

Phospholipase C β 4 signaling in the mammalian circadian system.

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Whether we wake or
we sleep,
Whether we carol
or weep,
The Sun
with his
Planets
in
chime,
Marketh
the
going
of Time.

~ Edward
Fitzgerald

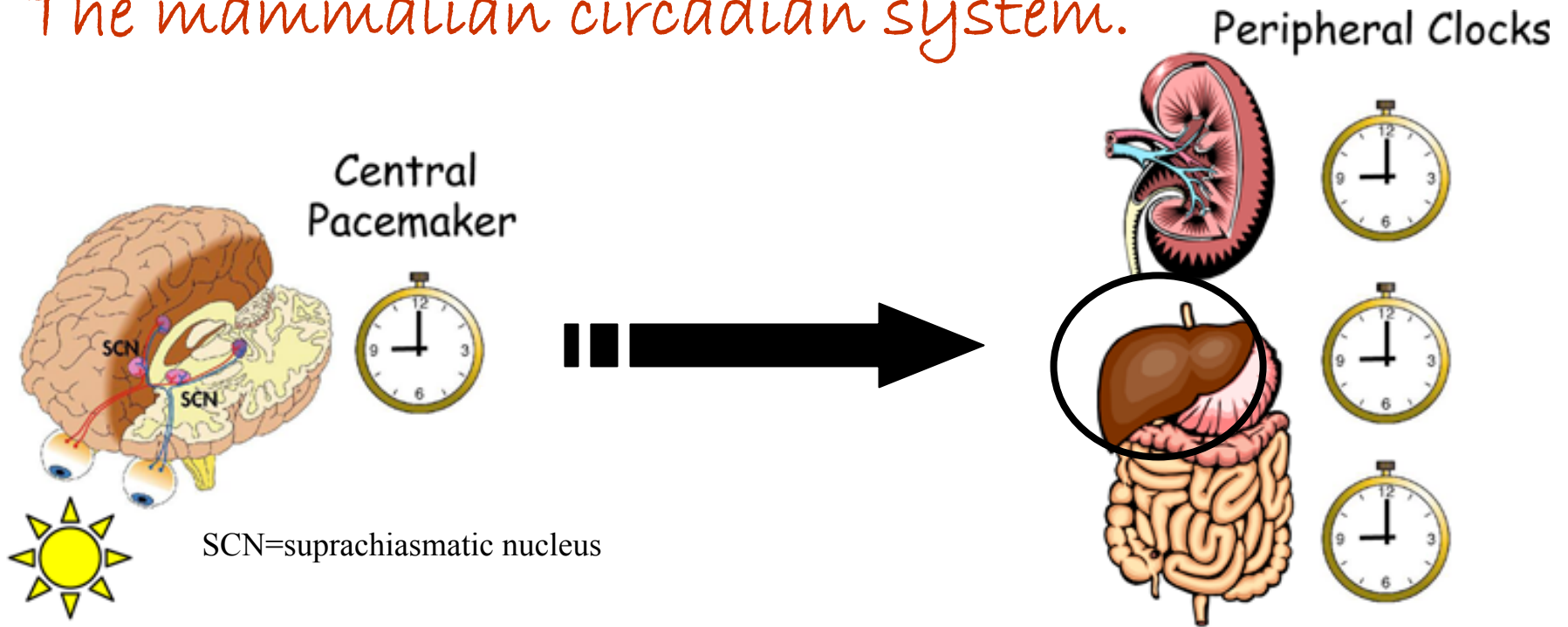
Health issues:

- sleep disorders
- depression
- Alzheimer's
- metabolic syndrome
- drug delivery

Quality of life issues:

- jet lag
- shift work

The mammalian circadian system.

