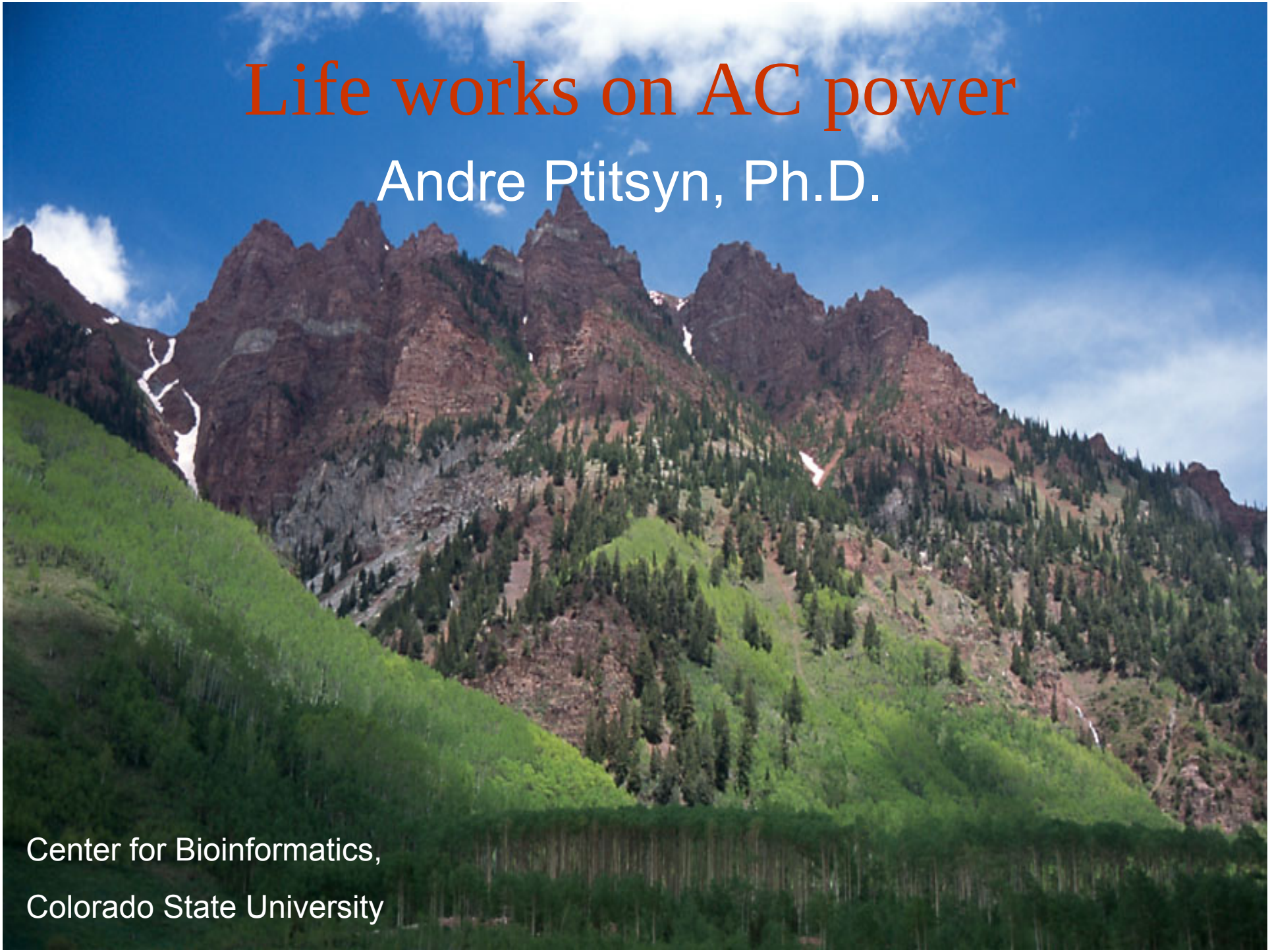


Life works on AC power

Andre Ptitsyn, Ph.D.

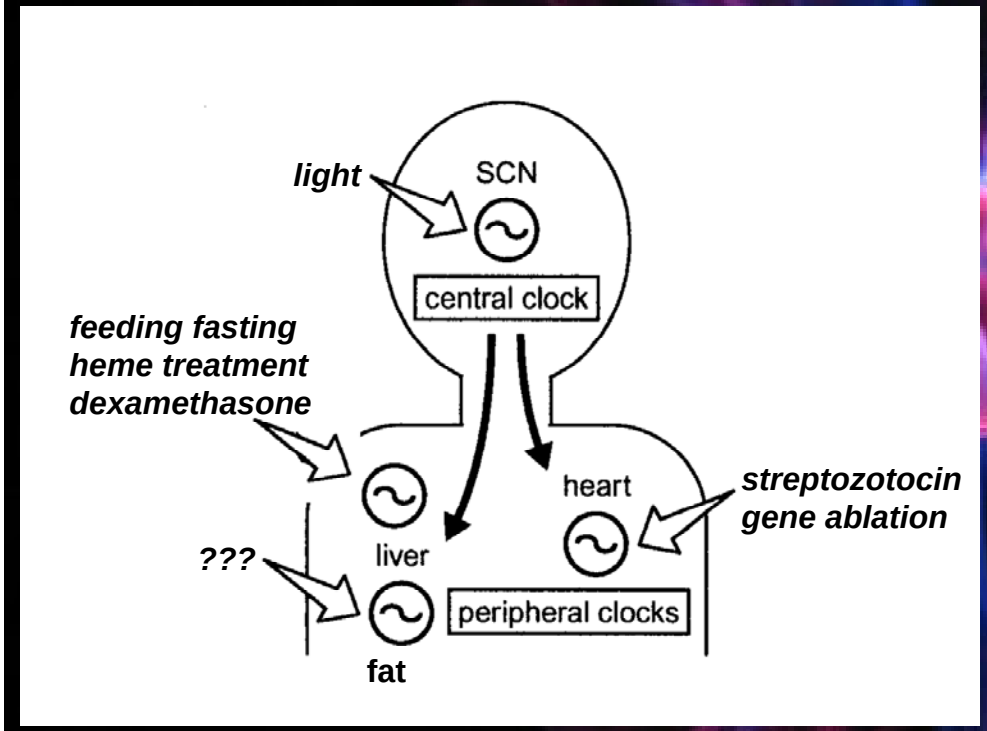
Center for Bioinformatics,

Colorado State University

Active research projects

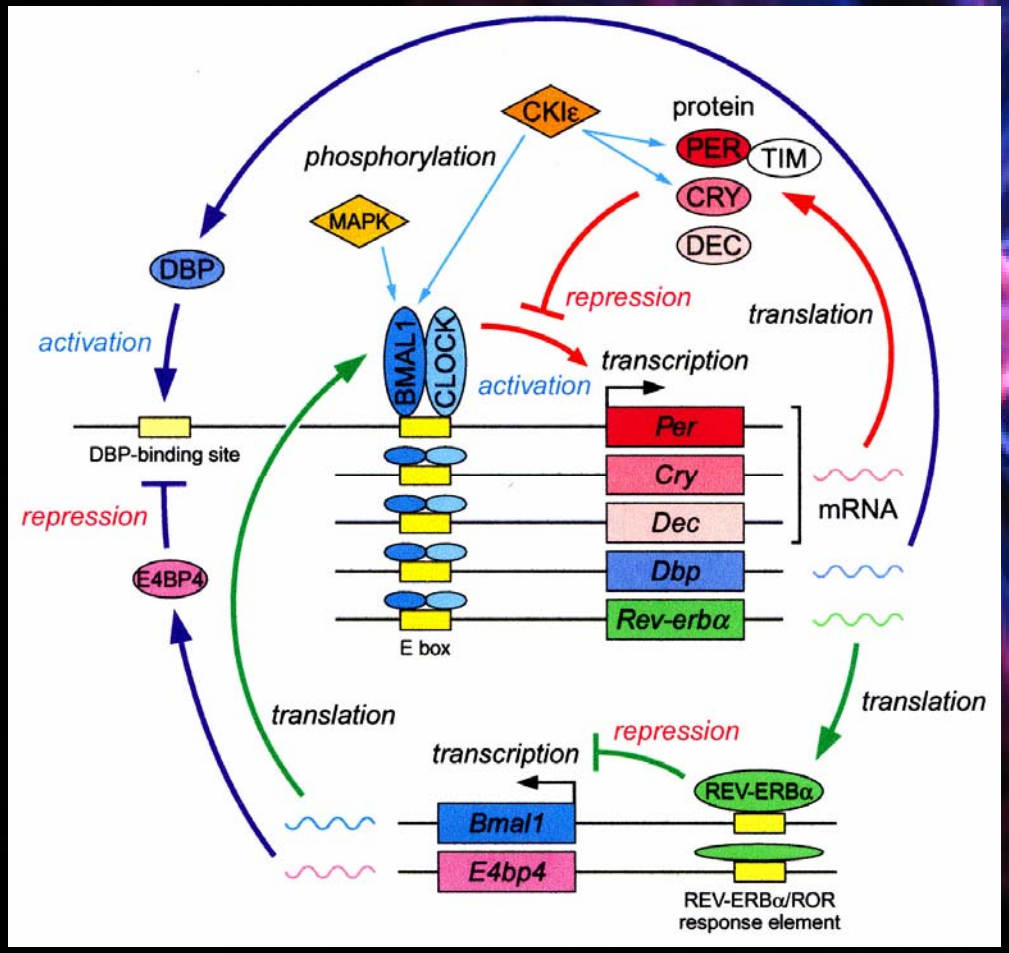
- Fragment match and assembly algorithms
- Algorithms for Microarray normalization, and identification of differentially expressed genes in context of biological pathways
- Molecular phenotyping of cancer
- Cluster analysis of stem cell molecular phenotypes
- High-performance libraries for bioinformatics
- Computational Analysis of insulin resistance in skeletal muscle
- Computational analysis of circadian periodicity in gene expression

