Potassium Flux and Leaf Movement in *Samanea saman*

II. Phytochrome Controlled Movement

R. L. SATTER, G. T. GEBALLE, and A. W. GALSTON
From the Department of Biology, Yale University, New Haven, Connecticut 06520

**ABSTRACT** Phytochrome, a membrane-localized biliprotein whose conformation is shifted reversibly by brief red or far-red light treatments, interacts with the rhythmic oscillator to regulate leaflet movement and potassium flux in pulvinal motor cells of *Samanea*. Darkened pinnae exposed briefly to red light (high \( P_{fr} \) level) have less potassium in motor cells in the extensor region, more potassium in motor cells in the flexor region, and smaller angles than those exposed to far-red light (low \( P_{fr} \) level). Increase in temperature from 24° to 37° increases the differential effect of the light treatments during opening (the energetic phase) but not during closure, implying that phytochrome controls an energetic process. It seems likely that phytochrome interacts with rhythmically controlled potassium pumps in flexor and extensor cells. During nyctinastic closure of white-illuminated pinnae, exposure to far-red light before darkening results in larger angles than does exposure to red. As in rhythmic opening, the angles of all pinnae and the differential effect of the light treatments increases with increasing temperature.

**INTRODUCTION**

*Samanea* leaflets, usually horizontal (open) in the light and vertical (closed) in the dark, oscillate between these two positions with a circadian rhythm during prolonged darkness (18). Both nyctinastic and rhythmic movement can be altered by brief irradiation with red (R) or far-red (FR) light (11, 32). The effects potentiated by R are fully reversible by subsequent FR and vice versa, indicating photoreception by phytochrome.

Leaflet movement in *Samanea* is controlled by turgor changes in pulvinal motor cells, and these turgor changes appear to be a consequence of \( K^+ \) flux through motor cell membranes. Rhythmic and white light-promoted changes in the distribution of \( K^+ \) ions in the pulvinus were described in an earlier paper (28). In the present investigation, we focus on the interplay of endogenous rhythms and phytochrome, which also exert joint control of leaf movements in *Phaseolus* (2), *Mimosa* (9), and *Albizia*, and \( K^+ \) flux in the latter
(27, 29), enzyme activity in Chenopodium (10), CO₂ output in Lemna (13), dormancy in many woody species (35), and floral initiation in photoperiod-sensitive plants (6, 8, 14, 16).

Phytochrome binds to cell membranes (17, 19, 23) and alters properties of natural (15, 20, 25-27, 29, 30, 33, 34) and model (22) membranes; recent evidence also suggests that changes in membrane properties play a central role in the operation of the biological clock (3, 7, 12, 28, 31). Changes in the Pf, level induce phase shifts in endogenous rhythms in Lemna (13) and Phaseolus (1, 2) implying that phytochrome interacts with the rhythmic oscillator in these plants. It therefore appears that studies of joint rhythmic and phytochrome control of a membrane-localized phenomenon such as K⁺ flux could reveal how these regulatory systems interact at the molecular level.

MATERIALS AND METHODS

Plant material, growing conditions, experimental techniques, and the electron microprobe (Acton Laboratories, Inc., Acton, Mass.) used for in situ K⁺ analyses have already been described (28). Light sources used to convert phytochrome to Pfr (4-min exposure, 1,200 ergs-cm⁻²-s⁻¹ at 600-690 nm) and Pfr (1.5-min exposure, 9,000 ergs-cm⁻²-s⁻¹ at 710-760 nm) have also been described (29).

RESULTS

Effect of R and FR Preirradiation on the Rhythmic Opening and Closure of Darkened Pinnae

Samanea plants grown under 16 h light-8 h dark cycles and transferred to continuous darkness, begin to open rhythmically at hours 7-8 of the dark period, remain open for 10-12 h, then begin rhythmic closure. To test the effects of the Pf, level on both opening and closure, we excised pairs of closed pinnae at hour 8 and open pinnae at hour 18, separated the paired members, removed laminar tissue (see Fig. 3 of [28]), briefly exposed one member to R (high Pf, level) and the other to FR (low Pf, level), then returned both to darkness at 24° or 37°.

Pinnae responded to the light treatments even though laminar tissue had been removed, indicating the presence of the phytochrome photoreceptor in the pulvinus or attached section of rachilla. R preirradiated pinnae opened less and closed more rapidly than those preirradiated with FR (Table I). Differences in the effects of R and FR on opening (an energetic process) were enhanced by high temperature, but the differential effects of the light treatments on closure were temperature independent. These data suggest that Pf, controls an energetic process an Samanea, as it appears to do in Albizzia (24).

