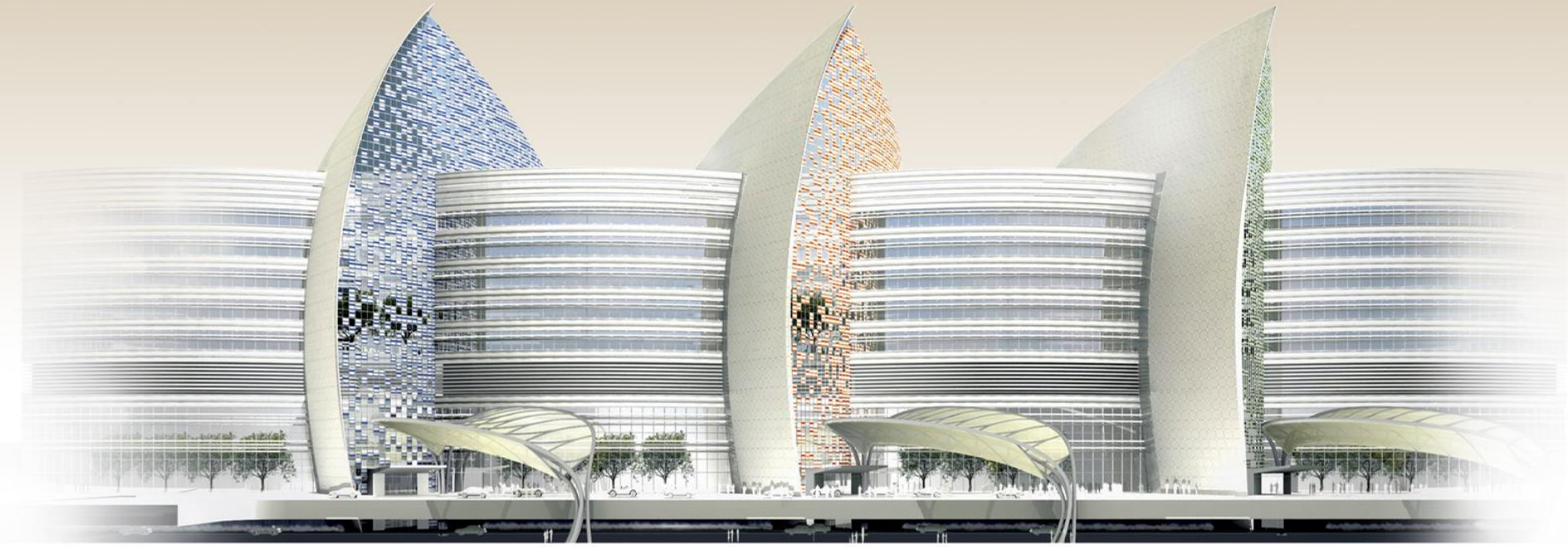
The Structure of Biological Pathways in Time

Life works on AC power



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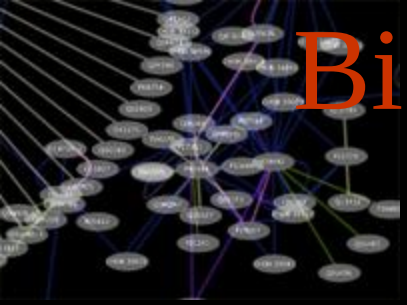
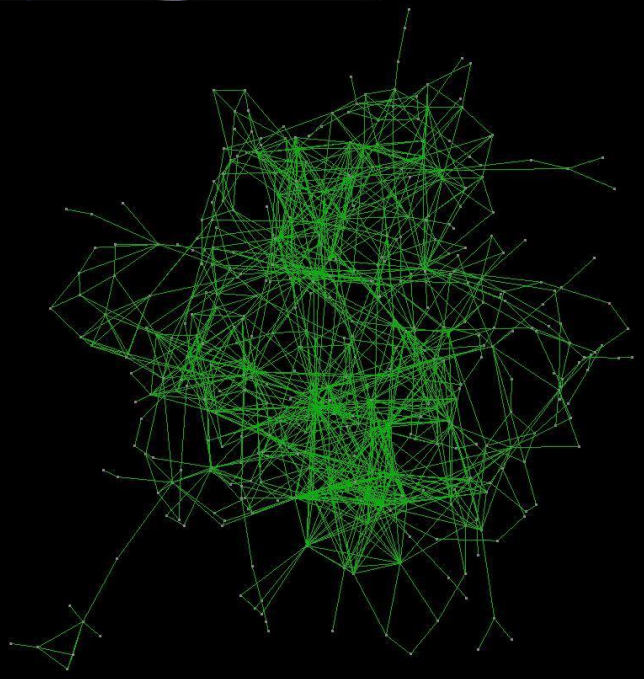
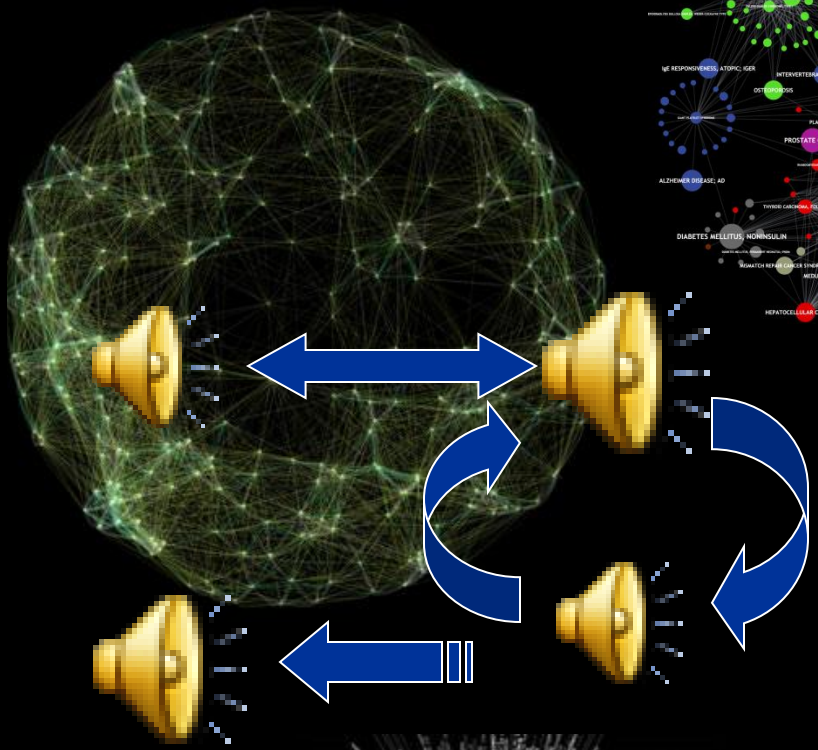
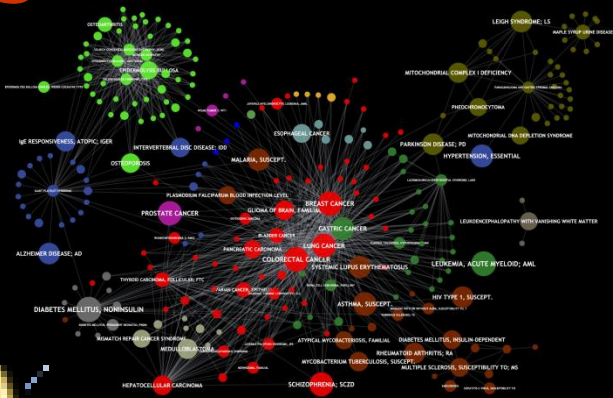
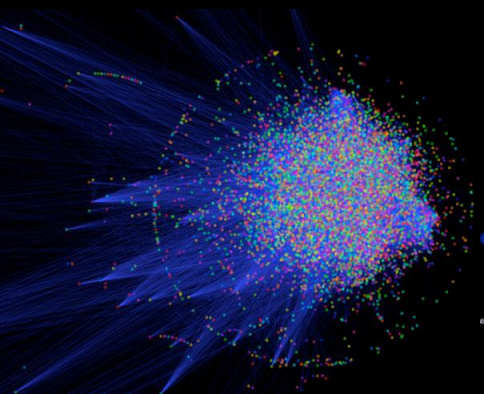
Biological pathways: untangling the hairballs

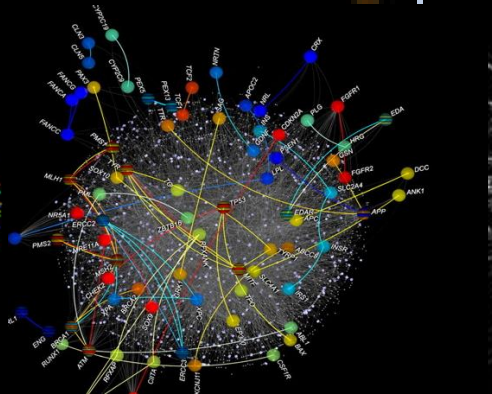


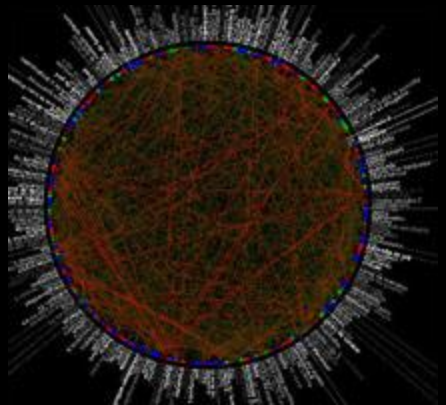


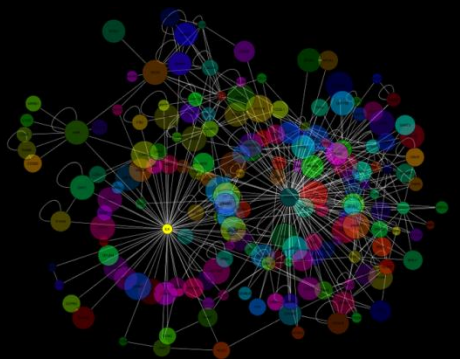




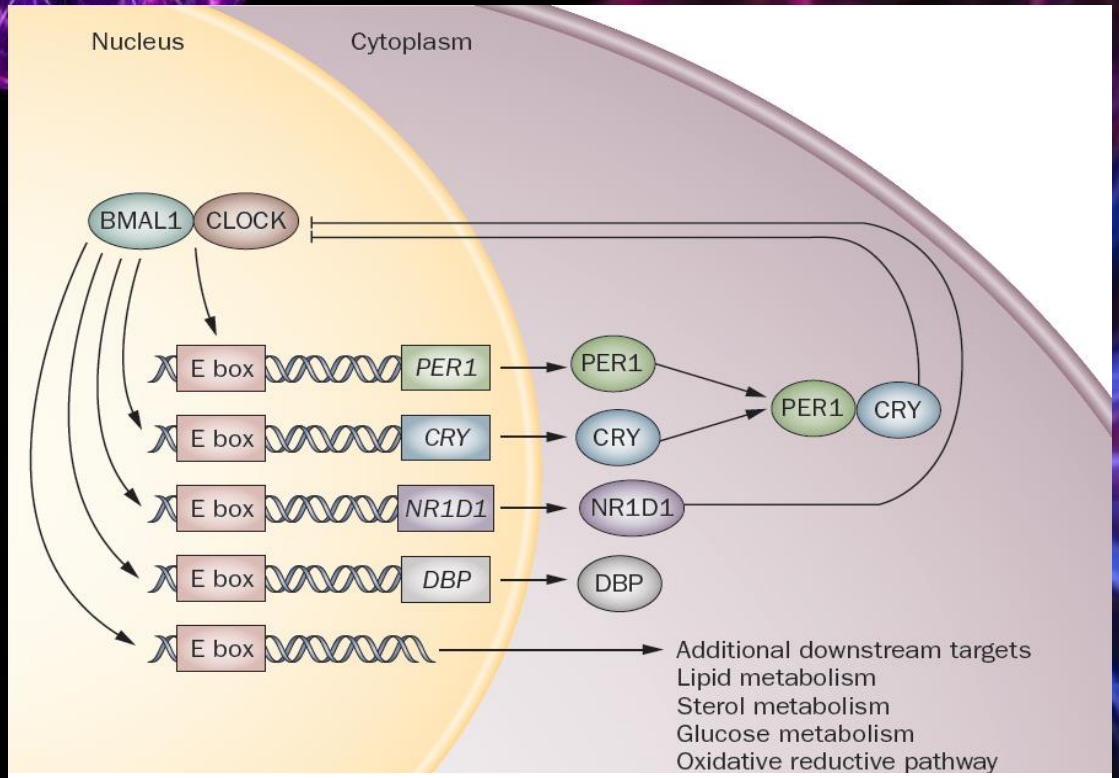


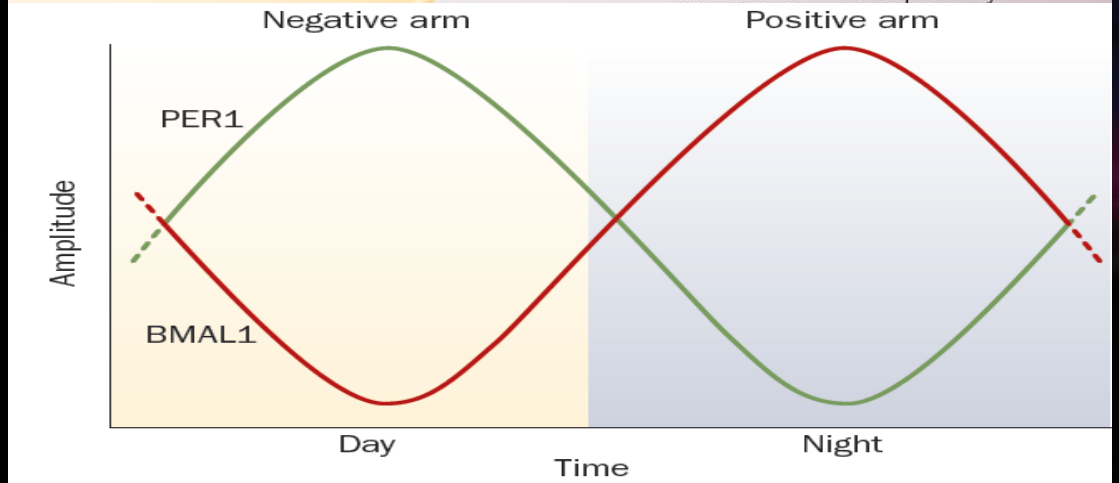






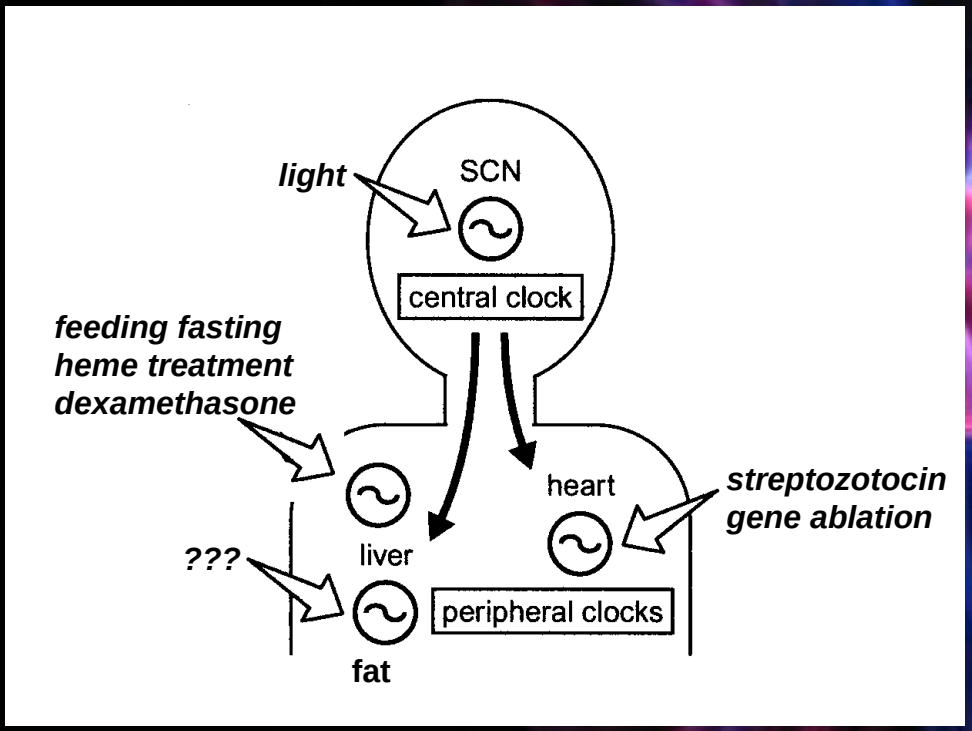
Mammalian molecular clock





- BMAL1 and CLOCK (NPAS2) form heterodimers that act as positive transcriptional regulators
- PERIOD (Per1, Per2, Per3) and CRYPTOCHROME (Cry1, Cry2) family members serve as negative transcriptional regulators
- downstream targets, such as the albumin D site binding protein (DBP), can further activate transcription while others, such as E4BP4, repress transcription
- The serine/threonine kinases, casein kinase Iε (CK1 ε) and glycogen synthase kinase 3β (GSK3 β), phosphorylate BMAL1, PER, and other proteins exposing them for degradathion through ubiquitin/proteasomal pathway