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

Mammalian molecular clock

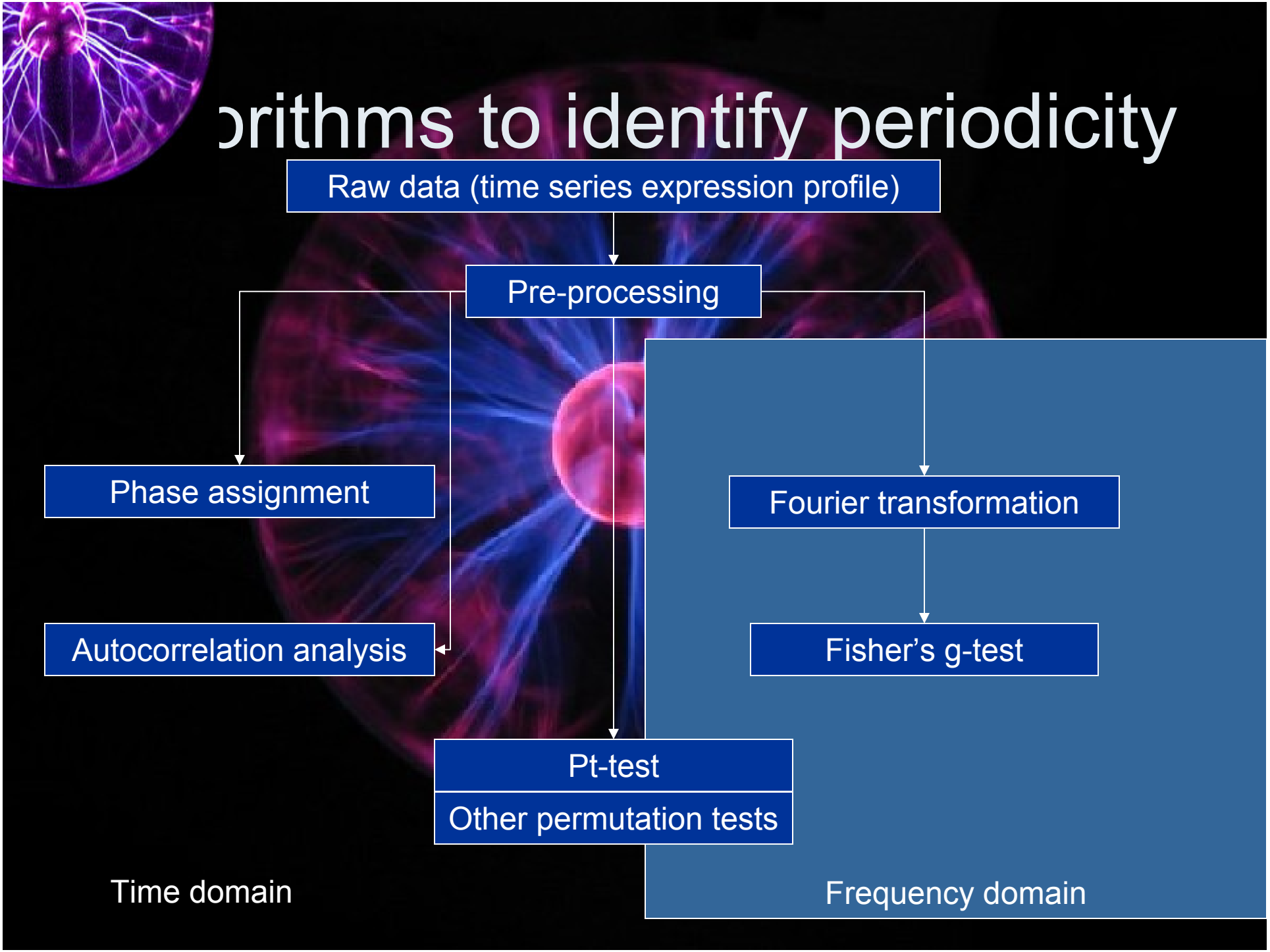


- BMAL1 and CLOCK (NPAS2) form heterodimers that act as positive transcriptional regulators
- PERIOD (Per1, Per2, Per3) and CRYPTOCHROME (Cry1, Cry2) family members serve as negative transcriptional regulators
- downstream targets, such as the albumin D site binding protein (DBP), can further activate 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

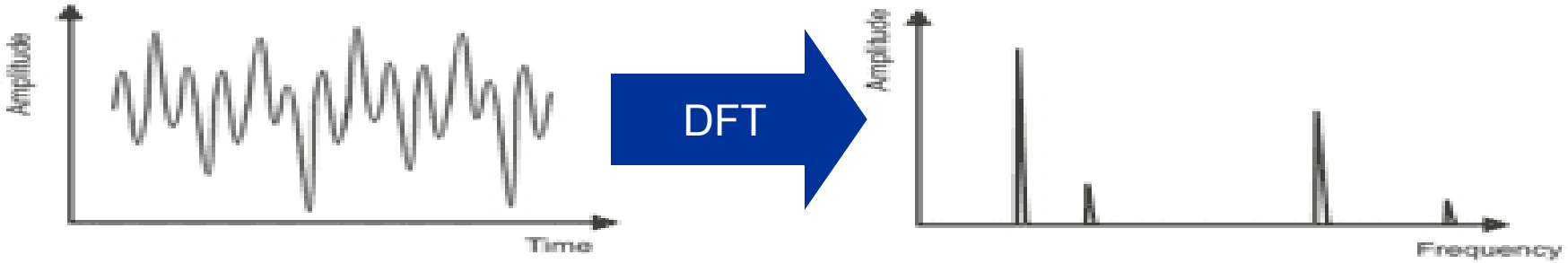
Data

- PBRC Data Set (Zvonic et al Diabetes 2006) AKR/J mice acclimated to a 12 hr light: 12 hr dark cycle, harvesting sets of 3-5 mice at 4 hr intervals. For each of liver, brown adipose and white adipose tissue we have over 22,000 time series gene expression profiles. Each time series consists of 2 periods sampled 6 times each, i.e. 12 time points.
- PBRC Bone data set (Zvonic et al. accepted in JBMR 2006).
- GNF Data Set (Panda et al. 2002, http://expression.gnf.org/cgi-bin/circadian/index.cgi). Time series expression profiles for murine liver, aorta, SCN and kidney. Nearly 10,000 genes in murine liver, measured at 4hr intervals over a 48hr period with 2 replicates for each time point. Mice entrained to a 12hr light/dark cycle, samples collected in complete darkness.
- Harvard Data Set (Storch KF et al. 2002). Derived from liver and heart samples, over 12,000 genes each. Each data set consisted of 12 time points collected at even intervals of 4 hours over a period of 48 hours., over Mice entrained to a 12hr light/dark cycle, samples collected in constant dim light.

orithms to identify periodicity



Fisher's g-test



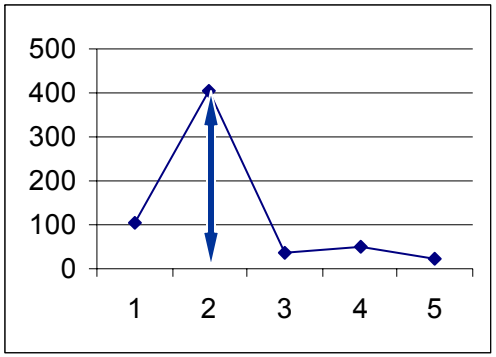
$$I(\omega) = \{\omega_0, \omega_1, \omega_2, \ldots \omega_{N/2-1}\}$$

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

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

Based on periodogram
Signal to noise ratio

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

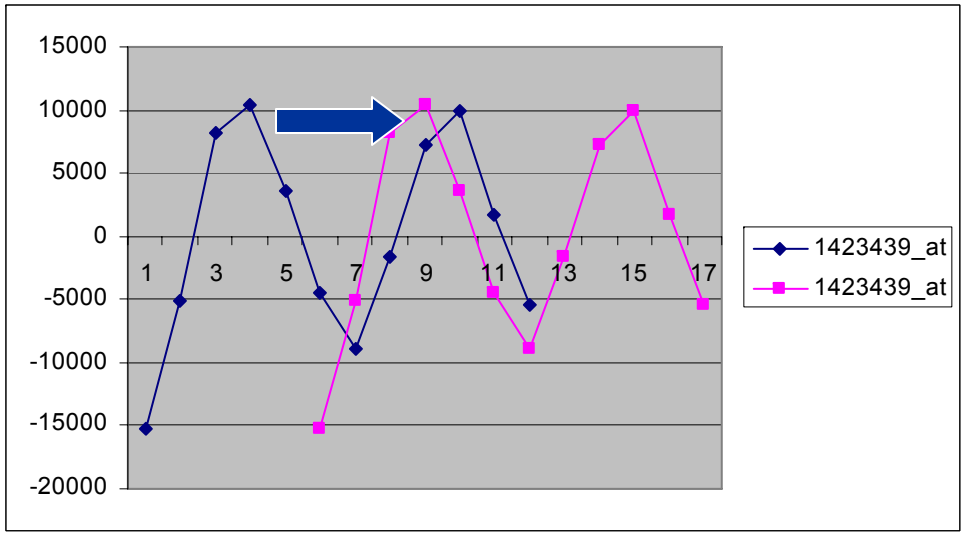


Fisher's formula produces p-value for significance of oscillation:

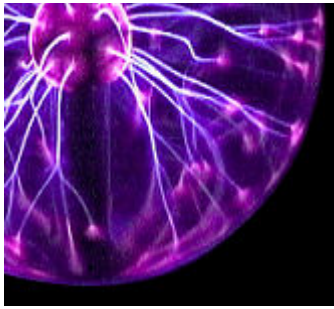
$$P(g > x) = \sum_{p=1}^{1/x}\left[(-1)^p \frac{n!}{p!(n-p)!}(1-px)^{n-1}\right],$$

Autocorrelation and Phase Assignment

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

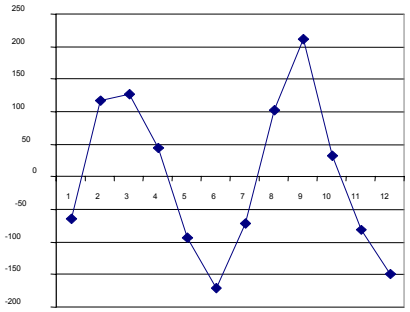


$$R(f) = \frac{\sum_0^{N-1} (x_i - \bar{x})(y_f - \bar{y})}{\sum_0^{N-1} (x_i - \bar{x})(y_i - \bar{y})} \quad \text{, where} \quad y_i = \cos\left(\frac{2\pi}{p} * i\right)$$