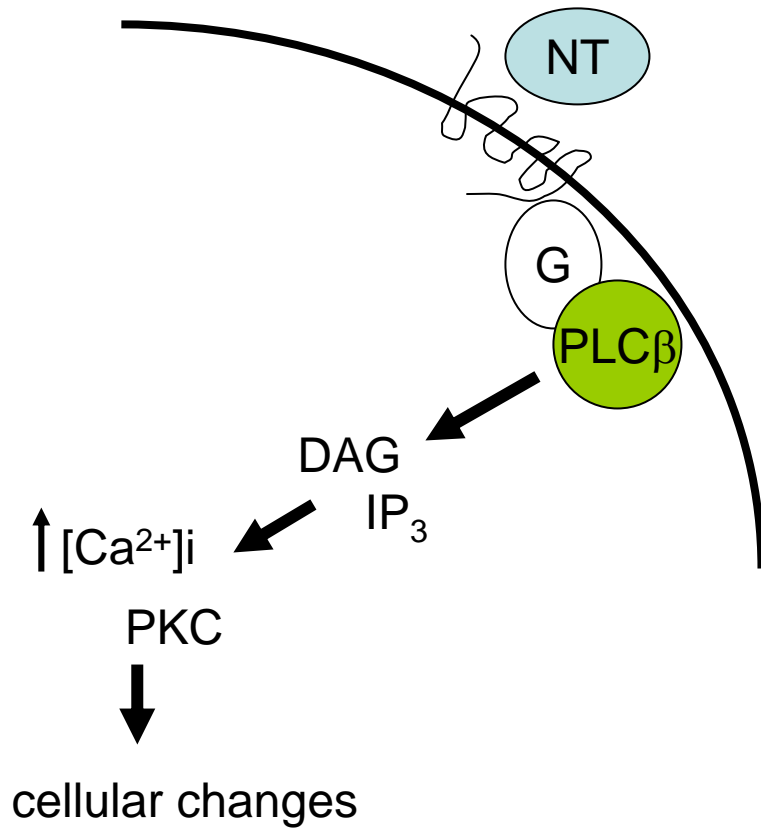


How does the central pacemaker convey temporal information to the peripheral clocks?

What are the signaling molecules involved in the output and input of these oscillators?

What kind of information are they conveying and what is the consequence for the cells?

Phospholipase C $\beta 4$ and the mammalian circadian system.



Linked to several receptors subtypes found in the SCN clock cells and hepatocytes.

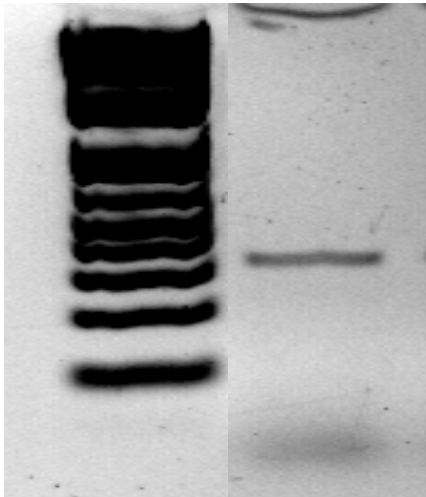
Knockouts have circadian behavioral phenotype.

Downstream effects of PLC β signaling are correlated with signaling events in the clock.

PLC $\beta 4$ has been localized to cells in the SCN (but not in hepatocytes)

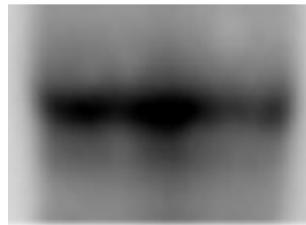
Is PLC β 4 expressed in the hepatocytes of mice?

