Phase Response Analysis of the Circadian Clock in *Neurospora crassa*

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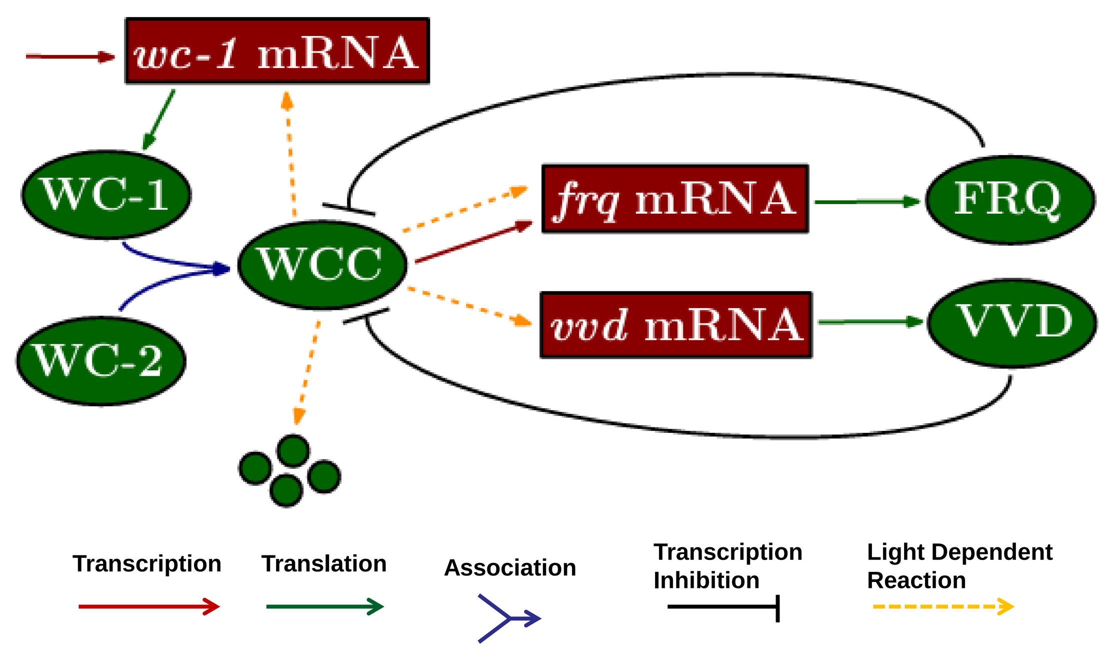
Introduction

Circadian rhythm plays a vital role in maintaining the daily activities of ~24 hours in many organisms. Malfunction of the circadian clock can be dangerous to an organism, and even life threatening. Disorders associated with circadian malfunction include *sleep disorders*, *jet lag*, and even *cancer*. In this research, mathematical models simulate the circadian clock of the fungus *Neurospora crassa*, specifically focusing on the phase of the clock in response to light. Our results suggest a crucial balance of molecular reactions to light is necessary for optimal phase response. The results from this research may provide useful information for treating circadian related diseases.

