An ultradian clock shapes genome expression in yeast
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Cell biology has traditionally focused on where rather than when things happen inside cells. The fundamental biochemical activities of the cell have been sorted into spatial domains, with functions allotted to subcellular compartments that can either promote or inhibit molecular interaction. Important exceptions to this prominence of three dimensions have arisen in the areas of cell division cycle research and studies of circadian (~24-hr) rhythm. Here the emphasis has been on an apparently time-oriented, and recent efforts have made use of powerful new strategies to develop temporal maps of gene expression and macromolecular assembly. Microarray analyses have exposed deeply rooted, cell-autonomous programs of timed gene activity, and in both areas sequential control is extensive. Circadian rhythms are associated with the ordered expression of 10% or more (see below) of the genome in Arabidopsis, Drosophila, and the mouse, and a comparable fraction of the genome appears to be regulated by the cell cycle in yeast (1–12). An even more pervasive system of temporal organization is now the subject of a study in this issue of PNAS by Klevecz et al. (13), who follow the yeast Saccharomyces cerevisiae. An initial search for clusters of gene expression that might support a well known ~40-min respiratory cycle has instead revealed that ~40-min rhythms are a genomewide phenomenon. Truly constitutive expression may not apply to any gene’s activity in continuously grown, aerobic cultures of yeast.

Respiratory oscillations have been observed in budding yeast for at least 30 years and are readily detected at higher cell densities in continuous culture. It has been proposed that a stage of ethanol metabolism may supply diffusible factors that effect synchrony (14). In addition to periodic fluctuations in the concentration of dissolved oxygen, NAD(P)H and reduced glutathione concentrations are rhythmic. Perturbations of the reduced glutathione concentration reset the phase of the respiratory cycle, indicating that intracellular redox potential influences the respiratory oscillation (15).

To better understand the genetic control of the respiratory rhythm, Klevecz et al. (13) isolated RNA samples from continuously cultured yeast at time points encompassing three respiratory cycles. The samples then were used to interrogate DNA microarrays with representative sequences from ~6,400 yeast genes. Of these, 5,329 were detectably expressed in the experiments. Rather than carving out a small group of genes central to organizing the respiratory rhythm, this study uncovered oscillations throughout the arrays. Three “permissive” intervals of gene activity were observed in each ~40-min respiratory cycle. Fewer than 200 genes, including favored candidates for constitutive housekeeping activities, were most abundantly expressed in all three permissive zones. For these genes, regular dips in expression were associated with each “nonpermissive” interval. In contrast, the vast majority of expressed genes exhibited a more restricted pattern in which activity was largely confined to a single permissive zone in the respiratory phase of the ~40-min cycle (650 genes), or within one of two permissive zones connected to the reductive phase of each cycle (4,679 genes).

Aside from their description of a stunning rhythm of genome activity, Klevecz et al. (13) demonstrate that these oscillations are ultimately coupled to cell division. Because cell doubling times in continuous culture are on the order of 8–10 hr, any attempt to connect division to a 40-min cycle might seem tenuous. However, older assessments of yeast growth under a variety of culture conditions had indicated quantized division times that could represent multiples of a fundamental ~40-min oscillation. By using flow cytometry, Klevecz et al. followed the changing DNA contents of cultures synchronized with respect to the respiratory cycle. Although <10% of a culture moved into S phase during any given turn of the respiratory cycle, DNA replication in those cells assumed a constant phase with respect to the 40-min cycles of respiration measured for the culture as a whole. DNA replication begins precipitously at the end of the respiratory phase, continues throughout the reductive phase, and ends as respiration returns. The authors speculate that temporal coupling of DNA synthesis to the reductive phase of the respiratory cycle may have evolved to minimize the exposure of single-stranded DNA to agents that would promote oxidative damage.