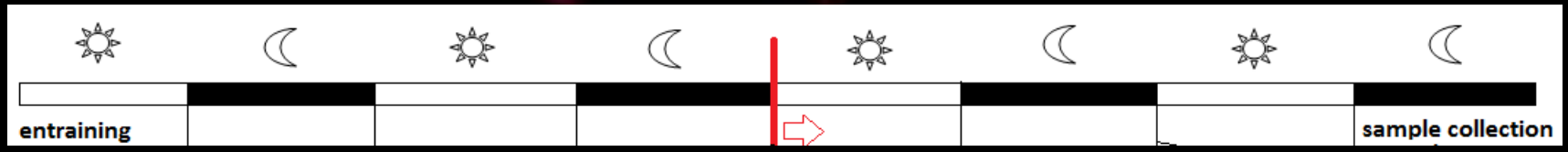
Circadian rhythms



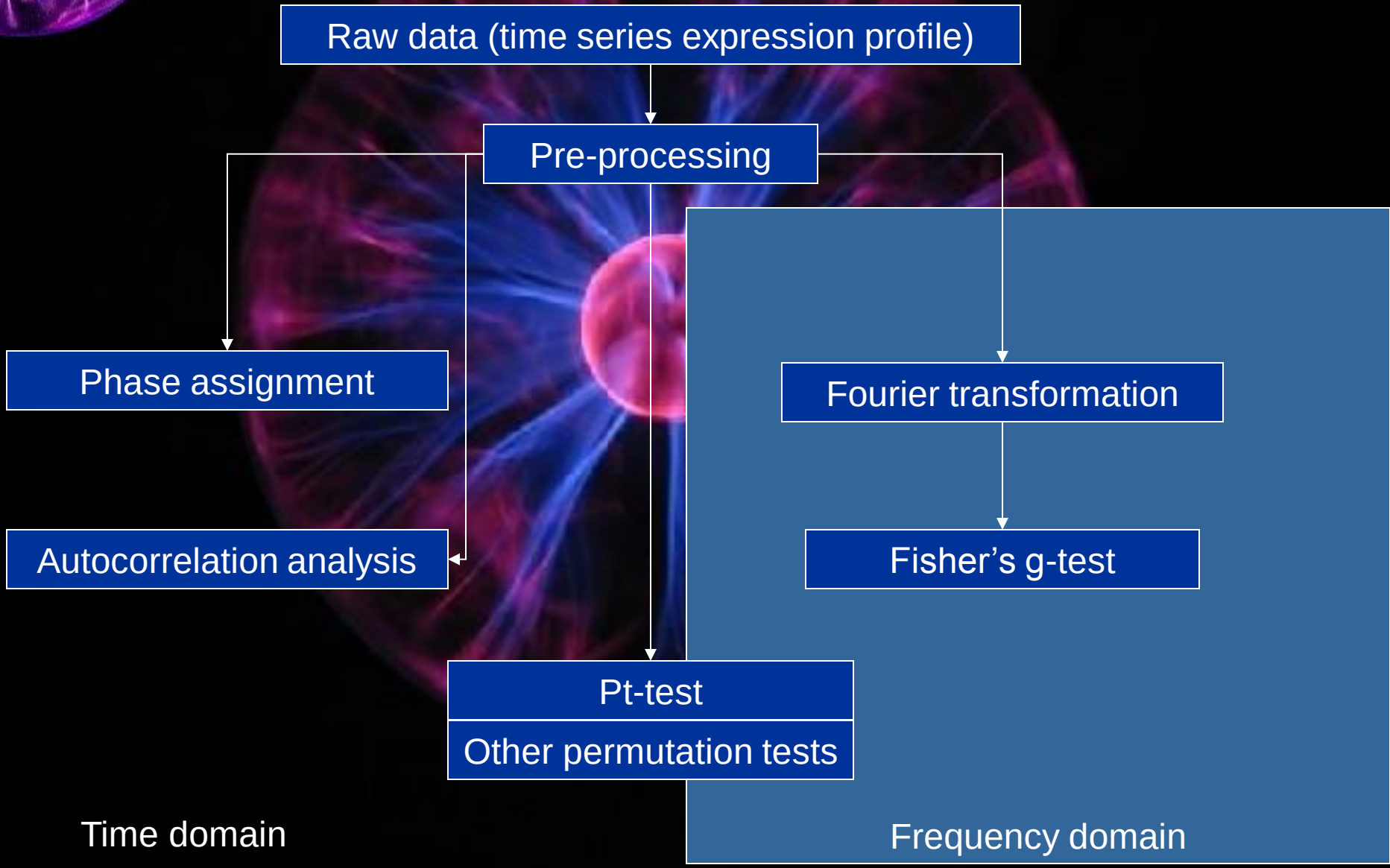
- Physiological rhythms respond to environmental cues
- Suprachiasmic nucleus (SCN) of the brain is the body's central oscillator
- SCN responds to light/dark cycle
- Daily activity rhythm continues even in total darkness
- The central circadian oscillator may act through sympathetic outputs and controlled secretion of circulating glucocorticoids, melatonin, and other mediators, thereby "synchronizing" the circadian rhythms of the body's tissues and organs

First experiment

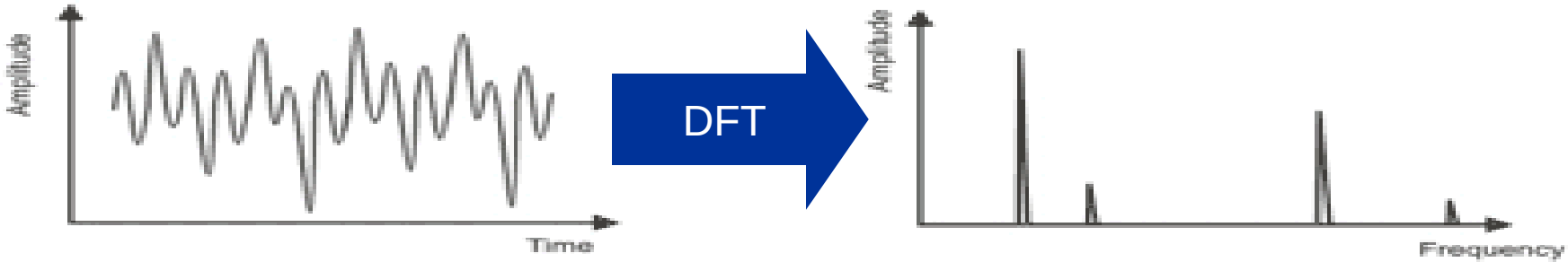
- Age-matched male Black 6 mice on chow
- entrained to 12/12 alternation of lighting
- samples collected for every 4h starting from 8am
- total of 12 samples collected to represent a 48h expression profile
- 3-5 mice are sacrificed at each time point, samples pooled
- liver, white fat and brown fat are collected (bone is added later in a similar experiment
- entraining conditions are kept throughout data collection period



Algorithms to identify periodicity



Fisher's g-test



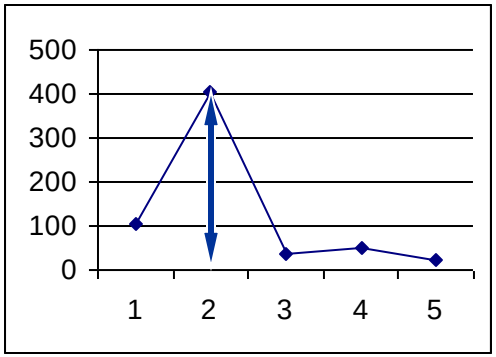
$$I(\omega) = \{\omega_0, \omega_1, \omega_2, \ldots \omega_{N/2-1}\}$$

$$I(\omega) = \frac{1}{N}\left|\sum_{t=0}^{N-1} x_t e^{(-i\omega t)}\right|^2, \omega \in [0, \pi]$$

$$Y = \{x_0, x_1, x_2, \ldots x_{N-1}\}$$

Based on periodogram
Signal to noise ratio

$$g = \frac{\max_k I(\omega_k)}{\sum_{k=1}^{N/2} I(\omega_k)},$$

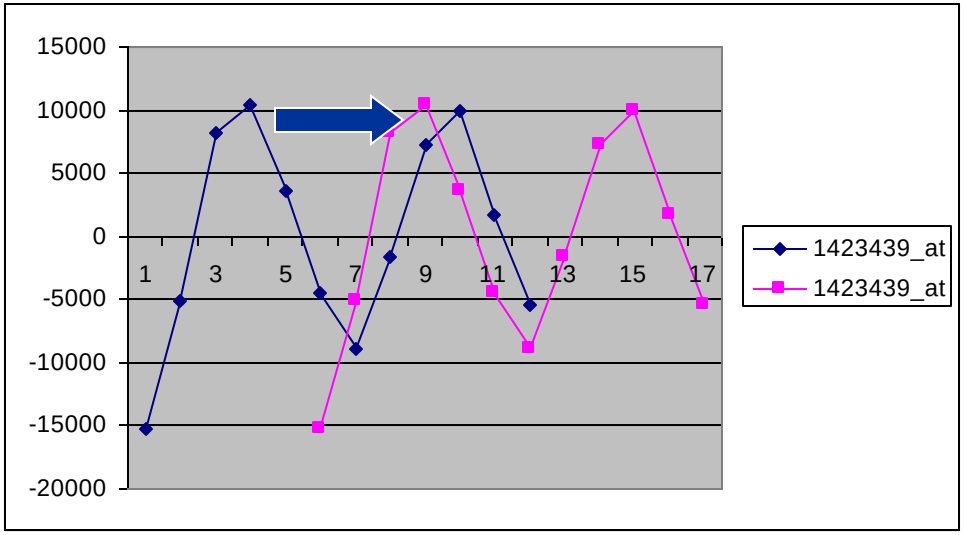


Fisher's formula produces p-value for significance of oscillation:

$$P(g > x) = \sum_{p=1}^{1/x}\left[(-1)^p \frac{n!}{p!(n-p)!}(1-px)^{n-1}\right],$$

Autocorrelation and Phase Assignment

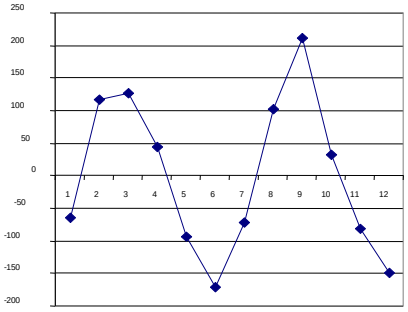
$$R(f) = \frac{\sum_0^{N-1} (x_i - \bar{x})(x_f - \bar{x})}{\sum_0^{N-1} (x_i - \bar{x})^2}$$



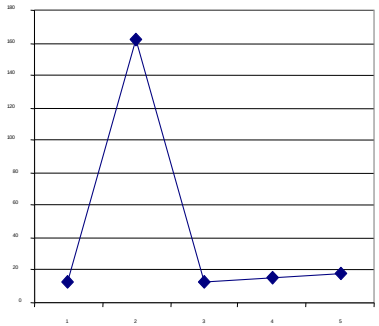
$$R(f) = \frac{\sum_0^{N-1} (x_i - \bar{x})(y_f - \bar{y})}{\sum_0^{N-1} (x_i - \bar{x})(y_i - \bar{y})}$$, where $$y_i = \cos\left(\frac{2\pi}{p} * i\right)$$

Pt-test

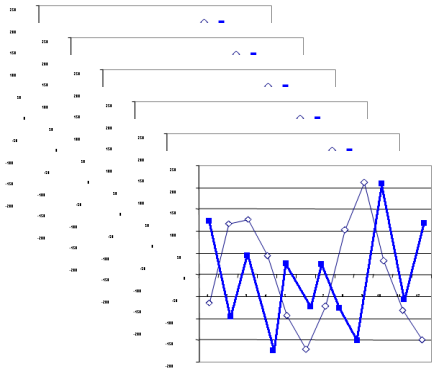
Original profile



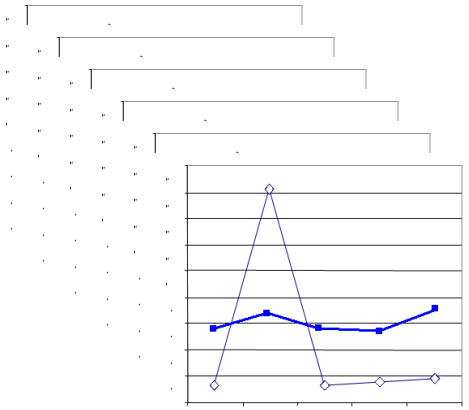
Original periodogram



Permutated profiles

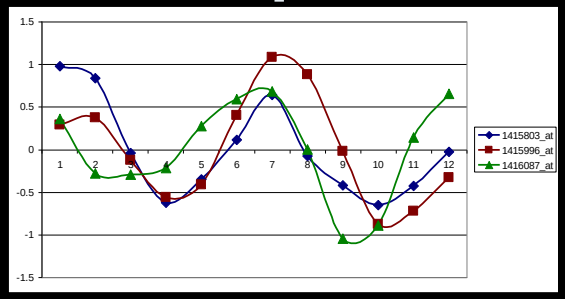


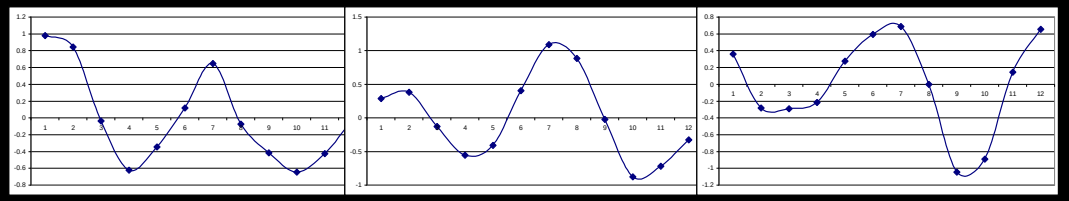
Permutated periodograms

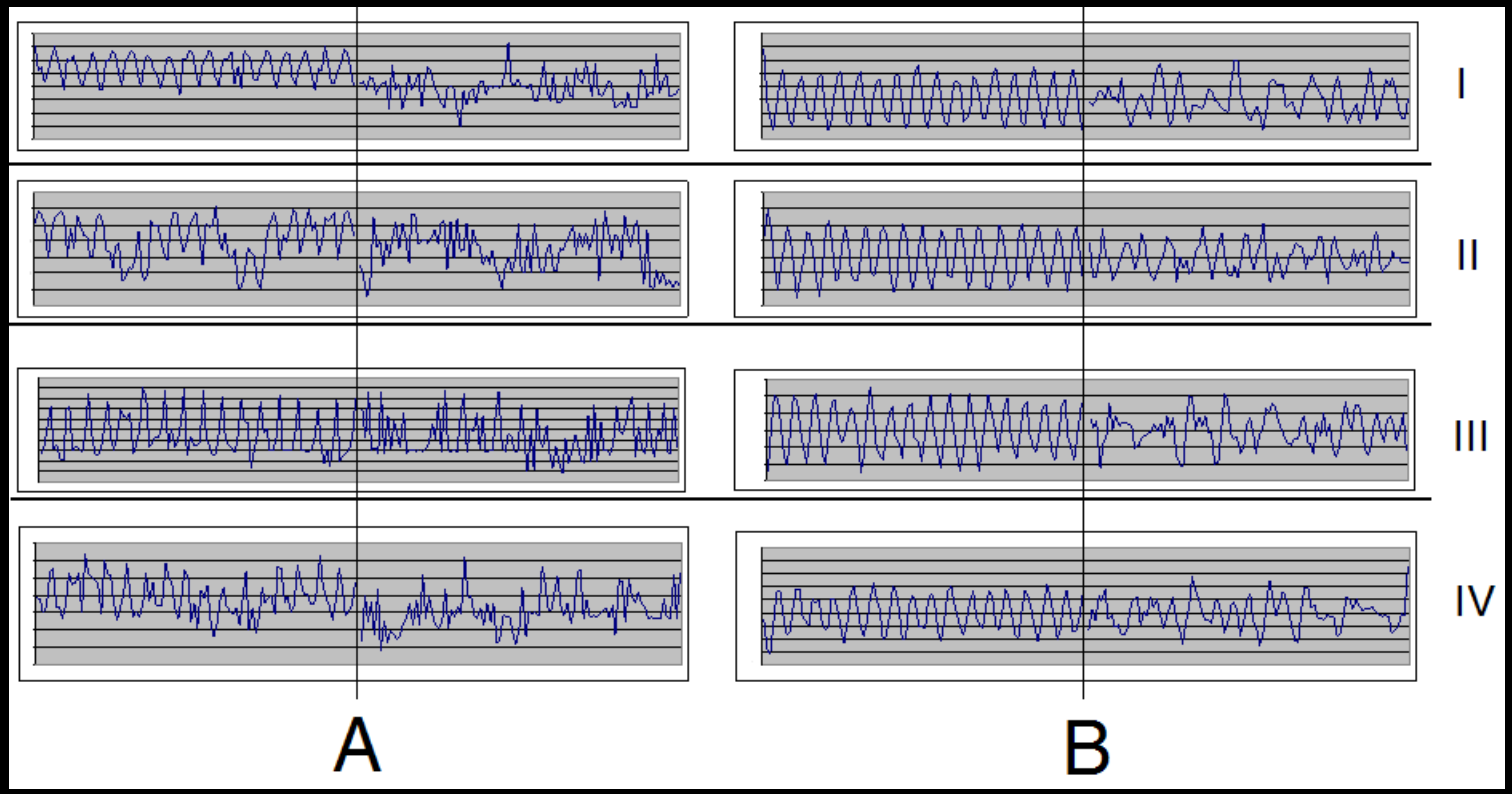


Significance is estimated by comparing specific frequency peak in original and multiple randomized periodograms

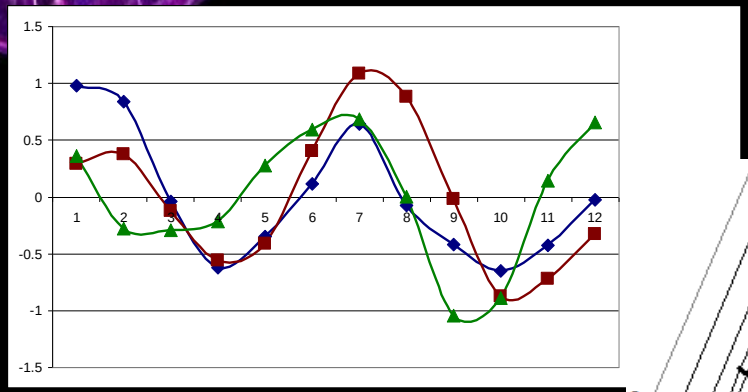
Analysis of circadian signal in phase continuum (real data)

