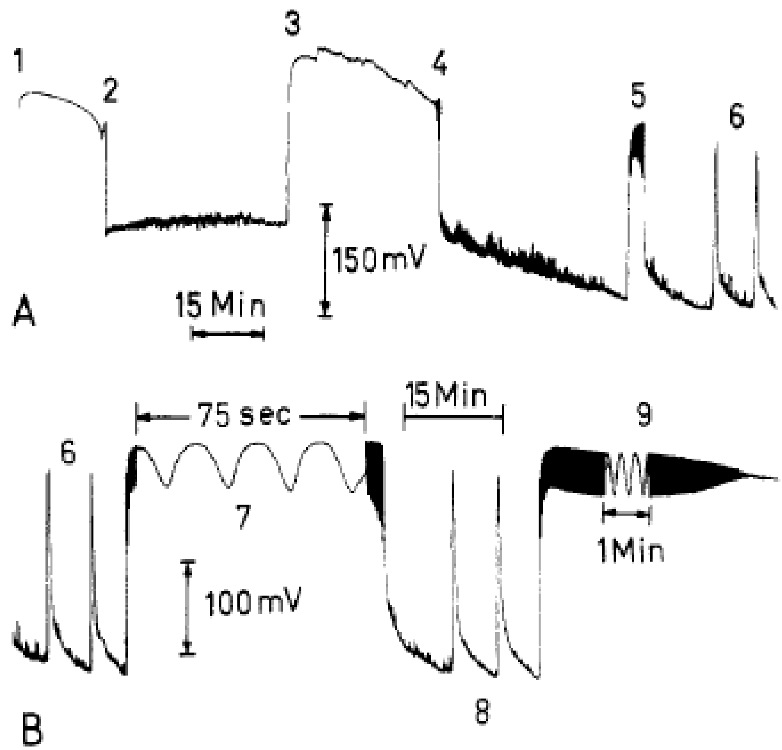


Figure 1. Oscillatory behavior of a ferroin-catalyzed methylmalonic acid BZ system. Initial concentrations: $[H_2SO_4]_0 = 0.5$ M; $[MeMA]_0 = 0.3$ M; $[NaBrO_3]_0 = 0.1$ M; $[KBr]_0 = 0.1$ M. Parts A and B represent a continuation of the same run with an overlap of one "spike". (A) 1: addition of KBr; 2: after bromine color has disappeared, the ferroin is added; 3: after staying for a while in the reduced state, the system goes spontaneously into the oxidized state; The color of the solution changes from red to blue; 4: at the beginning of the oscillating period, oscillations of high frequency and increasing amplitude are observed in the oxidized state; 5: train of small-amplitude oscillations in the oxidized state; 6: oxidizing excursions or "spikes". Note also the considerable "noise" which is observed in the reduced state. (B) 6 and 8: oxidizing excursions; 7: train of small-amplitude oscillations in the oxidized state. Period length in the expanded region is 20 s; 9: at the end of the oscillating region, small-amplitude damped oscillations in the oxidized state are observed (period length in the expanded region is 19 s).

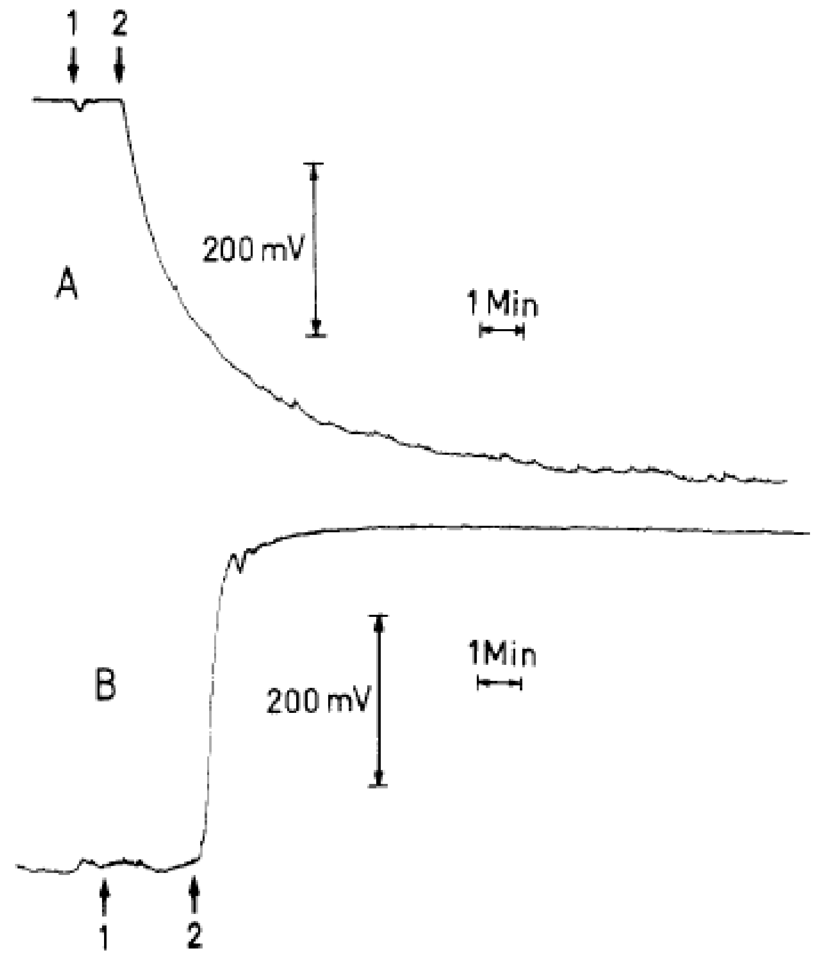


Figure 2. Bistability at the end of the oscillating region in the ferroin-catalyzed methylmalonic acid BZ reaction. The composition corresponds to that at the end of the time in Figure 1. (A) The system starts in the oxidized state. 1: one drop (31 μL) of a 0.004 M KBr solution (2.5×10^{-6} M in 50 mL) shows that the steady state is stable. 2: two drops of a 0.004 M KBr solution drive the system to the reduced state. The color of the solution changes from blue to red. (B) The system starts in the reduced state. 1: five drops of 0.004 M $AgNO_3$ solution (1.3×10^{-5} M) shows that the steady state is stable. 2: eight drops of 0.004 M $AgNO_3$ solution (2.0×10^{-5} M) drives the system to the oxidized state.

Amplified Oregonator

SCHEME 1. Pseudoelementary processes selected for model representation.

$BrO_3^- + Br^- + 2H^+ \rightarrow HBrO_2 + HOBr$	(P1)
$HBrO_2 + Br^- + H^+ \rightarrow 2HOBr$	(P2)
$BrO_3^- + HBrO_2 + H^+ \rightleftarrows 2BrO_2 + H_2O$	(P3a)
$BrO_2 + M^{(n)+} + H^+ \rightleftarrows HBrO_2 + M^{(n+1)+}$	(P3b)
$2HBrO_2 \rightarrow BrO_3^- + HOBr + H^+$	(P4)
$HOBr + Br^- + H^+ \rightleftarrows Br_2 + H_2O$	(P5a)
$RH + Br_2 \rightarrow RBr + Br^- + H^+$	(P5b)
$HOBr + R\cdot \rightarrow ROH + Br\cdot$	(P6a)
$RH + Br\cdot \rightarrow Br^- + H^+ + R\cdot$	(P6b)
$RH + M^{(n+1)+} \rightarrow M^{(n)+} + H^+ + R\cdot$	(P7a)
$2R\cdot + H_2O \rightarrow RH + ROH$	(P7b)

Amplified Oregonator

SCHEME 2. Amplified Oregonator model.

$$A + Y \rightarrow X + P \qquad (O1)$$
$$v_1 = k_1[A][Y]$$
$$X + Y \rightarrow 2P \qquad (O2)$$
$$v_2 = k_2[X][Y]$$
$$A + X + C \rightarrow 2X + Z \qquad (O3)$$
$$v_3 = k_3[A][X]$$
$$2X \rightarrow A + P \qquad (O4)$$
$$v_4 = k_4[X]^2$$
$$P \rightarrow L \qquad (O5)$$
$$v_5 = k_5[P][Y]$$
$$P \rightarrow Y \qquad (O6)$$
$$v_6 = k_6[Z]^{1/2}[P]$$
$$Z \rightarrow C \qquad (O7)$$
$$v_7 = k_7[Z]$$

$A \equiv BrO_3^-$ $\qquad X \equiv HBrO_2$

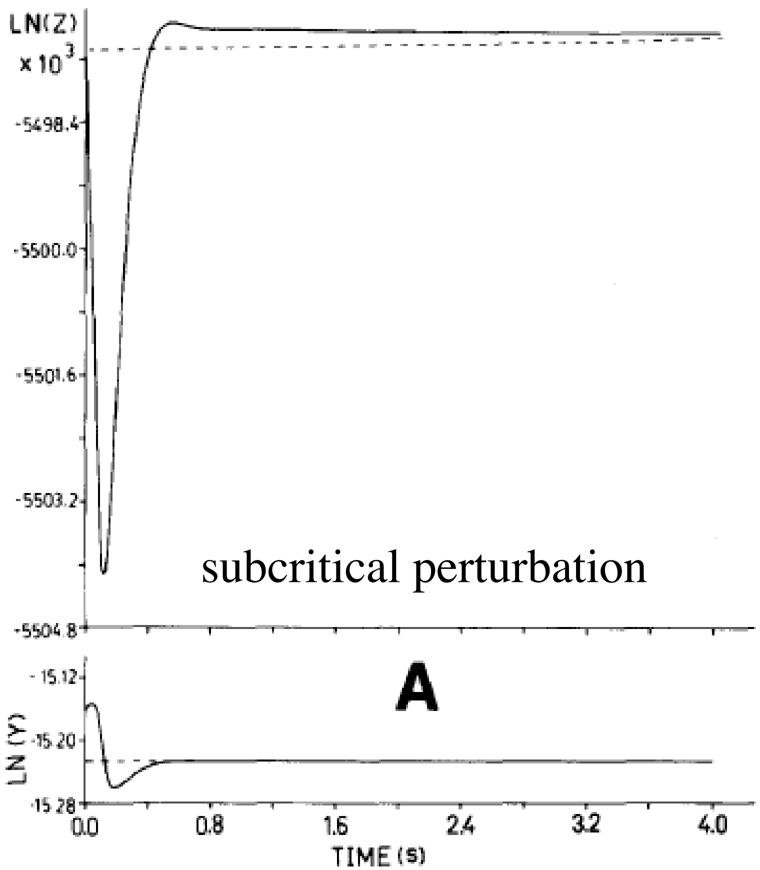
$L \equiv RBr$ $\qquad Y \equiv Br^-$

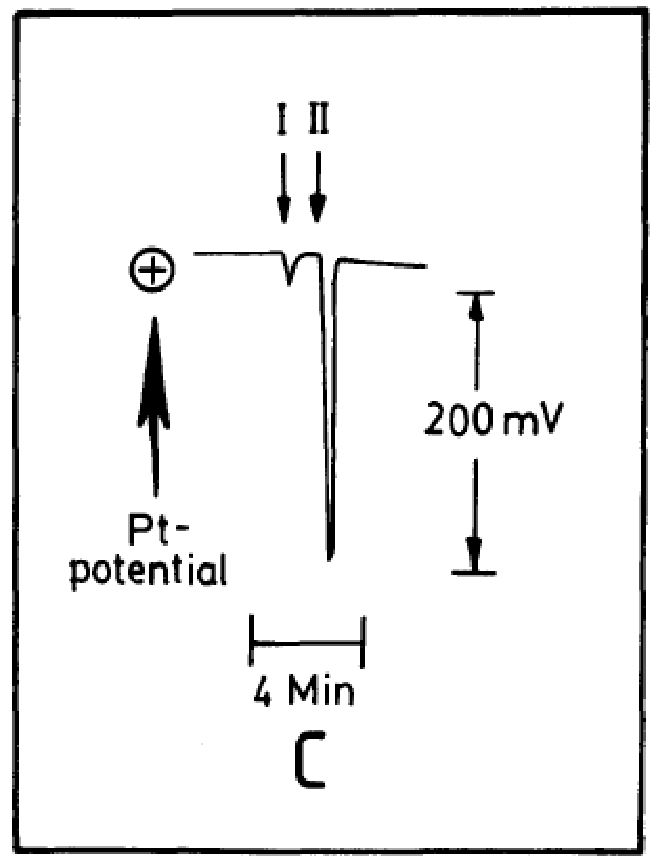
$P \equiv HOBr$ $\qquad Z \equiv 2M^{(n+1)+}$

