Illuminating the Circadian Clock in Monarch Butterfly Migration
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The present data show uncoupling of leading- and lagging-strand synthesis, implying the continuation of fork opening despite a block in one strand. Uncoupling of simultaneous strand synthesis may occur without disruption of the dimeric Pol III core assembly. When the lesion resides in the lagging strand, a new priming event may enable lagging-strand synthesis to continue, generating a gapped plasmid and a complete double-stranded plasmid molecule from lagging and leading strands, respectively. When the block resides in the leading strand, the lagging-strand Pol III core gets ahead of the leading-strand core, generating a complete double-stranded plasmid from the lagging strand. A partially double-stranded molecule with a single-stranded region (~1 kb) extending from the lesion site to the end of the plasmid is formed from the leading strand. Plasmids of larger size will be required to determine how far DnaB helicase can travel before the whole fork stops. In both orientations, TLS can repair the partially replicated molecule with similar efficiency and a 50-min delay. This delay strongly depends on the chemical nature of the blocking lesion (8). Alternatively, the partially replicated intermediates may be processed by regressed fork formation (10–13). In E. coli and in yeast, genetic data have indicated the bypass of specific lesions to require multiple polymerase switches and specific combinations of TLS polymerases (7, 14–21). The strategy implemented here will be useful to unravel the complex biochemistry of various TLS pathways in vivo, thus providing a powerful complement to in vitro approaches.

References and Notes

4. Materials and methods are available as supporting material on Science Online.
8. V. Pagès, R. P. Fuchs, unpublished data.
23. Supported by CNS and a grant from the Human Frontier Science Program (RG0351/1998-M). We thank V. Gasser for excellent technical support, N. Koffel-Schwartz (UPR 9003) and B. Michel (Institut National de la Recherche Agronomique, Jouy en Jonc).
ferred in phase from each other by 6 hours. When entrained to a light:dark condition that was close to the fall outdoor lighting cycle [lights on from 0700 to 1900 hours, eastern standard time (EST)], migrants exposed to the outdoor Sun oriented significantly to the southwest, toward Mexico, with a mean vector (α) of 233° (n = 14, r = 0.82, P < 0.001) (Fig. 2, upper panel). Migrants entrained to the advanced light:dark cycle (lights on from 0100 to 1300 hours) also oriented significantly, but to the southeast, with α of 118° (n = 18, r = 0.53, P < 0.01) (Fig. 2, middle panel). The direction and magnitude of the orientation difference between the two groups (a counterclockwise shift of 115°; P < 0.001) are those expected for a time-compensated Sun compass that has been advanced by 6 hours (3, 11).

Constant light was used to examine the effects of circadian clock disruption on time-compensated flight orientation. Migrants previously housed under the two light:dark cycles were placed in constant light and evaluated 5 days later. The flight behavior of individuals was still directional in the constant light-exposed migrants. But the orientation direction did not differ between the two light:dark groups of origin (n = 7 in each group; P > 0.05), indicating loss of circadian control. Instead, the combined analysis of the two groups showed highly synchronized flight orientation, with the migrants flying toward the Sun (α = 161°, n = 14, r = 0.73, P < 0.001; Fig. 2, lower panel) (12). These results show that the circadian clock is necessary for time-compensated navigation. The residual orientation in the butterflies exposed to constant light may be a positive phototactic response to the Sun itself.

We next investigated whether monarch navigational and circadian behaviors have differential light requirements. We first examined whether monarch migrants use ultraviolet (UV) light for navigation, given that UV light is used for the navigational activities of other insects (13). This was evaluated in migrants that were placed in the flight simulator outdoors under sunny skies. Once video monitoring indicated that a migrant had initiated continuous flight for at least 1 min, the simulator was covered with a UV-interference filter (which blocked light at wavelengths <394 nm) (14). All of the 13 butterflies that had initiated flight stopped flying completely or flew only intermittently when the filter was applied. Once the filter was removed, 85% (11 out of 13) of the individuals quickly reinitiated continuous directional flight over the next 5-min period of study. Nine of the butterflies that reinitiated directional flight were migrants that had been housed in outdoor lighting conditions and had a mean flight vector on recovery that was similar to that expected for migrants heading to Mexico (α = 212°, n = 9, r = 0.83, P < 0.001) (Fig. 3A) (15). The two remaining animals that flew directionally after removal of the UV block were from the constant-light-exposed group. Each of those butterflies flew directionally only when the UV-interference filter was removed, suggesting that they were indeed orienting toward the Sun (Fig. 3B).