The Pf, level of plant tissue decreases in the dark, presumably through enzymatic destruction of Pf, thermal reversion of Pf, to Pf, or both (5). The rate
of decrease varies with plant and experimental conditions; for example, the half-life of $P_f$, in etiolated maize is 75 min (5), while in light-grown cauliflower, it is 5 h (4). In our experiments, $P_f$ appears to be relatively stable during the first 8 h of the dark period, since pinnae behaved similarly (i.e., opened to the same angle) whether irradiated with R at the beginning of the dark period or 8 h later.

**K+ Redistribution During Rhythmic Opening of Pinnae Preirradiated with FR**

A closed pulvinus excised at hour 5 of the dark period was prepared for microprobe analysis of K+ in dorsi-ventral longitudinal sections. Its paired member was left on the plant and at hour 7, just before rhythmic opening, the plant was given a brief exposure to FR; 5 h later, when the pulvinus had opened to 120°, it was excised and similarly prepared for microprobe analysis.

As Fig. 1 reveals, K+ is high in the ventral motor tissue and low in the dorsal motor tissue of closed pulvini. Opening was accompanied by a large increase in the K+ content of the dorsal motor cells, and a small decrease in the K+ content of the ventral motor cells. The midcortex of both open and closed pulvini has a higher K+ content and less of a dorsi-ventral gradient than the motor region, consistent with data from previous experiments (28).

Another darkened pulvinus that had also opened to 120° after brief exposure to FR was excised and prepared for microprobe analysis of transverse sections (Fig. 2). K+ is maximal in the midextensor region and minimal in the midflexor region. The ratio of K+ in midextensor to K+ in midflexor is

---

1 Extensor cells expand during opening and contract during closure, while flexor cells act in the reverse manner but change shape more than size. The line separating extensor and flexor regions is displaced approximately 30° from that separating dorsal and ventral regions in transverse sections (26).
FIGURE 1. K⁺ distribution in longitudinal, dorsi-ventral sections of a darkened closed pulvinus (top) and one that opened 120° in the dark 5 h after brief exposure to FR (bottom). K was analyzed in the circled regions with an Acton electron microprobe. The measured regions were in the motor tissue (outer cortex, 30 μm from the epidermis), and in the midcortex (100 and 225 μm from the epidermis). Measured value are scintillations during 15 s. Each datum is an average of 24 measurements. The average standard deviation is 22%. There is no evidence of a longitudinal gradient.

6.5 while the ratio of K⁺ in middorsal to K⁺ in midventral is less than 2. Thus sections that do not indicate the flexor-extensor gradient (for example, Fig. 1) do not always reveal the full extent of K⁺ flux accompanying pinna movement.

Plant material of the same size, age, and physiological behavior was used for Fig. 2 and for Figs. 6–9 of (28). Microprobe analysis techniques were also similar; thus, data from these two papers can be directly compared. The K⁺ distribution in a closed, darkened pulvinus is shown in Fig. 6 of (28); Fig. 2 reveals changes in K⁺ during opening after conversion of Pr to Pf. Opening is accompanied by a large increase in K⁺ in the extensor region and decrease in K⁺ in the flexor region.

Fig. 7 of (28) reveals the distribution of K⁺ ions in a pulvinus that opened in white light. K⁺ is high and constant throughout the extensor region, and low and constant throughout the flexor region. Although the angle of opening and pattern of K⁺ distribution are generally similar to that of a FR pretreated pulvinus that opened in darkness (Fig. 2), there are two important differences: (a) FR pretreated dark pulvini have very high K⁺ values in a narrow portion of the extensor region; K⁺ is 30% higher under these condi-
Satter et al. potassium flux and leaf movement in Samanea saman

Figure 2. K⁺ distribution in a transverse section of a pulvinus that opened 120° in the dark 5 h after brief exposure to FR. K⁺ was analyzed with an electron microprobe, 30 μm from the epidermis (circled regions of Fig. 5 [28]). Measured values are scintillations during 15 s. The abscissa indicates circumferential distance from a middorsal point.

