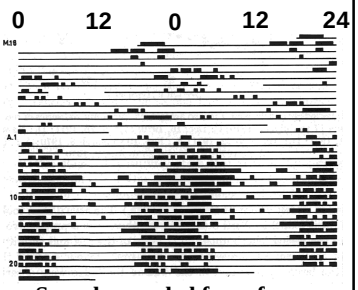
How Does a Circadian Clock Work?

Circadian Rhythm

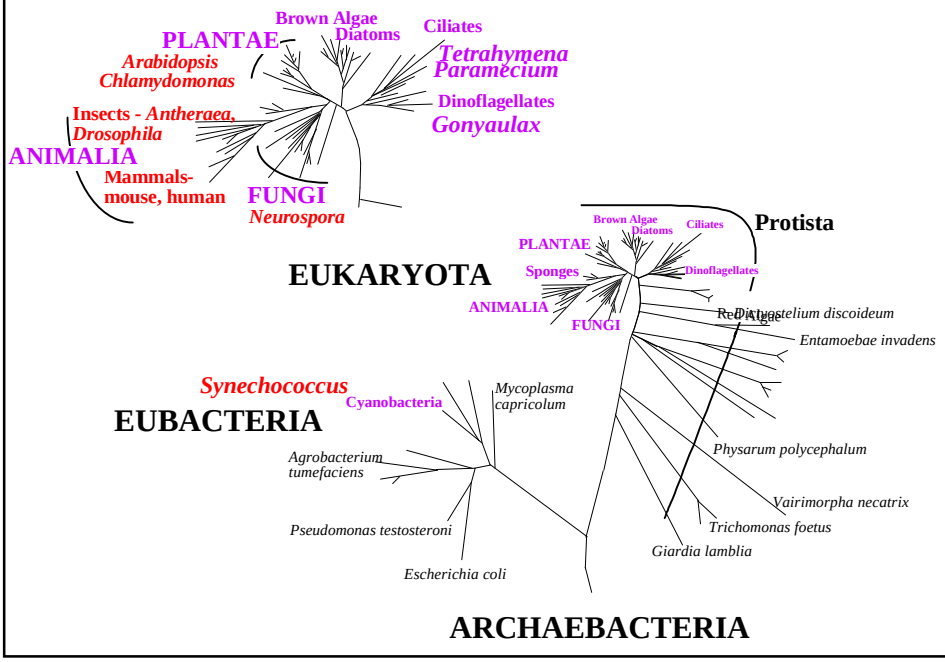
- freerunning, sustainable oscillation with an approximately 24 hour period
- universally associated with light and temperature perception
- entrainment to daily environmental changes
- clock operates within defined physiological limits



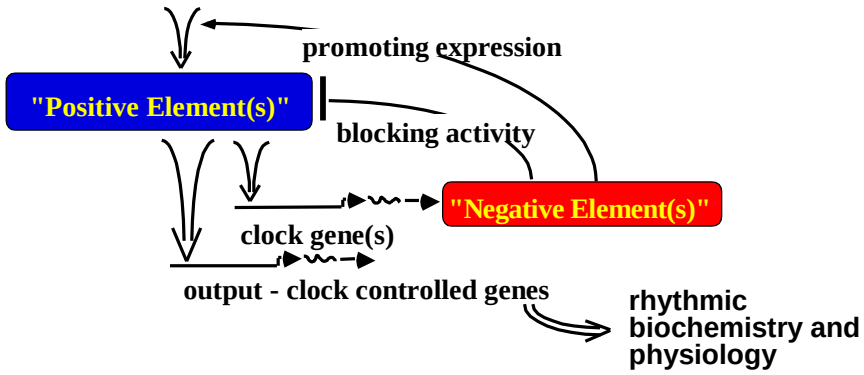
Sound recorded from four
Castor canadensis
March 18 - April 22, 1971
115°03'W, 51°02'N

- period length of rhythm is compensated against changes in temperature or metabolic state

Circadian Systems in the Universal Tree of Life



Common Themes and Elements in Eukaryotic Circadian Feedback Loops

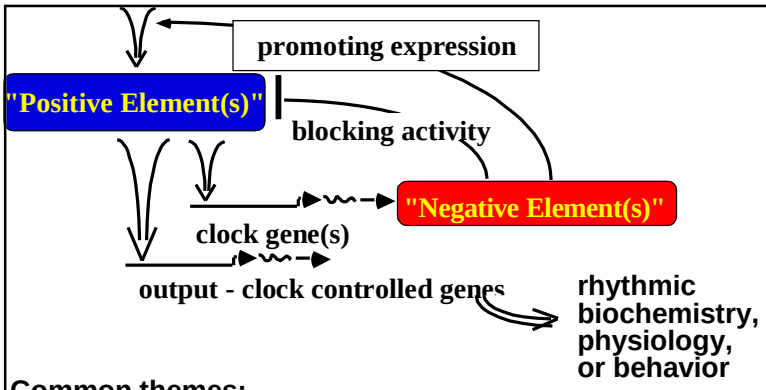


Positive elements in circadian loops:

- WHITE COLLAR-1 & WC-2 in Neurospora
- CLK & CYC in Drosophila
- CLOCK & BMAL1 in mammals

Negative elements in circadian loops:

- FREQUENCY in Neurospora
- PERIOD and TIMELESS in Drosophila
- PER1, 2, and 3 , CRY1 and CRY2 in mammals



Common themes:

Transcription/translation based feedback loops, with transcription factors as positive elements and proteins that block the activity of these factors as negative elements, are central.
Rhythmic expression is seen in transcripts and proteins corresponding to the "negative elements."
"Positive elements" are heterodimers comprised of two proteins interacting via PAS domains. Of proteins comprising the heterodimer, one is constitutive and one is rhythmically expressed.
Extended sequence similarity links some of the positive elements among all of the systems.
At least one of the "negative elements" has a dual role in the cycle, performing both the negative function in feedback and also a positive function in promoting the expression of one of the heterodimer partne

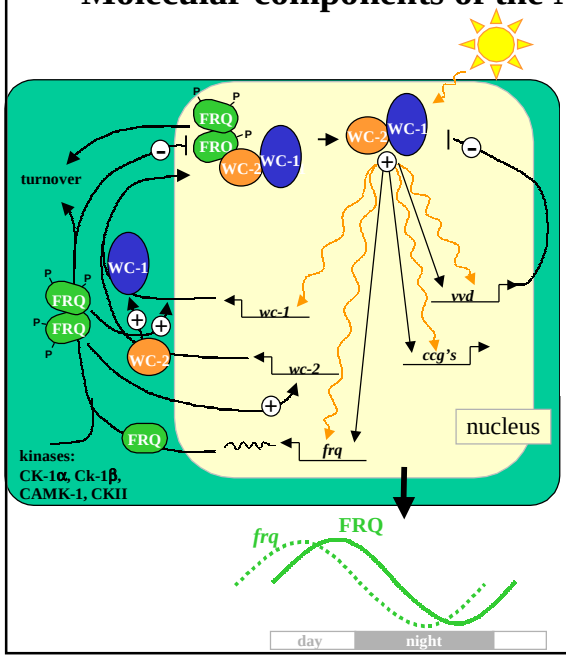
Positive elements in eukaryotic circadian loops:

- WHITE COLLAR-1 & WC-2 in Neurospora
- CLK & CYC in Drosophila
- CLOCK & BMAL1 in mammals

Negative elements in eukaryotic circadian loops:

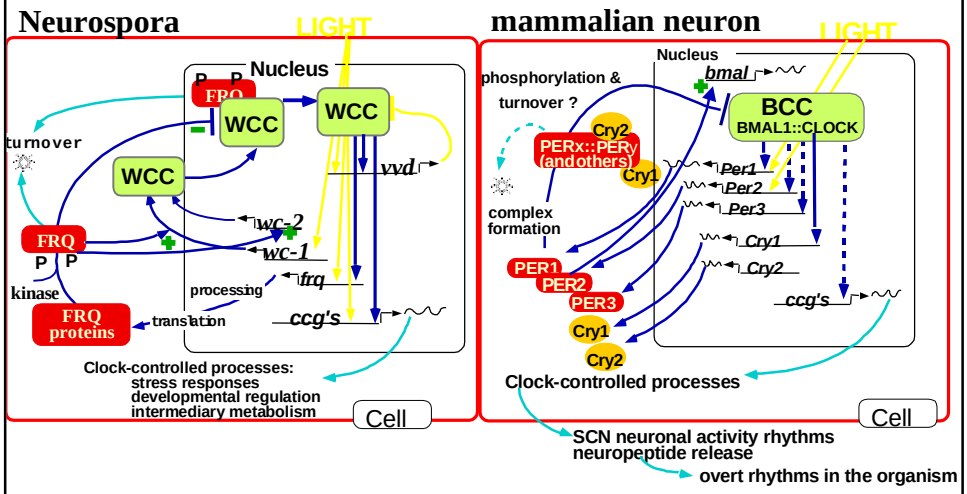
- FREQUENCY in Neurospora
- PERIOD and TIMELESS in Drosophila
- PER1, PER2, PER3 , CRY2, and CRY2in mammals

Molecular components of the Neurospora clock

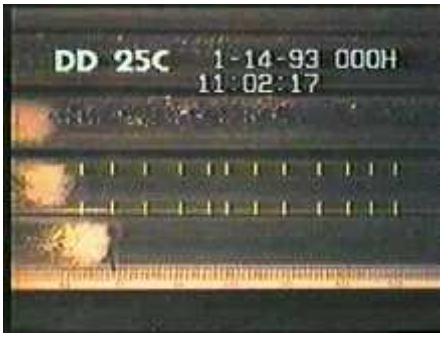


- WC-1 and WC-2 act positively on the production of *frq* transcript.
- FRQ acts negatively on the production of its own transcript. (negative feedback loop)
- FRQ acts positively on the production of *wc-2* transcript and WC-1. (positive feedback loops)
- FRQ becomes phosphorylated which leads to its turnover
- The *frq*/FRQ oscillations are essential for the generation of all circadian rhythms in Neurospora.
- Changes in *frq*/FRQ levels are directly translated into changes in the circadian rhythms in Neurospora.

