Modelling the plant circadian clock

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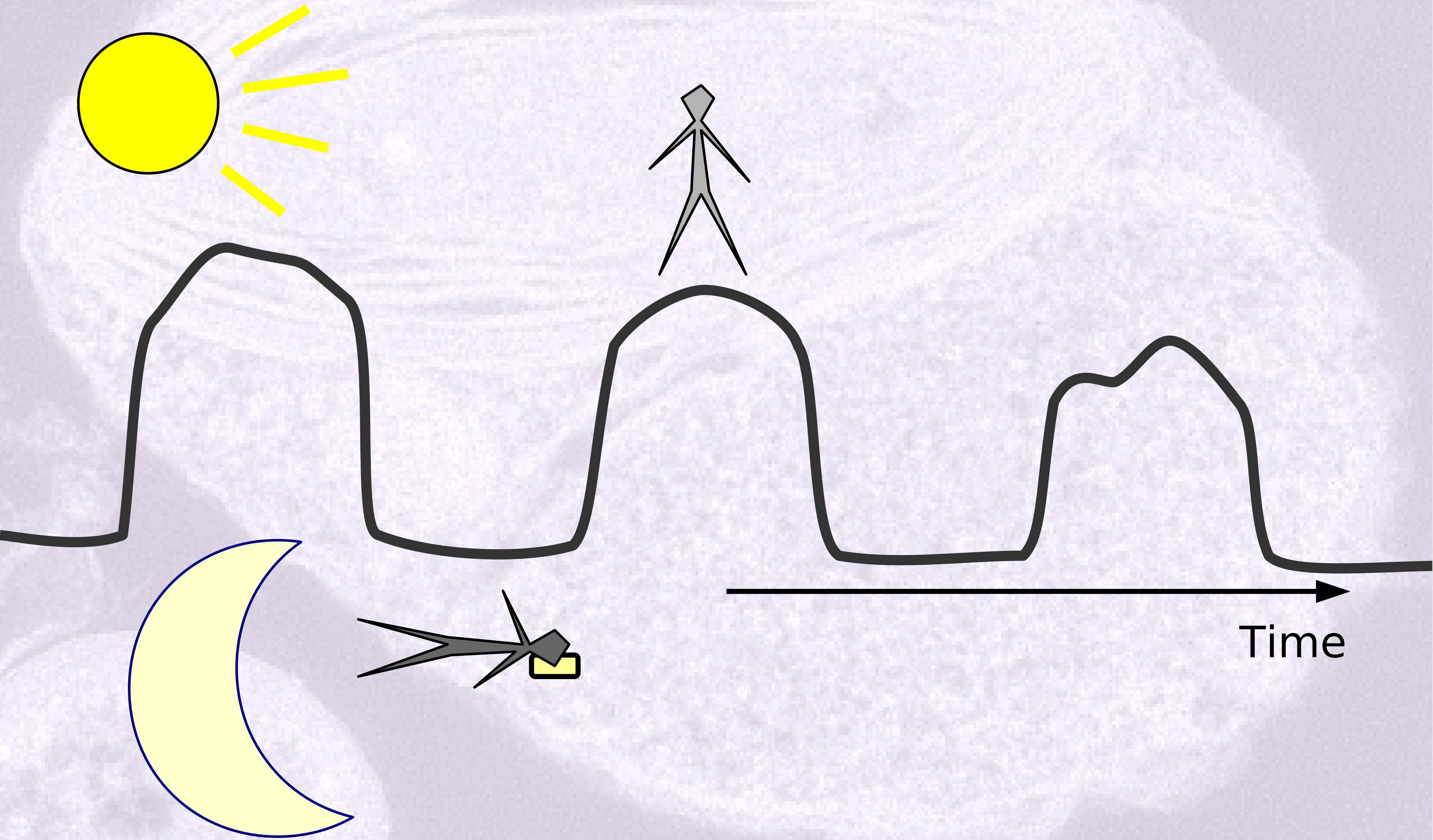
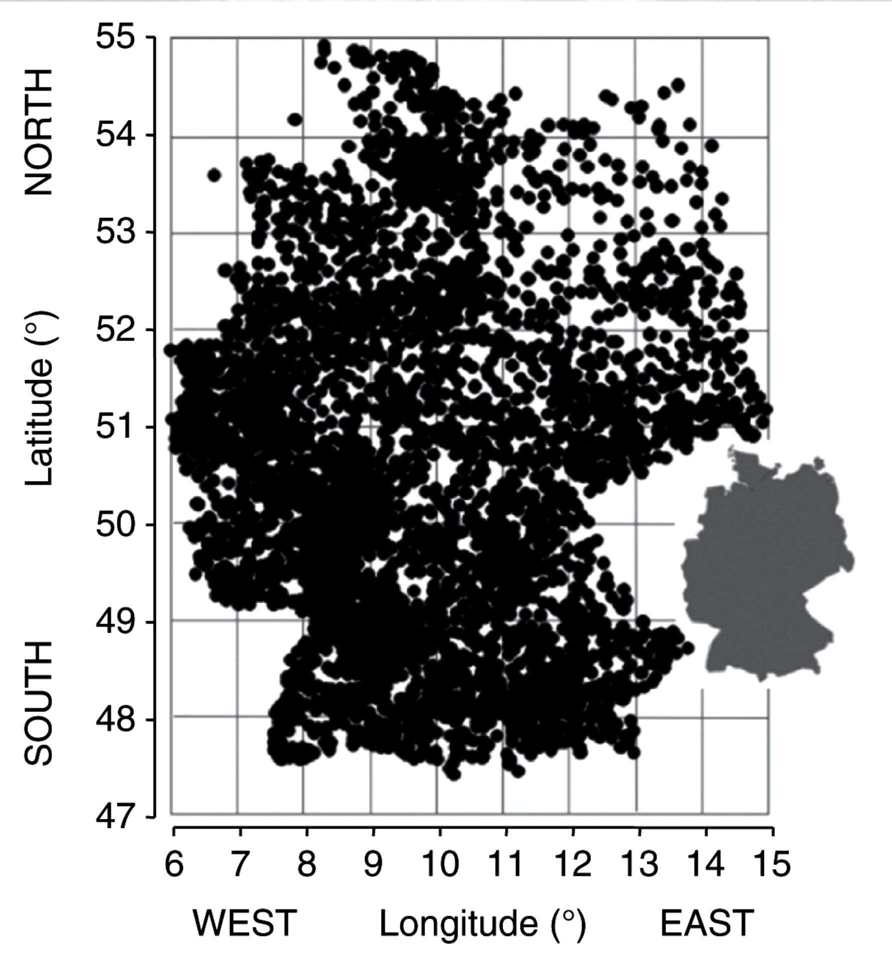
Daily rhythms