Figure 2 shows the distribution of K⁺ ions in a darkened, rhythmically open pulvinus. These plants were exposed to cool white fluorescent light (high Pf, level) before darkness. Thus, comparison with Fig. 2 (Pf, level is low) reveals differences in the K⁺ distribution attributable to differences in the phytochrome state. It appears that Pf, reduces K⁺ in the extensor cells and increases K⁺ in the flexor cells, i.e. Pf, inhibits or acts to antagonize the K⁺ fluxes that presumably promote pinna opening.

Pf, control of K⁺ flux was also tested in another experiment with excised pinnae (Figs. 3–5). Closed paired pinnae were excised and separated at hour...
Figures 3–5. The K+ distribution in transverse sections of paired darkened excised pulvini that opened 40° after brief exposure to R (Fig. 3) or 90° after exposure to FR (Fig. 4). Pinnae excised at hour 8 of the dark period were incubated at 37°C for 2.5 h. K+ was analyzed with an electron microprobe, 30 μm from the epidermis. Measured values are scintillations during 15 s. The broken line in Fig. 3 indicates a region of instability. The abscissa indicates circumferential distance from a middorsal point. Figs. 3 and 4 are superimposed in Fig. 5.

8 of the dark period; one member was exposed briefly to R, the other to FR, and both were returned to darkness and incubated at 37°C. Two and one-half h later, when the R-treated pinna had opened to 40° and the FR-treated pinna to 90°, pulvini were prepared for microprobe analysis of transverse sections. It is apparent that the light treatments are responsible for major differences in the distribution of K+ ions in the extensor and flexor cells of both excised and intact pinnae.

*Samanea* pinnae move in two planes, i.e., dorsi-ventral (toward and away from the rachis) and inward-outward (toward and away from each other).
If K⁺ fluxes are the basis for pinna movement, as our previous Albizzia and Samanea experiments suggest (25–29), then phytochrome photoconversion should affect movements in both planes. Data presented on pinna movement (Table I and Fig. 6) are based on measurement of the angle between the pinna and rachis, and thus reveal only movement in the dorsi-ventral plane. Additional experiments are required to analyze movement in the other plane.

**Effect of P_{fr} on Nyctinastic Closure**

We excised paired pinnae soon after they had opened in white light, then exposed one member to R and the other to FR before incubating them in the dark at 24° or 37°. Fig. 6 shows that P_{fr} promotes nyctinastic pinna closure in Samanea, as well as pinnule closure in Samanea (32) and Albizzia (2). Pinnae
DISCUSSION

Nyctinastic and Rhythmic Movement

Although leaflet angle increases during rhythmic opening and decreases during nyctinastic closure, there are similarities in the effect of $P_{fr}$ on each type of movement (Table I and Fig. 6). In both cases: (a) Pinnae exposed to $R$ have smaller angles than those exposed to $FR$. (b) The angles of $FR$-treated leaflets are larger at $37^\circ$ than at $24^\circ$, and the differential effect of the $R$ and $FR$ treatments also increases with temperature. (c) Phytochrome photoconversions have more of an effect on rhythmic movement early in the open phase (hours 8–11) than 10 h later (Table I). Sweet and Hillman similarly found (32) that $R$ and $FR$ treatments have a greater effect on nycti-
Phytochrome and Rhythms

In earlier papers we proposed that rhythmic leaflet movement in *Albizzia* (24, 26, 27) and *Samanea* (28) is due to rhythmic changes in the properties of motor cell membranes. The open period is characterized by active transport of K⁺ ions into extensor cells and possibly out of flexor cells, while the closed period is characterized by K⁺ diffusion away from a high K⁺ area in the middle of the flexor region. Rhythmic leaflet movement may thus result from rhythmic change in active potassium transport, potassium leakage, or both.

Phytochrome interacts with the rhythm to control leaflet movement and the K⁺ flux that is presumably responsible for such movement. Reduction in the $P_f$ level by FR increases the K⁺ content of the extensor region, decreases that of the flexor region, and increases pinna angle (Figs. 1-5). The effect of $P_f$ on K⁺ flux provides an osmotic explanation for its effect on motor cell turgor in all our experiments. Nevertheless, other mechanisms whereby phytochrome might influence leaflet movement, for example by altering permeability to water as reported for *Mougeotia* (34), should also be investigated.

