The role of circadian rhythm in mammary function in the cow

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Circadian clocks are responsive to the environment

- Master clock
  - sets circadian rhythms
  - lies in the suprachiasmatic nuclei
- Circadian rhythms
  - Allow organisms to prepare for environmental changes
  - Are entrained by external cues including food availability and light
- 3-10 % of the transcripts are under circadian control

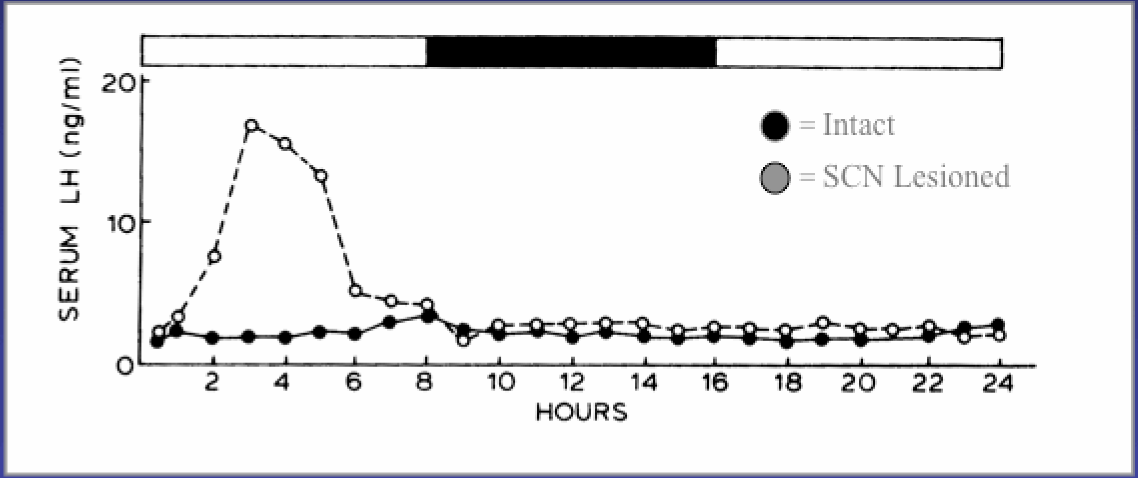
Circadian clocks influence many biological processes

- Metabolic Hormones
  - insulin, glucagon
  - adiponectin, corticosterone, leptin

- Enzymes
  - glycogen phosphorylase
  - cytochrome oxidase
  - lactate dehydrogenase
  - acetyl-CoA carboxylase
  - malic enzyme
  - fatty acid synthase

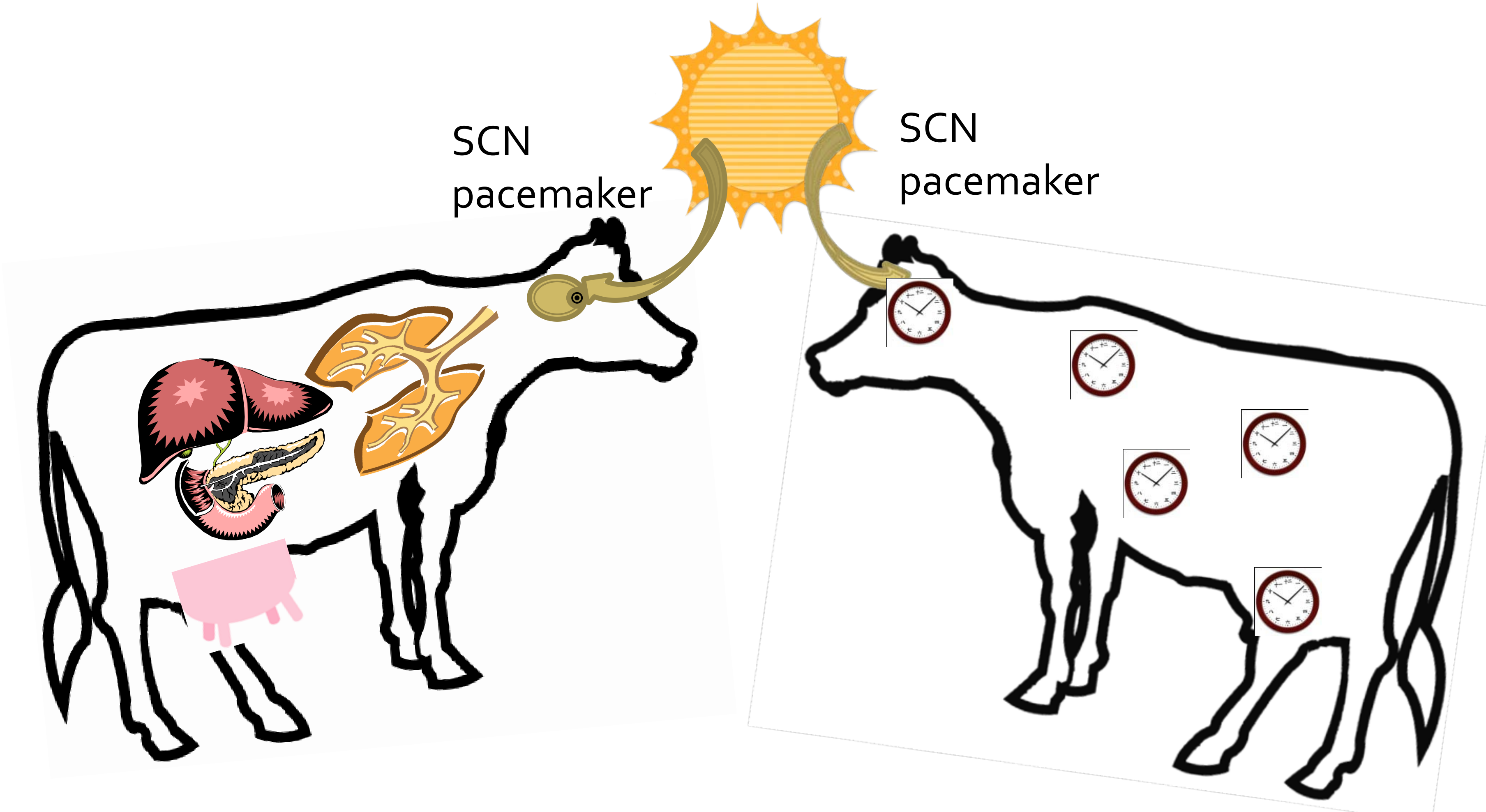
- SREBF1 may also exhibit circadian oscillation

