Right on time: the biochemistry behind the human circadian clock

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Introduction

The daily rotation of the Earth causes the 24 hour light-dark cycle, and this is arguably the Earth's most ancient evolutionary pressure. Since it's been around for so long, it is unsurprising that almost all life on Earth exhibits some sort of adaptation to this cycle in the form of circadian rhythms.

In mammals, the central driver of these rhythms consists of a biochemical feedback loop that is present in all of your cells (Figure 1). This loop is able to synchronize with the 24 hour light-dark cycle, and cause physiological and behavioral changes (Figure 2). But how does the circadian clock actually do this?

Since genes control many bodily functions, we utilized a new technique called ribosome profiling to identify genes that are turned on and off by the circadian clock. We found that genes are turned on at two distinct times of day, and unexpectedly, we also found that the circadian clock is able to regulate other very basic cellular functions that we didn't know about before.

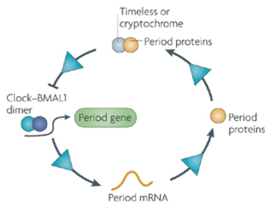

Figure 1. A simplified model of the molecular basis for circadian rhythm generation. Adapted from [1].

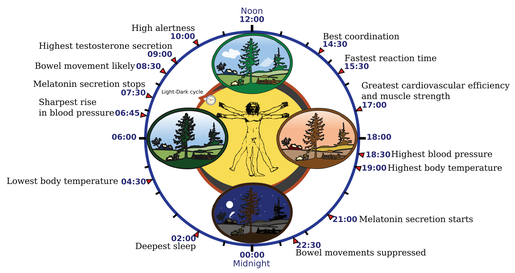

Figure 2. Some of the physiological and behavioral changes that the circadian clock is responsible for over 24 hours.

Materials & Methods

In our experiments, we used a new powerful new experimental technique called ribosome profiling [2]. This technique relies on modern high-thoroughput DNA sequencing technologies to determine which genes are active over a 24 hour period.

We conducted these experiments in both normal (wild-type) and circadian clock deficient human cells. By doing this, we can determine if any changes in gene activity that we see are truly controlled by the circadian clock.

Results

Genes Controlled by the Circadian Clock are Produced at Two Distinct Times of Day

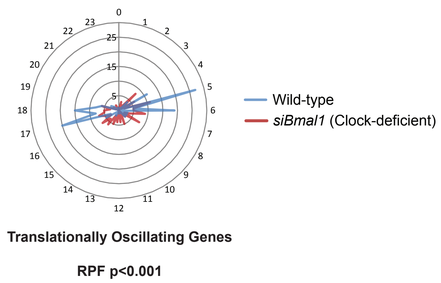

Figure 3. The time of peak gene expression level in both wild-type and clock-deficient human cells is plotted on a radarplot.

Above, our results show that in wild-type cells, cycling genes are turned on at two times of day.

In cells that don't have a functioning circadian clock, these genes don't seem to be temporally controlled anymore, and are expressed at all times over 24 hours.

Taken together, this tells us that the circadian clock is responsible for controlling when cycling genes are expressed over the course of a given day.

Identification of Genes Controlled by the Circadian Clock

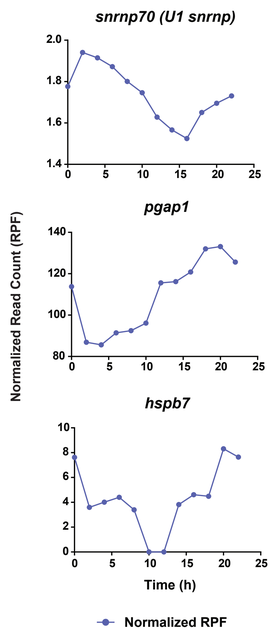

Figure 4. A search for genes that exhibited oscillations in wild-type cells but not in clock-deficient cells yielded a number of candidates. Gene expression of some of these candidates is plotted above as a function of time.

From our results, we found genes that cycled in normal cells over 24 hours, but did not in the clock-deficient cells. This implies that the cycling of these genes are controlled by the circadian clock.

At left, we have shown some of these genes, and their expression over 24 hours.

Biochemically, the role of these genes is very diverse, ranging from controlling general gene expression, to helping proteins fold properly in the cell.

Processing Body Formation is Regulated by the Circadian Clock

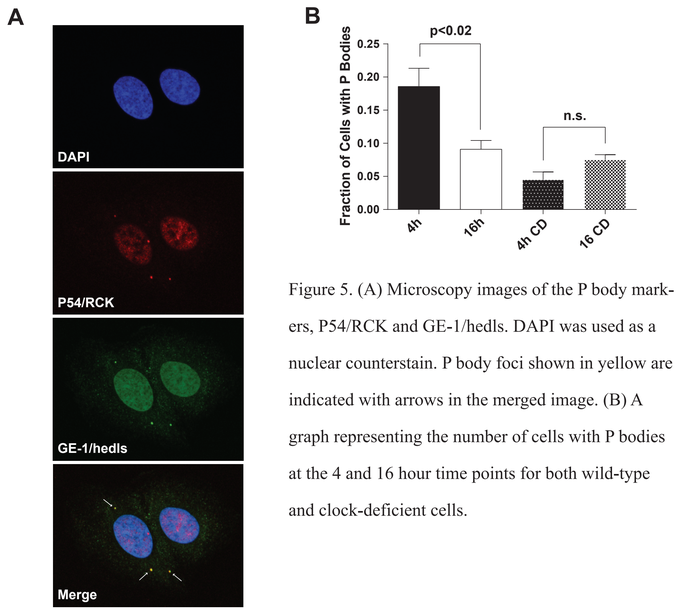

Figure 5. (A) Microscopy images of the P body markers, P54/RCK and GE-1/hedls. DAPI was used as a nuclear counterstain. P body foci shown in yellow are indicated with arrows in the merged image. (B) A graph representing the number of cells with P bodies at the 4 and 16 hour time points for both wild-type and clock-deficient cells.

Under normal conditions, cells recycle unwanted molecules in small foci known as processing bodies, or P bodies. These P bodies play an important role in proper cellular maintenance. You can see them in the microscopy images above, shown in yellow.

In the bargraph, we show that the formation of these P bodies normally cycles over 24 hours. However, this cycling is lost in clock-deficient cells.

This implies that the circadian clock controls the formation of P bodies, and thus controls an important aspect of cellular maintenance.

Conclusions

- Rhythmic clock-dependent gene expression occurs twice per day
- Circadian genes are implicated in a wide variety of important biochemical roles
- The circadian clock is required to regulate P body dynamics, and may play a role in regulating proper cellular maintenance

References & Acknowledgements

1. Herzog, E. D. Neurons and networks in daily rhythms. Nature reviews. Neuroscience 8, 790-802, doi:10.1038/nrn2215 (2007).
2. Ingolia, N. T. Ribosome profiling: new views of translation, from single codons to genome scale. Nature reviews. Genetics 15, 205-213, doi:10.1038/nrg3645 (2014).

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