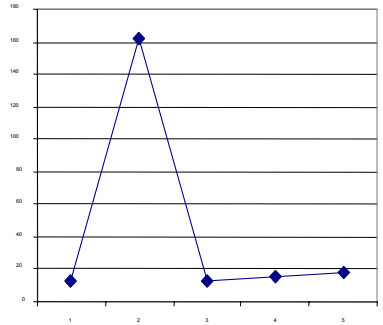


Pt-test

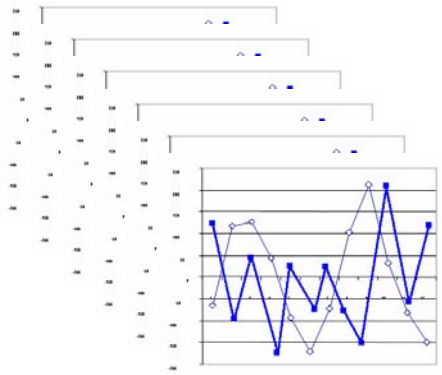
Original profile



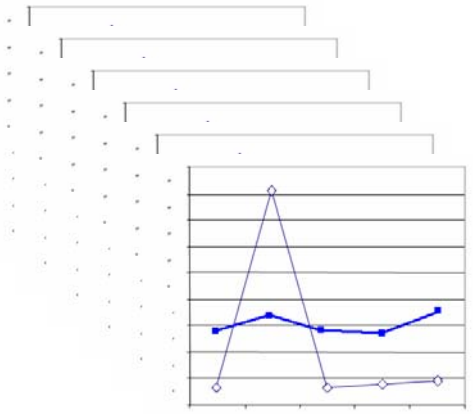
Original periodogram



Permutated profiles

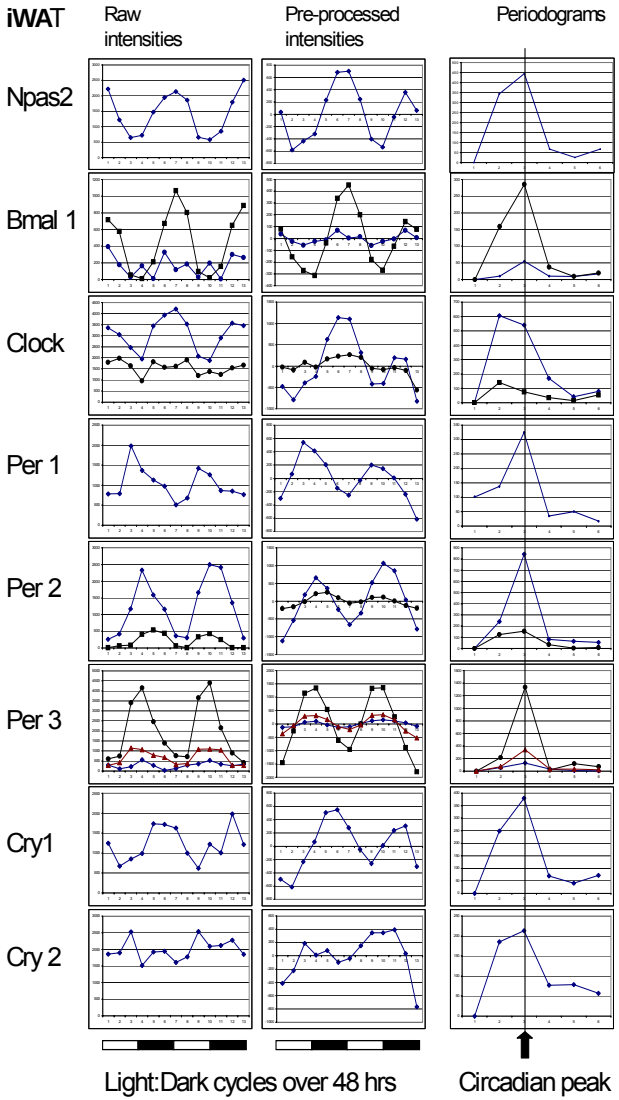


Permutated periodograms



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

Circadian clock in peripheral tissues are active



1425099_a_at
P_f=0.13
P_p=0.001
r=0.81

1421036_at
P_f=0.0
P_p=0.14
r=0.58

1421037_at
P_f=0.0
p_p=0.001
r=0.88

1418659_at
p=0.35
p_f=0.002
r=0.77

1418660_at
P_f=0.88
p_p=0.068
r=0.30

1449851_at
P_f=0.0
P_p=0.008
r=0.73

1417602_at
P_f=0.0
P_p=0.0
r=0.87

1417603_at
P_f=0.12
p_p=0.02 r=0.24
r=0.77

1460662_at
P_f=0.0
P_p=0.001
r=0.83

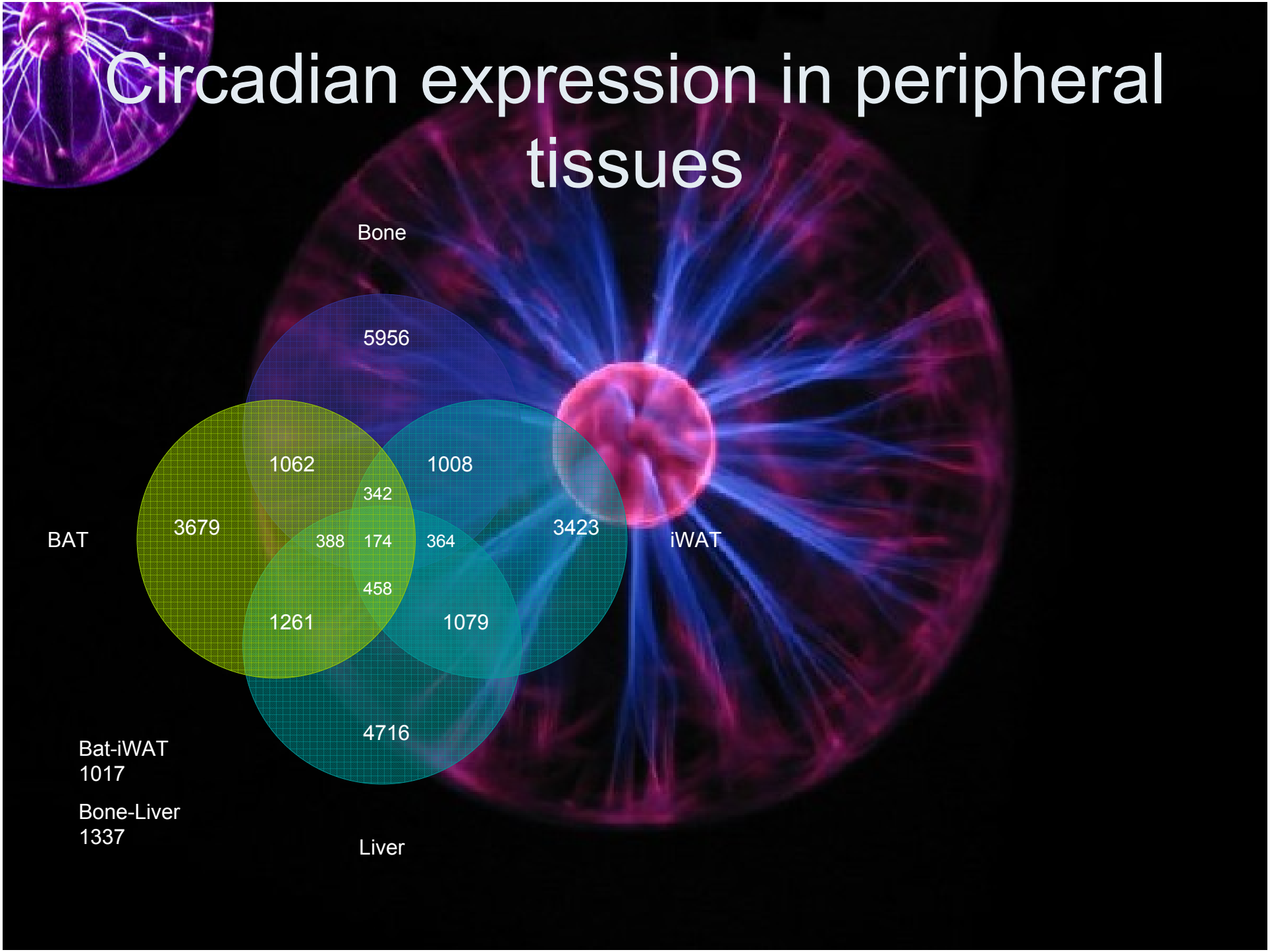
1421086_at
P_f=0.0
p_p=0.038
r=0.45

1421087_at
P_f=0.0
p_p=0.01
r=0.98

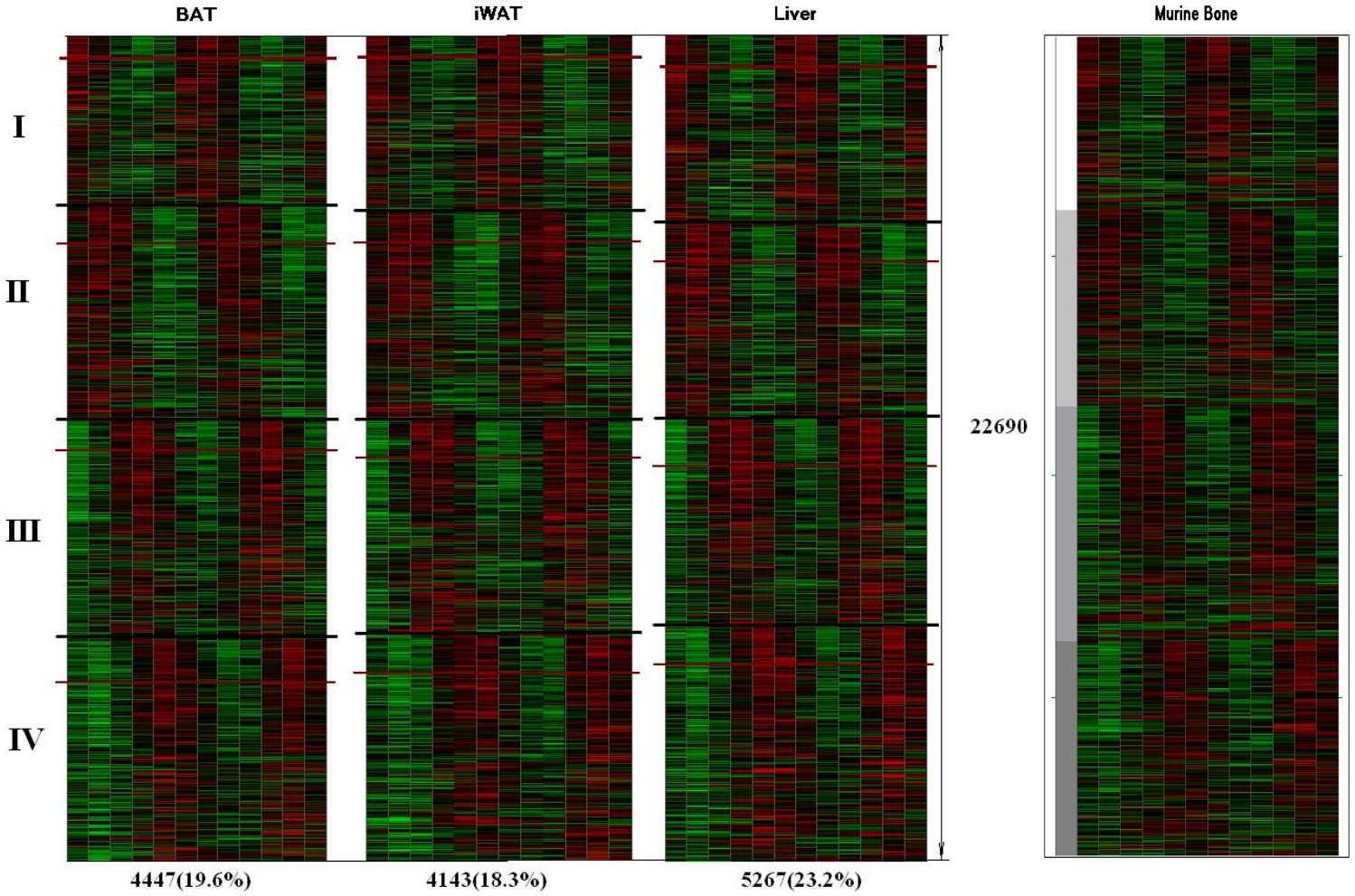
1433733_a_at
P_f=0.14
P_p=0.004
r=0.57

1426383_at
P_f=0.46
P_p=0.47
r=0.51

Circadian expression in peripheral tissues

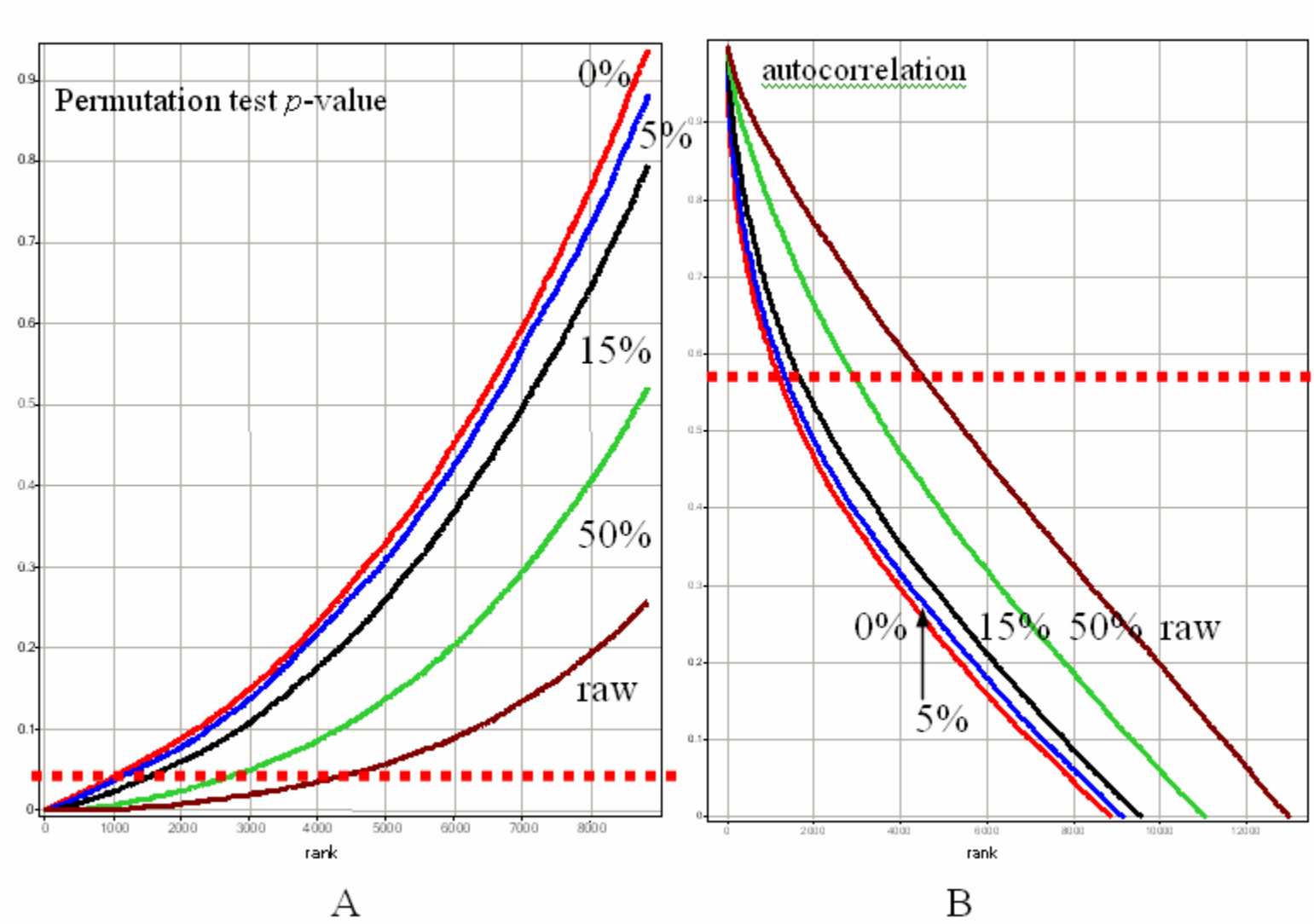