The Brain CLock is Necessary for Ovulation in Rodents

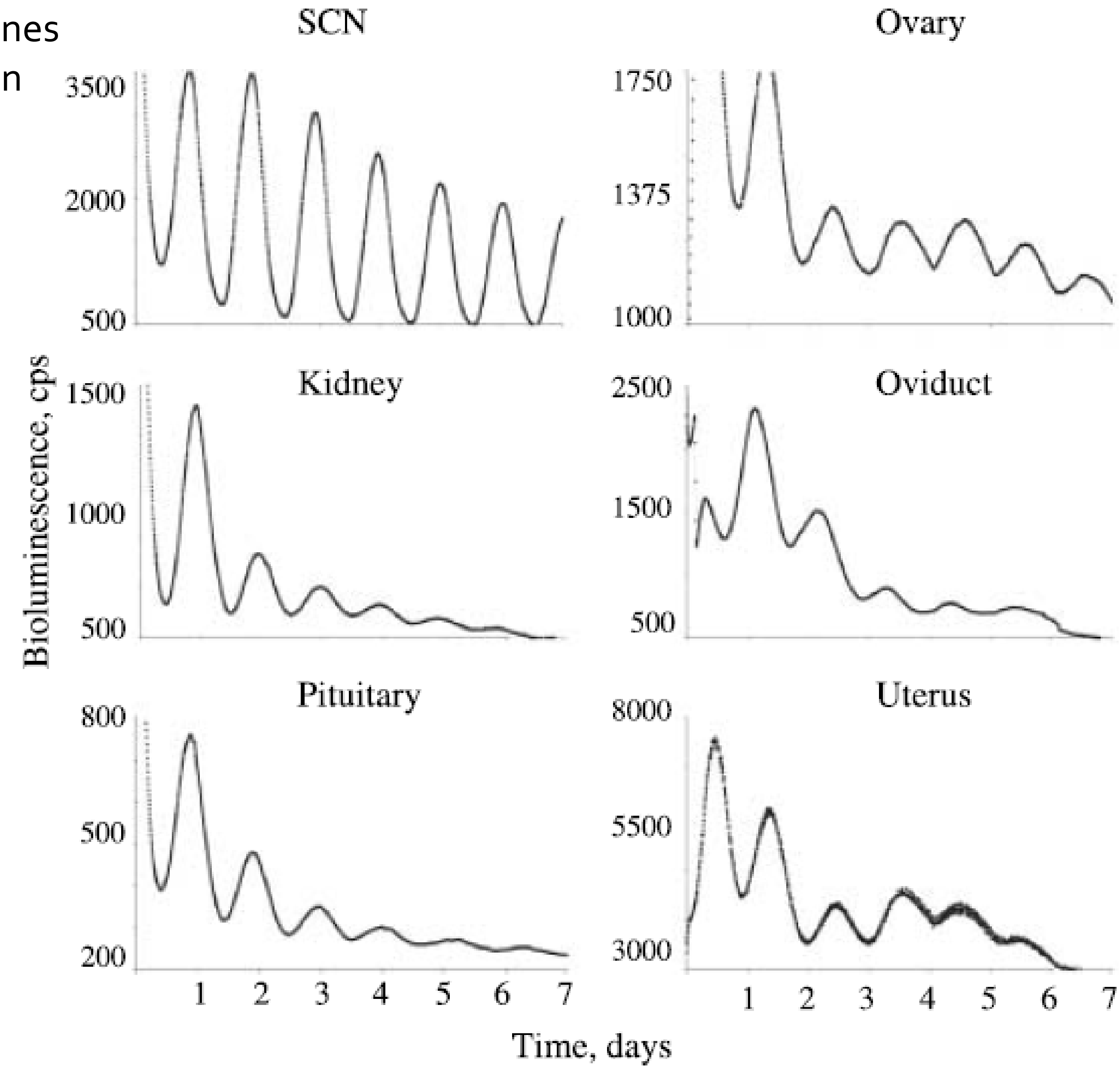


Turek, Swann, & Earnest, 1984

Peripheral tissues have their own clocks



Oscillation of circadian genes in vitro organ culture



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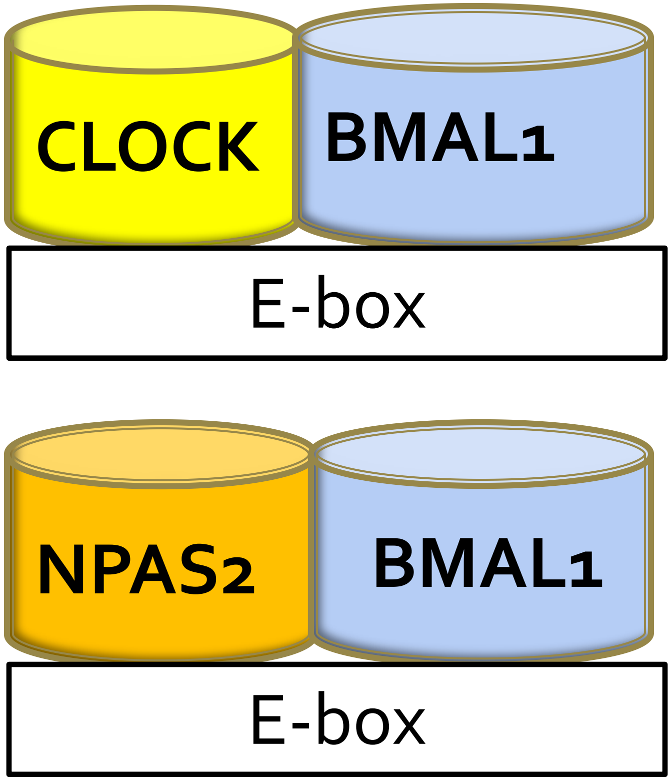
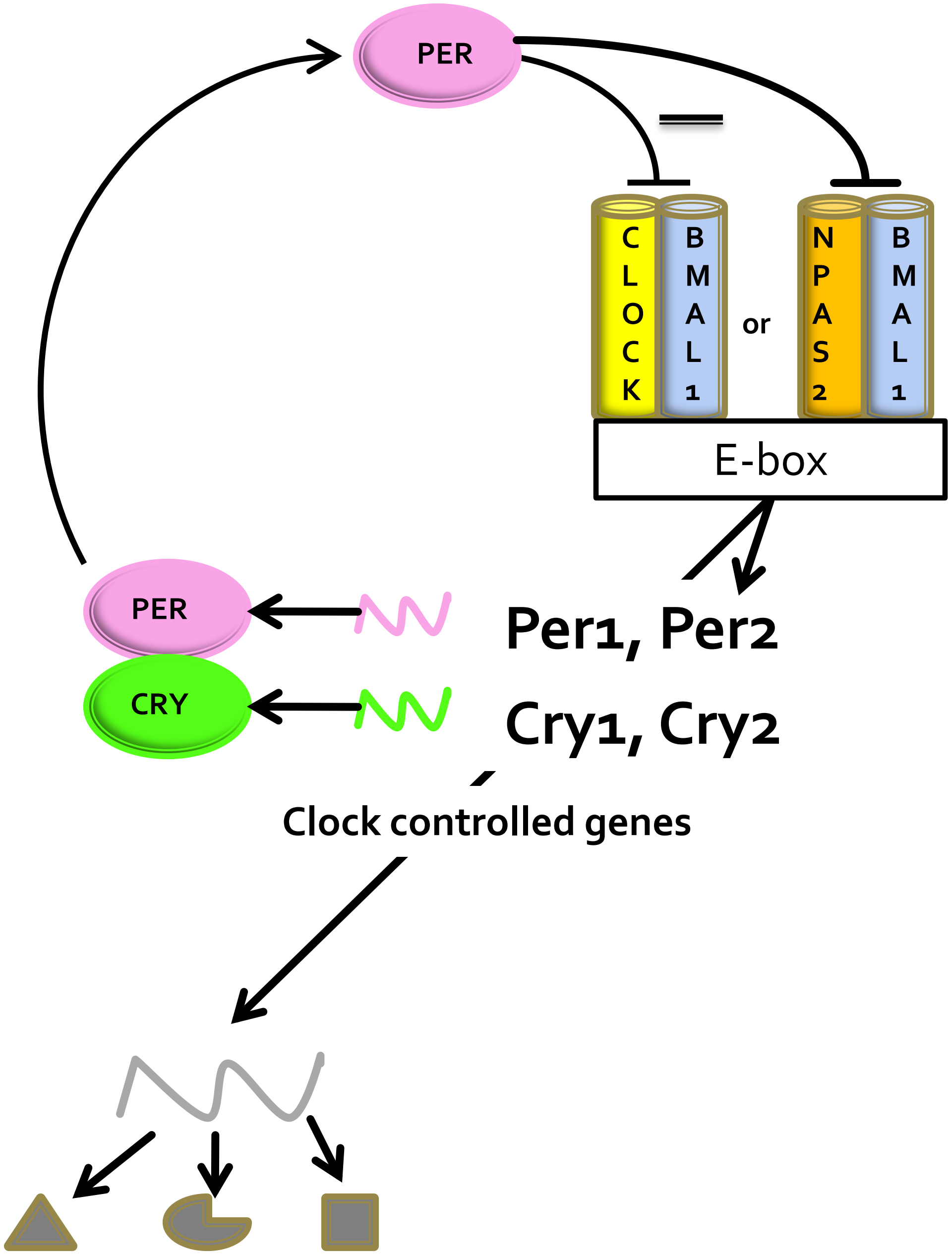
Major clock genes

- Positive Loop
  - CLOCK
  - BMAL1 (ARNTL) and
  - NPAS2
- Negative Loop
  - Pers
  - Crys
- Nuclear Receptors
  - ROR-α
  - Rev-erbα





Core molecular clock genes contain positive and negative elements that form auto-regulatory feedback loops.

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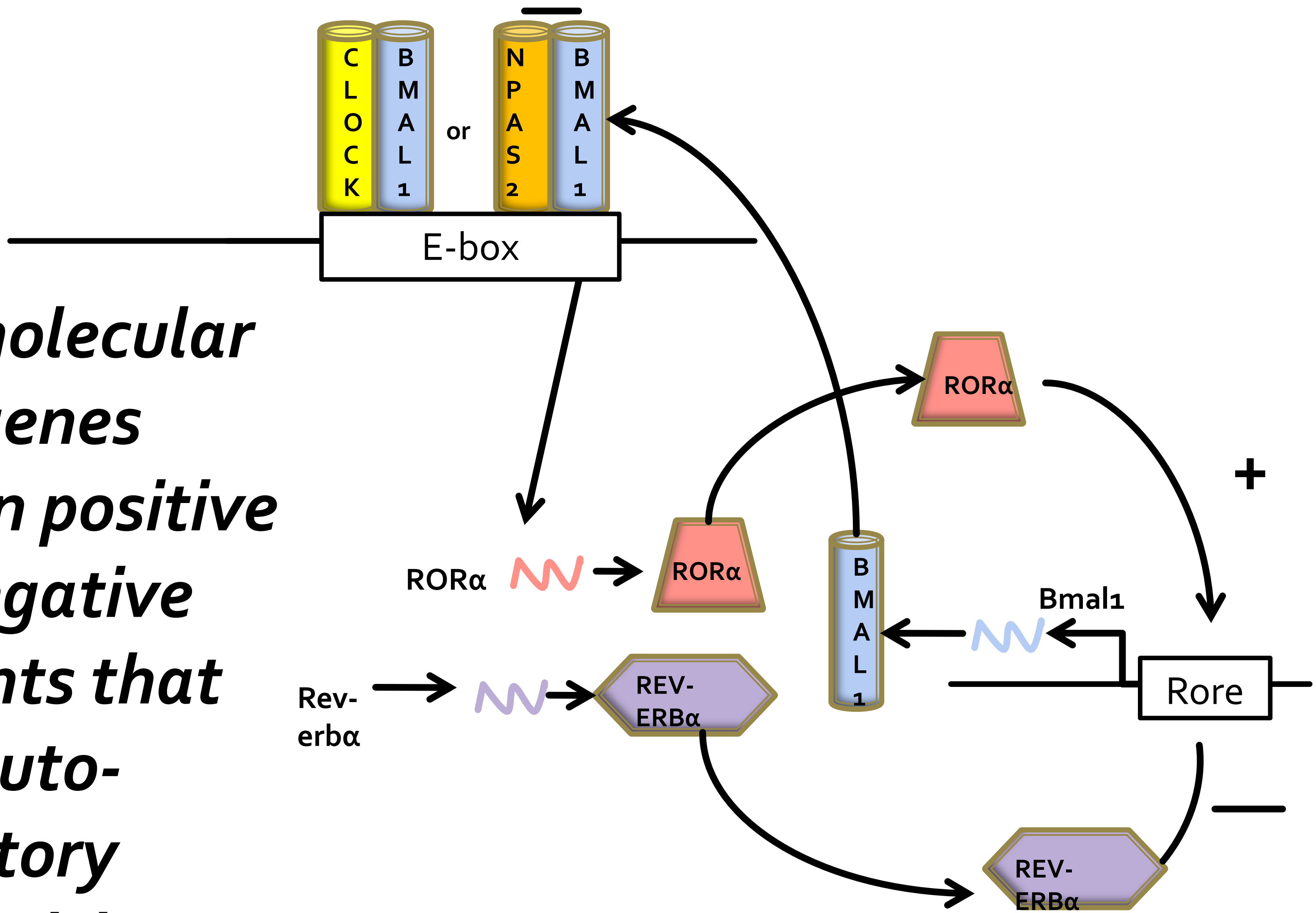
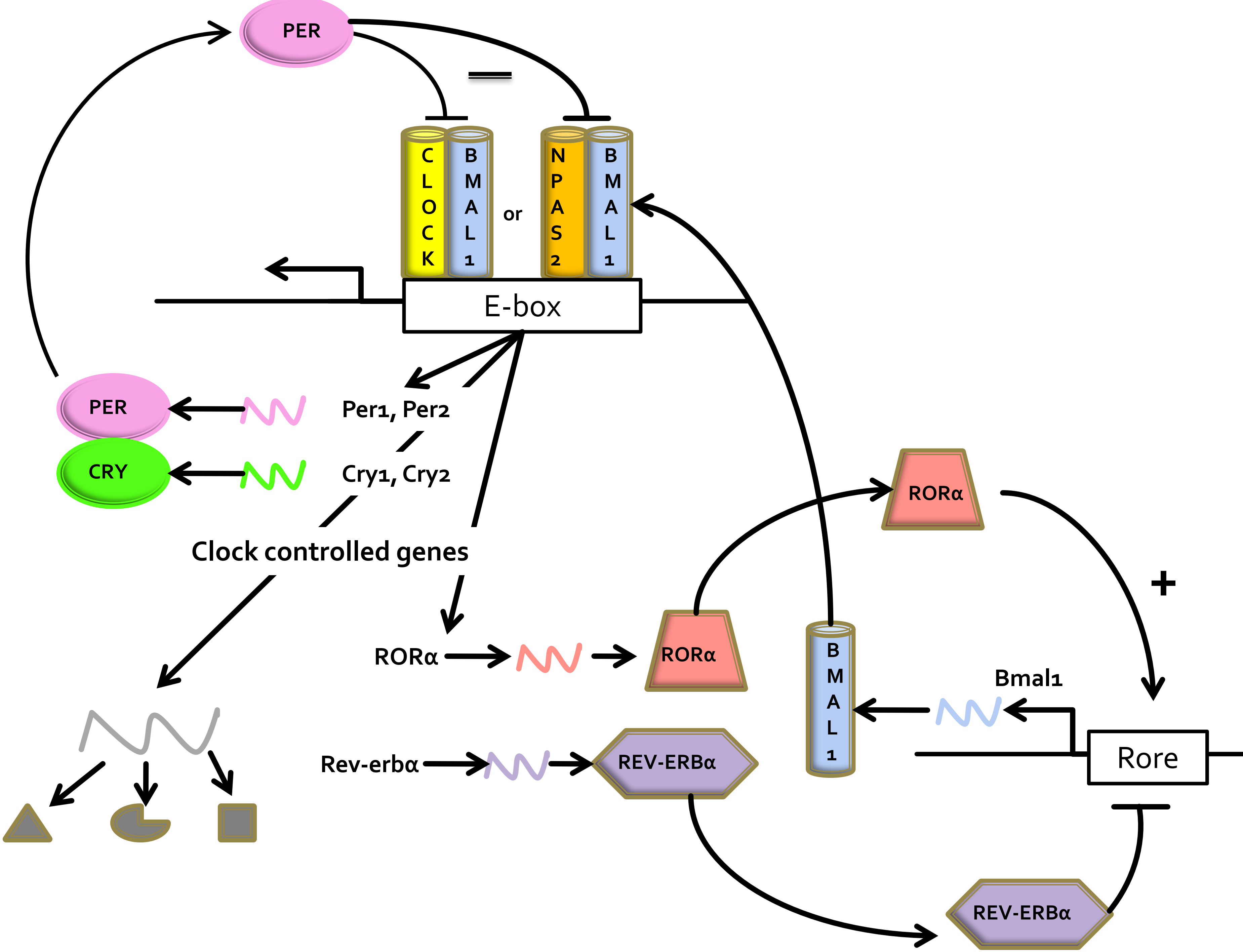



