PLANT SCIENCE

Circadian regulation of sunflower heliotropism, floral orientation, and pollinator visits

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Young sunflower plants track the Sun from east to west during the day and then reorient during the night to face east in anticipation of dawn. In contrast, mature plants cease movement with their flower heads facing east. We show that circadian regulation of directional growth pathways accounts for both phenomena and leads to increased vegetative biomass and enhanced pollinator visits to flowers. Solar tracking movements are driven by antiphasic patterns of elongation on the east and west sides of the stem. Genes implicated in control of phototropic growth, but not clock genes, are differentially expressed on the opposite sides of solar tracking stems. Thus, interactions between environmental response pathways and the internal circadian oscillator coordinate physiological processes with predictable changes in the environment to influence growth and reproduction.

Most plant species display daily rhythms in organ expansion that are regulated by complex interactions between light- and temperature-sensing pathways and the circadian clock (1). These rhythms arise in part because the abundance of growth-related factors such as light signaling components and hormones (e.g., gibberellins and auxins) are regulated by both the circadian clock and light (2–4). A further layer of regulation is afforded by circadian gating of plant responsiveness to these stimuli, with maximal sensitivity to light during the day (5) and to gibberellin and auxin at night (6, 7).

Because the direction and amount of solar irradiation undergo predictable daily changes, light capture might be optimized by links between the circadian clock and directional growth pathways. One such growth pathway is phototropism, in which plants align their photosynthetic organs with the direction of incoming light. Phototropism was first recognized by Charles Darwin (8) and is mediated by the perception of blue light by phototropin photoreceptors that then trigger asymmetric growth via the auxin signaling pathway (9). Heliotropism, or solar tracking, is a more dynamic form of phototropism, with aerial portions of the plant following the Sun's movement throughout the day. Some heliotropic plants such as sunflowers also reorient during the night so that their leaves and apices face east before sunrise (10, 11). Here we show that heliotropism in the common sunflower, Helianthus annuus, is generated by the coordinate action of light-signaling pathways and the circadian clock

and enhances plant performance in the natural environment.

Sunflower stems exhibit heliotropic movement such that their shoot apices shift from facing east at dawn to facing west at dusk as they track the Sun's relative position. Shoot apices then reorient at night to face east in anticipation of dawn (Fig. 1A and movie S1) (12, 13). We disrupted this process in two ways, either by rotating potted plants every evening so that they faced east at nightfall (and thus faced west each morning after directional nighttime growth) or by tethering plant stems to solid supports to limit their tracking movements. In multiple trials, we detected ~10% decreases in both dry biomass and leaf area of the manipulated plants relative to controls in both types of experiments (Fig. 1B and fig. S1; significance assessed using linear mixed-effect models with treatment as fixed effect, trial and leaf number as random effects), demonstrating that solar tracking promotes growth.

The nighttime reorientation of young sunflowers in the absence of any obvious environmental signal suggests involvement of the circadian system; however, an alternative explanation would be an hourglass-like timing mechanism. To distinguish between these possibilities, we examined the kinetics of nighttime reorientation near the summer solstice and the fall equinox. The rate of apical movement at night was higher at midsummer [16 hours light: 8 hours dark (16L:8D)] than during the longer nights of autumn (12L:12D), so that in each case plant apices face fully east just before dawn (Fig. 1C). We next investigated whether plants continue rhythmic tracking movements in the absence of directional light cues. Sunflower plants grown in pots in the field in 14L:10D conditions were heliotropic (Fig. 1D). When moved to a growth chamber with constant, fixed overhead lighting, these plants maintained their directional growth rhythms for several days. Plants reoriented toward

the east during the subjective night and toward the west during the subjective day, with times of maximal inclination corresponding to subjective dawn and dusk. As is true for many types of circadian outputs after withdrawal of environmental signals, rhythmic movements dampened over time (Fig. 1D).

Another way to distinguish rhythms regulated by the circadian clock from those directly induced by environmental cues is to maintain organisms in light-dark cycles with total period lengths (T-cycles) that differ from 24 hours (14). We examined heliotropism in a growth chamber with four directional blue LED (light-emitting diode) lights sequentially turned on and off to mimic the Sun's daily path. After several days in a 24-hour T-cycle (16L:8D), plants bent toward the light source during the day so that "westward" movement culminated at dusk. Anticipatory "eastward" movement then occurred throughout the dark period (Fig. 1E and fig. S2). Upon transfer to a 30-hour T-cycle (20L:10D), maximal "westward" inclination no longer occurred at the light-dark transition, and directionality of nighttime movement was erratic. The return to a 24-hour T-cycle reestablished anticipatory nighttime movement that began when lights were turned off (Fig. 1E and fig. S2). The complex patterns in 30-hour T-cycles suggest uncoordinated growth controlled by both environmental response pathways and the circadian clock. Thus, the circadian clock guides solar tracking in sunflowers.