$C \equiv 2M^{(n)+}$

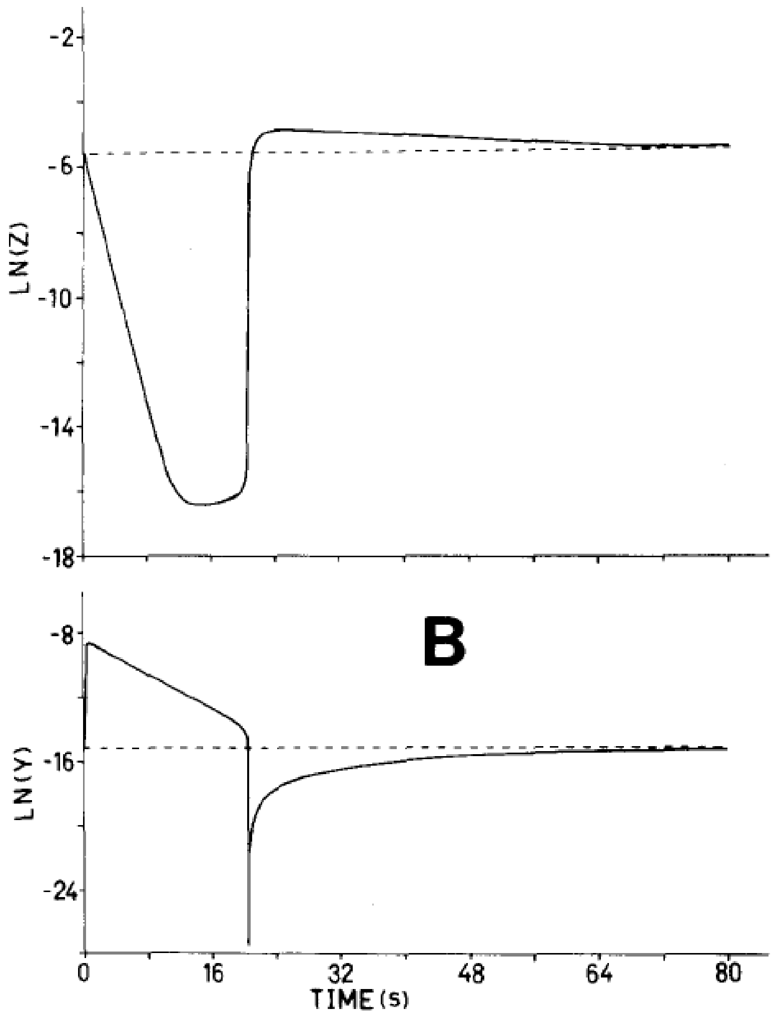
Bromide-Ion Induced Excitability (Reduction Spikes)

1416 P. Ruoff and R. M. Noyes: Amplified Oregonator model



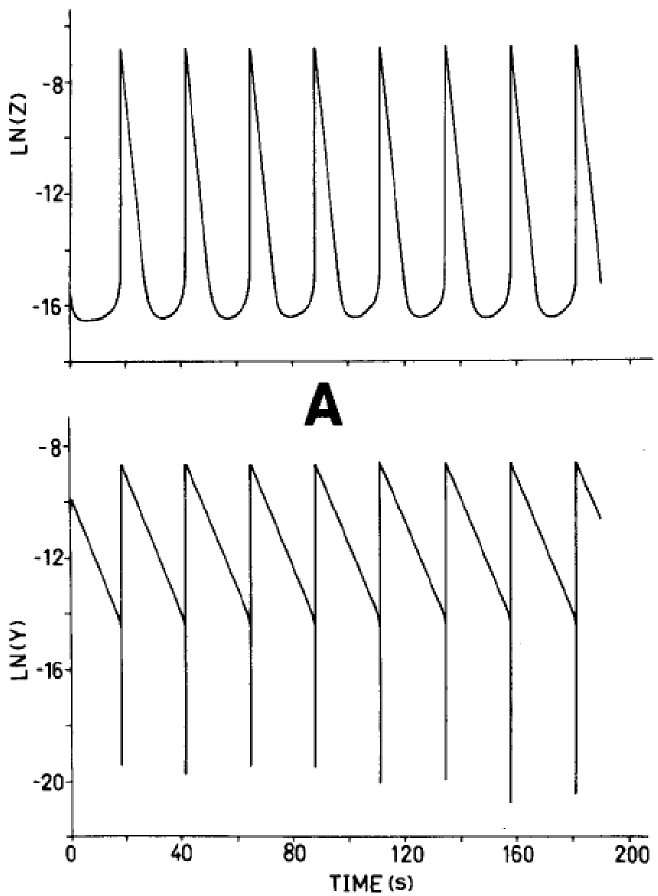


Bromide-Ion Induced Excitability (Reduction Spikes)

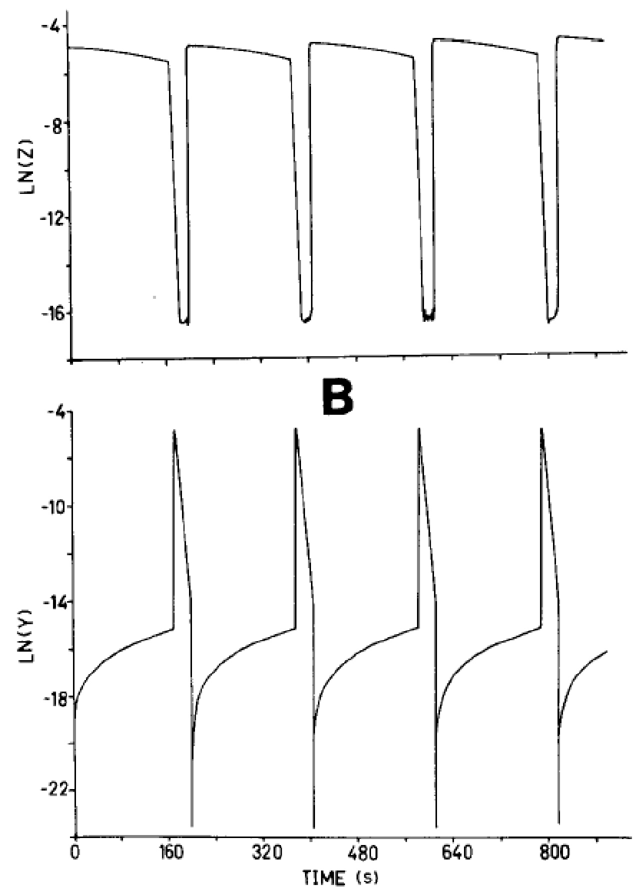


supercritical perturbation

Oxidation and Reduction Spikes in the Amplified Oregonator

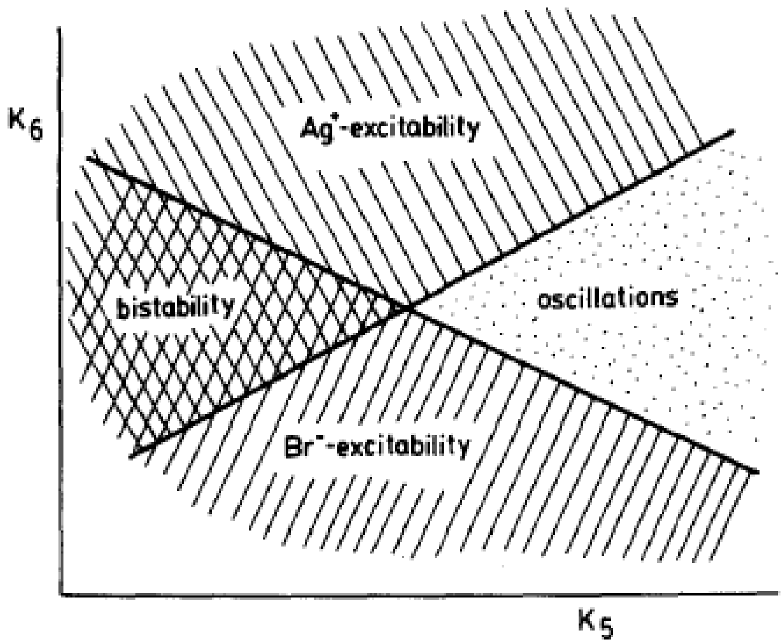


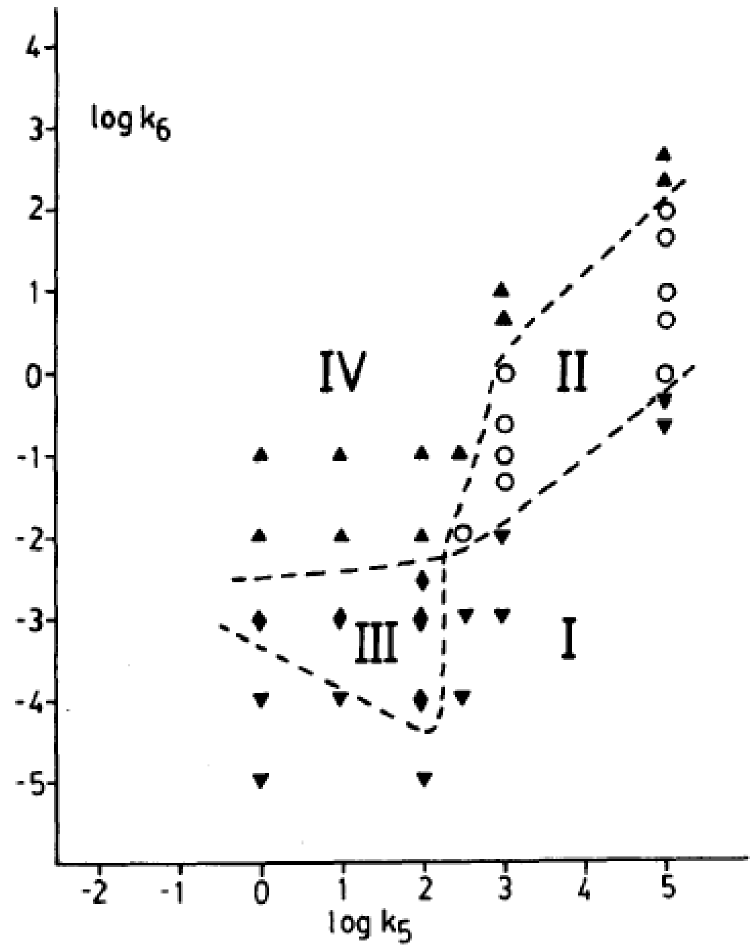
Large k_{O5}, k_{O6}



Low k_{O5}, k_{O6}

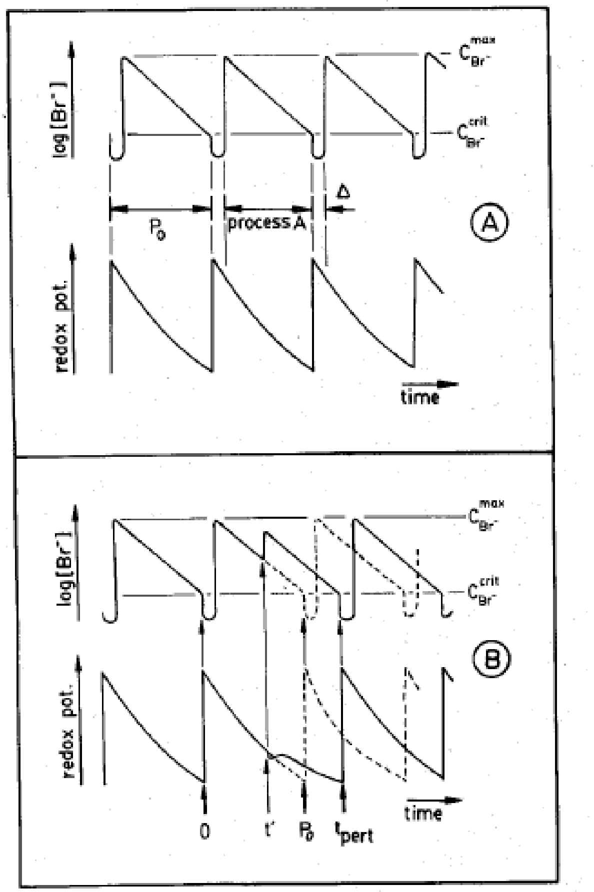
Regions of oscillations, bistability, and Ag^+ and Br^- excitable steady states of amplified Oregonator in k_5 - k_6 space



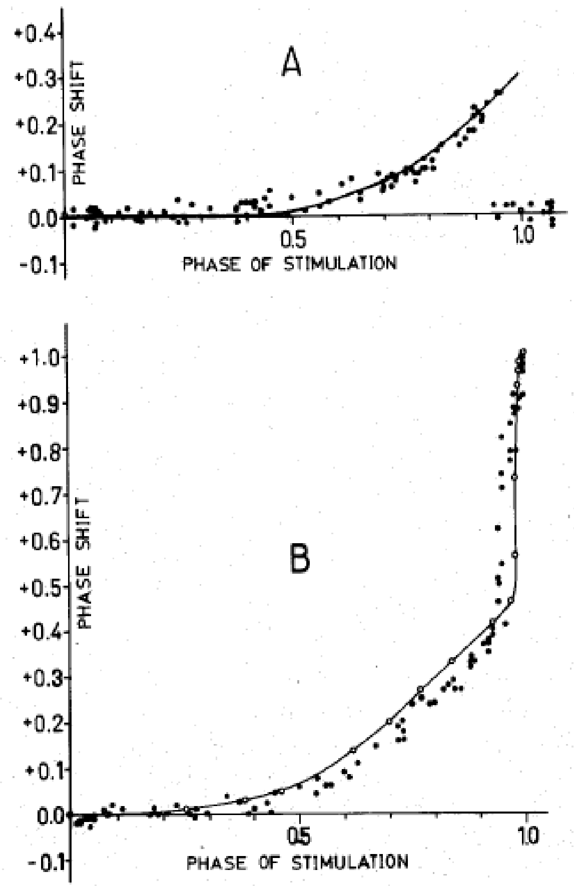


Phase response curves in the BZ reaction (oxidation spikes)