PER
CLOCK
BMAL1
or
NPAS2
BMAL1
E-box
Per1, Per2
PER
CRY
Cry1, Cry2
Clock controlled genes
RORα
RORα
RORα
RORα
+
BMAL1
Bmal1
Rore
Rev-erbα
REV-ERBα
REV-ERBα

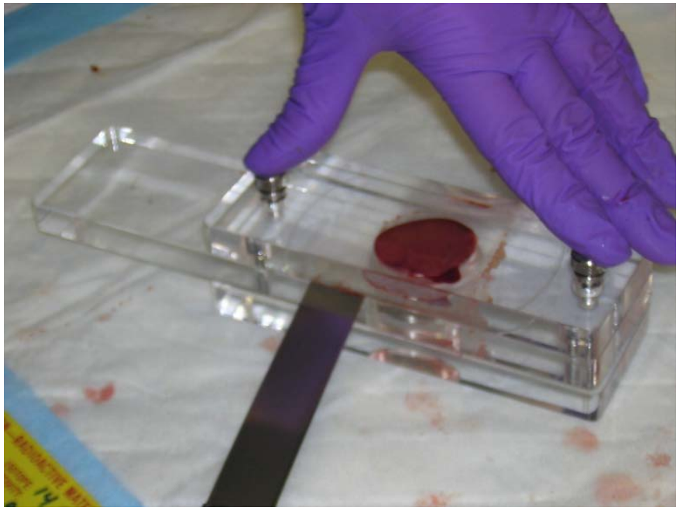
How are tissues coordinately regulated during the transition from pregnancy to lactation?

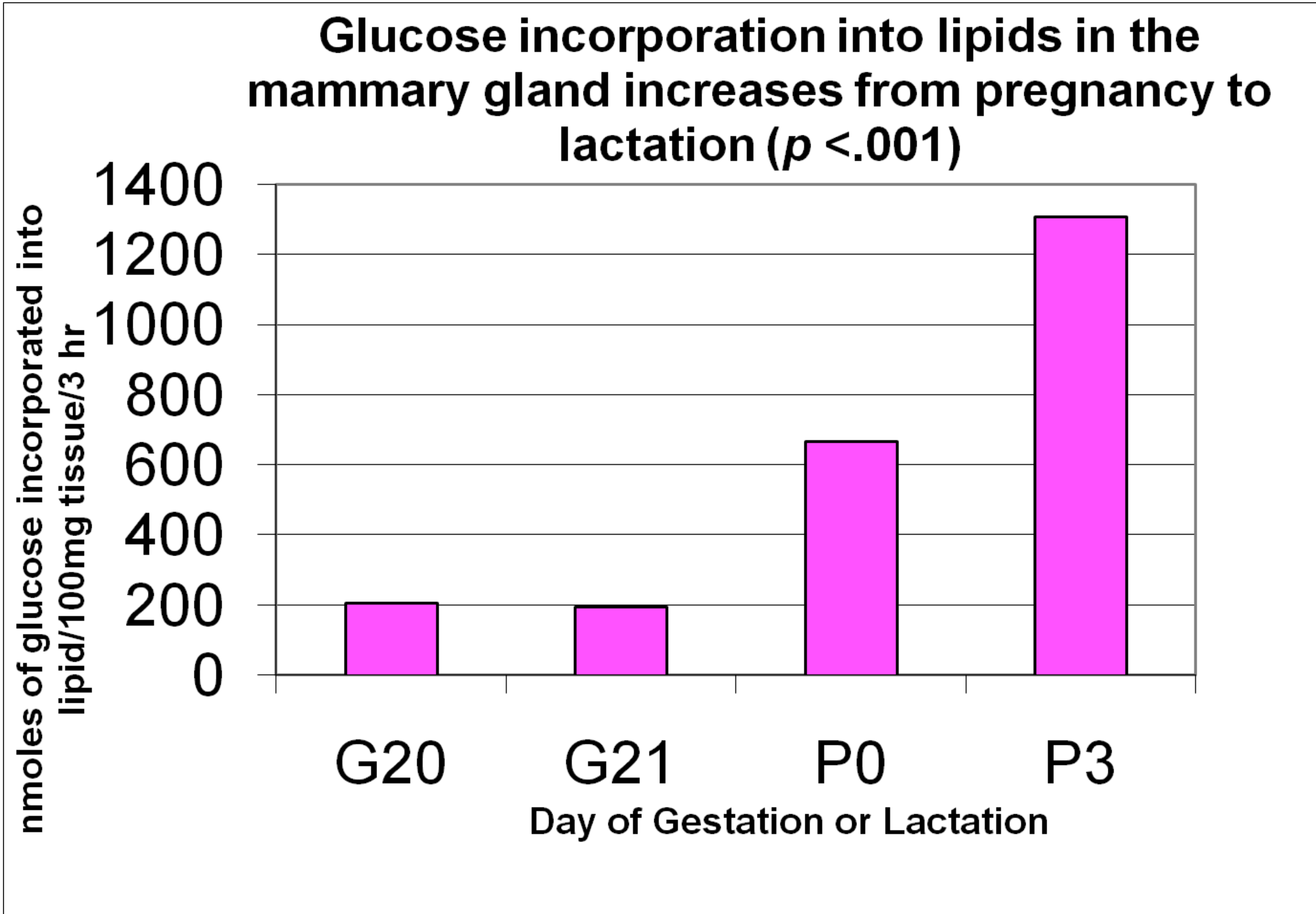
- Is there a circadian clock in the mammary gland?
- Are clocks altered during the transition from pregnancy to lactation?
- Are they synchronized among tissues?

Sprague-Dawley rats

Measured changes in gene expression and rate of lipid synthesis from pregnancy to lactation

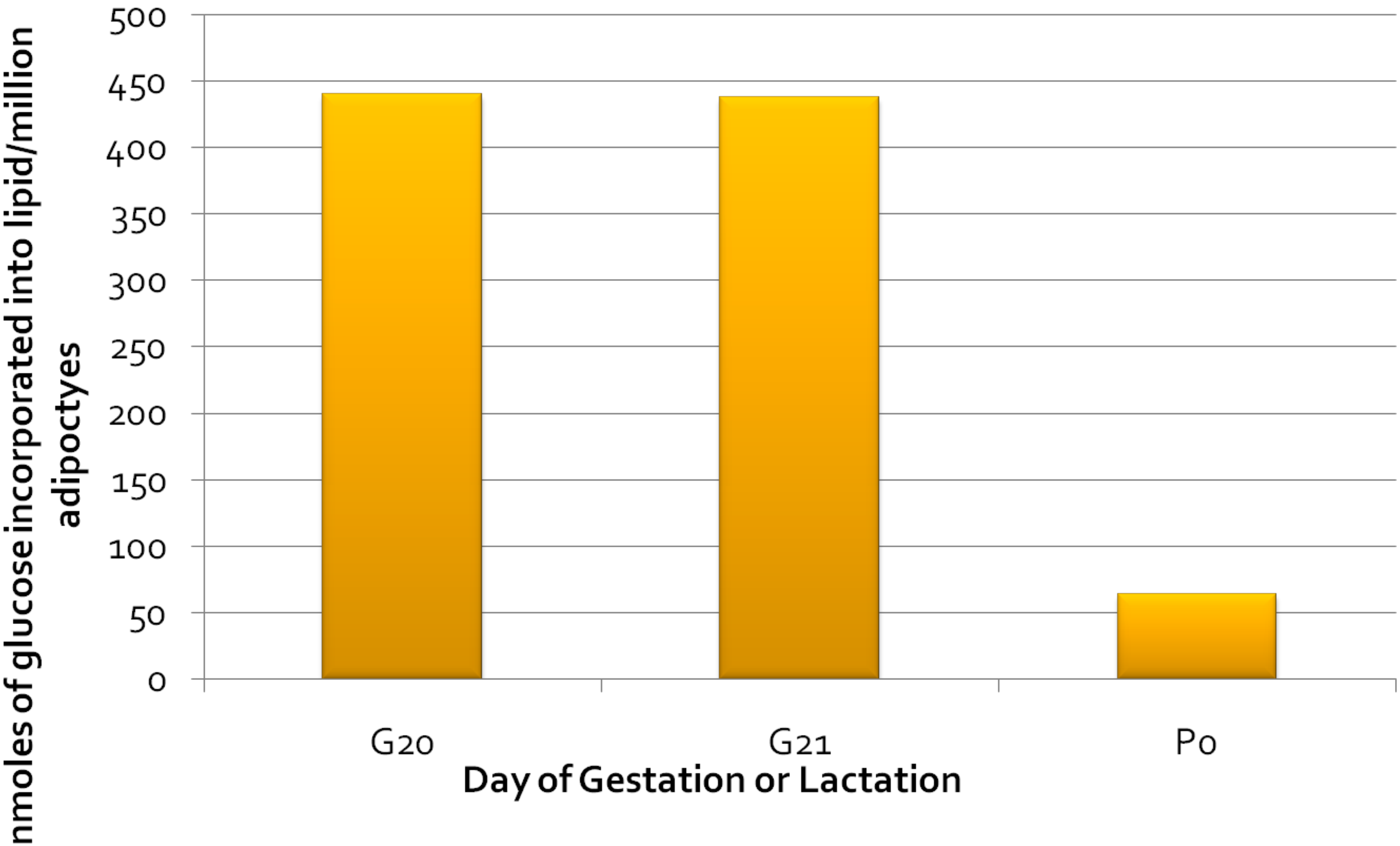
- Mammary, liver and adipose tissue removed during pregnancy and lactation (n=8/trt)
- Glucose incorporation into lipids measured with U-^{14}C labeled glucose
- RNA prepared from each tissue (n=5/trt)
- Gene expression measured using Affy rat gene chip 230.2