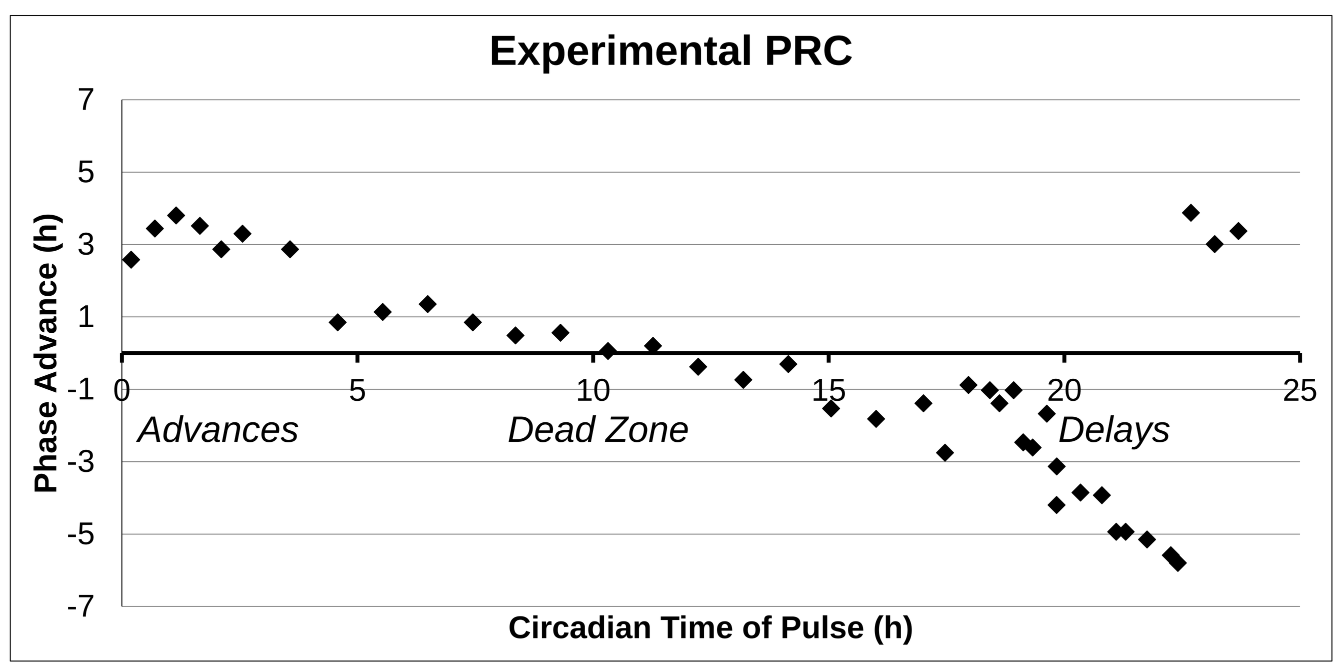
Neurospora Circadian Clock

The negative feedback loop between *frequency* (*frq*) and the transcription factor White Collar Complex (WCC) is the core mechanism of the Neurospora circadian clock that produces robust oscillations. Reactions to light include induced transcription of *frq*, *wc-1*, and *vvd* as well as degradation of WCC.