PCR

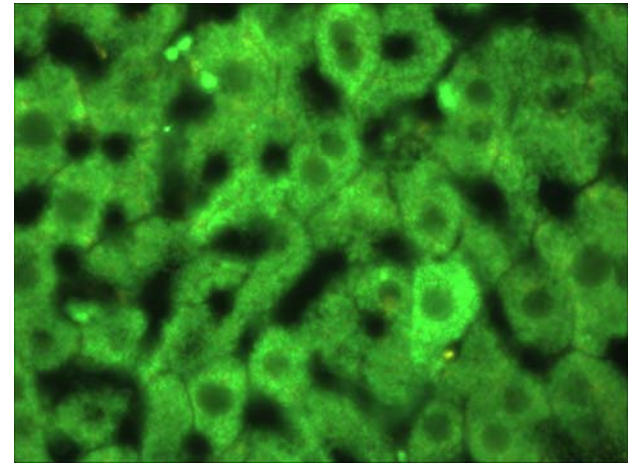


← 324 bp

Western blotting

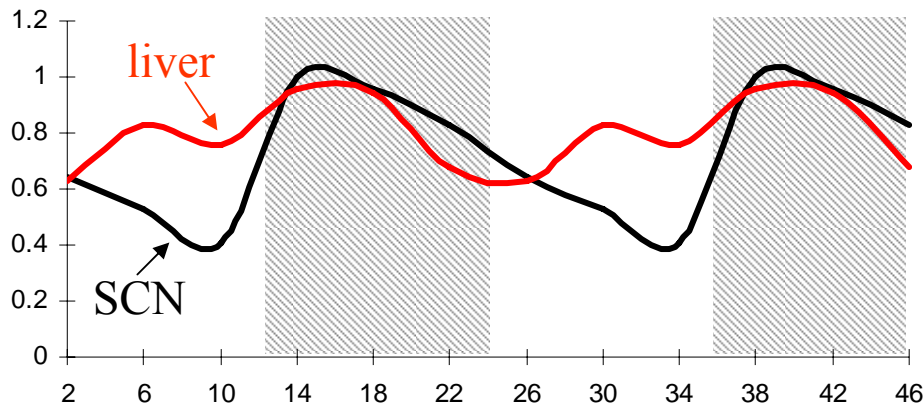


← 134 Kda



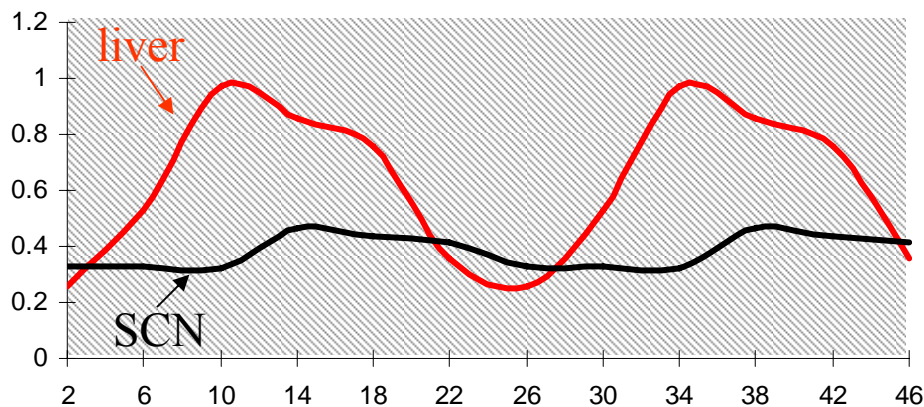
immunofluorescence

Does the PLC β 4 protein oscillate over the course of the day in central and/or peripheral clocks?



PLC β 4 undergoes a significant oscillation in mouse SCN and liver.