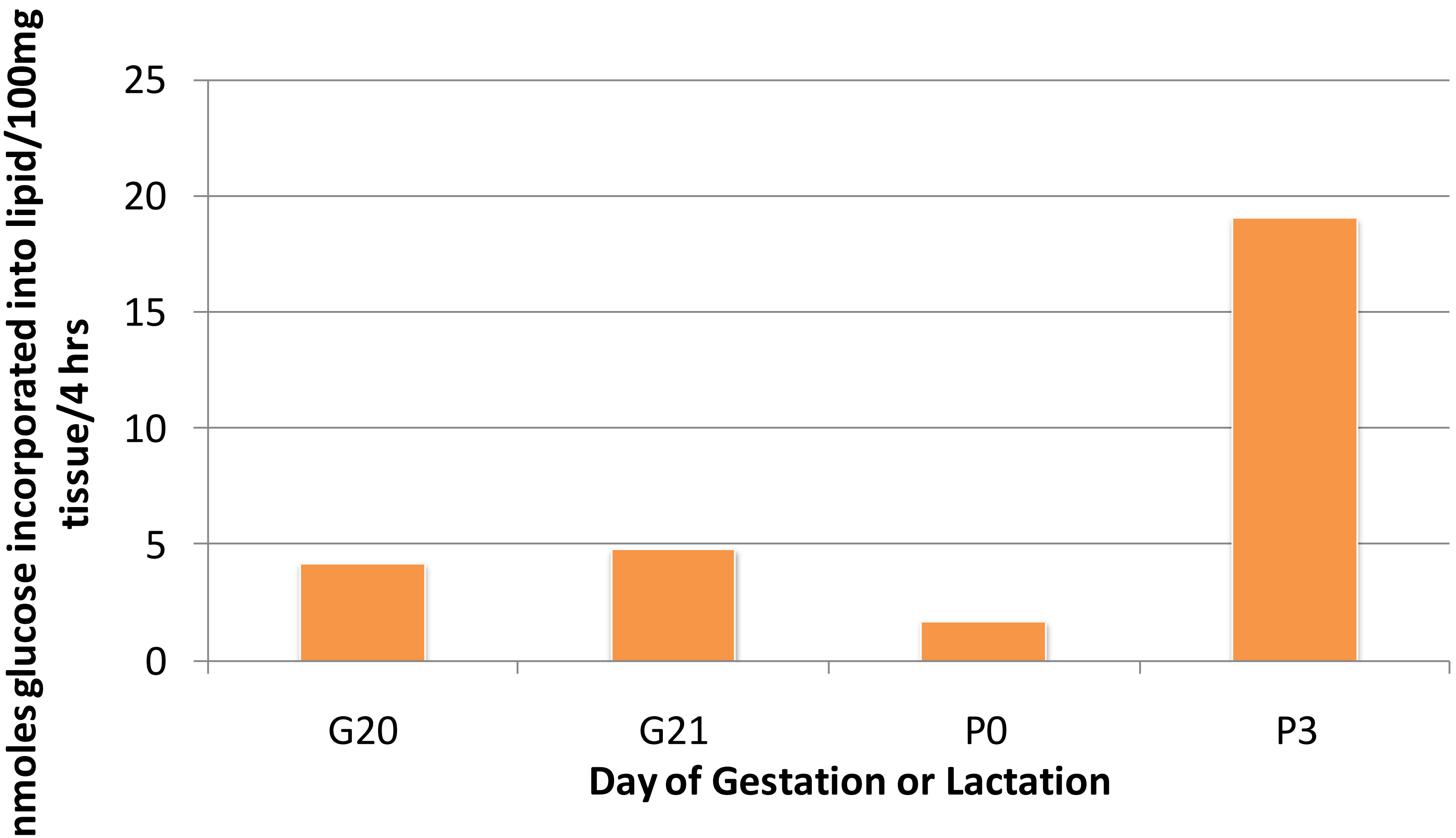


Glucose incorporation into lipids in the mammary gland increases from pregnancy to lactation (p <.001)
nmoles of glucose incorporated into lipid/100mg tissue/3 hr
1400
1200
1000
800
600
400
200
0
G20
G21
P0
P3
Day of Gestation or Lactation

Glucose incorporation into Lipid decreases in adipose tissue (p <.001) during lactation



Glucose incorporation into lipids is low during pregnancy and increases during lactation in rat liver (p <.001)



Microarray analysis
Ingenuity Pathways Analysis (IPA)

- Data processing
  - BioConductor version 2.0 software
  - Robust Model Analysis (RMA) for normalization
  - Differential gene expression was tested using MAANOVA Ingenuity Pathways Analysis (IPA) program (Ingenuity Systems, Mountain View, CA)
  - Filters for data analyses were set with $\geq$1.3 fold difference in expression and $P < 0.1$.
- Differentially expressed genes were mapped into functionally relevant networks.

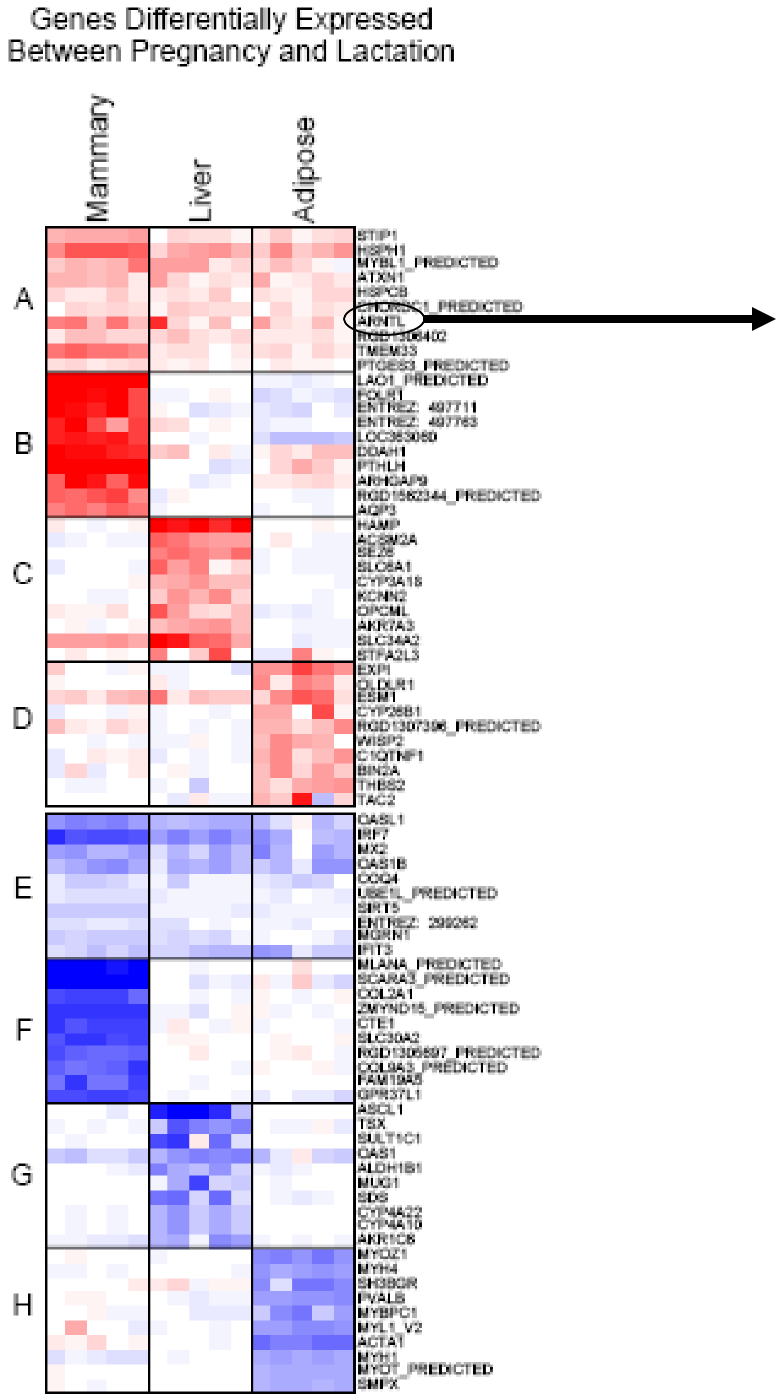
Table 1. Genes differentially expressed from P20 to L1 (P<0.001; FDR<0.005) in GO: Lipid Biosynthesis

Symbol	MG	LIV	ADI
Insig1	3.51672	-3.5591	-3.2246
Cyp51	3.15384	-2.9915	-3.2003
Prkaa2	3.12062	-3.0043	-3.7256
Fads1	2.86381	-2.9074	-2.9808
Abhd5	2.41232	-2.7197	-1.8899
Idi1	2.1234	-1.4565	-2.0421
Idi1	2.01467	-1.4153	-1.9458
Acsl5	1.82593	-1.9512	-1.5995
Scd	1.82284	-3.0439	-2.1075
Srebf2	1.65104	-1.3198	-1.2057
Fdps	1.56935	-1.6184	-1.3622
Fads2	1.48995	-2.974	-1.5232
Seli	1.38541	-1.6247	-1.2808
Hmgcr	1.35633	-1.6867	-1.3258
Acss2	1.35167	-1.4104	-1.4721
Elovl5	1.34906	-1.3311	-1.066
Gpsn2	1.13484	-1.3942	-1.3714
Etnk1	1.12099	-1.0724	-0.8586
Impa1	1.03758	-0.736	-0.7048
Sc5dl	0.87237	-1.233	-1.0136
Hsd17b12	0.82417	-0.7501	-0.624
Agpat6	0.77681	-0.7148	-0.7641
Acaca	0.76232	-0.8188	-0.8753
Mvd	0.76208	-0.6844	-0.8787
Hsd17b7	0.76153	-0.7029	-0.5377
Gne	0.76031	-0.8225	-0.7413
Serinc1	0.66892	-0.638	-0.8108
Agpat6	0.62586	-0.6675	-0.5922
Agps	0.62461	-0.6652	-0.731
Agpat1	0.56442	-0.5616	-0.6164