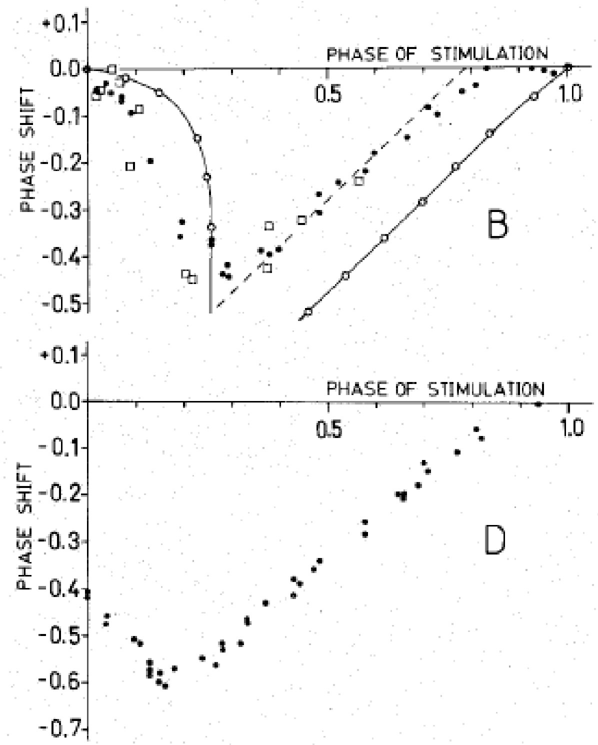
Phase shift = peak time (after perturbation) - peak time (no perturbation)



Outline of experiment

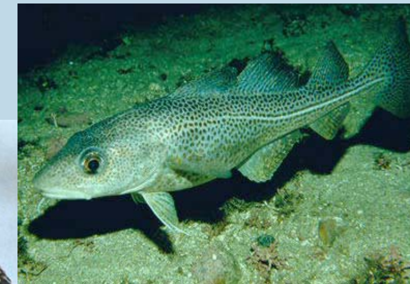
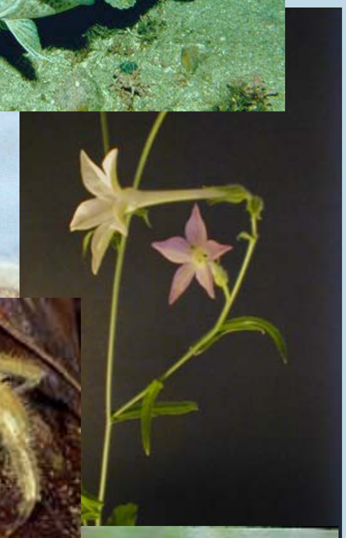


Perturbation by Br^-

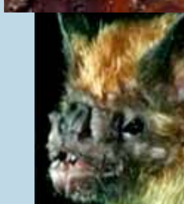


Perturbation by Ag^+

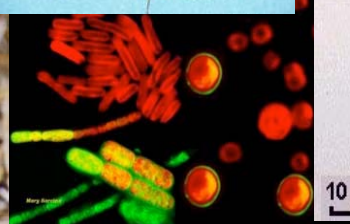


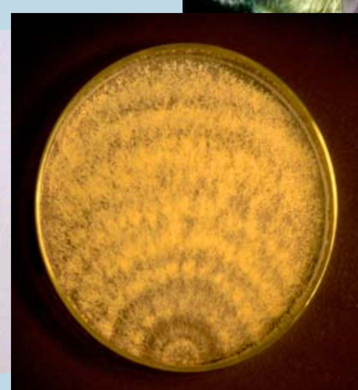




Circadian rhythms are important in daily and seasonal adaptations of organisms to their environments

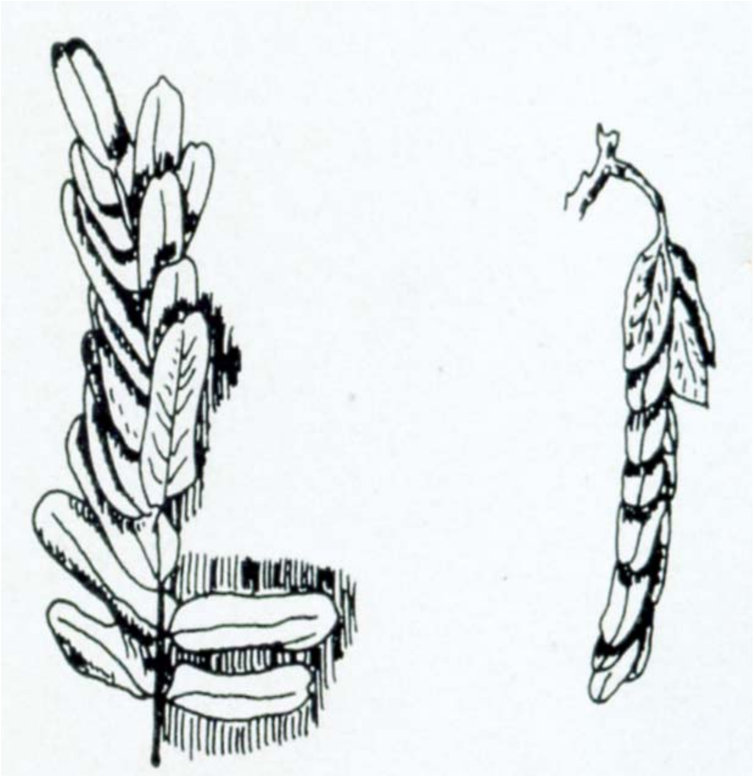







History of Circadian Rhythms. 325 BC:
Androsthenes (from Thasus) observes that plants ”sleep”



day night

The French astronomist De Mairan finds in 1729 that leaf movements in *Mimosa* plants continue in darkness

Credit: National Library of Medicine

OBSERVATION BOTANIQUE.

ON ſçait que la Senſitive eſt *héliotrope*, c'eſt-à-dire que ſes rameaux & ſes feüilles ſe dirigent toûjours vers le côté d'où vient la plus grande lumiére, & l'on ſçait de plus qu'à cette propriété qui lui eſt commune avec d'autres Plantes, elle en joint une qui lui eſt plus particuliére, elle eſt Senſitive à l'égard du Soleil ou du jour, ſes feüilles & leurs pédicules ſe replient & ſe contractent vers le coucher du Soleil, de la même maniére dont cela ſe fait quand on touche la Plante, ou qu'on l'agite. Mais M. de Mairan a obſervé qu'il n'eſt point néceſſaire pour ce phénoméne qu'elle ſoit au Soleil ou au grand air, il eſt ſeulement un peu moins marqué lorſqu'on la tient toûjours enfermée dans un lieu obſcur, elle s'épanoüit encore très-ſenſiblement pendant le jour, & ſe replie ou ſe reſſerre réguliérement le ſoir pour toute la nuit. L'expérience a été faite ſur la fin de l'Eté, & bien répétée. La Senſitive ſent donc le Soleil ſans le voir en aucune maniére; & cela paroît avoir rapport à cette malheureuſe délicateſſe d'un grand nombre de Malades, qui s'apperçoivent dans leurs Lits de la différence du jour & de la nuit.

Il ſeroit curieux d'éprouver ſi d'autres Plantes, dont les feüilles ou les fleurs s'ouvrent le jour, & ſe ferment la nuit, conſerveroient comme la Senſitive cette propriété dans des lieux obſcurs; ſi on pourroit faire par art, par des fourneaux plus ou moins chauds, un jour & une nuit qu'elles ſentiſſent; ſi on pourroit renverſer par là l'ordre des phénomenes du vrai jour & de la vraye nuit, &c. Mais les occupations ordinaires de M. Mairan ne lui ont pas permis de pouſſer les expériences juſque-là, & il ſe contente d'une ſimple invitation aux Botaniſtes & aux Phiſiciens, qui pourront eux-mêmes avoir d'autres choſes à ſuivre. La marche de la véritable Phiſique, qui eſt l'Expérimentale, ne peut être que fort lente.

Hamburgisches

Magazin,

oder

gesammlete Schriften,

zum

Unterricht und Vergnügen

aus der Naturforschung

und den

angenehmen Wissenschaften überhaupt.

Des ersten Bandes erstes Stück.

Hamburg, bey G. C. Grund, und in Leipzig
bey A. H. Holle, 1748.

* *

IV.

Von dem Schlafe der Pflanzen.

Die besondere Eigenschaft verschiedener Pflanzen, welche vom Herrn Archiater Linnäus, und andern Kräuterkennern der Schlaf der Pflanzen genannt wird, hat jederzeit die Aufmerksamkeit aller Naturkündiger auf sich gezogen. Unter dieser Benennung versteht man diejenige Eigenschaft der Pflanzen, da verschiedene derselben die Nacht durch eine solche Veränderung äußern, wodurch ihre Blätter eine ganz andere Lage bekommen, als diejenige ist, welche wir des Tages über an ihnen wahrnehmen. Es ist nämlich eine bekannte Erfahrung, daß bey verschiedenen Pflanzen gegen Abend und die Nacht durch, die vorhin ausgebreiteten Blätter sich gegen einander neigen und zusammen legen, oder auch sonst auf eine andere Weise ihre des Tages über gehabte Gestalt und Lage verändern, so daß einige, die vorher flach ausgebreitet waren, sich nun in die Höhe richten, und gegen den Stamm oder Ast sich neigen; andere hingegen nun vielmehr unter sich hängen. Die meisten Pflanzen, bey welchen man einen dergleichen Schlaf bemerket, haben zusammengesetzte Blätter, da an einem gemeinschaftlichen Stiele viel Blättchen hängen; doch

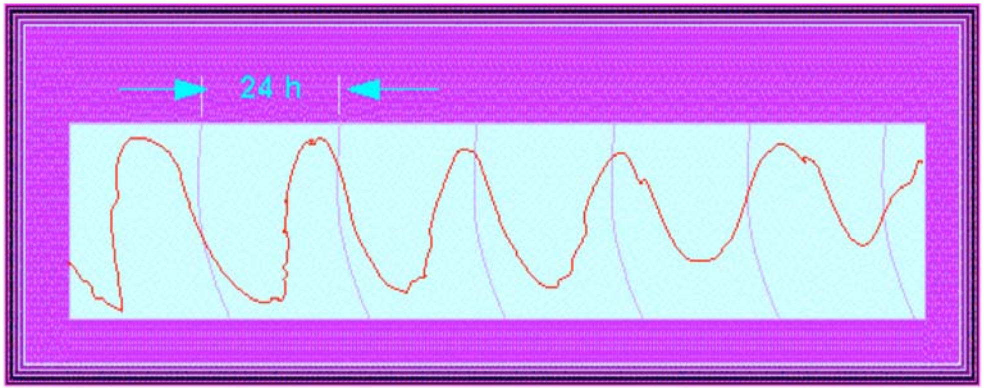
äußert

Linné's flower clock

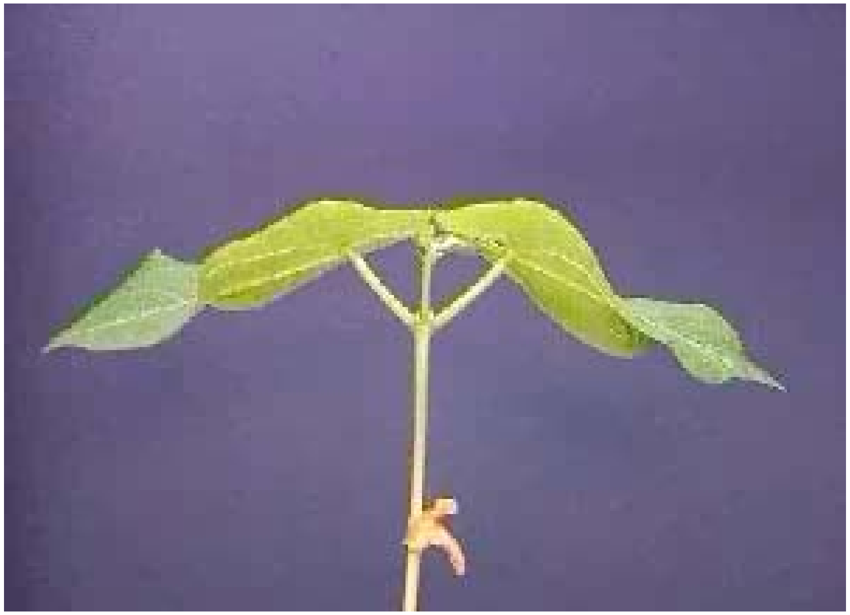
CARL von LINNÉ
(1707-1778)

"Tiger Jaw" (*Faucaria tigrinis*, South Africa, open flowers ≈ 5 pm, closes ≈ 8 pm)

Leaf movements under free-running conditions



Phaseolus coccineus. Typical course of the circadian leaf movements under constant light (weak intensity). The phase shifts within six days by roughly 17 hours compared to the normal day. The length of one period is thus about 27 hours (circles in 24-hour-intervals; E. BÜNNING and M. TAZAWA, 1957).