Because sunflowers lack pulvini, the specialized motor organs that mediate solar tracking in some species (15), we hypothesized that regulated stem elongation might drive solar tracking. We observed a gradual reduction in the amplitude of heliotropic movements as plants reached maturity, correlating with cessation of stem elongation (Fig. 2, A and B, and Fig. 3A). To further investigate the involvement of stem elongation in heliotropism, we examined solar tracking and stem growth rates in dwarf2 (dw2) sunflowers, which are deficient in the production of gibberellin growth hormones (16). In the absence of exogenous gibberellin, dw2 plants have very short stems and no perceptible heliotropism (movie S2). Treatment with exogenous gibberellin transiently restored normal elongation (Fig. 2A) and heliotropism (Fig. 2B) in the mutant. Between days 7 and 14 after the last gibberellin application, stem elongation and the amplitude of solar tracking rhythms coordinately diminished by ~35% (Fig. 2, A and B). Thus, stem elongation is essential for heliotropism.

Many plant species show daily rhythms in nondirectional stem and leaf growth (1). We hypothesized that heliotropism results from differential elongation on opposite sides of stems. Indeed, the growth pattern on the east side of solar tracking sunflower stems was different from that on the west side (Fig. 2C). Growth rates on the east side were high during the day and very low at night, whereas growth rates on the west side were low during the day and higher at night. The higher growth rate on the east versus west side of the stem during the day