More than expected

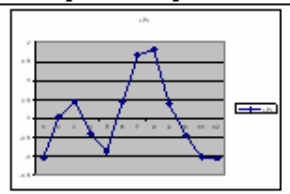
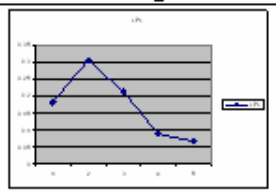
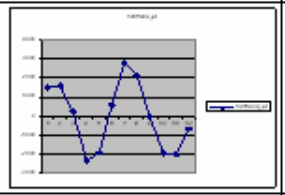
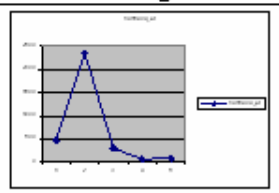
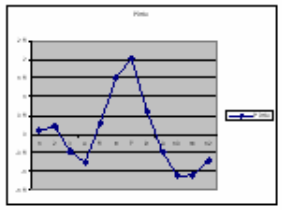
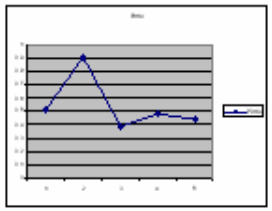
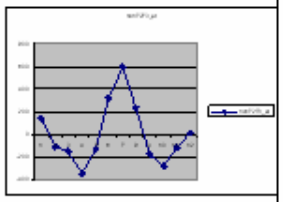
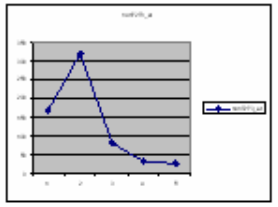
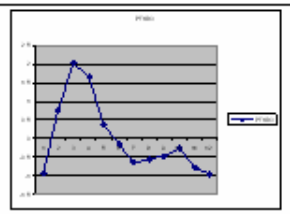
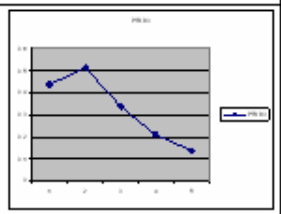
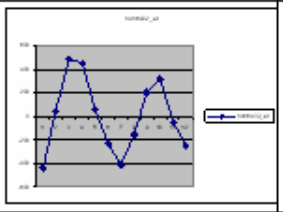
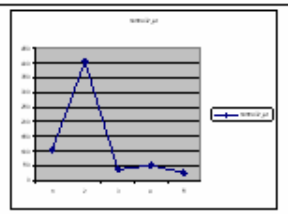
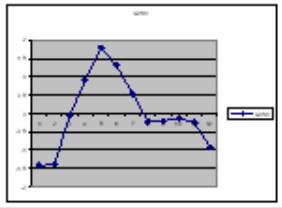
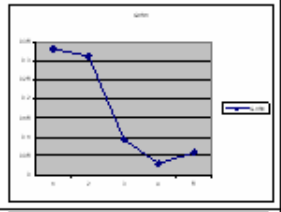
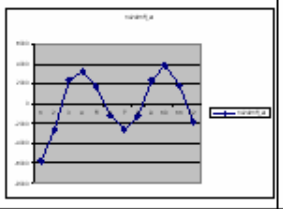
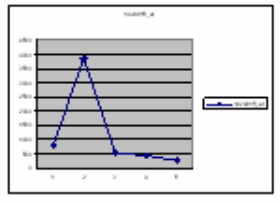
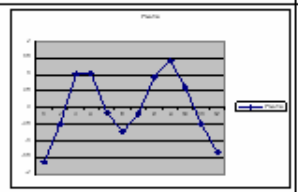
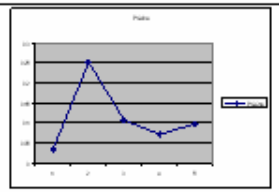
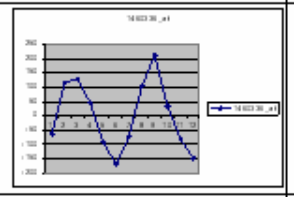
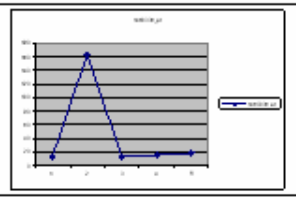
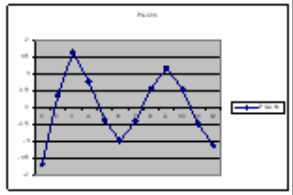
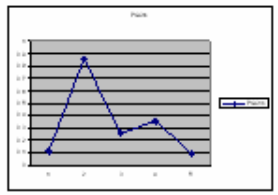
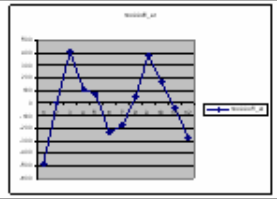
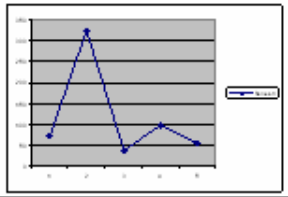


Is there a non-oscillating fraction at all?