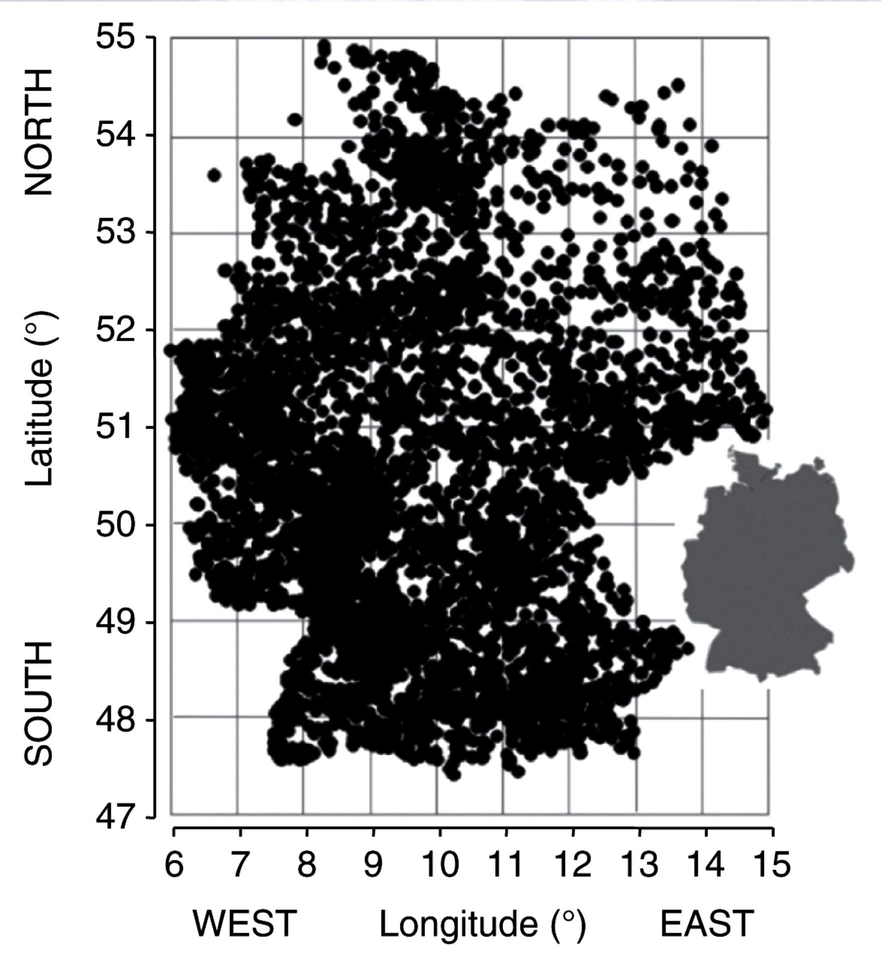


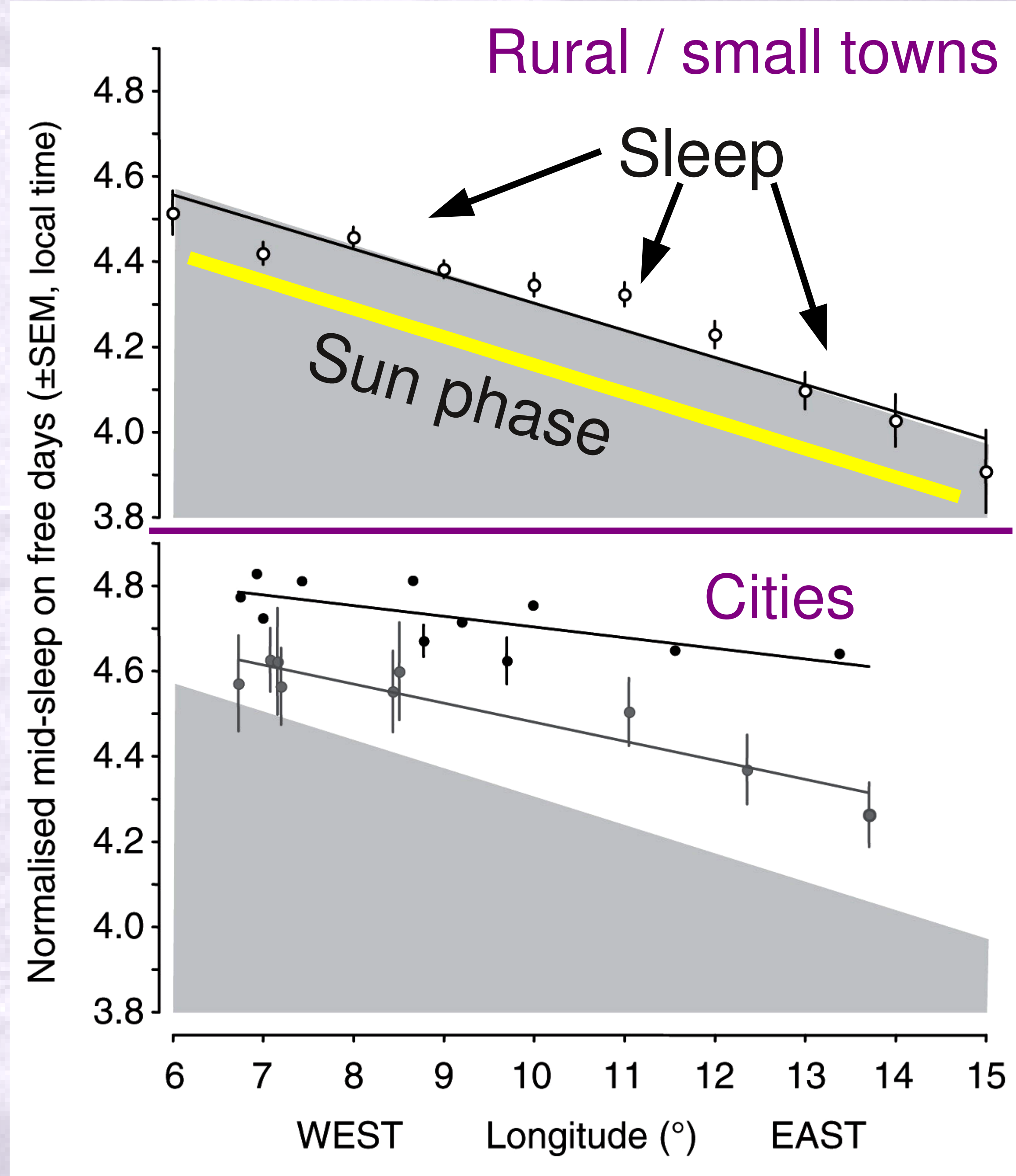
What sets the time in humans?



Roenneberg *et al.*, *Current Biology* (2007)

Sunlight sets our clocks





Roenneberg *et al.*, *Current Biology* (2007)

Circadian clock

- Oscillator with period about 24 hours
- Entrains to light input
- Adapts to photoperiod (long/short days)
- Highly robust (to temperature etc.)

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- Oscillator with period about 24 hours
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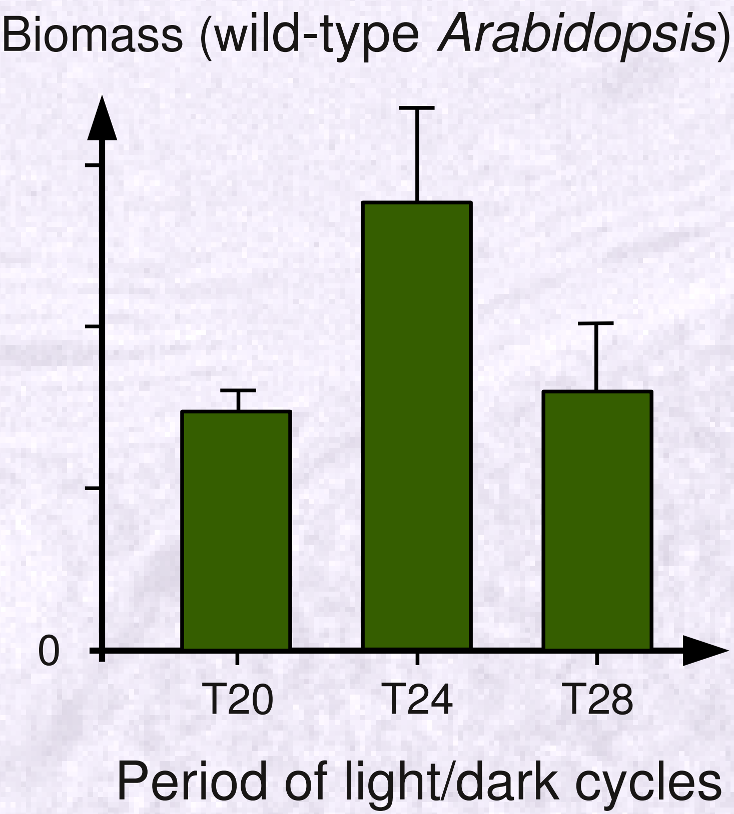
- Clocks are everywhere:

  - Animals, plants, fungi, cyanobacteria...

Arabidopsis thaliana

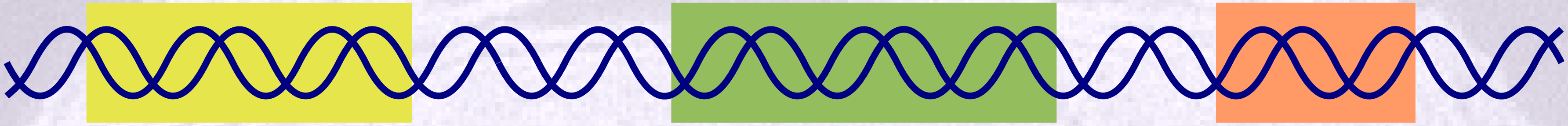
Circadian timing is crucial to plants



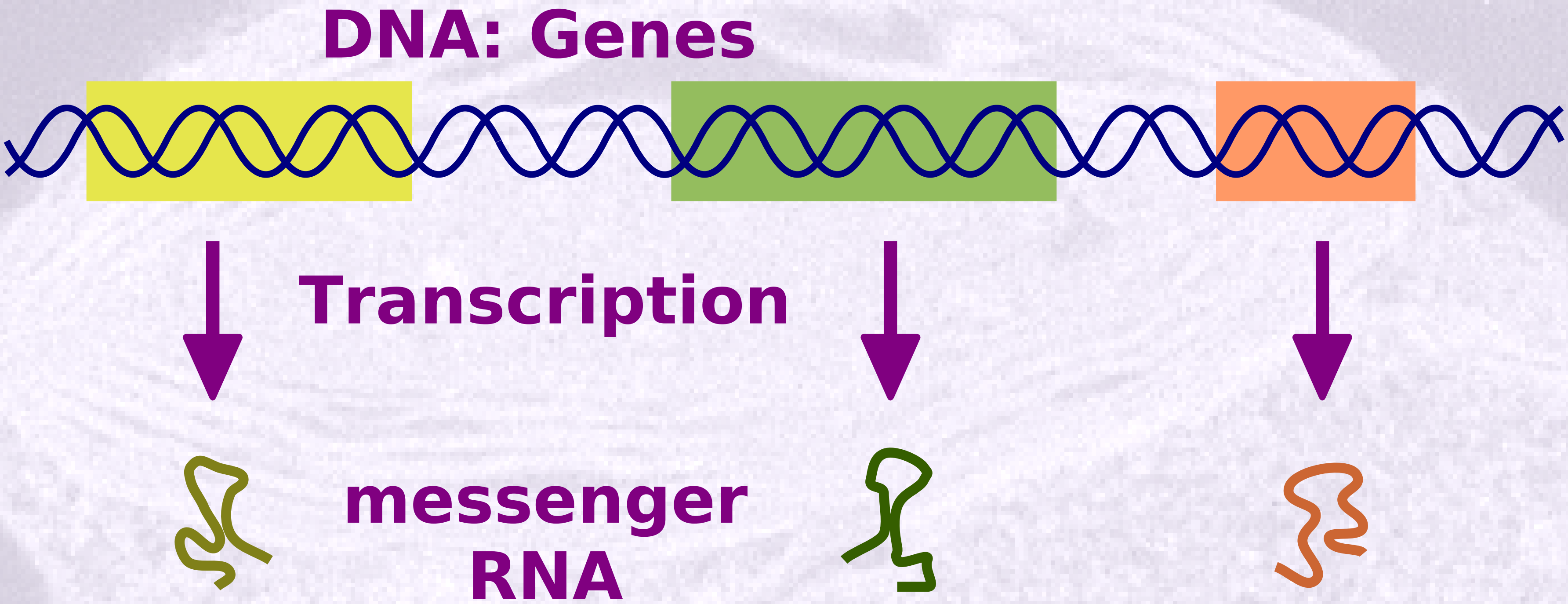
Dodd *et al.*, Science 309 (2005)