Time lapse movie of leaf movements in bean (*Phaseolus*) seedlings by Roger P. Hangarter, Indiana University.

Flashing and glow circadian rhythms in *Gonyaulax polyedra*

(von der Heyde *et al*. (1992) JBR 115-123)

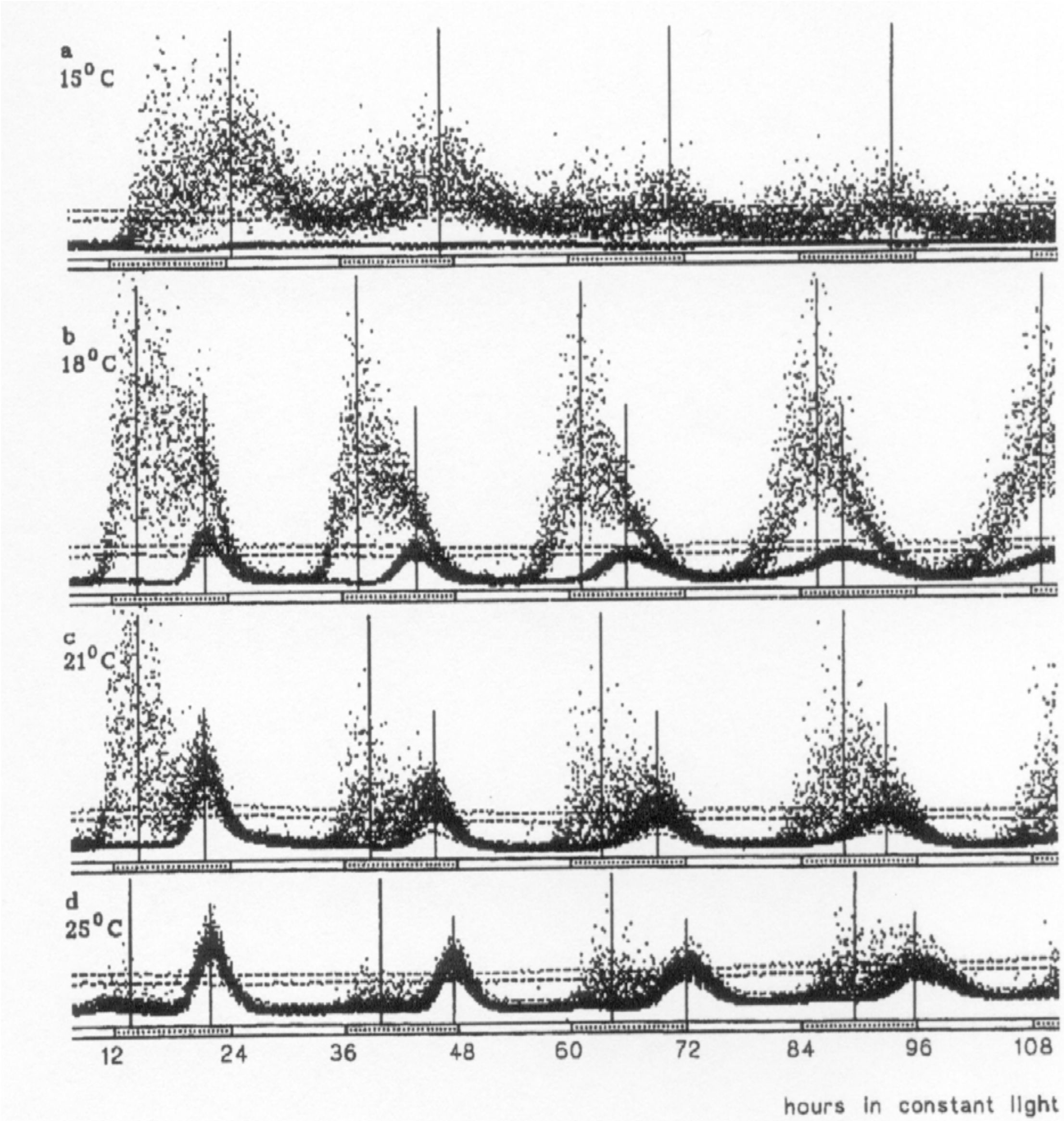
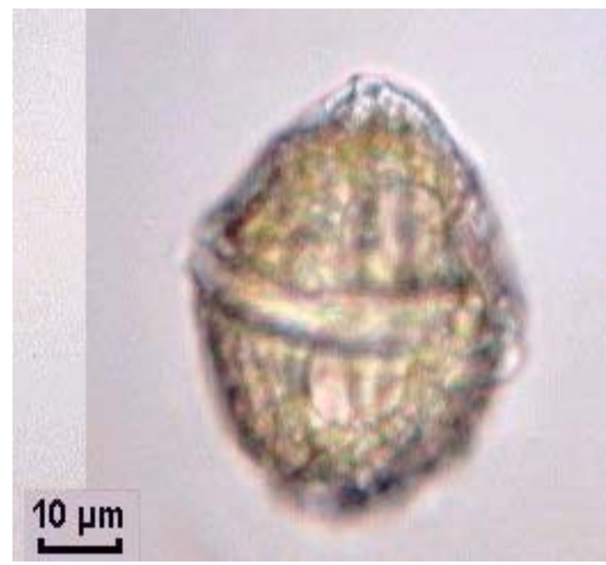


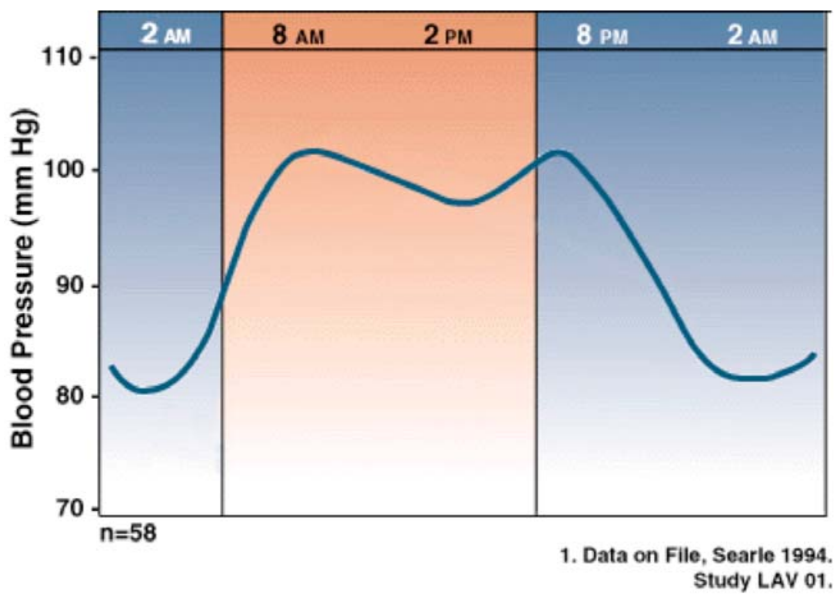
FIGURE 2. Bioluminescence rhythms at different termperatures (dim LL 1000 lux). Vertical lines indicate circadian glow and flashing maxima fitted by eye; for other details, see Figure 1.

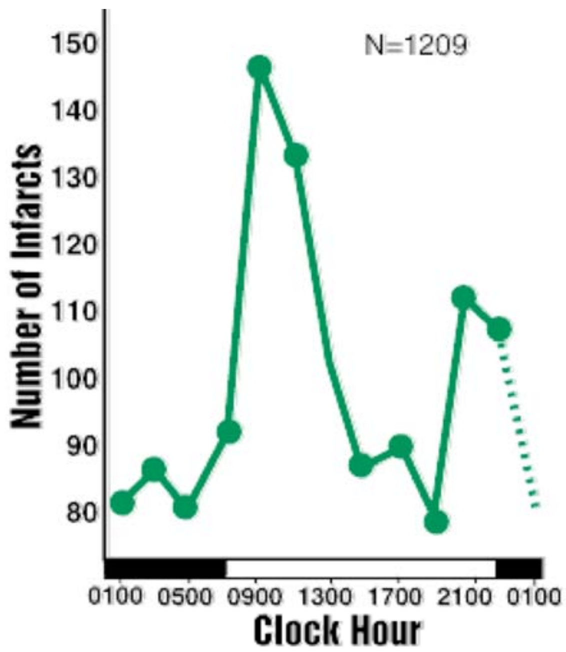
With increasing temperature flashing and glow rhythms change their amplitudes in opposite directions.



Gonyaulax polyedra

Circadian variation in blood pressure and heart attacks





daily variation of blood pressure

Variation of heart attacks during the day

Properties of circadian rhythms

• circadian rhythms are generated already within a single cell and can occur under constant environmental conditions (**free-running conditions**). We call these rhythms for **endogeneous rhythms**.

• they can be **phase shifted** by sudden changes in light, temperature, drugs, etc.

• they can track external periodic variations such as "lights on/off" or temperature variations. This property is called **entrainment.**

• the circadian period is unaffected by different (but constant) temperatures. This property is called **temperature compensation**. **Chronobiologists require this property for calling an oscillator 'a clock'.**

Examples of temperature compensated rhythms

(from E. Bünning: The Physiologcal Clock, Berlin, 1964)

Table 2. *Periplaneta americana, Running Activity* (BÜNNING, 1958a)

Temperature °C	Length of Periods hrs.
18	24 — 25
19—20	24.4 ± 0.1
22—23	24.5 ± 0.1
27—28	25.0 ± 0.3
29	25.8 ± 0.7
31	24 — 27

Table 3. *Gonyaulax polyedra, Rhythm of Luminescence* (HASTINGS and SWEENEY)

Temperature °C	Length of Periods hrs.
15.9	22.5
19	23.0
22	25.3
26.6	26.8
32	25.5

Table 4. *Phaseolus multiflorus, Leaf Movements* (LEINWEBER)

Temperature °C	Length of Periods hrs.
15	28.3 ± 0.4
20	28.0 ± 0.4
25	28.0 ± 1.0

Table 5. *Lizards (Lacerta sicula), Running Activity* (HOFFMANN, 1957)

Temperature °C	Length of Periods hrs.
16	25.20
25	24.34
35	24.19

The 1957 suggestion by Hastings and Sweeny how temperature compensation may occur

University of Stavanger

Practically all physiological reactions, enzyme catalyzed reactions, elementary reactions, etc. are quite dependent upon temperature. Their rates increase by a factor of 2-3 when temperature is increased by 10°C (“Van’t Hoff’s rule”).

This makes it difficult pinpointing a certain process as **the** candidate how temperature compensation may arise.

Hastings and Sweeney proposed in 1957 a general mechanism based on opposing reactions, **suggesting that temperature compensation is a systemic property**.

Importance of genetics to study the mechanisms of the circadian clock

• In the beginning of the 1970's reseachers showed using the fly ***Drosophila*** and the fungus ***Neurospora crassa*** that circadian properties such as period length, temperature compensation, and entrainment behavior are **inherited** and determined by genes.

• Genes were identified, where **alleles** of these genes showed different properties of the circadian clock.
Examples of these genes are *period* and *timeless* in *Drosophila* and *frequency* in *Neurospora*.

• This was the start of using **model organisms** in circadian rhythm research

Circadian clock properties are inherited

RESEARCH NEWS

JANUARY 12, 2001

First Human Circadian Rhythm Gene Identified

Researchers exploring the genetic basis of a rare syndrome that causes people to fall asleep and awaken earlier than normal have pinpointed the first human gene that controls circadian rhythm. The finding establishes a link between the human circadian system and that of animal models such as *Drosophila*, mice and hamsters, say the researchers. It also raises the possibility of treating jet lag, as well as sleep problems in adolescents, the elderly and shift workers.

A research team that included Howard Hughes Medical Institute investigator Louis J. Ptacek reported that a mutation in a gene called h*Per*2 is responsible for familial advanced sleep-phase syndrome (FASPS) in members of a Utah family. This syndrome typically causes sleep onset around 7 p.m., and spontaneous awakening around 2 a.m., in affected family members. The research was published online by the journal *Science* on January 12, 2001. The article will also appear in print in a future issue of *Science*.

