observable in molecular beam experiments of angular distributions for this reaction at energies well below that of the conical intersection (*8*). However, other calculations that did not account for the geometric phase gave good agreement with measured angular distributions for this reaction at the same collision energies, which suggests that the effect would not be detectable at these energies (*9*).

More recent time-independent quantum mechanical calculations by Kendrick (*10*) gave an intriguing result: Geometric phase effects did occur for individual J values, but the numerical results for odd and even J canceled out when computing the angular distributions at collisional energies below that of the conical intersection. Therefore, no overall geometric phase effect was predicted in the observable quantities for these energies. The result also held for isotopic substitution of the hydrogen atoms by deuterium atoms in the reaction. These findings were confirmed in calculations that used a time-dependent quantum dynamical theory of Althorpe (*11*). The agreement of these two sets of results is important because they were obtained using two totally different scattering theories.

An explanation was needed, however, as to why the effect of the geometric phase for scattering solutions of odd and even J cancels out at the energies considered. These solutions are obtained from highly complex and computationally time-consuming numerical calculations of the nuclear Schrödinger equation that do not provide a simple physical picture for the even-odd cancellation. The paper by Juanes-Marcos *et al*. provides this explanation.

Juanes-Marcos *et al*. exploit the seemingly abstract mathematical mapping of a pair of connected Möbius strips onto a double cylinder. This topological trick enables them to show that the nuclear wave function around a conical intersection separates into even and odd components with a sign that depends on the geometric phase. As shown in the figure, there are two possible paths to form $H_2 + H$ products. For energies below the conical intersection, most reaction paths pass through just one transition state that corresponds to the even or "direct" component. A very small proportion of reactions paths can also pass through two transition states, and these correspond to the odd or "loop" components. These odd and even components can quantum-mechanically interfere.

To understand why there is a cancellation of even and odd J solutions to the scattering problem, Juanes-Marcos *et al*. examine the direct and loop scattering amplitudes of the angular-dependent components of the scattering wave function at large values of the H_2-H separations. The scattering amplitudes for low J contribute mainly to angular distributions in which the reaction products are scattered backward with respect to the $H + H_2$ direction of approach, and the amplitudes for high J contribute to angular distributions largely in the sideways direction. Classical trajectory calculations show that, for low J, the direct paths scatter mainly in the backward region, whereas the looping paths are concentrated in the forward region. Thus, the direct and looping scattering amplitudes do not interfere in this case. This explains why, for smaller values of J, no geometric phase effect is observed when summing the squares of the scattering amplitudes over even and odd values of J. For larger values of J the analysis is more involved, as the direct and looping components do interfere. However, by making use of a theory of angular distributions developed by Connor and co-workers (*12*), it is possible to explain why the even and odd J solutions cancel in this case also.

One of the findings of Juanes-Marcos *et al*. is that the interference between the direct and looping reaction paths should become more pronounced at collision energies close to the conical intersection, leading to an observable geometric phase effect. This is also found in the quantum dynamical calculations (*11*). The effect of the upper excited electronic surface could also become important at these energies. It would be of interest to compare measurements of the angular distribution in this energy region with the calculations obtained with and without consideration of the geometric phase. The group of Zare has compared their measurements with calculations on this benchmark reaction (*13*), and groups so equipped might be able to perform such experiments at the required collision energies. It is noteworthy that the findings are general and will be applicable to many other chemical reactions beyond $H + H_2$ when conical intersections occur (*14*).

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10.1126/science.1117201

PHYSIOLOGY

Biological Clocks Coordinately Keep Life on Time

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Eating, sleeping, seasonal migration, cell proliferation—a few examples of the many behaviors and life processes that are driven by biological clocks. These "chronometers" coordinate the passage of time with orchestrated cycles that extend from molecular through cellular and systems levels. Timekeeping is part of the very fabric of life, and the clocks that regulate life processes do so over a broad range of time scales: from millisecond oscillations of neuronal activity to seasonal changes that mark shifts in the relative amount of daylight over the course of a year. These timekeeping mechanisms have traditionally been studied in isolation. But a new era in chronobiology is emerging as unexpected interactions among these clocks are discovered, raising interesting questions about why life processes are organized in this way *(1)*.