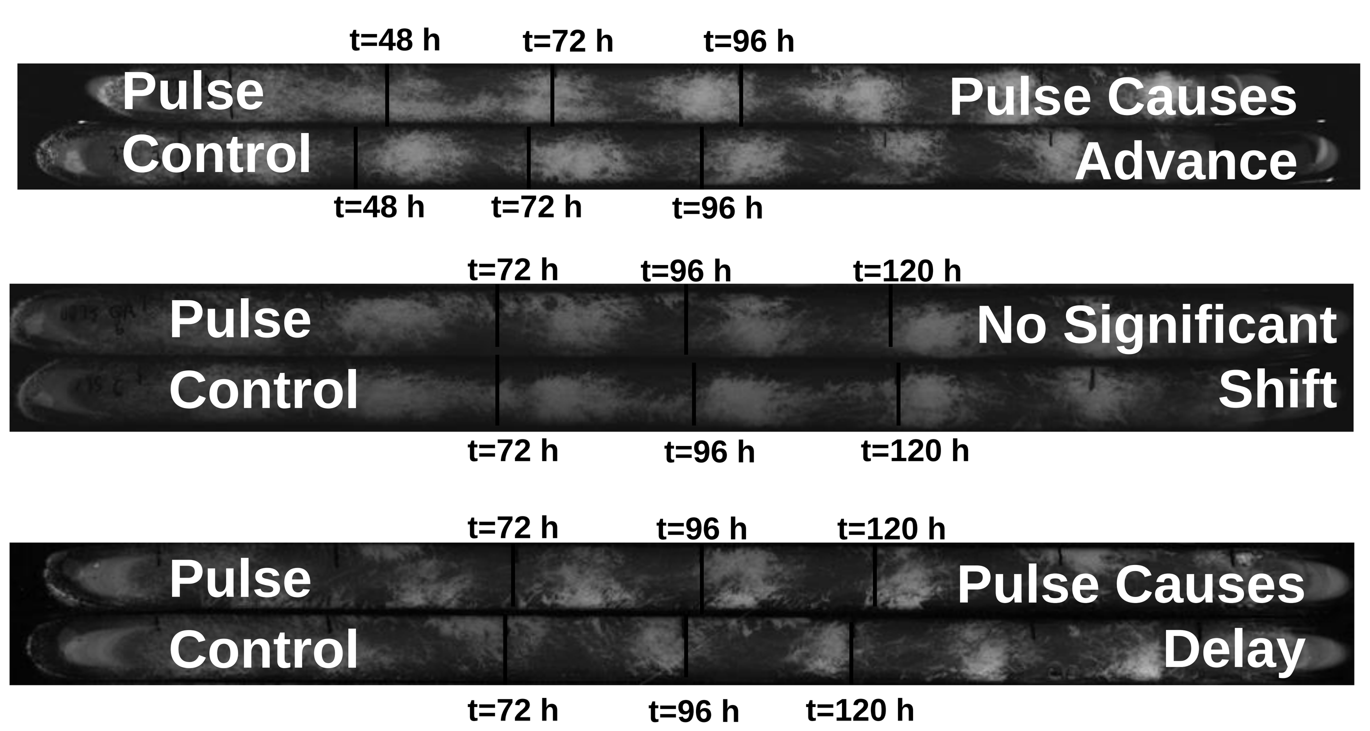


Experimental Reactions to light in the Neurospora Circadian Clock

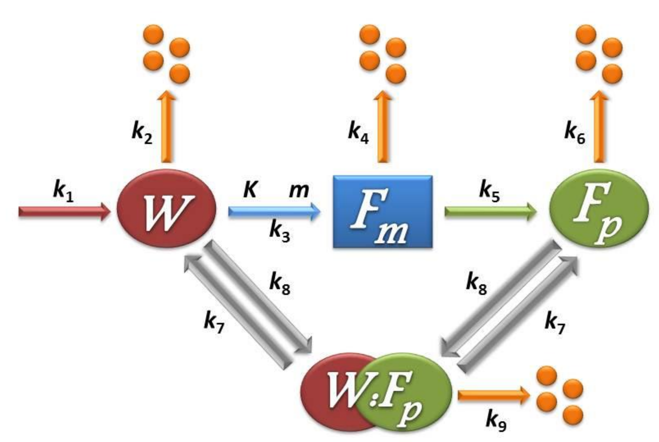
The plot of the change in phase against the time of an applied pulse is known as a phase response curve (PRC). An experimental PRC of Neurospora in response to light can be found below [1].



Below are examples of a phase advance, no shift, and a phase delay in a phase shifting experiment with the fungus, *Neurospora crassa.*



A Basic Circadian Model

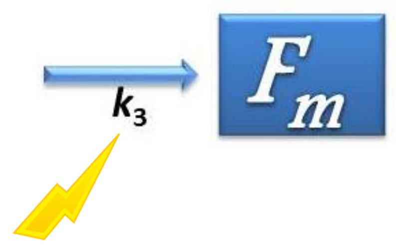


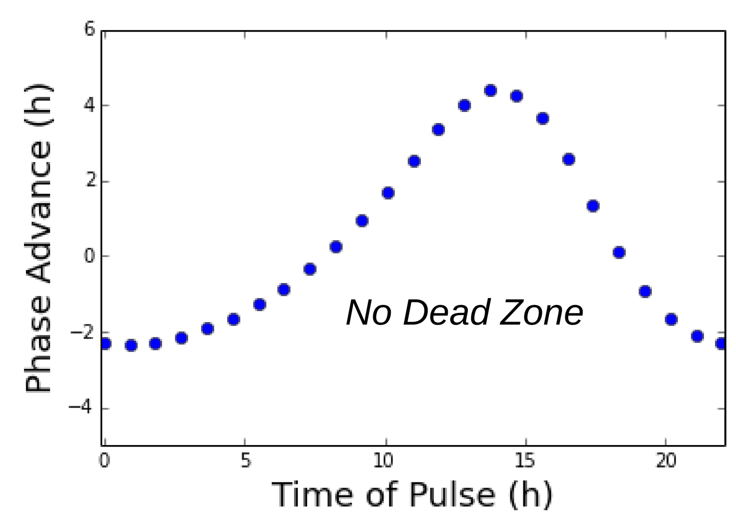
$$\frac{d[W]}{dt} = k_1 - k_2[W] + k_7[W{:}F_p] - k_8[W][F_p]$$
$$\frac{d[F_m]}{dt} = k_3\frac{[W]^m}{K^m+[W]^m} - k_4[F_m]$$
$$\frac{d[F_p]}{dt} = k_5[F_m] - k_6[F_p] + k_7[W{:}F_p] - k_8[W][F_p]$$
$$\frac{d[W{:}F_p]}{dt} = k_8[W][F_p] - k_7[W{:}F_p] - k_9[W{:}F_p]$$