We have considered two possible explanations for joint rhythmic and
phytochrome control of the K+ flux which appears to underlie leaflet movement. In the first, phytochrome and the rhythm affect K+ movement at different loci on motor cell membranes. This hypothesis was incorporated into a model that correlates leaflet movement with K+ flux in *Albizzia* (29). It envisions two types of K+ pumps in the ventral motor cells: rhythmically controlled pumps, unaffected by phytochrome photoconversion, that transport K+ ions to the cellular interior, and Pfr-dependent pumps that transport K+ ions in the opposite direction. We further proposed that the effect of Pfr on the K+ content of the dorsal cells was an indirect consequence of the phytochrome state in that a high Pfr level reduced the K+ content of the ventral motor cells, which in turn increased the size of the K+ pool and thus the K+ content of the dorsal motor cells. The experiments which led to this hypothesis indicated that Pfr has a large depressive effect on the K+ content of the ventral motor cells and a minor promotive effect on the K+ content of the dorsal motor cells. Longitudinal dorsi-ventral sections were used in all of our *Albizzia* experiments, but the K+ analyses presented in this paper reveal that such sections do not indicate the full magnitude of the potassium flux. Our *Samanea* data reveal that phytochrome exerts its major effect on K+ in the middle of the extensor and flexor regions rather than the middle of the dorsal and ventral regions. Furthermore, *Samanea* experiments indicate that the Pfr level affects K+ distribution throughout the pulvinus. These data have led us to favor an explanation in which phytochrome and the rhythm affect K+ flux at the same locus, i.e., either at an active pump or at a site of K+ diffusion through the membrane. We consider the pump the more likely site, since the light treatments have a greater effect on the energy-requiring opening phase than on the nonenergetic closure phase. Also, the differential effects of R and FR increase with temperature, as would be expected for an ATP-dependent process.

It is clear that additional experiments are required to test the validity of the theories described above. Hopefully, techniques recently developed for isolating membrane-bound phytochrome from etiolated tissue (17, 19) can be adapted to green plants, to permit in vitro tests of phytochrome-rhythmic interactions.

Dr. Galston was supported in part by National Science Foundation grant no. GB 22613.

Received for publication 4 February 1974.