One of the most studied biological clocks at the genetic, cellular, and molecular levels is that which regulates the dynamic process of eukaryotic cell division (mitosis). Cells of different types and/or sizes spend different amounts of time in different parts of the cell cycle. We know that control of cell cycle progression requires that key proteins, the cyclins, undergo post-translational modifications, including phosphorylation, proteolysis, and spatial targeting. The cell assesses successful completion of critical events at "check points," when decisions are made whether to pro-

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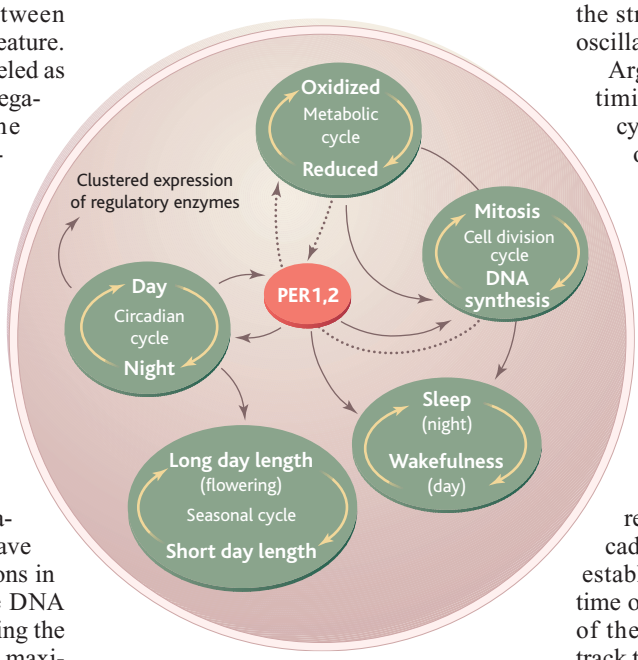
ceed. Is this timing mechanism influenced by other cellular timekeepers? For example, yeast cells oscillate between a reductive and oxidative phase of metabolism and interestingly, yeast cell replication is restricted to the reductive phase of this metabolic cycle. Is this just a coincidence or are the cell division and metabolic cycles meaningfully linked? Because similarly linked metabolic and mitotic oscillations have been observed in high-density cultures of human cells, a functionally relevant interface between these two clocks may be a general feature. The cell division cycle has been modeled as a relaxation oscillator of embedded negative-feedback loops involving the cyclins (*2*), based on studies of budding yeast (*3*). Coupling of this model with metabolic models could reveal how these clocks are coordinated.

Interestingly, yeast undergo a genome-wide, high-frequency (~40 min) oscillation in transcription that is synchronized with respiration (*4*). Transcripts encoding proteins that are necessary for reductive versus respiratory (oxidative) functions are made at opposite points in the ensuing metabolic cycles. Dividing cells may have become tuned to metabolic oscillations in ways that permit unwinding of the DNA during cell division to take place during the reductive metabolic phase in order to maximize faithful replication.

Cycles of cell division and metabolism also appear coordinated with the circadian pacemaker, which has been well studied at the cellular and genetic levels (*5*). The circadian clock is an innate timekeeping mechanism that governs a rhythmic activity cycle, based on (roughly) 24-hour intervals, that is exhibited by many organisms. Like the cell cycle, the cell-based circadian clock is governed by a control point (*6*), where a decision is made whether to proceed with the circadian cycle. And, like the cell cycle, the check point appears to involve posttranslational modification of a clock element. The circadian clock in mammals is primarily located in a region of the brain called the suprachiasmatic nucleus (SCN). Many circadian clock genes encode proteins that are transcription factors—for example, BMAL, PERIOD, CRYPTOCHROME, and CLOCK—that regulate their own transcription. Transcriptional-translational and posttranslational regulation of core circadian clock genes forms feedback loops that generate circadian time (*5*).