PULSE | **PRC**

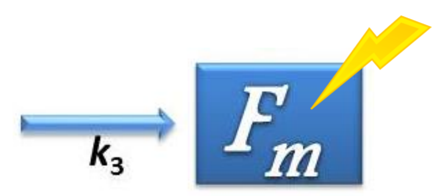
frq Transcription Pulse (Multiplicative)

$$k_3 \rightarrow k_3 + p(t)$$

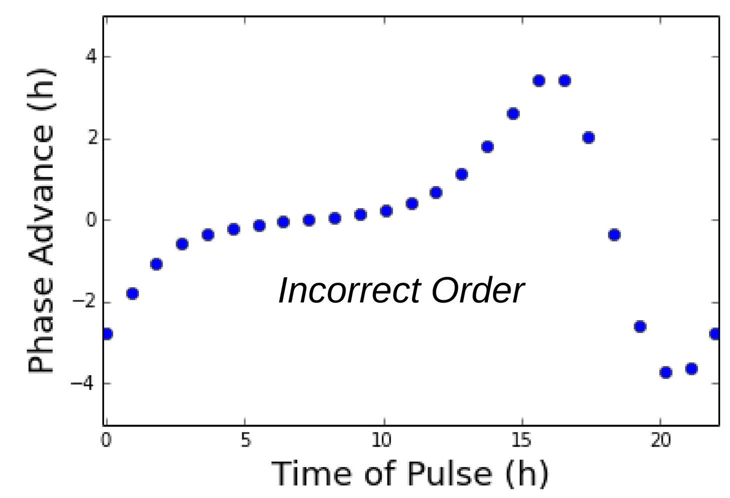




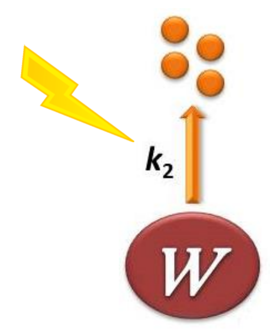
frq Transcription Pulse (Additive)



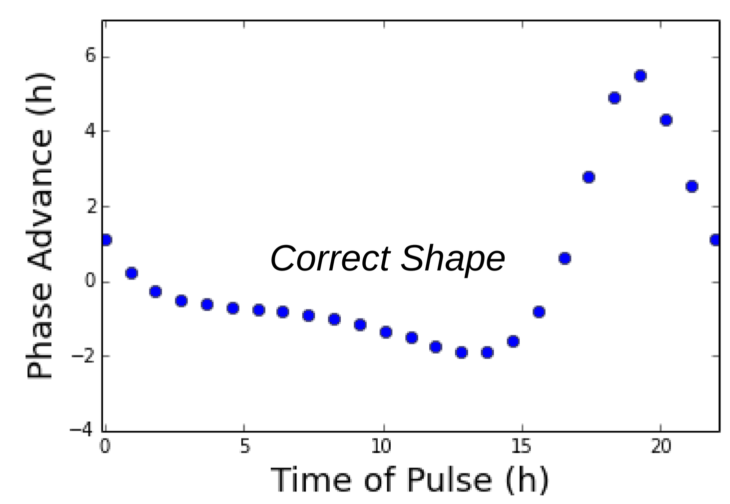
$$\frac{d[F_m]}{dt} \rightarrow \frac{d[F_m]}{dt} + p(t)$$