Genes associated with lipid metabolism were significantly altered during transition from pregnancy to lactation

Mammary Tissue	Microarray Fold expression	Q PCR Fold Expression
Acetyl CoA carboxylase	2.3	5.6
ATP citrate lyase	3.0	3.3
Fatty acid synthase	4.4	14.8
SREBP1	1.7	7.1

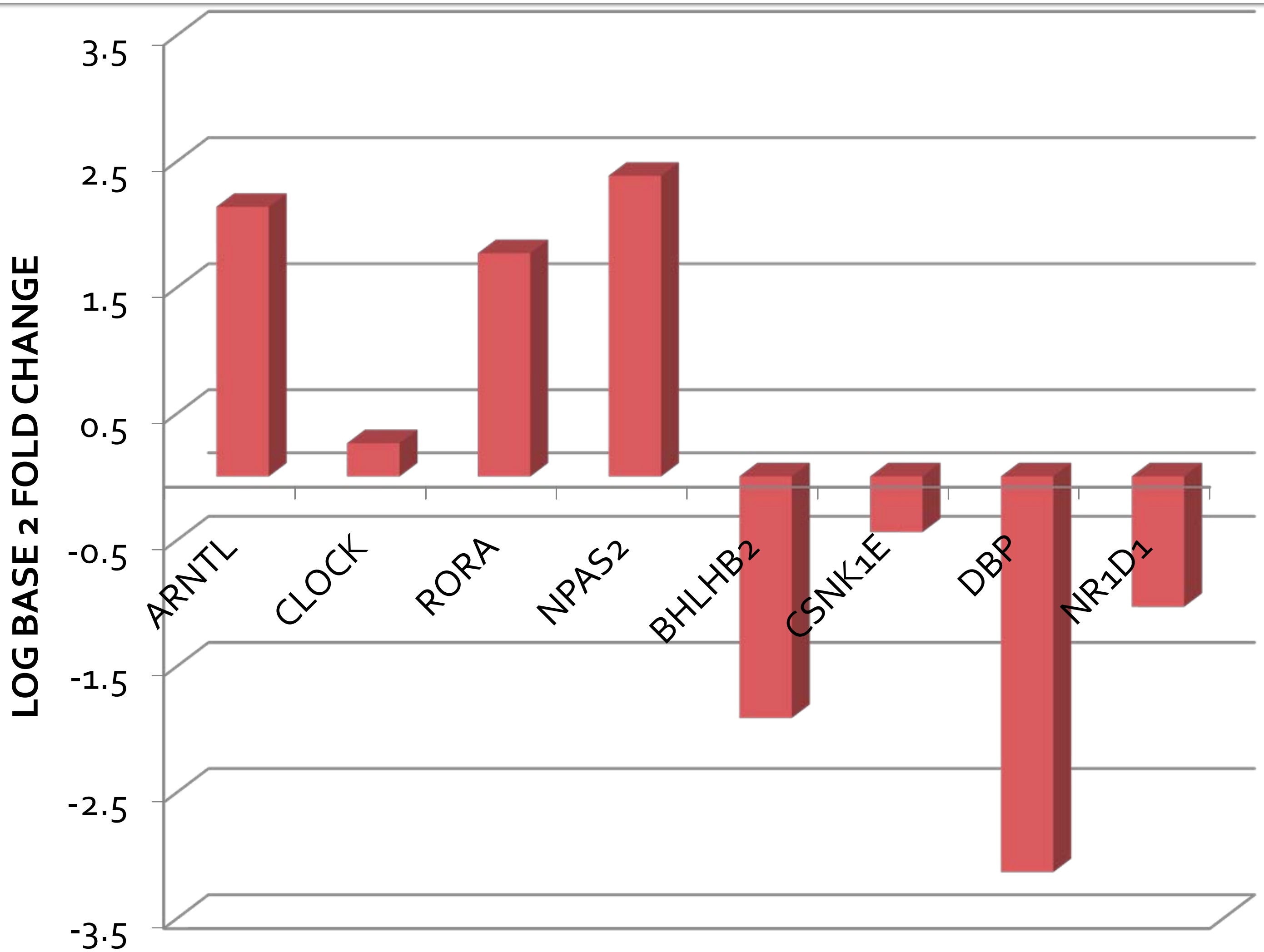
Adipose Tissue	Microarray Fold expression	Q PCR Fold Expression
Acetyl CoA carboxylase	0.4	0.4
ATP citrate lyase	0.7	0.7
Fatty acid synthase	0.3	0.5
SREBP1	1.3	1.8



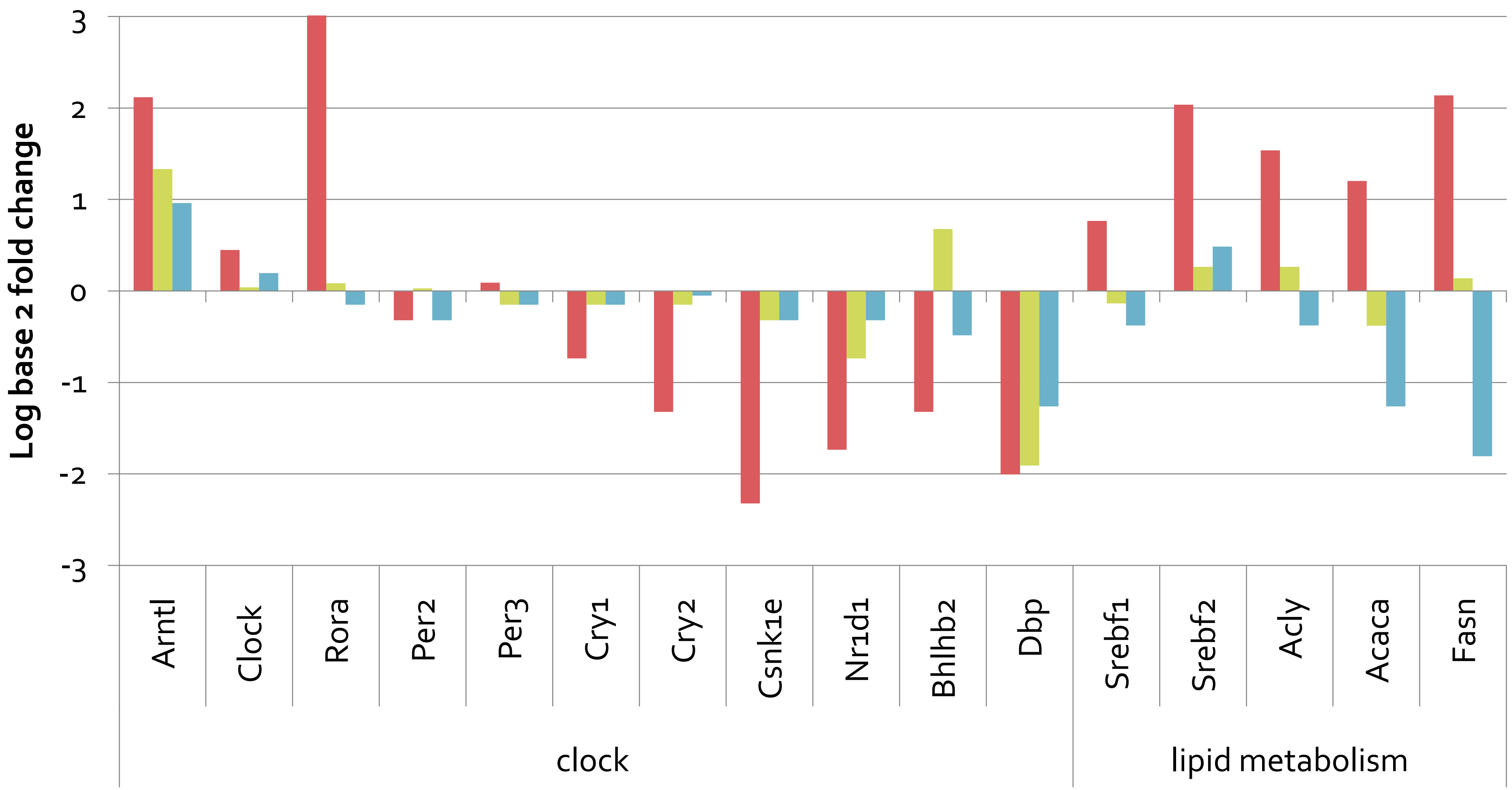
Coordinate Regulation

***Arntl* expression was among the top 10 genes induced during the P→L transition in all mammary, liver and adipose tissues.**

Clock genes are expressed in the mammary gland during the pregnancy to lactation transition

