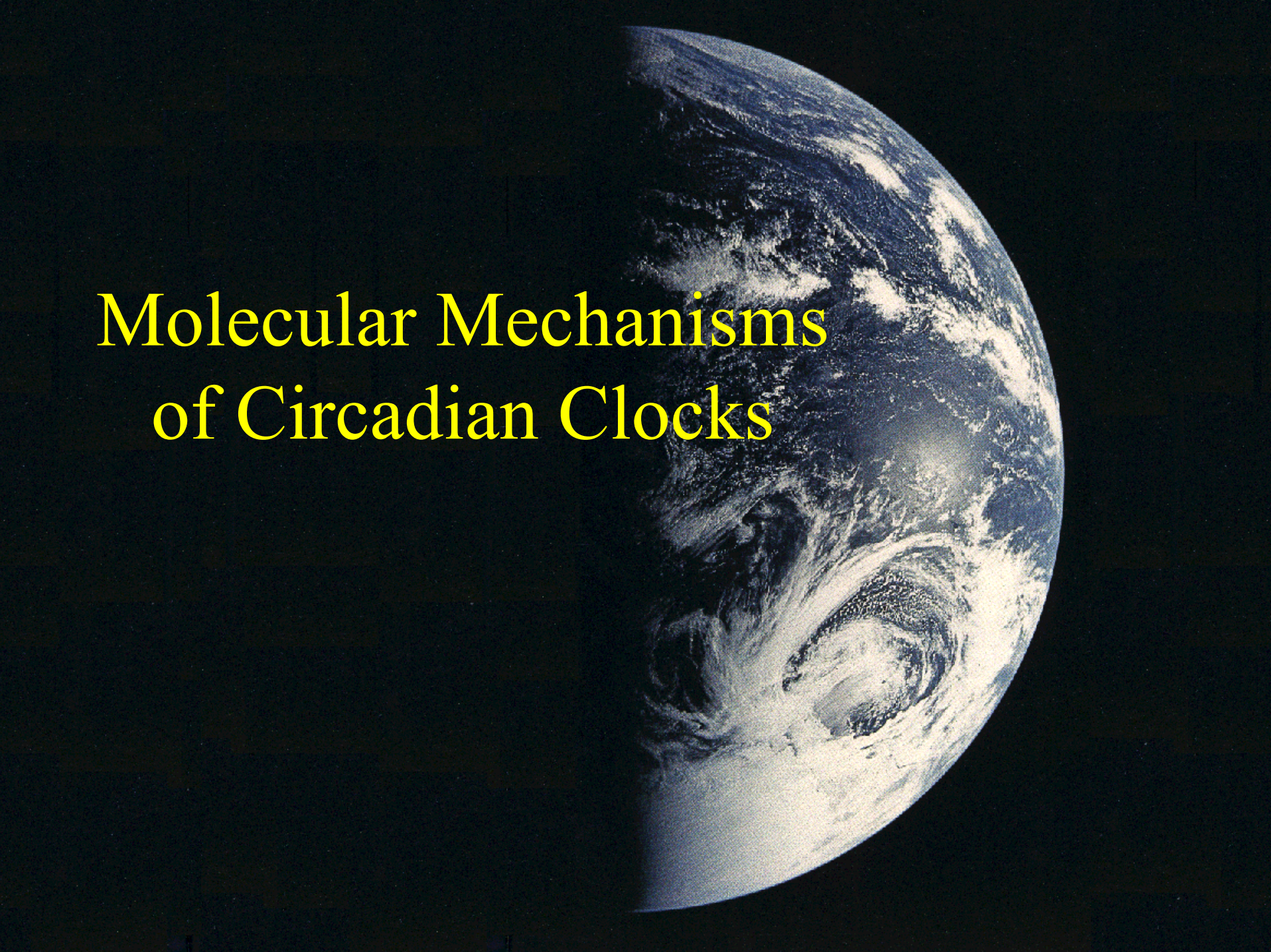
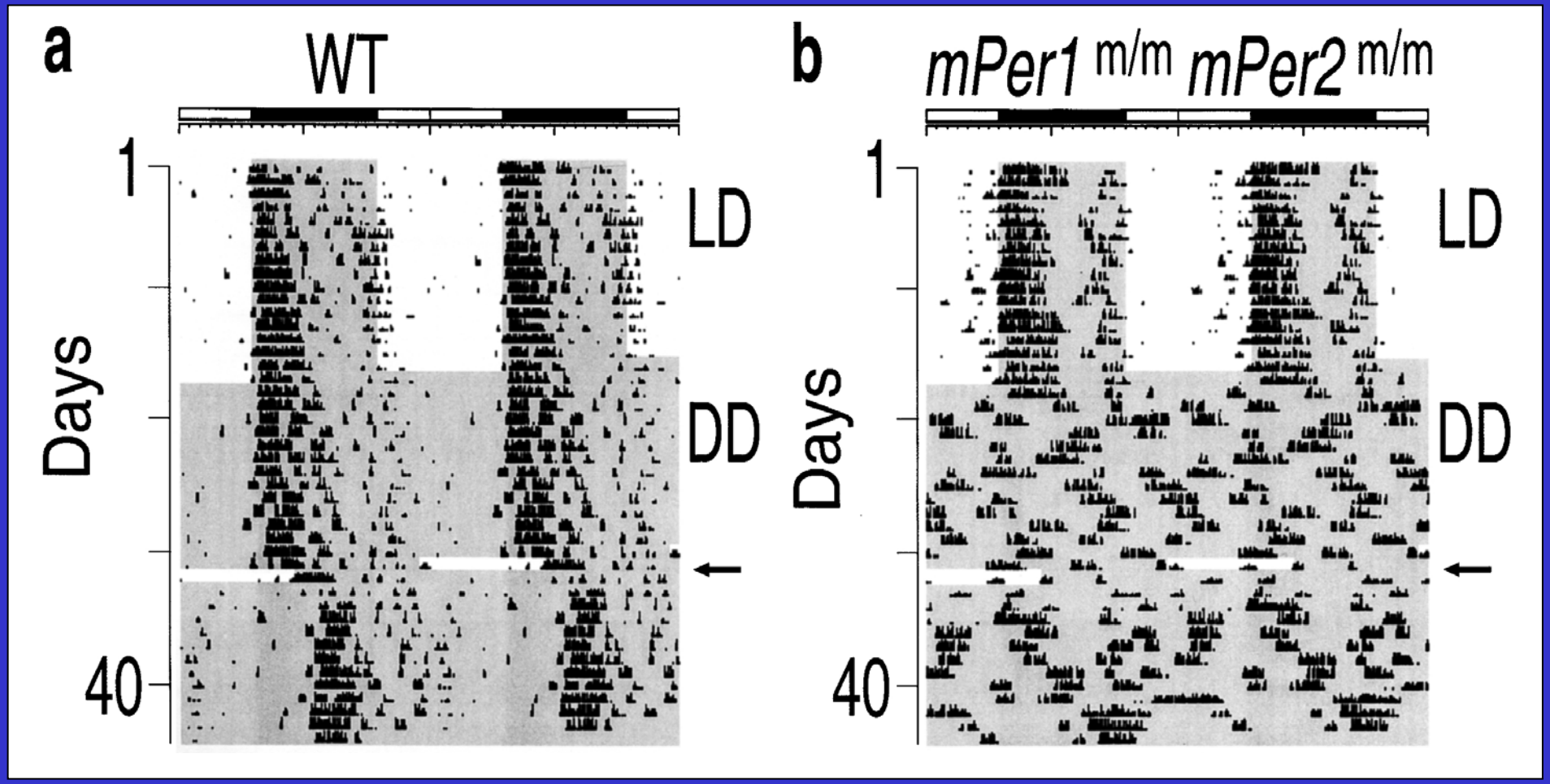