WC-1 Degradation Pulse

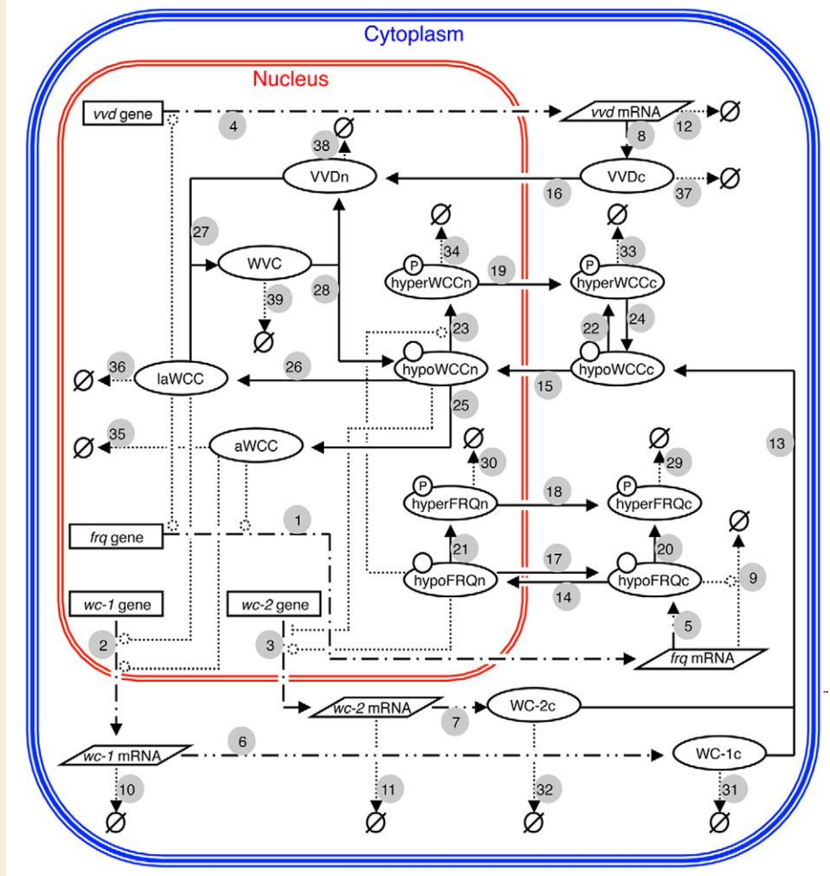


$$k_2 \rightarrow k_2 + p(t)$$

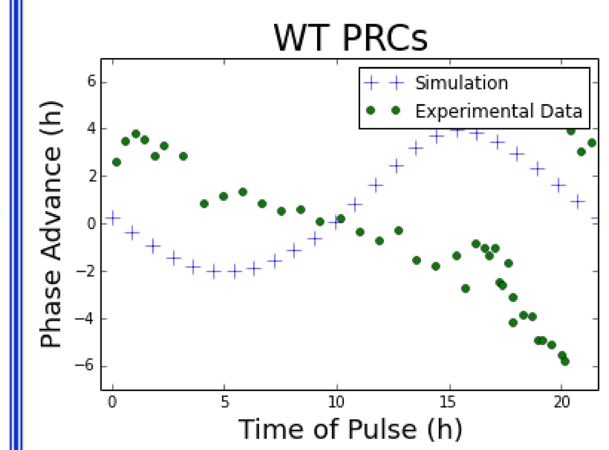


- It is generally believed that *frq* induction is responsible for causing the Neurospora circadian clock to phase shift accurately [2]. Above, phase response analysis of the basic circadian oscillator suggests that *frq* induction alone is not sufficient to correctly shift the clock. These results are consistent with more basic and more complex models.
- Although it is known that WC-1 becomes phosphorylated and degraded in response to light [3], this response is not usually modeled as the primary phase shifting mechanism. It is evident that pulsing the degradation of WC-1 in the basic model results in a more accurate PRC shape than pulsing *frq* transcription.

A Complex Circadian Model [4]

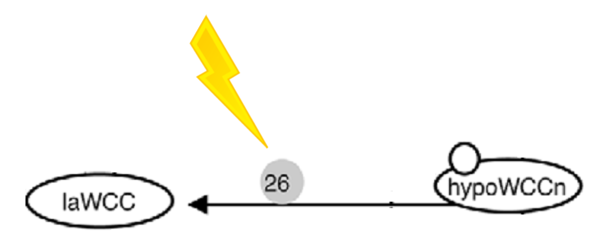


The model to the left [4] produces an incorrect PRC shape with the original parameter set, as seen below.

