Biological clocks in Drosophila

- Drosophila circadian rhythm is a daily 24-hour cycle of rest and activity in the fruit flies of the genus Drosophila. The biological process discovered in model species Drosophila melanogaster is best understood as of date. Because, D. melanogaster has two unique daily behaviours, namely regular movement and hatching from the pupa, called eclosion. Locomotor activity is on daily basis with two peaks, while eclosion occurs at dawn.
- Drosophila circadian rhythm was discovered in 1935 by German zoologists, Hans Kalmus and Erwin Bünning.

Outline of lecture

- Model organism
  - Definition
  - Current models
- Characteristics of Drosophila
- Drosophila in Research
- Homology with humans, Conserved genes
- Locomotor activity monitoring
- Genetic basis of the clock in flies
- The transcriptional feedback loop of the Drosophila clock

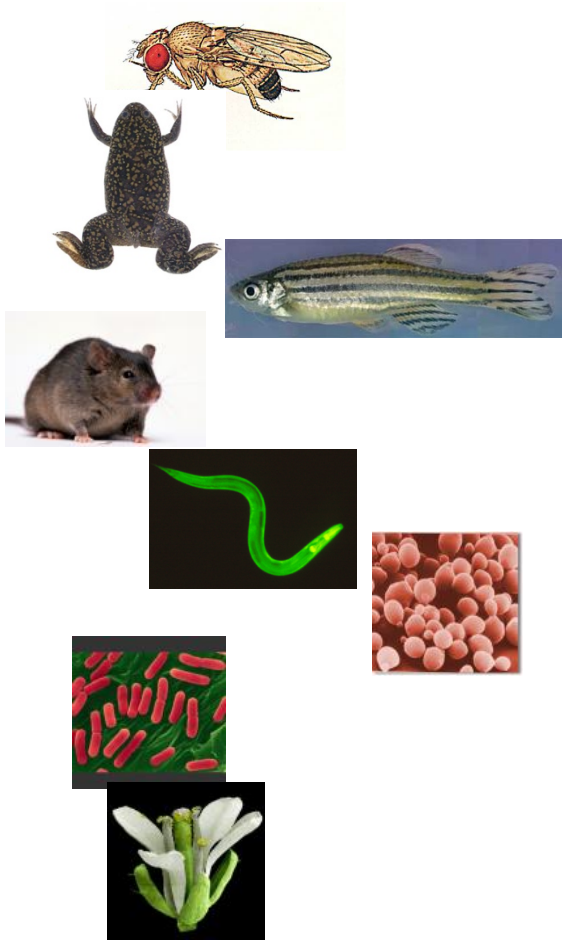
- Limitations of fly models
- Summary
-

Model Organism

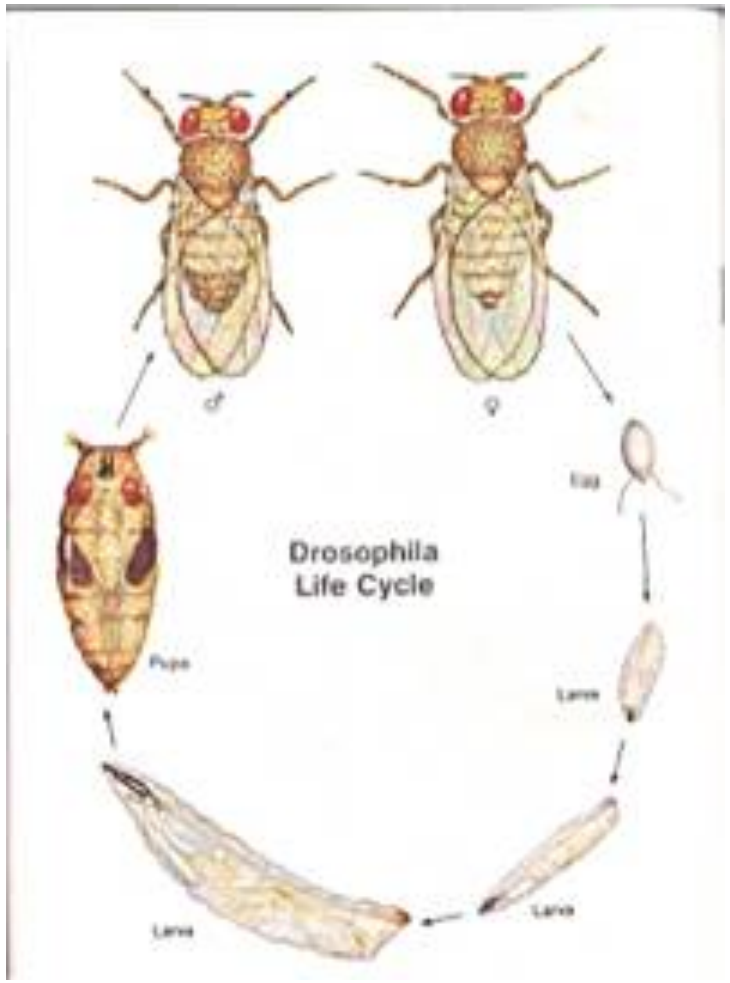
- Specific species
- research laboratories take up more frequently
- Aimed at understanding
  - Cellular function
  - Developmental details
  - Abberations
- Knowledge acquired can be extented to other organisms