two eukaryotic circadian oscillators



A Circadian Rhythm in Development on Racetubes



Van D. Gooch
University of Minnesota

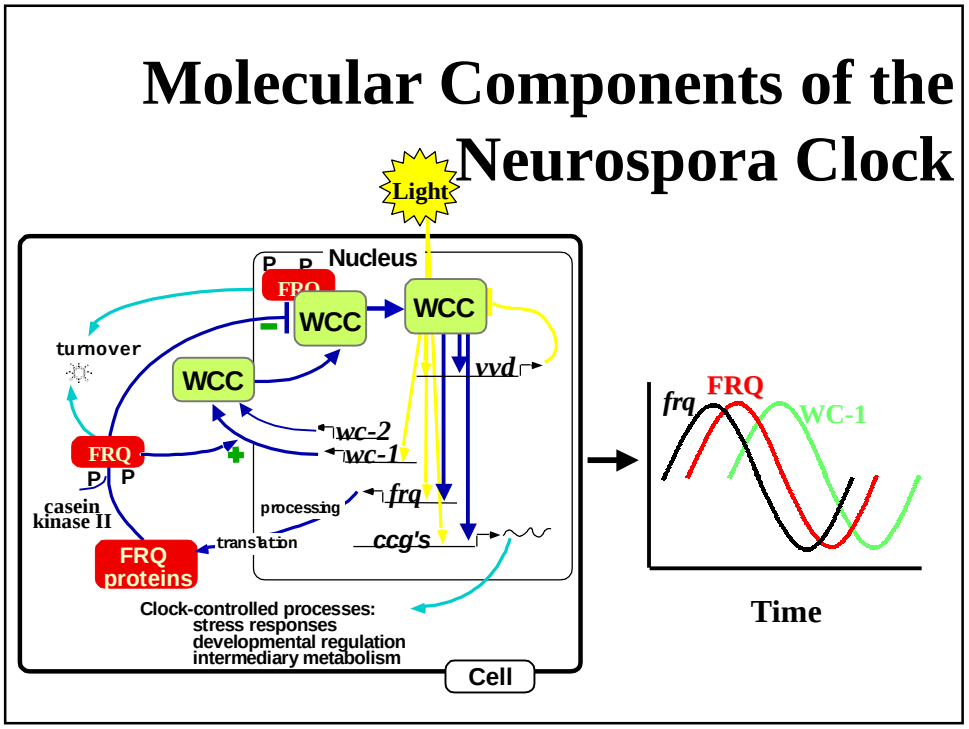
Circadian rhythm

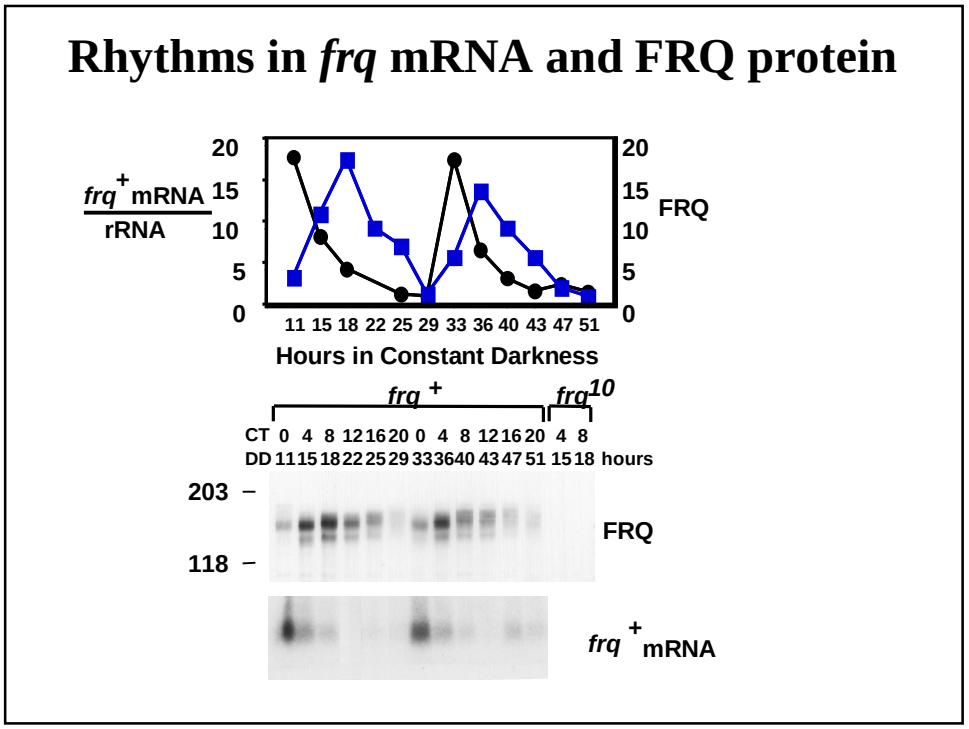
- **Biological rhythm with a period of ~24 hours**
- **Endogenous and self-sustaining under constant environmental conditions**
- **Temperature compensated within the physiological range of the organism**
- **Environmental signals (light and temperature changes) can entrain or reset the rhythm**
- **Organized at the cellular level**

ENTRAINMENT

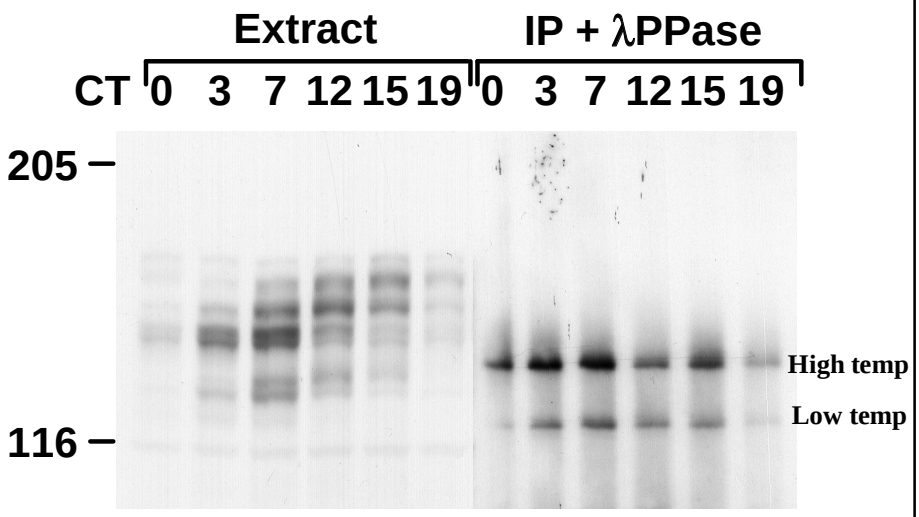
Light or temperature treatment results in changes in the timing of conidial banding.

Van D. Gooch
University of Minnesota

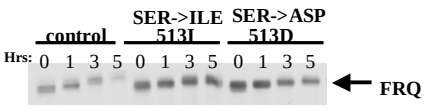

Molecular Components of the Neurospora Clock
Light
Nucleus
P P
FRQ
WCC
WCC
WCC
vvd
turnover
wc-2
wc-1
FRQ
P P
casein kinase II
processing
frq
translation
ccg's
FRQ proteins
Clock-controlled processes:
stress responses
developmental regulation
intermediary metabolism
Cell
frq
FRQ
WC-1
Time

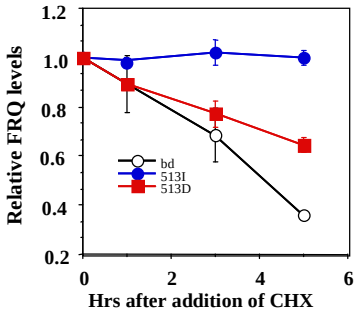

Rhythms in frq mRNA and FRQ protein
frq+ mRNA / rRNA
20
15
10
5
0
FRQ
11 15 18 22 25 29 33 36 40 43 47 51
Hours in Constant Darkness
frq +
frq10
CT 0 4 8 12 16 20 0 4 8 12 16 20 4 8
DD 11 15 18 22 25 29 33 36 40 43 47 51 15 18 hours
203
118
FRQ
frq + mRNA

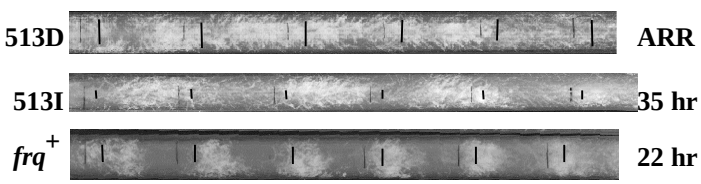
FRQ is Phosphorylated in a Time-of-Day-Specific Manner



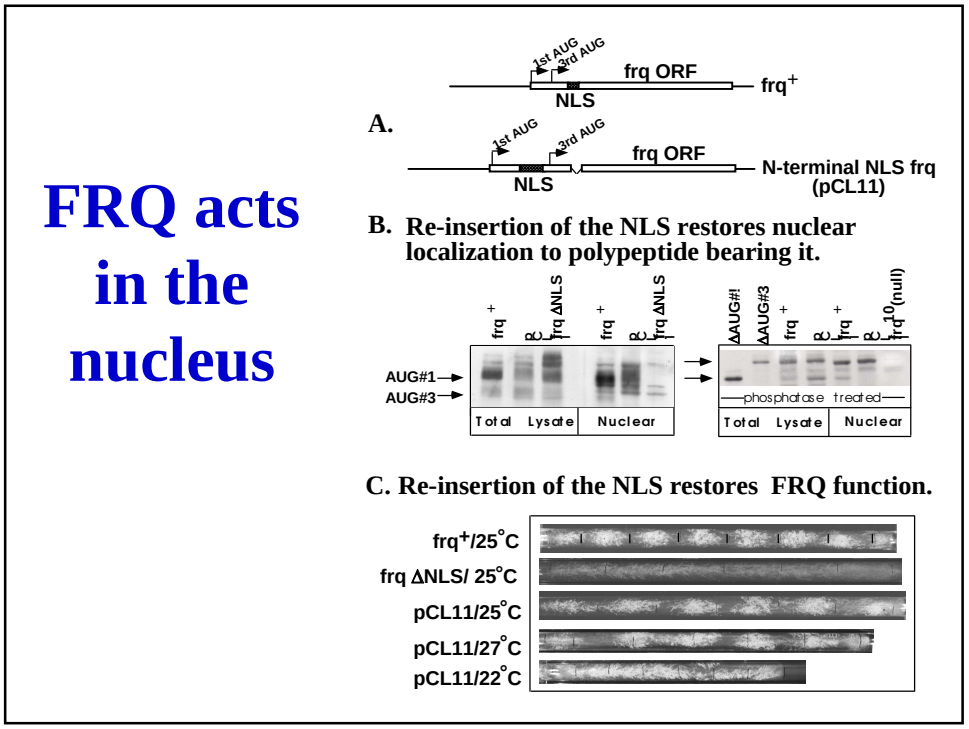
Phosphorylation site at aa 513 is important for FRQ degradation