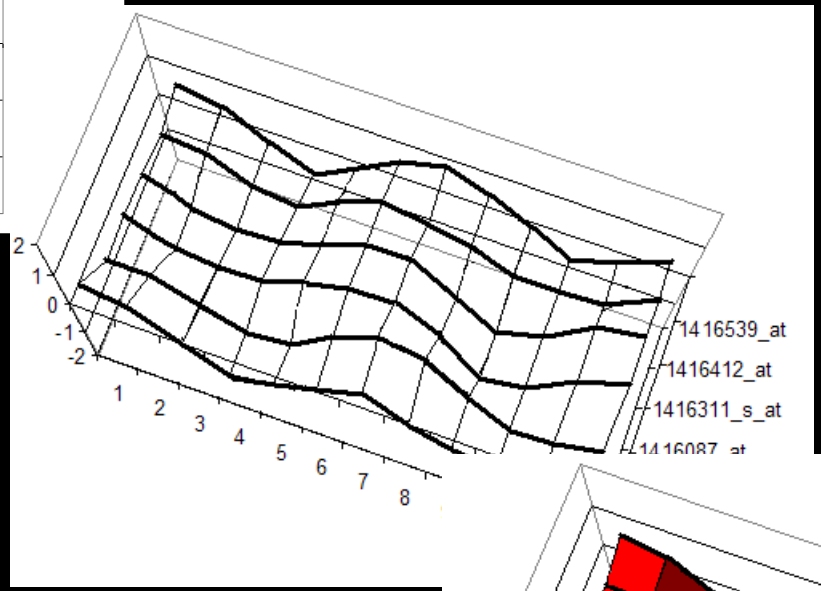


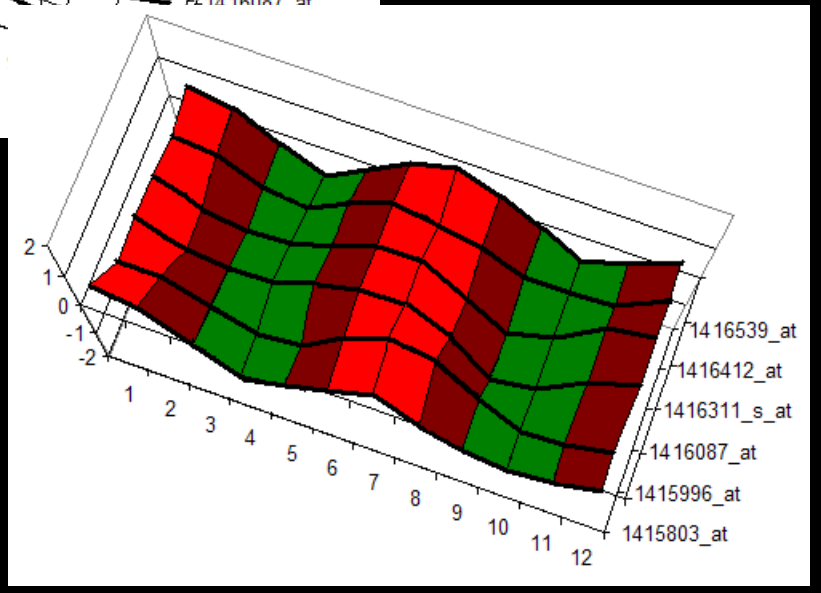




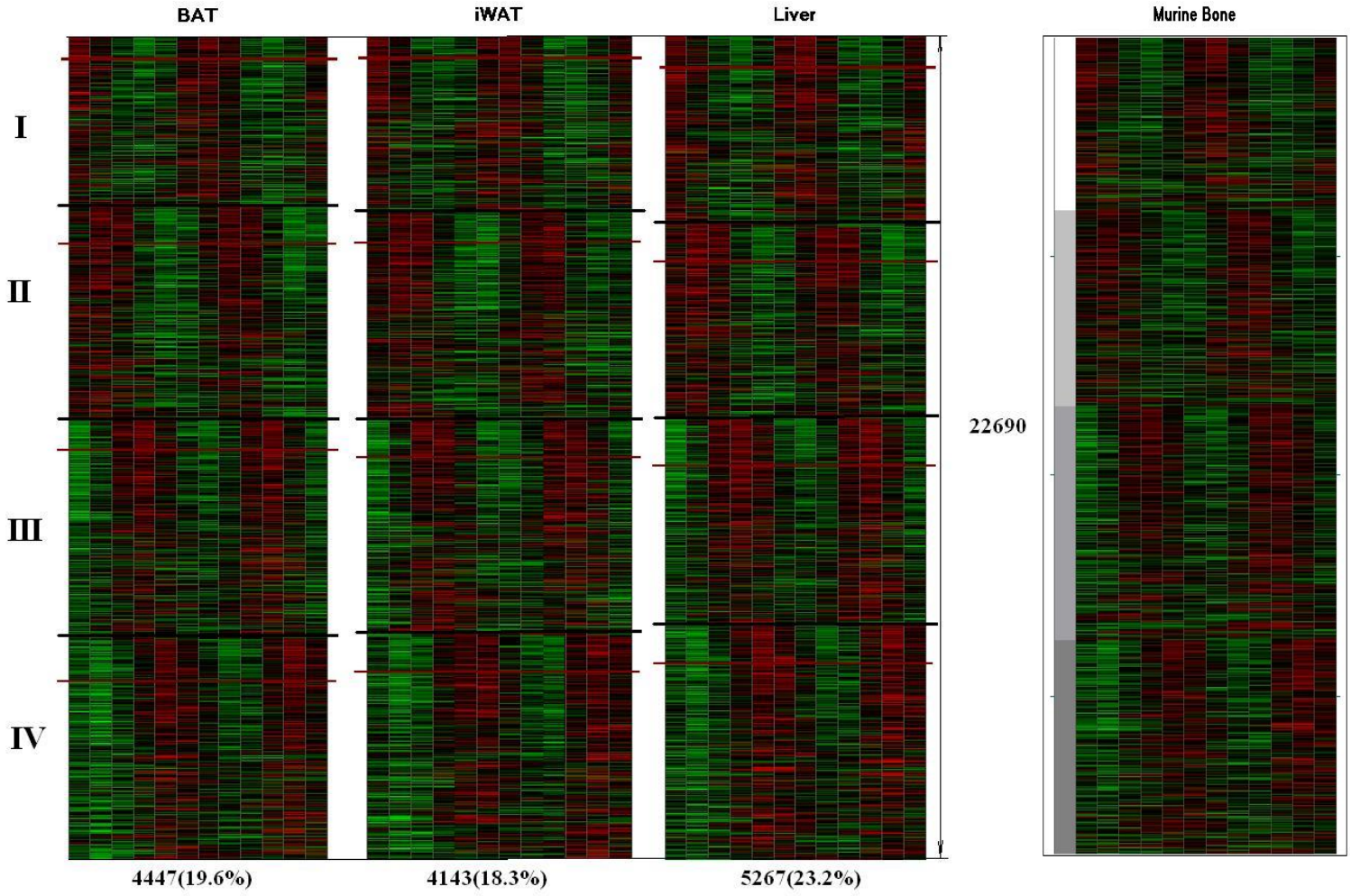
Heat map Visualization





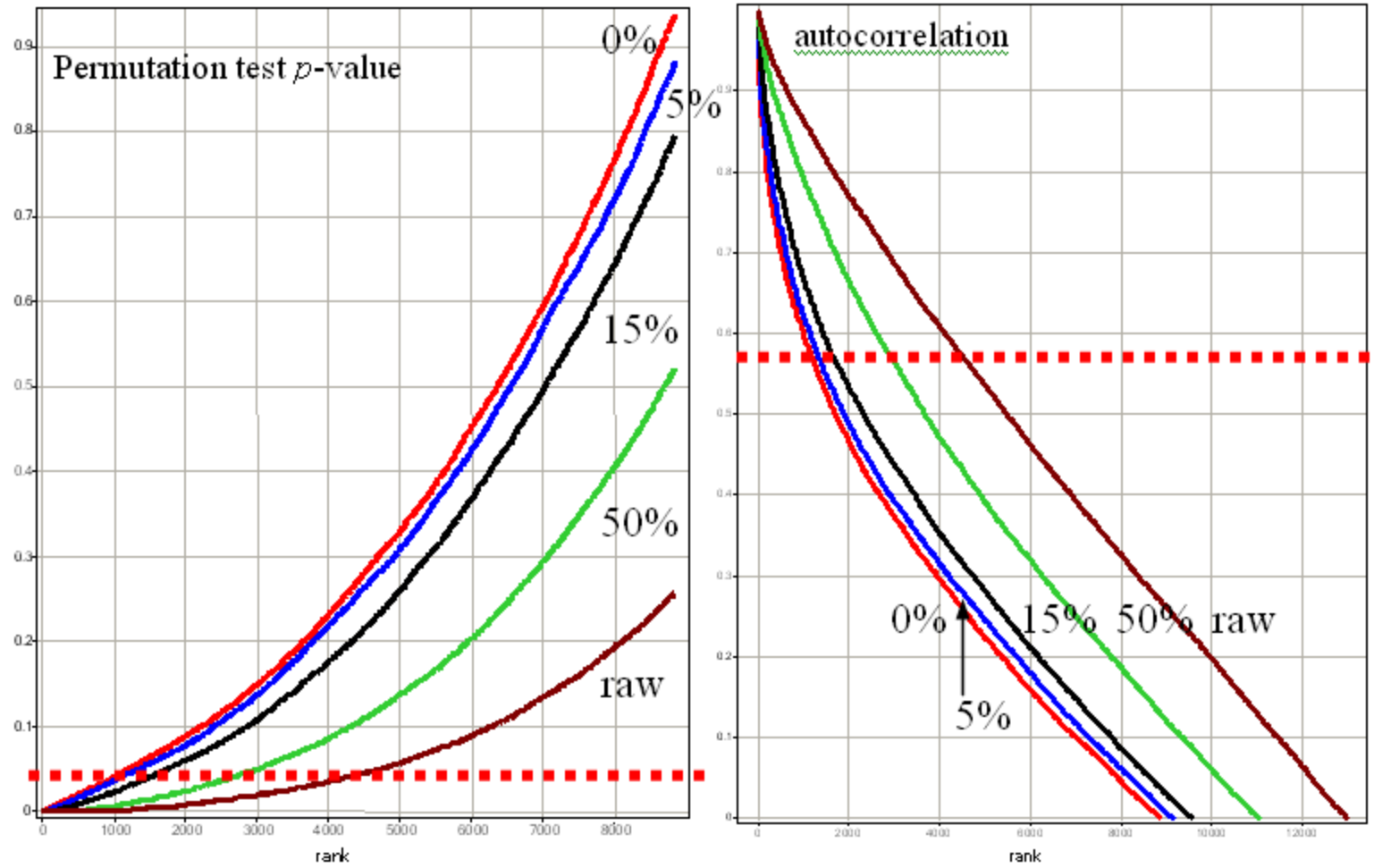


Murine transcriptome

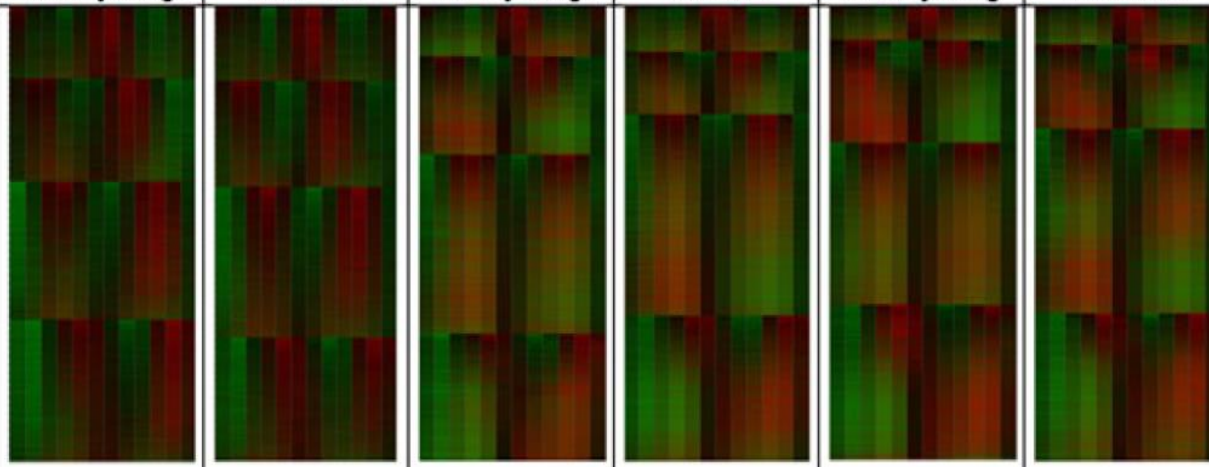


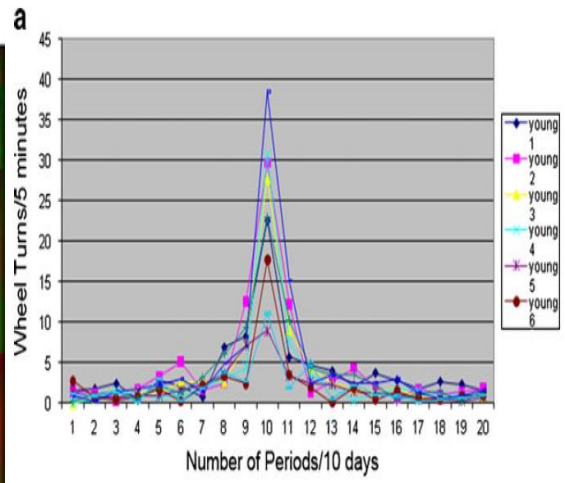
Is there a non-oscillating fraction at all?

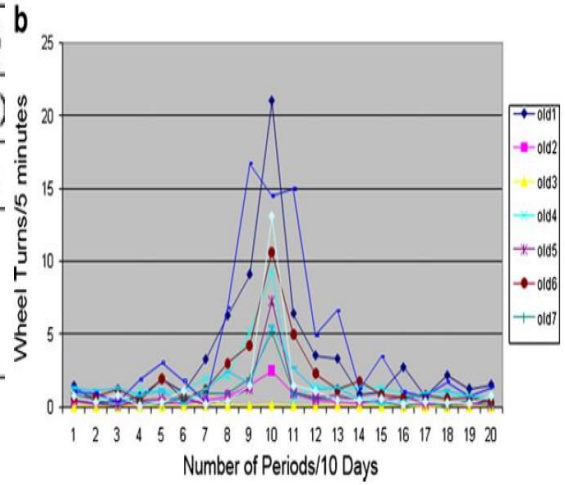
Simulation experiment



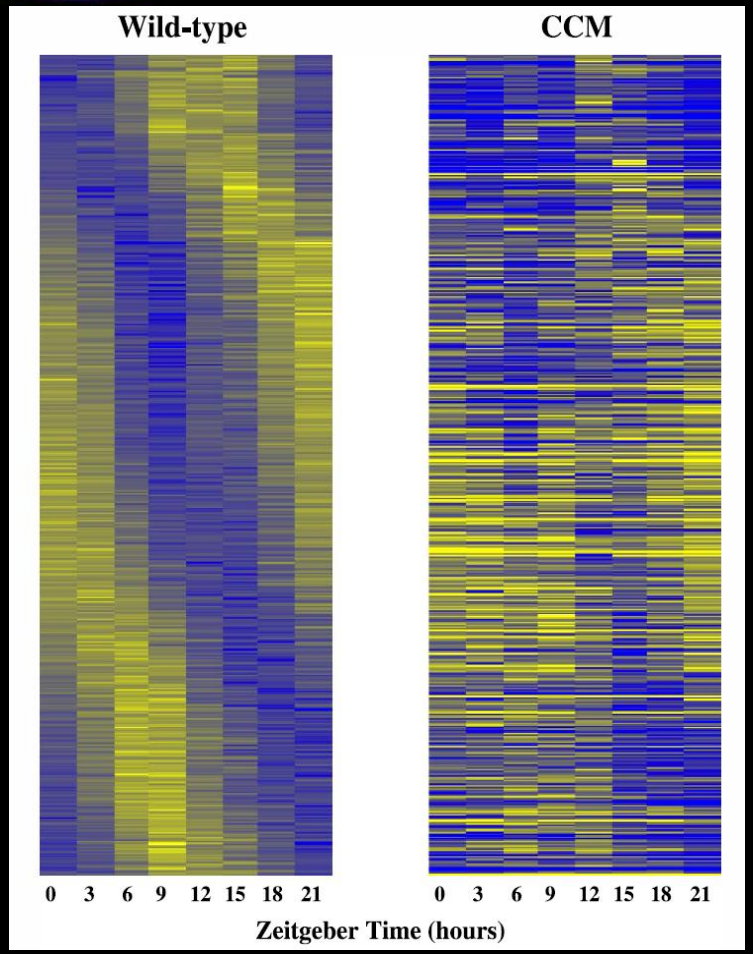
Does aging affect the rhythm?

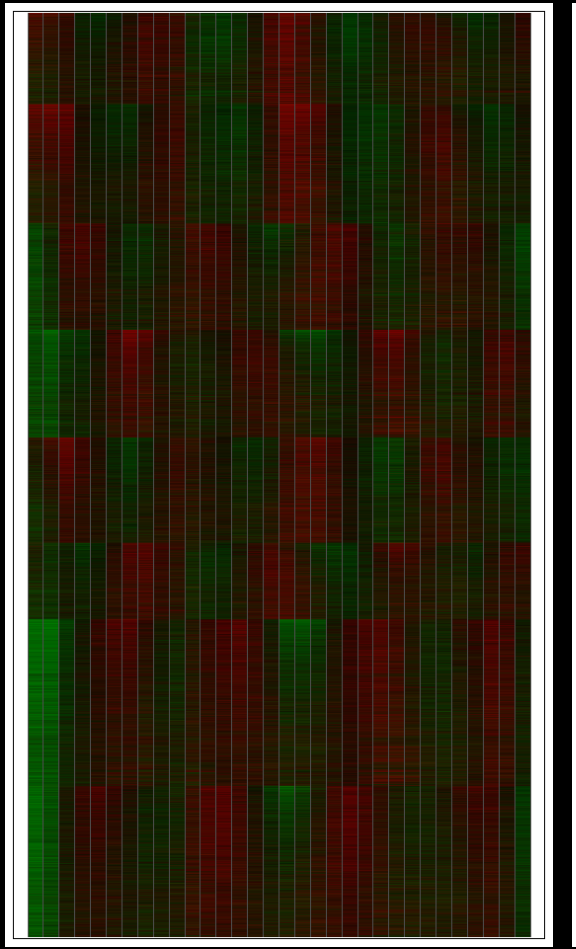
	Liver young	Liver old	BAT young	BAT old	iWAT young	iWAT old
Overview of the expression pattern for the entire transcriptome						
Pt-test	8744 (19%)	9472 (21%)	3381 (7.5%)	3525 (7.8%)	2765 (6.1%)	3283 (7.2%)
Fisher's g-test	12452 (28%)	12826 (28%)	4778 (10.6%)	4906 (10.8%)	4106 (9%)	4640 (10.3%)
autocorrelation	1672 (3.7%)	2215 (4.9%)	778 (1.7%)	889 (2%)	598 (1.3%)	816 (1.8%)
Oscillating in the same phase with age	583		152		267	
Changed phase with age	1812		616		717	

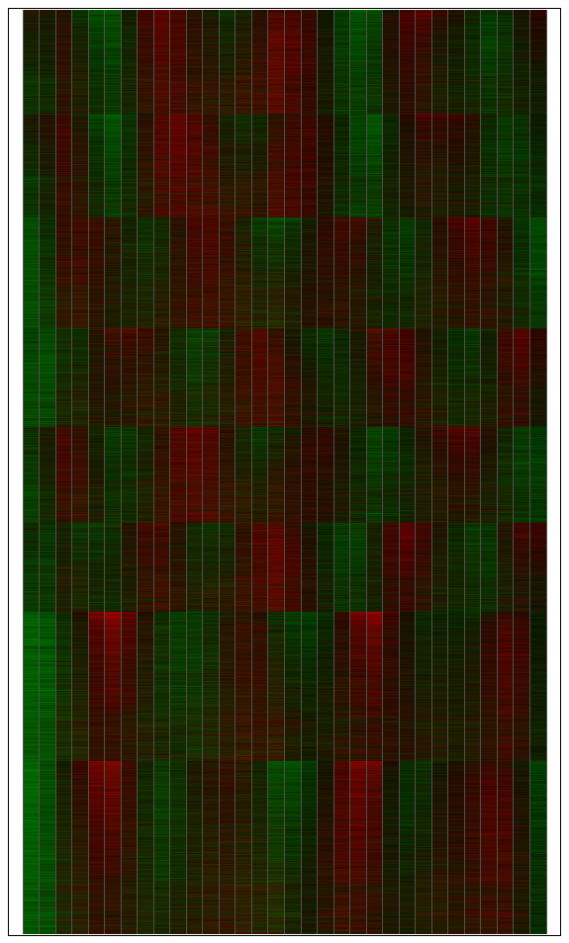




Can we knock out the rhythm?