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enables the shoot apex to move gradually from east to west. At night, the higher growth rate on the west side culminates in the apex facing east at dawn. We postulated that one of these growth patterns might be similar to the overall growth rhythms of sunflower plants not manifesting heliotropism. We therefore monitored plants maintained in 16L:8D cycles in a growth chamber with overhead lighting. Consistent with reports in pea and zinnia (17, 18), stem elongation growth rates were higher at night than during the day under these controlled conditions (Fig.
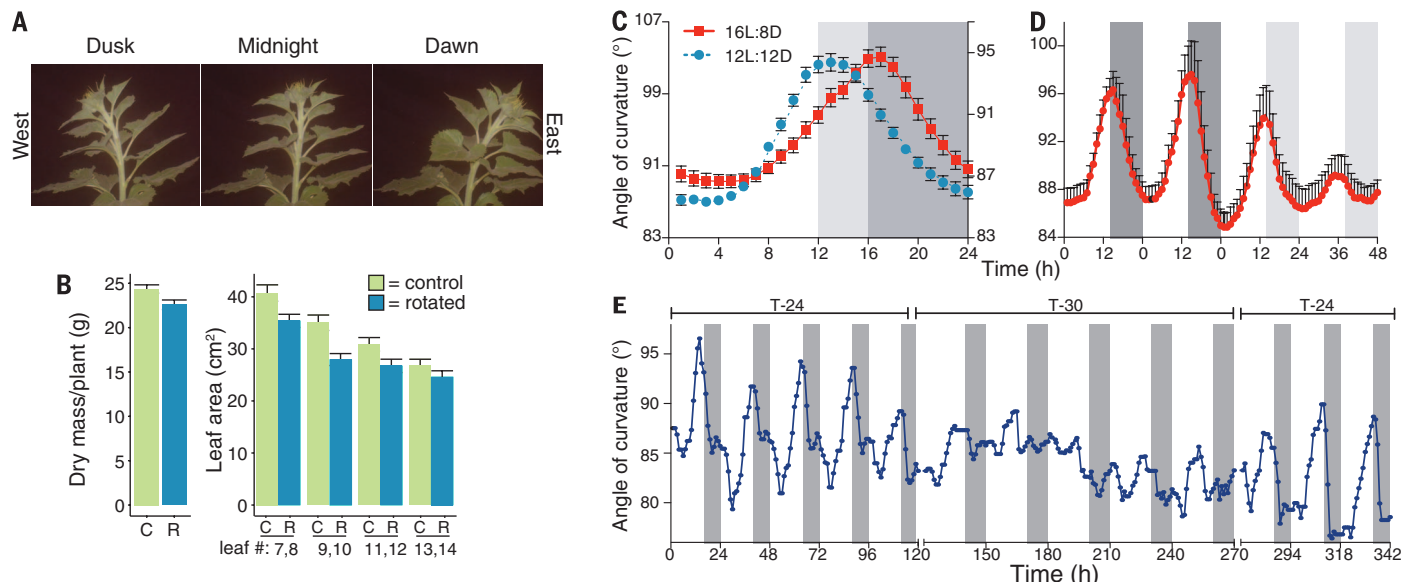
Fig. 1. The circadian clock regulates solar tracking. (**A**) Nighttime reorientation of stem and shoot apex. (**B**) Disruption of solar tracking by daily evening 180° rotation of experimental plants results in a 7.5% reduction in biomass (left) and an 11% reduction in leaf area (right) compared with 360°-rotated control plants [mean ± SEM (error bars), n = 80 plants, P = 0.01 (biomass) and 1 × 10⁻⁶ (leaf area), mixed effect linear regression models]. Numbers refer to leaf pairs. C, control; R, rotated. (**C**) Changes in orientation anticipate dawn and dusk transitions in both fall (left y axis) and summer (right y axis) [mean ± SEM, n = 10 plants]. (**D**) Persistence of rhythmic move-ments after transfer from field to continuous light and temperature conditions. In (C) and (D), white areas denote daytime; dark and light gray areas represent night and subjective night, respectively [mean ± SEM, n = 3 plants]. (**E**) The onset of "eastward" movement in a growth chamber equipped with four directional lights is consistently phased with lights being turned off in 24-hour T-cycles (left and right) but is erratic in 30-hour T-cycles (center). Time zero indicates dawn [(C) and (D)] or the beginning of the first T-cycle (E). Angles <90° and >90° represent inclination toward east and west, respectively. White areas, day; gray, night.
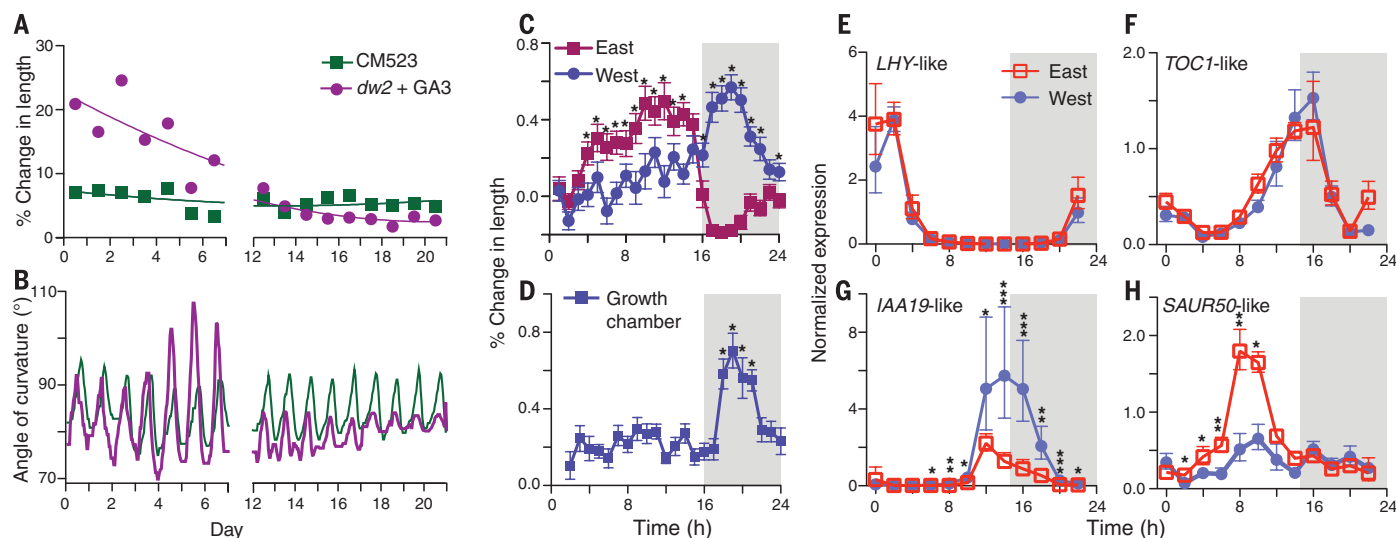