Molecular Mechanisms
of Circadian Clocks

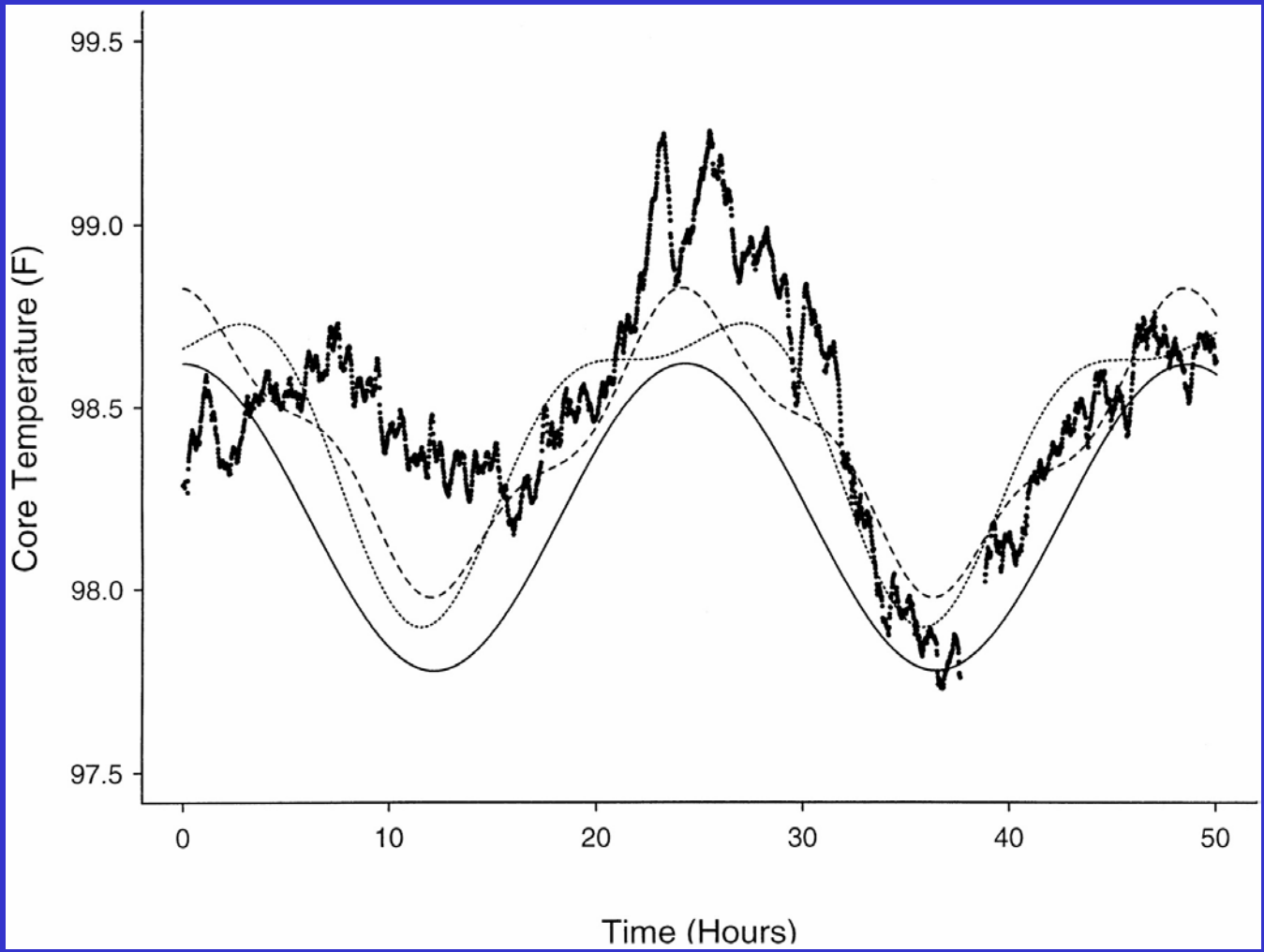
Circadian Rhythms

- Endogenous rhythm which persists under constant conditions
- Period of approximately 24 hours
- Entrainable (reset) by environmental cues such as light and temperature
- Temperature compensated - period stays about the same at different temperatures