Molecular biology (1/4)

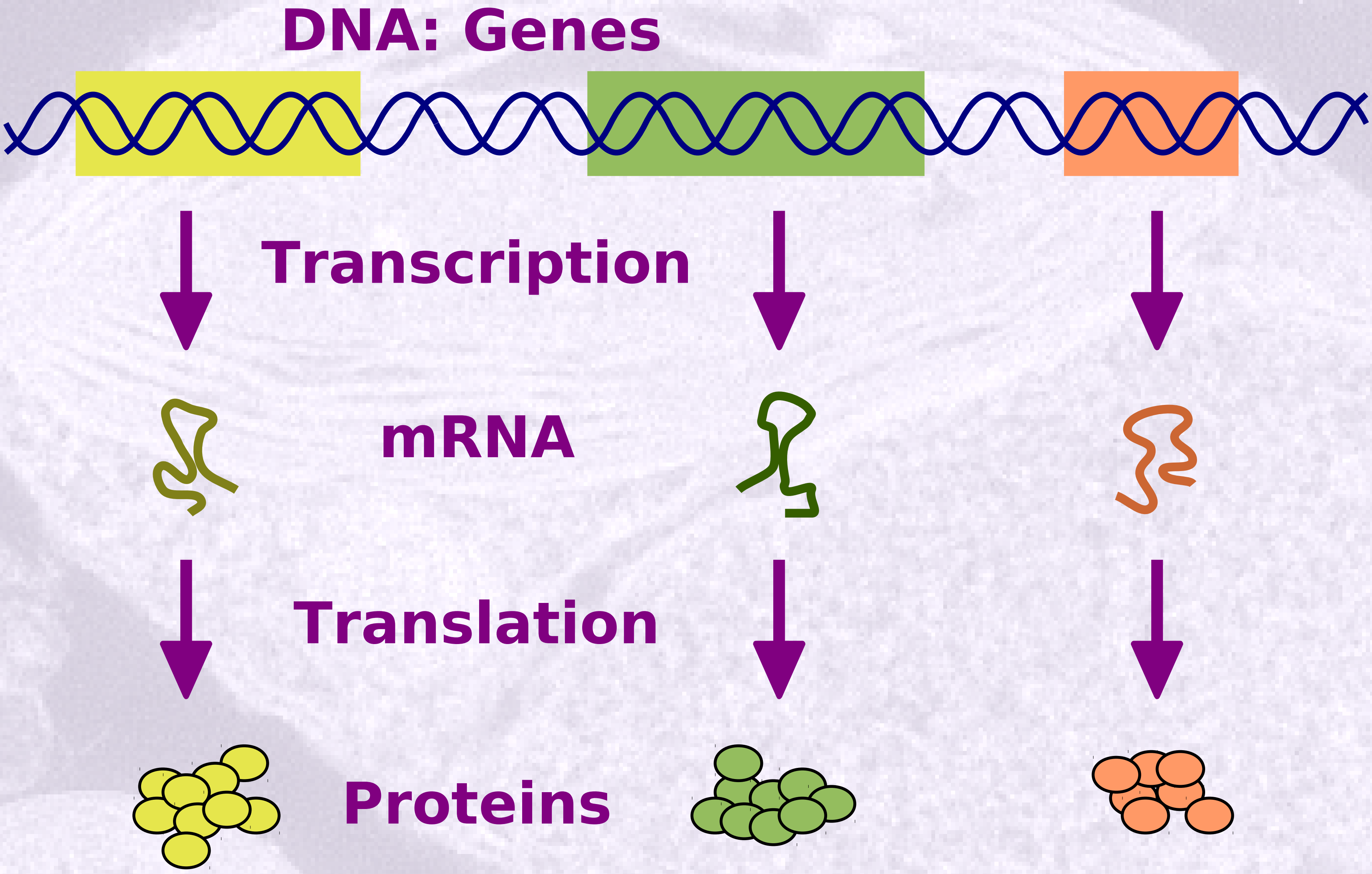
DNA: Genes



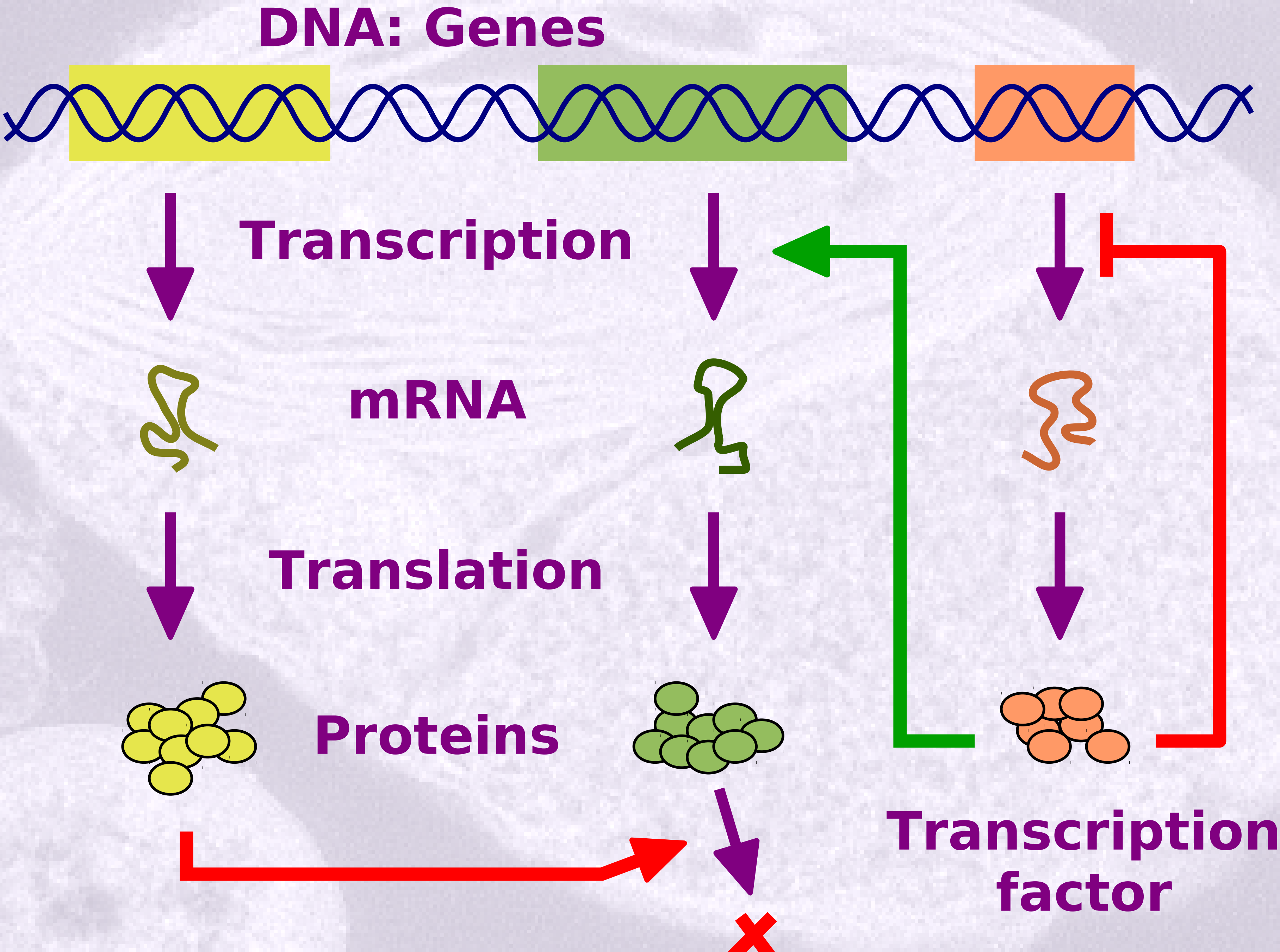
Molecular biology (2/4)