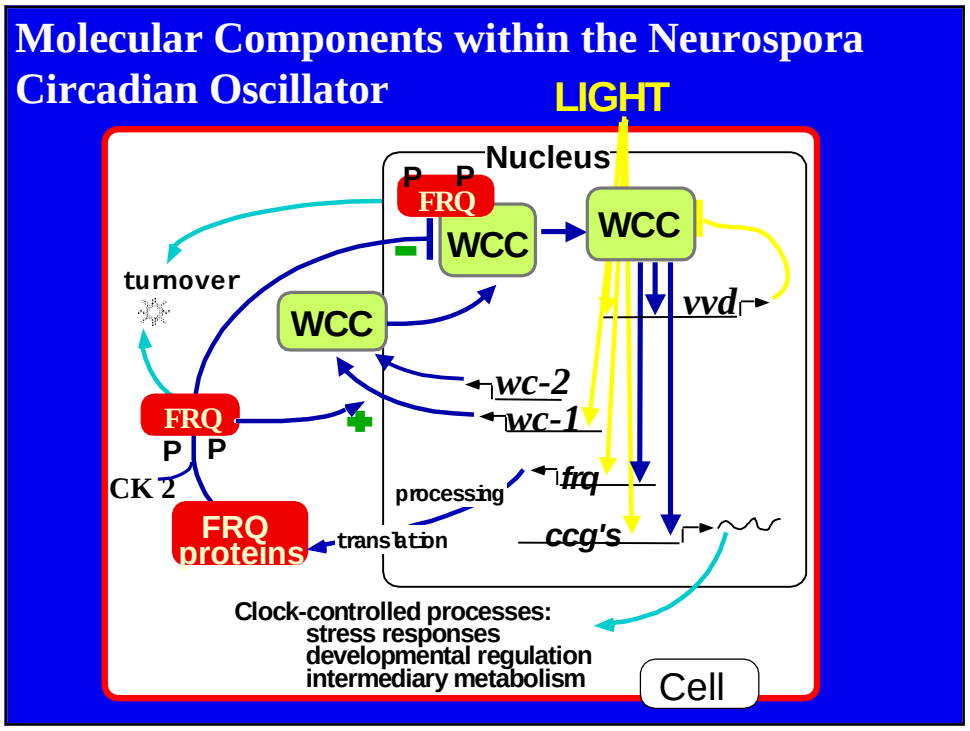






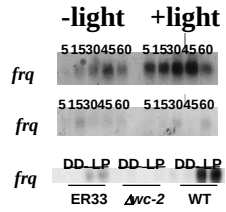
YL 5/99


FRQ acts in the nucleus
A.
1st AUG
3rd AUG
frq ORF
frq+
NLS
1st AUG
3rd AUG
frq ORF
N-terminal NLS frq (pCL11)
NLS
B. Re-insertion of the NLS restores nuclear localization to polypeptide bearing it.
+ frq
frq ΔNLS
+ frq
frq ΔNLS
ΔAUG#1
ΔAUG#3
+ frq
+ frq
frq10 (null)
AUG#1
AUG#3
phosphatase treated
Total Lysate
Nuclear
Total Lysate
Nuclear
C. Re-insertion of the NLS restores FRQ function.
frq+/25°C
frq ΔNLS/ 25°C
pCL11/25°C
pCL11/27°C
pCL11/22°C

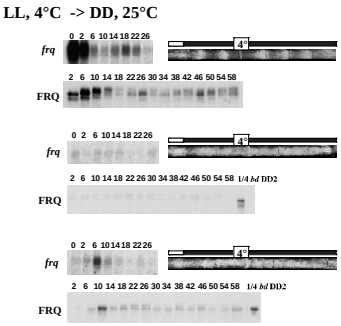

Molecular Components within the Neurospora Circadian Oscillator
LIGHT
Nucleus
P P
FRQ
WCC
WCC
turnover
WCC
vvd
wc-2
wc-1
FRQ
P P
CK 2
processing
frq
FRQ proteins
translation
ccg's
Clock-controlled processes:
stress responses
developmental regulation
intermediary metabolism
Cell

WC-1 and WC-2 are part of the Neurospora clock

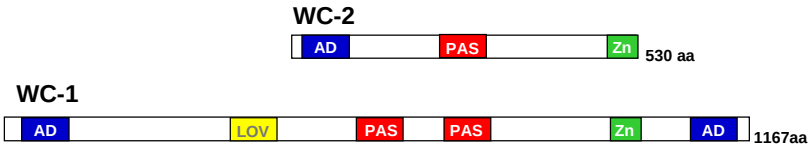
2 ' light pulse



dark

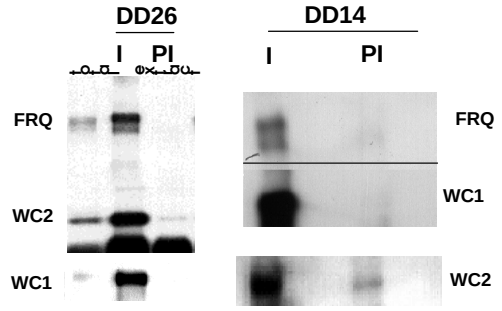


White Collar-1 (WC-1) and White Collar-2 (WC-2)

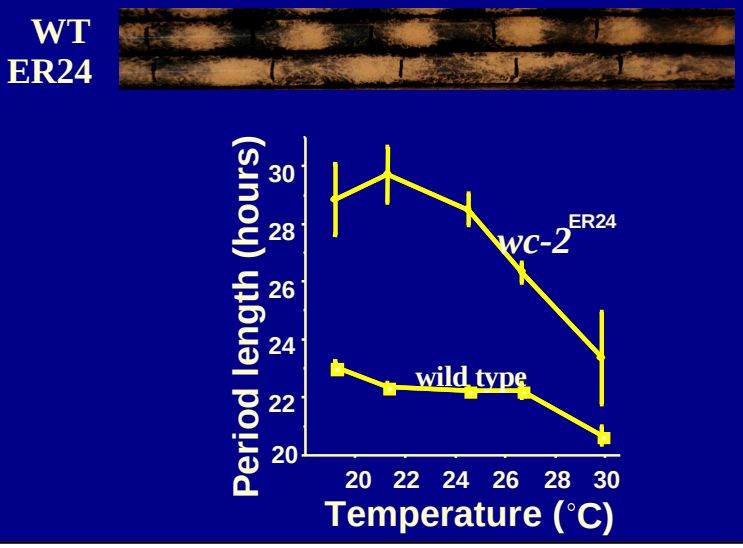


- WC-1 and WC-2 are required for the light induction of *frq* transcript.
- WC-1 and WC-2 heterodimerize via their PAS domains.
- WC-1 and WC-2 contain zinc-finger (Zn) DNA binding domains and activation domains (AD).

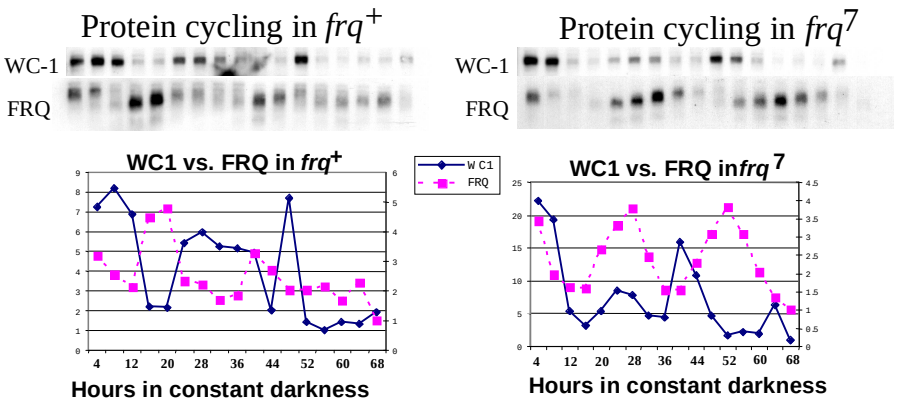
Anti-WC2 co-immunoprecipitates FRQ and WC1



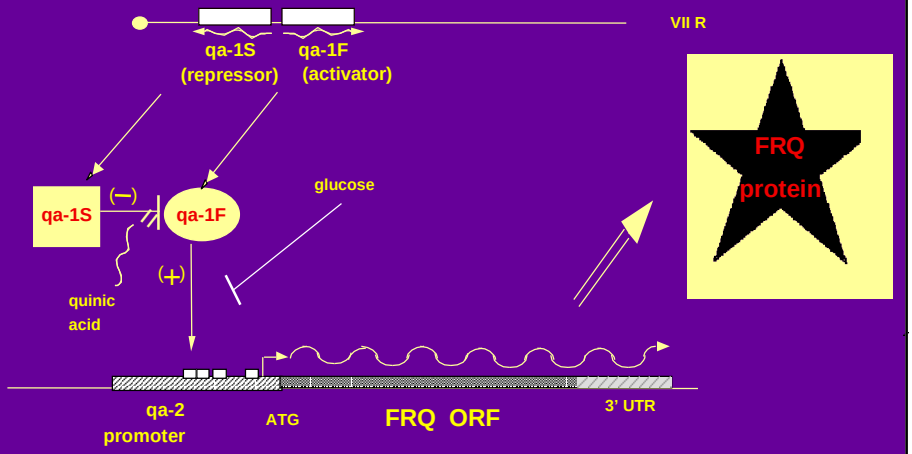
A Partially Functional Allele of *wc-2* Displays Altered Period Length and Temperature Compensation