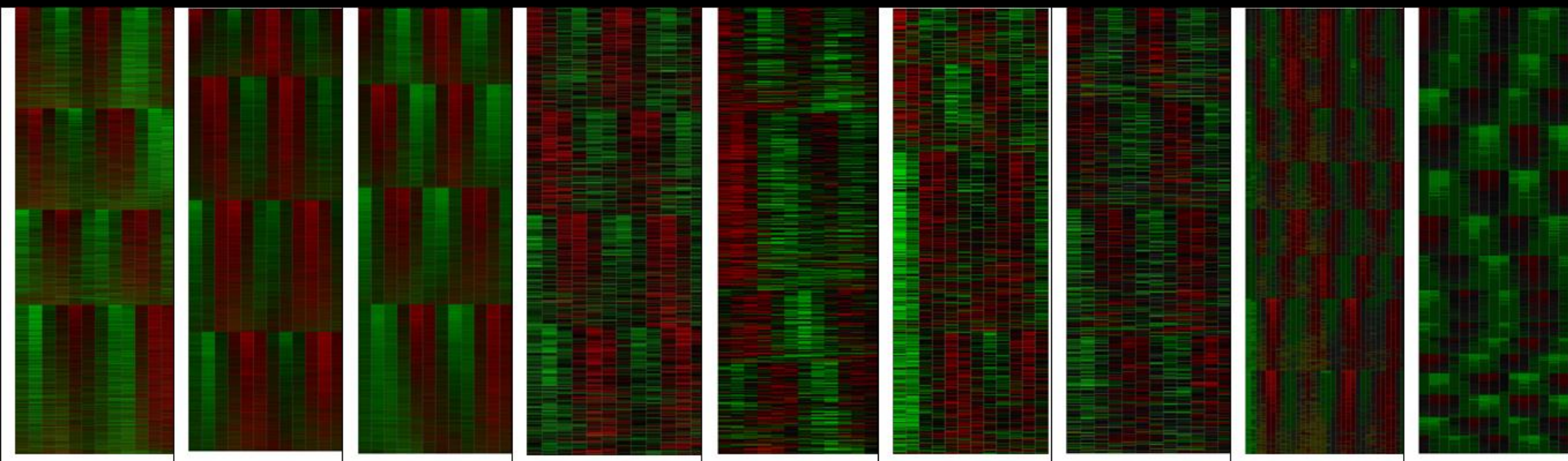


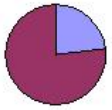
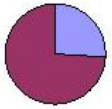
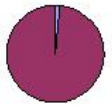




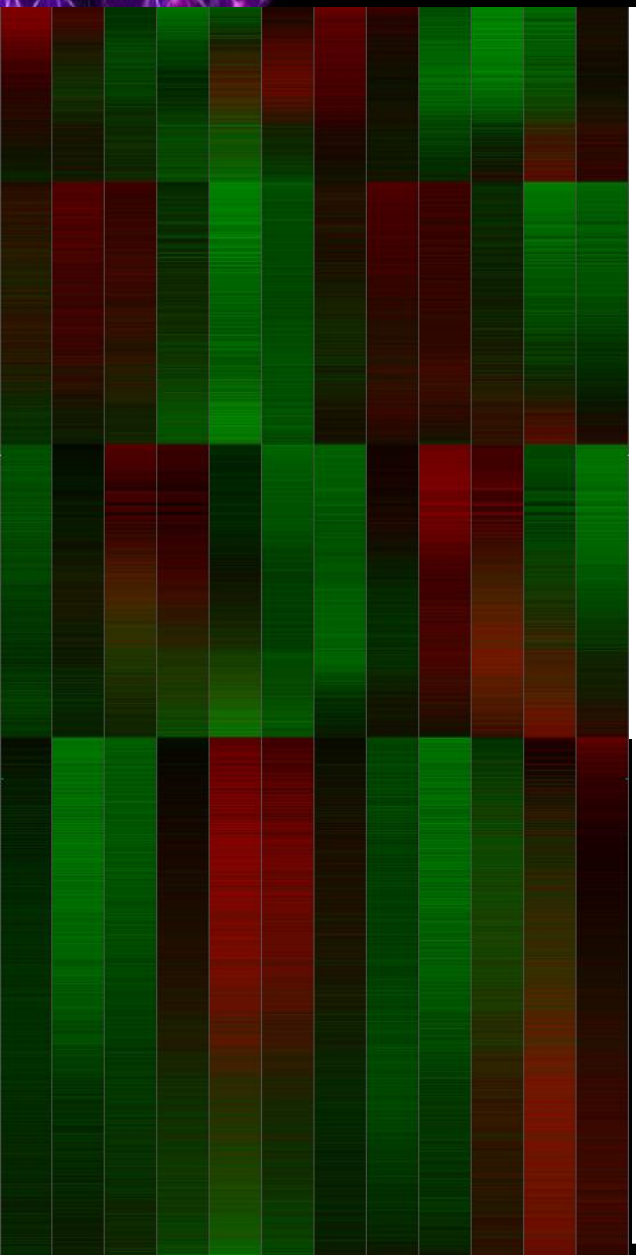
Bray, M., et al., Disruption of the circadian clock within the cardiomyocyte influences myocardial contractile function, metabolism, and gene expression *Am J Physiol Heart Circ Physiol* 294:1036-1047, 2008.

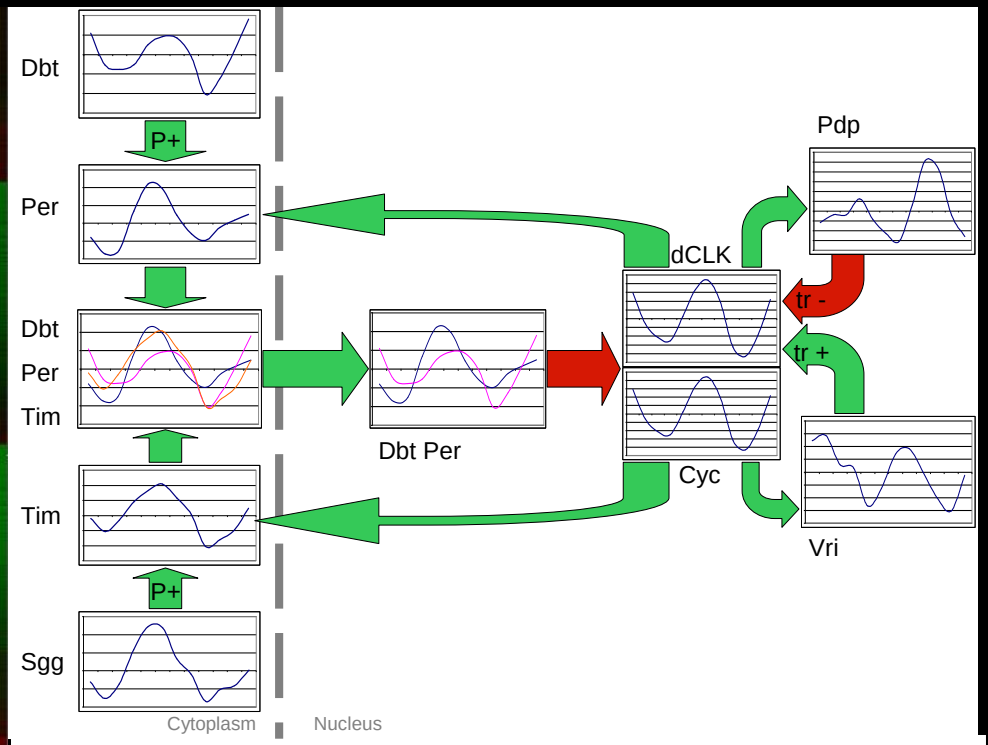
Reanalysis vs. original report

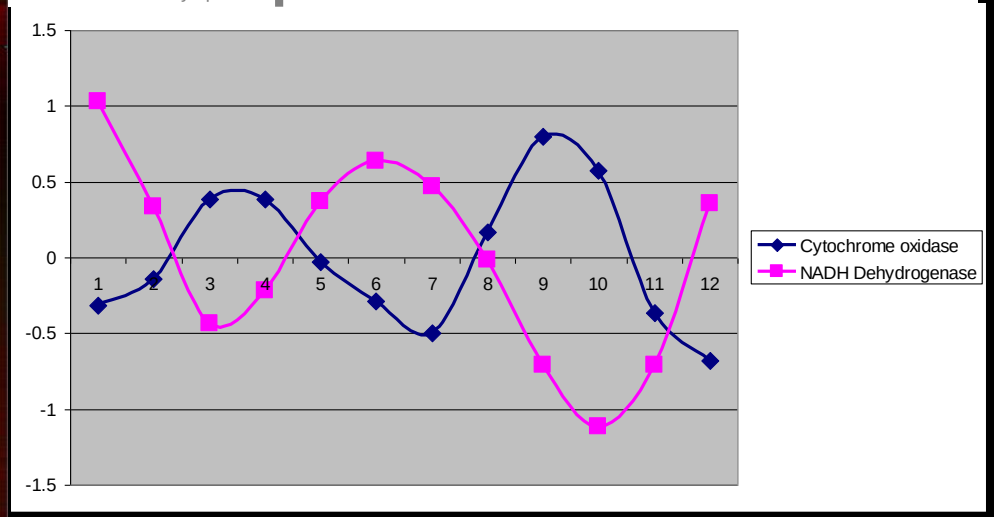


Drosophila melanogaster (heads)	*Arabidopsis thaliana*	*Arabidopsis thaliana*	*Mus musculus* (liver)	*Mus musculus* (liver)	*Mus musculus* (liver)	*Mus musculus* (bone)	*Mus musculus* (heart)	*Homo sapience* (milk fat globule)
1.2%	7%*	15.4%*	23.8%*	4.6%	1.5%	26%*	1.6%	4.6%
								

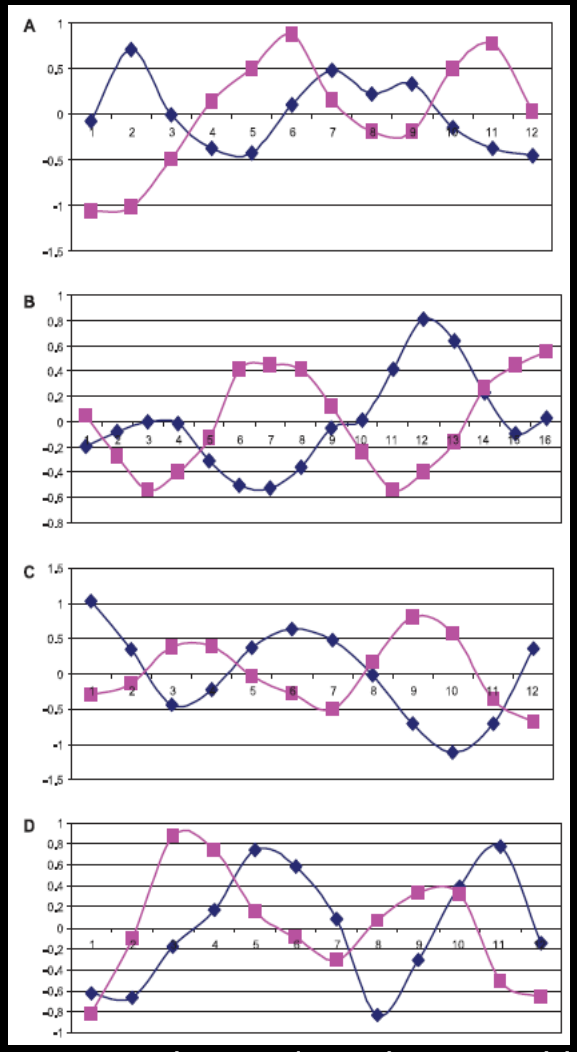
Mosquito




Dbt
P+
Per
Dbt
Per
Tim
Tim
P+
Sgg
Cytoplasm
Nucleus
Dbt Per
dCLK
Pdp
tr -
tr +
Cyc
Vri


1.5
1
0.5
0
-0.5
-1
-1.5
1
2
3
4
5
6
7
8
9
10
11
12
Cytochrome oxidase
NADH Dehydrogenase

Are those rhythms only driven by light?