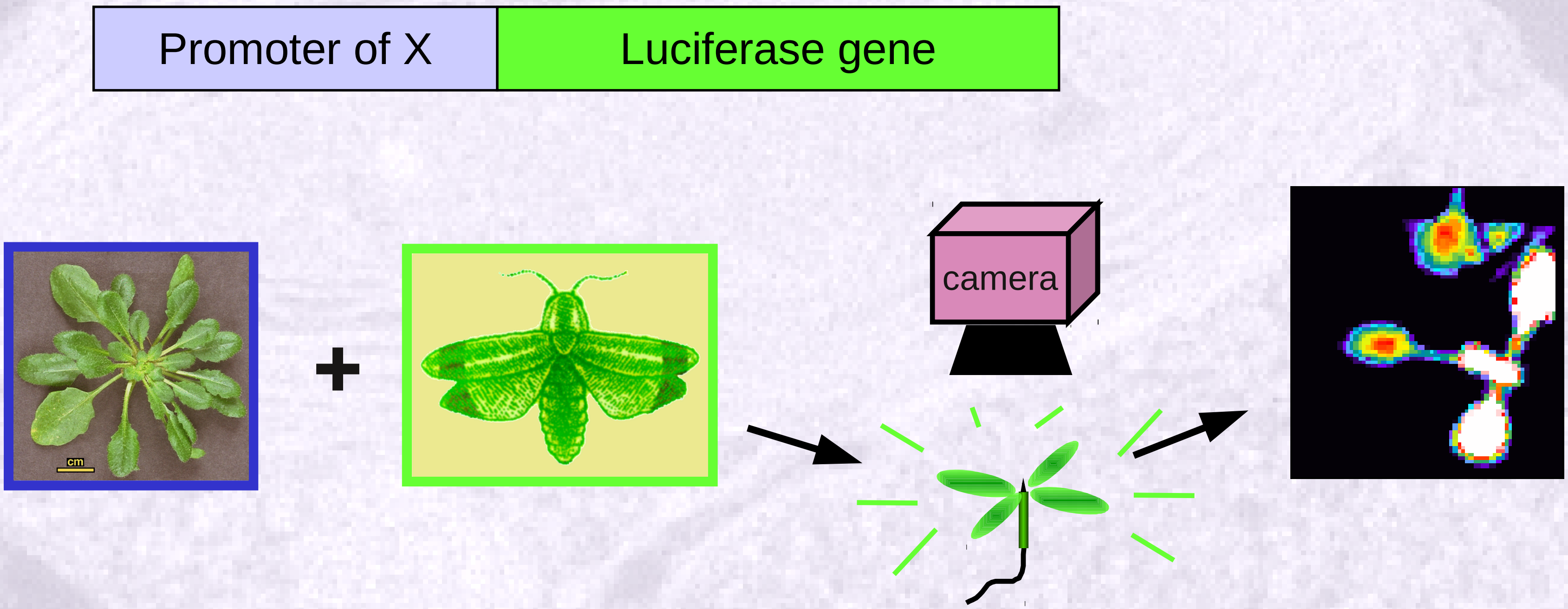
Molecular biology (3/4)



Molecular biology (4/4)



Measuring gene expression with luciferase

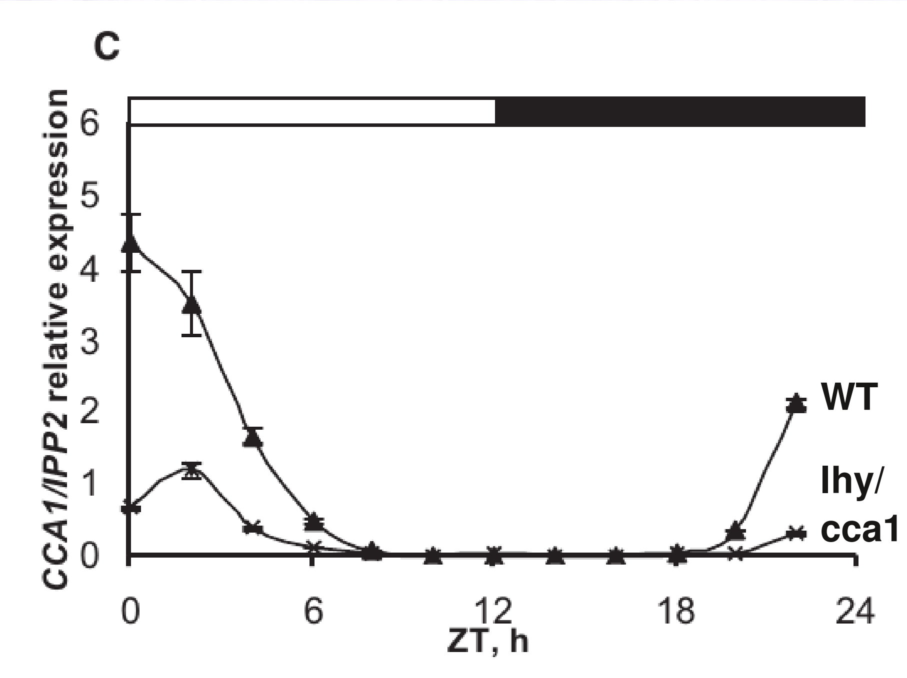


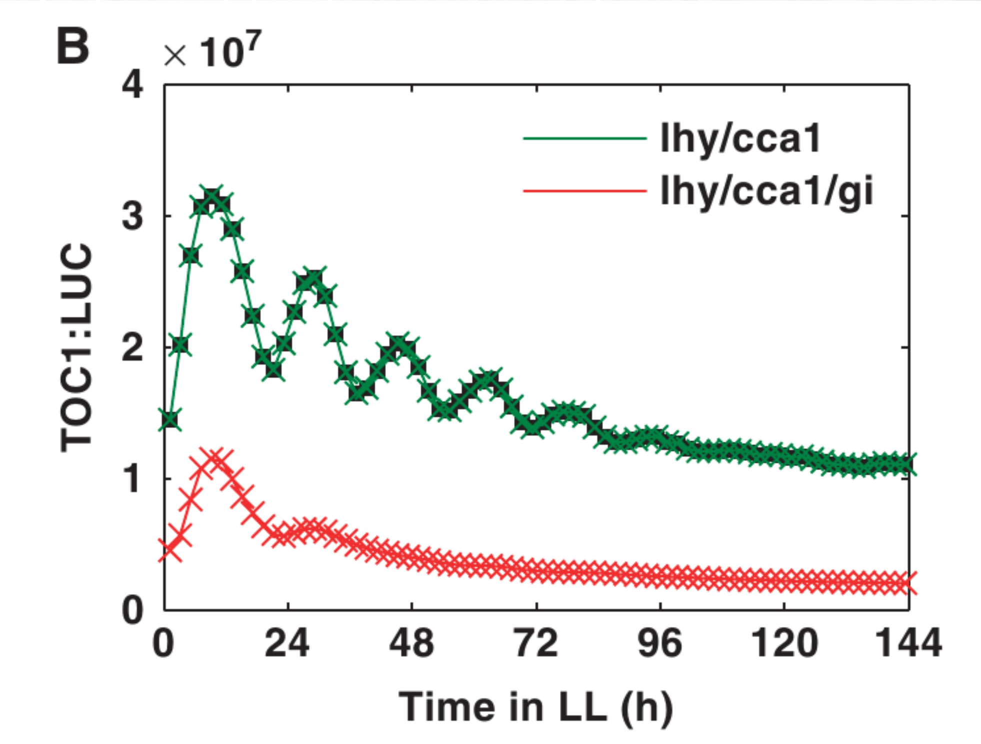
- Luciferase (LUC) produces light in fireflies
- Also works in plants
- Regulation of gene X -> luciferase protein
- Quantitative, real-time readout of gene expression

Probing the biological system

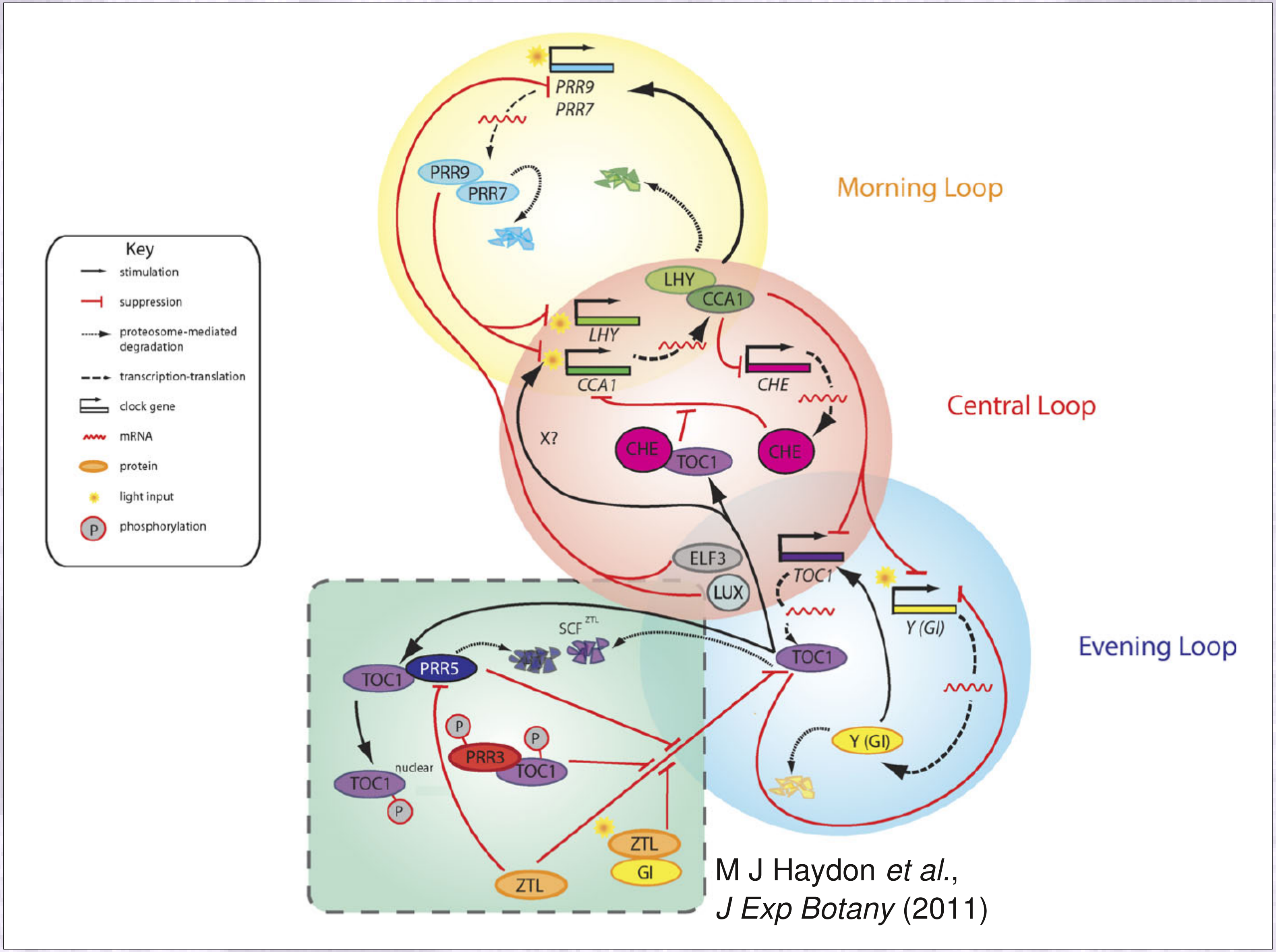
- Clock mutants (single, double...)
- Light/dark (LD) or constant light (LL)
- Light quality