The imposition of ~40-min periodicity on gene activity, DNA replication, and respiration indicates the presence of a novel biological clock in yeast. This oscillator could act as the fundamental timekeeping device in the organism, with all of the manifest rhythms listed above coordinated as its driven components. Klevecz et al. (13) suggest that a similar organization might emerge from temporal studies of animal cells. Although there is presently no evidence for regulation of this sort in the ultradian domain (minutes to hours), wide-ranging metabolic oscillations have in fact been described in circadian studies of plants and animals. For example, circadian changes in cellular redox were described in plants several decades ago (16), and robust 24-hr oscillations of reduced glutathione are commonly observed in mammalian cells (17). It may be significant that restricted feeding, which acutely alters reduced glutathione levels, resets the phases of circadian clocks in many mammalian tissues (17–19). Recall that perturbing the concentration of reduced glutathione has been shown to phase-shift the respiratory rhythm. That elements of cell division in yeast can be associated with recurring ultradian gates reminds us that, in organisms that possess them, circadian clocks regularly set the pace of the cell cycle. Studies of Euglena and Chlamydomonas have demonstrated that circadian clocks establish temporal windows that dictate progression through specific stages of the cell cycle (20, 21), and close coupling of cell division to the circadian clock has been extended to mammals. For instance, surgical removal of a portion of the liver induces a widespread proliferation among residual hepatocytes, leading to replacement of lost...
mass within a few days. Whereas maximal DNA synthesis occurs at a fixed time after surgery, the resulting G2 cells will go on to enter mitosis only at a particular phase of the circadian cycle. Thus, depending on the time of day surgical ablation takes place, cell division can be delayed by up to 24 hr (22).

Perhaps most relevant to any comparison of the biology determined by \( \sim 40 \)-min rhythms in yeast and the circadian clocks of animal cells are the microarray data that have been collected on a circadian time scale from flies and mice. Gene expression profiles in Drosophila show that regulatory steps governing flux through foremost pathways of glycolysis and gluconeogenesis are enforced with a circadian rhythm. Glucose-6-phosphate 1-dehydrogenase, the enzyme gating entry into the pentose-phosphate pathway, is encoded by a gene that fluctuates with a robust circadian cycle. Activity is maximal toward the end of the day. Opposing this activity, fructose-bisphosphatase, whose performance determines rates of gluconeogenesis, is rhythmic but with a phase that is shifted to dawn (2). Potentially antagonistic pathways mediating glucose metabolism thus have been segregated into distinct temporal compartments. Because the pentose-phosphate pathway affords the major route for metabolic conversion of \( \text{NAD(P)} \) to \( \text{NAD(P)H} \), \( \text{NAD(P)H} \) levels should cycle in Drosophila. Perhaps the \( \sim 40 \)-min \( \text{NAD(P)H} \) oscillations described for yeast do have a counterpart in animal cells, but one that is adapted to a circadian rhythm rather than an ultradian clock. Of special interest here are the studies of Rutter et al. (23), and Dioum et al. (24), who have shown that the functions of key transcription factors composing the animal circadian clock are sensitive to concentrations of gas (e.g., CO) and \( \text{NAD(P)H} \). McKnight and colleagues (25) have hypothesized that these metabolite-sensing proteins evolved in primitive cells to couple transcriptional networks to diurnally enforced rhythms of metabolism. Early adaptations of this sort might have prompted the evolution of self-sustaining programs of circadian rhythmicity that tightly determine metabolic flux (25). Presumably, shorter metabolic cycles, such as those found in yeast, would favor emergence of ultradian rather than circadian clocks. It would be fascinating to search for comparable reciprocating control in yeast as its ultradian clockworks are exposed.

In contrast to Drosophila, microarray studies in mice benefit from the easy separation of cells representing the major organ systems. When the identities of genes falling under the control of circadian clocks in diverse tissues are compared, differences far outweigh similarities, in contrast to the molecular organization of the clock itself, which is largely invariant. To better illustrate this point, consider one listing of clock-controlled genes from liver and a portion of the hypothalamus (suprachiasmatic nucleus) that included \( \sim 300 \) responsive genes in each tissue. Fewer than 30 genes were common to both lists (7). Another study comparing heart and liver found a similar bias toward divergent circadian control (8). Because the average roster of clock-controlled genes represents \( \sim 10\% \) of the expressed genes sampled in each tissue, one can safely guess that most mouse genes would be found to fall under circadian control in at least one cell type if the analysis were extended to just a few more tissues. Whereas the yeast \( \sim 40\)-min clock must pack all this control into a single cell type, multicellularity brings with it the opportunity for sorting temporal control according to cell identity.

Are there clues about the composition of the new yeast pacemaker? Klevecz et al. (13) point out that mutations have been recovered by others that substantially alter the period of the respiratory oscillation. Just as genetic screens have cracked open the circadian clockworks (26), mutations affecting the yeast timer will surely expose its mechanism. In fact, it is already claimed that a first gene affecting the yeast rhythms carries a short sequence that previously tied together the \( \text{period} \) and \( \text{frequency} \) genes of the Drosophila and Neurospora circadian clocks (27). It is time to take a closer look.