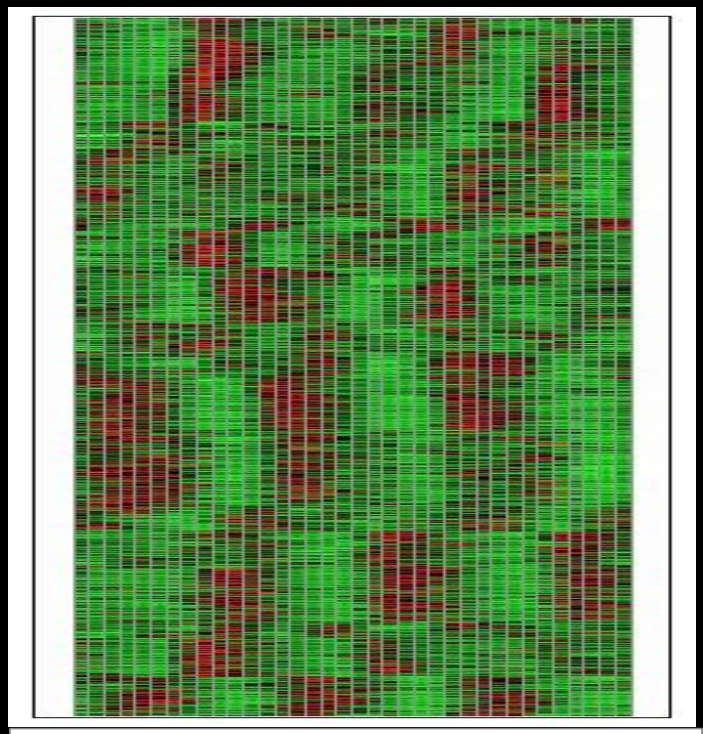
Plant

Human

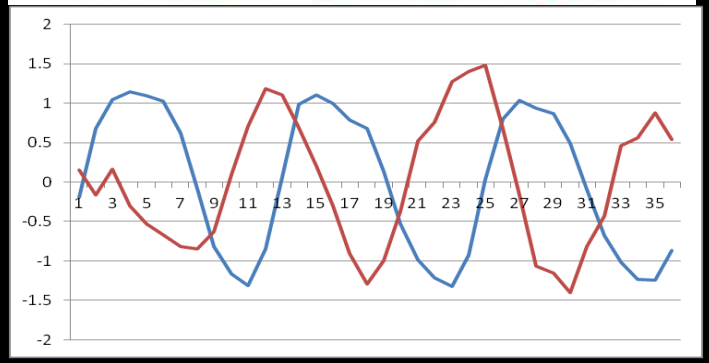
Mosquito

Mouse

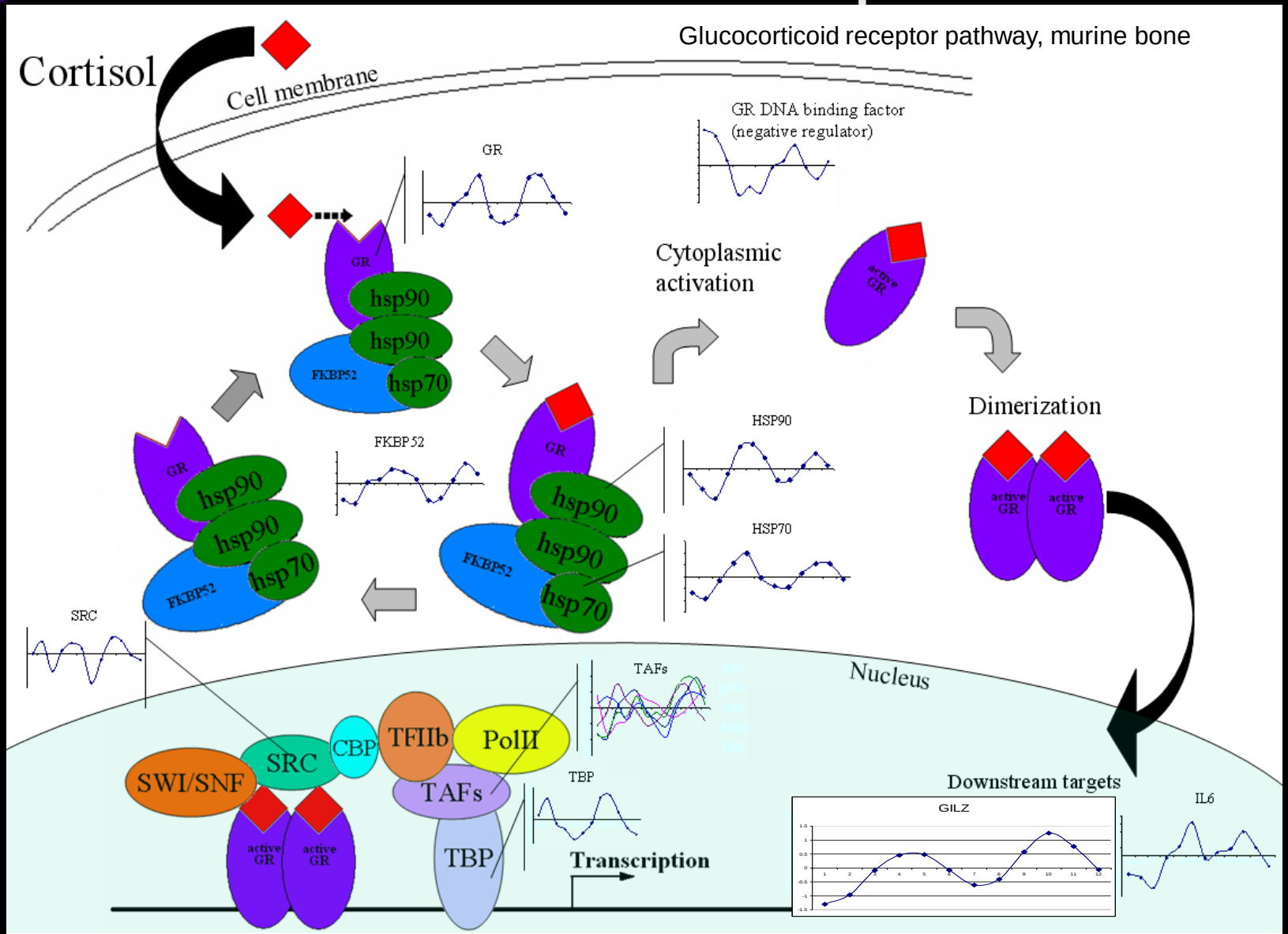
NADH reductase/cytochrome oxidase



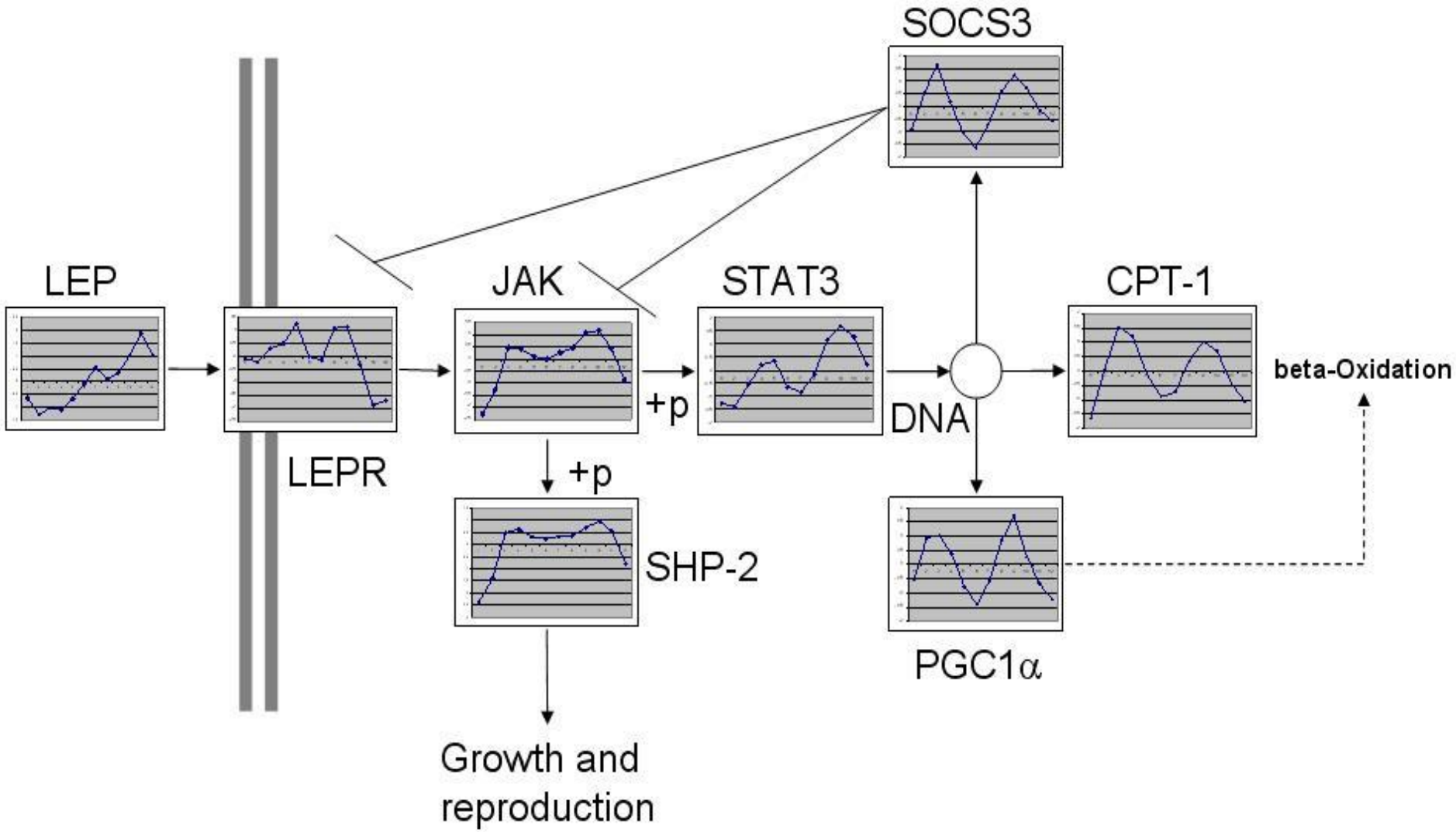
Yeast



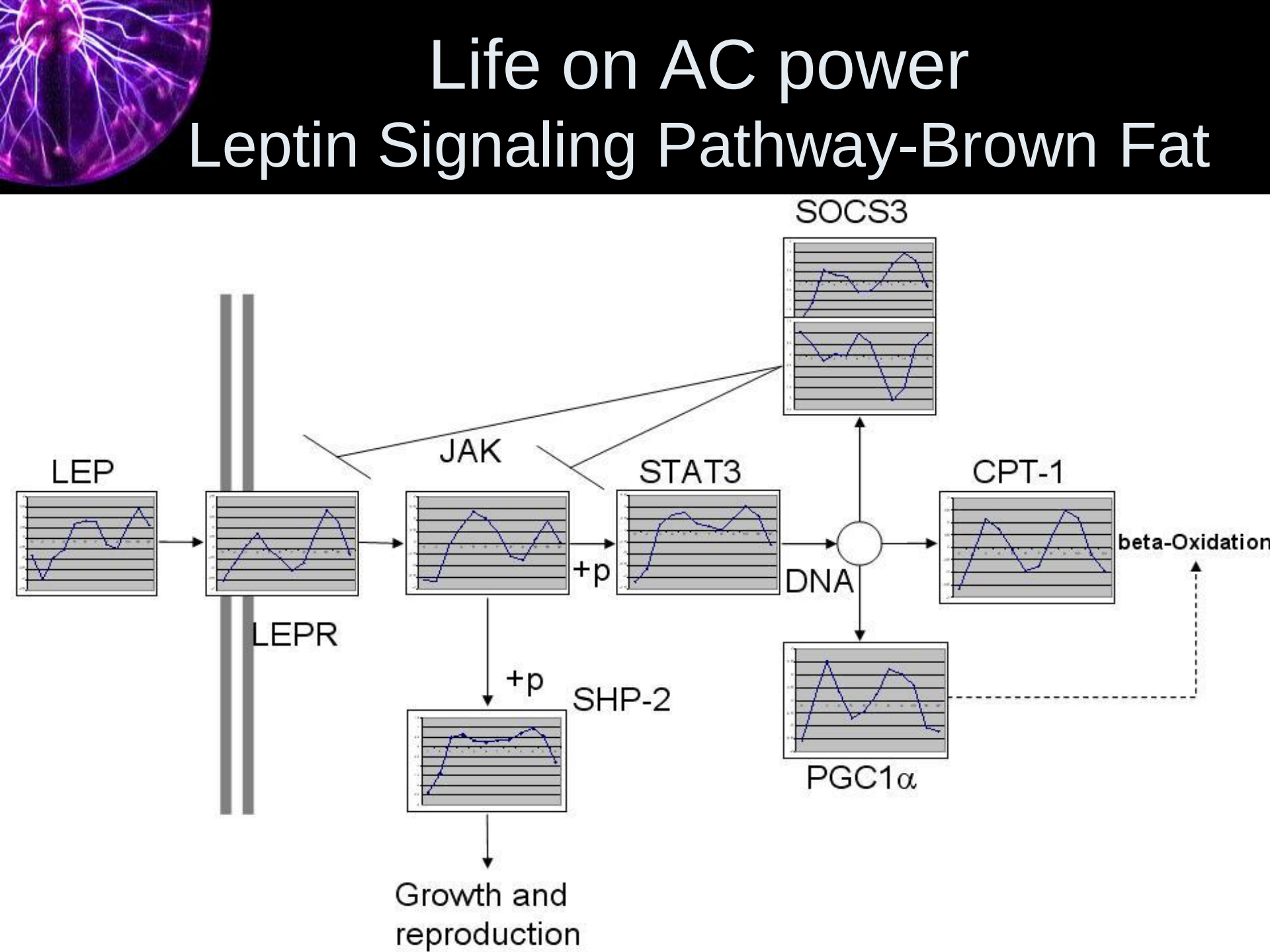
Oscillation in Biopathways: Life works on AC power



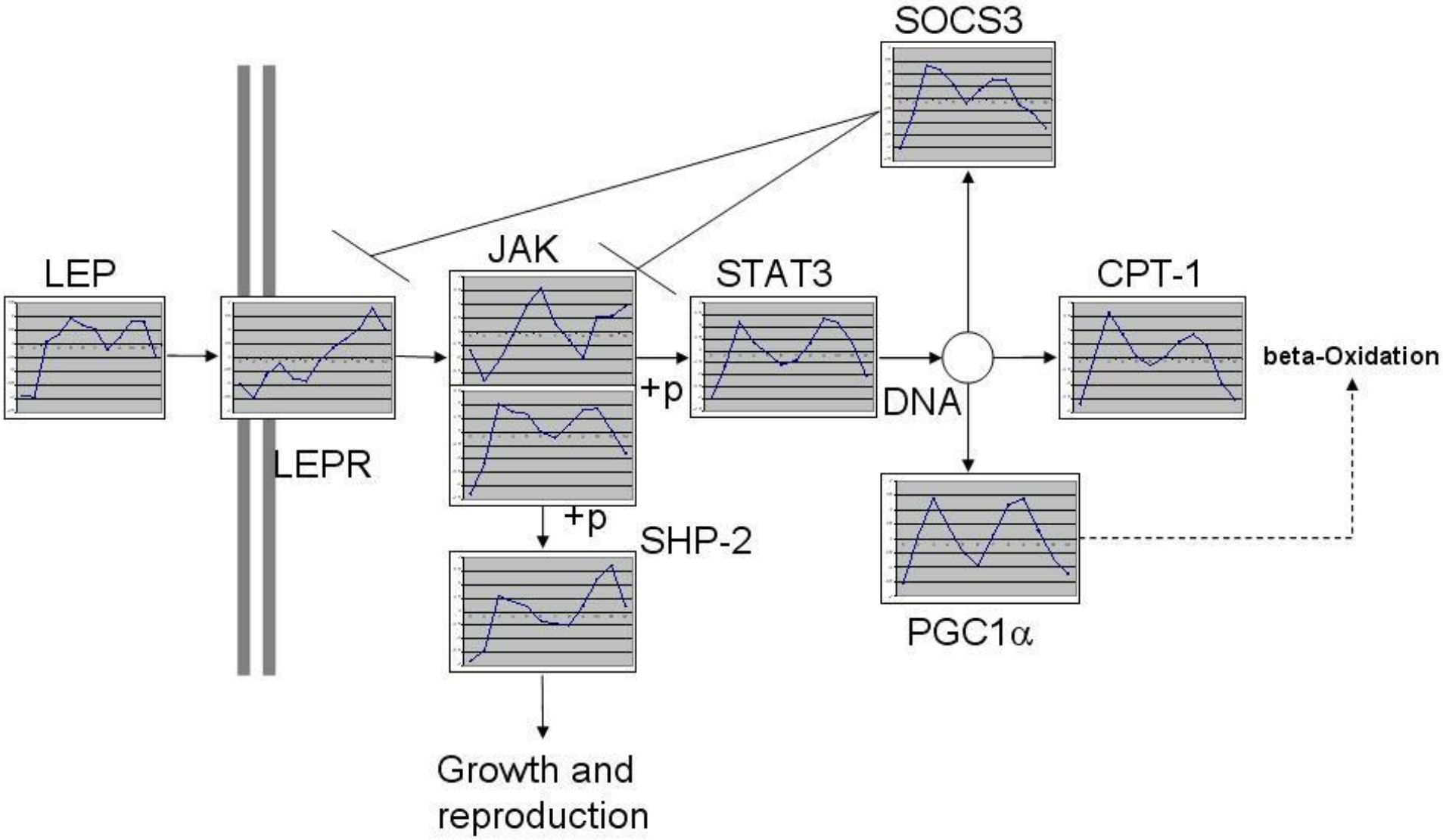
Life on AC power
Leptin Signaling Pathway-Liver



Life on AC power
Leptin Signaling Pathway-Brown Fat



Life on AC power
Leptin Signaling Pathway-White Fat



Mathematical model

$$\begin{cases} \dfrac{dn_1}{dt} = pr_p - r_{d1}; \\ \dfrac{dn_2}{dt} = (1-p)r_p - r_{d2}; \end{cases} \Rightarrow \begin{cases} \dfrac{dn_1}{dt} = pa\sin(\omega t + \alpha_1) - b\sin(\omega t + \alpha_2); \\ \dfrac{dn_2}{dt} = (1-p)a\sin(\omega t + \alpha_1) - c\sin(\omega t + \alpha_3); \end{cases}$$

$$n_1(t) = A\cos(\omega t + \beta_1); \qquad n_2(t) = B\cos(\omega t + \beta_2);$$

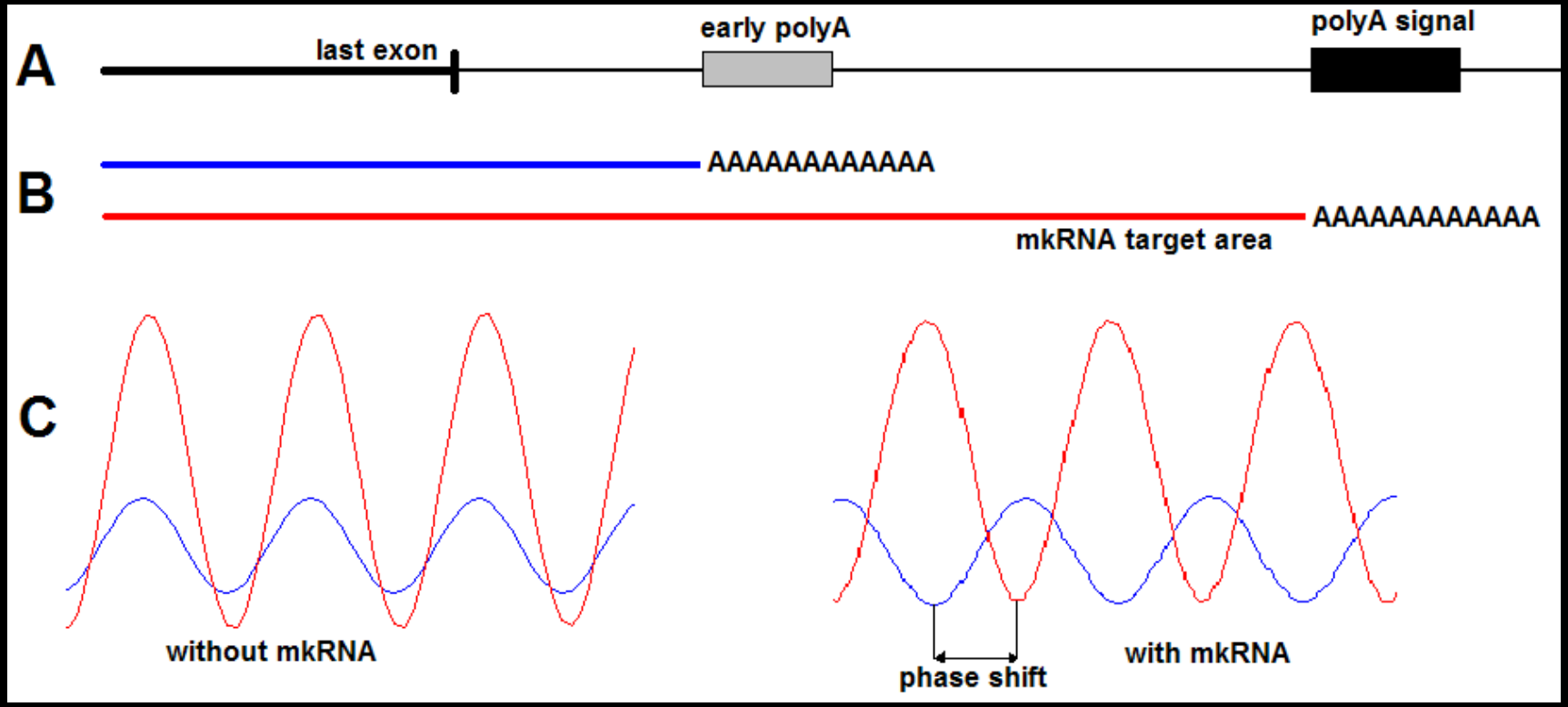
$$A = \sqrt{\left(\frac{pa}{\omega}\right)^2 - \left(\frac{b}{\omega}\right)^2 - \frac{2pab}{\omega^2}\cos(\alpha_1 - \alpha_2)}$$

$$B = \sqrt{(1-p)^2\left(\frac{a}{\omega}\right)^2 - \left(\frac{c}{\omega}\right)^2 - \frac{2(1-p)ac}{\omega^2}\cos(\alpha_3 - \alpha_1)}$$

$$\beta_2 - \beta_1 = arctan\left(\frac{c\sin\alpha_3 - (1-p)a\sin\alpha_1}{c\cos\alpha_3 - (1-p)a\cos\alpha_1}\right) - arctan\left(\frac{b\sin\alpha_2 - pa\sin\alpha_1}{b\cos\alpha_2 - pa\cos\alpha_1}\right)$$

The phase lag between isoforms may have values varying between 0 and 2p. In the middle of this range, when b_2-b_1=p the amplitude of n is reduced to 0.