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VERSIÓN EN ESPAÑOL

HHMI INVESTIGATOR

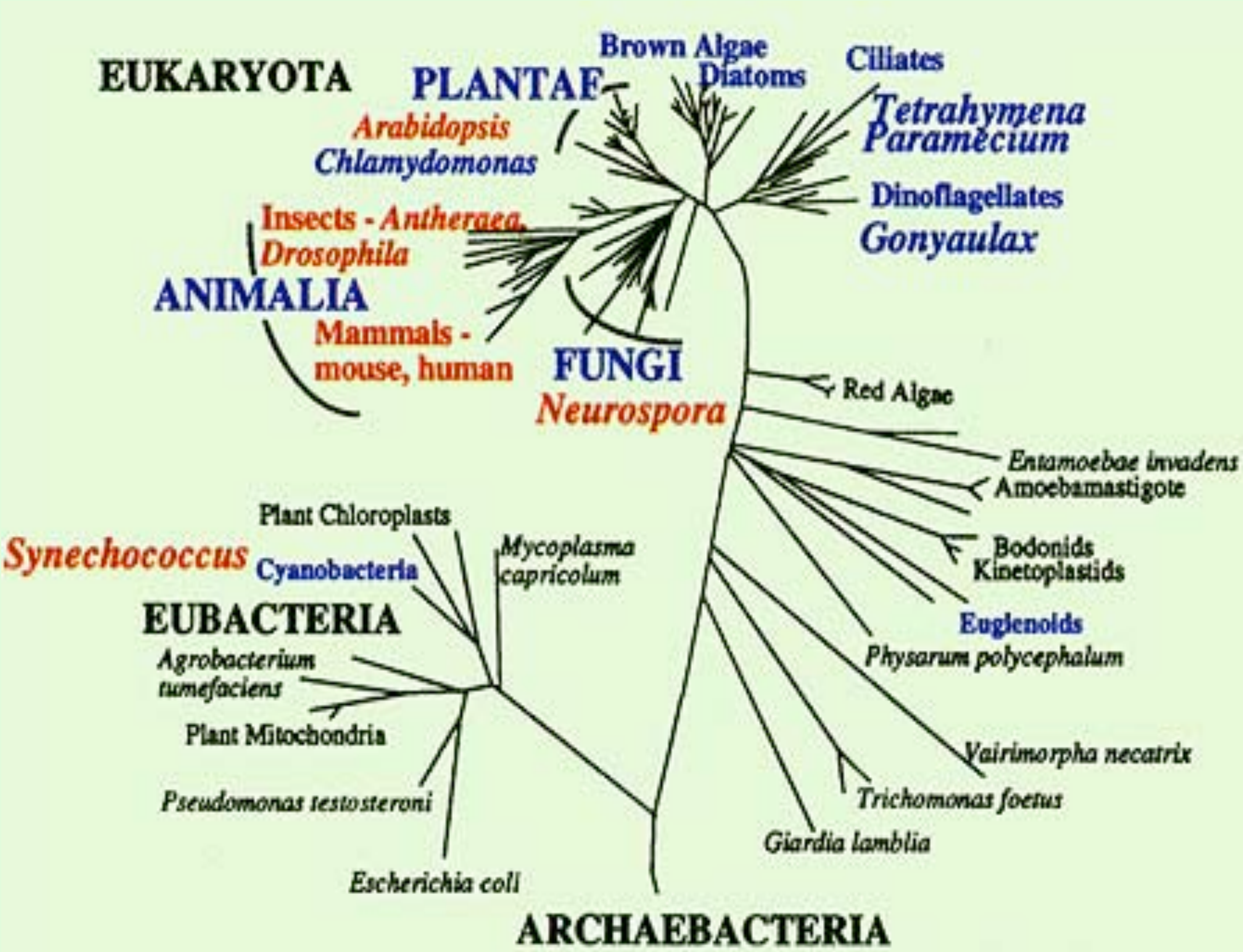
Louis J. Ptacek

ABSTRACT:

Understanding Brain Function Through Study of Inherited Traits in Humans

RELATED LINKS

Model organisms in circadian rhythm research



Neurospora crassa:
A model organism to study genetics and circadian rhythms

- eukaryotic filamentous fungus
- growths rapidly
- nonpathological
- easy to handle
- many mutants available
- circadian (conidiation) rhythm is easy assayable

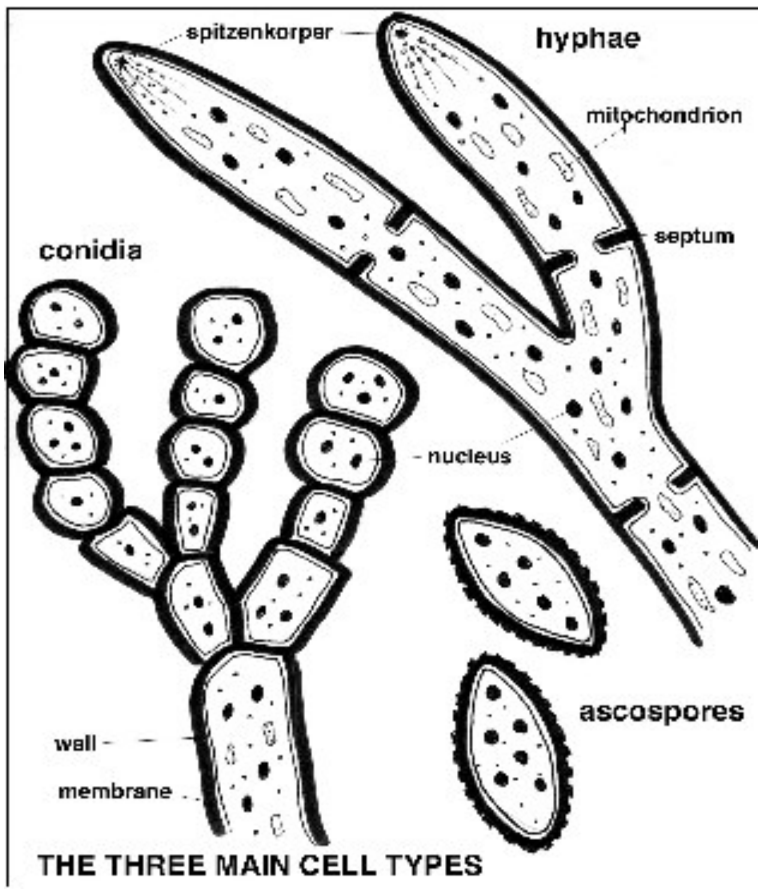
web resource:
Fungal Genetics Stock Center

Neurospora in nature

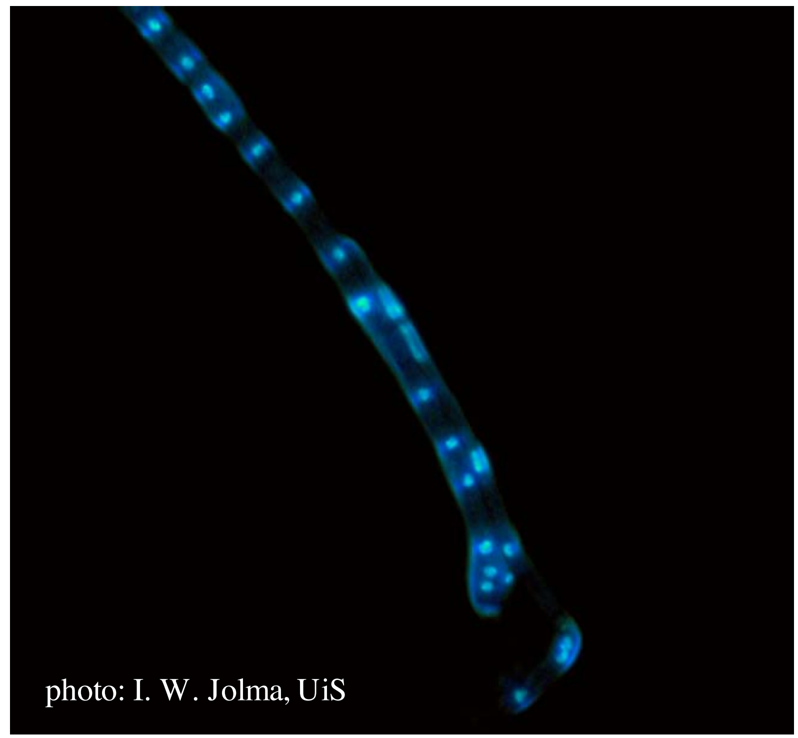
- grows in the tropics/subtropics
- has also been found in New Mexico, Alaska, Spain, Portugal, Switzerland.

- Genome sequenced in 2003
- 39,225,835 bp
- 9,826 genes
- 7 chromosomes

Neurospora's three main cell types



(from Fungal Genetics Stock Center)



DAPI stain of nuclei

for more pictures or videos, click here

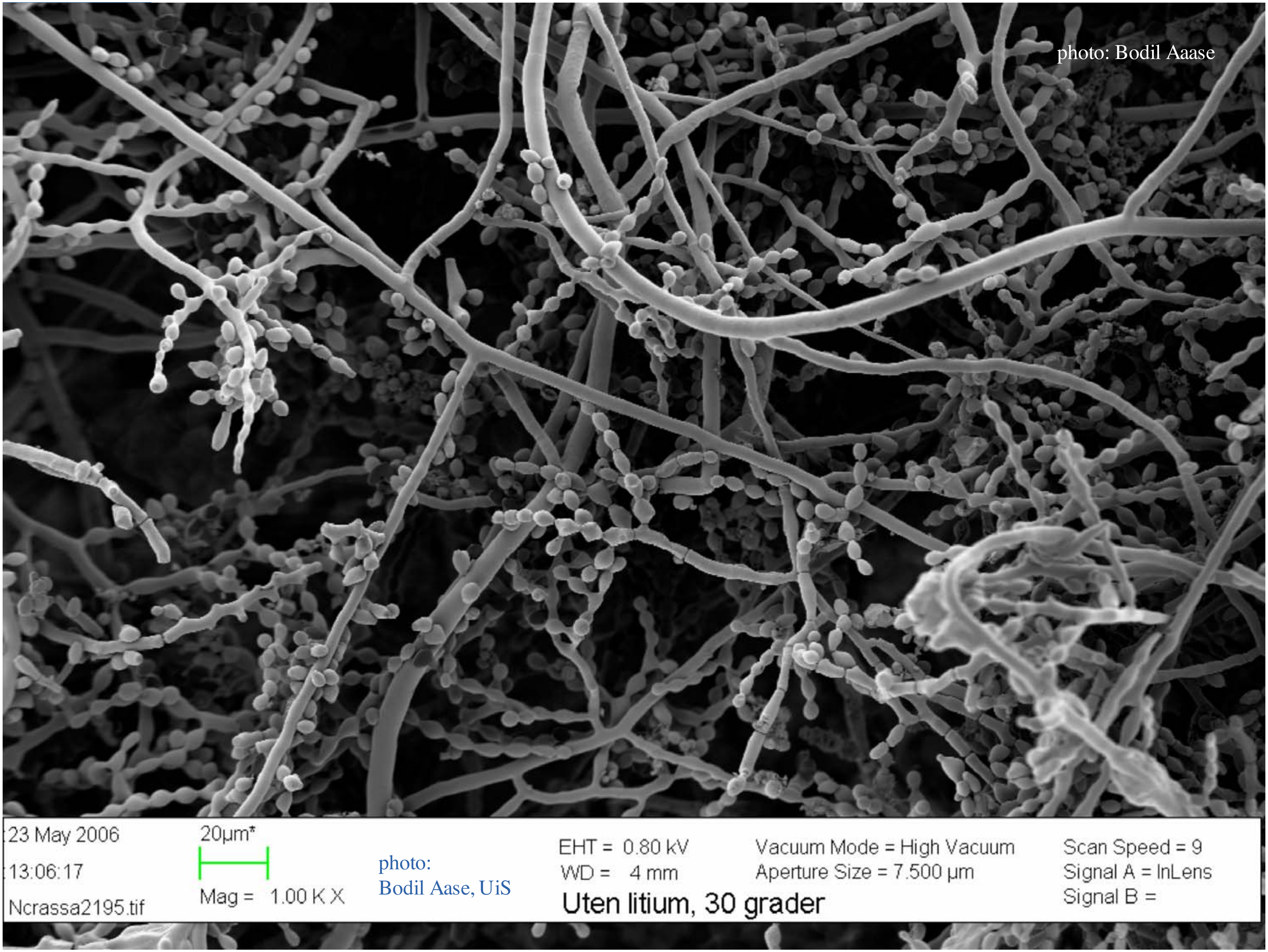
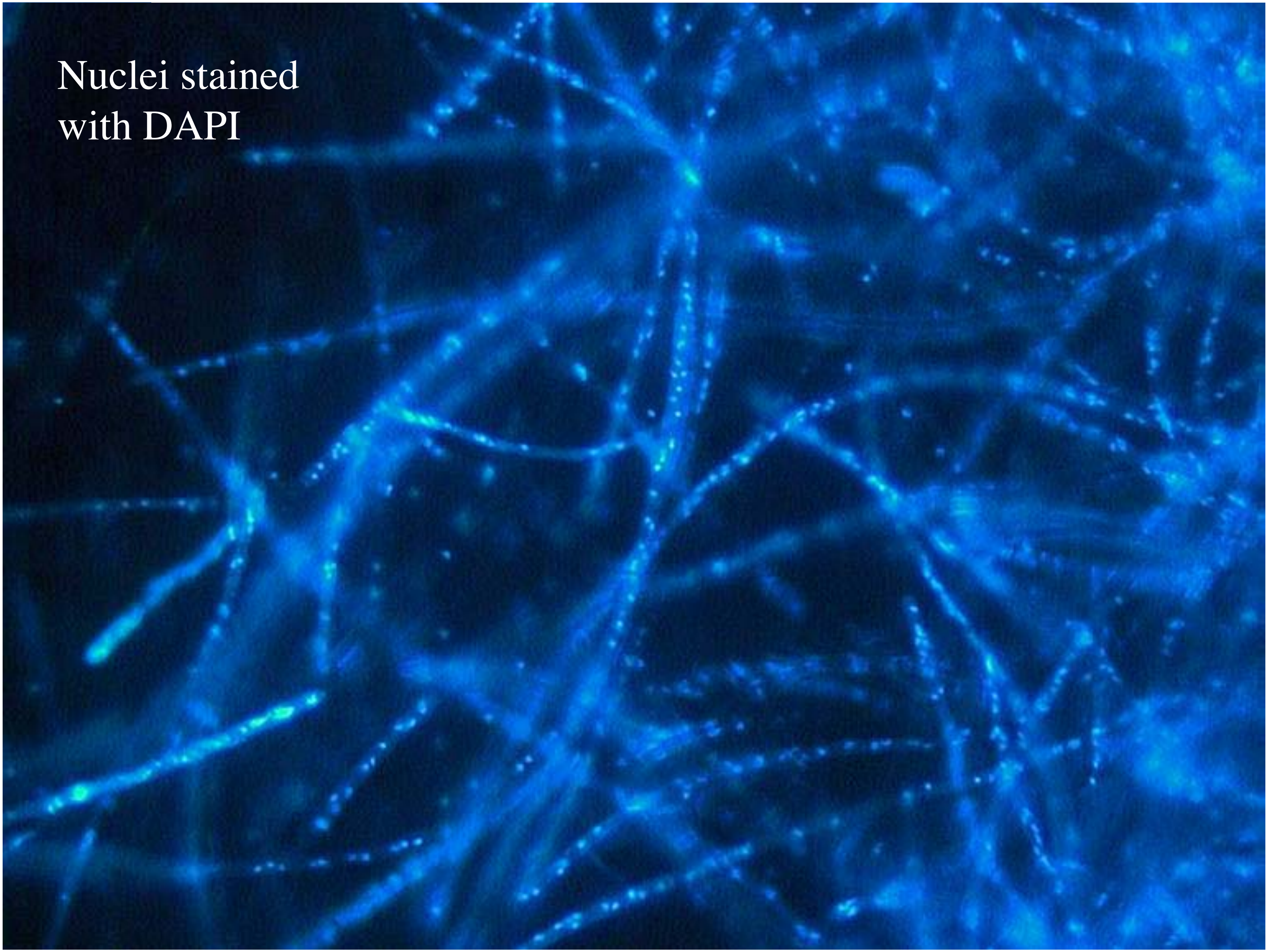