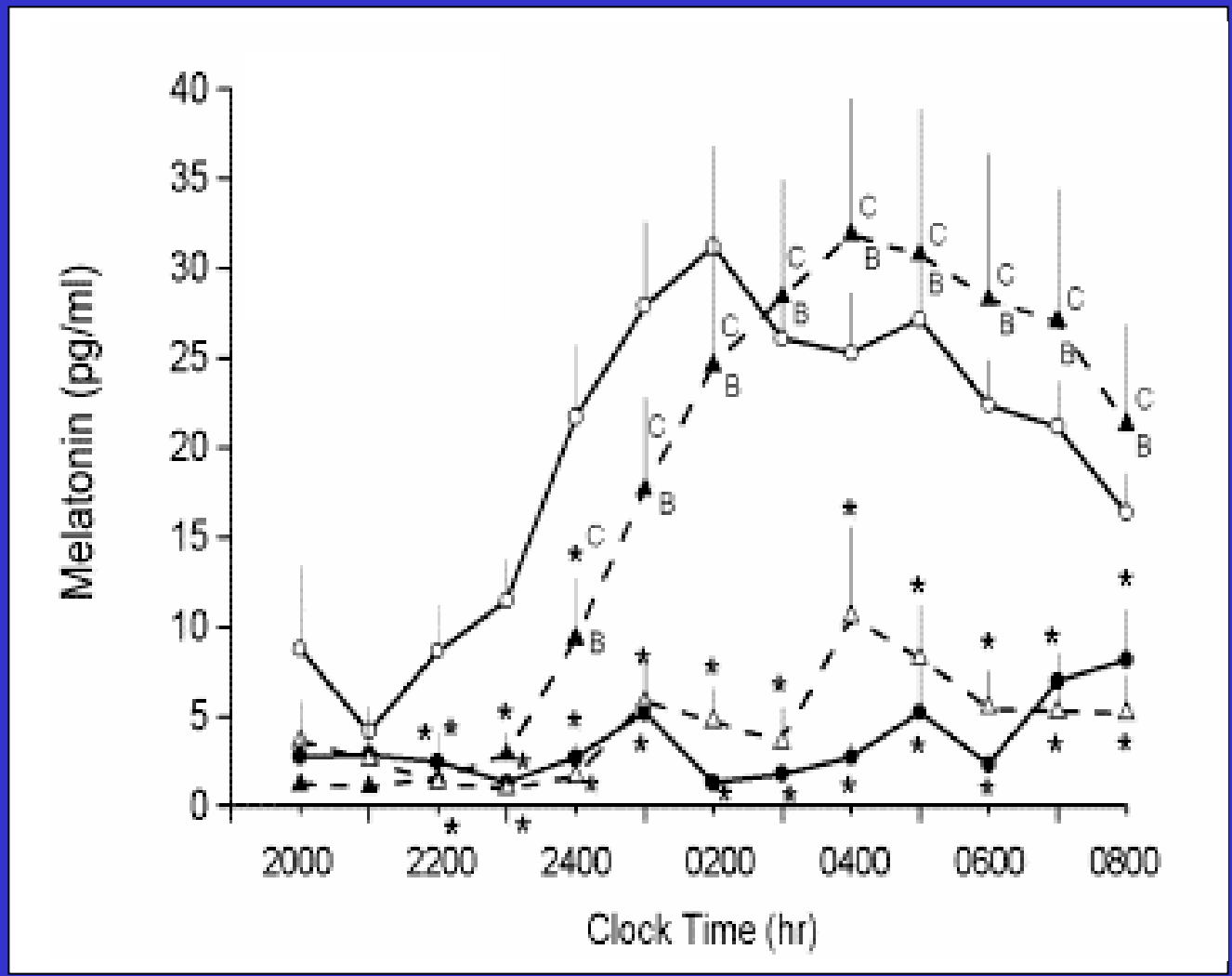
Circadian rhythm of locomoter activity



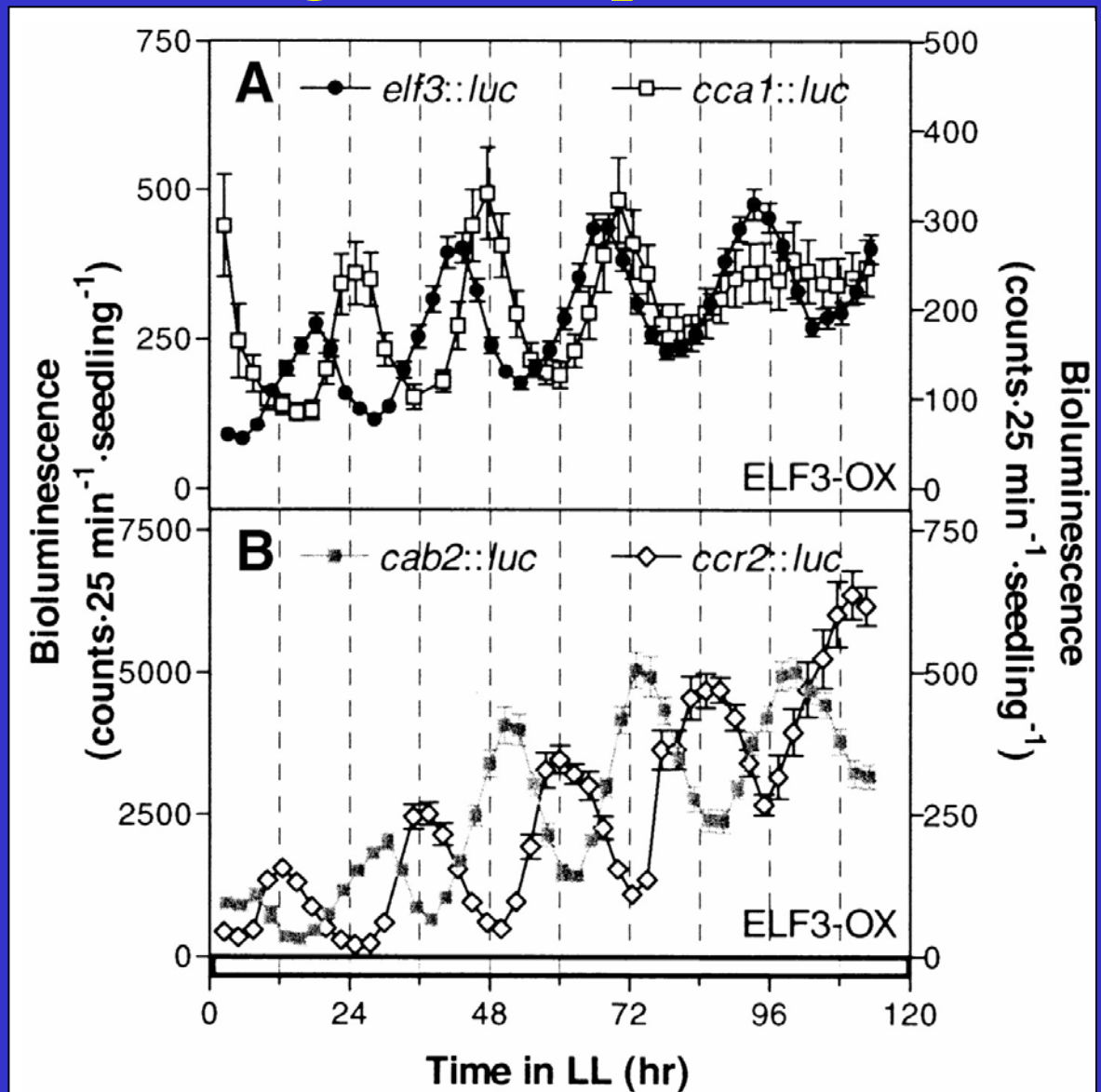
Body temperature cycle



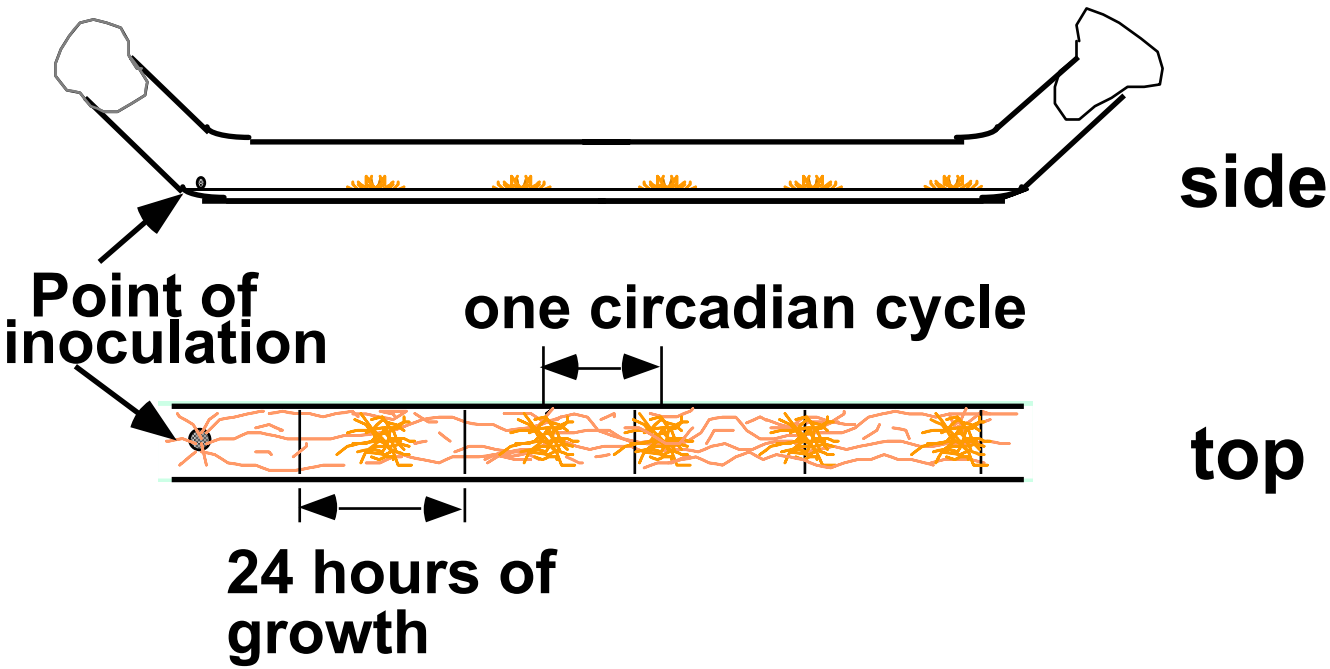
Circadian rhythm of melatonin secretion



Circadian rhythms of photosynthetic genes in plants

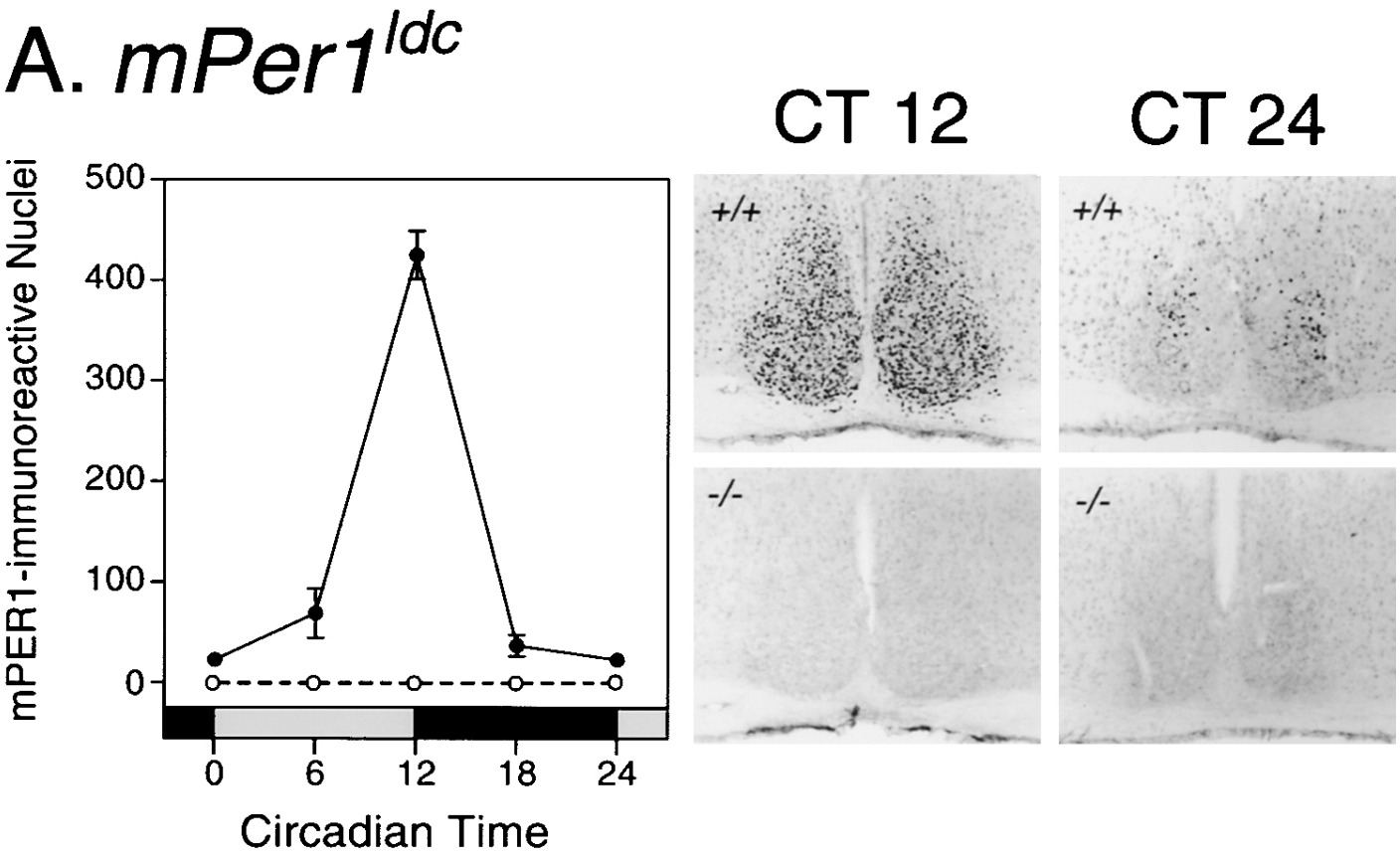


Circadian rhythm of asexual reproduction in fungi



Neurospora

Suprachiasmatic Nucleus (SCN), the Master Pacemaker in Mammals



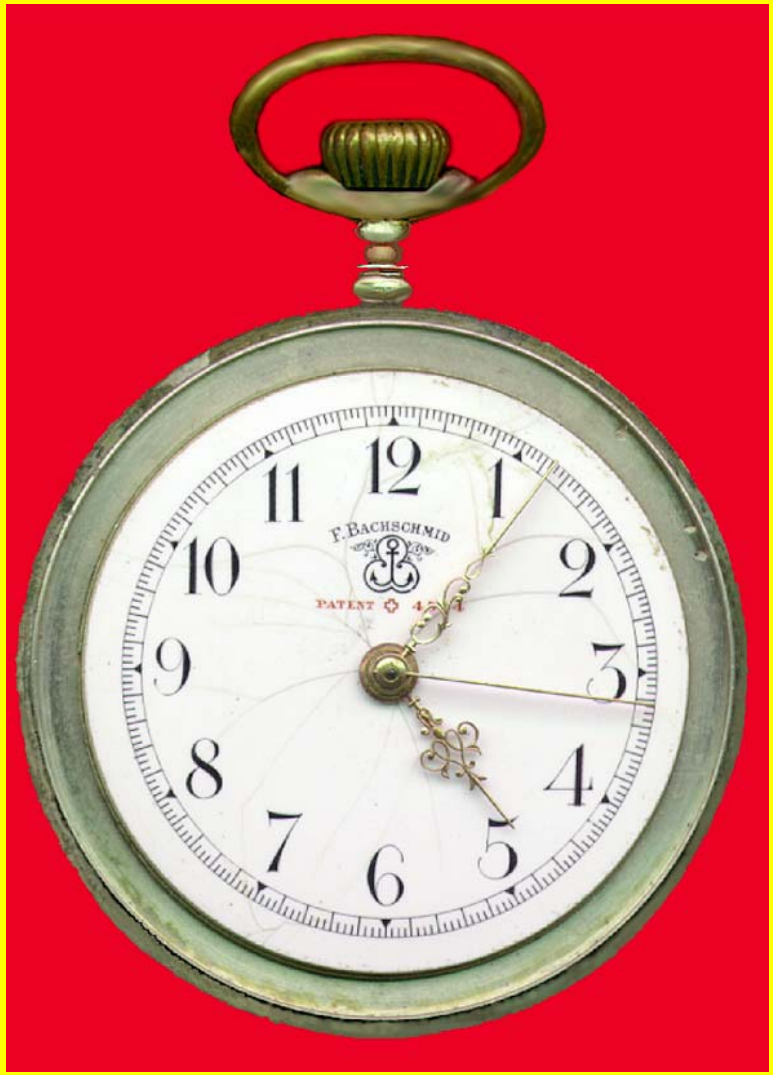
Science 2001 Feb 9;291(5506):1040-1043

An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome (FASP).