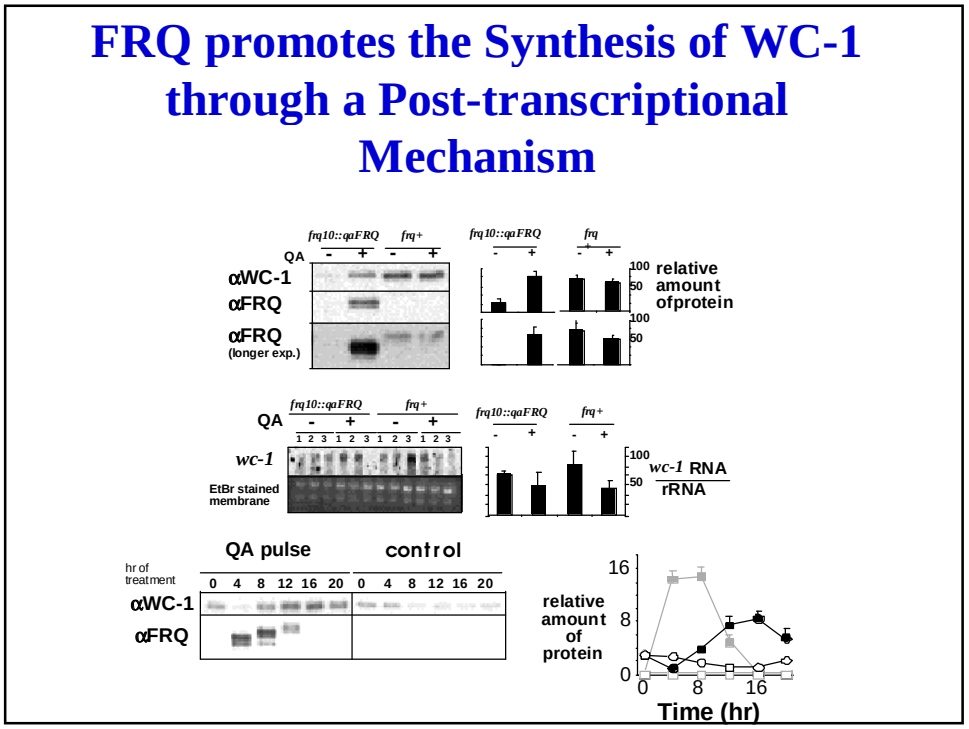


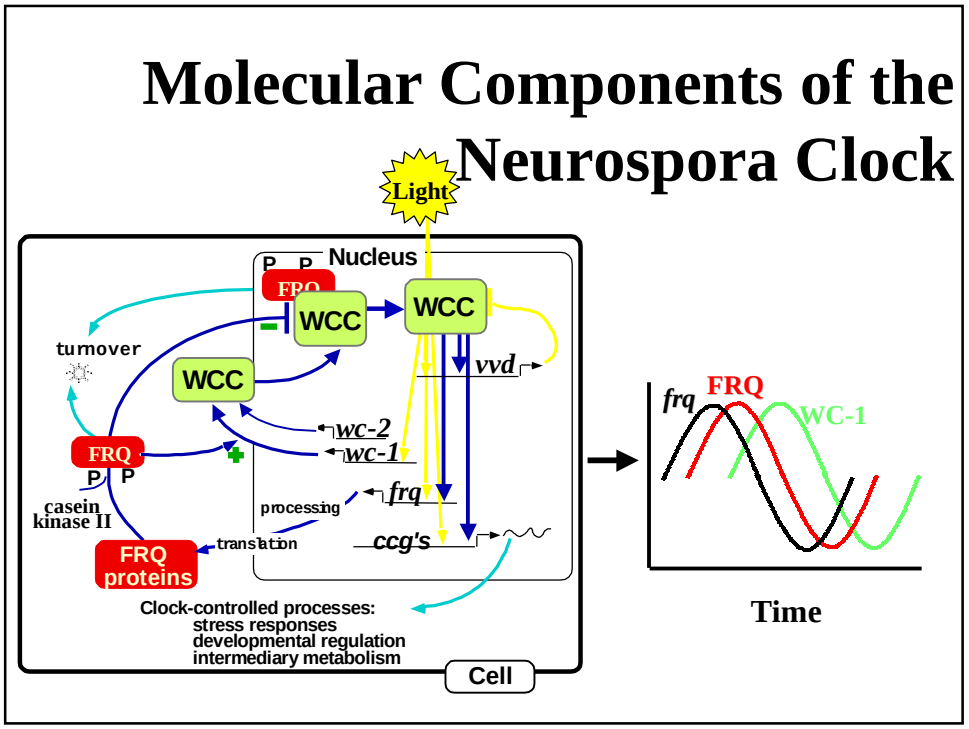
Levels of WC-1 and FRQ cycle in Antiphase to One Another



Inducible FRQ Protein Production




FRQ promotes the Synthesis of WC-1 through a Post-transcriptional Mechanism
frq10::qaFRQ
frq+
QA
αWC-1
αFRQ
αFRQ (longer exp.)
relative amount ofprotein
wc-1
EtBr stained membrane
wc-1 RNA / rRNA
QA pulse
control
hr of treatment
0 4 8 12 16 20
relative amount of protein
Time (hr)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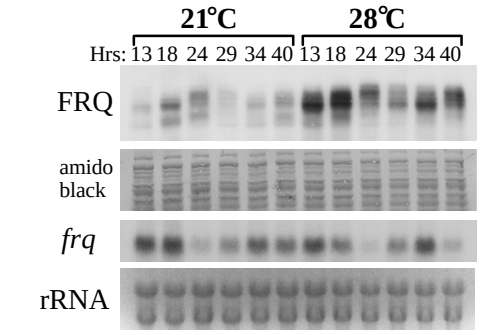
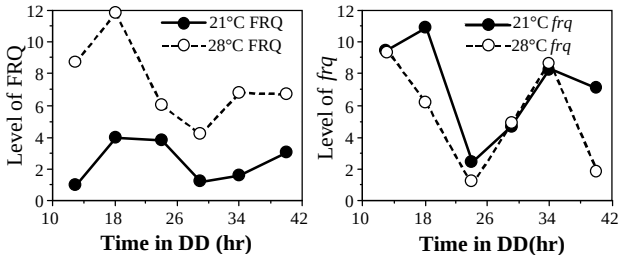
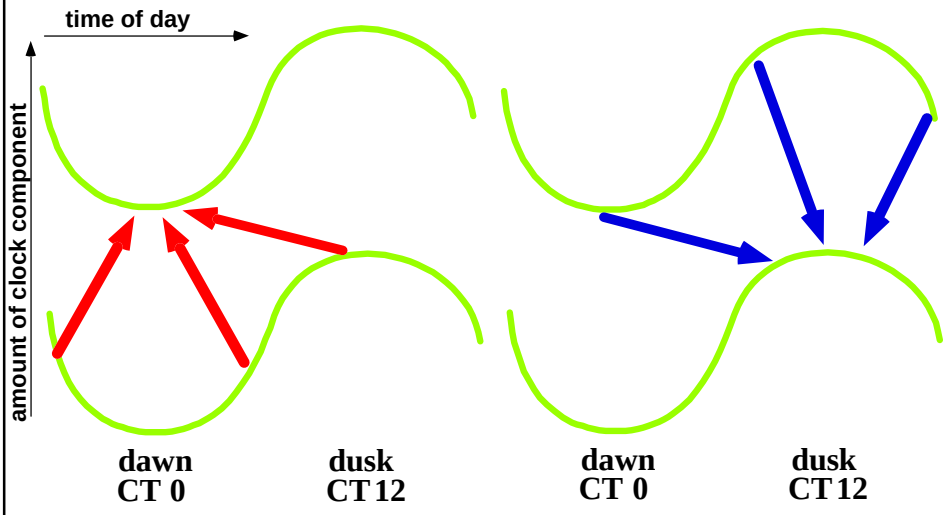
FRQ
WCC
WCC
turnover
WCC
vvd
wc-2
wc-1
FRQ
P P
casein kinase II
processing
frq
translation
ccg's
FRQ proteins
Clock-controlled processes:
stress responses
developmental regulation
intermediary metabolism
Cell
frq
FRQ
WC-1
Time

FRQ Oscillates around a Higher Level at Higher Temperatures





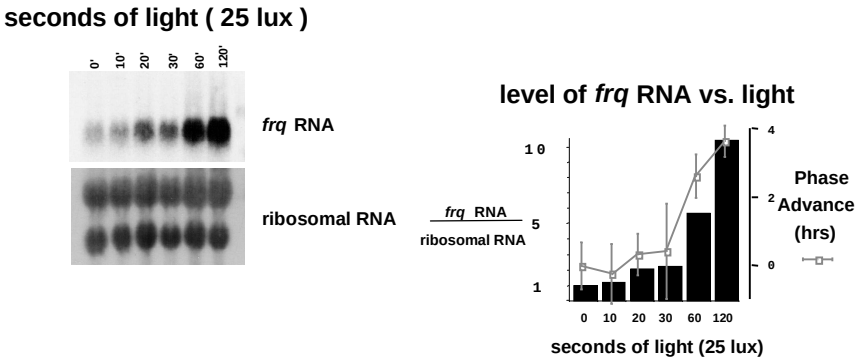
Clock Resetting by Temperature Steps



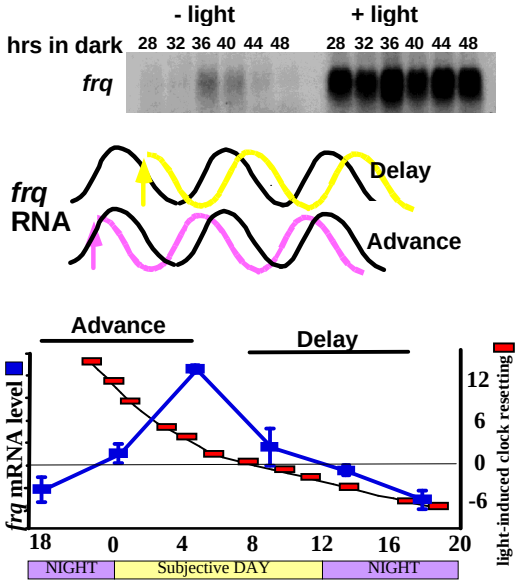
STEP UP: All points on the low temp curve are low compared to the high temp curve, so the clock is reset to the time corresponding to the low point in FRQ - dawn.

