

Research Summary
Henry Mirsky

The circadian clock is an endogenous, intracellular oscillator found widely in prokaryotes and eukaryotes. It provides the biological mechanism whereby organisms keep time, allowing for the separation of incompatible events, the simultaneity of events that need to occur together, and the ordering of events that must occur in fixed sequence. In angiosperms, for example, the clock “tells” flowers when to open and how to track the sun with their leaves. In mammals, the clock sets the sleep/wake schedule, controls the onset of hunting and foraging behavior, and underlies daily cycles in physiology.

At the molecular level, the clock is manifested as a set of genes, transcripts, and proteins forming interlocked negative- and positive-feedback loops -- though the precise structure of these loops varies by organism. In mice, the core of the clock is composed of the genes *Clock*, *Bmal1*, the *Pers*, and the *Crys*. The protein products from *Clk* and *Bmal1* dimerize and activate the *Per* and *Cry* genes. The protein products from the *Pers* and *Crys*, in turn, dimerize and repress their own transcription. Thus, repression and release alternate, resulting in oscillations. (See Figure 1).

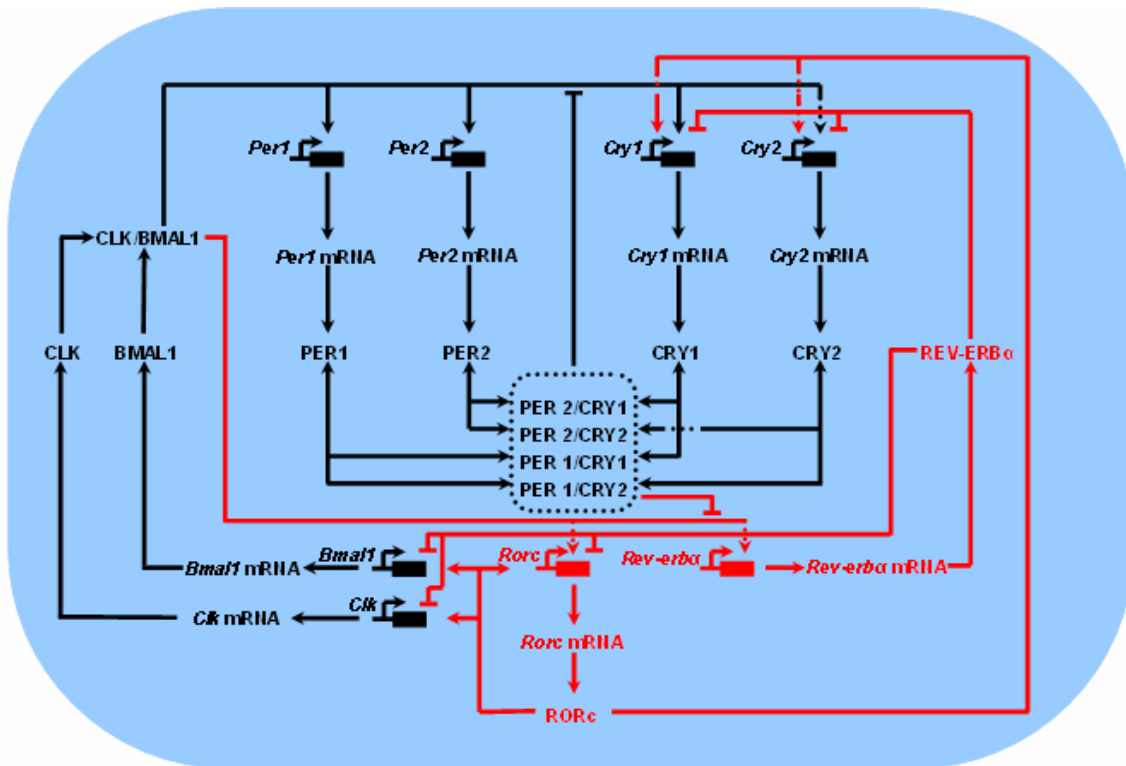


Figure 1. Schematic of the intracellular mouse circadian clock, showing the “core” (in black) consisting of *Clock*, *Bmal1*, *Pers*, and *Crys*, their transcripts, their protein products, and various heterodimers. Several other components (in red) play a subsidiary role.

Mathematical models can be constructed to accord with the oscillation patterns found experimentally (i.e. to mimic the phase relationships among components and, to a lesser extent, their concentrations). We have built a model as a system of 21 ordinary

differential equations that produces the oscillations shown in Figure 2. These oscillations are very close to those seen in cells. Moreover, the model was used to predict the effect on phenotype (i.e retention of rhythmicity) and expression levels in nine knockout mutants. The model correctly predicts phenotype in all nine knockouts and change in expression level in eleven of thirteen species in five knockouts.

The production of a mathematical model that is highly predictive builds confidence in its output. Biologists can use the model to simulate outcomes of experiments that would take months to perform and restrict their actual experimentation to the set of *in silico* changes that produce interesting results.