Null mutation of the mouse circadian *Period* clock genes, m*Per1* and m*Per2* disrupts circadian rhythms of behavior (*7*). Because these animals also displayed abnormal regulation of cell proliferation with increased hyperplasias, a link between the cell cycle and circadian clocks was proposed. Moreover, mice lacking m*Per2* express altered levels of cell cycle regulators such as c-Myc. The BMAL-CLOCK heterodimeric transcriptional complex of the circadian clock also directly controls the expression of WEE1, a regulator of the mammalian cell cycle (*8*). Microarray analysis of m*Per1, 2*-null mice for changes in other clock-controlled genes identified *Alas1*, which encodes a rate-limiting enzyme for mitochondrial heme biosynthesis (*9*). Interestingly, PER2 has a region called the PAS domain that binds to heme. Furthermore, treatment of cultured mammalian cells with heme synchronizes circadian clock gene expression. Hence, key regulators of cell division, circadian timekeeping, and metabolic state appear coupled, suggesting that these three cycles may interact such that the state of one system can alter regulators in another (see the figure).



How timekeepers interact with each other. The cell division cycle takes place during the reduced phase of the metabolic cycle (in yeast). The circadian clock regulates cell division cycle, metabolism, and restorative sleep through the transcription factors PER1 and PER2. The length of the day within the circadian cycle controls seasonal changes in plants, such as flowering.

A recent dramatic revelation about the circadian cycle links it directly to the metabolic cycle (*10*). Surprisingly, the transcriptional regulators of the circadian pacemaker control clusters of genes that encode rate-limiting enzymes in intermediary metabolism (*11*). Those clock proteins with PAS domains are regulated, in turn, by metabolites that oscillate over the circadian cycle. Clustered gene expression is observed during the metabolic cycle of yeast, and this regulatory logic can be extended to mammalian metabolic cycles. Metabolic cycles can regulate circadian cycles through sensors such as embedded heme moieties in clock proteins, suggesting that lessons learned regarding temporal clustering of gene expression in yeast may be fundamental and provide insights into the structures of circadian and metabolic oscillatory processes (*10*, *11*).

Arguably the most familiar oscillatory timing system in organisms is the daily cycle of sleep and wakefulness. Studies of human sleep, performance, and quantitative electroencephalograms indicate that the sleep-wake cycle is regulated by dual brain mechanisms (*12*). The "two-process model" of sleep regulation integrates (i) the drive to sleep, called sleep homeostasis, that increases with time awake and is restorative during rest, and (ii) the circadian process that is regulated by the ~24-hour clock in the SCN and organizes sleep and wakefulness with respect to night and day. Whereas circadian clock genes in the SCN have well-established roles in determining internal time of day, in other brain areas expression of these core clock elements appears to track time spent awake and allow for adaptability of sleep when food is restricted (*13*). Sleep homeostasis is linked directly to loss and restoration of the brain's energy stores. These findings raise the possibility that circadian clock genes may play an important, unanticipated role, interfacing with cellular metabolism in neurons that contribute to sleep homeostasis.

The sleep-wake cycle also controls a cascade of oscillatory electrical activity in the brain. These neural oscillations take place on time scales ranging from seconds for slow oscillations and brain rhythms to hours for the regulation of slow wave sleep and rapid eye movement sleep. Traditionally, neuroscientists view sleep oscillations through the lens of electrical recordings, but growing evidence suggests that gene regulation is involved. The low-threshold calcium currents that are activated during sleep oscillations allow a massive influx of calcium ions into the dendrites of cortical neurons of the brain. These calcium fluxes may prepare the way for changes in gene regulation and remodeling of dendrites and synapses during the subsequent phase of slow wave sleep (*14*). The relationship of these calcium fluxes to cellular metabolism and clock gene function is unexplored.

Biological systems must adapt on even longer time scales. Seasonal variations in light and nutritional environments regulate cyclic processes in cells that affect behavioral and developmental change. Cyanobacteria, obligate phototrophs that produce 40% of the oxygen in Earth's atmosphere, exhibit a mitotic rate that depends on light intensity. Organisms in which the period of circadian rhythm matches the period of the environmental light-dark cycle are most nutritionally competitive and less exposed to light stress. Light intensity may determine the state of their redox pool within the metabolic cycle, altering circadian clock elements and global transcription (*15*).