Step Down: All points on the high temp curve are high compared to the low temp curve, so the clock is reset to the time corresponding to the high point in FRQ - late day.

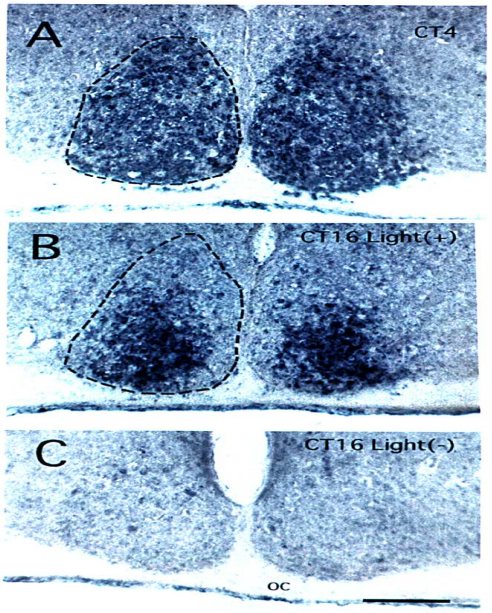
Light-Induced Clock Resetting Increases in Proportion to Light Induction of *frq*



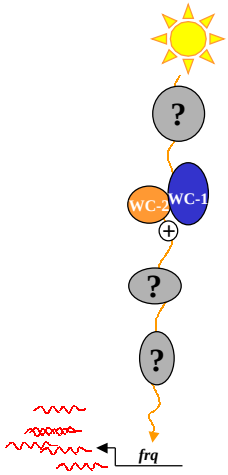
Resetting the Neurospora Clock with Light



mPer1 Expression in the SCN is Regulated by Time-of-Day, Light, and Location

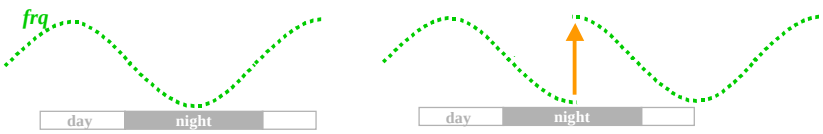


Shigeyoshi et al, Cell, 1997

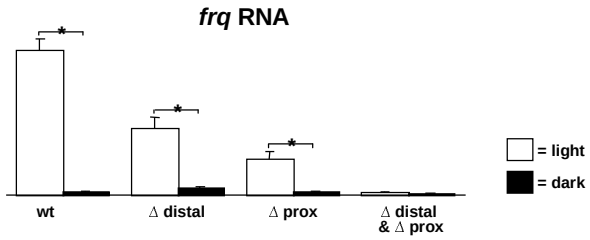


How are light signals perceived and transduced to the clock?

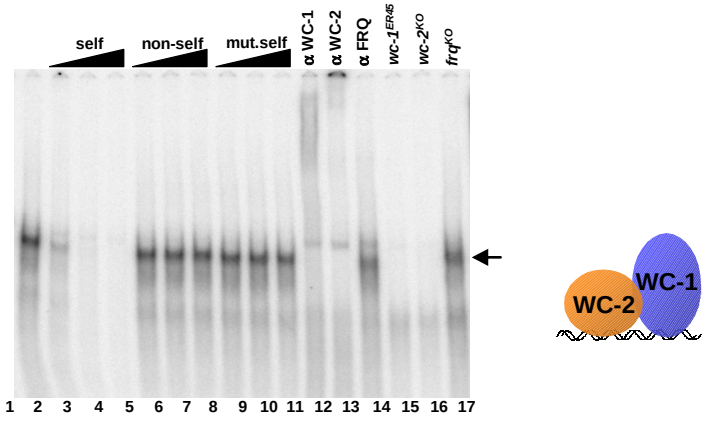
- ***frq*** **transcript is rapidly and highly induced in response to light.**
- **WC-1 and WC-2 are required for the light induction of *frq* transcript.**



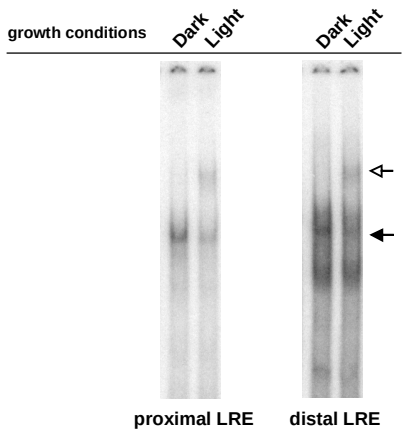
The *frq* promoter contains two Light Response Elements (LRE) necessary and sufficient for light induction



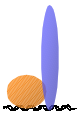
The LREs are bound by a WC-1/WC-2 containing complex



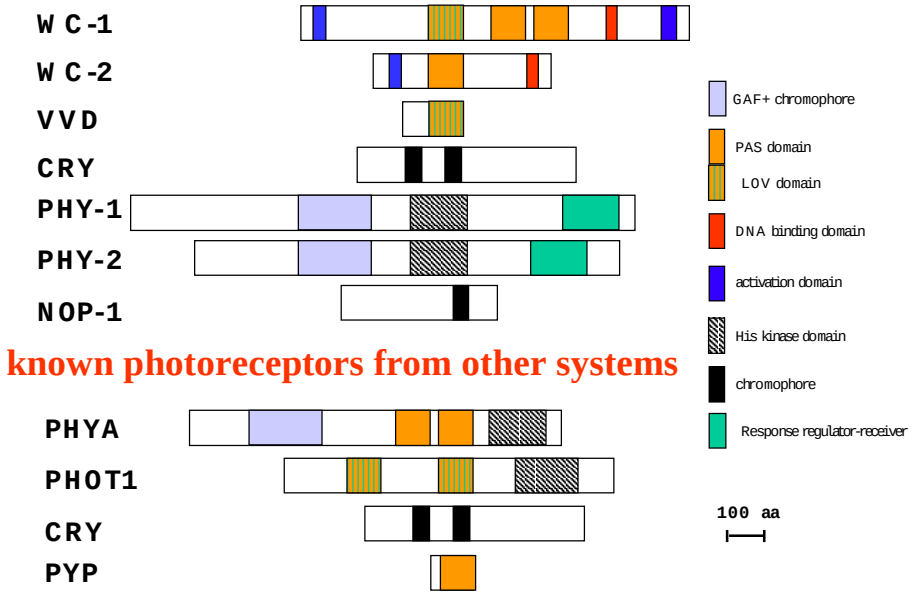
The distal and proximal LREs are bound by distinct complexes in the light and dark



What causes the change in mobility of the WCC/LRE complex in the light?

- phosphorylation of WC-1/WC-2 
- conformational change of WC-1/WC-2 
- binding of additional factor(s) 
- combination of the above
- other?

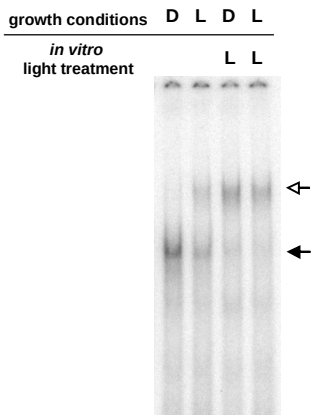
Real and Potential Neurospora Photoreceptors



known photoreceptors from other systems

Nuclear Protein Extracts are Photoreactive

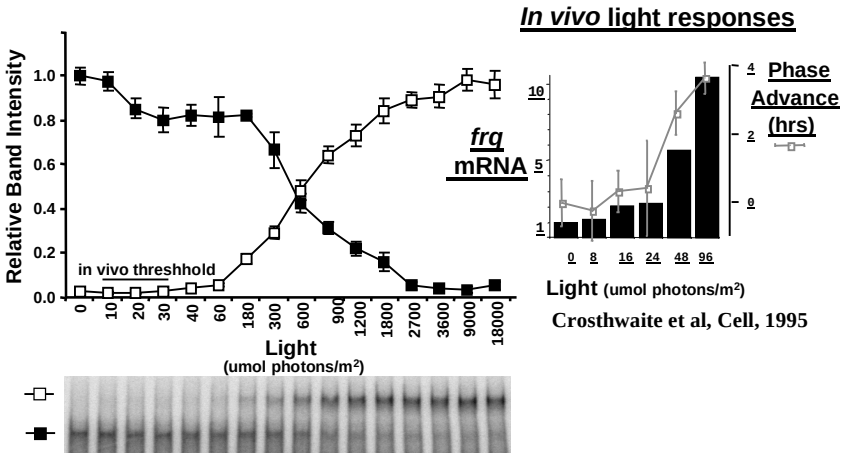
Protein extractions, binding reactions and gel running performed in the dark (red lighting).