-> lots of information





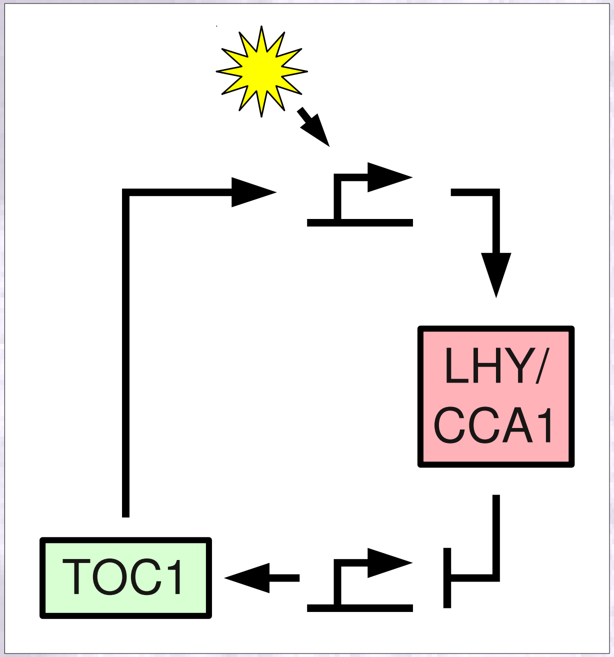
We want to model the clock, but:



M J Haydon *et al.*, *J Exp Botany* (2011)

The system is highly complex

Arabidopsis clock model (J. Locke *et al.*, 2005)

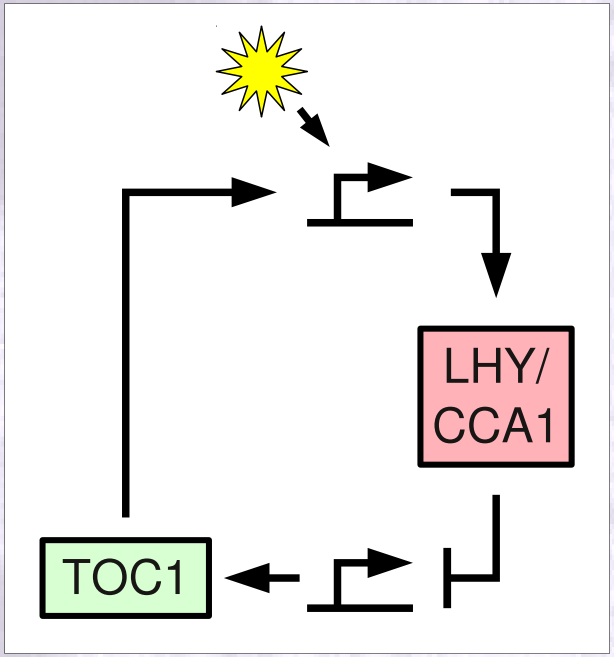


Negative feedback loop with light input

↓

Oscillations with entrainment to light

Arabidopsis clock model (J. Locke *et al.*, 2005)



$$\frac{\mathrm{d}c_L^{(m)}}{\mathrm{d}t} = L(t) + \frac{n_1 c_T^{(n)a}}{g_1^a + c_T^{(n)a}} - \frac{m_1 c_L^{(m)}}{k_1 + c_L^{(m)}},$$

$$\frac{\mathrm{d}c_L^{(c)}}{\mathrm{d}t} = p_1 c_L^{(m)} - r_1 c_L^{(c)} + r_2 c_L^{(n)} - \frac{m_2 c_L^{(c)}}{k_2 + c_L^{(c)}},$$

$$\frac{\mathrm{d}c_L^{(n)}}{\mathrm{d}t} = r_1 c_L^{(c)} - r_2 c_L^{(n)} - \frac{m_3 c_L^{(n)}}{k_3 + c_L^{(n)}},$$

$$\frac{\mathrm{d}c_T^{(m)}}{\mathrm{d}t} = \frac{n_2 g_2^b}{g_2^b + c_L^{(n)b}} - \frac{m_4 c_T^{(m)}}{k_4 + c_T^{(m)}},$$

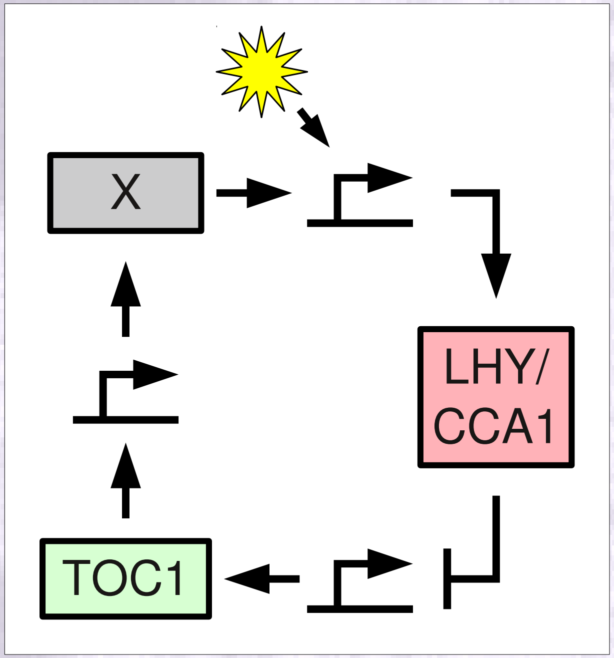
$$\frac{\mathrm{d}c_T^{(c)}}{\mathrm{d}t} = p_2 c_T^{(m)} - r_3 c_T^{(c)} + r_4 c_T^{(n)} - \frac{m_5 c_T^{(c)}}{k_5 + c_T^{(c)}},$$

$$\frac{\mathrm{d}c_T^{(n)}}{\mathrm{d}t} = r_3 c_T^{(c)} - r_4 c_T^{(n)} - \frac{m_6 c_T^{(n)}}{k_6 + c_T^{(n)}}.$$

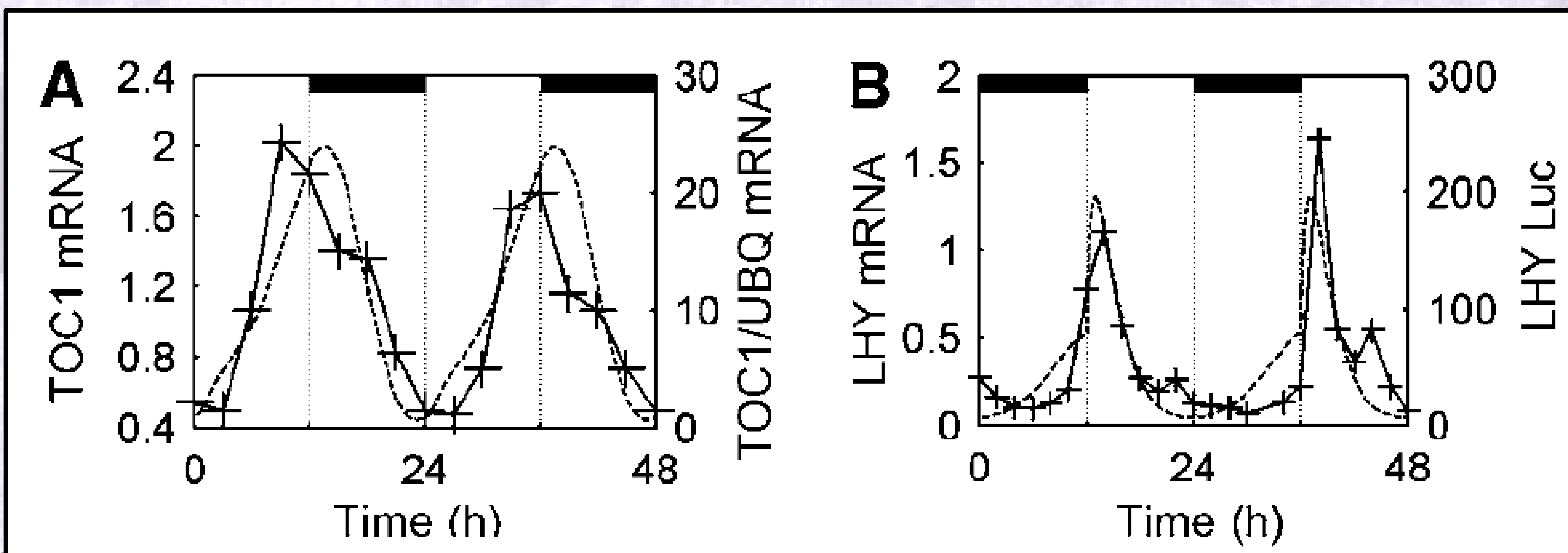
$$\frac{\mathrm{d}c_P^{(n)}}{\mathrm{d}t} = (1 - \Theta_{light}) p_3 - \frac{m_7 c_P^{(n)}}{k_7 + c_P^{(n)}} - q_2 \Theta_{light} c_P^{(n)},$$