Fig. 2. Solar tracking is driven by opposing growth rhythms on the east and west sides of stems. (**A**) Changes in stem elongation and (**B**) the angle of curvature of the shoot apex relative to the horizon in control (green) and gibberellin-deficient dw2 plants (purple). dw2 mutants were treated twice with 2 μM of the gibberellin GA₃ (gibberellic acid), with the last treatment on day 0. Data in (A) were fitted to centered second-order polynomial equations to aid visualization. (**C**) Timing of elongation for east and west sides of stems of solar tracking field-grown plants [mean ± SEM, n = 42 plants]. (**D**) Timing of stem elongation of plants growing vertically in a top-lit environmental control chamber [mean ± SEM, n = 9 plants]. Asterisks indicate that the east and west sides of the stem (C) or the daytime and nighttime means (D) significantly differ (*P < 0.05, Student's t test). (**E** to **H**) Differential gene expression on the east and west sides of solar tracking stems assessed by quantitative reverse transcription polymerase chain reaction (*P < 0.05, **P < 0.01, ***P < 0.001; orientation by time-point effect, single linear mixed model). Time zero indicates dawn [(C) and (E) to (H)] or when lights were turned on (D). White areas, day; gray, night.
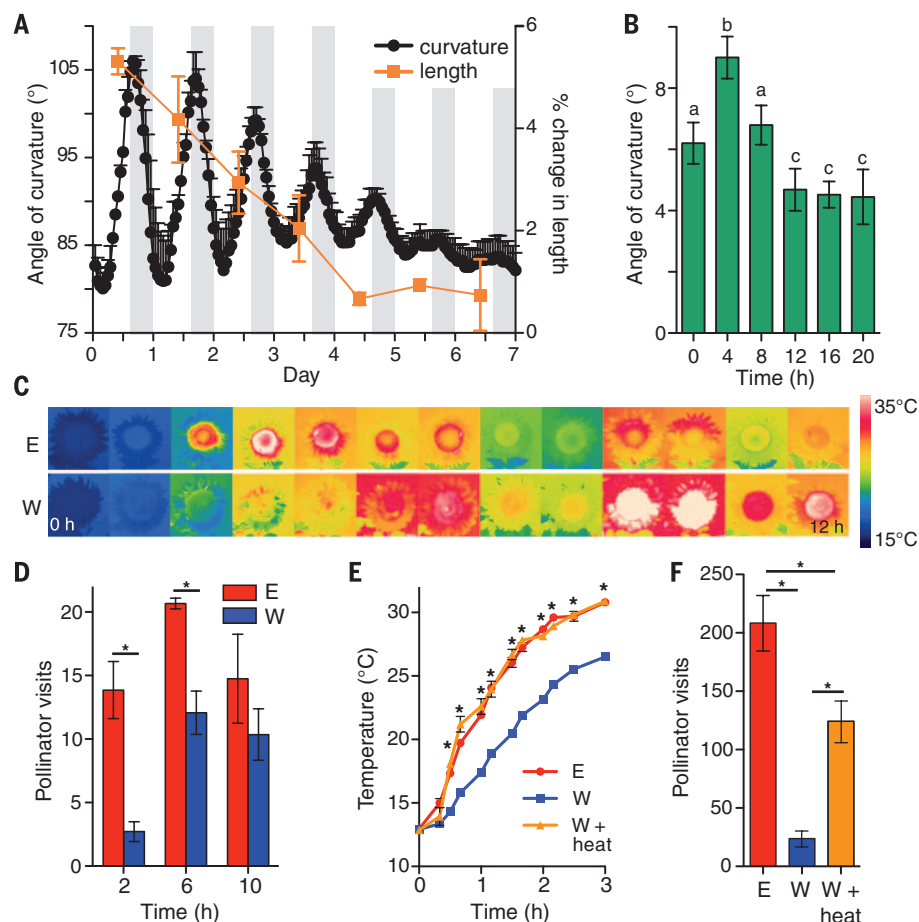
Fig. 3. Eastward orientation of sunflower heads after anthesis is due to gating of light responses by the circadian clock and enhances pollinator visits. (**A**) Amplitude of solar tracking and changes in stem growth of mature plants nearing floral anthesis. Petals were first observed during day 5 (mean ± SEM, $n = 2$ plants). (**B**) Stem curvature of juvenile plants entrained in 16L:8D cycles and then exposed to unidirectional blue light for 4 hours at the indicated times (mean ± SEM, $n = 11$ plants). Different letters indicate significantly different curvature values in pairwise comparisons ($P < 0.05$, Student's t test). (**C**) FLIR images of east-facing (E) and west-facing (W) floral disks at hourly intervals. (**D**) Pollinator visits to east- and west-facing plants (mean ± SEM, $n = 4$ days, eight plants per treatment) during 45-min intervals at three times of day. (**E**) Temperature (mean ± SEM, $n = 6$ plants per condition) of sunflower disks with east or west (with or without supplemental heat) orientations. (**F**) Pollinator visits in the morning to the inflorescences with temperatures reported in (E). In (D) to (F), *$P < 0.05$, t test with unequal variances; comparisons are for: (D) E versus W, (E) W versus W + heat, and (F) all three values. Time zero indicates dawn [(A) and (C) to (E)] or lights on (B).