Current Models

- Drosophila
- Xenopus
- Zebrafish
- Mouse
- C. elegans
- Yeast
- E. coli
- Arabidopsis



Characteristics of Drosophila



- Small, easy and cheap to maintain and manipulate
- Short lifespan
- Produce large numbers of offspring
- Development is external
- Availability of mutants
- Lots of history/previous experiments and discoveries
- Genome is sequenced
- Homologues for at least 75 % of human disease genes
- Exhibit complex behaviours
- Fewer ethical concerns

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Drosophila in Research

- Another circadian behavior in Drosophila is courtship between the male and female during mating.
- In Drosophila there are two distinct groups of circadian clocks, namely the clock neurones and the clock genes. They act concertedly to produce the 24-hour cycle of rest and activity.

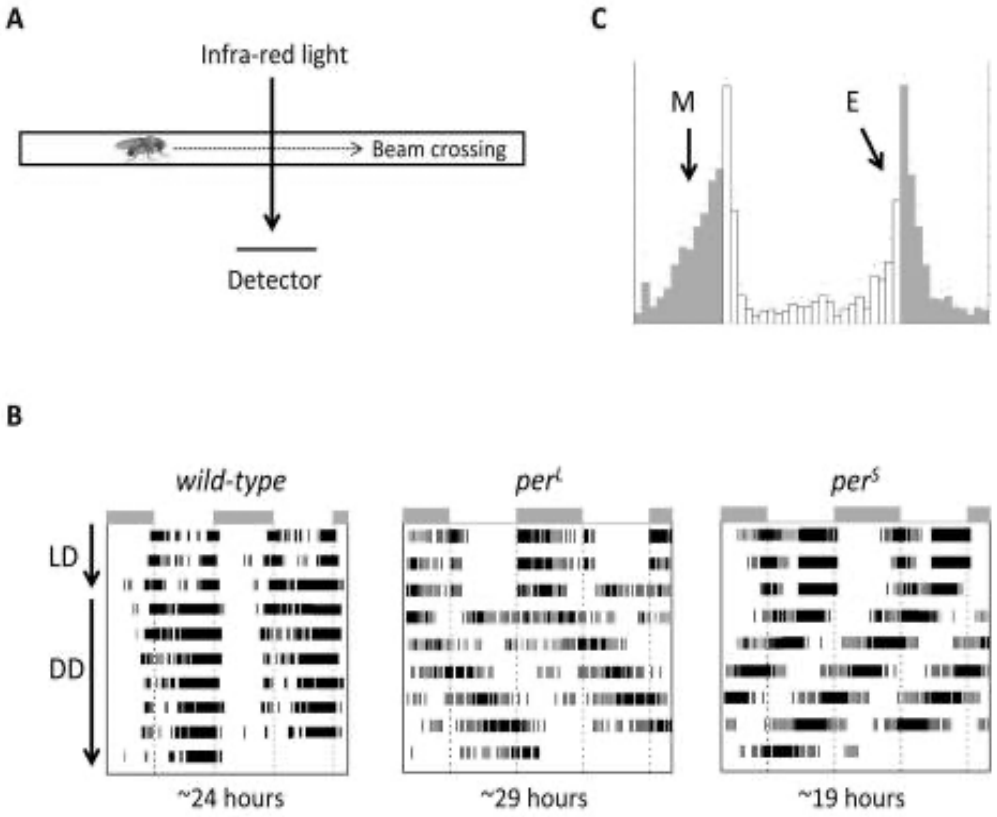
More about Drosophila

- Another circadian behavior in Drosophila is courtship between the male and female during mating.
- In Drosophila there are two distinct groups of circadian clocks, namely the clock neurones and the clock genes. They act concertedly to produce the 24-hour cycle of rest and activity.

Homology with humans, Conserved genes

Human gene	**Drosophila gene**	**Affect when mutated**
Hox genes	Hox genes	Alteration of anterior-posterior identities
PAX6	*eyeless*	Defects of the eyes
SALL1	*salm or salr*	Defects of the auditory system
TWIST1	*twist*	Malformations of mesodermal derivatives
NKX2-5	*tinman*	Defects in heart specification and function