- ODEs
- 7 variables
- 23 parameters

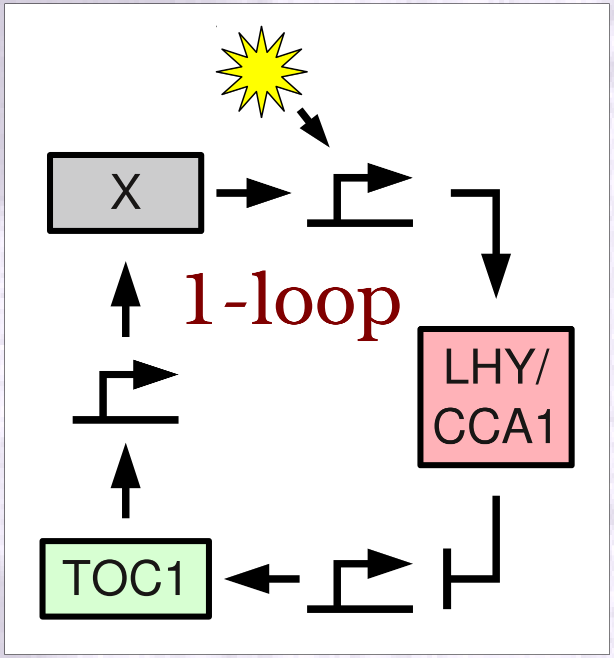
Arabidopsis clock model (J. Locke *et al.*, 2005)



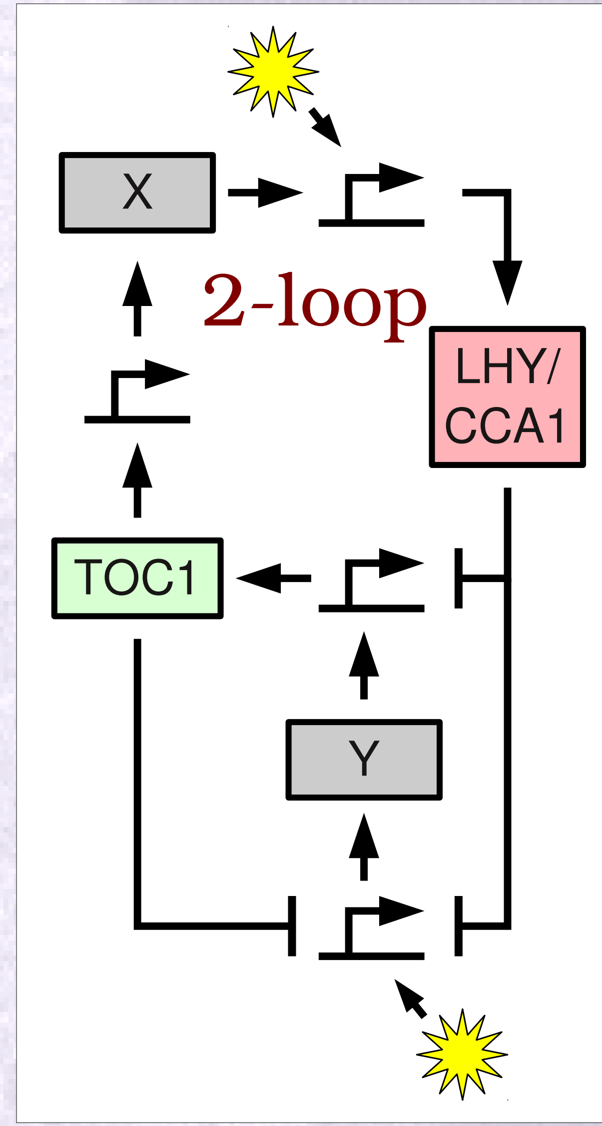
- Model did not agree with data
- Adding a third gene helped
- Could that gene be identified?

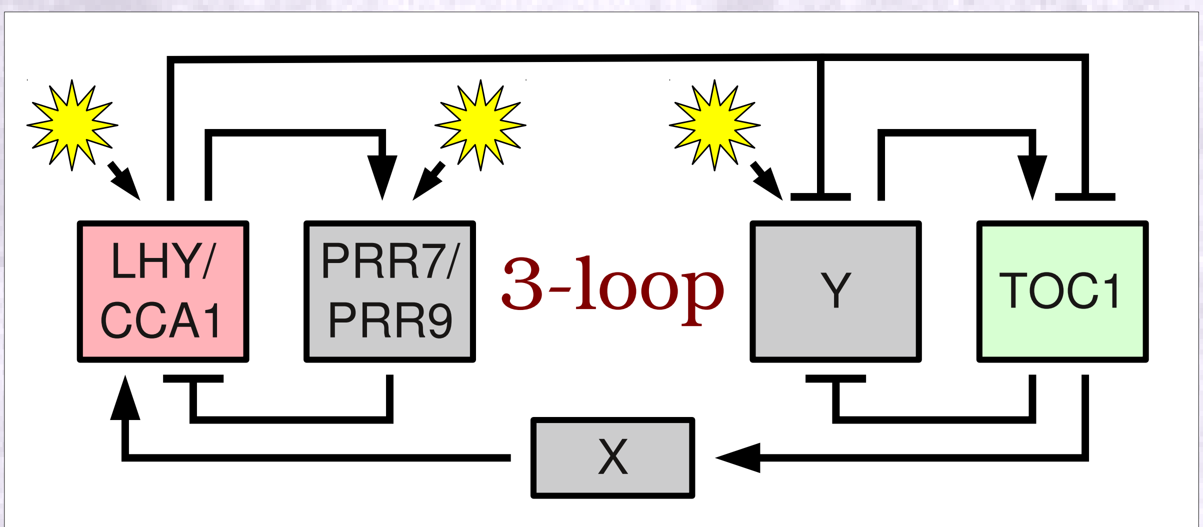


Arabidopsis clock models (A. Millar *et al.*)



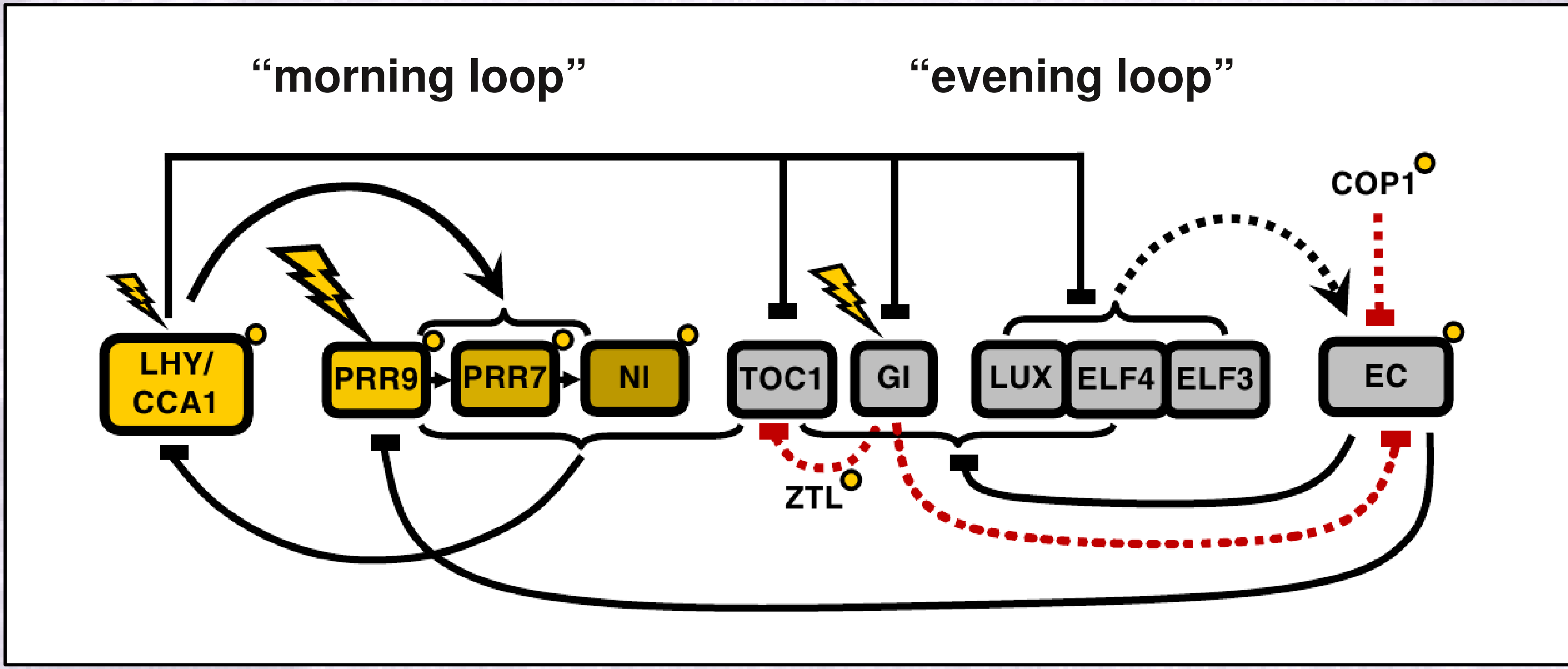
Model refinement → more loops added





The most recently published model

(Pokhilko *et al.*, MSB 2012)