Toh KL, Jones CR, He Y, Eide EJ, Hinz WA, Virshup DM, Ptacek LJ, Fu YH.

Department of Human Genetics, University of Utah

How does a clock work?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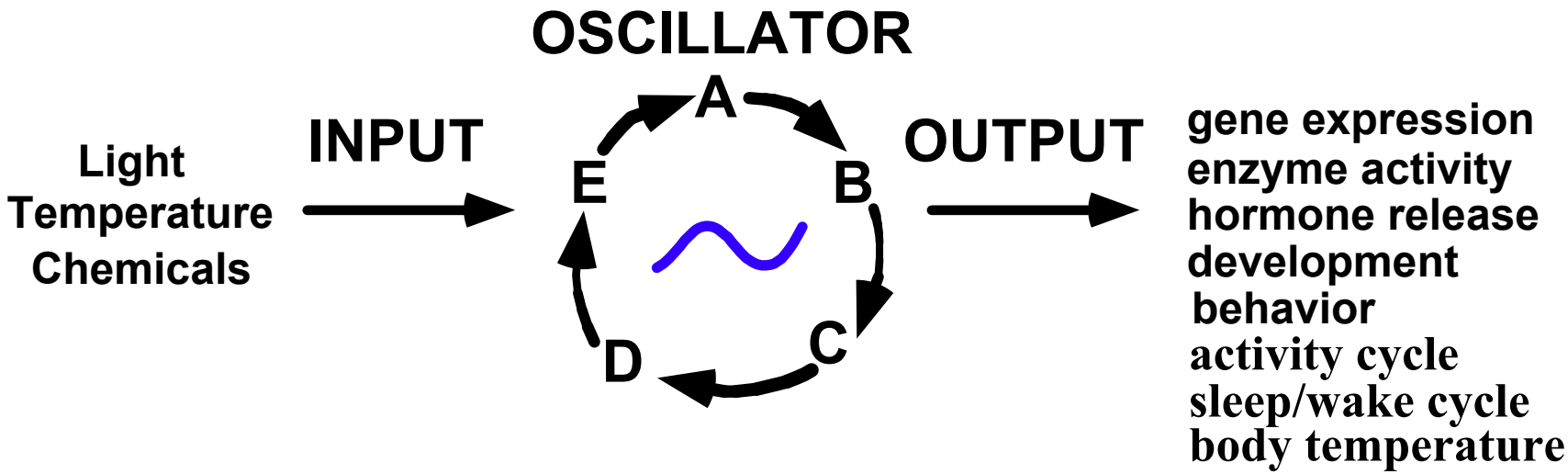

OSCILLATOR
INPUT
Light
Temperature
Chemicals
A
B
C
D
E
OUTPUT
gene expression
enzyme activity
hormone release
development
behavior
activity cycle
sleep/wake cycle
body temperature

Mutations Affecting the *Neurospora* Clock

Clock Genes
- isolated in the context of circadian rhythms

allele	linkage	period length	dominance/ recessivity	other clock properties affected
frq^{1}	VII R	16 hrs	semidominant	
frq^{*2}	VII R	19.3 hrs	semidominant	
frq^{3}	VII R	24 hrs	semidominant	temperature compensation
frq^{*4}	VII R	19.3 hrs	semidominant	
frq^{*6}	VII R	19.3 hrs	semidominant	
frq^{*7}	VII R	29 hrs	semidominant	temperature compensation, cycloheximide resetting
frq^{*8}	VII R	29 hrs	semidominant	temperature compensation
frq^{9}	VII R	arrhythmic/ uncompensated	recessive	temperature compensation, nutritional compensation,** entrainment
frq^{10}	VII R	arrhythmic/ uncompensated	recessive	temperature compensation, nutritional compensation,** entrainment
chr	VI L	23.5 hrs	semidominant	temperature compensation
prd-1	III C	25.8 hrs	recessive	temperature compensation
prd-2	V R	25.5 hrs	recessive	
prd-3	I C	25.1 hrs	recessive	temperature compensation
prd-4	I R	18 hrs	dominant	temperature compensation
cla-1	I R/VII R	27 hrs	semidominant	temperature compensation

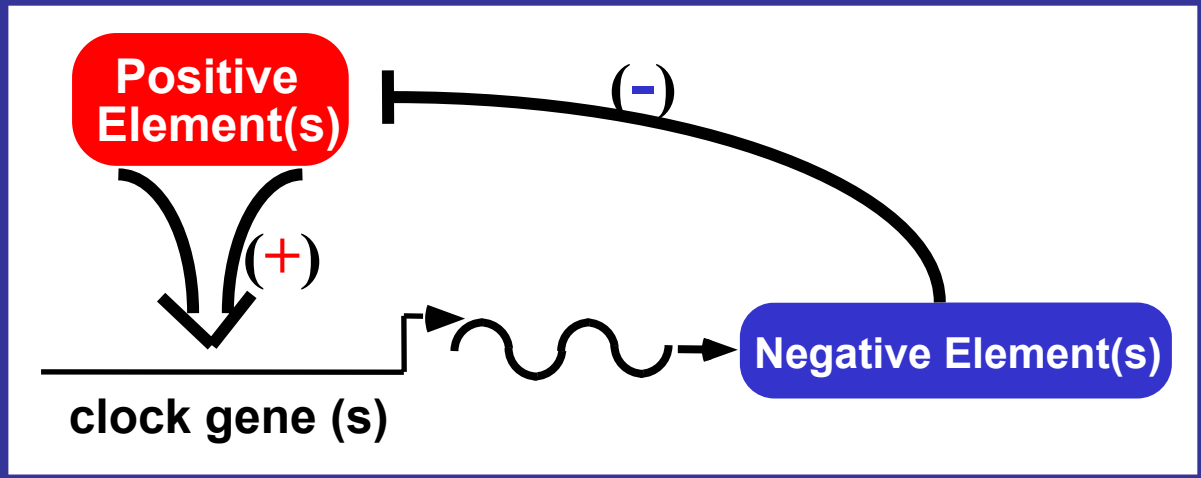
* Genetically $frq^{2} = frq^{4} = frq^{6}$ and $frq^{7} = frq^{8}$

Other mutations affecting clock period length

allele	linkage	period length	dominance/ recessivity	other clock properties affected
arg-13**	I R	21 - 19	recessive	
cel**	IV R	20 - 40		temperature compensation
cys-4	IV R	19		
cys-12	I R	19		
glp-3(ff-1)	II R	19		
phe-1	I L	19	recessive	
mitochondrially related				
[mi-1]		18 - 19		
cya -5	IV R	19		
cyb-2		18		
cyb-3	II L	20	semidominant	
cyt-4	I R	20		
oli^{r}	VII R	18 - 20		

** Extent of period effect is dependent on the growth medium

Common theme among circadian oscillators



Negative elements in circadian feedback loops:

Synechococcus:	kaiC
Neurospora:	FREQUENCY
Drosophila:	PERIOD and TIMELESS
Mouse:	mCRYs and mPERs

Positive elements in circadian feedback loops

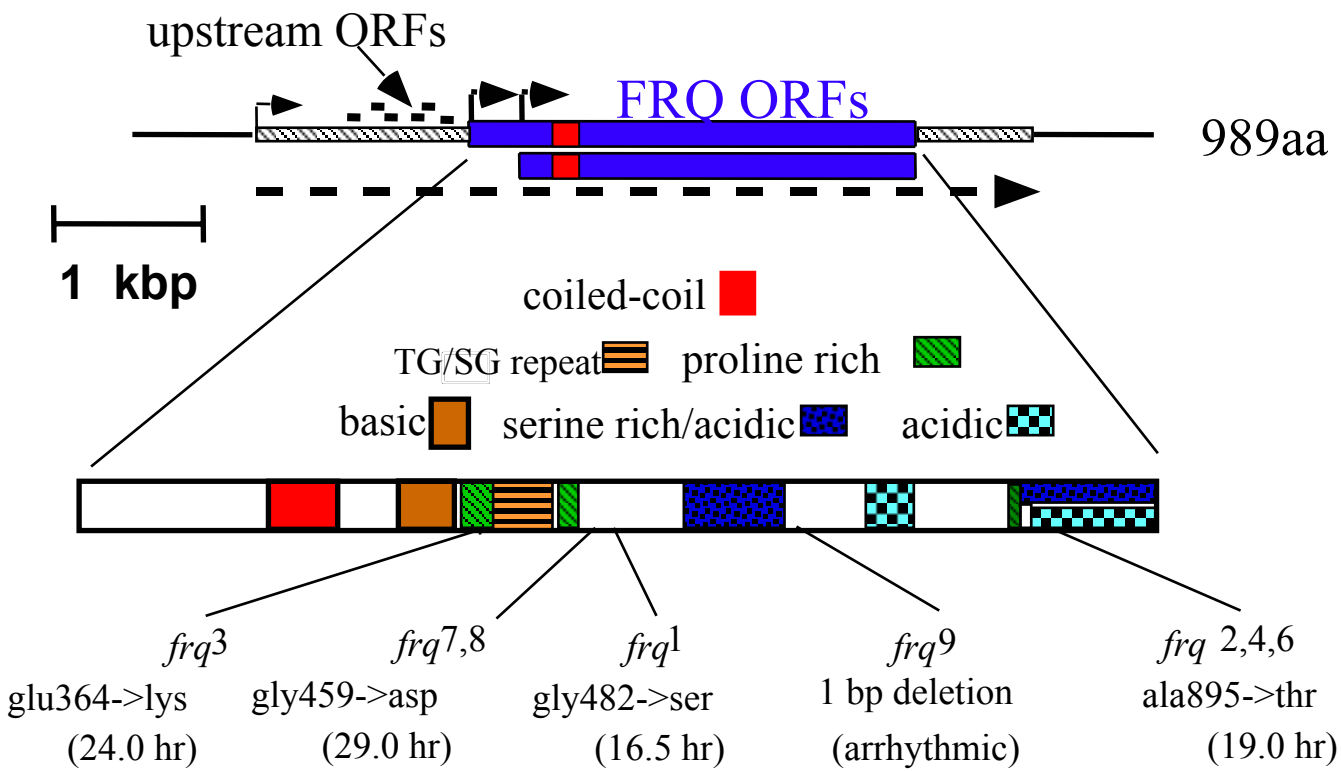
Synechococcus:	kaiA
Neurospora:	WHITE COLLAR-1 and WC-2
Drosophila:	dCLOCK and CYCLE
Mouse:	CLOCK, NPAS2 and BMAL1