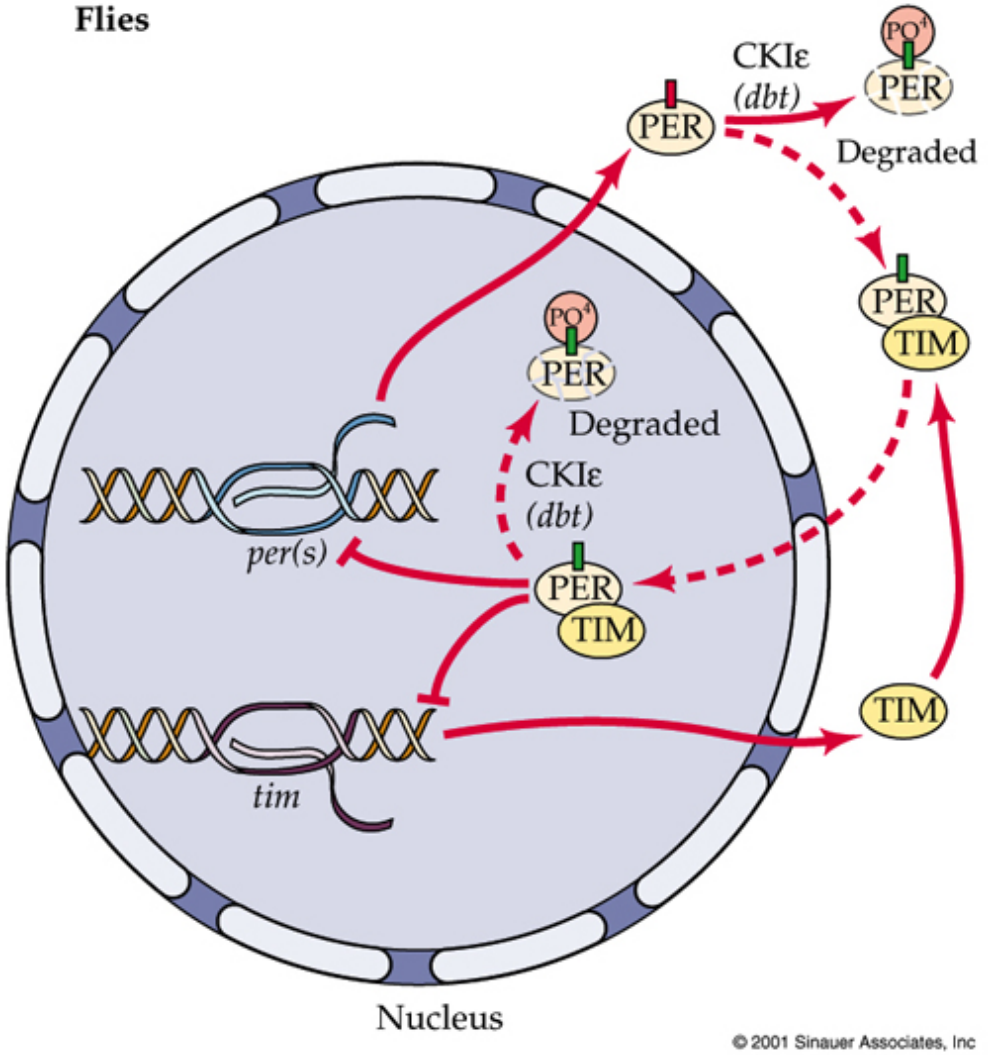
Locomotor activity monitoring



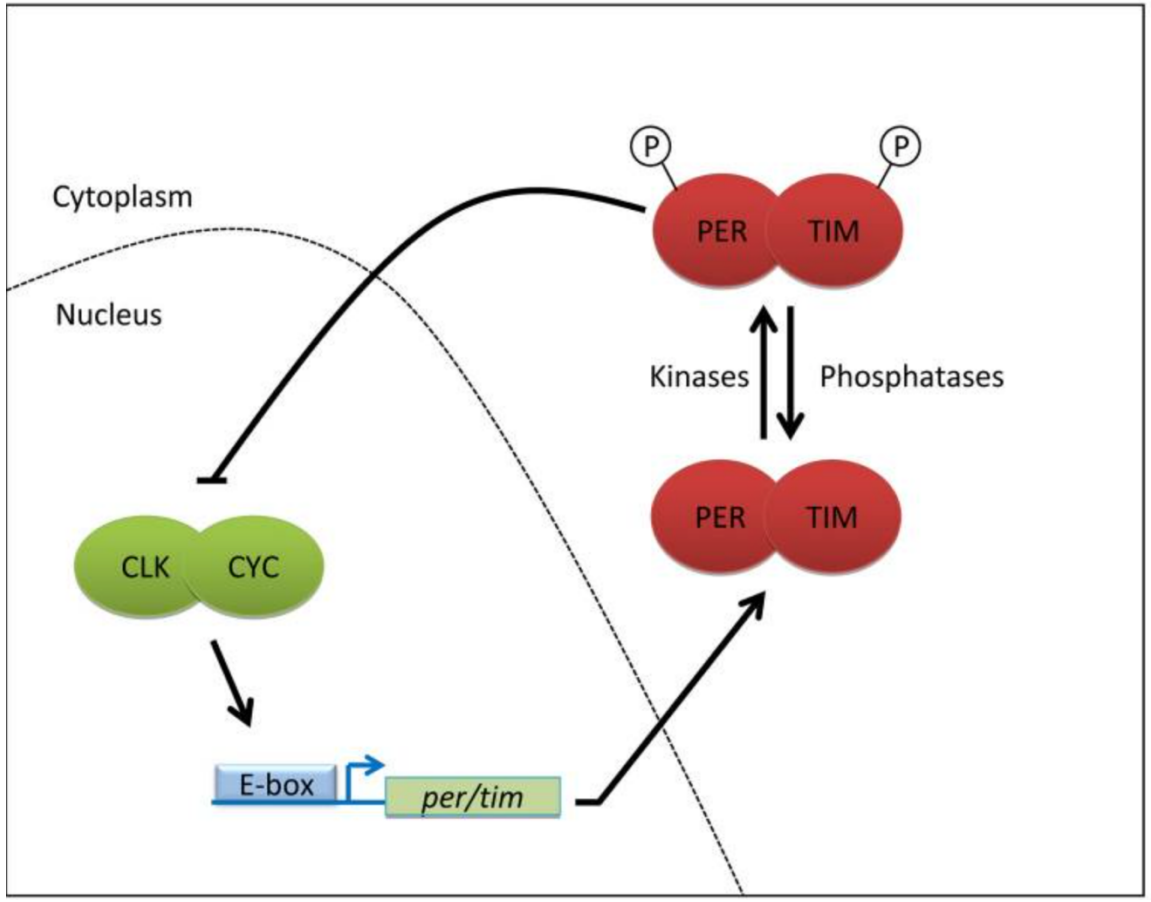
- A) Fly locomotion is detected when a fly breaks an infra-red beam crossing the small glass tube in which it is housed (B) Double-plotted actogram showing the activity of flies entrained to a 12/12-hr LD cycle and then released in constant darkness for period determination. Each day is plotted twice, first on the right and duplicated on the left half of the next line, except for the first day. Note the progressive drift of circadian behavior in constant conditions in per mutant flies, corresponding to long and short periods (C) Eduction plot of fly activity after entrainment to an LD cycle. The Morning (M) and Evening (E) anticipatory behavior driven by the circadian clock are shown with arrows.