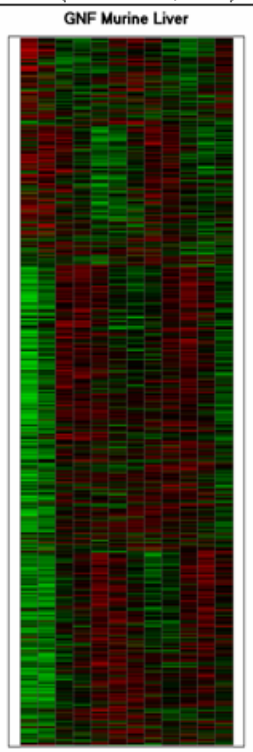
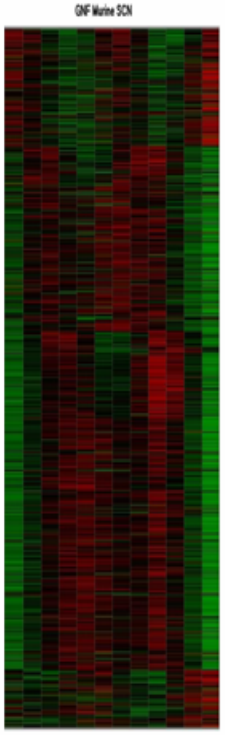
Simulation experiment



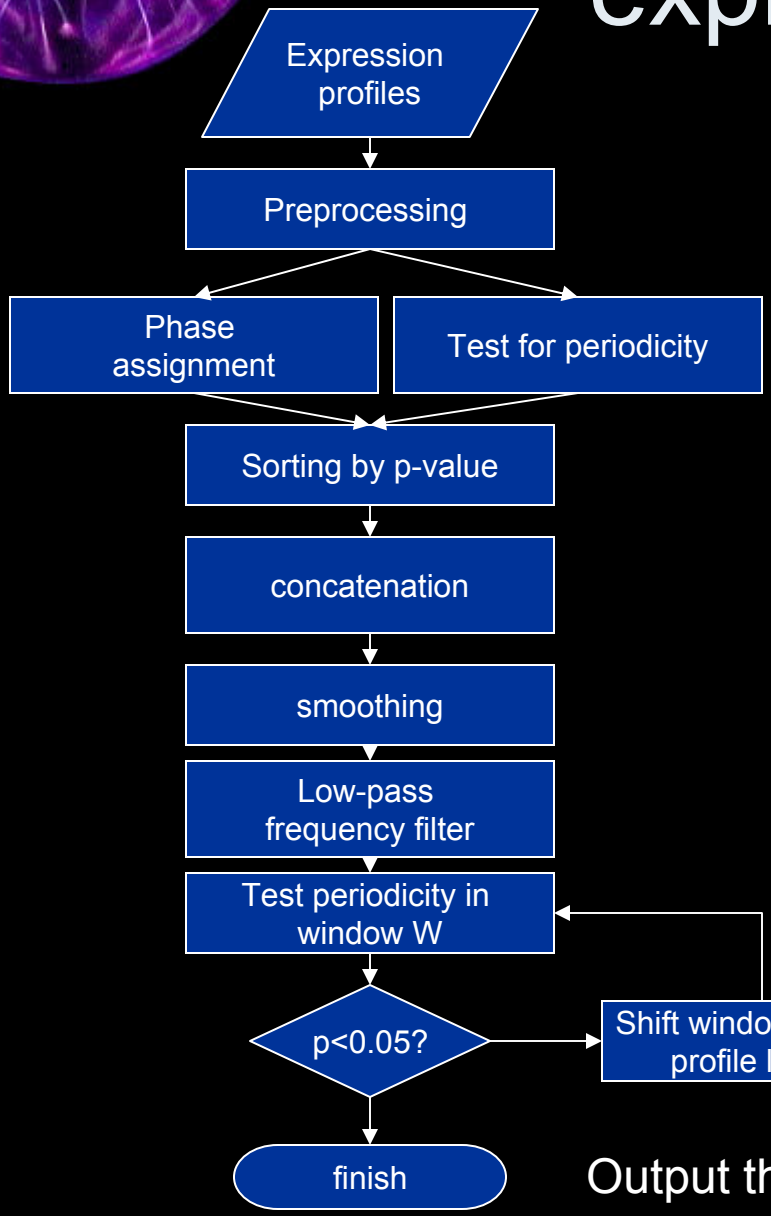
Verification

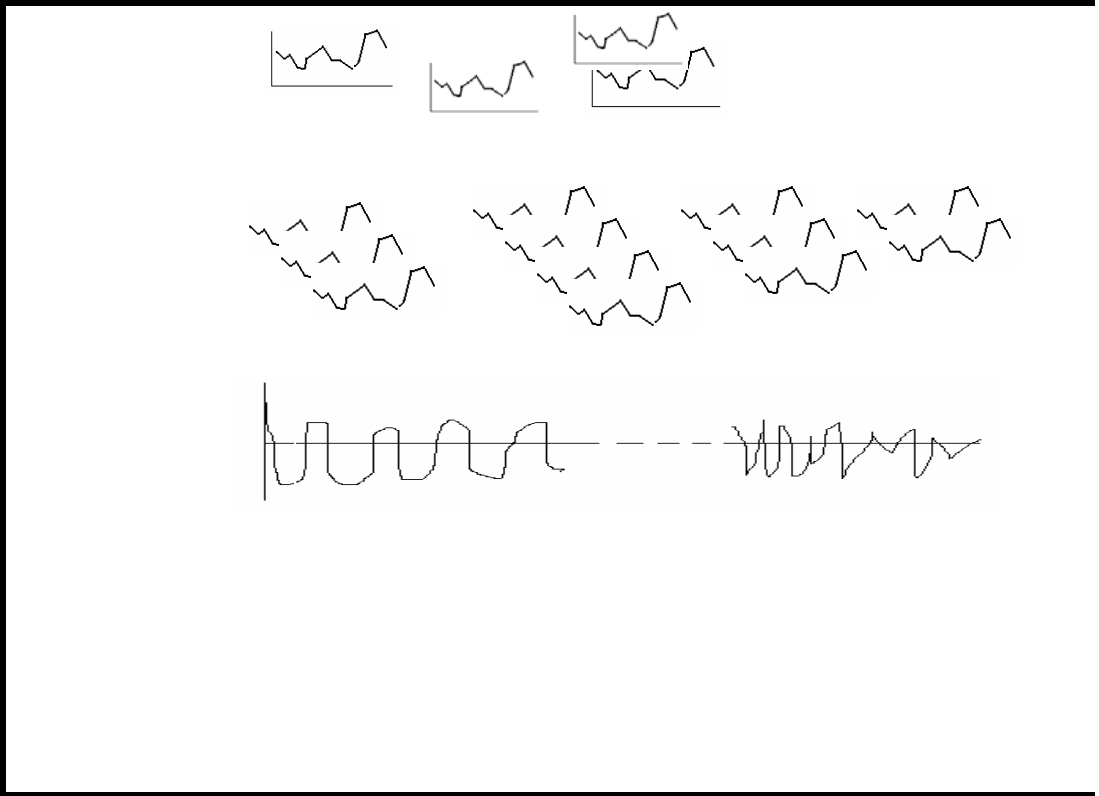
	qRT-PCR			Microarray		
	Expression profile	Periodogram	Test results	Expression profile	Periodogram	Test results
LPL (415904_at)			Pfg=0.425 Pt=0.076			Pfg=0 Pt=0
PDK4 (1417273_at)			Pfg=0.514 Pt=0.032			Pfg=0 Pt=0
PFKFB3 1416432_at			Pfg=0.581 Pt=0.114			Pfg=0 Pt=0
GYS 1424815_at			Pfg=0.346 Pt=0.192			Pfg=0 Pt=0
PGC1a 1460336_at			Pfg=0.174 Pt=0.003			Pfg=0 Pt=0.009
PGC1b 1449945_at			Pfg=0 Pt=0			Pfg=0 Pt=0.09

Reanalysis vs. original report

	Liver (Panda et al., 2002)*	SCN (Panda et al., 2002)	Liver (Storch et al., 2002)*	Liver (Zvonic et al., 2006)*	Yeast (Tu et al., 2005)
Heatmap					
Gene expression profiles	9968	9971	12486	22689	9333
Time points	48	46	24	24	36
Fisher's *g*-test	5.1%	6.9%	3.2%	9.3%	27.1%
Autocorrelation	11%	24.6%	2.1%	19.5%	60.2%
Pt-test	42.9%	32.6%	18.2%	21%	84.7%
Reported by authors, $p \leq 0.05$	1.5%	0.8%	4.6%	23.8% (3 algorithms)	38.1%