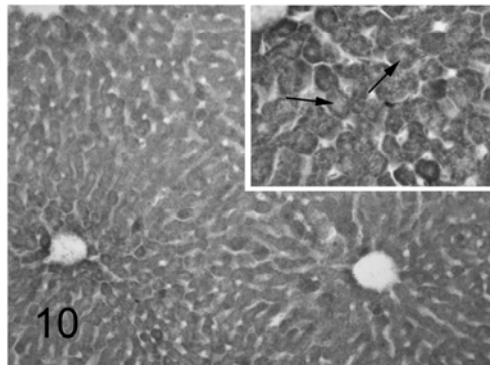
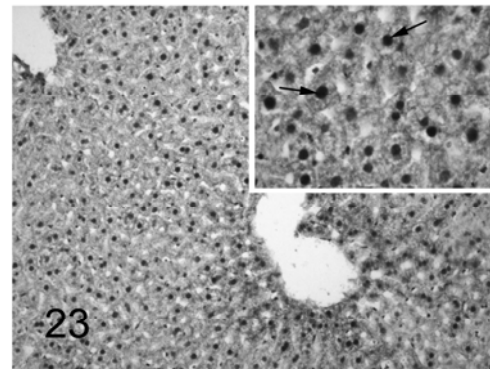
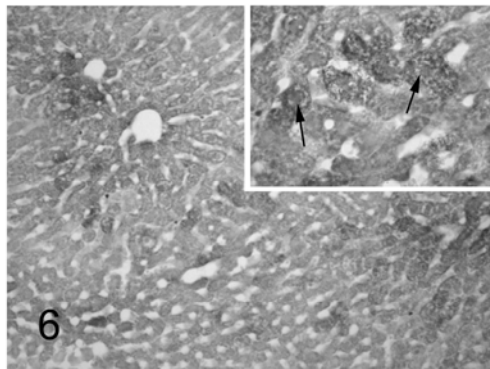
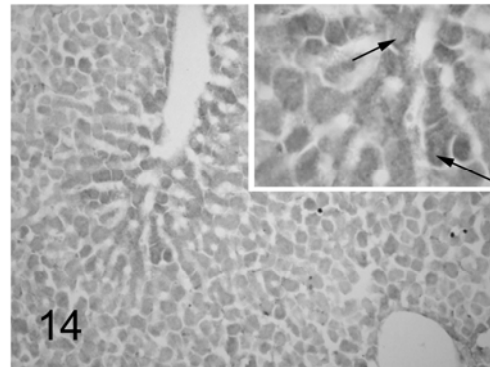
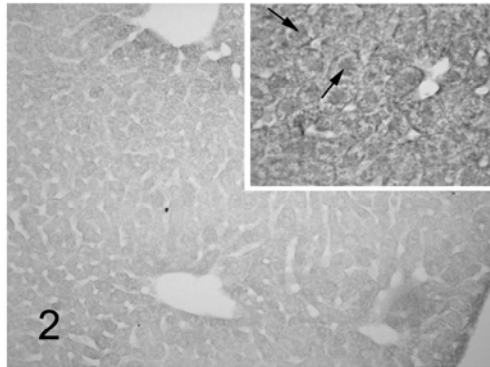
In both tissues, protein levels peak in the early night time hours.



The PLC β 4 oscillation in the SCN is primarily regulated by the photoperiod.

The rhythm in the liver persists in constant darkness, and is thus, circadian in nature.

What is the distribution of PLC β 4 in the liver?