} PAS domain containing transcription factors

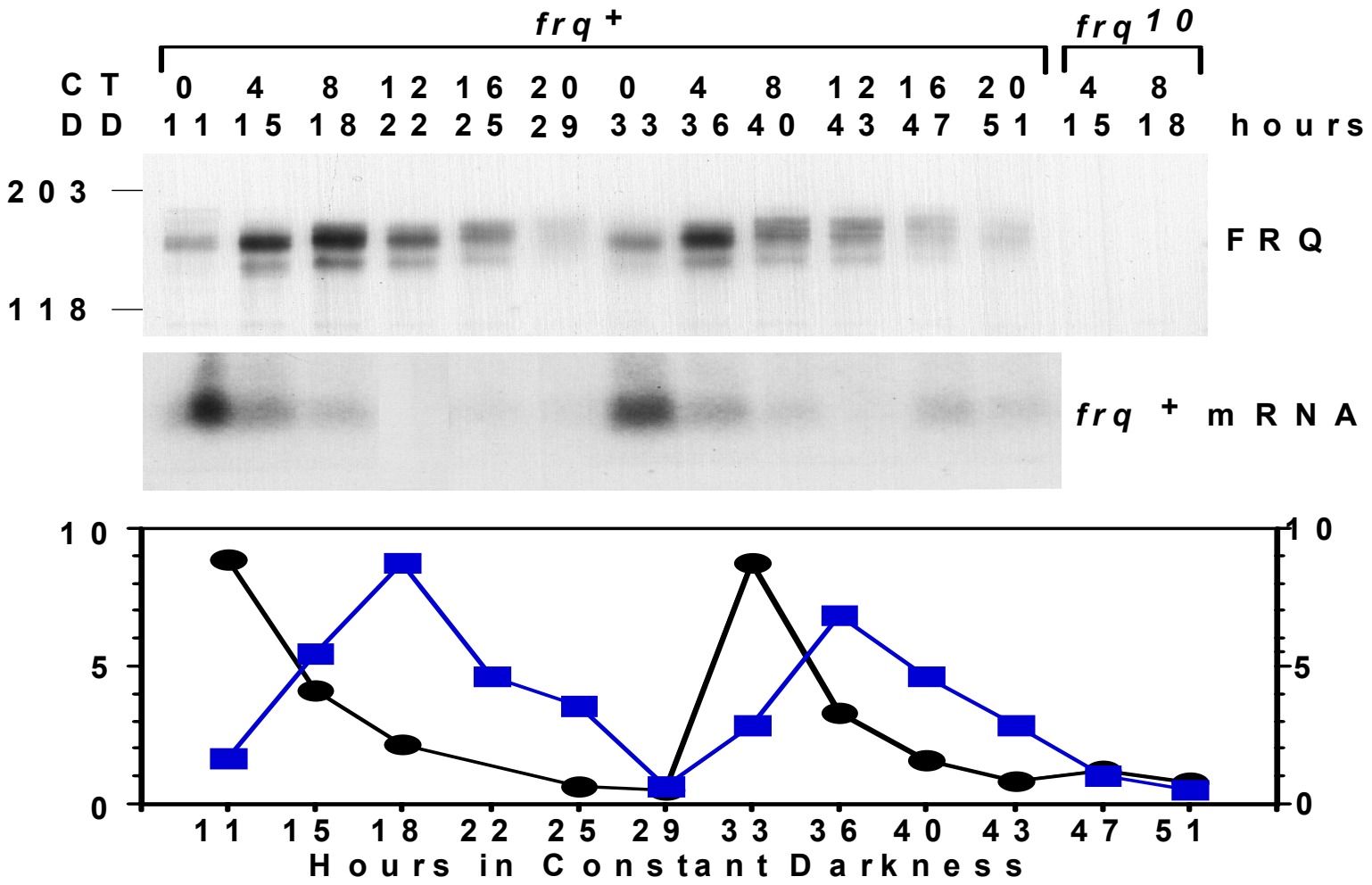
Definitions of a central component of a circadian clock

- Mutations result in long, short period, or arrhythmic phenotypes
- Deletion of the locus abolishes the rhythms
- Both mRNA and protein cycle
- Constitutive expression eliminates the clock
- Feeds back negatively on its own expression
- Change in levels resets the clock

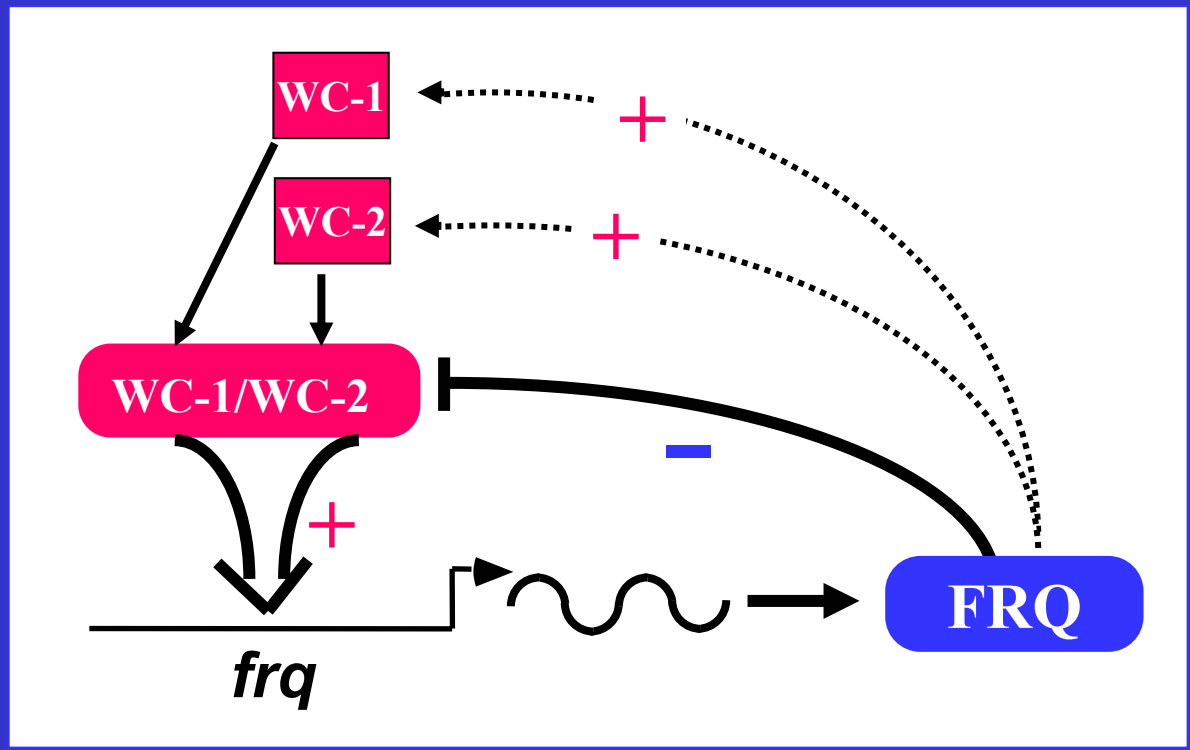
Transcripts and Domains in *frq* and FRQ