photo: Bodil Aaase
23 May 2006
13:06:17
Ncrassa2195.tif
20µm*
Mag = 1.00 K X
photo:
Bodil Aase, UiS
EHT = 0.80 kV
WD = 4 mm
Uten litium, 30 grader
Vacuum Mode = High Vacuum
Aperture Size = 7.500 µm
Scan Speed = 9
Signal A = InLens
Signal B =


Nuclei stained
with DAPI

Conidia

Conidia

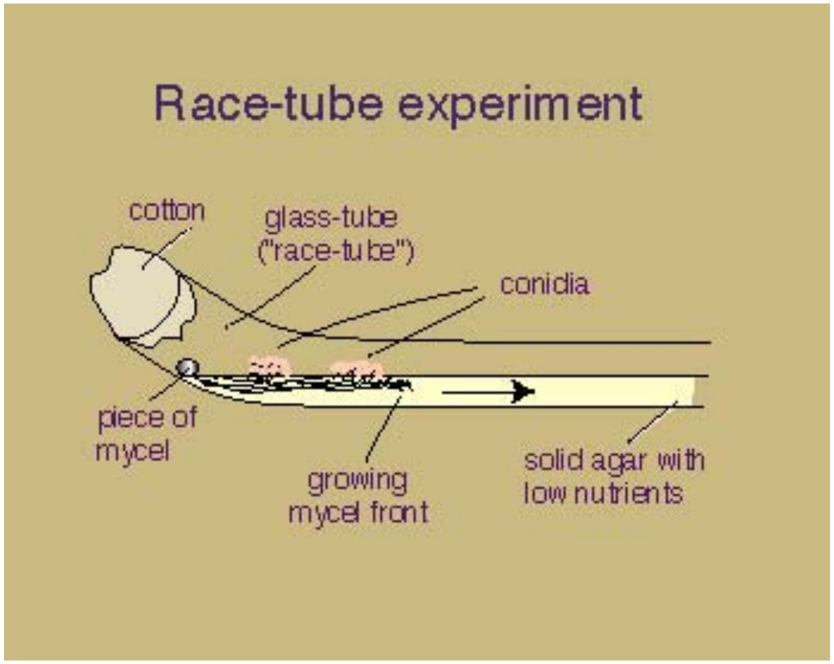
Neurospora circadian rhythm in a Petri dish

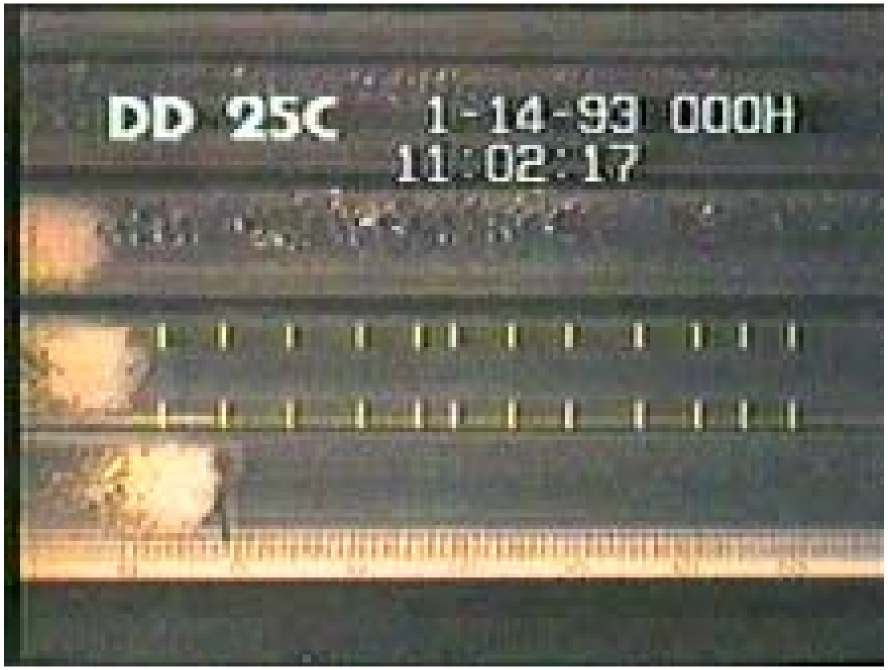
- Period length ≈ 22 h
- Rhythm shows temperature and pH compensation

University of Stavanger

In *Neurospora,* the circadian rhythm is easily seen in so-called “race tubes”

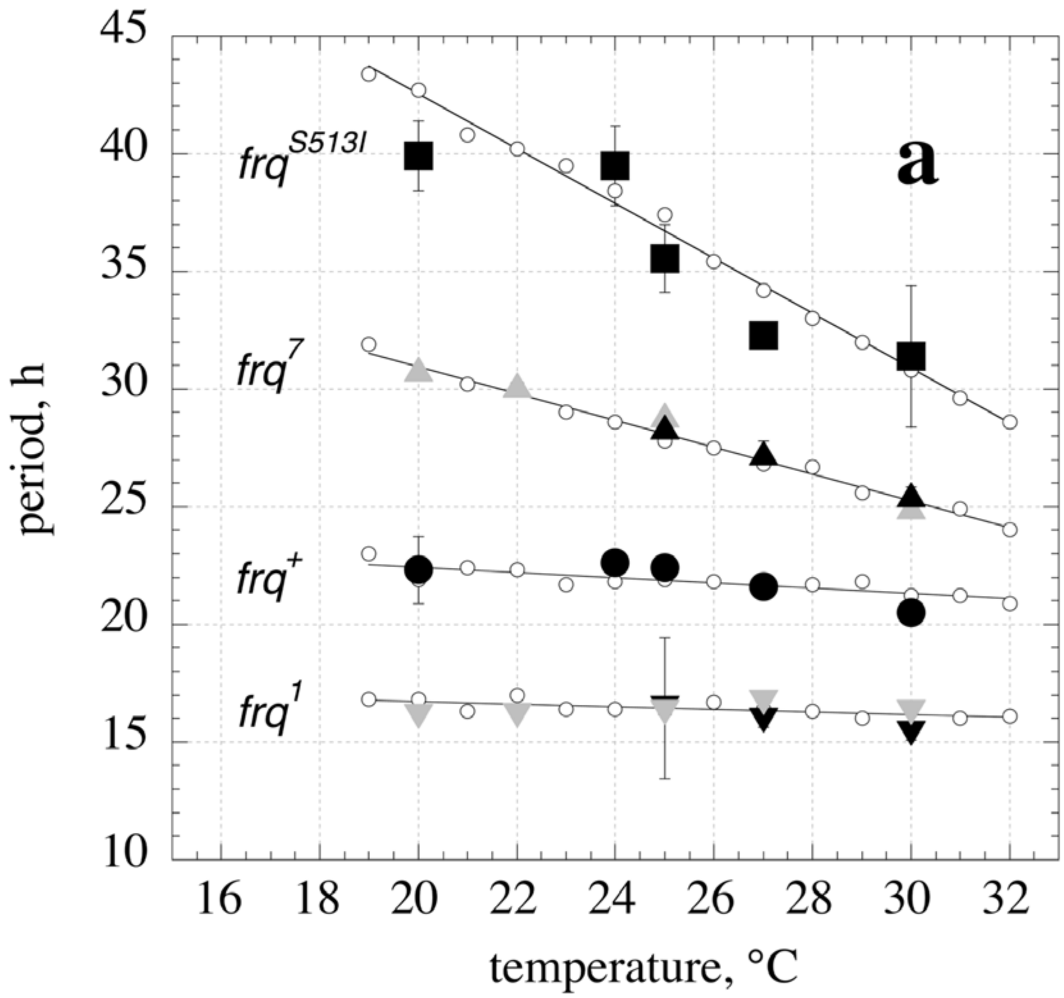
Race tube assay of *Neurospora's* circadian rhythm



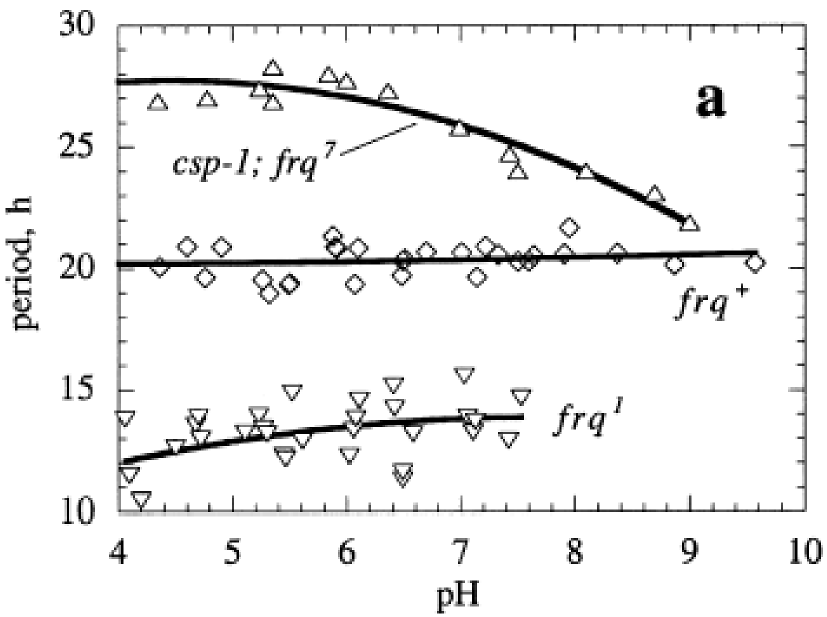


Jason C. Thoen and Van Gooch:
"Time Lapse Video Showing an Internal Circadian Clock in Mold (*Neurospora*) Growth"