Function of miRNA



Affy target sequences

```
11101 cacacaagga gccaaacaca gccaataggc agagagttga gggattcacc caggtggcta
11161 caggccaggg gaagtggctg caggggagag acccagtcac tcaggagact cctgagttaa
11221 cactgggaag acattggcca gtcctagtca tctctcggtc agtaggtccg agagcctcca
11281 ggccctgcac agccctccct tctcacctgg ggggaggcag gaggtgatgg agaagccttc
11341 ccatgccgct cacaggggcc tcacgggaat gcagcagcca tgcaattacc tggaactggt
11401 cctgtgttgg ggagaaacaa gttttctgaa gtcaggtatg gggctgggtg gggcagctgt
11461 gtgttggggt ggctttttc tctctgtttt gaataatgtt tacaatttgc ctcaatcact
11521 tttataaaaa tccacctcca gcccgccct ctccccactc aggccttcga ggctgtctga
11581 agatgcttga aaaactcaac caaatcccag ttcaactcag actttgcaca tatatttata
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11701 aaaaaaaaaa gtgacttgag
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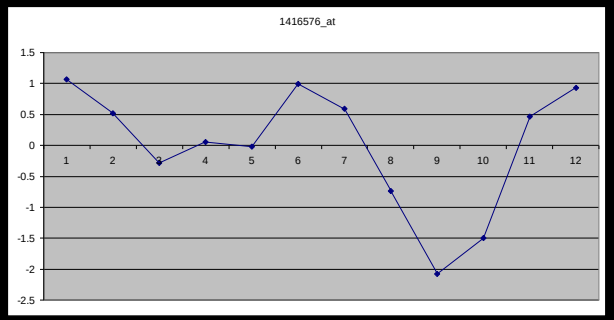
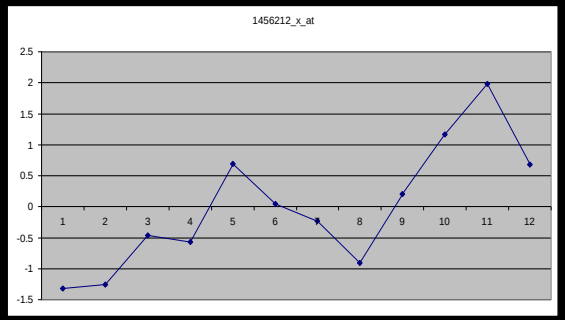
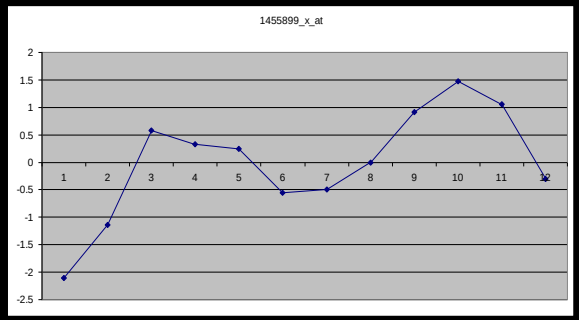
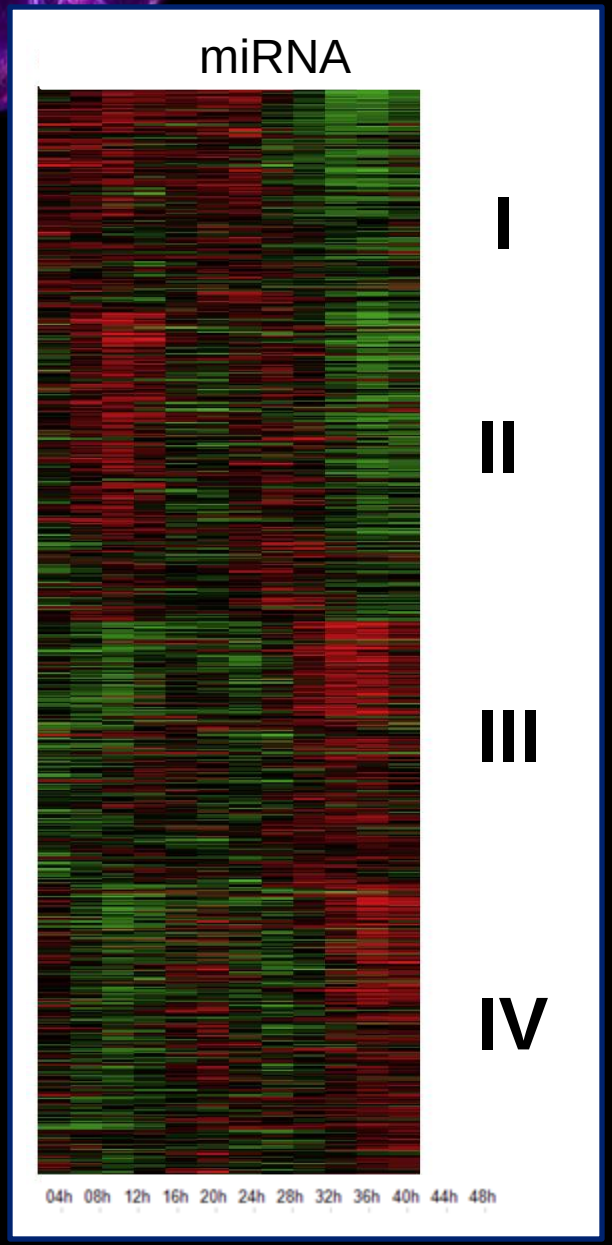


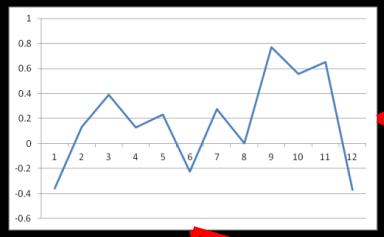


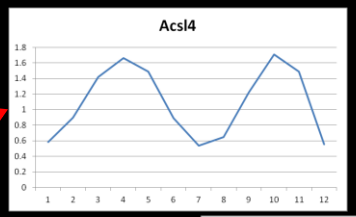


miRNA expression patterns

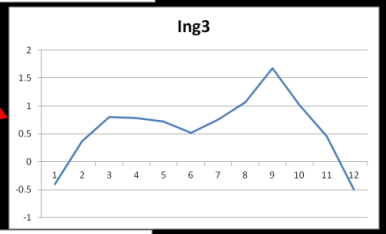


miR-187

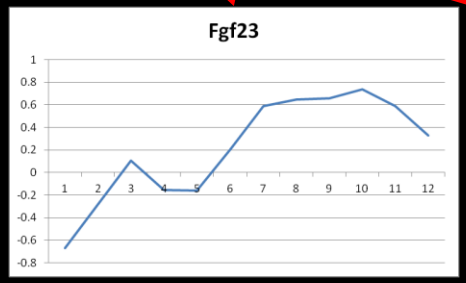




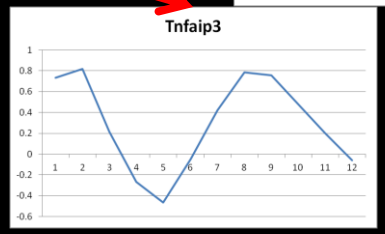
Acyl-CoA synthetase



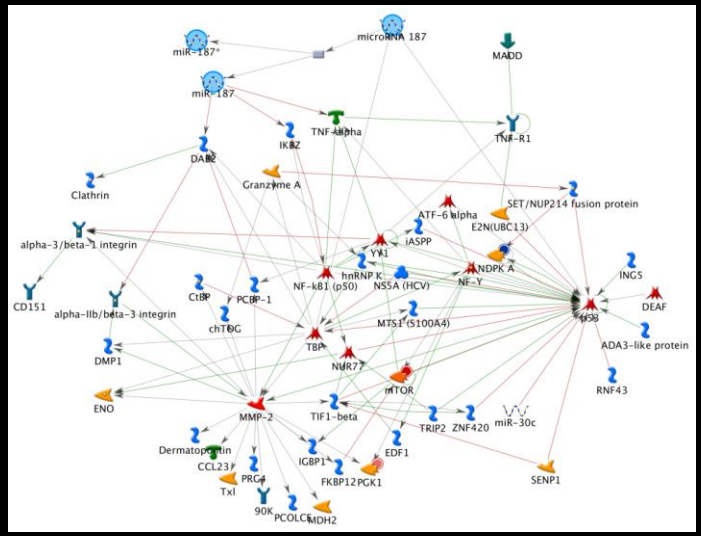
Inhibitor of growth



Fibroblast growth factor 23



Tumor necrosis factor, alpha-induced protein 3



Math again

Let x_p be rate of transcription, x_d- rate of mRNA degradation and x- abundance of transcript.

$$x_p(t) = \sin(\omega t + \alpha_1);\ x_d(t) = \sin(\omega t + \alpha_2);$$

$$\frac{dx}{dt} = \sin(\omega t + \alpha_1) - \sin(\omega t - \alpha_2) = 2\cos\left(\omega t + \frac{\alpha_1 + \alpha_2}{2}\right)\sin\left(\frac{\alpha_1 - \alpha_2}{2}\right)$$

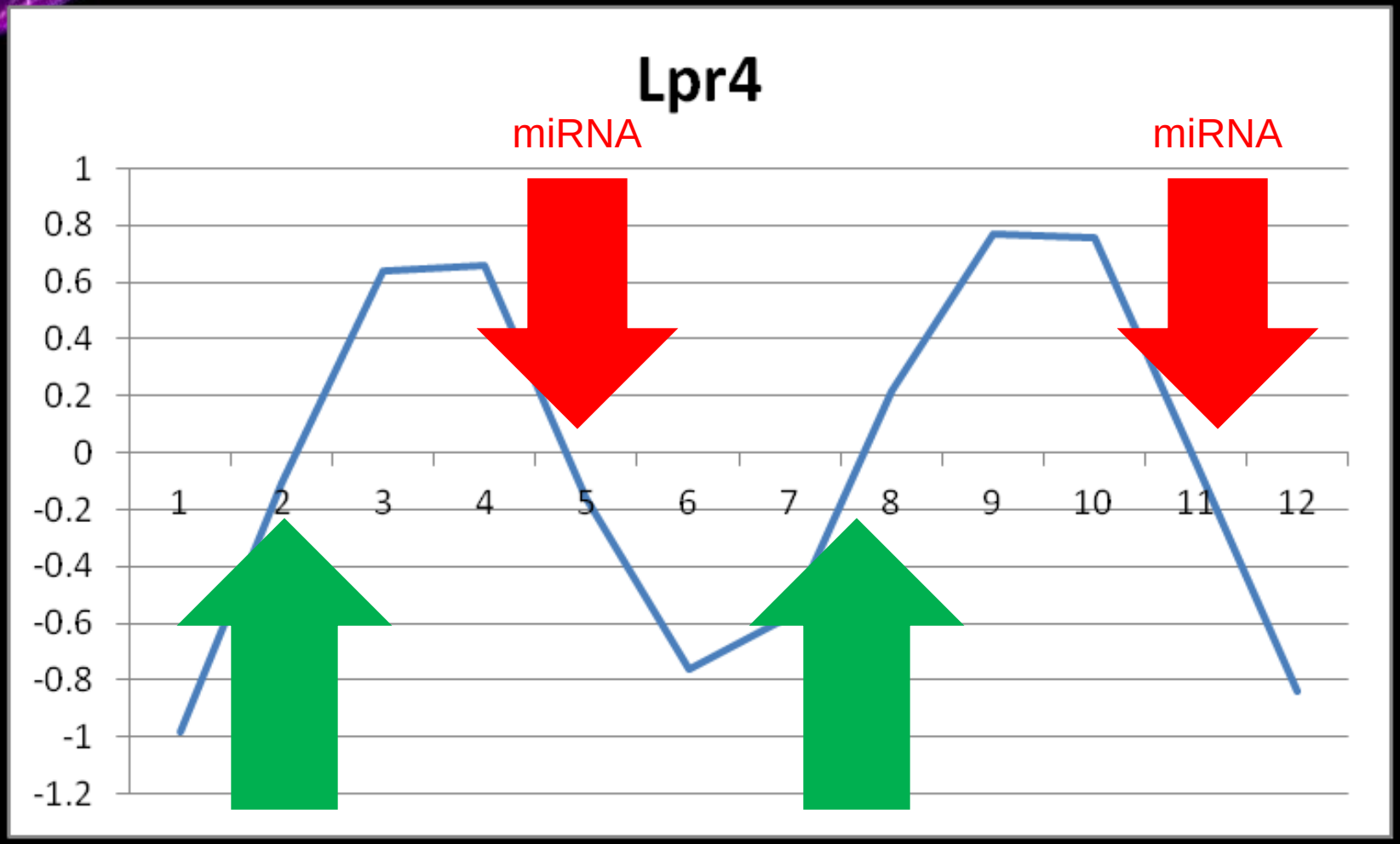
$$= 2\sin\left(\frac{\alpha_1 - \alpha_2}{2}\right)\cos\left(\omega t + \frac{\alpha_1 + \alpha_2}{2}\right)$$

$$x(t) = \int 2\sin\left(\frac{\alpha_1 - \alpha_2}{2}\right)\cos\left(\omega t + \frac{\alpha_1 + \alpha_2}{2}\right)dt$$

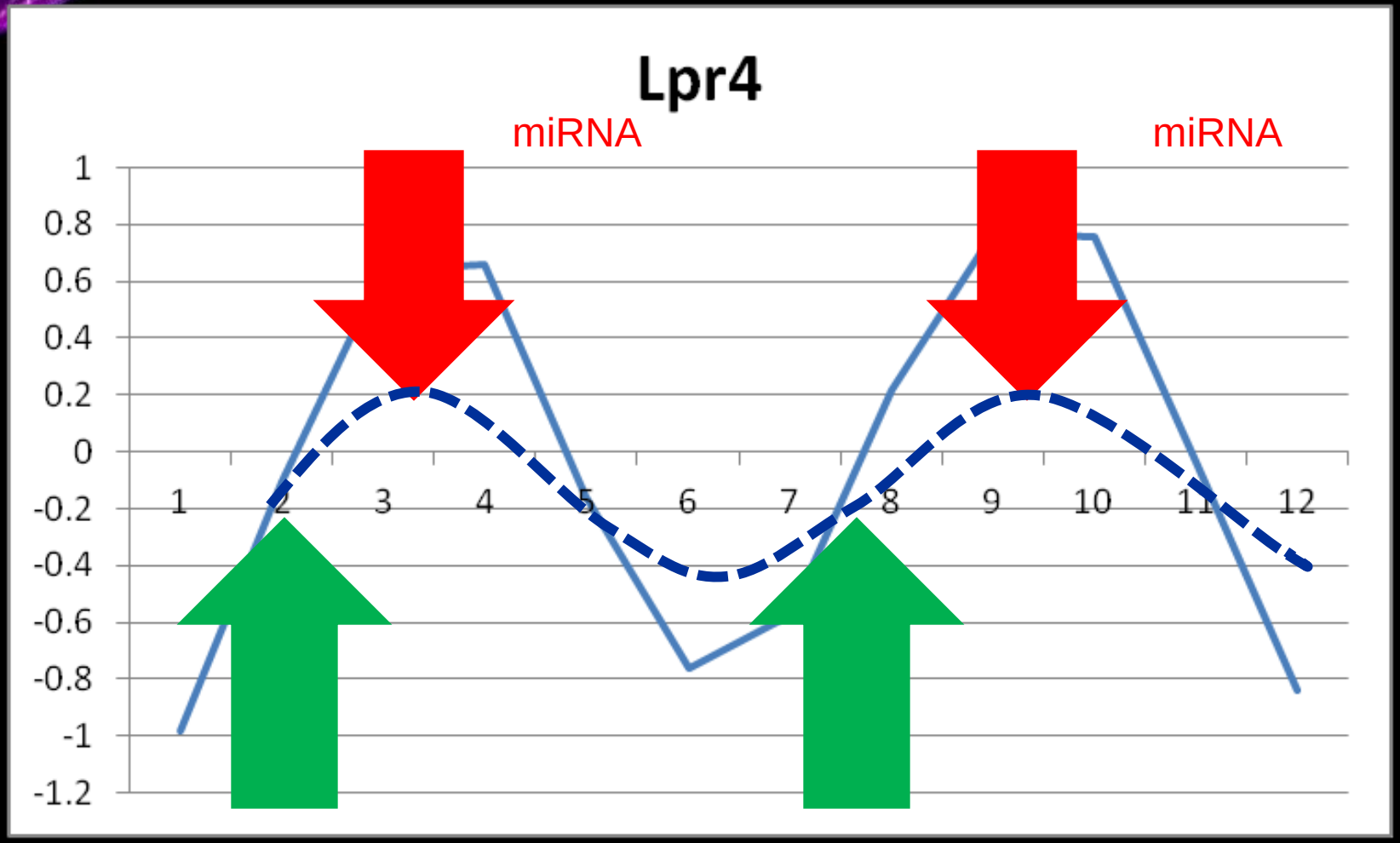
$$x(t) = \frac{2}{\omega}\sin\left(\frac{\alpha_1 - \alpha_2}{2}\right)\sin\left(\omega t + \frac{\alpha_1 + \alpha_2}{2}\right) + C$$

The new amplitude reaches peak when $\frac{\alpha_1 - \alpha_2}{2} = \frac{\pi}{2}$

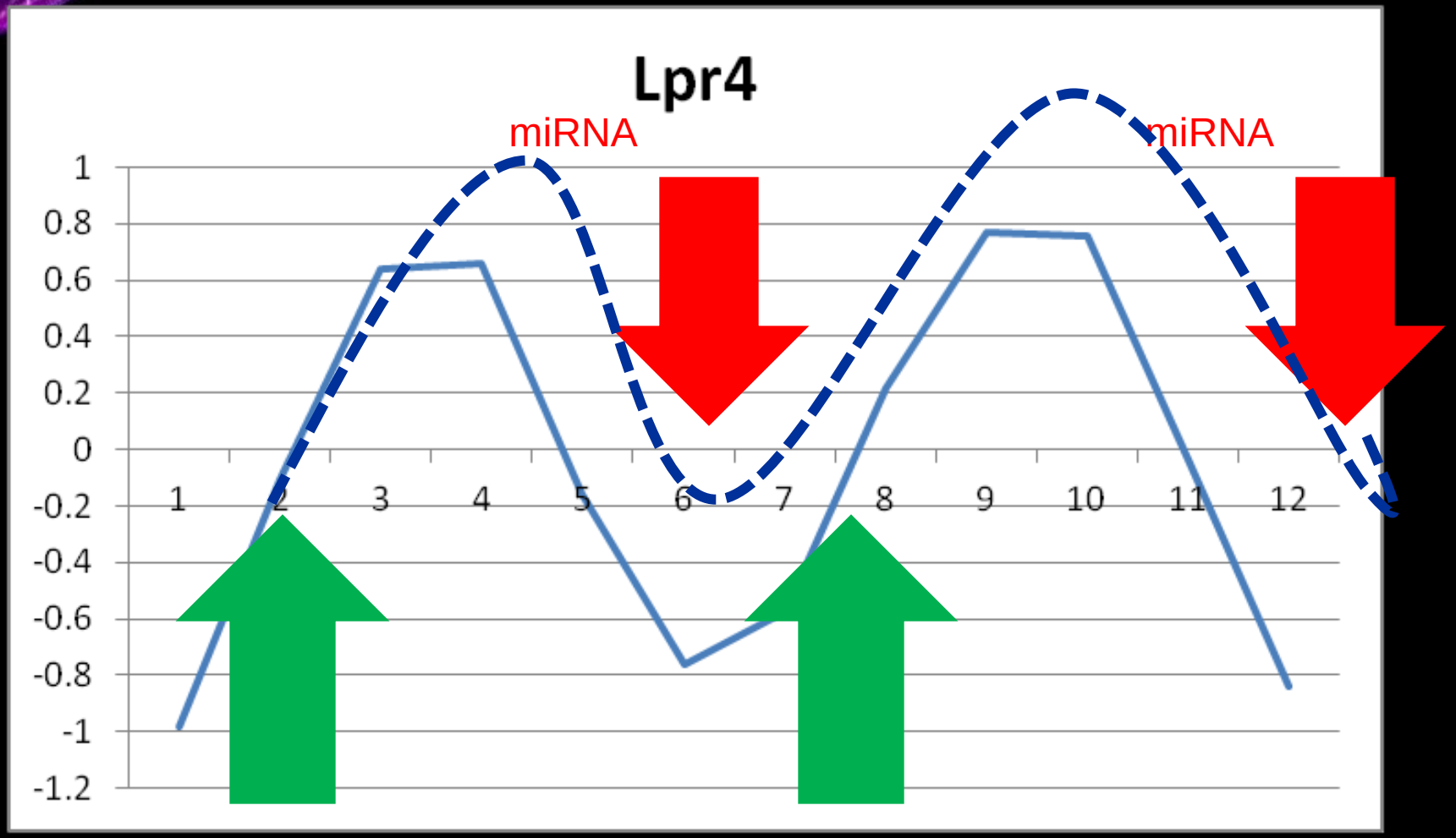
Function of miRNA



miRNA too early



miRNA too late



Summary of observations

- Oscillation of baseline expression is an immanent property of all genes, not a function of some;
- Phase and amplitude of expression are important characteristics of expressed genes and specific to tissues and conditions;
- Alternative expression variants can have different oscillatory properties;
