Broken circadian clocks: a clock gene mutation and entrainment by feeding

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Many animals have evolved the ability to anticipate periodic food availability. In mammals, this ability is due to a circadian clock that still functions after ablation of the suprachiasmatic nucleus (SCN), although such lesions abolish (or severely disrupt) virtually all circadian rhythms in behavior and physiology when food is available ad libitum. Restriction of meals to a certain time of day elicits anticipatory behavior, as well as anticipatory changes in physiology, and these are clearly under the control of a circadian oscillator. Despite many attempts to find the locus of the feeding-entrainable oscillator (FEO) by central nervous system (CNS) lesions, endocrine gland extirpation, etc., the locus of the FEO is still unknown. Several reviews may be consulted for background information on the FEO (5, 8, 9).

The cloning of mammalian clock genes has provided the opportunity to study circadian mechanism at a molecular level in new and exciting ways. One is to investigate the consequence of mutations in clock genes, as exemplified by a paper in this issue of the American Journal of Physiology-Regulatory, Integrative and Comparative Physiology (7). Another is to monitor rhythms of gene expression, briefly discussed below. Mice with the clk/clk mutation are able to entrain activity rhythms to light-dark cycles but have an abnormally long period in constant darkness and may eventually become arrhythmic (2). In some sense, it appears that the SCN becomes a damped, rather than a self-sustained, oscillator. A damped oscillator requires some periodic input to maintain the amplitude of the oscillations. Whether the FEO is a damped or self-sustained oscillator has not been fully resolved, although some evidence indicates the latter. Food anticipatory activity persists during food deprivation for up to 5 days, it reappears during food deprivation at the previously entrained phase even after weeks of ad libitum feeding, and, in some instances, prolonged free-runs are observed when the period of food availability is outside the range of entrainment (5, 8, 9).

As far as is known, the clock genes found in many brain structures and in peripheral tissues are the same as those found in the SCN. If the FEO uses these same genes, it seems reasonable to expect that the clk/clk mutation would affect entrainment to periodic meals. Pitts et al. (7) report that mutant mice show food anticipatory activity (FAA) to restricted meal time and that FAA reappears at the entrained phase during food deprivation after 2 days of ad libitum feeding. They suggest that perhaps the FEO does not require the normal CLOCK protein and may be based on a different molecular mechanism. This is an intriguing hypothesis; however, it is not unequivocally supported by this experiment. Although it is not possible to food deprive mice for many days, a much longer intervening ad libitum feeding period might reveal whether the FEO had been damped, i.e., FAA would not reappear, or whether the mutation produced an abnormally long period in the FEO, i.e., FAA would appear at a delayed phase position. One interesting finding is that the onset of FAA occurred earlier in clk/clk mutants than in wild-type mice, especially under a light-dark cycle. This could be interpreted to mean that CLOCK does play some role in the entrainment of FAA. It is of interest to note that periodic meals entrain clock gene expression in the heart and that the amplitude of mPer2 and BMAL1 mRNAs was actually greater in clk/clk mutants than in wild types (6). Clearly, this mutation is not incompatible with some clock functions. Needless to say, one looks forward to additional experiments using various clock gene mutants or knockouts. Hopefully, such studies will lead to a better understanding of the elusive FEO.

In a broader context, it should be noted that circadian rhythms in gene expression in many peripheral tissues become entrained to meal time. The liver seems to be particularly responsive to a change in meal time from the dark to the light phase, showing a complete phase shift after 2 days (10). A transgenic rat model in which the mouse Per1 promoter has been linked to a luciferase reporter has proven to provide interesting insights into rhythms of gene expression in vitro, as well as the effects of periodic meals on the phase of gene expression in CNS and peripheral tissues. Of all the CNS and peripheral tissues examined, only the SCN seems capable of a self-sustained rhythm in gene expression (up to 32 days), whereas rhythms in other tissues are damped over two to seven cycles (1, 11). In liver and lung, after damping, a rhythm can be reinstated by a change in the medium (11). This is consistent with the hypothesis that damping is the result of gradual desynchronization among individual cellular clocks, thus reducing the overall amplitude of the rhythm. In the SCN, dye coupling among cells and the presence of connexin32, a protein that forms gap junctions, suggest that cellular oscillators may maintain synchrony via gap junctions (3). If the FEO is indeed a...
self-sustained oscillator, its locus has not been identified by these in vitro studies.

A recent report indicates that gene expression rhythms in liver, stomach, colon, and esophagus, although entrained by restricted feeding, can become dissociated from FAA. During food deprivation after ad libitum feeding, FAA appears at the previously entrained phase, whereas the phase of gene expression in these organs was shifted back to the phase observed in ad libitum feeding. Furthermore, FAA to two meals per day was not reflected in gene expression, i.e., no bimodal rhythms were observed and all tissues were entrained only to the meal presented at night (4).

Thus many unanswered questions remain. Where is the FEO, how does it communicate with other central and peripheral clocks, and is the molecular mechanism the same as in other clocks? Clearly, this is an exciting time to be a circadian rhythm researcher.

REFERENCES