2D), resembling the growth pattern of the west side of solar tracking stems. These data suggest that heliotropism is mediated by both the default pattern of growth on the west sides of stems and an environmentally imposed growth pattern on the east sides.

Because our data implicate the circadian clock in solar tracking movements, we examined the expression of sunflower homologs of central clock genes on the east and west sides of solar tracking stems. Although a *LATE ELONGATED HYPOCOTYL*–like gene and a *TIMING OF CAB EXPRESSION1*–like gene displayed the expected rhythmic patterns of daily expression, these genes were not differentially expressed on the opposite sides of stems (Fig. 2, E and F). However, expression of two homologs of genes implicated in phototropism (19, 20)

differed on the opposite sides of solar tracking sunflower stems, with an *INDOLE-3-ACETIC ACID19*–like gene more highly expressed on the west side at night (Fig. 2G) and a *SMALL AUXIN-UPREGULATED50 (SAUR50)*–like gene more highly expressed on the east side during the day (Fig. 2H). Homologs of these genes are induced by auxin in many species (21), and SAUR proteins promote cell elongation (22). Directional growth toward a light source is thought to be instigated by the phototropin-triggered redistribution of auxin across plant stems (9), whereas the circadian clock regulates both auxin levels and plant responsiveness to exogenous auxin (4, 7). It is plausible that solar tracking rhythms are generated by coordinate regulation of auxin signaling by blue-light photoreceptors and the circadian

clock on the opposite sides of a radially symmetrical organ, the sunflower stem.

In nature, only young sunflower plants exhibit heliotropic movements. At the final stage of floral development, or anthesis, sunflower apices stop tracking the Sun and acquire a permanent eastward orientation (12). Close examination of the growth dynamics over this period revealed that as stem elongation slows, likely accounting for the overall reduction in movement, the apices move less and less to the west each day, although they return to face east by morning (Fig. 3A and movie S3). This gradual cessation of westward movement might be explained by circadian gating of plant responsiveness to light, with plants responding more strongly to activation of the phototropin blue-light photoreceptors in the morning than at other times of day. We tested this possibility by entraining young plants in 16L:8D cycles and measuring their bending response after exposure to unidirectional blue light at different times of the day or night. Plants exposed to light during the first part of the day showed a stronger tropic response than those stimulated late in the day or at night (Fig. 3B), consistent with studies in potato (23). These data suggest that lower competence of plants to respond to directional light in the afternoons and evenings (Fig. 3B), combined with progressively reduced elongation rates as plants approach maturity, likely accounts for the progressive loss of daily stem movements toward the west and can explain the eastward orientation of sunflower disks at anthesis.

We next investigated whether this eastward orientation provides any ecological advantage. Because floral orientations that elevate floral temperature enhance pollinator visitation in alpine plants (24, 25), we hypothesized that an eastward orientation may promote sunflower attractiveness to pollinators through increased morning interception of solar radiation, coincident with the daily timing of anther and stigma exsertion. Sunflowers were grown in pots in the field; just before the appearance of ray petals, half of these plants were rotated to face west. Hourly recording of disk temperature by forward-looking infrared (FLIR) imaging and more continuous monitoring with thermocouples revealed that east-facing heads warmed up more quickly in the morning than west-facing heads (Fig. 3C and fig. S3). In these early morning hours, pollinators visited east-facing heads fivefold more often than west-facing heads (Fig. 3D and movie S4). This differential was observed only when east-facing flowers were warmer than west-facing flowers. With the exception of one trial where plants flowered during a period of inclement weather, these observations were consistent across trials, years, and field sites (fig. S3). Notably, west-facing flowers warmed with portable heaters so that their morning surface temperatures matched east-facing flowers received significantly more pollinator visits than nonheated west-facing flowers (Fig. 3, E and F, and fig. S3, F and G), albeit fewer than east-facing flowers.