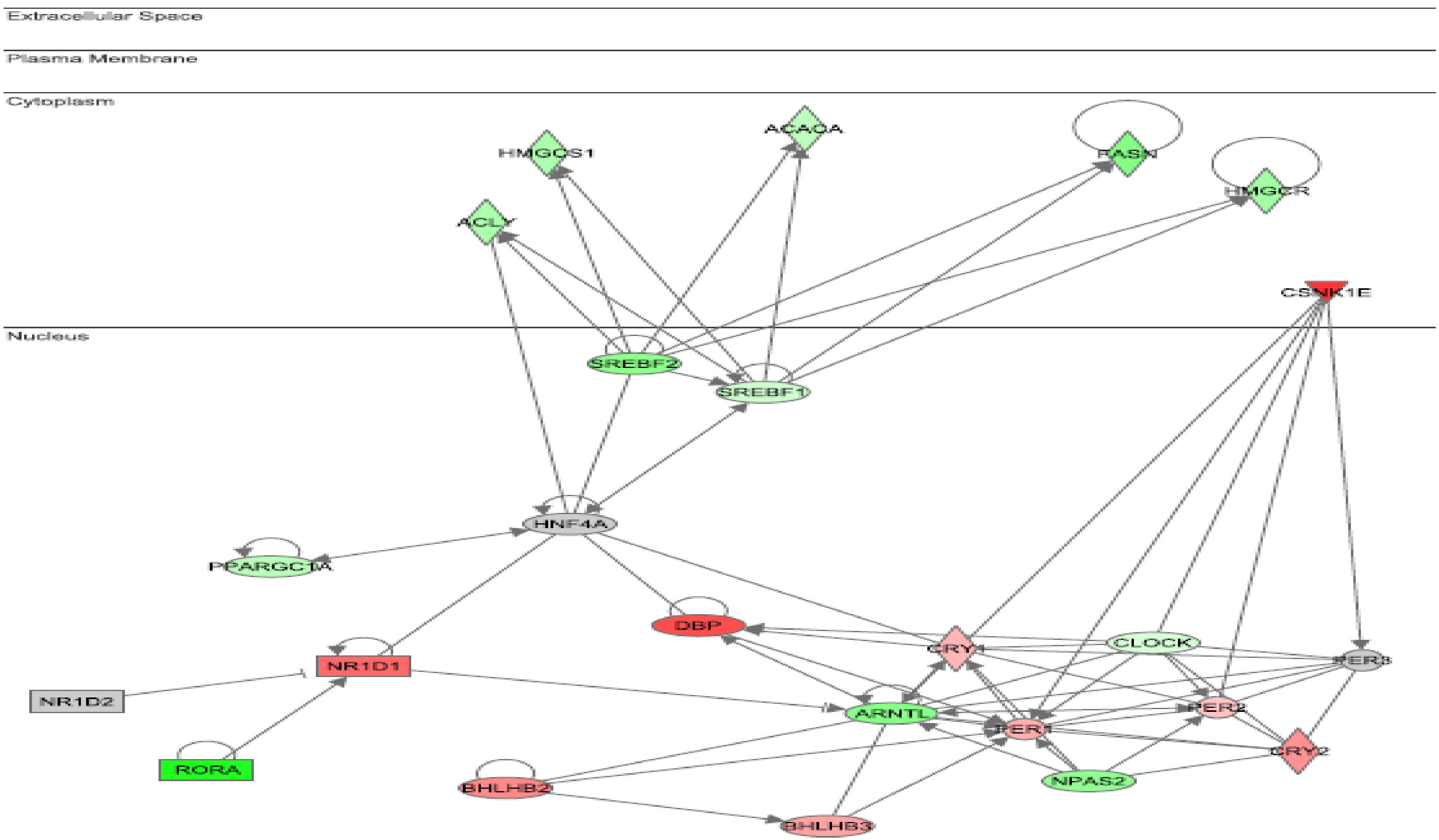


Changes in core clock genes and lipid metabolism genes during pregnancy to lactation transition

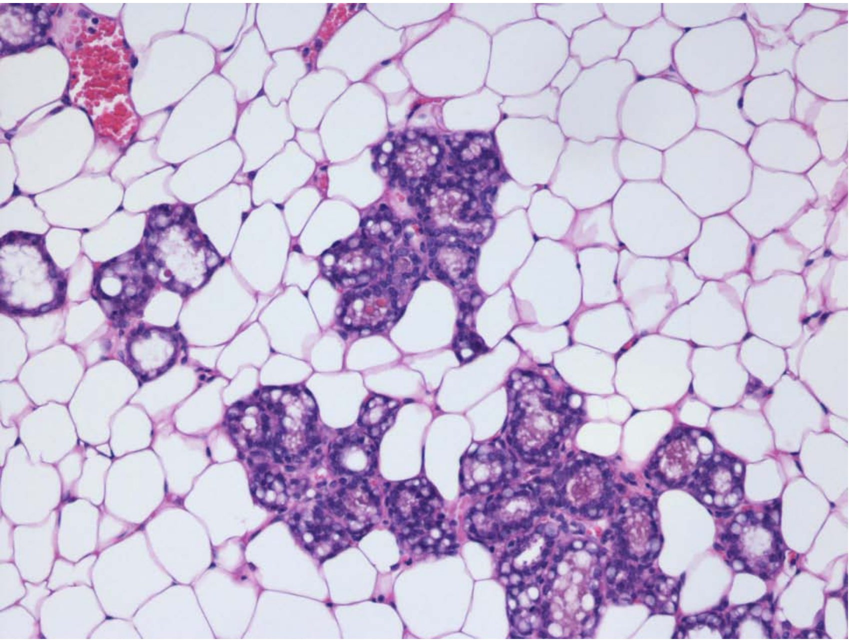


mammary (red) liver (green) adipose (blue) tissues.

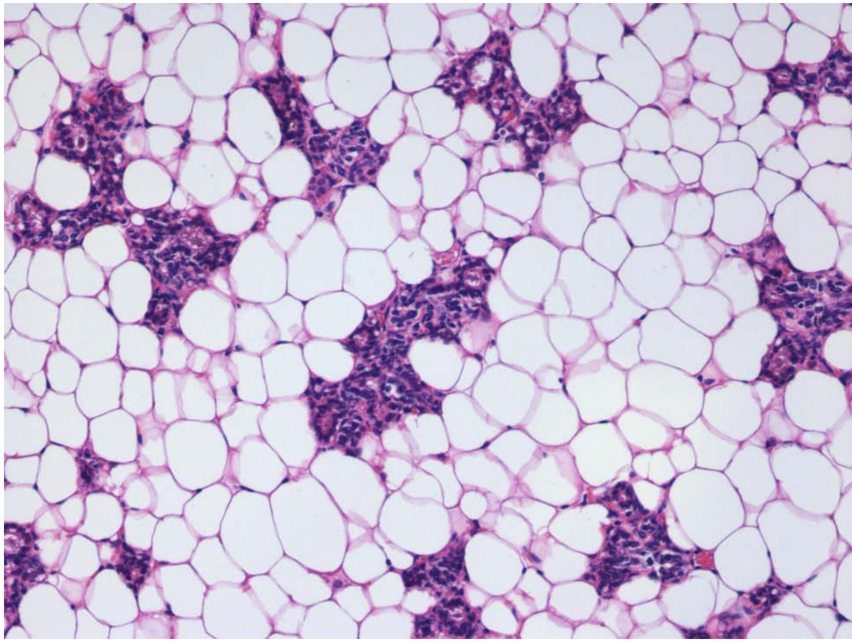
IPA network showing known interactions of circadian rhythm genes and lipid metabolism genes



Clock mutant mice show less mammary parenchyma on day 18 of pregnancy and a reduced ability to lactate



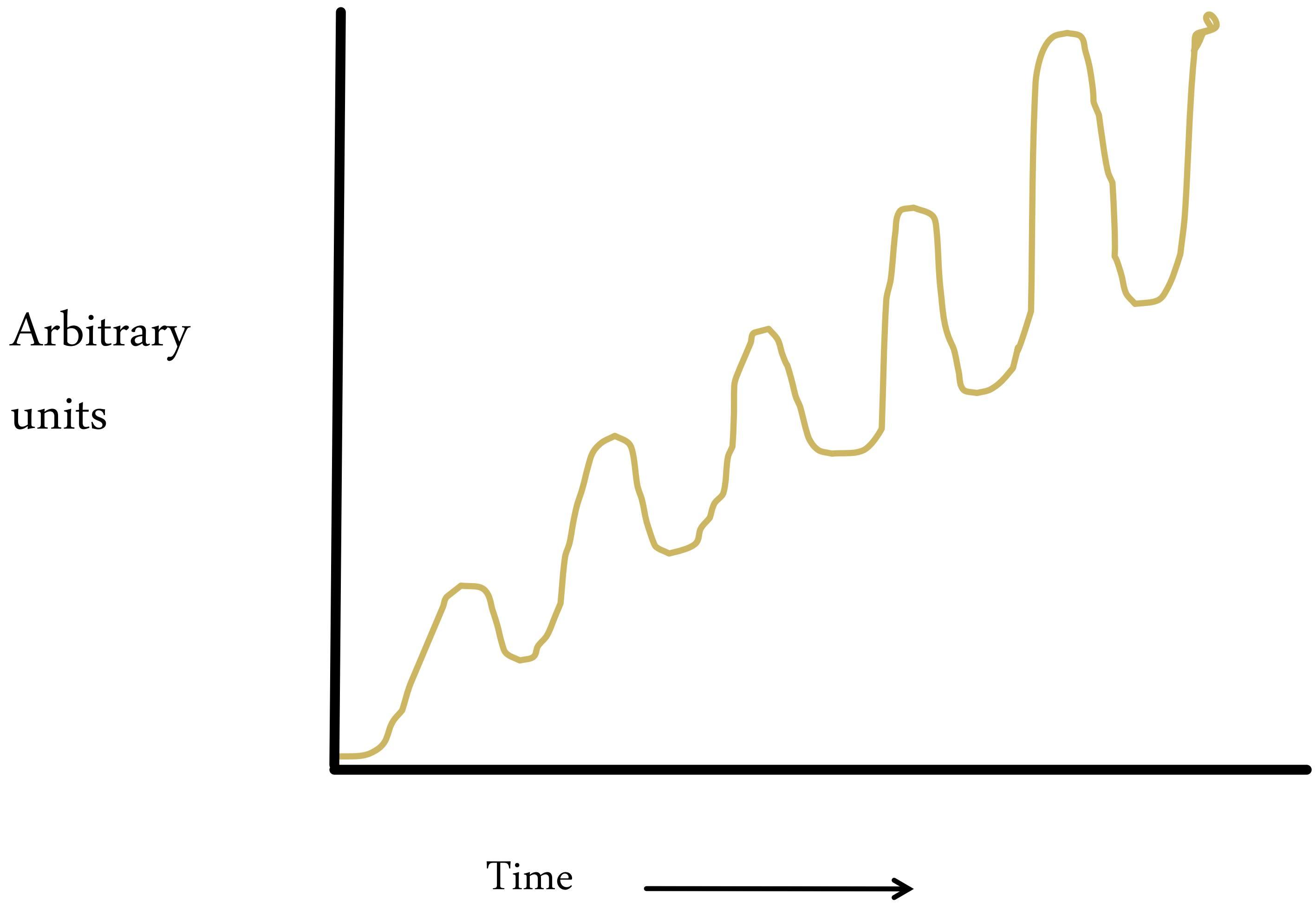
Wild type



clock/clock

Hematoxylin and eosin stained 5 μm sections of mammary gland no. 4 from WT C57BL/6J mice and C57BL/6J *Clock/Clock* mutant mice on pregnancy day 18