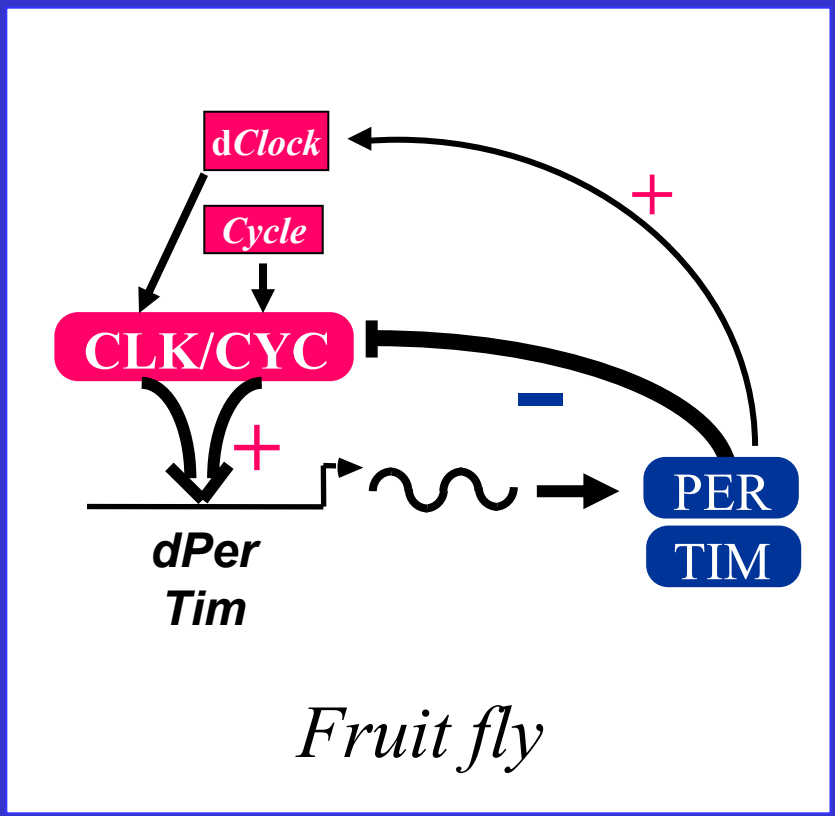
Both FRQ protein and *frq* RNA cycle

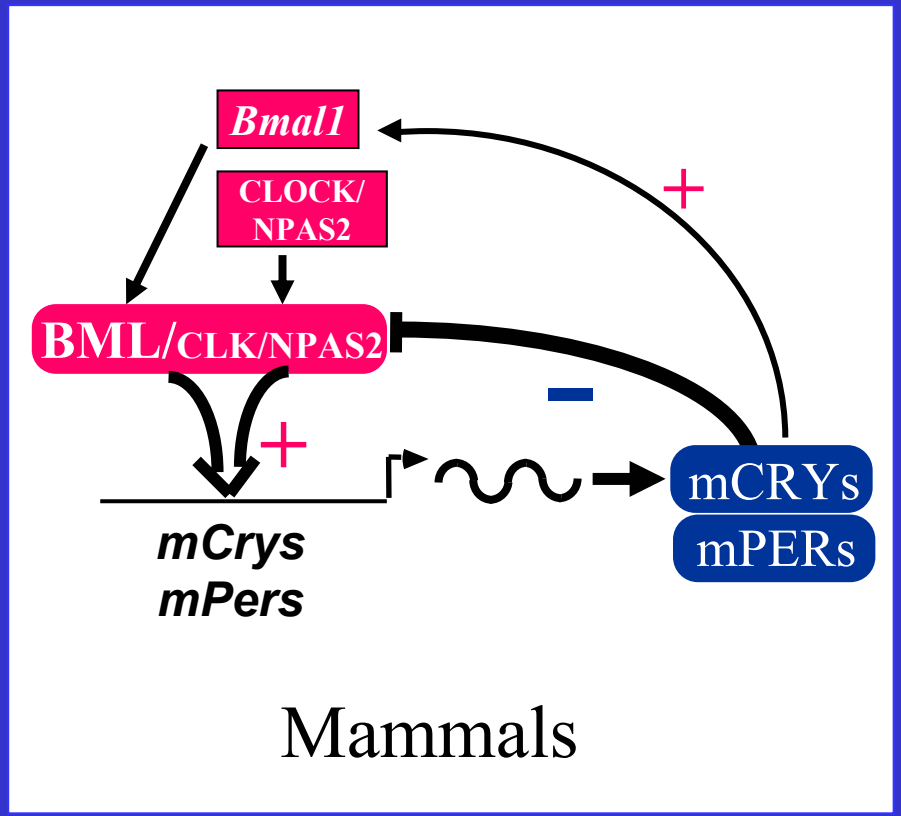


The *Neurospora* (fungal) circadian feedback loops

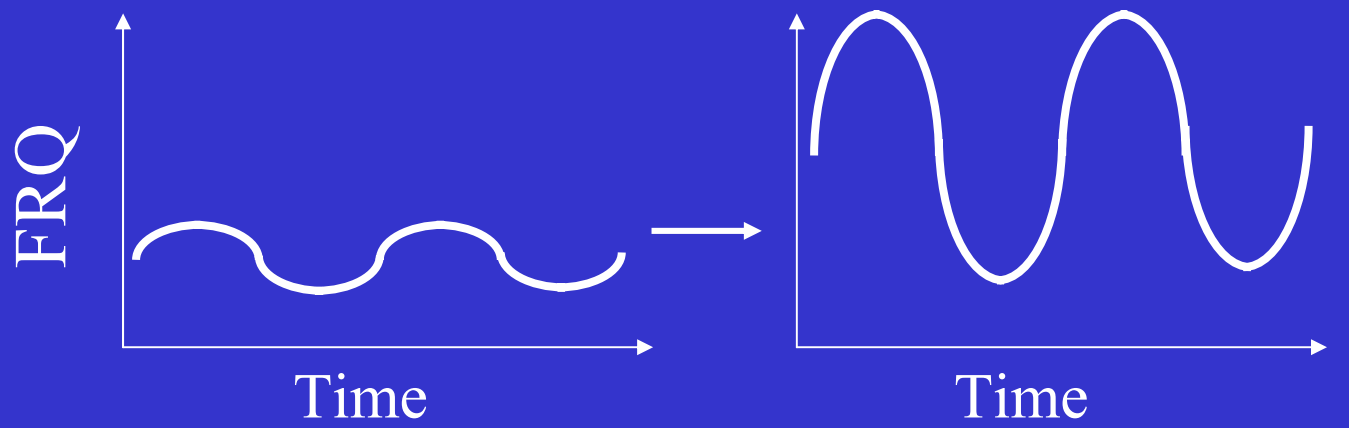


Circadian interlocked feedback loops in fruit fly and mouse





The positive feedback loops are important for the robustness of the clock



