

# A Model of the Uncoupled Mammalian Circadian Clock

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The circadian clock produces diverse behaviors with ~24-hour periodicity in numerous organisms, leading, for example, to sleep/wake cycles in animals [1] and leaf movement in plants [2]. In mammals, the postulated “master” clock is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus [3]. This clock then directs putative “slave” clocks throughout the body in miscellaneous organs such as liver and kidney [3].

Manipulation of environment and observation of the resultant changes in wheel-running behavior in rodents led to the initial understanding of clock performance [4]. While these experiments continue to be useful, they have been supplemented by application of biochemical techniques to cells in the SCN, thereby uncovering many facets of the clock’s *molecular* operation. Specifically, the mammalian clock functions as an oscillator because the principal clock components (*Per* and *Cry*) negatively regulate their own production. Beyond *Per* and *Cry*, various elements feed into the clock as activators and repressors, adding subsidiary feedback loops to the circadian system and increasing its complexity [1,3]. This advancing understanding of the clock has led to the development of several mathematical models at the molecular level. To date, the most sophisticated of these are the deterministic model produced by Leloup and Goldbeter [5] and the deterministic and stochastic models produced by Forger and Peskin [6,7].

Though these representations produce oscillations of clock components with correct periodicity, they were constructed from a meld of cellular, tissular, and organismal data. It has been argued, however, that the correct phenotypes driven by intracellular (i.e. core) clock networks can be observed only when individual neurons are decoupled from one another [8]. Indeed, specific manifestations of level-specific clock behavior can be seen in both the broader spread of circadian period and the greater cycle-to-cycle variation in circadian period evident in decoupled neurons when compared to SCN explants [8]. Furthermore, a recent work shows that dispersed neurons with *Per1* and *Cry1* knockouts exhibit arrhythmic behavior, in contrast to the previously-reported results, developed from experiments involving interacting cells, which indicated merely altered rhythms [9].

Here, we take the phenotypes of the true, uncoupled oscillator and develop a mathematical model of the single-cell circadian clock. Two versions of the period gene (*Per1* and *Per2*), both versions of the cryptochrome gene (*Cry1* and *Cry2*), and also the *Bmal1*, *Rev-ErbA*, and *RorC* genes are included. These genes and their products are linked in a network of interactions with numerous positive- and negative-feedback loops. We show that the model correctly predicts a 24-hour period and also maintains accurate phase relationships among the components (i.e. four-hour leads of *Rev-ErbA* over *Per1* and *Per2*, four-hour leads of *Per1* and *Per2* over *Cry1*, *Cry2*, and *RorC*, four-hour leads of *Cry1*, *Cry2*, and *RorC* over *Bmal1*, and a twelve-hour lead of

*Bmall* over *Rev-Erba*). Furthermore, the model accurately predicts a lead of mRNAs over proteins of approximately four to six hours for model components.

Project supported by the Institute for Collaborative Biotechnologies through Grant DAAD19-03-D-0004 from the U. S. Army Research Office.

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