~28 variables

~100 manually(!) fitted parameters

Note that TOC1 now *represses* LHY/CCA1

A general modelling problem/cycle:

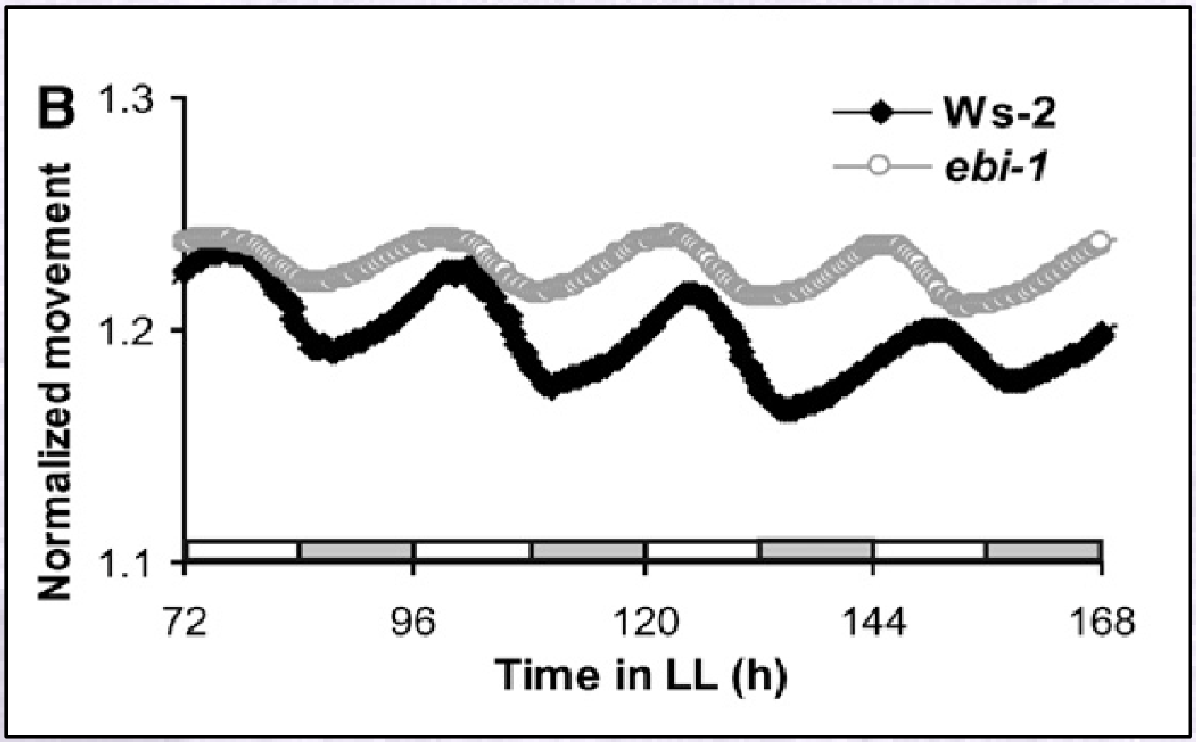
- Model seems to work fairly well
- New data show gaps/errors in model
- New components/interactions are proposed
- How much of the old model is valid in the context of the new data?
- Propose modifications to the model. Are there any non-trivial predictions?

Test them!

Current project: *EARLY BIRD (EBI)*

w/ Maria Eriksson (Umeå / Cambridge) + Karl Fogelmark

ebi-1 is a short period mutant (in constant light)

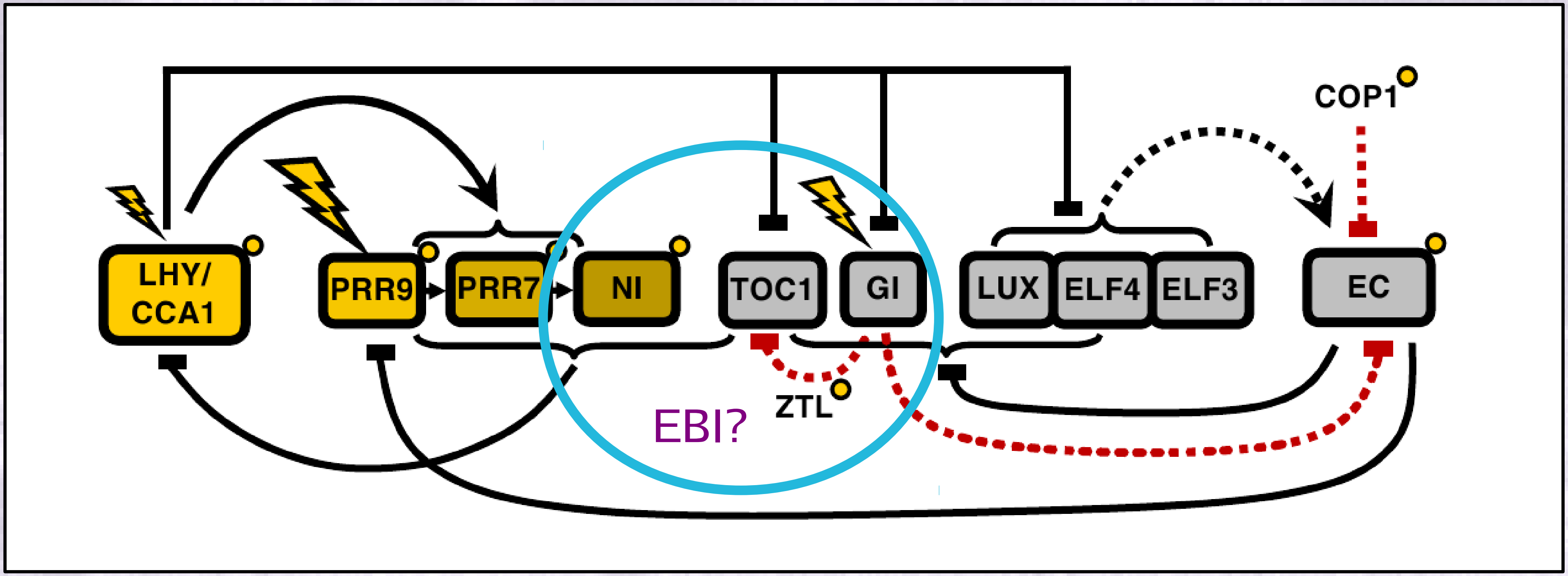


(M Johansson *et al.*, Plant Phys 2011)

Partners in Time: EARLY BIRD Associates with ZEITLUPE and Regulates the Speed of the Arabidopsis Clock[1[W][OA]]

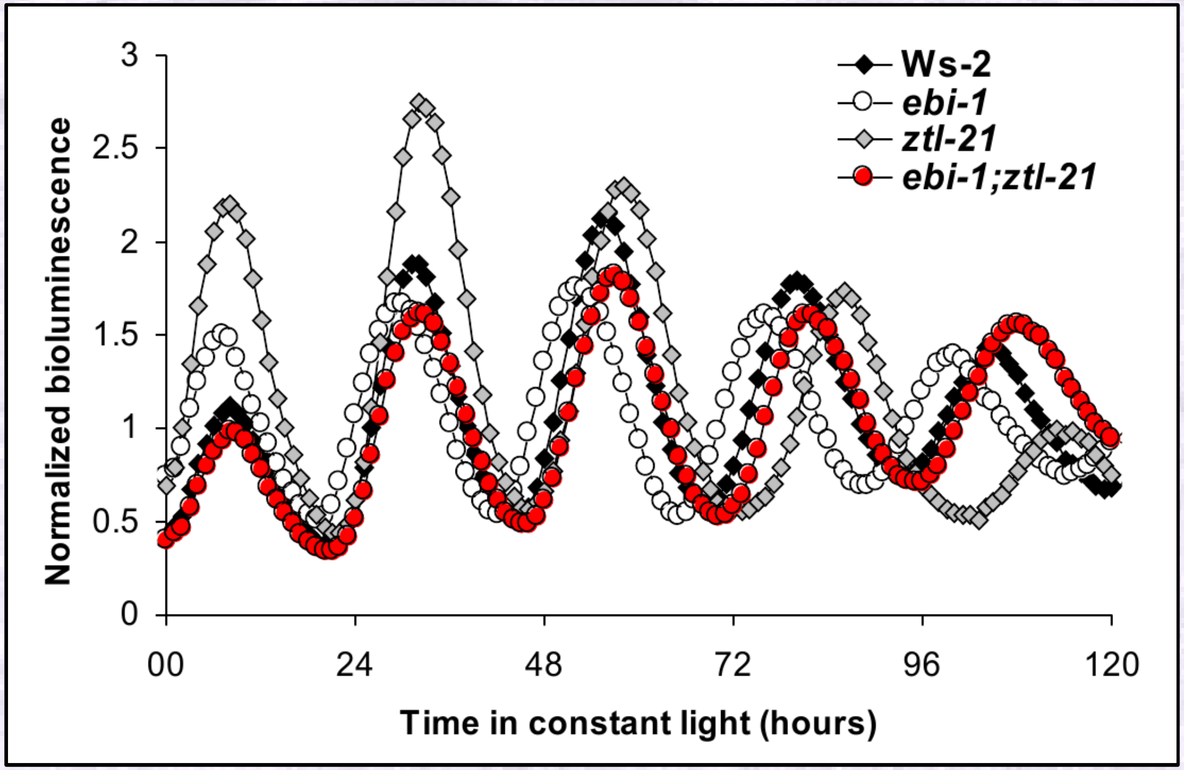
Mikael Johansson[2], Harriet G. McWatters[2], László Bakó, Naoki Takata, Péter Gyula, Anthony Hall, David E. Somers, Andrew J. Millar, and Maria E. Eriksson*

