Temperature acts to synchronize the metabolism of yeast in a highly systematic way like what was first demonstrated by HOFFMANN, when he put lizards into temperature cycles of different length, showing that they would entrain later as cycles became shorter (3, 4). This phenomenon turns out to be one of the circadian rules that even contributes to the explanation of chronotype, the distinct entrained phase of an individual. In the general population, there is a distribution of chronotypes (5) and this is thought to be due to differences in genetic background resulting in differences in free-running period (and probably other circadian properties, such as zeitgeber input pathways, as well). Indeed, in the 1970s, the concept of zeitgeber strength as it regulates chronotype was demonstrated in hamsters (6).

Obviously, the environmental cycle is the cycle to which the biological one must adjust. If the biological clock would simply respond with a switch-like mechanism (becoming active when the sun comes up, for instance), then the organism would need no biological clock. A circadian clock, however, allows differential entrainment according to season, for instance, thus allowing gating of seasonal behaviors such as reproduction (7, 8). The experiments described in this paper use these basic ideas, namely that the biological clock must adjust. If the biological clock would simply synchronize with each other and entrain each other.

This phenomenon is comparable to circadian rhythms synchronizing to the external zeitgeber cycle. The physical cycles need to communicate in some way with the biological ones (those of the circadian clock). Most entrainment mechanisms have been defined using light as a zeitgeber; it is an important zeitgeber for most circadian systems and it is experimentally facile to use. In the case of the work here, the external cycle uses temperature as a zeitgeber. There is virtually nothing known concerning the mechanisms by which temperature entrains circadian rhythms. It may be changes in biochemical reactions in the cell or it may be via specialized temperature sensors, akin to photoreceptors. Regardless of the mechanisms, temperature is apparently also a universal zeitgeber for circadian systems. In the case of homeotherms, low-amplitude temperature cycles are effective synchronizers (2). In the case of poikilotherms, the experienced temperature cycles are typically much higher.
Fig. S1. Short, nonresonating $T$ cycles show fixed (driven) phase angles in the concentration of $\text{H}^+$ ions in the media. Temperature cycles were imposed on the yeast fermentor cultures, with a cycle length of 7 h (Upper) or 6.5 h (Lower). The temperature cycle is indicated in two ways on the graph: the gray panels indicate cold phase and the open panels show the warm phase; the actual temperature cycle is plotted at the Top of the Upper graph, showing the gradual, 1-h transitions between the high (28 °C) and low (21 °C) temperature phases. The scale for the oscillations in $\text{H}^+$ ion concentration is indicated. The data are smoothed using a 2-h window and are not trend corrected.

Fig. S2. High-amplitude temperature cycles show an altered waveform and phase angle. A 24-h temperature cycle of 15 °C to 30 °C was applied to fermentor cultures. Here, the oscillation in protons from the high-amplitude cycle (Lower) is compared with that from the 21/28 °C temperature cycle from Fig. 1 (Upper). Two sequential days of stable entrainment are shown.
Fig. S3. Oscillations following release to constant conditions. In two experiments, the pH did not change substantially on release from a 24-h temperature cycle to constant conditions. In these cases, the period of the nonentrained, free-running oscillation was closer to 24 h and did not appear to be unstable (although it did damp rapidly). These two examples contrast what is more typically observed in the fermentor cultures (see Fig. 3 and note the reproducibility therein between experiments). (A) 18/25 °C temperature cycle with a release to 25 °C. (B) 21/28 °C cycle with a release to 28 °C.

Fig. S4. RNA expression during entrainment with temperature. MEP2 (A) and GAP1 (B) mRNA levels were determined in a 24-h 21/28 °C temperature cycle. In each graph, two separate experiments are shown, with MEP2/GAP1 RNA as dashed and dotted lines (representing the two experiments). The oscillations in proton concentrations are shown in the same graphs (solid lines). The lines with squares as markers correspond to the same experiment; the lines with triangles and diamonds are samples from the same experiment. The gray and open bars at the top of the graphs indicate cold and warm phases of the entraining cycle, respectively. Tubulin was used for normalization; for each time series, the high value is set to 1.