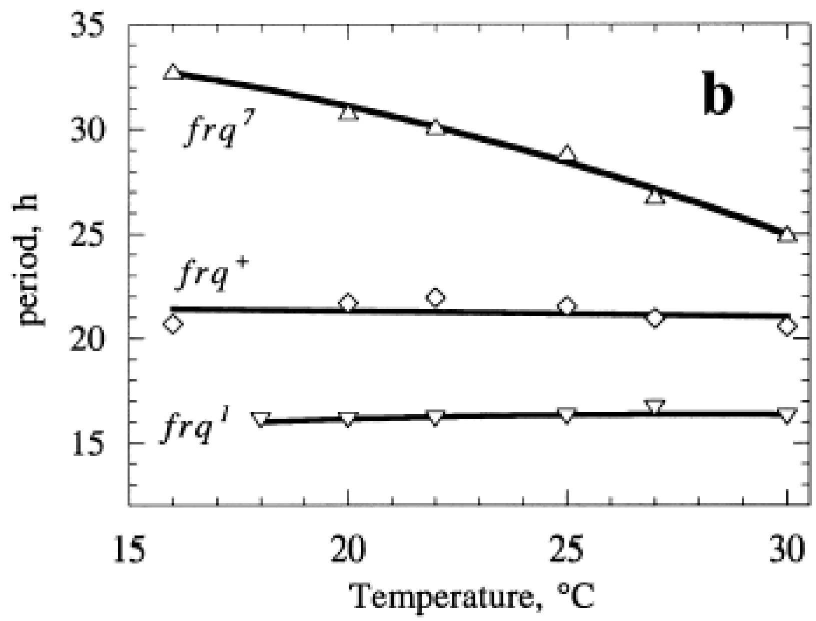
Temperature Compensation in different *frq* mutants



pH Compensation compared to temperature compensation in different *frq* mutants



pH compensation



temperature compensation

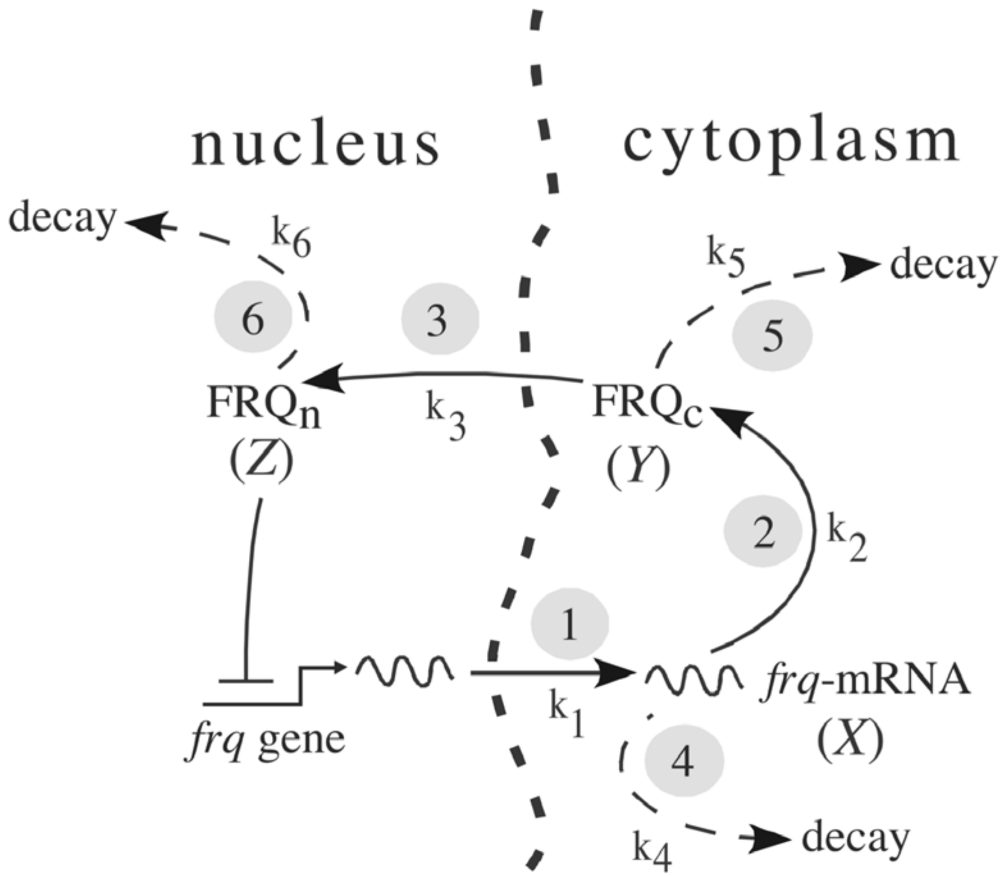
University of Stavanger

FREQUENCY (FRQ) protein

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

```
MADSGDKSQGMRPPPFDSRGHPLPRRASPDKSITLENHRLARDTSSRVTS   start of long FRQ
SSALGVTESQPQLKSSPTRRNSSGESEPTNWFNQSNRNPAAAFHDESHIM   start of short FRQ
EVDPPFYQKETDSSNEESRYPPGRNPVHPPGGVQLPGFRPVAAHSTAADD
YRSVIDDLTVENKRLKEELKRYKQFGSDVMRKEKLFEIKVHGLPRRKKRE   coiled-coil
LEATLRHFAASLGDSSESTSQRRKTGRHGTAVYSSGVSLSKHDSSSSSRS
RPVDSAYNSMSTGRSSHAPHSSGPSLGRPSLTRAKSVGTQKVENYLRDTP
DGLLPHHIVMTDKEKKKLVVRRLEQLFTGKISGRNMQRNQSMPSMDAPLA
PEGTNMAPPRPPPEGLREACIQLQDGDNPRKNRSSKDNGSASNSGGDQTE
LGGTGTGSGDGSGSGGRTGNNTSPPGAIAPDQRPTRPRDLDPDRVQIPSE   potential GSK-3 P-site
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
```

Minimal Model of the Circadian Rhythms in *Neurospora crassa*



$$\frac{dX}{dt} = k_1 f_{inhib} - k_4 X$$

$$\frac{dY}{dt} = k_2 X - (k_3 + k_5) Y$$

$$\frac{dZ}{dt} = k_3 Y - k_6 Z$$

frq: frequency

A theory for temperature compensation: The antagonistic balance equation

$$P = P(k_1, k_2, \ldots, k_i, \ldots, k_N).$$

$$\frac{\partial P}{\partial T} = \sum_i \left(\frac{\partial P}{\partial k_i}\right)\left(\frac{\partial k_i}{\partial T}\right).$$

$$k_i = A_i e^{-\frac{E_i}{RT}},$$

$$\frac{\partial P}{\partial T} = \sum_i \left(\frac{\partial P}{\partial k_i}\right)\frac{E_i}{RT^2} k_i = \sum_i \left(\frac{\partial P}{\partial \ln k_i}\right)\frac{E_i}{RT^2}. \qquad [12]$$

Multiplying Eq. **12** by $1/P$ and observing that $\partial P/P = \partial \ln P$, Eq. **12** can be written as

$$\frac{1}{P}\frac{\partial P}{\partial T} = \frac{\partial \ln P}{\partial T} = \frac{1}{RT^2}\sum_i \left(\frac{\partial \ln P}{\partial \ln k_i}\right) E_i = \frac{1}{RT^2}\sum_i C_i^P E_i.$$

This approach suggests that temperature compensation is a systemic property as suggested by Hastings and Sweeney in 1957.

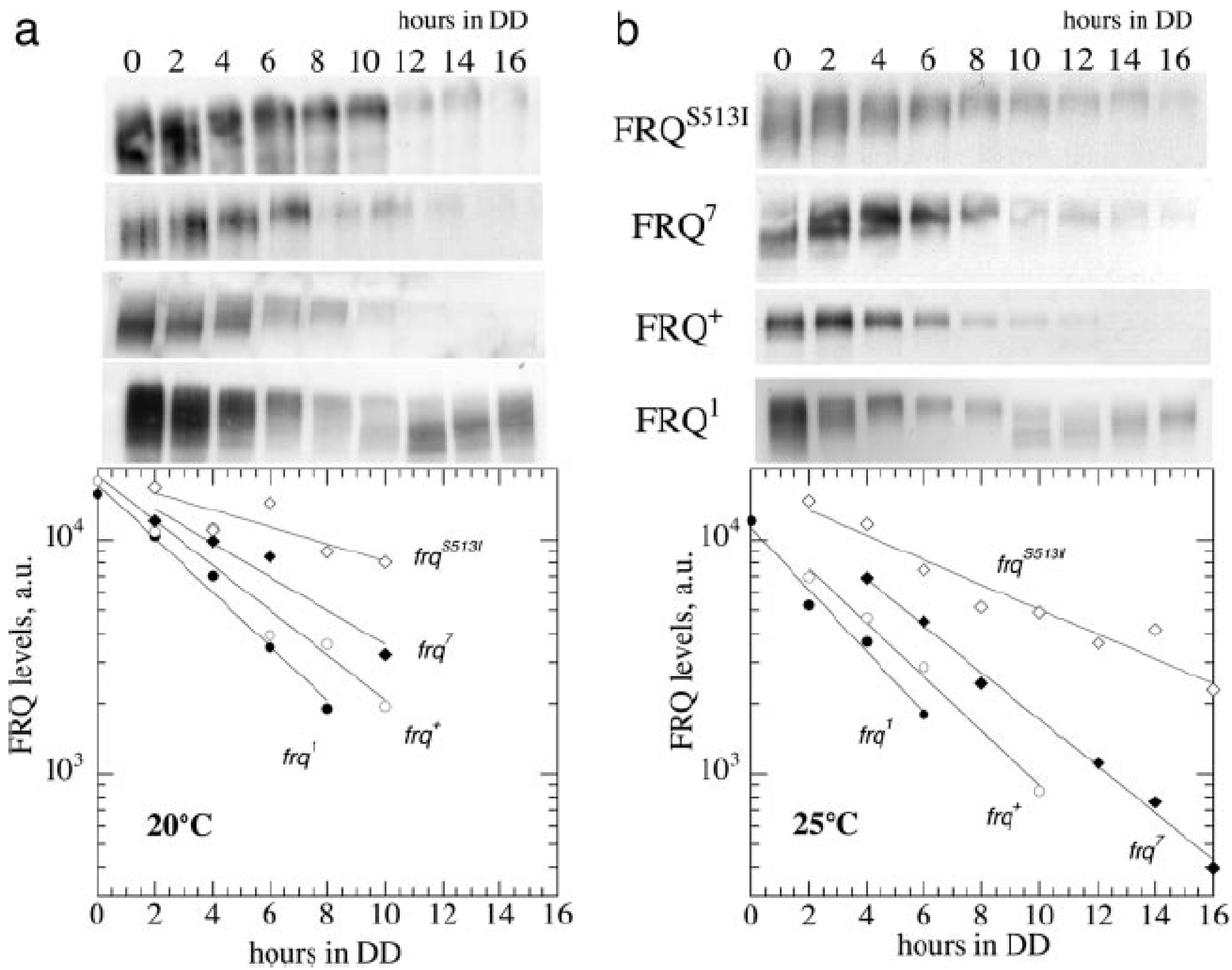
Table 1. Rate constants, control coefficients, and activation energies used in the model calculations

Reaction i	Rate constant k_i, h^{-1}	C_i^P (15% variation of the k_is)	E_i, kJ/mol	$C_i^P E_i$, kJ/mol
1	0.3	0.131	190	24.9
2	0.3	0.093	190	17.7
3	0.3	−0.040	190	−7.6
4	0.27	−0.470	30	−14.1
5	0.2*	−0.131	30†	−3.9
6	0.2*	−0.628	30†	−18.8
		$\Sigma i C_i^P = -1.045$		$\Sigma i C_i^P E_i = -1.8$

The given rate constant values are defined for $T_{ref} = 292$ K. Rate constants k_5 and k_6 (with asterisk) differ for the various *frq* mutants. Initial concentrations (a.u.) used in all calculations: $X = 6.124 \times 10^{-2}$, $Y = 8.452 \times 10^{-2}$, $Z = 5.245 \times 10^{-1}$. Threshold for *frq* transcription inhibition, $Z_{max} = 0.1$ a.u.; threshold for reactivating *frq* transcription, $Z_{min} = 0.05$ a.u. (used in all calculations).

*k_5, k_6 values for frq^+ at $T_{ref} = 292$ K. The following k_5, k_6 values (defined at $T_{ref} = 292$ K) have been used in the calculations for the other mutants: frq^1, 0.320 h^{-1}; frq^7, 0.124 h^{-1}; frq^{S5131}, 0.080 h^{-1}.