Additional evidence for pleiotropy in circadian clock function and time scale of action is emerging in the responsiveness of the plant *Arabodopsis thaliana* to light signals, leaf movement, and regulation of flowering time. Induction of flowering is a developmental change that does not occur daily, but rather annually, when the photoperiod lengthens (*16*). Transition from producing leaf-after-leaf to stalk-borne flowers occurs within the shoot meristem upon temporal coincidence between duration of day length and the circadian clock. The clock controls timing of the expression and the turnover of a key clock transcription factor called timing of chlorophyll a/b binding protein (TOC), which is a clock-controlled factor. During long days, the circadian pattern of TOC protein expression in the meristem moves into daytime, when it can interact with FT, a flowering transcription factor, to induce homoeotic genes that pattern flower formation (see the figure). Remarkably, these findings at the molecular level support Bünning's 1936 "coincidence model" of photoperiodism for how circadian timing controls photoperiodic timing.

Biological oscillations likely emerged from the earliest cyclic life processes on Earth—those of opposing metabolic states imposed by the presence versus absence of solar energy and light-induced stress. Reciprocal regulation of the circadian clock mechanism and the metabolic cycle would ensure that these cycles, and interfacing cell division, mesh in alignment with the external cycle of light and darkness. As organisms evolved and became more complex, physiological and developmental systems also became organized around the day and night cycle, and may have incorporated elements of the ancestral cycles in new ways for their regulation. A consequence of these insights is that the separate computational models that have been developed for each of these biological clocks will need to be integrated.

It is time to focus on the interrelationships between the many cyclical processes in organisms and how they interact across a wide range of temporal and spatial scales. While keeping watch over life's diverse cyclic processes, nature's clocks are not oscillating in isolation.

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10.1126/science.1111420

PHYSICS

Freezing and Melting: Action at Grain Boundaries

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We all have experience with liquids that freeze and melt. Yet the detailed mechanisms underlying this apparently simple phase transition are still not fully understood. Experiments on simple liquids such as argon or water are difficult because the molecules are small and move rapidly. A more successful approach examines the freezing and melting of a colloid, which can be followed directly by video light microscopy (*1*); micrometer-sized Brownian particles suspended in a liquid typically join or leave a colloidal crystal in about 1 second. Furthermore, colloidal particles that interact through a steep repulsive potential mimic the theorists' ideal of an assembly of hard spheres, the simplest system to show a freezing/melting transition. On page 1207 of this issue, Alsayed *et al.* (*2*) describe the melting of colloidal crystals of thermosensitive gel particles that interact almost as if they were hard spheres. Through beautiful video images, they provide what is probably the clearest direct evidence to date that the process of melting starts by "premelting" at defects such as vacancies, dislocations, and particularly grain boundaries.

The freezing transition of hard spheres was discovered by computer simulation in 1957 (*3*) and confirmed some 30 years later by experiments on colloidal suspensions (*4*). The transition is driven by entropy—paradoxically, the apparently ordered crystal has a higher entropy than the metastable fluid from which it grows, for example (*5*, *6*)—and is controlled by just one variable, the concentration by volume, or volume fraction, of the particles in the suspension. As the concentration is increased, spheres in the fluid become increasingly crowded by their neighbors. By crystallizing, they gain more freedom for local motions: While ordered on the large scale, a crystal is locally disordered. Above the melting concentration (volume fraction 0.545) the entropy loss associated with large-scale ordering is more than offset by the entropy gain associated with increased local freedom.

Studying the melting of hard spheres is difficult because the volume fraction of rigid colloidal particles cannot be changed continuously in a single sample: Both the number of particles and their size are fixed. Alsayed *et al.* cleverly overcome this difficulty by using colloidal particles whose size, and therefore volume fraction, changes with temperature. The particles are spherical "droplets" of a temperature-sensitive, cross-linked polymer gel. Suspended in water, these microgel droplets are themselves mostly water, containing only a few percent polymer. On heating, the polymer becomes less soluble, and the particles expel water and shrink. A small increase in temperature, from 25° to 30°C, causes the volume fraction of the suspension to decrease by almost 50%, while the interaction between the particles remains steeply repulsive.

Although a number of researchers have studied the phase behavior and flow properties of suspensions of these temperature-sensitive microgels, for example (*7*) (see the first figure, lower left), Alsayed *et al.* are the first to exploit the temperature dependence of the volume fraction for a detailed study of melting. They monitored the positions of the

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