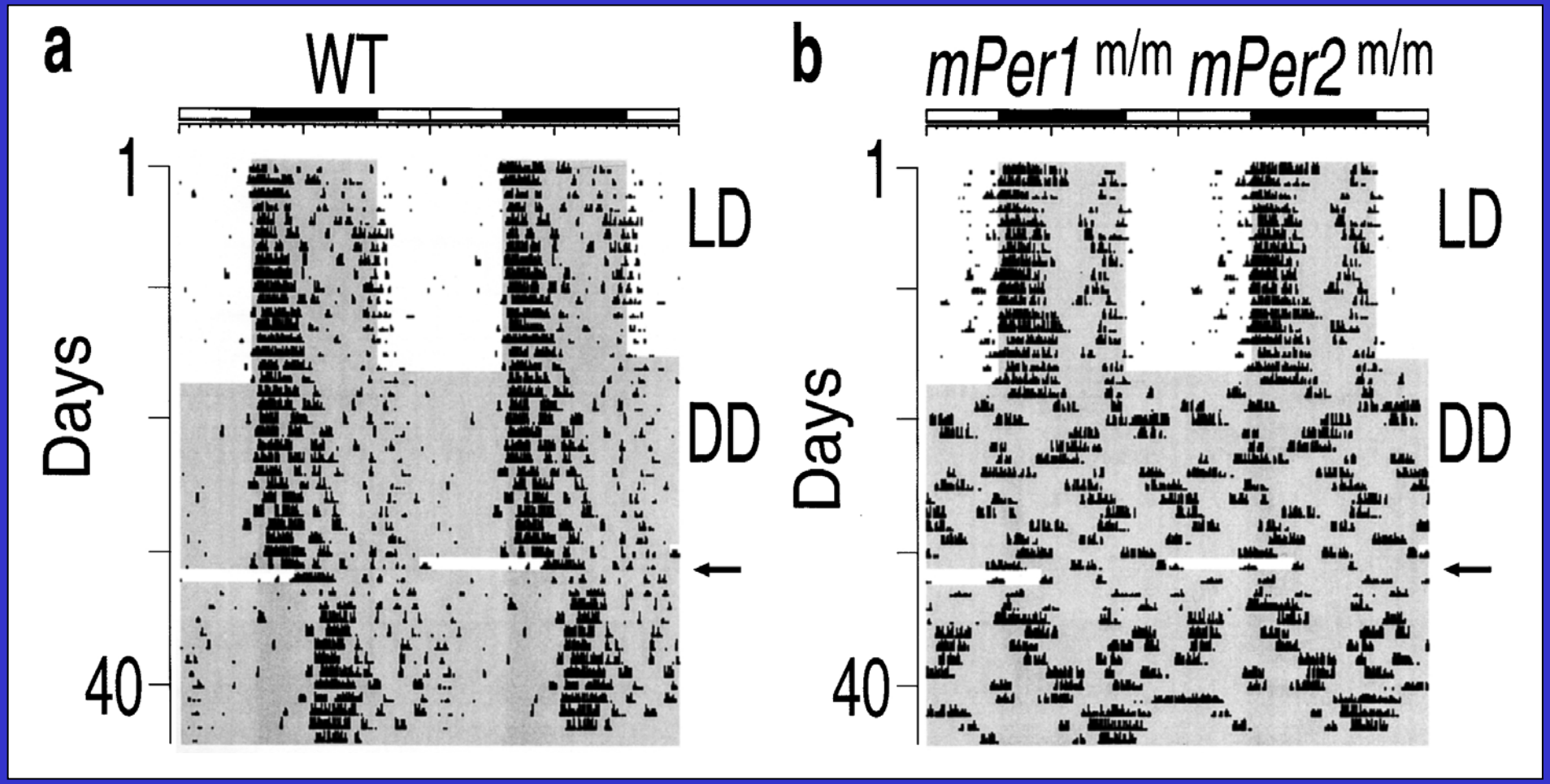
low levels of WCs

high levels of WCs

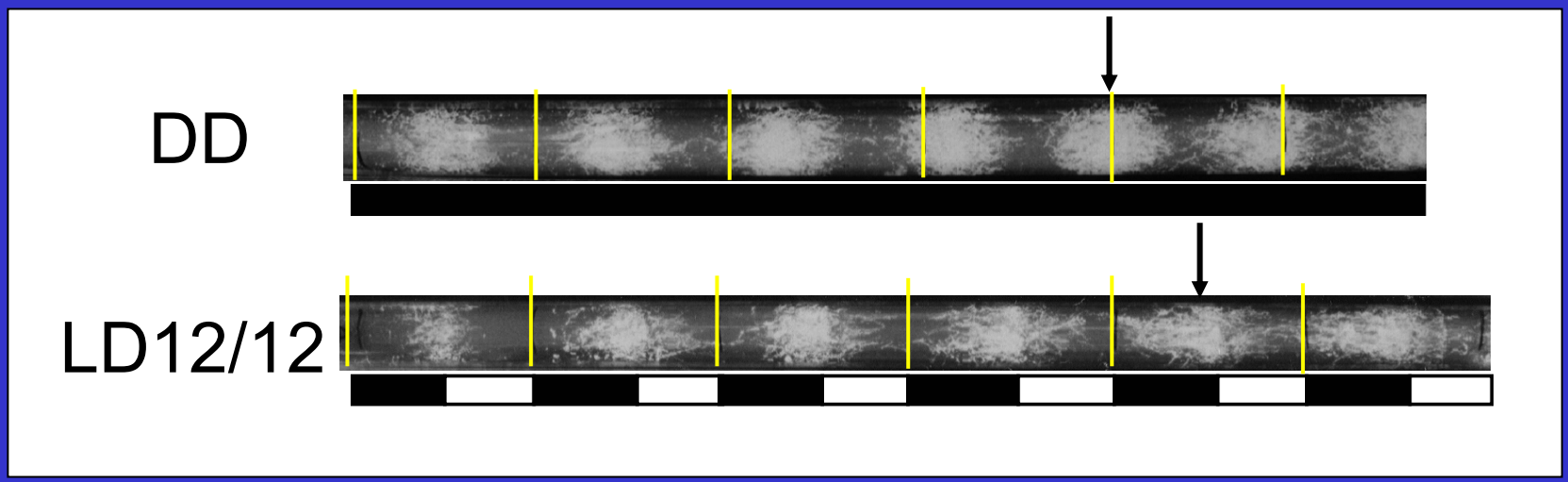
How do environmental signals reset clocks?

Two most important signals: Light and Temperature

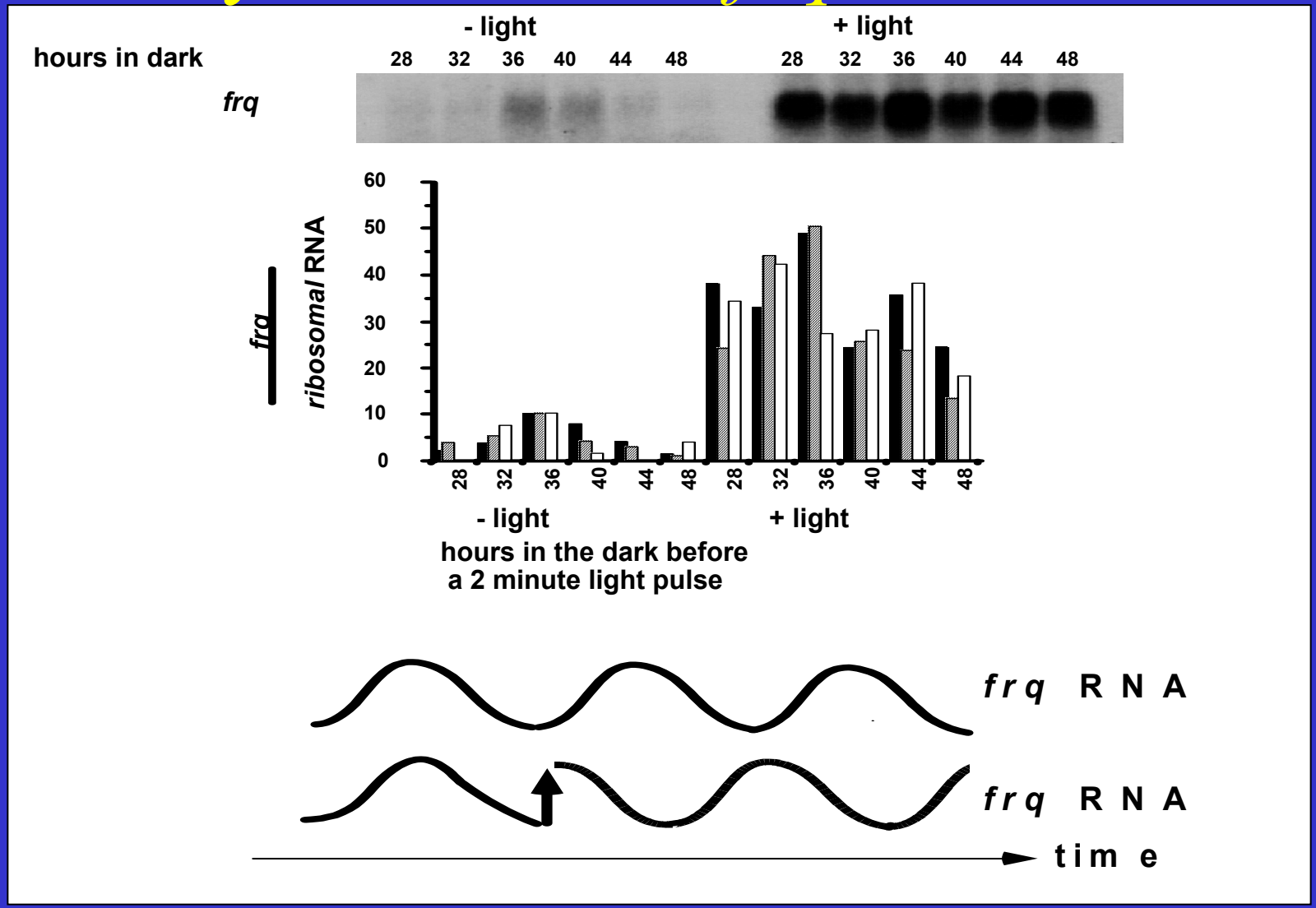
Circadian rhythm of locomoter activity



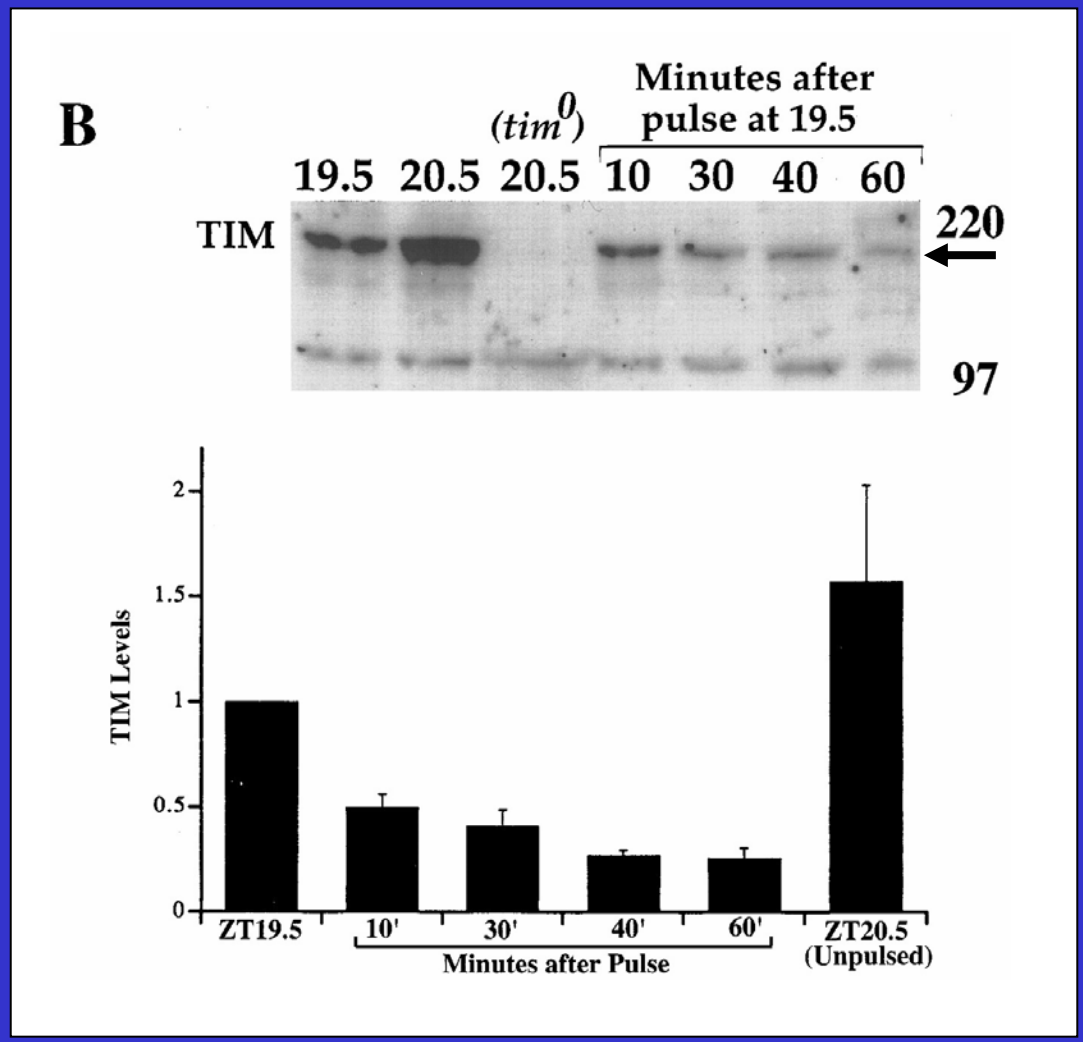
Light resetting of the *Neurospora* clock



