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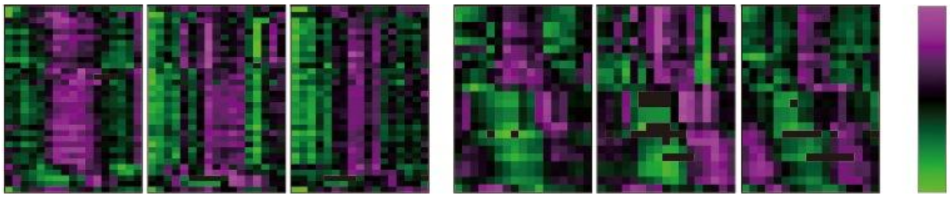
The body's clock from a blood sample

August 29, 2012 – Our bodies have a daily activity cycle that tracks closely with the natural day-night rhythm of the rotating earth, and helps regulate such physiological functions as hormone secretion, blood pressure, and sleep. The importance of these biological clocks can be seen from the many diseases, such as asthma, myocardial infraction, and even some allergies, that show clear tendencies to worsen or occur at specific times of day. Misalignment between internal time and the outside environment is also tied to other problems, such as jetlag, hormone dysregulation, cognitive problems, and affective disorders, all of which make the ability to make clear and accurate measurements of the body's clock an important research goal.

Now, Takeya Kasukawa and others in the Functional Genomics Unit and Laboratory for Systems Biology (both headed by Hiroki R. Ueda) reveal a convenient, non-invasive method for calculating body time in humans. This work, conducted in collaboration with researchers from Keio University, the National Center of Neurology and Psychiatry, and Hokkaido University, and published in the Proceedings of the National Academy of Sciences, shows how a timetable of oscillating metabolites provides a readout of the internal clock of potentially great clinical value.



Changes in metabolite levels in blood samples from three human subjects over a 24-hour period. Positive ion metabolites shown to left, negative ion metabolites to right. (Green indicates lower and magenta higher abundances)

Previously published methods for telling internal time have been based on measurements of known cycling molecules such as melatonin or cortisol, but these necessitated the isolation and monitoring of the subject, taking multiple samples over an extended period, making them labor-intensive to researchers and burdensome to patients. Taking a hint from an observation made by Carl von Linné (better known as Linnaeus), who noted that as different flowers bloom at different times of day it should be possible to tell the time simply by seeing which flowers are in bloom, Kasukawa et al looked for a way to read a molecular version of this "flower clock" in humans. The Ueda lab had previously developed a method for doing just that in mouse, by developing and referring to a 24-hour timetable showing the cycling levels of a large number of hundreds of metabolites.

In this latest study, Kasukawa sought to apply those same techniques in humans, beginning by measuring the rise and fall of metabolite levels over the course of a 24-hour day in three human subjects following predetermined activity routines, in which the subjects stayed in light- and temperature-controlled environments, receiving specific calorie amount every two hours and giving blood samples on the hour over a day and a half period. This so-called constant routine minimizes the effects of external influences on internal state making it possible to extract readouts of the unperturbed endogenous levels of metabolites in the blood. These fluctuating abundances were measured by liquid chromatography and mass spectrometry, making it possible to construct a timetable plotting metabolite levels against body time.

The next step was validation. Blood samples were taken at arbitrary times from three different subjects, analyzed for the same metabolites, and compared to the molecular circadian schedule. Samples were also taken from the six subjects subjected to shift routines, in which external and internal time signals are purposely uncoordinated. Comparing the results from all these groups, Kasukawa et al. found that the readouts matched neatly with the subjects' actual internal time, with a maximum margin of error of only three hours. The success of this method provides an accurate new way to evaluate biological time in humans without requiring multiple blood draws over extended periods.

The Ueda lab's technique has great potential for uses in the study of rhythm disorders and development of effective treatments. “We hope to improve the accuracy and ease of use of this metabolite timetable approach in the future,” says Kasukawa, “and to show its potential for clinical application by using it to analyze actual patient blood samples as well.”