Genetic basis of the clock in flies

- *per* and *tim* genes are turned on by *clock* and *cycle*
- PER and TIM proteins build up inside the cell during dark
- *dbt* codes for an enzyme that degrades PER & adds time delay
- Cryptochromes absorb blue light and activate *cry* gene expression
- TIM protein is degraded by CRY protein



The transcriptional feedback loop of the *Drosophila* clock

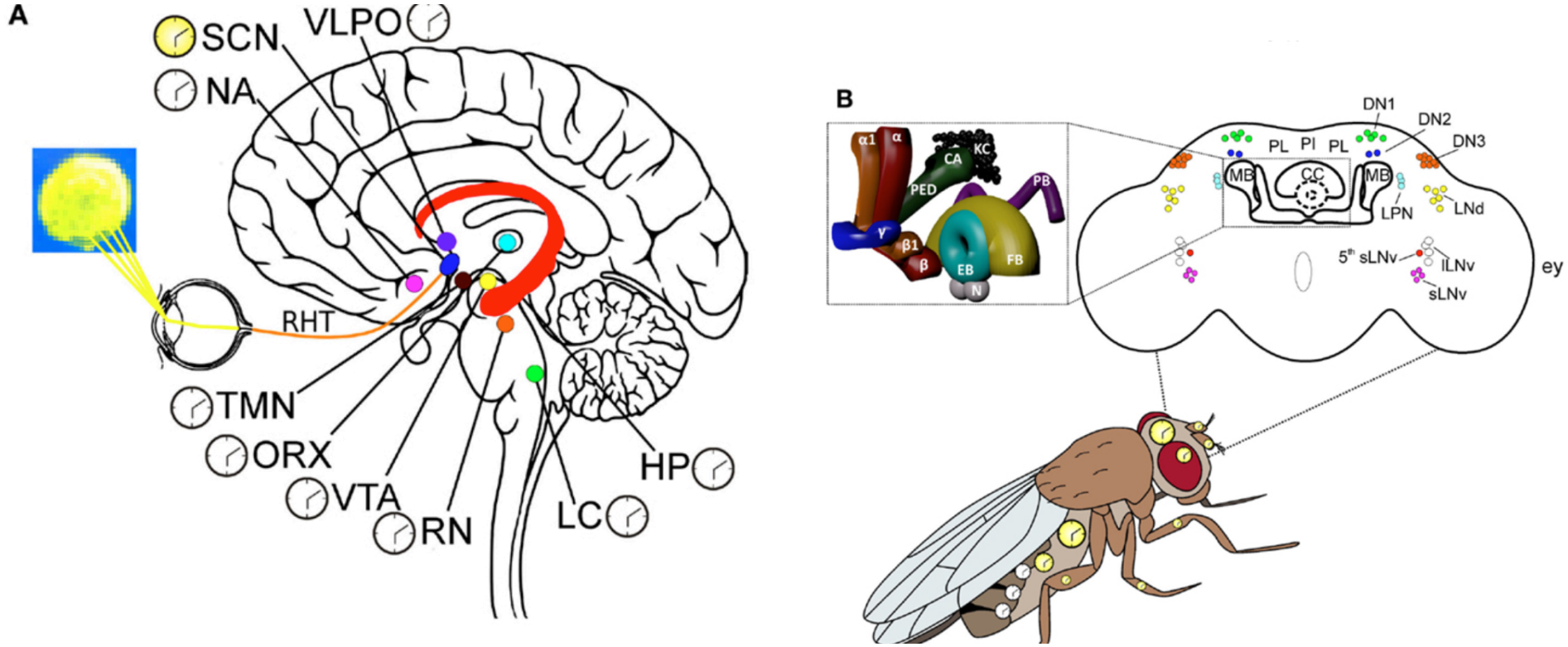


CLK/CYC drive expression of their own repressors PER and TIM. PER/TIM go through various modifications during the day, until they are eventually turned over to release CLK/CYC from repression, starting the next cycle.