†E_5 and E_6 values for frq^+. The following E_5, E_6 values ($E_5 = E_6$) have been used in the calculations for the other mutants (see also Table 2): frq^1, 29 kJ/mol; frq^7, 46 kJ/mol; frq^{S5131}, 59 kJ/mol.


a
hours in DD
0 2 4 6 8 10 12 14 16
FRQ levels, a.u.
frq^S513I
frq^7
frq^+
frq^1
20°C
hours in DD
b
hours in DD
0 2 4 6 8 10 12 14 16
FRQ^S513I
FRQ^7
FRQ^+
FRQ^1
FRQ levels, a.u.
frq^S513I
frq^1
frq^+
frq^7
25°C
hours in DD

Estimation of activation energy for k_5 and k_6

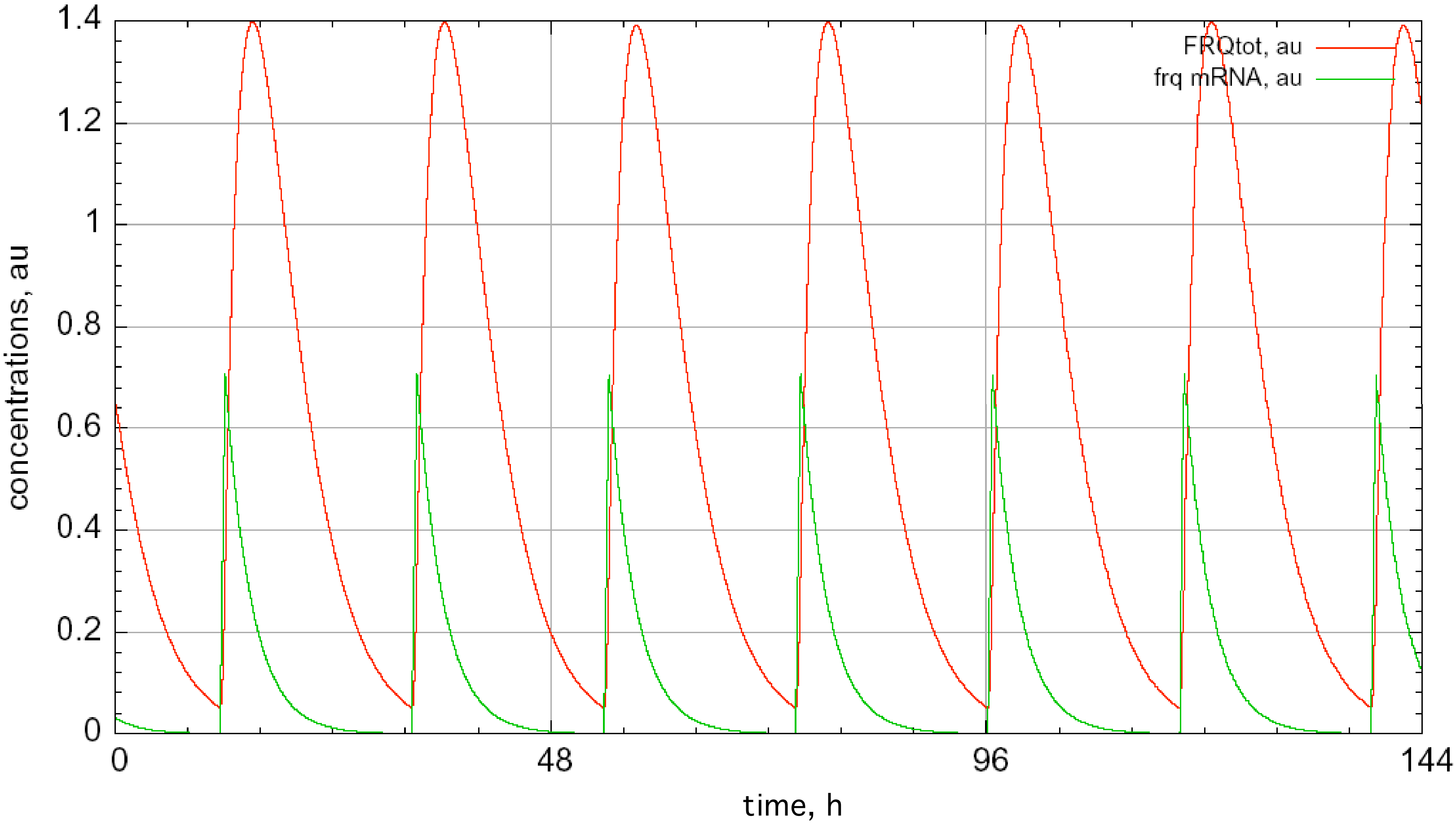
$$E_a = \frac{R \times \ln\left(\frac{k^{25°C}}{k^{20°C}}\right)}{\frac{1}{293K} - \frac{1}{298K}}.$$

Table 2. Experimental (Exp) and theoretical (Theor) FRQ degradation rate constant values at 20°C and 25°C

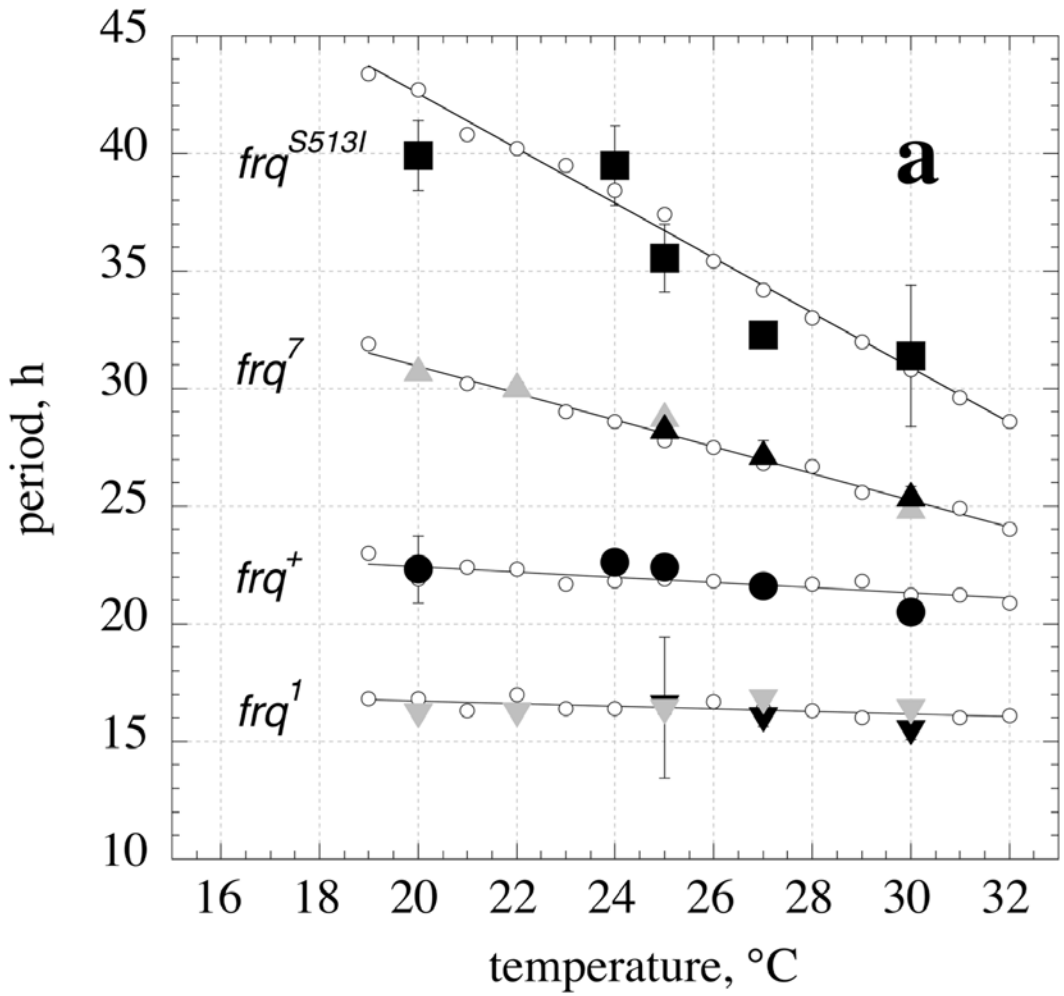
	FRQ^1		FRQ^+		FRQ^7		FRQ^{S5131}	
	Exp	Theor	Exp	Theor	Exp	Theor	Exp	Theor
k (20°C)*, h^{-1}	0.27	0.33	0.22	0.21	0.16	0.13	0.08	0.09
k (25°C)*, h^{-1}	0.33	0.41	0.27	0.26	0.22	0.18	0.12	0.13
E_a (kJ/mol)	29		30		46		59	

*The theoretical k values at 20°C and 25°C are calculated from the (theoretical) k_5 (=k_6) FRQ degradation rate constants given in Table 1 by using the experimentally determined activation energies E_a and Eq. **1.**

Model shows relaxation type of oscillations in *frq*-mRNA: Short transcription phase long FRQ-protein degradation phase



Temperature Compensation in different *frq* alleles



Applying the antagonistic balance equation: The Oregonator

$$A + Y \xrightarrow{k_1} X + P \qquad (O1)$$

$$X + Y \xrightarrow{k_2} 2P \qquad (O2)$$

$$A + X \xrightarrow{k_3} 2X + Z \qquad (O3)$$

$$2X \xrightarrow{k_4} A + P \qquad (O4)$$

$$Z \xrightarrow{k_5} fY \qquad (O5)$$

Table 3

Oregonator period as a function of temperature and activation energies of component processes O1–O5

E_1 (J/mol)	E_2 (J/mol)	E_3 (J/mol)	E_4 (J/mol)	E_5 (J/mol)	$\Sigma^+ - \Sigma^-$ [a] (J/mol)	Period				
						at 15°C	at 20°C	at 25°C	at 30°C	at 35°C
17848	46785	4691	6181	3903	0	356	354	354	353	352
36477	32867	59217	24424	875	0	353	355	358	363	368
25819	59088	56071	56764	2027	0	354	355	357	359	362
8717	38527	29507	42515	1317	0	355	355	355	356	357
1033	42946	44286	14315	9946	0	356	355	353	350	347
40943	35921	49142	17867	385	0	353	355	359	362	367
70000	70000	70000	70000	70000	−71435	585	355	219	138	87
50000	0	0	0	70000	−75739	605	355	213	130	82
40943	80000	80000	17867	385	10498	329	355	383	410	438
26487	59389	10186	36803	14252	−13477	391	355	323	296	271
27337	53948	31776	20429	7195	− 3062	362	355	347	341	334
51192	31300	30705	7055	55569	−55062	523	355	245	170	121
55745	47569	29048	56544	30476	−37416	458	355	277	217	172
38202	17177	47318	26272	30000	−31884	441	355	288	235	193
46122	39461	15733	27625	58358	−60116	543	355	235	159	109
30433	35003	36866	34186	54216	−52591	515	355	248	176	126
26098	4432	35971	3082	41158	−39767	469	355	271	209	163
9905	33336	46833	56358	47904	−45234	489	355	261	193	145
16440	13245	8106	4103	44278	−42523	482	355	265	200	153

[a] $\Sigma^+ - \Sigma^- = (0.1629E_2 + 0.1075E_3) - (0.1964E_1 + 0.1528E_4 + 0.9417E_5)$

The (closed) Belousov-Zhabotinsky reaction is not temperature compensated

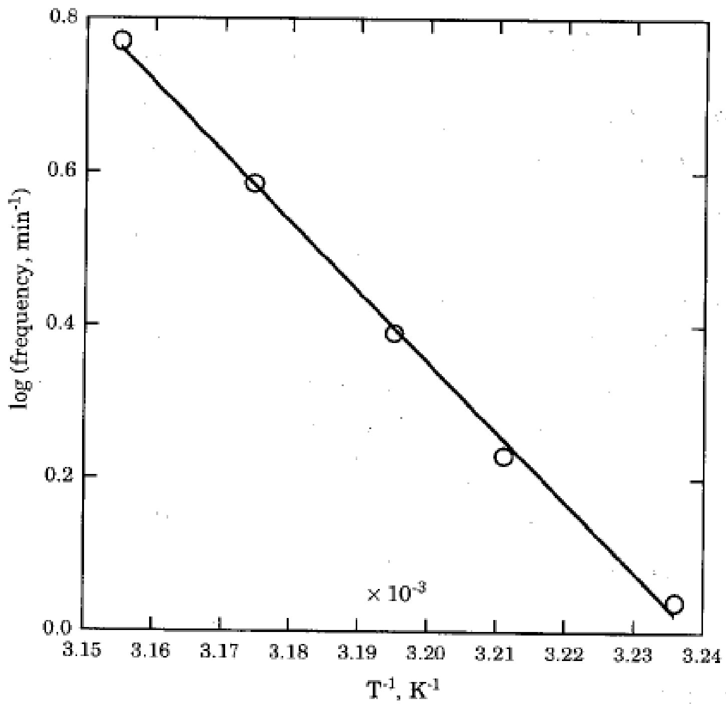


Fig. 1. Arrhenius plot (here: natural logarithm of inverse of period length versus inverse of absolute temperature) of a batch Belousov–Zhabotinsky reaction. Reaction volume is 100 mL. Initial reagent concentrations: malonic acid 0.3 M, potassium bromate 0.1 M, $(NH_4)_2Ce(NO_3)_6$ 2.1×10^{-3} M, sulfuric acid 1.0 M. The calculated $Q_{10} = (P_2/P_1)^{10/(T_1 - T_2)}$ is 2.5, where P_1 and P_2 are period lengths at temperatures T_1 and T_2, respectively. The calculated activation energy is 73 kJ/mol.

The Arrhenius plot to the left shows that the BZ reaction is not compensated. The overall E_a for the period is ca. 73 kJ/mol.

10302 *J. Phys. Chem. A* **2005,** *109,* 10302−10306

Temperature Compensation in the Oscillatory Bray Reaction

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Received: July 12, 2005; In Final Form: September 16, 2005

The influence of temperature on the oscillatory frequency of the hydrogen peroxide−iodate ion reaction is found to be two-sided: (i) the period length decreases with increasing temperature in most of the instances studied, (ii) or in some cases an opposite change is observed. A temperature-independent period length (temperature compensation) is also discovered experimentally in a rather wide temperature interval at a narrow concentration range of reactants both in a batch configuration and under flow conditions. A simple model was considered to simulate this behavior. Opposing effects of the composite reactions of the model on the calculated period length with changing temperature are shown to be responsible for temperature compensation or overcompensation.

PCCP

Temperature-compensation in pH-oscillators

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First published as an Advance Article on the web 20th September 2002

Temperature independent period length (temperature-compensation) in pH-oscillators has been simulated with a simple general model. Opposing effects of the composite reactions on the period length with changing temperature have been shown to be responsible for this peculiar phenomenon. Experiments have shown that temperature-compensation exists in the oscillatory hydrogen peroxide–sulfite ion–thiosulfate ion flow system in a narrow range of conditions. A simple mechanism with estimated activation energies of the steps was used successfully to simulate the phenomenon.

University of Stavanger

Thank you for your attention!