- miRNAs play important role is oscillatory regulation of gene expression;
- Accounting for the oscillatory properties of gene expression is essential for understanding and modeling of biological processes

Acknowledgements

- Jeffrey M. Gimble (PBRC)
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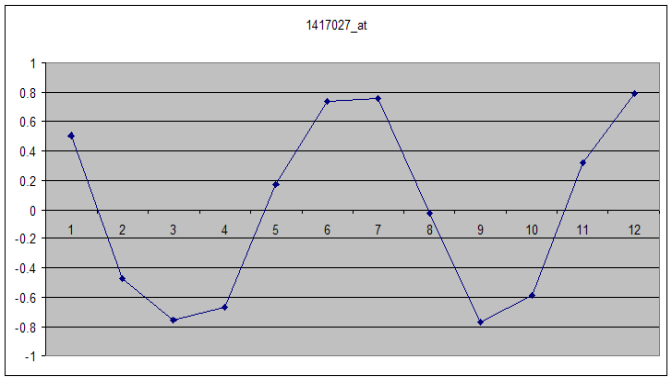
- Molly Bray (UAB)

Postulates for a better chronobiology paradigm

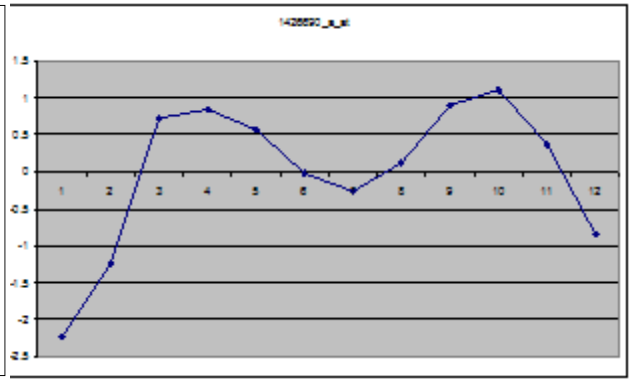
- The function of circadian clock is to synchronize multiple oscillators to environmental cues and temporary timekeeping
- Circadian molecular clock does not make gene expression oscillate
- All transcripts are expressed in oscillating pattern ever since the dawn of life
- Constant abundance of certain transcripts and steady production rate of corresponding proteins is an evolutional adaptation providing uniform response to a signal at any time
- At least one of the mechanisms creating such time-independent response is provided by alternative transcripts oscillating in counter-phase

Can it be disrupted?

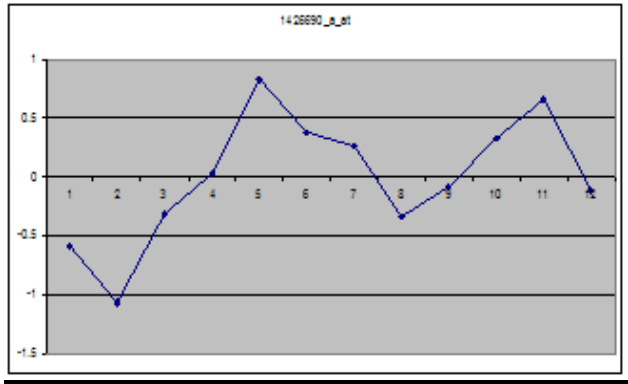
GAPDH



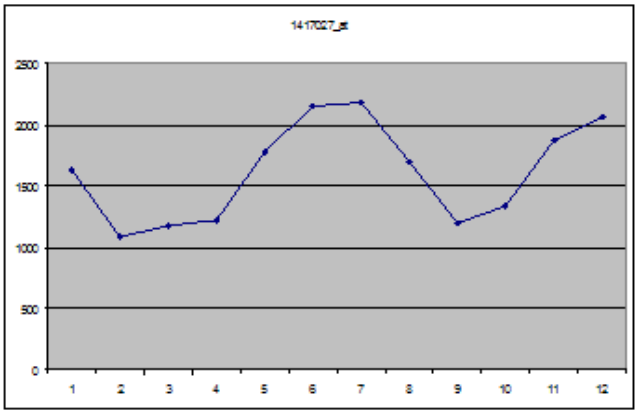
SREBP-white fat

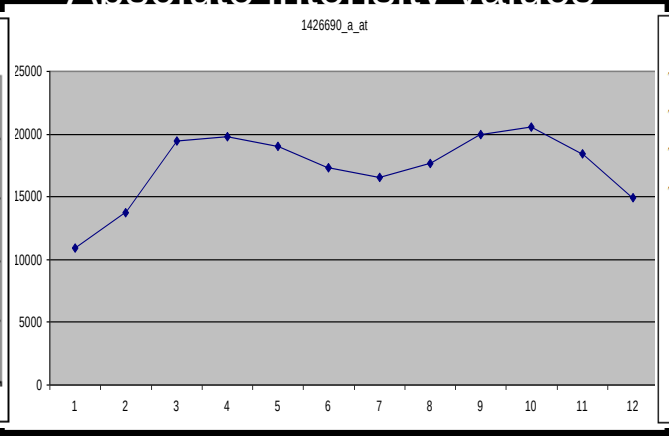


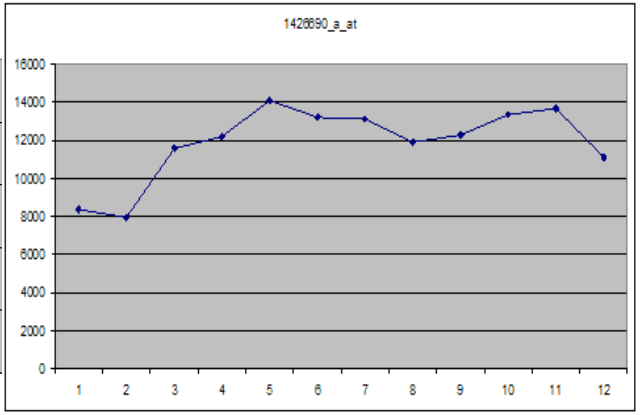
SREBP-liver



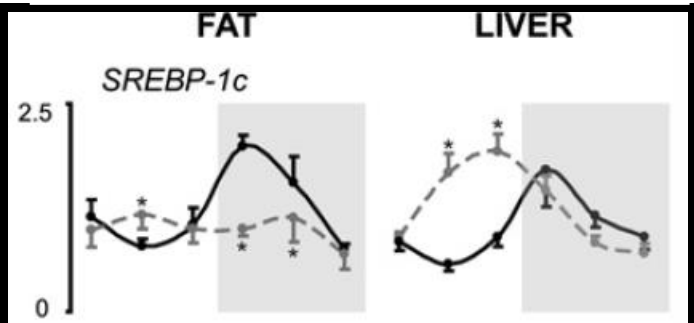
Absolute intensity values





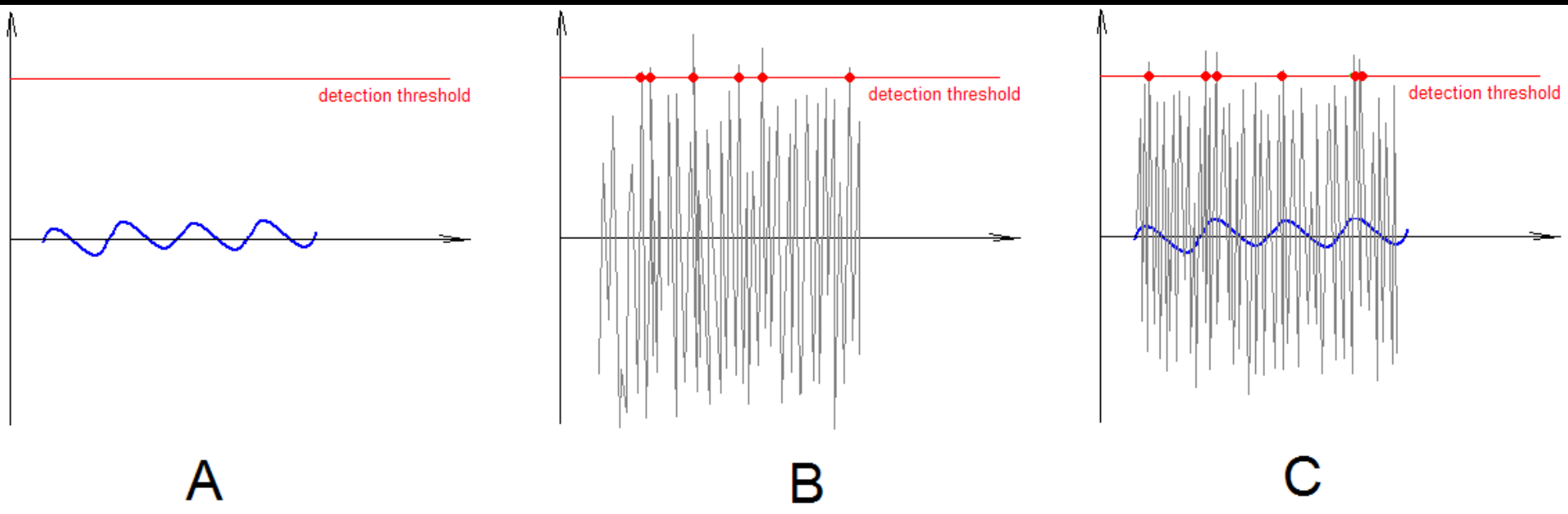


From Cell Metabolism:

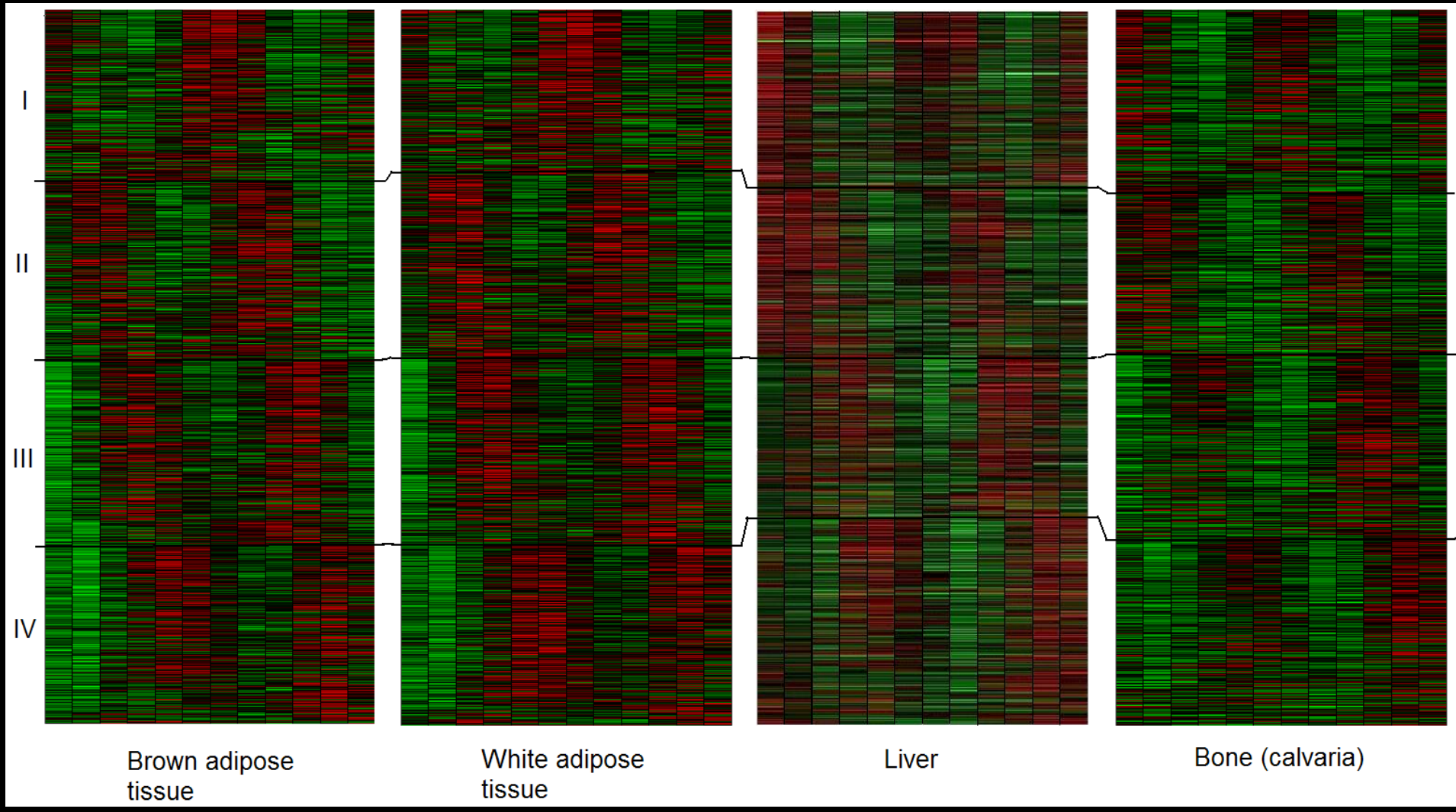


Kohsaka, A. *et al. Cell Metab.* 6, 414-421 (2007).

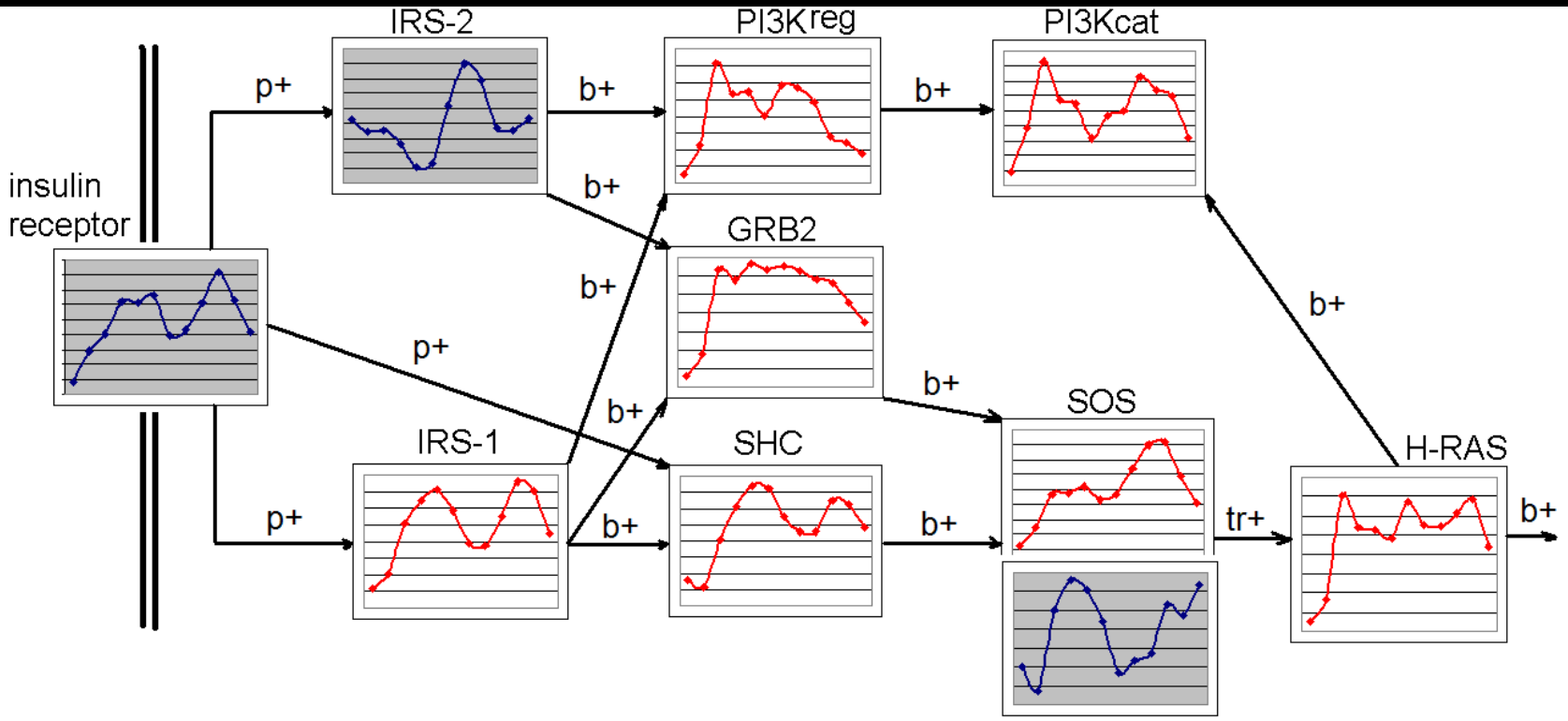
Stochastic resonance



Ghosts?

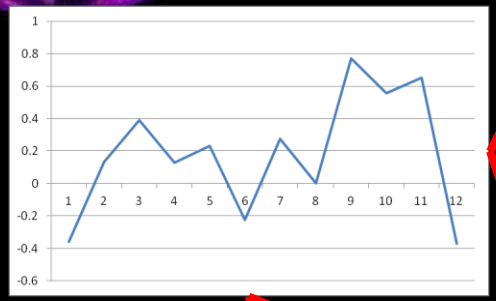

I
II
III
IV
Brown adipose tissue
White adipose tissue
Liver
Bone (calvaria)

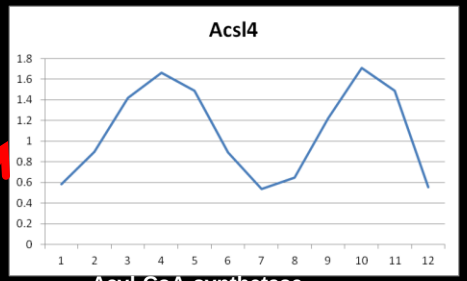
Team Spirits?



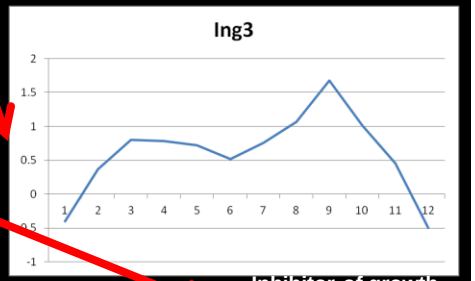
miRNA and target mRNA

miR-187

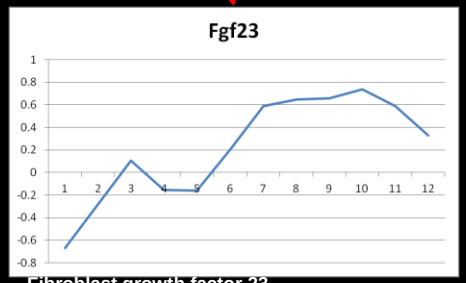




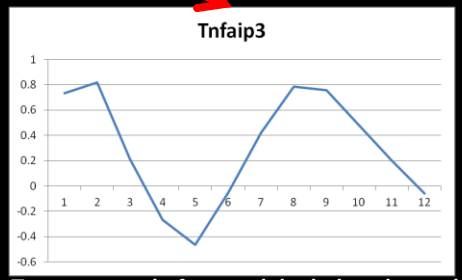
Acyl-CoA synthetase



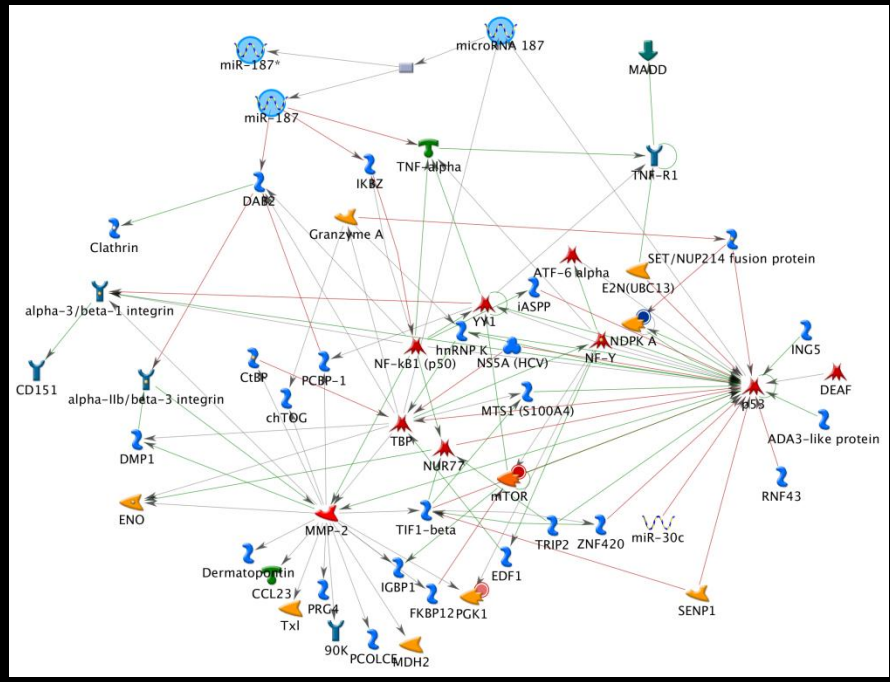
Inhibitor of growth



Fibroblast growth factor 23

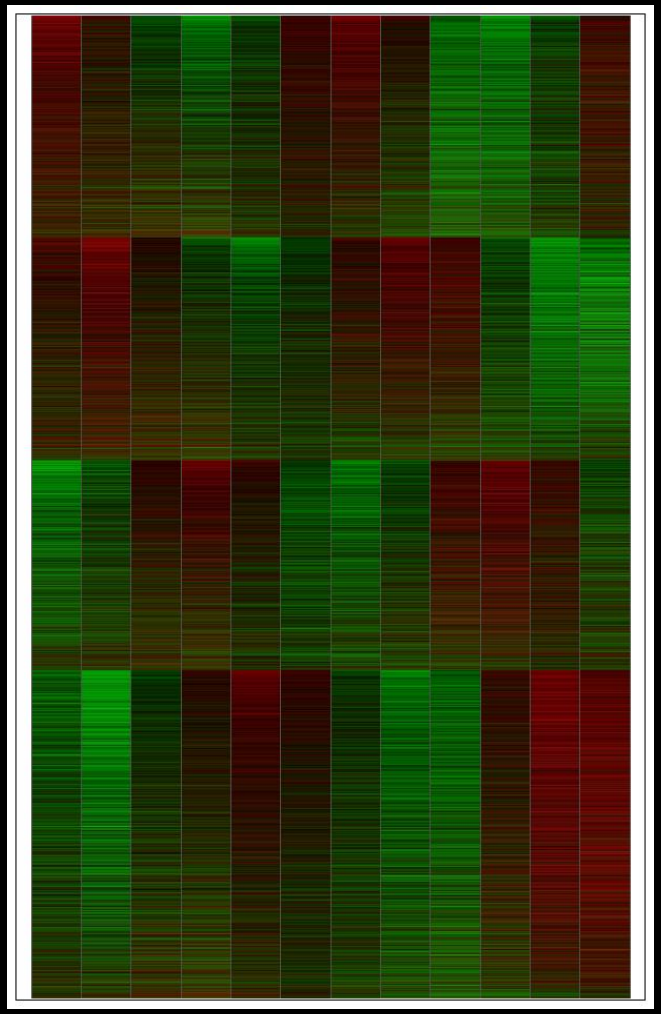


Tumor necrosis factor, alpha-induced protein 3



Regulation of ovarian cancer progression by microRNA-187 through targeting Disabled homolog-2.
IL-10-induced microRNA-187 negatively regulates TNF-?, IL-6, and IL-12p40 production in TLR4-stimulated monocytes.
miR-187 is an independent prognostic factor in breast cancer and confers increased invasive potential in vitro.

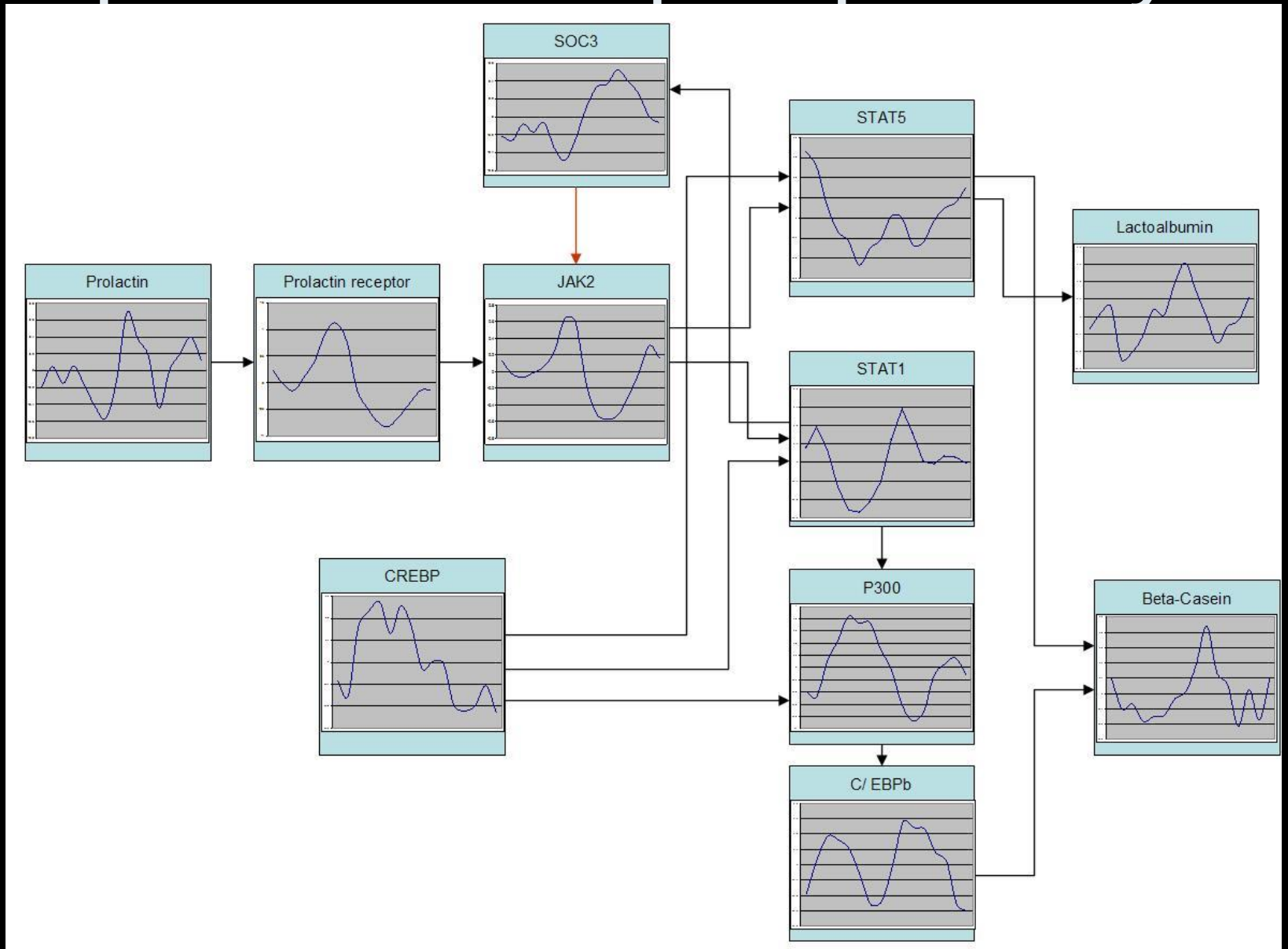
Circadian oscillation in *D. melanogaster* (heads)



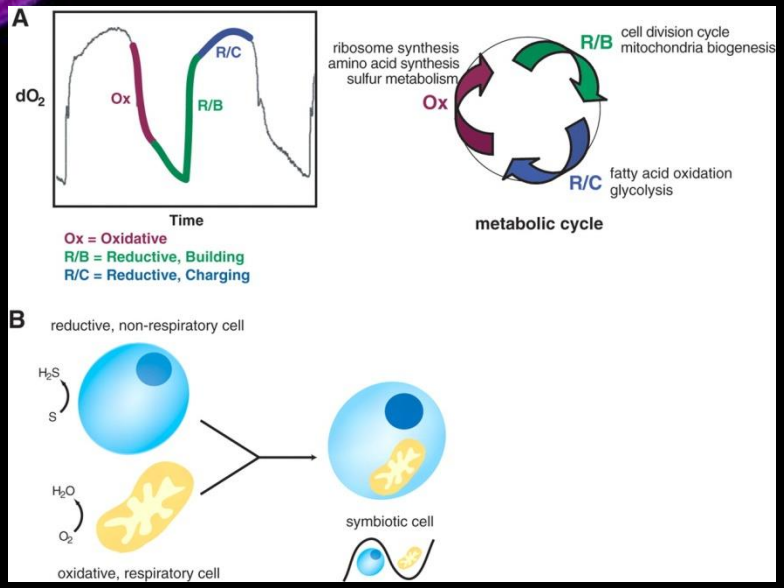
Original report: 172 “light-entrained” transcripts