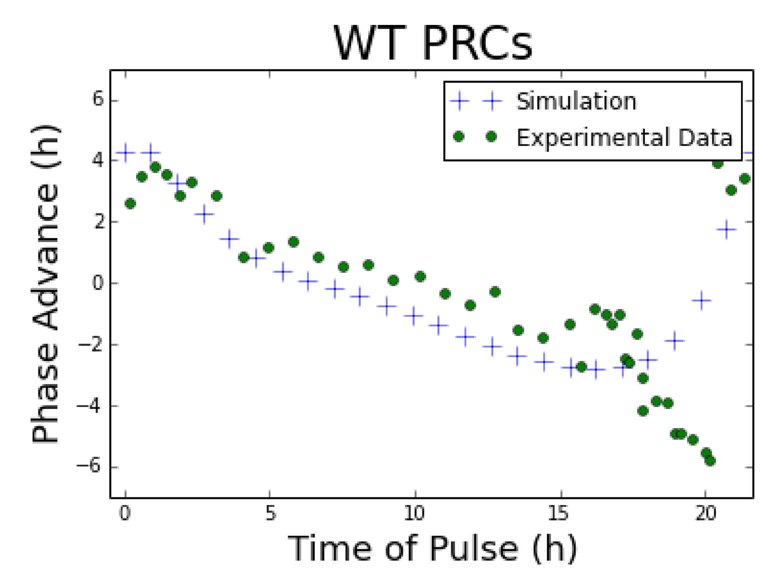


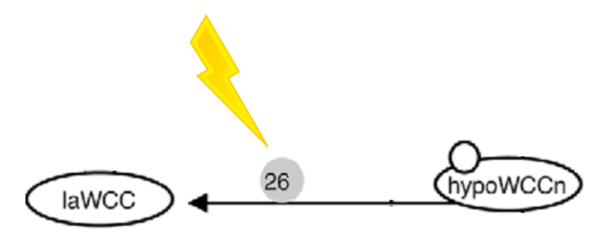
For this research, parameters affected by light in the complex model above have been modified to match experimental data from [1].

PULSE | **PRCs**

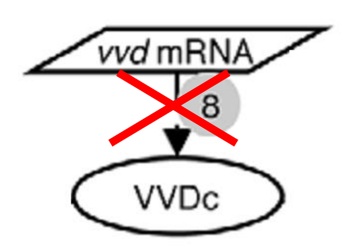


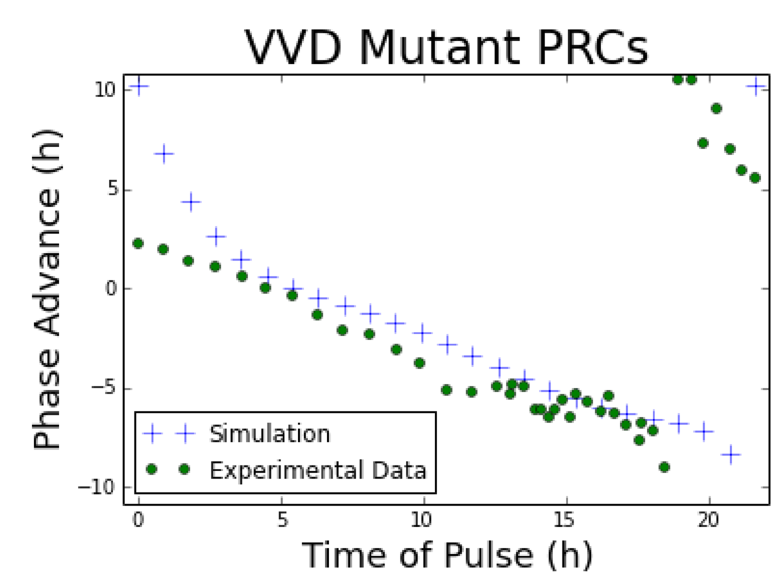
$$k_{26} \rightarrow k_{26} + p(t)$$





$$k_{26} \rightarrow k_{26} + p(t)$$





Conclusions

In this research, phase response analysis mathematically revealed that pulsing *frq* mRNA alone is insufficient for optimal phase response of the Neurospora circadian clock. In fact, it is likely that degradation of light activated WCC and transcriptional inhibition of WCC through VVD are also necessary for accurate phase shifting.

References

1. Heintzen et. al., Cell, 104:453-464 (2001)
2. Crosthwaite, S. Cell, 81:1003-1012 (1995)
3. Linden, The EMBO Journal, 18 (18) (1999)
4. Tseng, PLoS Computational Biology, 8 (3) (2012)

Acknowledgements

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