Digital signal processing for expression profiles

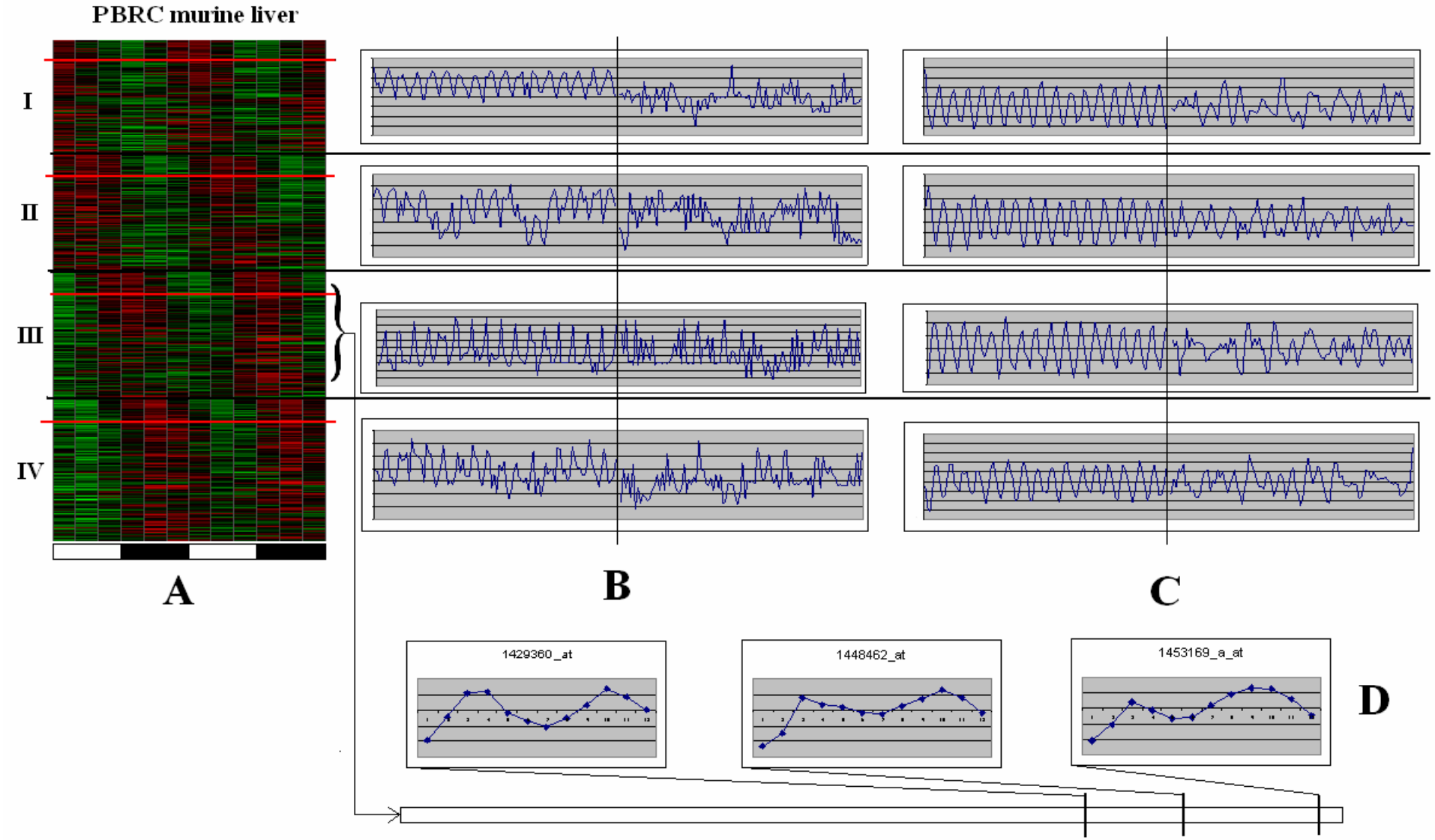




Kolmogorov-Smirnov Goodness of Fit Test between original and permutated periodograms

Output the number of iterations

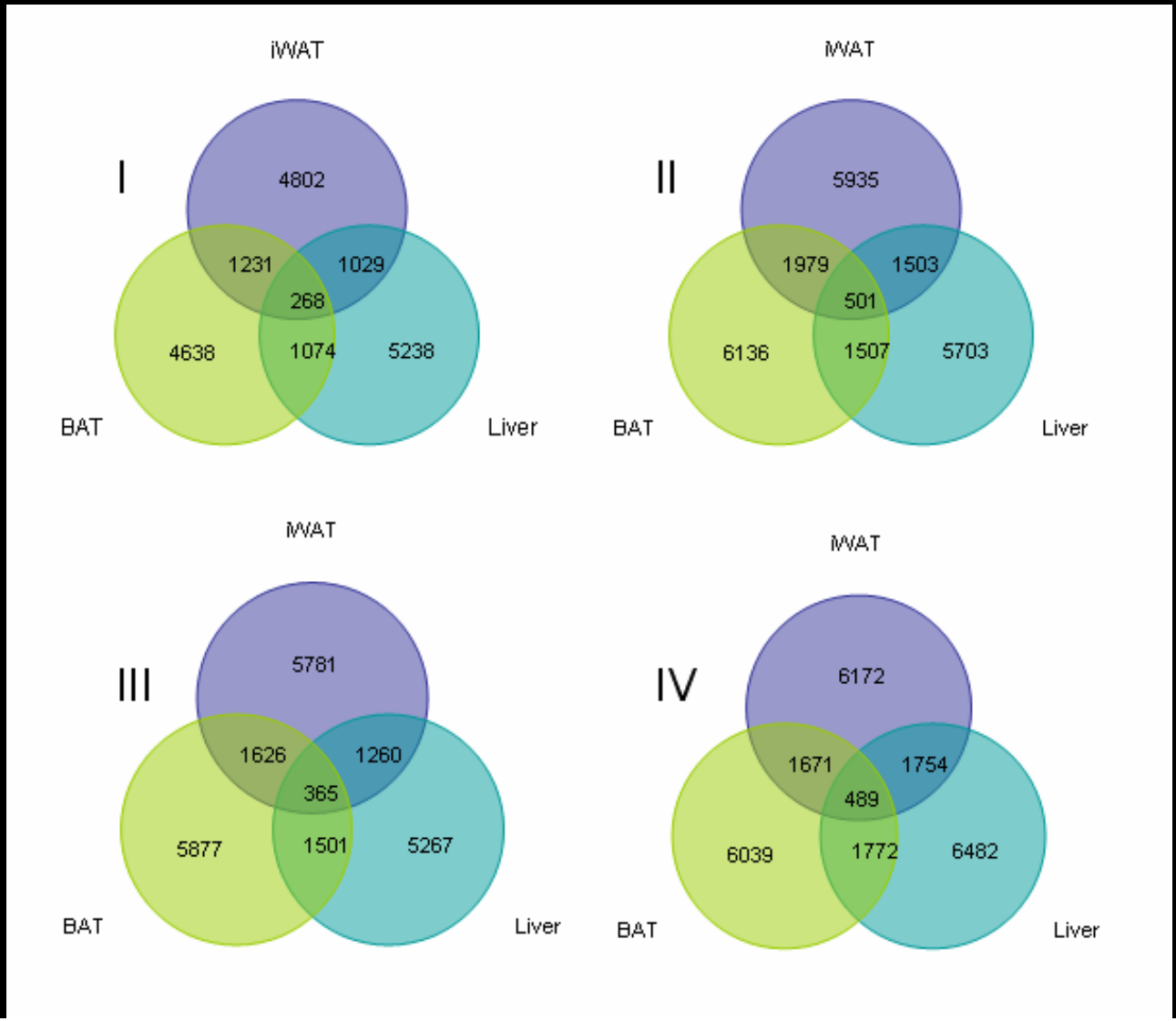
Analysis of real data



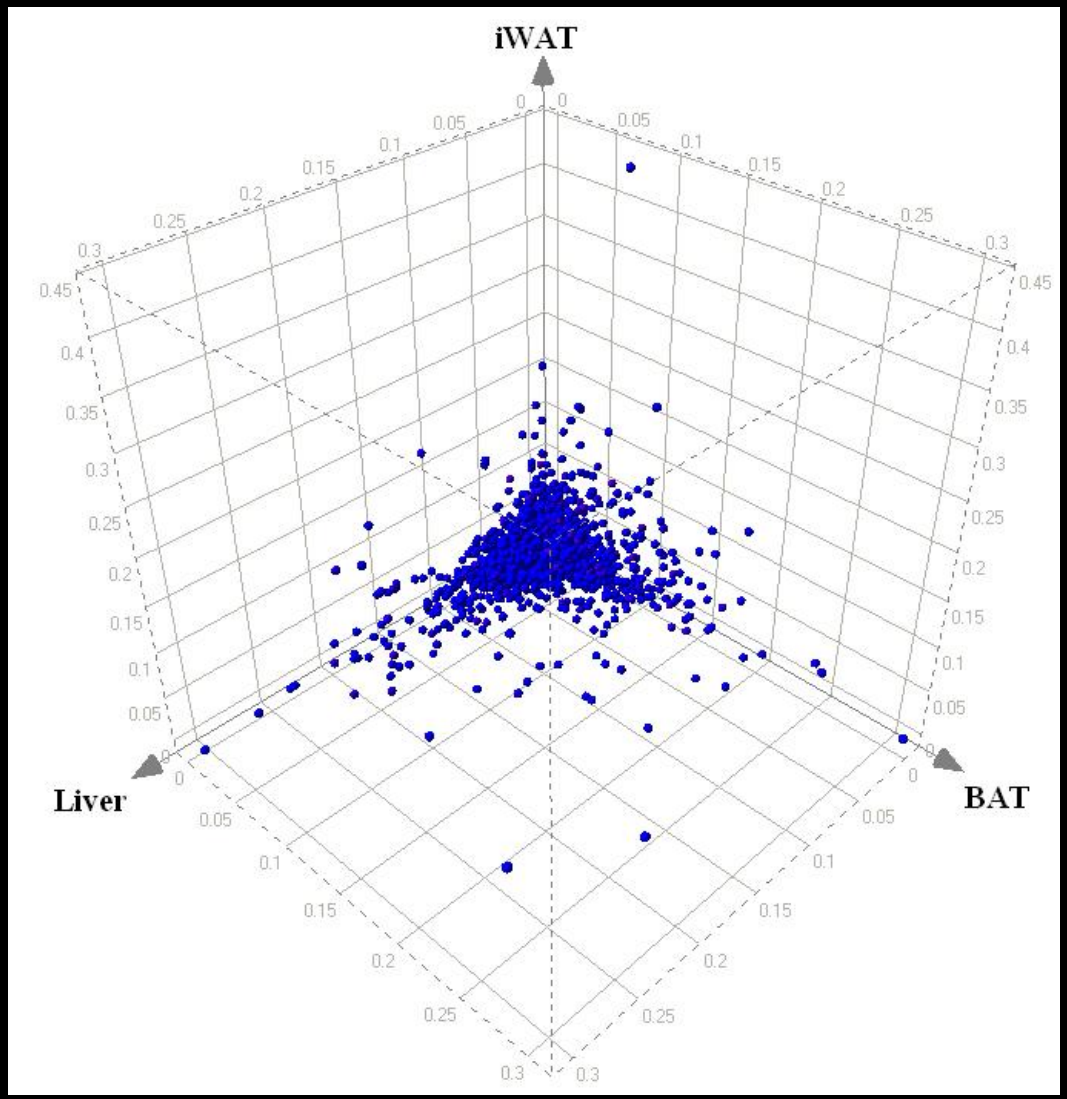
Effect of Digital Filters

	Autocorrelation	Fisher's g-test	KS-test
No filter	23.1%	10.2%	99.7%
Non-Recursive Average filter (NRAF)	20.9%	20.5%	99.8%
Polynomial Filter (Savitzky-Golay)	16.3%	43.4%	99.9%
Positional Centering Filter	99%	99%	52.5%