REFERENCES

1. Büning, E., and L. Lörcher, 1957. Regulierung und Auslösung endogen-tagesperiodischer Blattbewegungen durch verschiedene Lichtqualitäten. *Naturwissenschaften*. 44:472.
2. Bünning, E., and I. Moser. 1966. Response-Kurven bei der circadianen Rhythmik von Phaseolus. Planta (Berl.). 69:101.
3. Bünning, E., and I. Moser. 1972. Influence of valinomycin on circadian leaf movements of Phaseolus. Proc. Natl. Acad. Sci. U.S.A. 69:2732.
4. Butler, W. L., and H. C. Lane. 1965. Dark transformations of phytochrome in vivo. II. Plant Physiol. 40:13.
5. Butler, W. L., H. C. Lane, and H. W. Siegelman. 1963. Nonphotochemical transformations of phytochrome in vivo. Plant Physiol. 38:514.
6. Cumming, B. G., S. B. Hendricks, and H. A. Borthwick. 1965. Rhythmic flowering responses and phytochrome changes in a selection of Chenopodium rubrum. Can. J. Bot. 43:825.
7. Engelmann, W. 1972. Lithium slows down the Kalanchoe clock. Z. Naturforsch. Teil B. 27:477.
8. Evans, L. T. 1969. The Induction of Flowering: Some Case Histories. Cornell University Press, Ithaca, N. Y.
9. Fondéville, J. C., H. A. Borthwick, and S. B. Hendricks. 1966. Leaflet movements of Mimosa pudica L. indicative of phytochrome action. Planta (Berl.). 69:357.
10. Frosch, S., and E. Wagner. 1973. Endogenous rhythmicity and energy transduction. II. Phytochrome action and the conditioning of rhythmicity of adenylate kinase, NAD- and NADP-linked glyceraldehyde-3-phosphate dehydrogenase in Chenopodium rubrum by temperature and light intensity cycles during germination. Can. J. Bot. 51:1521.
11. Galston, A. W., R. L. Satter, J. J. Keirns, J. Freeman, M. W. Bitesney, P. B. Applewhite, and Y. S. Bau. 1973. Physiology of pinna movement in Samanea saman. Plant Physiol. 51 (Suppl.):16.
12. Halaban, R., and W. S. Hillman. 1970. Response of Lemna perpusilla to periodic transfer to distilled water. Plant Physiol. 46:641.
13. Hillman, W. S. 1971. Entrainment of Lemna CO₂ output through phytochrome. Plant Physiol. 46:770.
14. Hillman, W. S. 1973. Light, time and the signals of the year. Bioscience. 23:81.
15. Jaffe, M. J. 1968. Phytochrome-mediated bioelectric potentials in mung bean seedlings. Science (Wash. D. C.). 162:1016.
16. King, R. W., and B. G. Cumming. 1972. The role of phytochrome in photoperiodic time measurement and its relation to rhythmic time-keeping in the control of flowering in Chenopodium rubrum. Planta (Berl.). 108:39.
17. Marmé, D., J. Boisard, and W. R. Briggs. 1973. In vitro binding properties of phytochrome to a membrane fraction. Proc. Natl. Acad. Sci. U.S.A. 70:3861.
18. Palmer, J. H., and G. F. Asprey. 1958. Studies in the nyctinastic movement of the leaf pinnae of Samanea saman (Jacq.) Merrill. I. A general description of the effect of light on the nyctinastic rhythm. Planta (Berl.). 51:757.
19. Quail, P. H., D. Marmé, and E. Schäfer. 1973. Particle-bound phytochrome from maize and pumpkin. Nat. New Biol. 245:189.
20. Racusen, R., and K. Miller. 1972. Phytochrome-induced adhesion of mung bean root tip to platinum electrodes in a direct current field. Plant Physiol. 49:654.
21. Raschke, K., and M. F. Fellows. 1971. Stomatal movement in Zea mays: Shuttle of potassium and chloride between guard cells and subsidiary cells. Planta (Berl.). 101:296.
22. Roux, S., and J. Younabédé. 1973. Phoretive conductance changes induced by phytochrome in model lipid membranes. Proc. Natl. Acad. Sci. U.S.A. 70:762.
23. Rubenstein, B., K. S. Dehry, and R. B. Park. 1969. Evidence for bound phytochrome in oat seedlings. Plant Physiol. 44:105.
24. Satter, R. L., P. B. Applewhite, D. J. Kreis, Jr., and A. W. Galston. 1973. Rhythmic leaflet movement in Albizzia julibrissin: Effect of electrolytes and temperature alteration. Plant Physiol. 52:202.
25. Satter, R. L., and A. W. Galston. 1971a. Potassium flux: a common feature of Albizzia leaflet movement controlled by phytochrome or endogenous rhythm. Science (Wash. D. C.). 174:518.
26. Satter, R. L., and A. W. Galston. 1971b. Phytochrome controlled nyctinasty in Albizzia
III. Interaction between an endogenous rhythm and phytochrome in control of potassium flux and leaflet movement. Plant Physiol. 48:740.

27. SATTER, R. L., and A. W. GALSTON. 1973. Leaf movements: rosetta stone of plant behavior? Bioscience. 23:407.

28. SATTER, R. L., G. T. GEBALLE, P. B. APPLEWHITE, and A. W. GALSTON. 1973. Potassium flux and leaf movement in Samanea saman. I. Rhythmic movement. J. Gen. Physiol. 64:413.

29. SATTER, R. L., P. MARINOFF, and A. W. GALSTON. 1970. Phytochrome controlled nyctinasty in Albizia julibrissin. II. Potassium flux as a basis for leaflet movement. Am. J. Bot. 57:916.

30. SCHÄFER, E., and W. SCHMIDT. 1974. The temperature dependence of phytochrome dark reactions. Planta (Berl.). In press.

31. Sweeney, B. M. 1974. The potassium content of Gonyaulax polyedra and phase changes in the circadian rhythm of stimulated bioluminescence by short exposures to ethanol and valinomycin. Plant Physiol. 53:337.

32. SWEET, H. G., and W. S. HILLMAN. 1969. Phytochrome control of nyctinasty in Samanea as modified by oxygen, submergence, and chemicals. Physiol. Plant. 22:776.

33. Tanada, T. 1967. A rapid photoreversible response of barley root tips in the presence of 3-indoleacetic acid. Proc. Natl. Acad. Sci. U.S.A. 59:376.

34. Weisenseel, M. H., and E. SMEIBL. 1973. Phytochrome controls the water permeability in Mougeotia. Z. Pflanzenphysiol. 70:420.

35. Williams, B. J., Jr., N. E. Pellett, and R. M. Klein. 1972. Phytochrome control of growth cessation and initiation of cold acclimation in selected woody plants. Plant Physiol. 50:262.