Diurnal and longitudinal profile of milk fat and lactose synthesis in rat dams



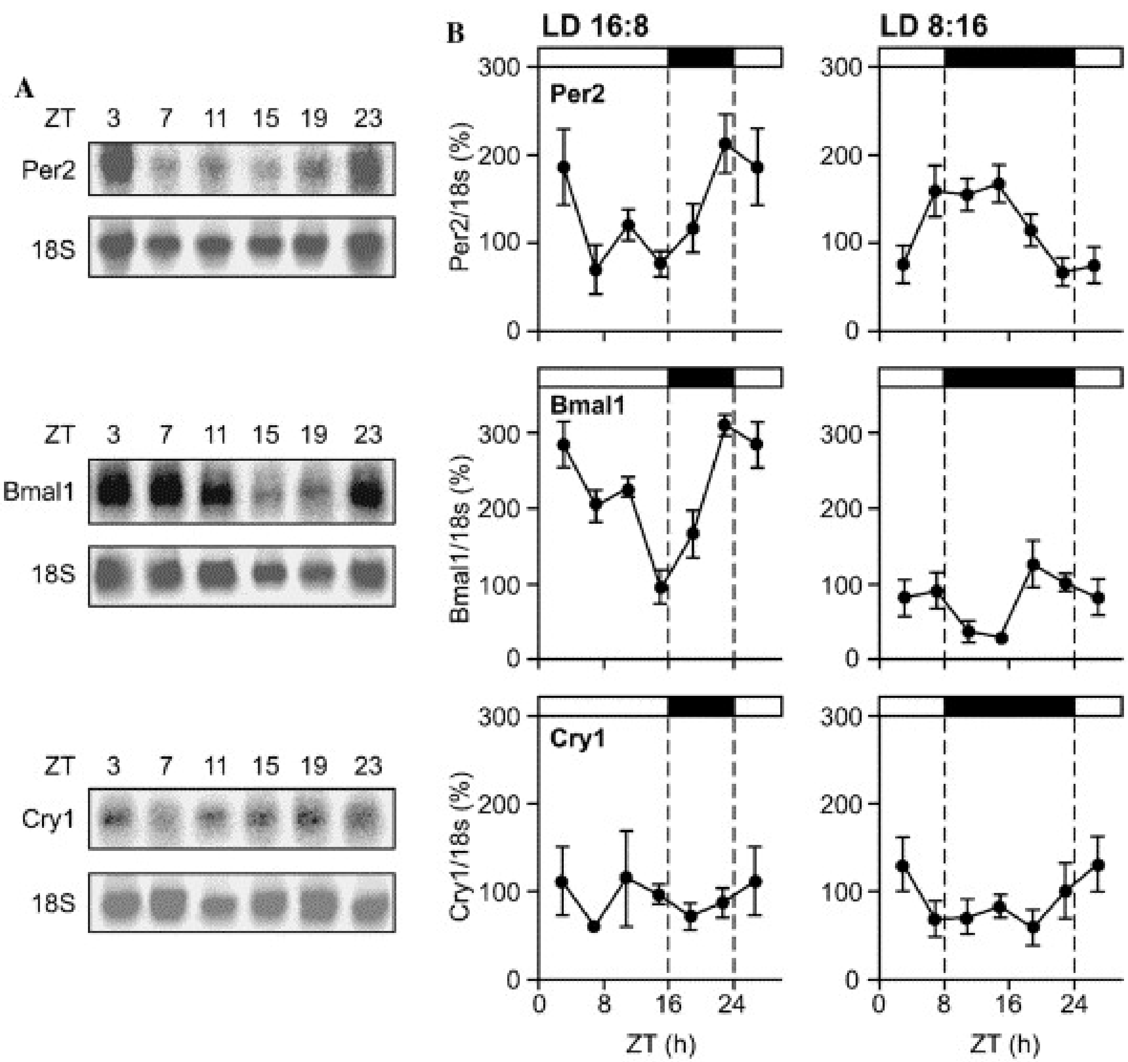
Kuhn, NJ et al., 1980. Lactose Synthesis the possibility of regulation. J Dairy Science.

What do we currently know about clock genes in cows?

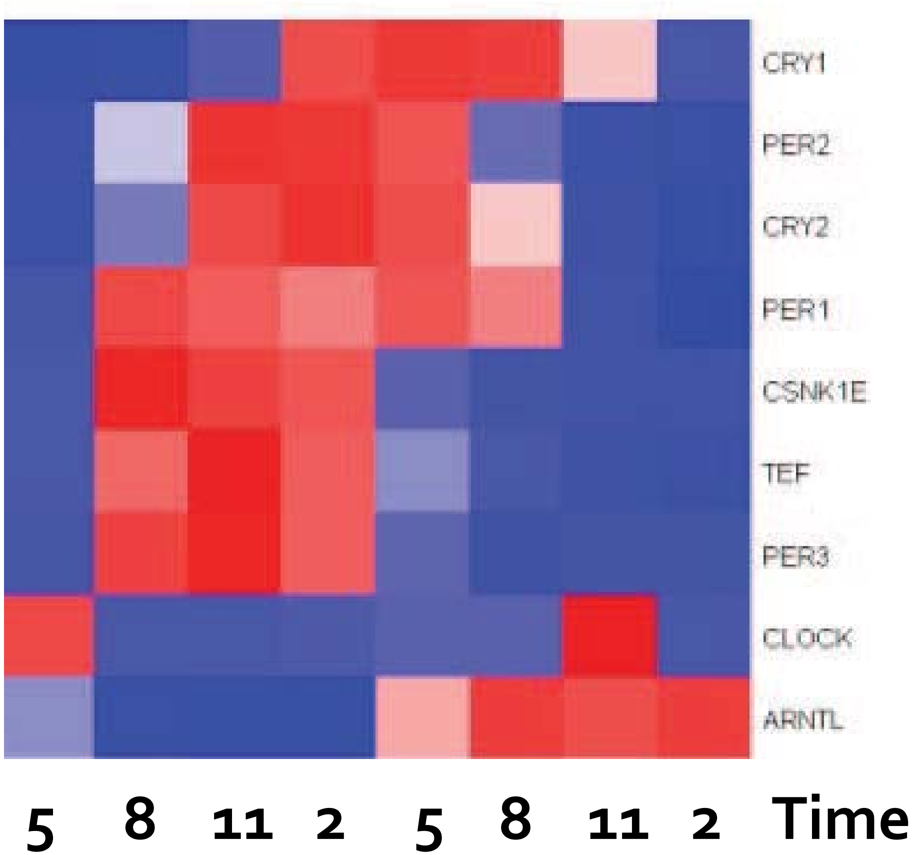
- Cows exhibit circadian rhythms
  - **Are there clock genes in the mammary gland?**
- Milk yield is influenced by length of photoperiod
  - Long days during the dry period: decrease milk yield
  - Long days during lactation: increase volume (Dahl)
- PRL and PRL-R change in response to photoperiod (Plaut – no change in milk Production)
  - **Do Clock genes respond to PRL?**

Core clock genes change in sheep liver in response to photoperiod

From anderrson et al. General and Comparative Endocrinology
Volume 142, Issue 3, July 2005, Pages 357-363. Shows that expression of core clock genes in sheep liver change with photoperiod manipulation….Per2 shows shift, BMAL1 shows amplitude change.



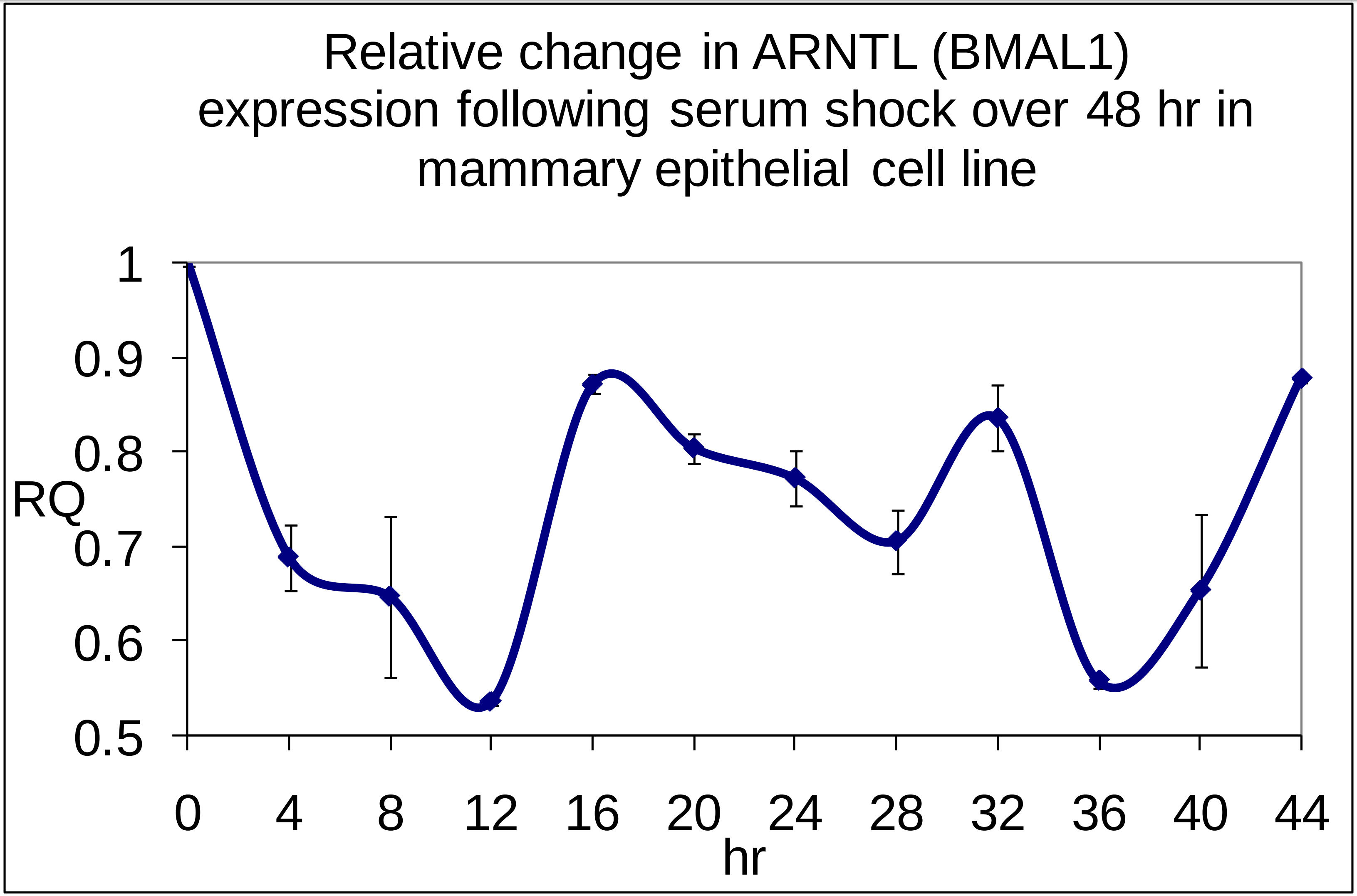
Human Mammary Epithelial Cells show circadian expression