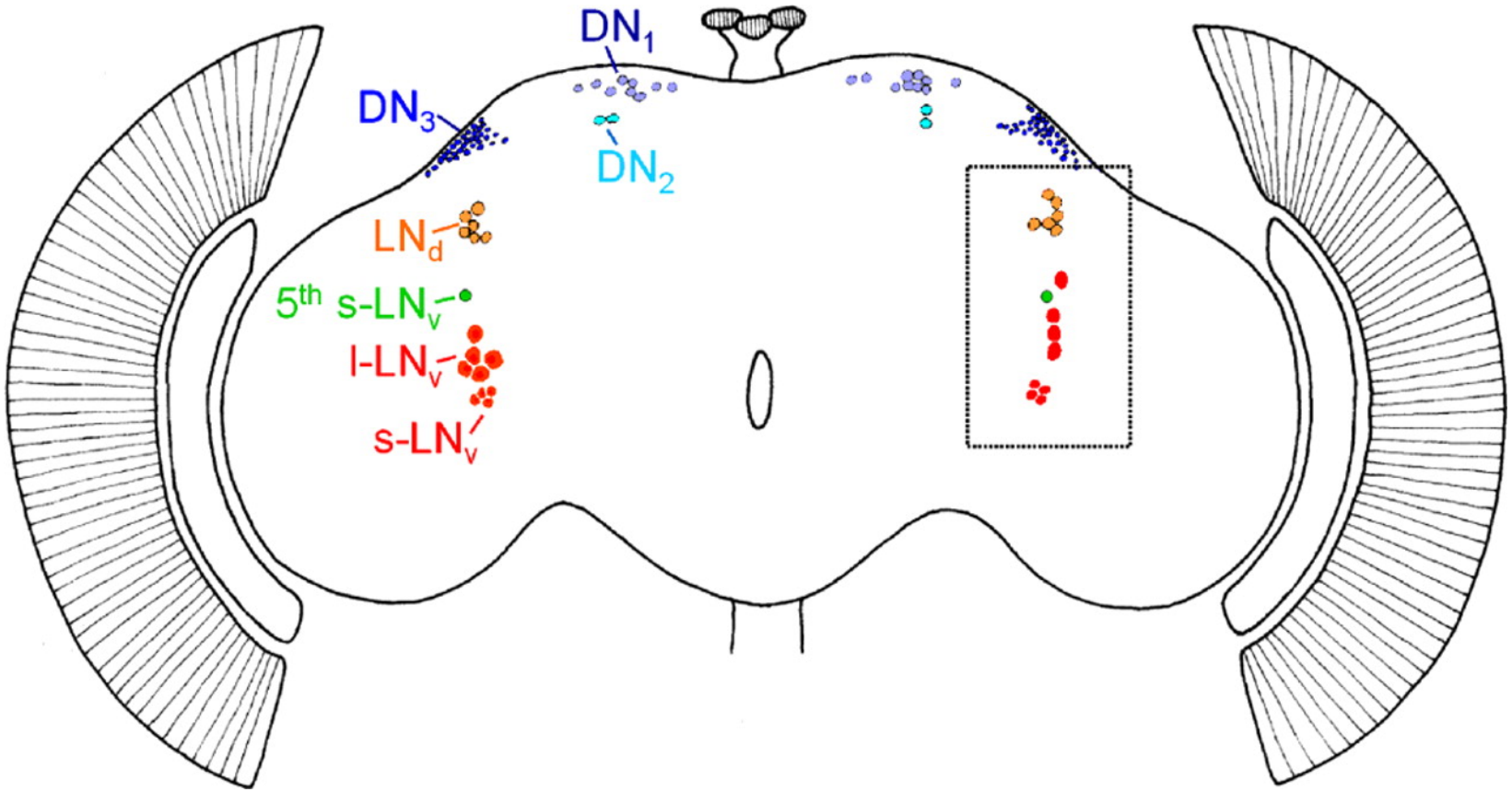
Tataroglu O, Emery P. Studying circadian rhythms in Drosophila melanogaster. Methods. 2014;68(1):140–150. doi:10.1016/j.ymeth.2014.01.001