To further examine the importance of UV light for monarch flight behavior, four migrants housed under diurnal lighting were examined indoors where a mercury arc lamp functioned as the light source (4). The purpose of this experiment was to confirm the importance of UV light for monarch flight in an experimental situation in which we could use a UV-pass filter (which blocked light at wavelengths from 428 to 500 nm), in addition to the UV-interference filter. Each animal that we tested continued to fly toward the artificial light filtered through a UV-pass filter, but each also stopped flying when the UV-interference filter was used (Fig. 3C and fig. S1). The combined results of the outdoor and indoor experiments show that a UV photoreceptor is required for sustained flight (16).

In contrast to the importance of UV light for navigation, UV light was not required for photic entrainment of the monarch circadian clock.
This was shown by analyzing the circadian timing of adult eclosion behavior. Newly formed pupae were exposed to one of two light-dark cycles, which differed in phase from each other by 6 hours, in which all light was filtered through the UV-interference filter. Circadian entrainment to the two lighting cycles that lacked UV light was evaluated by placing the pupae in constant darkness as the animals became mature enough to eclose. For each group, animals eclosing in constant darkness did so during the early portion of what would have been the light period of the lighting cycles (Fig. 3D). The mean peak times of eclosion, as monitored for 2 days in constant darkness, differed between the two groups by 5.5 hours (as compared with the 6.0-hour shift of the lighting cycles) (Fig. 3D). The data indicate that the circadian clock was properly entrained by light cycles (Fig. 3D). The data for both groups are plotted relative to EST. Open bars, light; black bars, dark; gray bars, subjective day.

Our results provide insights into time-compensated Sun compass navigation suggests that there are distinct light-input pathways for the stimulation of oriented flight behavior (UV-dependent) and entrainment of the circadian clock (UV-independent). Tracing these pathways into the brain should aid our understanding of the clock-compass interface and further illuminate the mechanisms of monarch butterfly migration.

References and Notes
4. Materials and methods are available as supporting material on Science Online.
5. D. S. Saunders, Insect Clocks (Pergamon, New York, 1982).
10. Outdoor experiments were performed under sunny conditions in Worcester, MA (latitude 42°16' N, longitude 71°49' W) in an outdoor arena between 0900 to 1300 hours EST from 21 September through 21 October 2002.
11. The Sun's azimuth varied from 16° to 22° per hour during the time of day of study, which would result in a 6-hour shift of between 96° and 132°.
12. The azimuth of the Sun during the constant-light study was between 16° and 160°.
14. The UV-interference filter was a long-wavelength pass filter with a photopic luminous transmission of 87% (E400 from Gentex, Carbondale, PA). Transmission values were as follows: 5% ≥ 394 nm, 50% ≥ 398 ± 6 nm, and 80% ≤ 415 nm.
15. We found no significant difference in flight orientation between the first 5 min of continuous flight as compared with those from 10- to 20-min flight records.
16. Based on studies in other migrating insects (13, 18), it is likely that the polarization pattern of skylight is involved in monarch navigation. In fact, the dorsal rim of the monarch butterfly eye contains some ommatidia with orthogonal microvilli (19), an anatomical hallmark of polarized skylight detection (13).
17. Based on studies in Drosophila, the major monarch circadian photoreceptor is likely to be a blue-light-sensing cryptochrome (20), which has been cloned (21).
22. We thank B. J. Frost for instructions in constructing the flight simulators and for helpful discussions, A. Allard for building the simulators, F. Gagnon for sharing his expertise, D. R. Weaver and F. C. Dyer for suggestions, and A. Chavda and K. Misztal for technical assistance.

Supporting Online Material
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Materials and Methods
Figs. S1 and S2
References
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