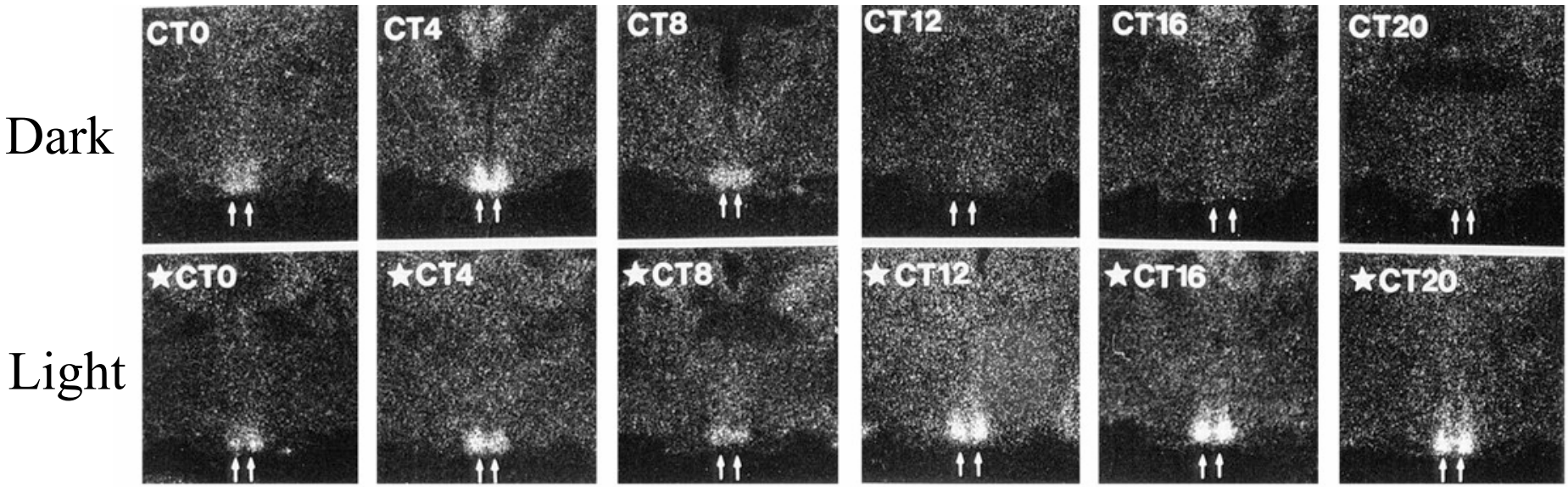
Light resets the *Neurospora* clock by induction of *frq* mRNA

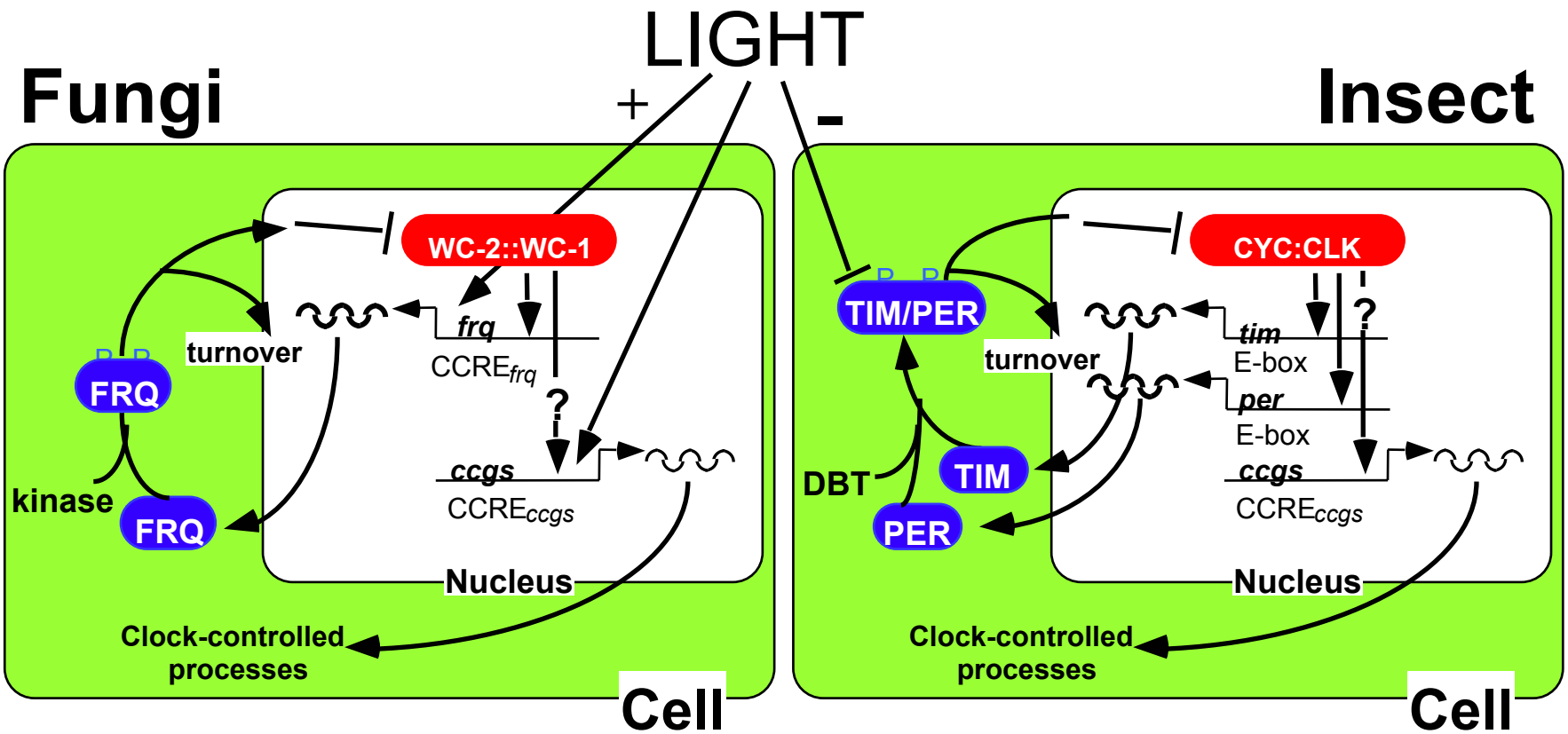


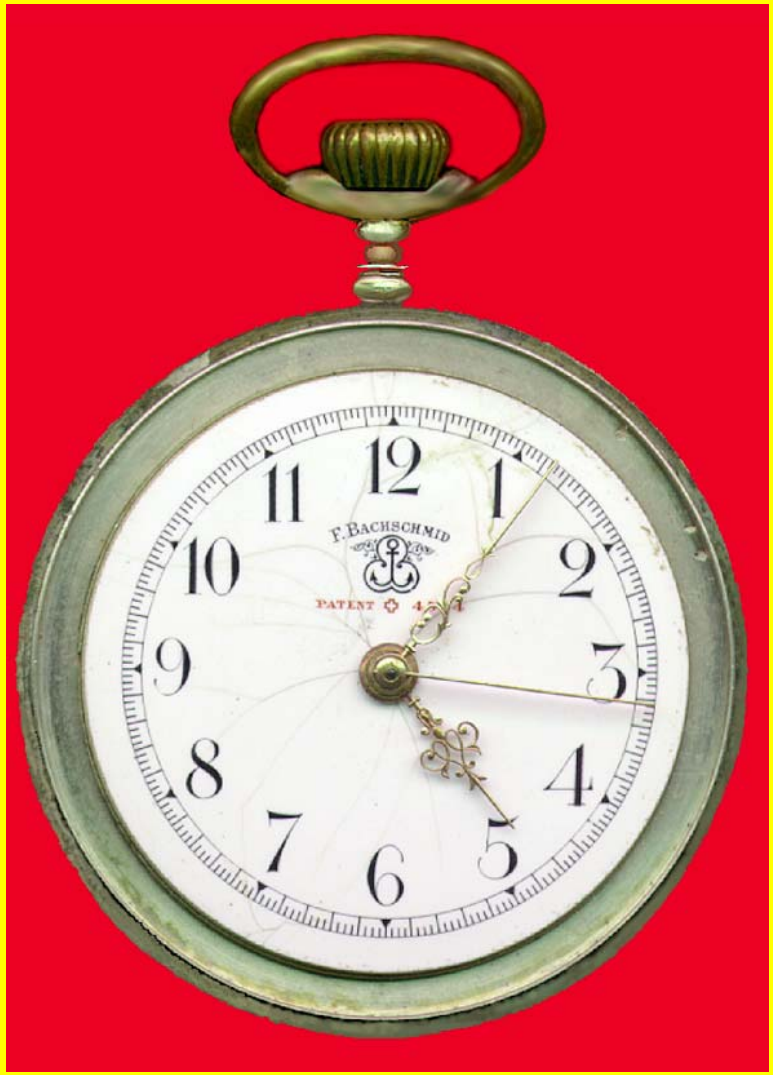
Light resets the *Drosophila* clock by degrading TIMELESS protein



Light resets the mammalian clock by inducing Per RNA expression




LIGHT
+
-
Fungi
Insect
WC-2::WC-1
frq
CCREfrq
?
ccgs
CCREccgs
turnover
FRQ
kinase
FRQ
Nucleus
Clock-controlled processes
Cell
CYC:CLK
TIM/PER
tim
E-box
per
E-box
ccgs
CCREccgs
turnover
DBT
TIM
PER
Nucleus
Clock-controlled processes
Cell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