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## Circadian Clock Genes Also Tick Outside the Brain

The clock genes that govern the 24-hour circadian rhythms of the body also function in similar cycles in peripheral cells outside the brain, researchers have found. Their findings hint that organs throughout the body, and not just the brain, keep time using their own internal genetic clocks.

The research team, which included Joseph S. Takahashi, a Howard Hughes Medical Institute investigator at Northwestern University, and David K. Welsh and Steve A. Kay of The Scripps Research Institute, published its findings November 23, 2004, in an immediate early publication in the journal *Current Biology*.

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The studies stand in sharp contrast to findings from previous experiments, which indicated that although the peripheral cells expressed the clock genes, they did not appear to function in a persistent circadian rhythm. "Since the first cloning of the clock genes, it had been known that they were expressed widely in many tissues," said Takahashi. "And many laboratories had found that they were expressed rhythmically in these cells. However, these studies indicated that only the clocks in the suprachiasmatic nucleus of the brain could have a persistent oscillation; whereas in the liver and other tissues, they appear to be damped."

The suprachiasmatic nucleus (SCN) was believed to be the site of the body's sole biological clock. Most biological clocks operate on a 24-hour, circadian (Latin for "about a day") cycle that governs functions like sleeping and waking, rest and activity, fluid balance, body temperature, cardiac output, oxygen consumption and endocrine gland secretion.

To explore whether such damping really existed at the single-cell level in peripheral cells, the researchers used fibroblast cells from mice in which they

had engineered a gene that produced a luminescent protein, luciferase, to be under the same control as the clock gene *Period2*.

According to Welsh, fibroblasts were chosen for three main reasons: Rhythmicity of non-SCN cells was first demonstrated in immortalized fibroblasts; the cells are relatively easy to grow in culture; and fibroblasts are found throughout the body. “A fibroblast is as close to a ‘generic’ mammalian cell as you’re likely to find,” said Welsh, who was an HHMI predoctoral fellow at Harvard University before moving to Scripps. “So if fibroblasts have self-sustained clocks, most other cells probably do, too.”

By measuring the level of bioluminescence emitted by fibroblasts over many days, the researchers could detect any oscillations in expression of the clock gene. In an earlier paper, the researchers showed that the peripheral tissues of mice did show oscillation in the genes. So, in the experiments reported in *Current Biology*, the researchers sought to explore whether that oscillation persisted at the level of the individual cells.

“We found that, when you look at the signal from the overt culture, it damps out, but the individual fibroblasts reveal a persistent, independent oscillation,” said Takahashi. He said that the findings thus indicate that in cell culture there appears to be no evidence for “coupling” between cells that would coordinate the oscillations. Such coupling has been seen in whole tissues, and would be key to coordinated circadian rhythms in organs, he said.

“Although we have known that peripheral tissues can show oscillations, it has never been shown that this is occurring in every cell,” said Takahashi. “However, in these cultures, every cell that expressed the *Period2* luciferase gene had a very robust circadian oscillation. If this is true in every tissue, then perhaps almost every cell in our body has a circadian oscillation that governs its timing. And that’s a very different view than we’ve had before, which held that the brain was the exclusive circadian oscillator.”

One mystery, said Takahashi, arises from the observation that, although each cell is oscillating with a consistent period length, those periods range from 22 hours to 30 hours, which is significantly outside the normal 24-hour circadian range seen in the whole organism.

“We’d like to know the origin of this variation,” said Takahashi. “Also, it suggests that maybe we need to be studying the properties of oscillation at the single-cell level when we are trying to understand the mechanism.”

Another central goal, said Takahashi, is understanding the mechanisms by which the rhythms of different cells are coupled in tissues. “Since such distinctive rhythms appear in a wide variety of tissues, it seems likely that there are going to be different coupling mechanisms for different tissues—for example in neurons, fibroblasts, liver, kidney and lung cells—all of which oscillate,” he said.

Although it is still too early to tell whether these findings could have an impact on the timing of drug delivery, for example, Welsh believes that they

raise interesting questions about the possible differences in rhythmicity of cells within tissues. “It is known that the sensitivity of tumor cells to chemotherapeutic agents can depend on circadian phase,” Welsh said. “If cells within a tumor are not identically phased, this may allow some cells to escape from the drug's effect. Perhaps synchronizing the cells prior to drug treatment would improve tumor eradication.”