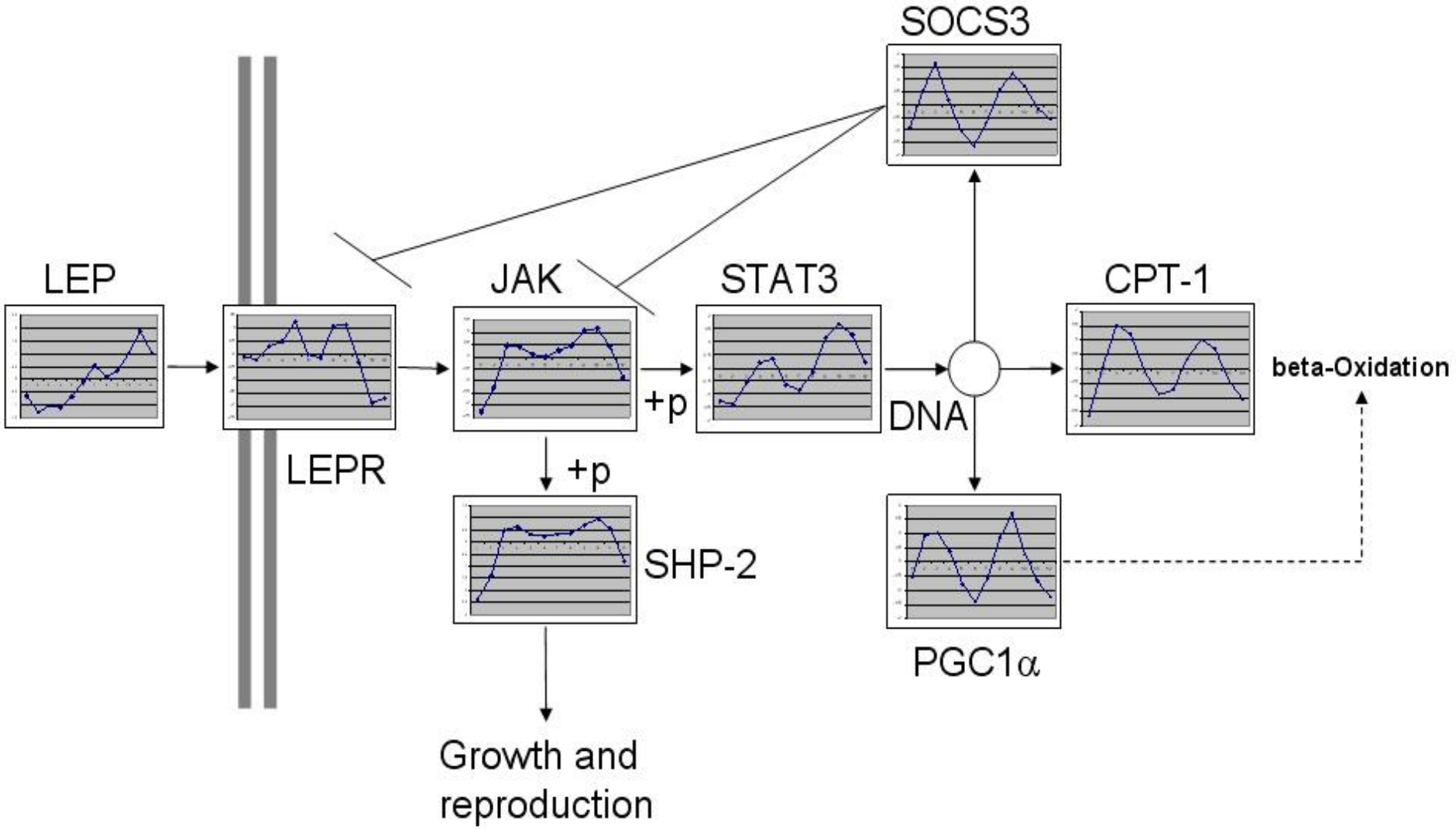
Phase of expression is tissue-specific



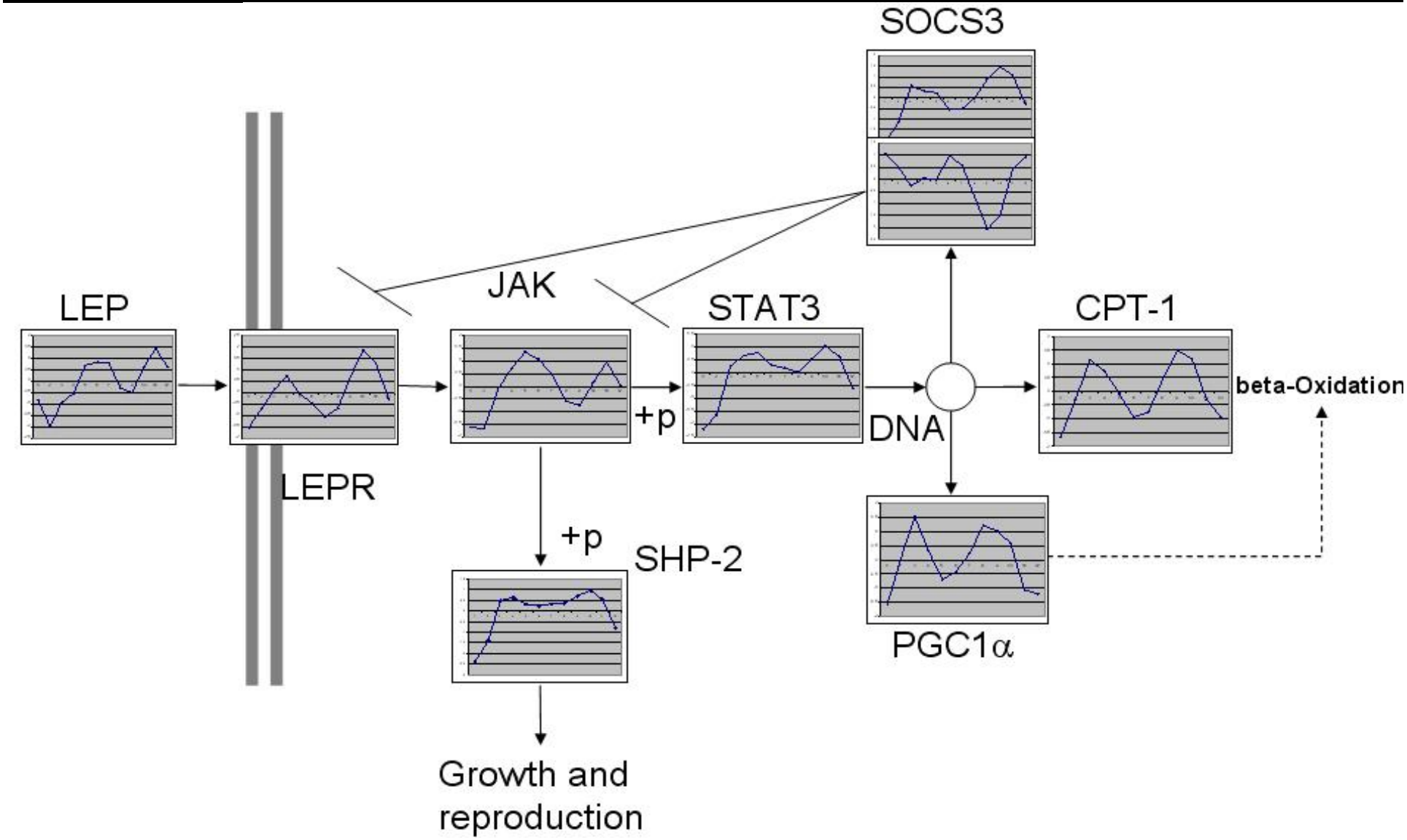
Amplitude of oscillation is tissue-specific



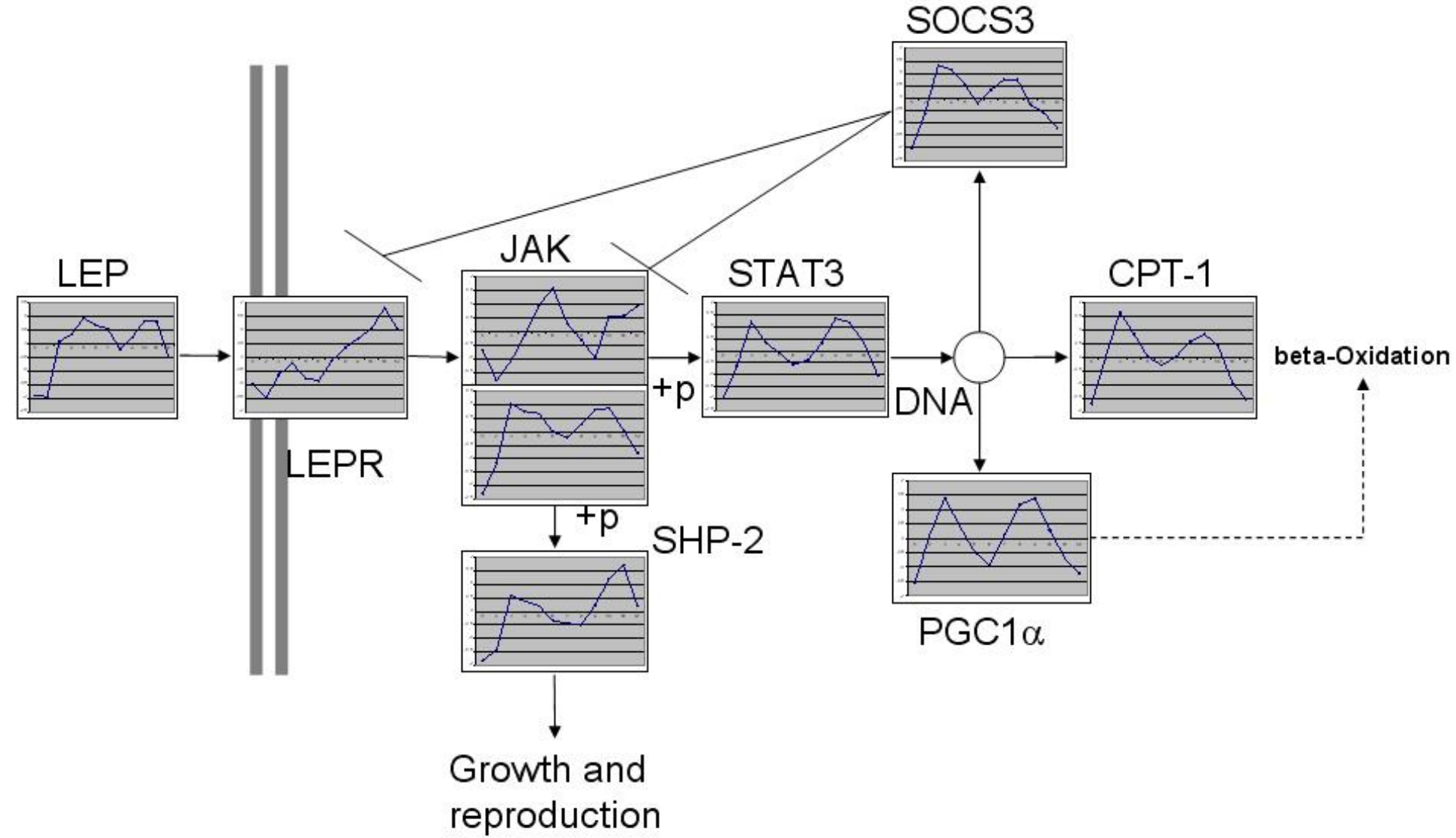
Oscillation in Leptin Signalling Pathway-Liver