EBI interacts with TOC1 and ZEITLUPE



- EBI can bind to TOC1 and ZTL
- ZTL is temporarily sequestered by GI in the dark.
- ZTL degrades TOC1
- PRR5 (NI) localizes TOC1 to the nucleus
- What does EBI actually do?

EBI acts in parallel with ZEITLUPE



The mutants *ebi-1* and *ztl-21* have opposite effects.
(*ebi-* and *toc1-* have short period, *ztl-* long period)
The double mutant is more similar to wild type.
Thus: EBI and ZTL appear to act independently.

EBI isn't trivial to add

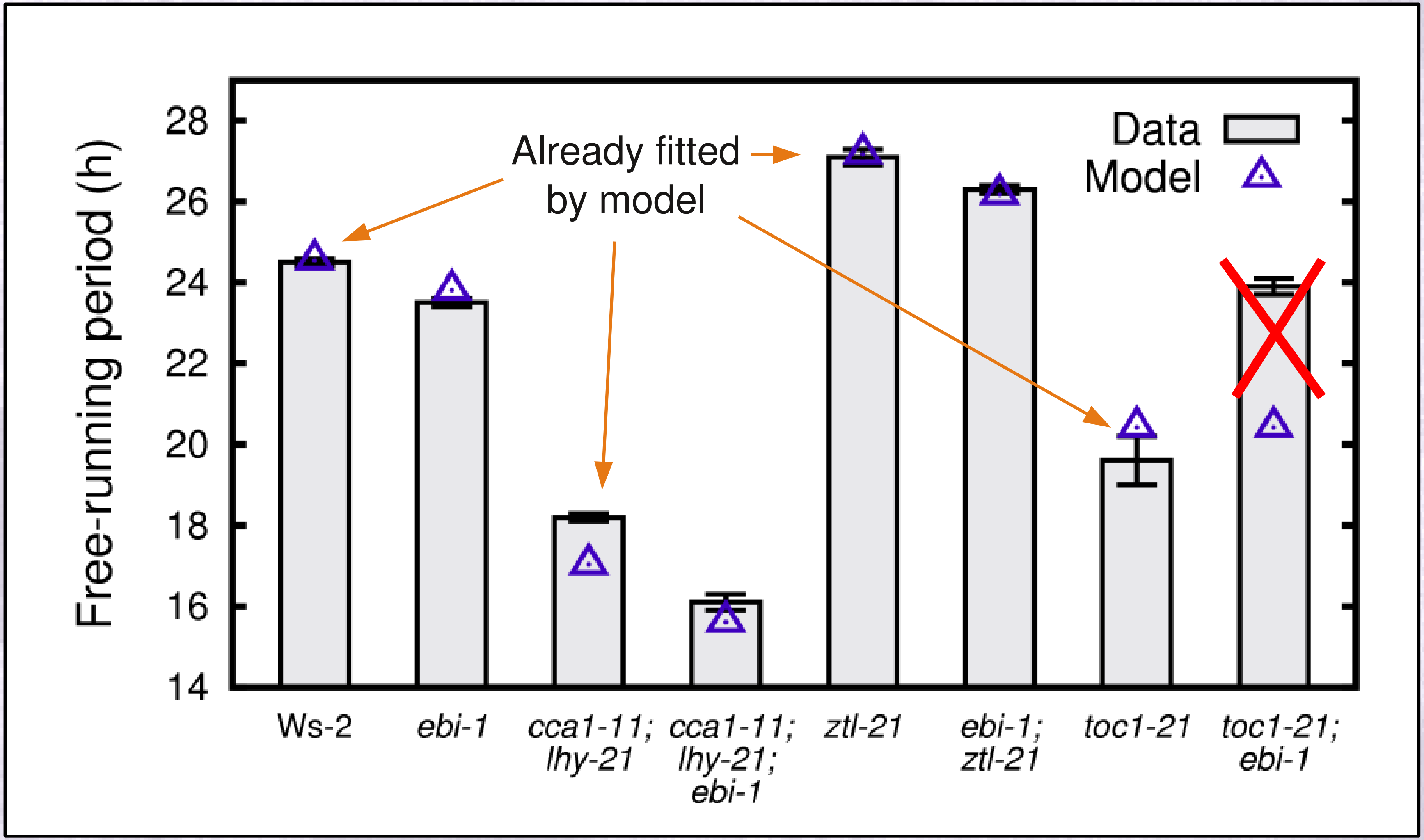
Systematically alter the model parameters, one at a time (for an earlier model).
Effects vary between genetic backgrounds.

	A	B	C	D	E	F	G	H	I	J	K
1		**wt**	24.5512	***ztl-21***	27.1788	***ztl-0***	28.25	***toc1***	20.4213	***lhy***	17.026
2		WT +10%	WT -10%	*ztl +10%*	*ztl -10%*	*ztl +10%*	*ztl -10%*	*toc +10%*	*toc1 -10%*	*lhy +10%*	*lhy -10%*
3	para	period	period	period	period	period	period	period	period	period	period
4	m1	23.8762	25.3625	26.5963	27.8113	27.5838	28.9763	19.6762	20.8388	17.0137	17.011
5	m2	24.5487	24.5538	27.1788	27.1788	28.2538	28.2538	20.4812	20.4738	17.0162	17.013
6	m3	24.1988	24.9613	26.8263	27.5338	27.8212	28.7063	19.4262	20.7787	17.0287	17.012
7	m4	24.3812	24.7662	26.9688	27.4038	27.9563	28.5712	20.3313	20.5287	17.0262	17.013
8	m5	24.0963	25.2312	26.8087	27.5162	28.2213	28.2712	20.4213	20.4213	16.3062	17.696
9	m6	24.2512	24.9563	26.9863	27.3588	28.2488	28.2538	20.4238	20.4713	16.6212	17.536
10	m7	24.5512	24.5488	27.1788	27.1788	28.25	28.25	20.4238	20.4238	17.0288	16.987
11	m8	24.3987	24.7238	26.9713	27.3688	28.2438	28.25		20.4688	16.8588	17.216
12	m9	24.4488	24.7113	27.0838	27.2938	28.2163	28.2888	20.4713	20.4812	16.9088	17.198
13	m10	24.3062	24.9387	27.0087	27.3688	28.2163	28.2938	20.4213	20.4213	16.4887	17.562
14	m11	24.5463	24.5587	27.1812	27.1762	28.2512	28.2538	20.4462	20.2375	17.0062	17.013
15	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Do any parameters changes behave like *ebi-1*?

Altering period is easy – how constrain it?

EBI as inhibitor of ZTL function



EBI can probably not be added trivially

Does EBI have to do with TOC1 localization?

```
#eq (11)
d c_T_m = n2*g4/(g4+c_EC) * g5^e/(g5^e + c_L^e) - m5*c_T_m;
#eq (12)
d c_T = p4*c_T_m -(m6 + m7*D) * c_T*(c_ZTL*p5 + c_ZG) - m8*c_T;
```

Must split into cytosol and nucleus (more parameters!)

```
#eq (11)
d c_T_m = n2*g4/(g4+c_EC) * g5^e/(g5^e + c_L^e) - m5*c_T_m;
# NI (PRR5) increases diffusive transport to the nucleus
let T_transp = p37*c_Tc*(1 + p38*c_NI*ni_ok);
#eq (12)
d c_Tn = T_transp - p39*c_Tn - (m6 + m7*D) * c_Tn * (p105*c_ZTLn + p200*c_ZG) - m43*c_Tn;
#eq (12.5)
d c_Tc = p4*c_T_m + p39*c_Tn - (m6 + m7*D) * c_Tc*(c_ZTLc*p5 + c_ZG*p200) - m8*c_Tc – T_transp;

and similarly for ZTL
```

Experimental validation

prr7 mutant known to exxagerate prr5 or prr9
- the model should predict this

Predict period of ebi/prr5 double mutant?

Experimental results emerging
- prr5/toc1 has long period?
- prr5/ebi long as well?

Vetenskapsrådet

HFSP

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Susanna Hammarberg (summer student '11)

Umeå:

Maria Eriksson

Mikael Johansson