Comparing Drosophila & mammalian brains (A)

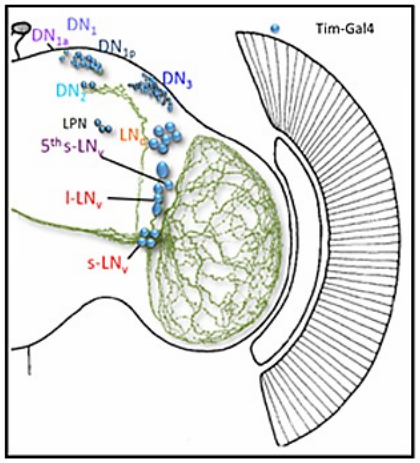
circadian system in Drosophila (B).

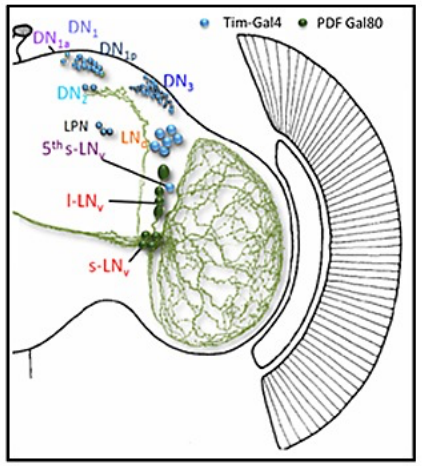


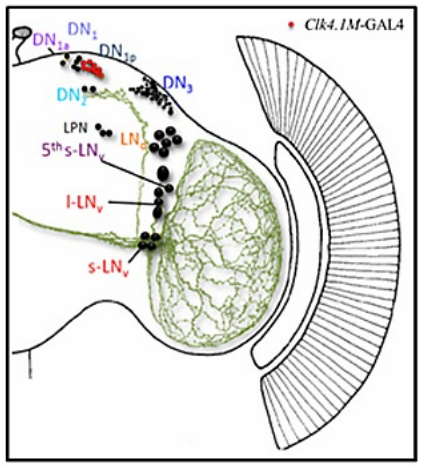
Brain circadian system in *Drosophila* (B).