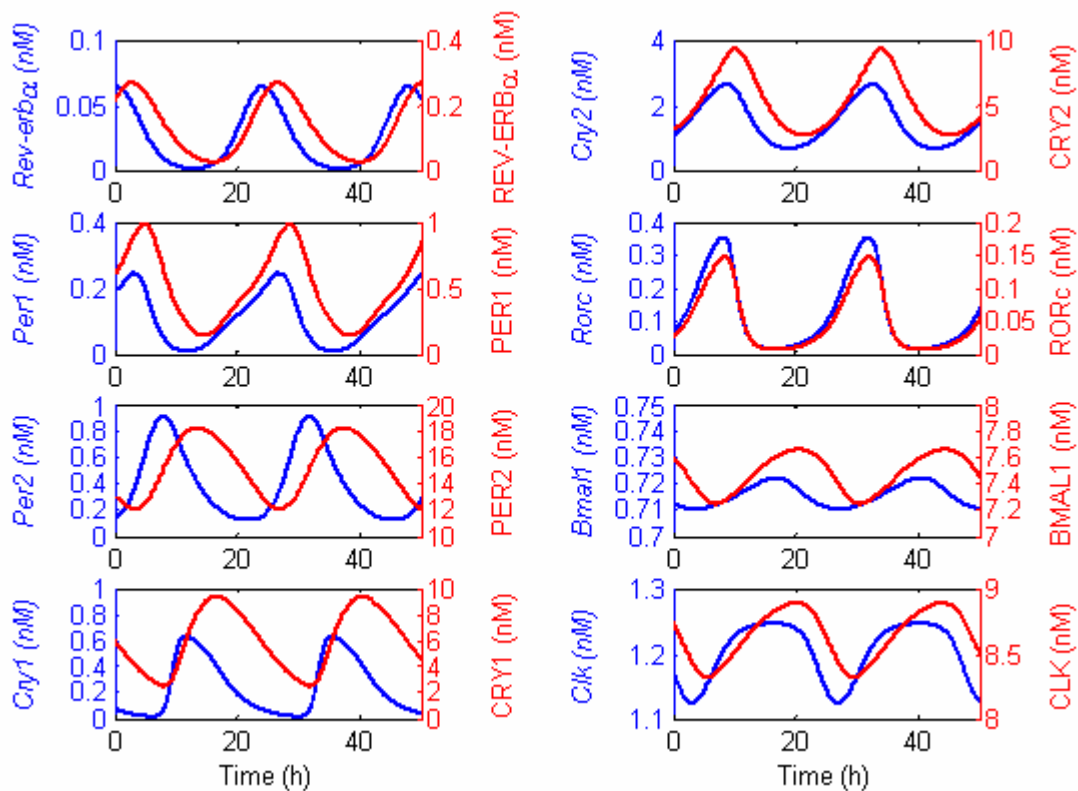


Figure 2. Time courses showing oscillation patterns for eight mRNAs and eight proteins in the mathematical model of the intracellular mouse circadian clock.

It would be interesting to know something about the effect of parameter perturbations on the performance of the clock. For example, how might a change in a particular kinetic constant (e.g. the binding rate of PER1 to CRY1) change the nature of these oscillations? Sensitivity analysis, widely employed in engineering, allows one to identify points in the clock that are robust and points that are fragile. Our analyses reveal that the clock is sensitive to perturbations in those parameters that are general (e.g. transcription, translation) and insensitive to perturbations that are clock-specific (e.g. association and dissociation of clock proteins). (See Figure 3). This conclusion makes biological sense:” nature has organized the clock such that its fragilities can be off-loaded to extra-circadian

cellular machinery while minimizing the need to maintain control over clock-specific kinetics.

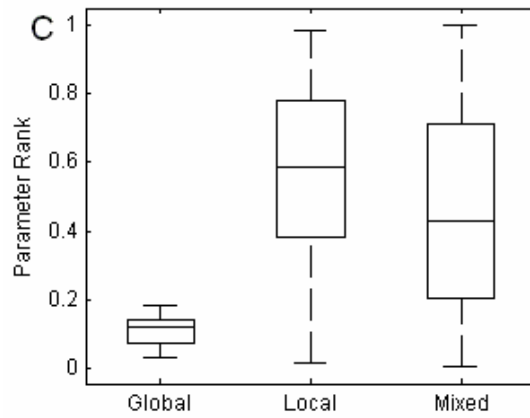


Figure 3. Sensitivity to parameter perturbation in the intracellular mouse circadian clock is retained in the “global” parameters that govern processes that are not clock-specific (e.g. transcription, translation). The clock, meanwhile, is robust to perturbation in “local” clock-specific processes like association and dissociation of clock proteins. Here, “0” signifies parametric sensitivity and “1” signifies parametric robustness.