Is the *in vitro* light-induced change in mobility of the WCC/LRE complex occurring at biologically relevant…

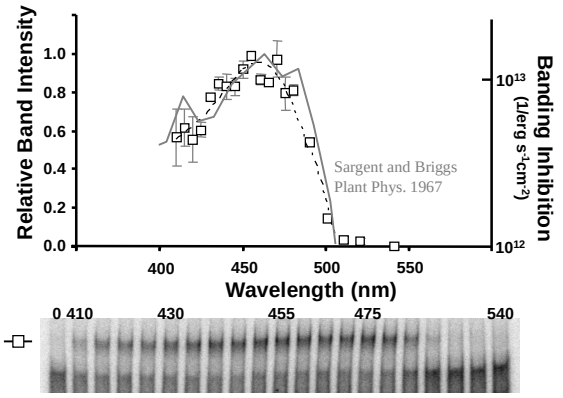
- **amounts of light (illuminance)?**
- **qualities of light (wavelength)?**

The *in vitro* light-induced WCC/LRE mobility change occurs at biologically relevant light intensities.

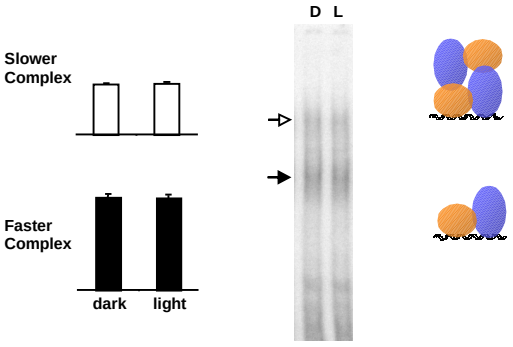


Crosthwaite et al, Cell, 1995

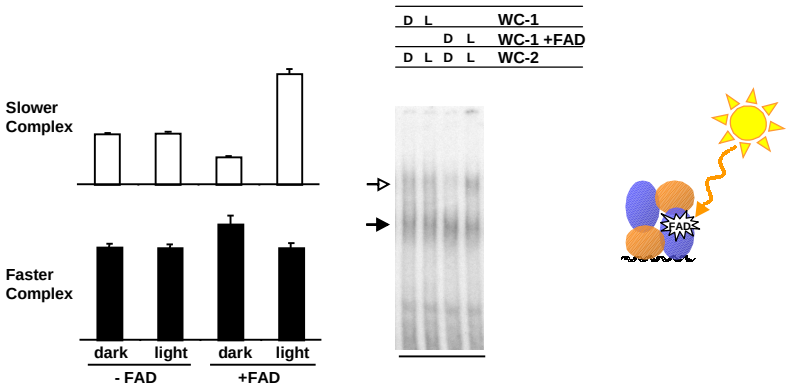
The *in vitro* light-induced WCC/LRE mobility change occurs at biologically relevant wavelengths.



WC-1 and WC-2 generated *in vitro* can bind to the LREs as both the faster and slower migrating complexes



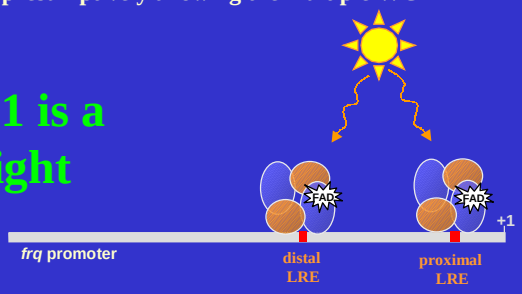
FAD imparts light regulation to the WC-1/WC-2 complex



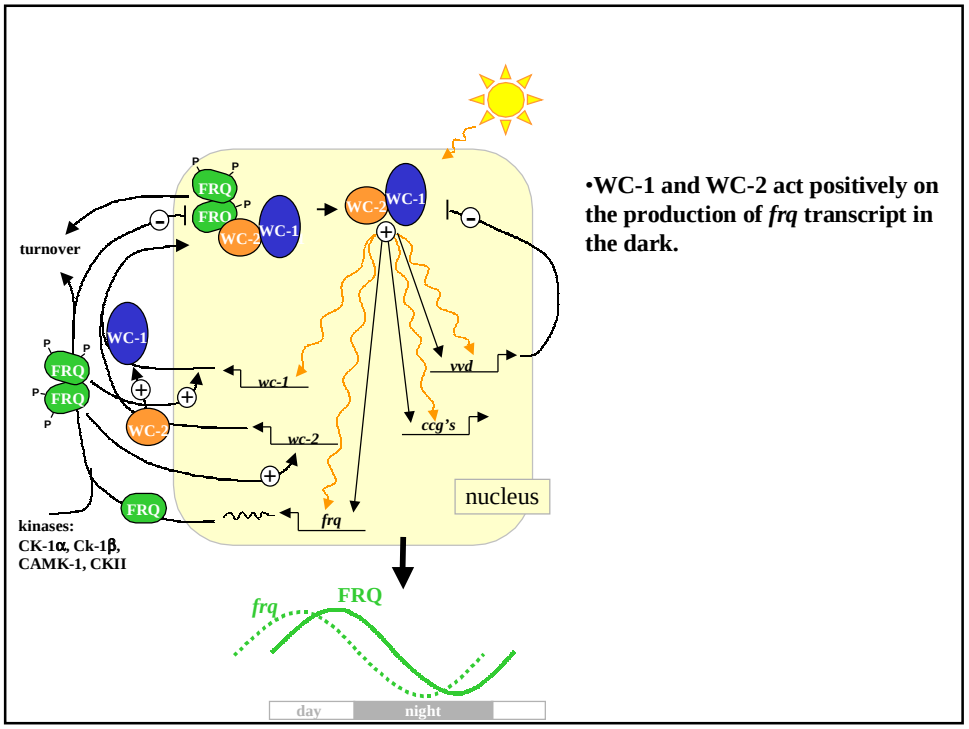
How are light signals perceived and transduced to the clock?

- *frq* transcript is rapidly and highly induced in response to light.
- The *frq* promoter contains two *cis*-acting Light Response Element (LREs).
- The LREs are bound by a WC-1/WC-2 heterodimeric complex in the dark.
- Absorption of light by a FAD chromophore bound by WC-1 triggers the multimerization of the WCs presumptively allowing the multiple WC activation domains to act.

Therefore, WC-1 is a circadian blue light photoreceptor.

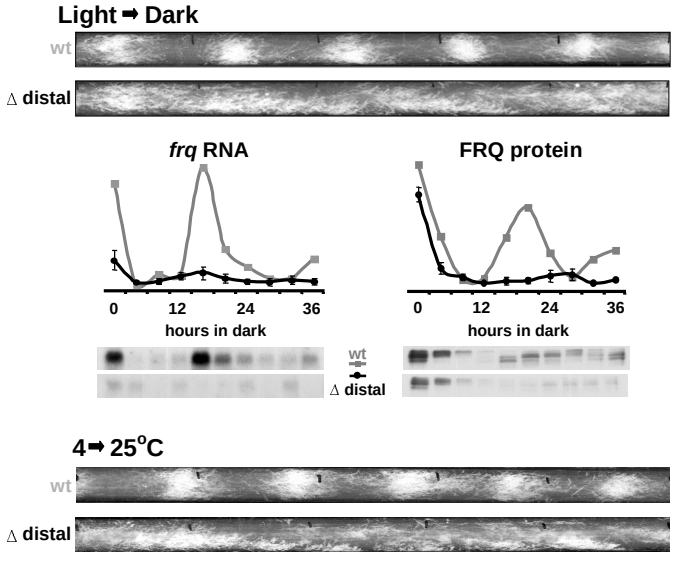


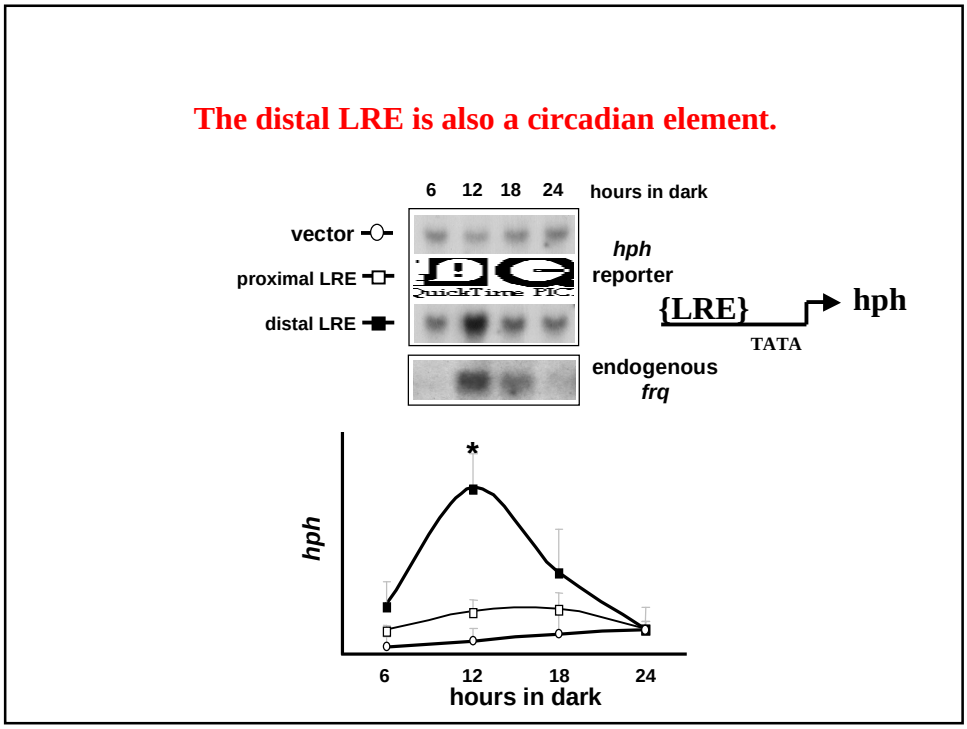
How Does a Circadian Clock Work?