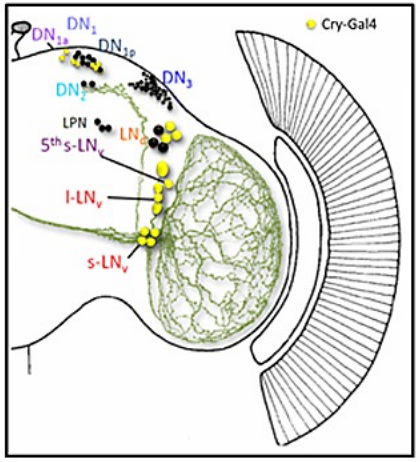


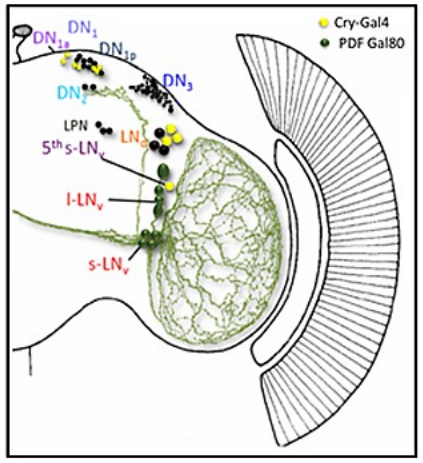
Details of brain in Drosophila







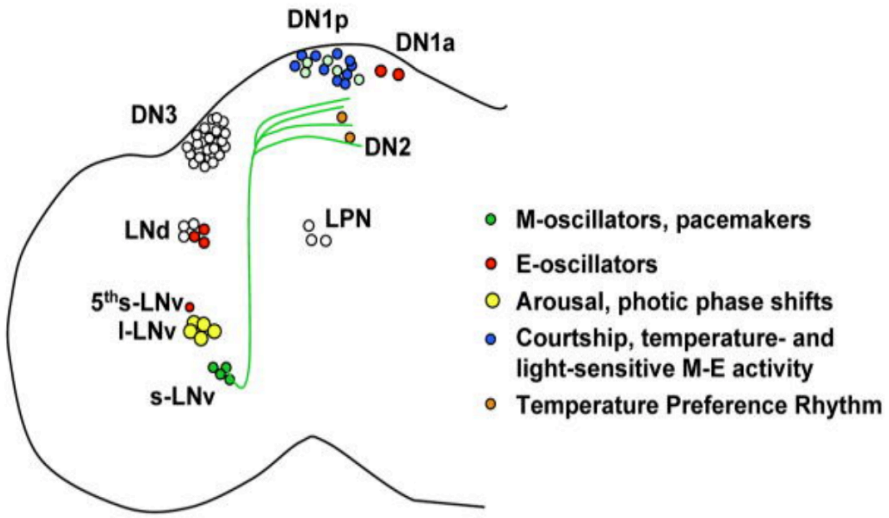




DN_{1a} Dorsal Neurons 1 anterior
DN_{1p} Dorsal Neurons 1 posterior
DN_2 Dorsal Neurons 2
DN_3 Dorsal Neurons 3
LPN lateral posterior neurons
LN_d lateral Neurons dorsal
l-LN_v large lateral neurons ventral
s-LN_v small lateral neurons ventral
5^{th} s-LN_v 5th small lateral neuron

Targeting circadian neurons

A



B

GAL4	Expression in
elav-GAL4 [153]	All neurons
repo-GAL4 [141]	All glia
tim-GAL4 [131]	All circadian tissues
Pdf-GAL4 [135]	s- and l-LNvs
R6-GAL4 [154]	s-LNvs
C929-GAL4 [148]	l-LNvs
Mai179-GAL4 [148]	s-LNvs, LNds, weak in DN1as
Clk4.1M-GAL4 [151]	ca. 10 DN1ps
cry-GAL4-13 [144, 155]	LNvs, LNds, DN1as, 2 DN1ps
cry-GAL4-39 [143]	LNvs, LNds, DN1as, most DN1s, some DN3s
Clk9M-GAL4 [3]	s-LNvs, DN2s

GAL80	Repression in
Pdf-GAL80 [149]	s- and l-LNvs
cry-GAL80 [149]	LNvs, LNds, DN1as

The circadian neurons of *Drosophila* and their best-known functions. Projection from the s-LNvs are shown in green. (B) Important driver and repressor transgenes and their expression patterns in circadian neurons.

References in brackets are supplementary readings

1. 3- PMC3470760
2. 131- Kaneko- J Comp Neurol. 2000
3. 135- Renn- Cell. 1999
4. 148- Grima Nature. 2004
5. 149- Stoleru Nature. 2004
6. 153- Luo- Genes Dev 1994;
7. 154- Helfrich-Forster. J Comp Neurol. 2007
8. 155-Shafer- J Comp Neurol. 2006

For more details click the link below

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4049855/

Sum up

43 years after the genetic identification of the *per* gene, and 30 years after its cloning, *Drosophila* remains a powerhouse for the study of circadian rhythms, from input pathways to circadian outputs. The combination of biochemical, genetic, genomic, neural, and behavioral approaches is permitting to understand with remarkable depth how circadian rhythms are generated. Greater advances in behavioral monitoring, neural imaging, genomics and proteomics will considerably accelerate the pace of discoveries in the upcoming years.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4049855/