Boothroyd CE, Wijnen H, Naef F, Saez L, Young MW (2007) Integration of light and temperature in the regulation of circadian gene expression in Drosophila. PLoS Genet 3(4): e54.doi:10.1371/journal.pgen.0030054

prolactin receptor pathway



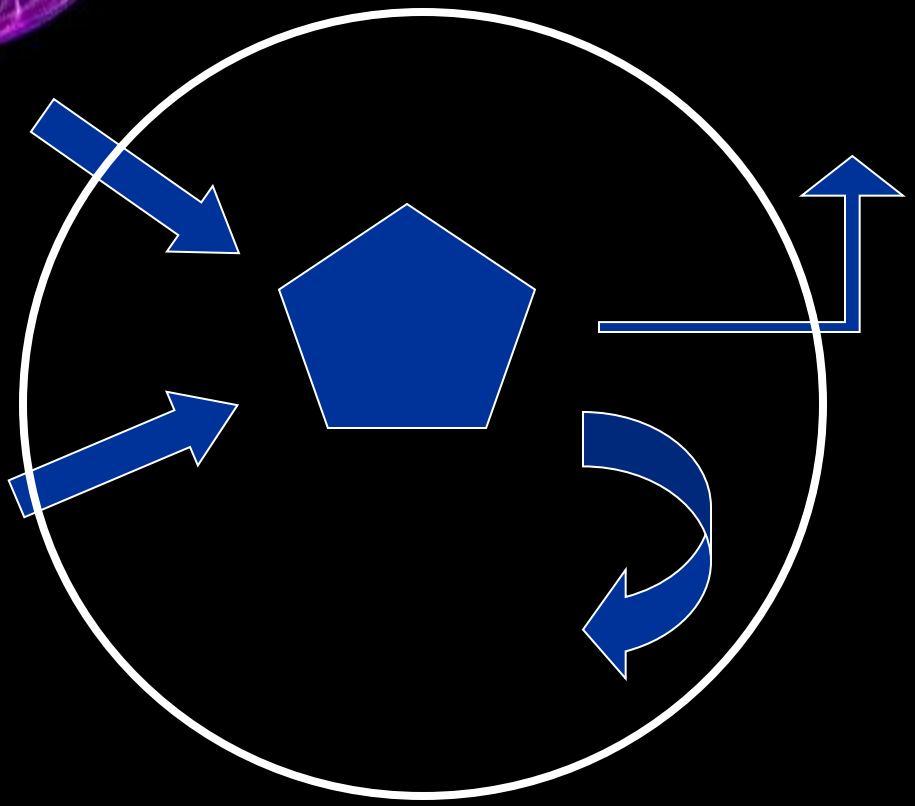
McKnight's hypothesis



Temporal compartmentalization in a simple eukaryote. (A) Key cellular processes are compartmentalized in time via the metabolic cycle. The ordered progression through distinct phases (Ox, R/B, and R/C) of the metabolic cycle allows temporal compartmentalization of numerous cellular and metabolic processes. (B) Proposed hypothesis for the evolution of metabolic oscillation. After a fusion event between a respiring bacterium and a nonrespiring eukaryotic host, the resulting symbiont evolved to carry out the distinct metabolic programs of the progenitors at separate times, forming the basis of a metabolic cycle. (Tu et al. 2005)

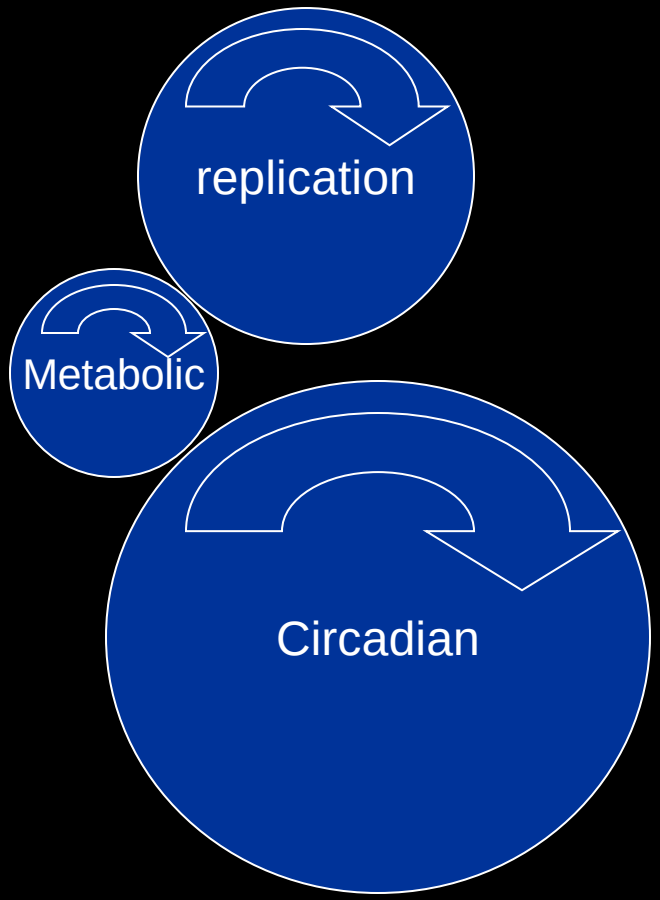
- Tu BP, Kudlicki A, Rowicka M, McKnight SL. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science. 2005 Nov 18;310(5751):1152-8.
- Selkov E., "On the Mechanism of Single-Frequency Self-Oscillations in Glycolysis. I. A Simple Kinetic Model," Eur. J. Biochem. 4(1), 79-86, 1968
- Sel'kov E., "Stabilization of Energy Charge, Generation of Oscillation and Multiple Steady States in Energy Metabolism as a Result of Purely Stoichiometric Regulation," Eur. J. Biochem. 59(1), 151-157, 1975.

A better paradigm:



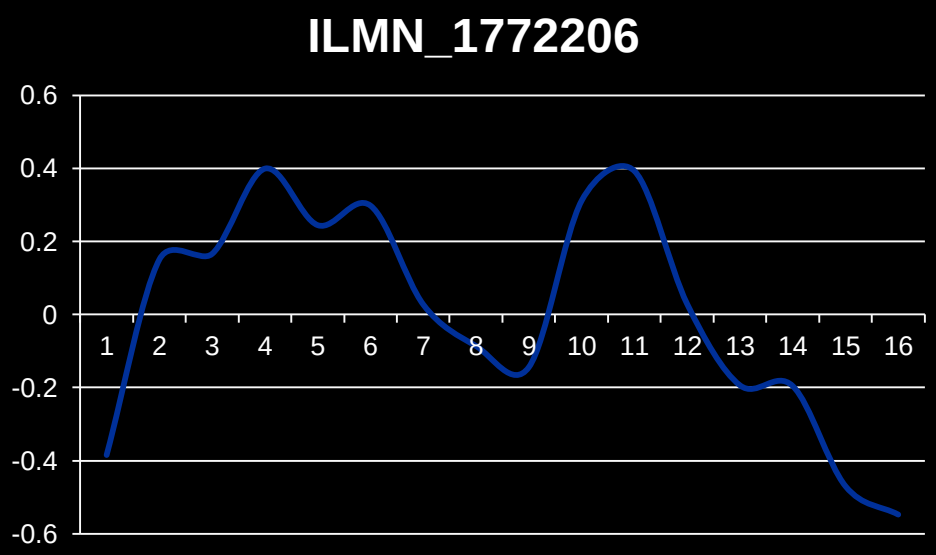
- First biochemical reactions were probably gated by periodic changes in light and temperature
- As soon as a set of self-reproducing ribozyme reactions became contained inside a proto-cell rhythm became an organizing principle for multiple, often mutually exclusive processes sharing the same intracellular compartment
- Introduction of peptide enzymes and structures increased complexity, adding structures reusable through a number of cycles, possibly created a mechanism for period multiplication

A better paradigm: multiple gears

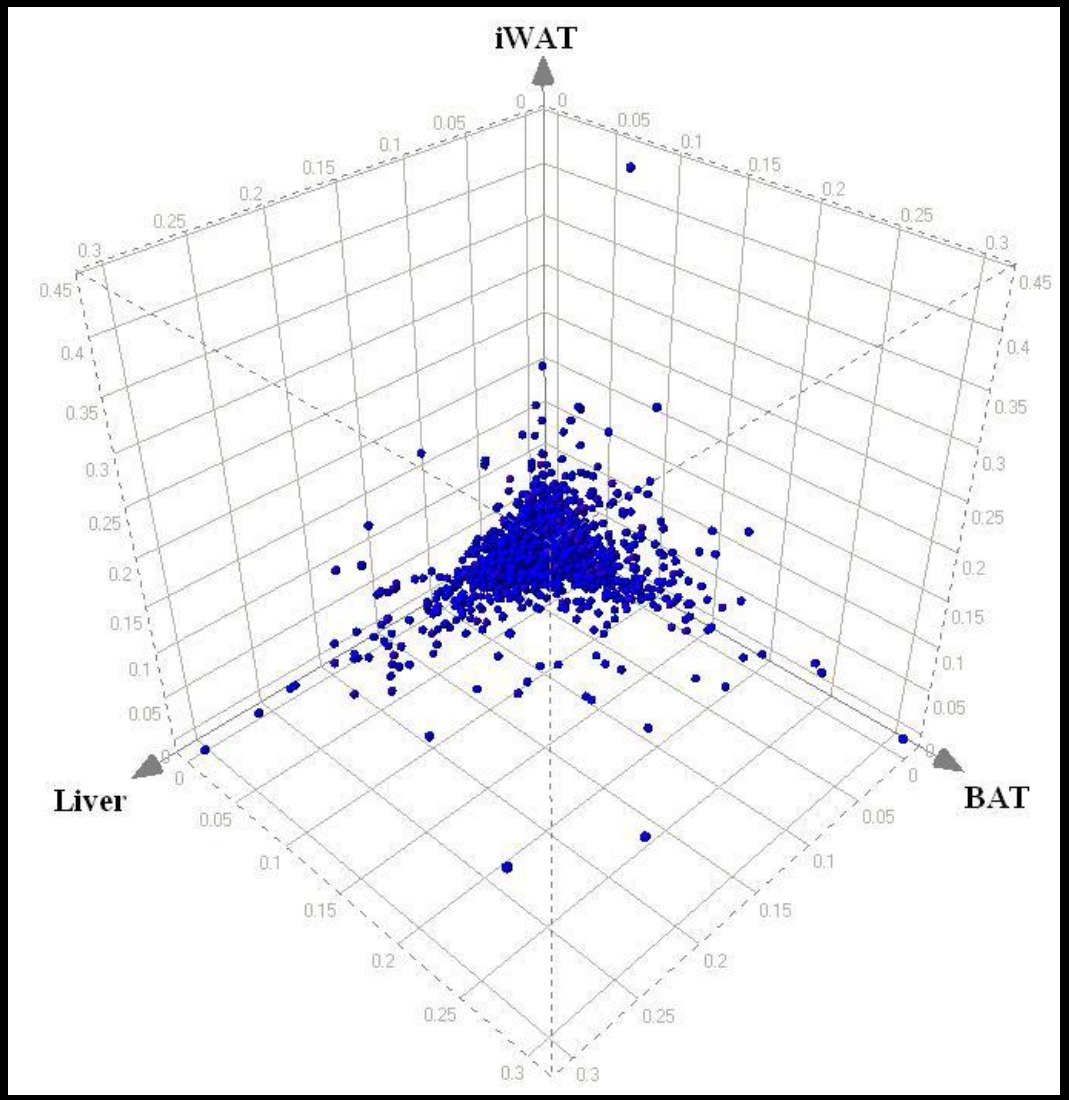


- Cell cycle is a derivative of metabolic cycle
- Circadian cycle is either equal or multiple of metabolic cycle, possibly through period duplication
- Circadian oscillation is an evolutional adaptation in organisms which can get advantage of being prepared to the oncoming periodic change of environment
- The need for preemptive response to the daily change appeared independently in different distantly related groups of organisms. In each case different genes, but the same general principle were recruited to form an internal temporary timekeeper – circadian molecular clock.

human CYP3A4



Amplitude of oscillation is tissue-specific



Phase of expression is tissue-specific