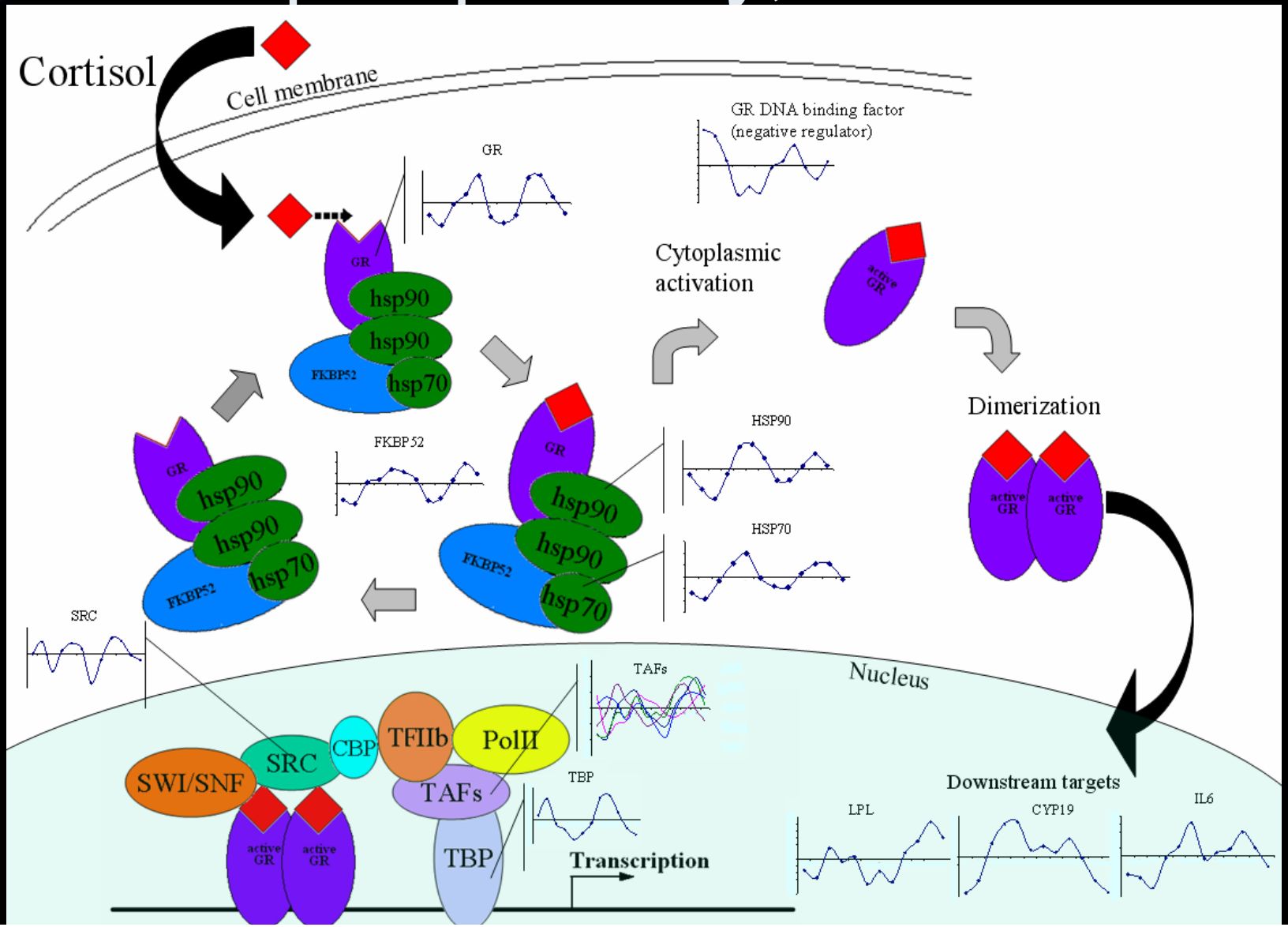
Oscillation in Leptin Signalling Pathway-Brown Fat



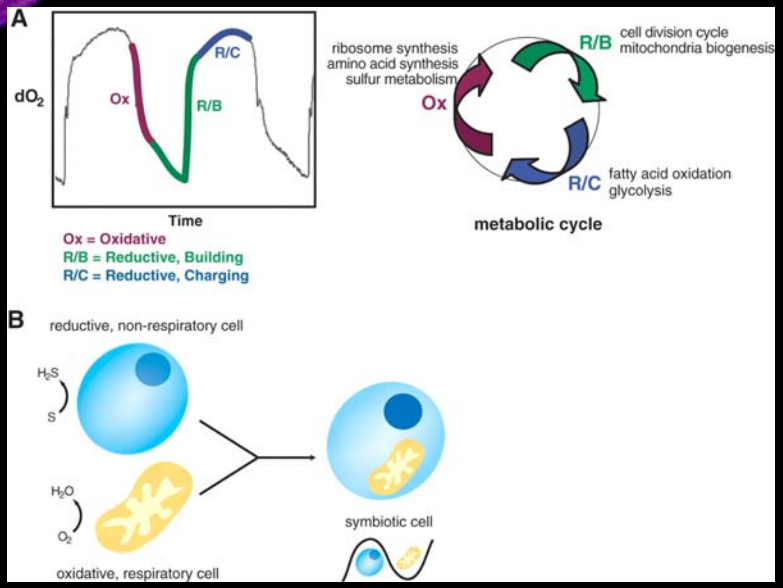
Oscillation in Leptin Signalling Pathway-White Fat



Oscillation in Glucocorticoid receptor pathway, murine bone



Hypothesis: Who's the driver?



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)

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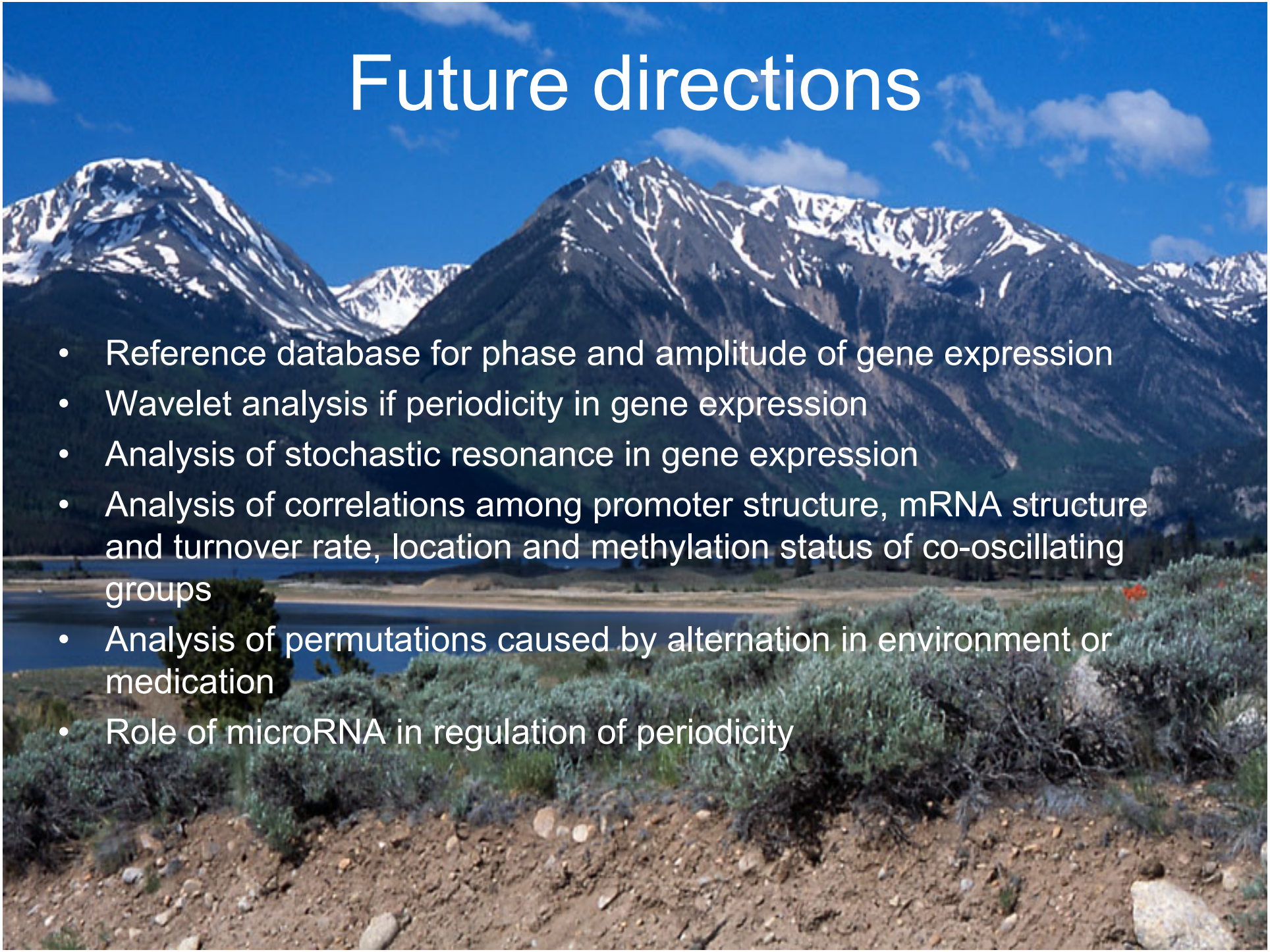
- Tu BP, Kudlicki A, Rowicka M, McKnight SL. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science. 2005 Nov 18;310(5751):1152-8.

Future directions



- Reference database for phase and amplitude of gene expression
- Wavelet analysis if periodicity in gene expression
- Analysis of stochastic resonance in gene expression
- Analysis of correlations among promoter structure, mRNA structure and turnover rate, location and methylation status of co-oscillating groups
- Analysis of permutations caused by alternation in environment or medication
- Role of microRNA in regulation of periodicity

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