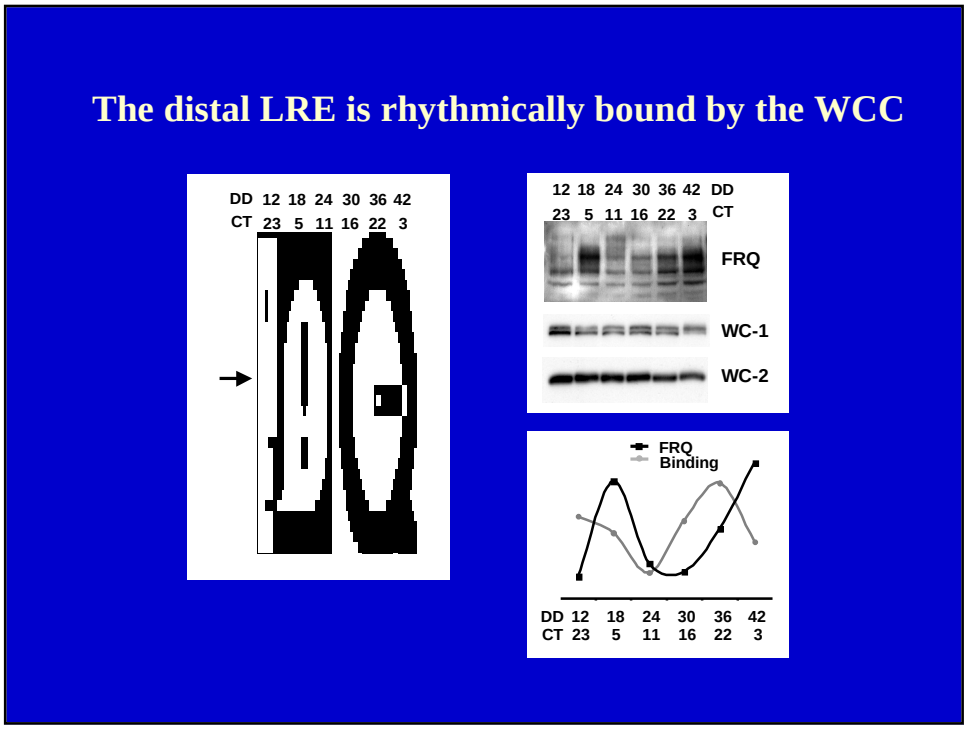


•WC-1 and WC-2 act positively on the production of *frq* transcript in the dark.

The distal LRE is necessary for overt and molecular rhythmicity



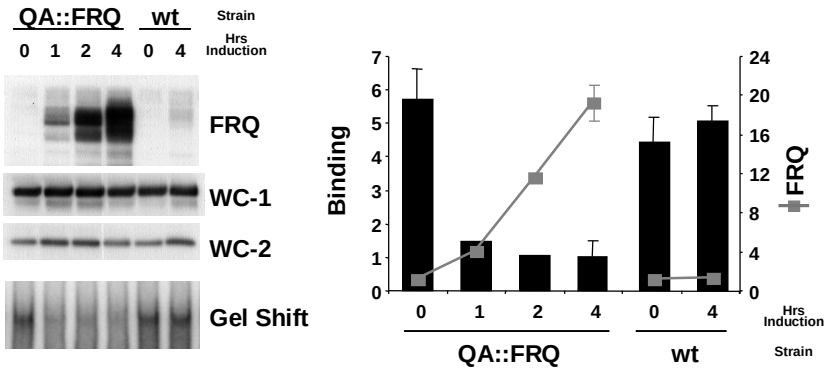

The distal LRE is also a circadian element.
6 12 18 24 hours in dark
vector
proximal LRE
distal LRE
hph reporter
{LRE}
TATA
hph
endogenous frq
hph
*
6 12 18 24
hours in dark

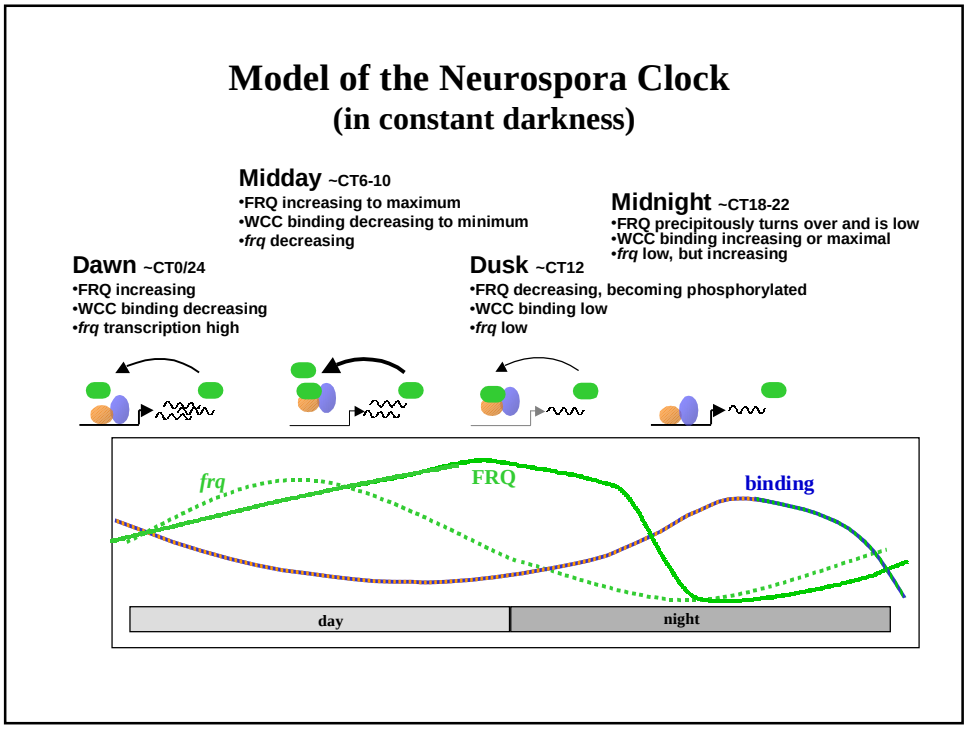

The distal LRE is rhythmically bound by the WCC
DD 12 18 24 30 36 42
CT 23 5 11 16 22 3
12 18 24 30 36 42 DD
23 5 11 16 22 3 CT
FRQ
WC-1
WC-2
FRQ
Binding
DD 12 18 24 30 36 42
CT 23 5 11 16 22 3

Does FRQ regulate WCC binding to the distal LRE?

- **FRQ negatively regulates its own transcript.**
- **FRQ physically interacts with WC-1 and WC-2.**
- **Oscillations in FRQ levels are phased appropriately and suggest an attractive means of generating oscillations in WCC binding.**