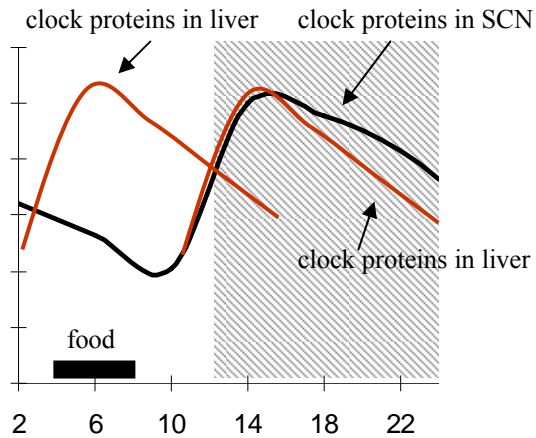


	ZT2	6	10	14	23
Nuclear	++	+	-	-	+++
Cytoplasmic	+	+	++	++	+

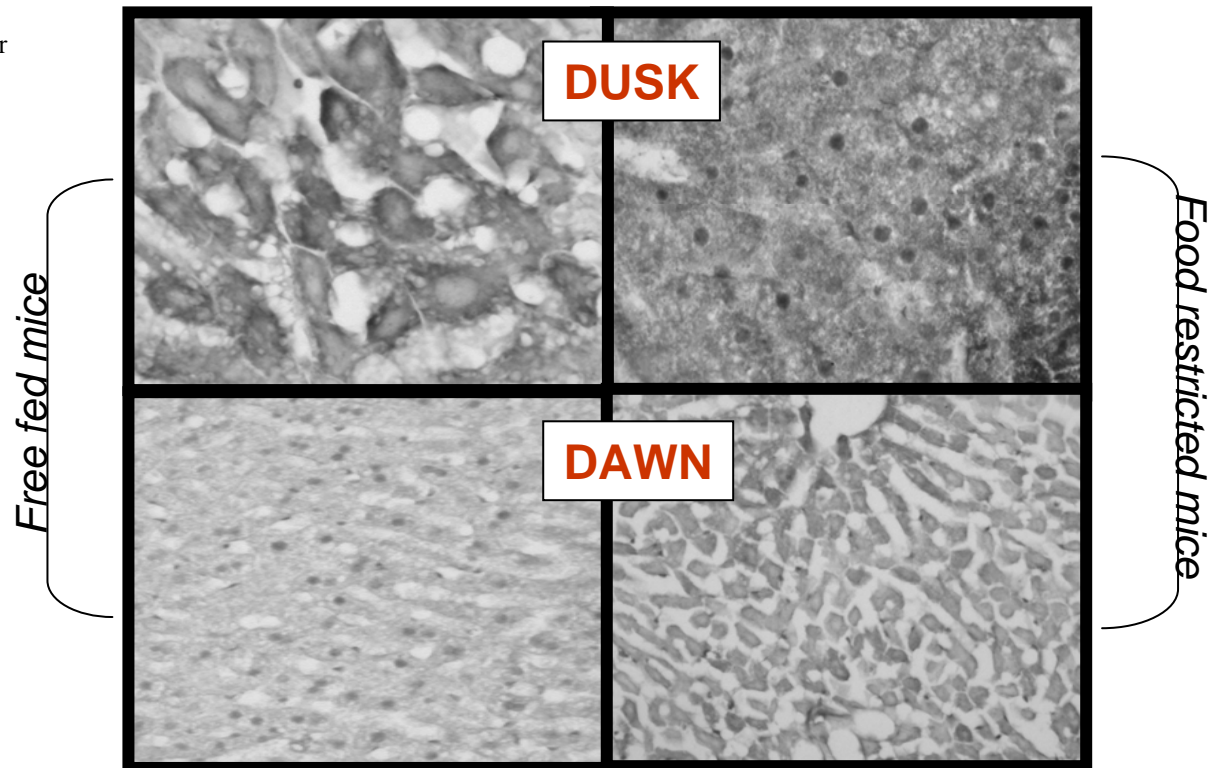
PLC β 1 protein has been shown to function as a nuclear signaling molecule to regulate hepatocyte growth and proliferation.

Core clock proteins translocate to the nucleus to negatively regulate their own transcription at night.

Effect of food availability on the circadian system.



Is the timing of PLC β 4 translocation altered by food availability?



What is the significance of all this?

The identification of a signaling pathway that oscillates over the course of the day in the SCN clock cells could underlie why certain stimuli such as light (glutamate and PACAP) and melatonin can reset the clock during the late day into night.

We have been able to localize this same enzyme to mouse hepatocytes where it was previously thought not to exist.

The levels of this protein oscillate, it translocates to the nucleus and responds to restricted feeding independently of the external photoperiod and in a manner similar to known clock proteins.

These data could have implication with regards to liver metabolism and cellular proliferation, both of which are known to be under circadian control.



Undergraduates:

- Ashley Nazario
- Rich Wooldrige
- Travis Jenkins
- Rachel Fuller
- Blakely Andrews
- Barbra Bannan
- Brittany Klein
- Andrea Chianella
- David Williams
- Kristin Martin
- Kim Christensen

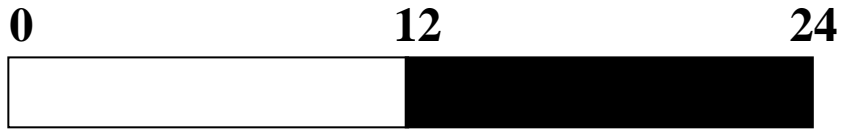
Funding:

SC-INBRE

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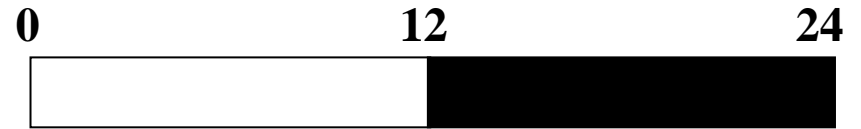
Food Entrainment Paradigm

Zeitgeber Time (ZT) (hours)



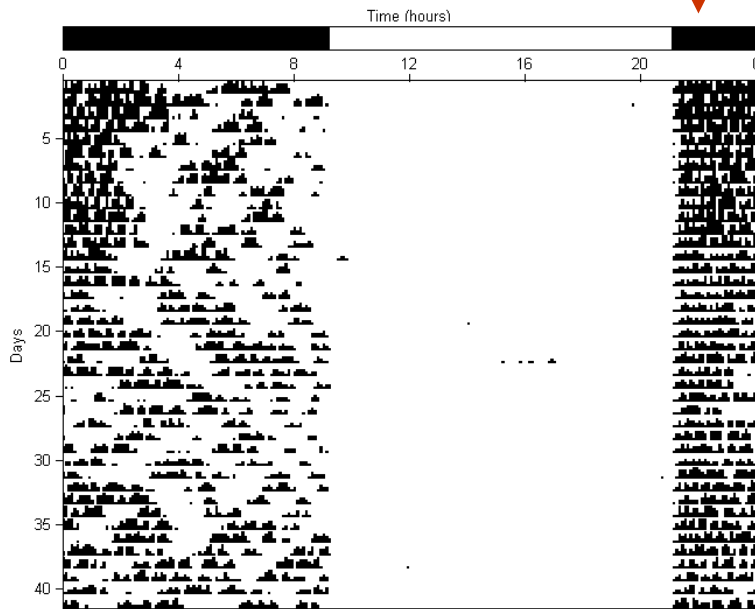
Food availability ZT5-ZT 9

Zeitgeber Time (ZT) (hours)



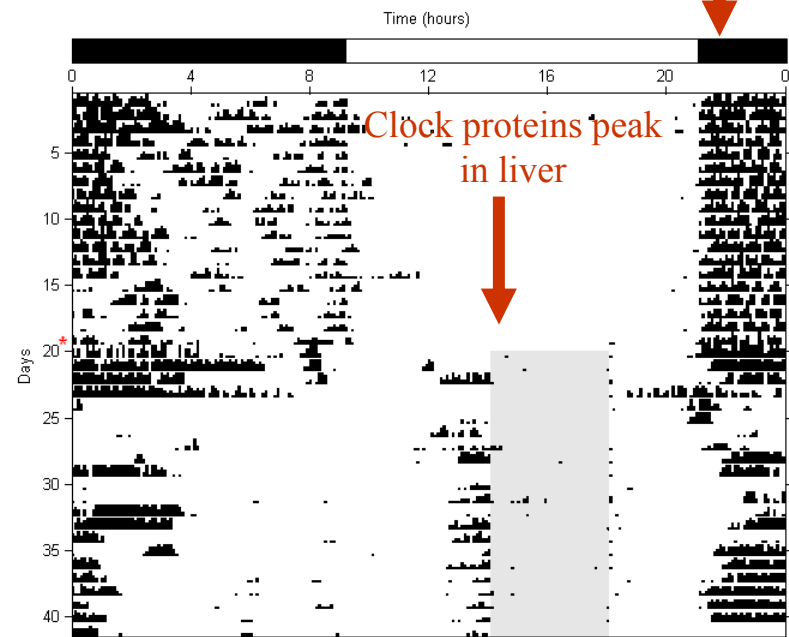
4, 5 11, 12 17 20 23
Times of Sacrifice

Free-fed mouse



Clock proteins peak in
SCN and liver

Food restricted mouse



Clock proteins peak
in SCN

Clock proteins peak
in liver

Is the oscillation being regulated at the level of transcription in either of these tissues?