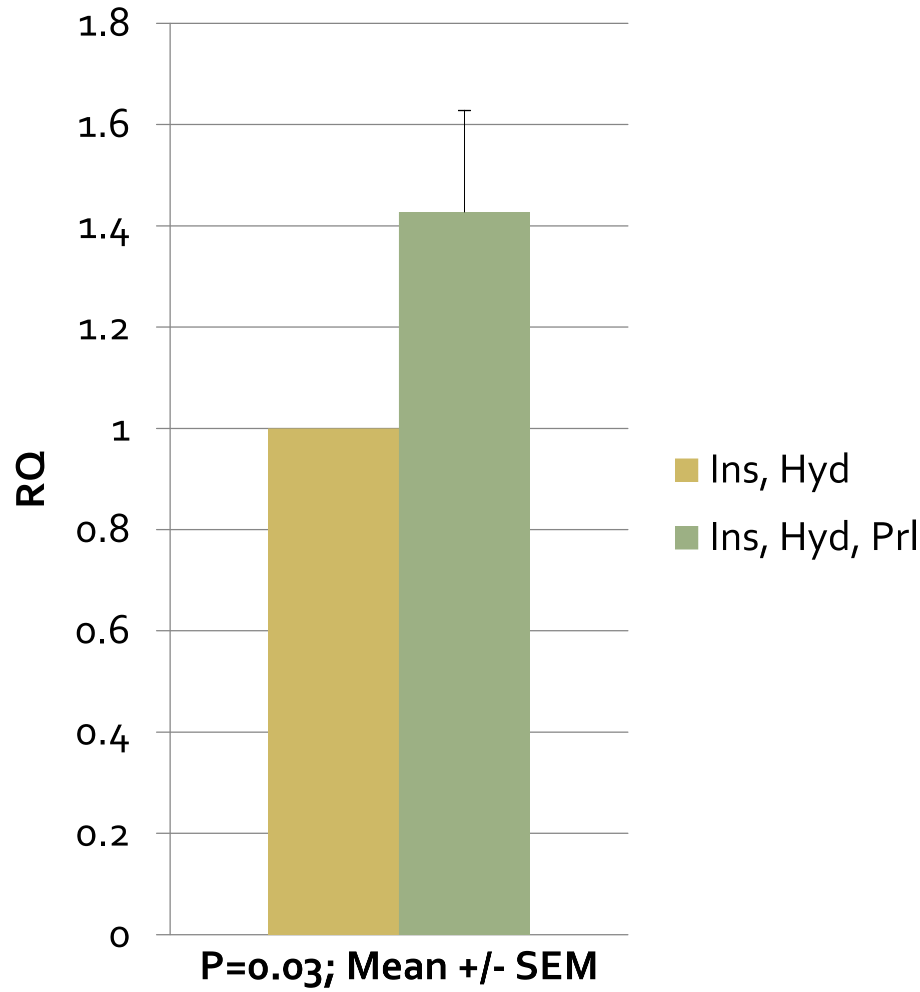
- Cells derived from milk fat of lactating women
- Milk samples taken every 3 hours over a 24 hour period
- 1,029 genes of 14,000 expressed exhibited a circadian rhythm

Mannigat et al.. Physiol Genomics 37: 12–22, 2009.

Bovine MAC-T cells express clock genes that exhibit a circadian pattern



PRL induces expression of BMAL-1 in bovine mammary explants

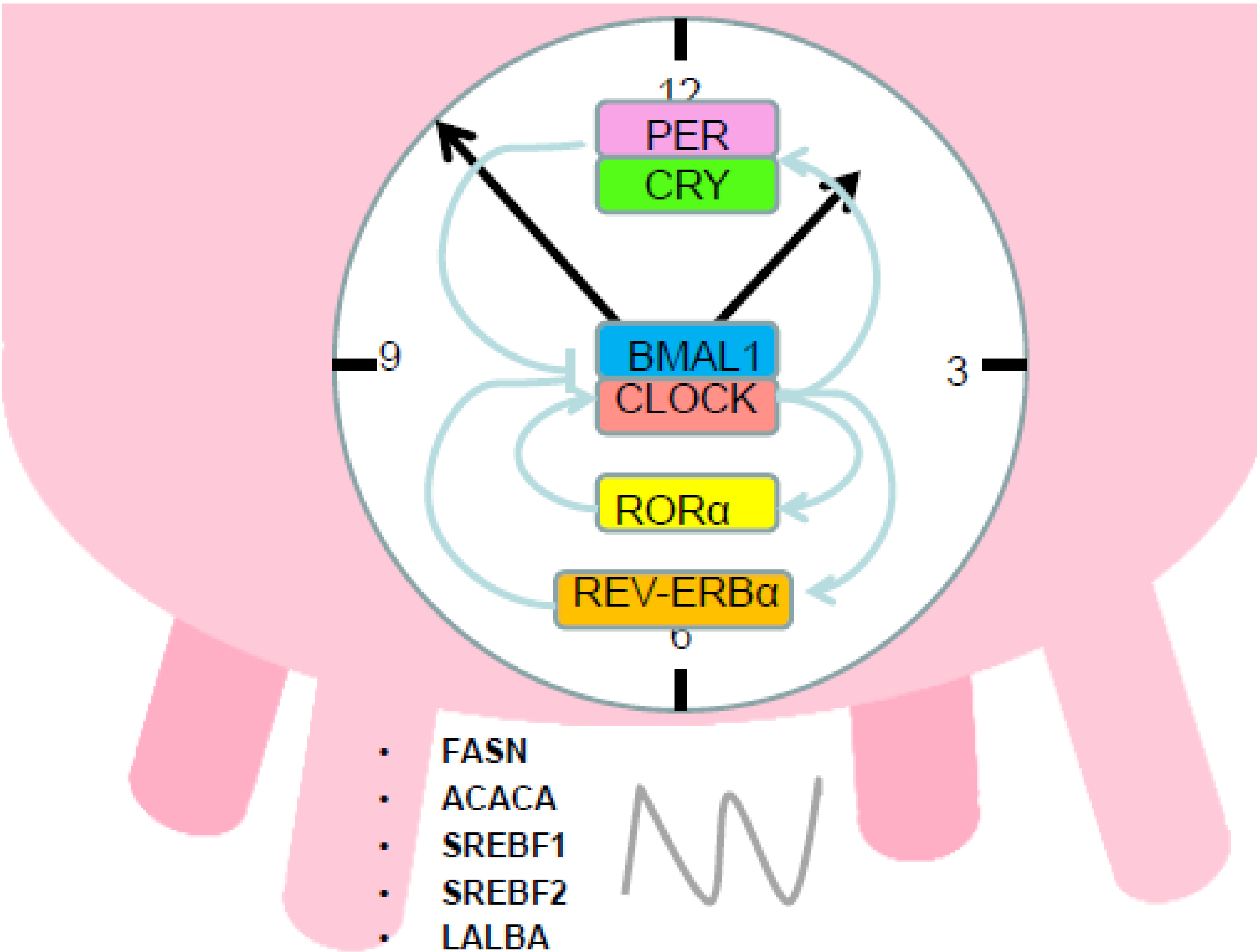


The expression of the core clock gene, BMAL1 was induced when prolactin was added to bovine mammary explant culture in the presence of insulin and hydrocortisone.

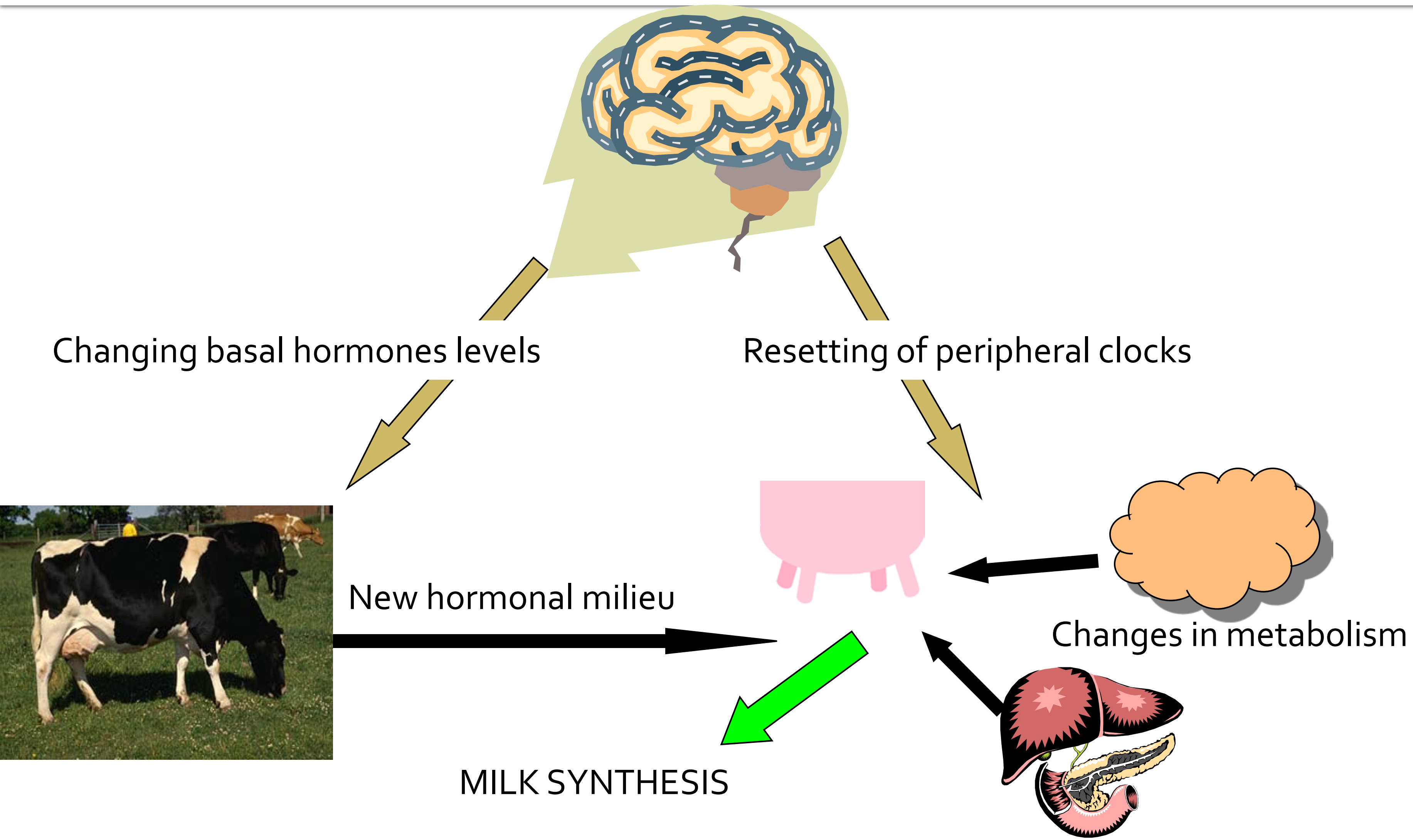
Role of Clock Genes in Mammary Function

- Mammary tissue, a peripheral tissue, has a functional clock that can be synchronized by physiological signals.
- Peripheral mammary clocks are responsive to prolactin, a major lactogenic hormone.
- Molecular clocks are coordinated among multiple tissues during the transition from pregnancy to lactation.
- Preliminary evidence suggests that molecular clocks may impact lactation performance

Transition from pregnancy to lactation induces changes in peripheral clocks



Resetting of master clock at onset of lactation results in…….



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