Increases in FRQ cause a decrease in WCC binding




Model of the Neurospora Clock
(in constant darkness)
Midday ~CT6-10
•FRQ increasing to maximum
•WCC binding decreasing to minimum
•frq decreasing
Midnight ~CT18-22
•FRQ precipitously turns over and is low
•WCC binding increasing or maximal
•frq low, but increasing
Dawn ~CT0/24
•FRQ increasing
•WCC binding decreasing
•frq transcription high
Dusk ~CT12
•FRQ decreasing, becoming phosphorylated
•WCC binding low
•frq low
frq
FRQ
binding
day
night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Clock Genes and Clock Mechanism Light Regulation
Neurospora
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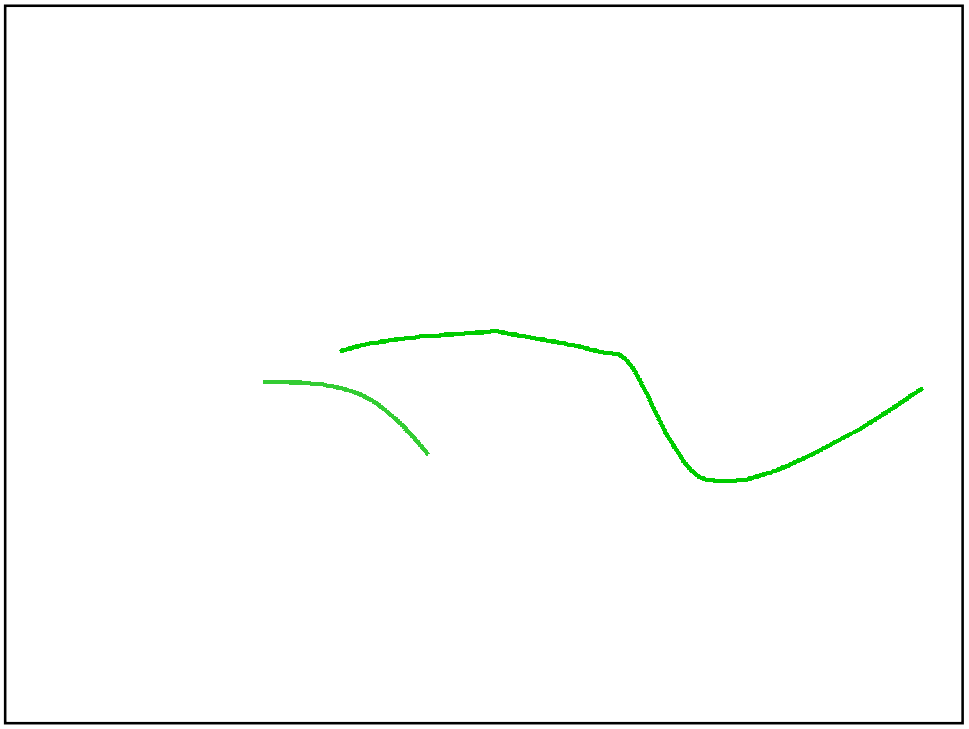
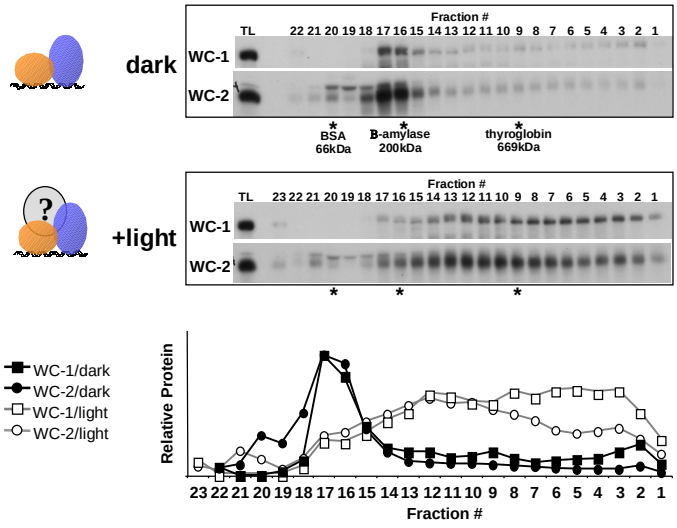
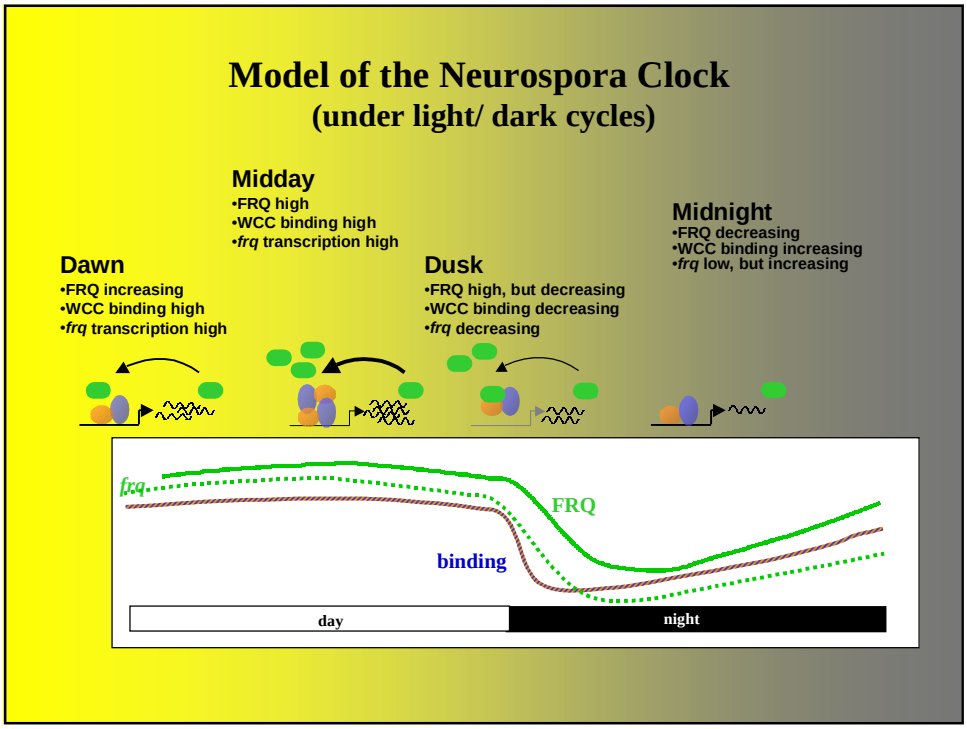
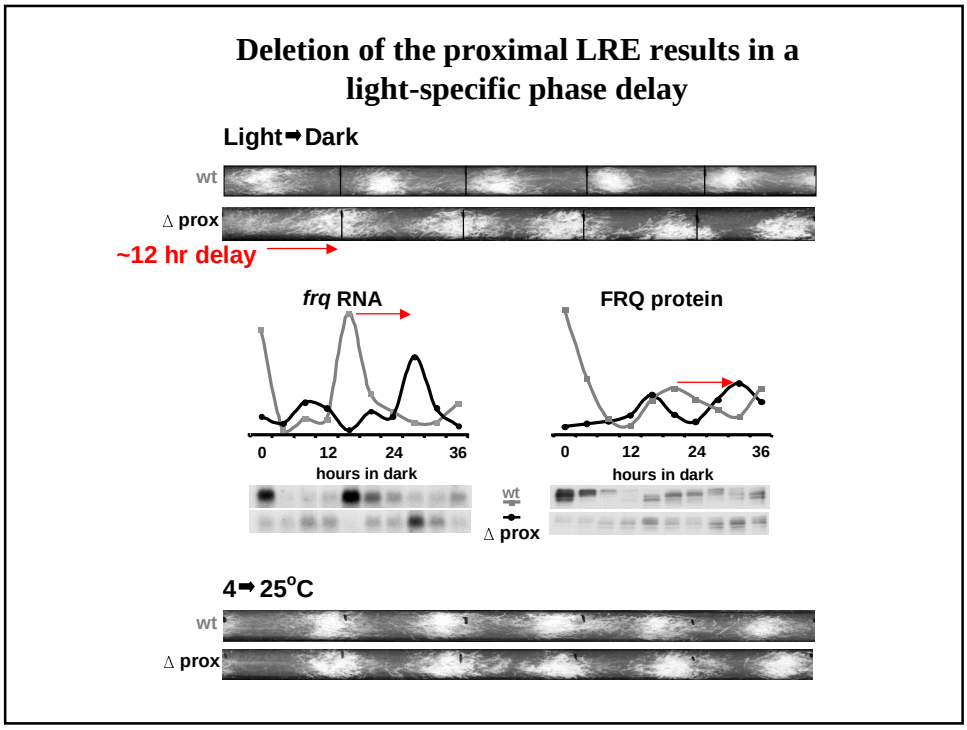
Jennifer Loros
Jay Dunlap



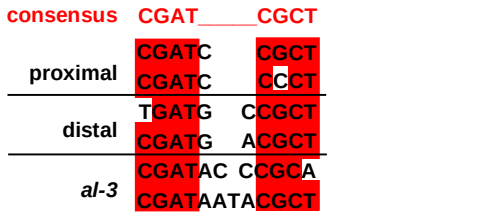
Light-Induced Shift of WC-1/WC-2 into a Higher Molecular Weight Complex




Model of the Neurospora Clock
(under light/ dark cycles)
Midday
•FRQ high
•WCC binding high
•frq transcription high
Midnight
•FRQ decreasing
•WCC binding increasing
•frq low, but increasing
Dawn
•FRQ increasing
•WCC binding high
•frq transcription high
Dusk
•FRQ high, but decreasing
•WCC binding decreasing
•frq decreasing
frq
FRQ
binding
day
night

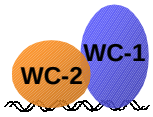

Deletion of the proximal LRE results in a light-specific phase delay
Light➡Dark
wt
Δ prox
~12 hr delay
frq RNA
FRQ protein
0
12
24
36
hours in dark
wt
Δ prox
4➡25°C
wt
Δ prox

Consensus sequence for WC-1/WC-2 binding

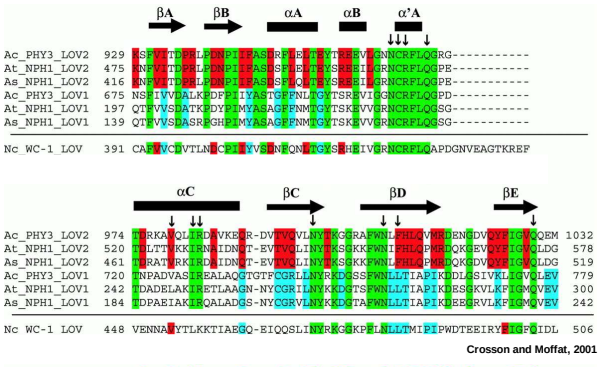


CGCAGAGGACCCTGAACTTT TCGATCCGCT TCGATCCCCT GGAA
proximal LRE

CGTC CTGATGCCGCTGCAAGA CCGATGACGCTGCAA AATTGAGATCTA
distal LRE



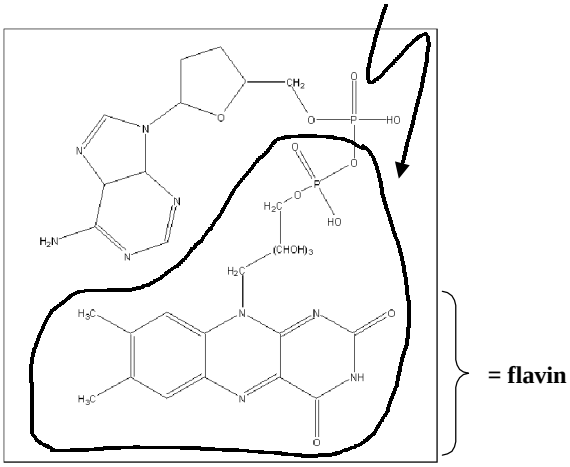
The WC-1 LOV domain shares homology with LOV domains found in blue light photoreceptors



Crosson and Moffat, 2001

Flavin mononucleotide

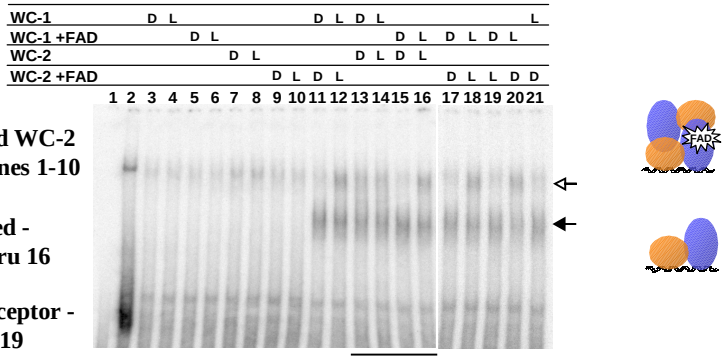
FMN



Flavin adenine dinucleotide

FAD

WC-1, bound to FAD, is the Neurospora circadian photoreceptor and does not require WC-2 for photoreception



Both WC-1 and WC-2 are needed - lanes 1-10

FAD is required - compare 11 thru 16

WC-1 is the receptor - compare 20 vs 19

How are light signals perceived and transduced to the clock?

- *frq* transcript is rapidly and highly induced in response to light.
- The *frq* promoter contains two *cis*-acting Light Response Element (LREs).
- The LREs are bound by a WC-1/WC-2 (WCC) containing complex.
- Light causes decreased mobility of the WCC/LRE.