Thus, temperature directly contributes to, but does not solely determine, the differential attractiveness of east- and west-facing flowers to pollinators. In the future, we will investigate how temperature affects floral physiology and interactions with pollinators.

Circadian oscillators enhance fitness by coordinating physiological processes with predictable changes in the environment (26, 27). Our findings demonstrate that such effects accrue in part through the coordinate regulation of directional growth by environmental response pathways and the circadian oscillator. Such coordination generates the heliotropic movement of young sunflowers, enhancing plant growth, and also leads to the eastward orientation of blooming sunflower disks, promoting a key component of reproductive performance.

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BIOMINERALIZATION

Macromolecular recognition directs calcium ions to coccolith mineralization sites

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Many organisms form elaborate mineralized structures, constituted of highly organized arrangements of crystals and organic macromolecules. The localization of crystals within these structures is presumably determined by the interaction of nucleating macromolecules with the mineral phase. Here we show that, preceding nucleation, a specific interaction between soluble organic molecules and an organic backbone structure directs mineral components to specific sites. This strategy underlies the formation of coccoliths, which are highly ordered arrangements of calcite crystals produced by marine microalgae. On combining the insoluble organic coccolith scaffold with coccolith-associated soluble macromolecules in vitro, we found a massive accretion of calcium ions at the sites where the crystals form in vivo. The in vitro process exhibits profound similarities to the initial stages of coccolith biogenesis in vivo.

Mineralized structures formed by organisms are hybrid materials, characterized by the intimate association of organic macromolecules within and/or around the inorganic phase (1–3). The hierarchical assembly of the organic and inorganic components is accountable for the superior materials properties that biominerals exhibit (4). Soluble organic macromolecules control mineralization by interacting with the developing mineral. Such interactions can affect the morphology of the growing crystal, stabilize a transient amorphous precursor phase, or inhibit precipitation in solution (5–7). The insoluble organic components of biominerals, usually forming scaffold structures, also have been shown to influence crystal nucleation and growth (8–10). These observations have led to the general view that the localization of crystallization is determined by direct interactions between nucleating macromolecules and the developing mineral.

One of the prominent examples demonstrating high degree of control over crystallization are the calcitic scales produced by coccolithophores (11, 12). These ubiquitous marine microalgae, which are the main calcifying phytoplankton, produce complex arrays of calcite crystals, termed coccoliths (13). Each coccolith is formed inside a specialized vesicle and upon completion, it is extruded to the cell surface to form an extracellular shell (Fig. 1, A and B) (11). Coccolith

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biogenesis starts with formation of an organic scale, called the base plate, inside the coccolith vesicle (14, 15). Calcite crystals nucleate on the periphery of the base plate with their crystallographic orientation being precisely controlled (13). Ultrastructural studies on coccoliths have led to the hypothesis that crystal nucleation is mediated by specific chemical moieties at the base-plate periphery (13, 16). The initial simple crystals grow and develop genus-specific, complex morphologies (15, 17). Acidic polysaccharides, which become tightly associated with the mineral phase during its formation, further affect crystal nucleation and growth (7, 18–20) (supplementary text).

We tested the proposed functions of the organic building blocks of coccoliths individually and in a holistic context in vitro. For this, we isolated coccoliths from live *Pleurochrysis carterae* cells using a mild harvesting procedure, preserving the coccolith-associated organic material as close as possible to its native state. *P. carterae* coccoliths consist of two types of morphologically distinct calcite crystals that are placed in an alternating order along the base-plate periphery. Previous characterization of the organic constituents of *Pleurochrysis* coccoliths have shown that the organic base plate is composed of cellulosic fibers and proteins, and that the soluble fraction is dominated by acidic polysaccharides (7, 17, 21–25) (supplementary text).

Atomic force microscopy (AFM) of isolated and dried base plates on a negatively charged mica surface showed a radial array of fibers, characteristic for the bottom side of the base plate (Fig. 1, C and D) (15, 17). When the mica surface was functionalized with positively charged polylysine, however, the base plates adsorbed

Editor's Summary

Searching for the Sun

The growth of immature sunflower plants tracks the Sun's movement. The young plants lean westward as the day progresses but reorient to the east each night. As the flowers mature and open, they settle into a stable east-facing orientation. Atamian *et al.* show how circadian rhythms regulate the east-west elongation of cells in the young plants' stems (see the Perspective by Briggs). They show that eastward-oriented flowers are warmer than westward-oriented flowers, and this warmth attracts pollinators. Auxin signaling pathways in the stem coordinate to fix the eastward orientation of the mature plant.

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