Potassium Flux and Leaf Movement in *Samanea saman*

II. *Phytochrome Controlled Movement*

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**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 *Albizzia*, 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 Pₕ (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 in Samanea, as it appears to do in Albizia (24).

The Pf level of plant tissue decreases in the dark, presumably through enzymatic destruction of Pfr, thermal reversion of Pfr to Pₕ, or both (5). The rate
TABLE I
RHYTHMIC MOVEMENT OF DARKENED, EXCISED PINNAE THAT WERE EXPOSED TO BRIEF R AND FR FOLLOWED BY INCUBATION AT 24° OR 37°

<table>
<thead>
<tr>
<th>(A) Rhythmic opening. Closed (0°) pinnae were excised at hour 8.</th>
<th>Temperature</th>
<th>24°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>FR</td>
<td>R</td>
</tr>
<tr>
<td>Angle (hour 11)</td>
<td>10°</td>
<td>28°</td>
<td>8°</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(B) Rhythmic closure. Open (60°) pinnae were excised at hour 18.</th>
<th>Temperature</th>
<th>24°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>FR</td>
<td>R</td>
</tr>
<tr>
<td>Angle (hour 20)</td>
<td>23°</td>
<td>33°</td>
<td>41°</td>
</tr>
</tbody>
</table>

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\(^1\) 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 values 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.

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 $P_f$ to $P_r$. 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-
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.

...than when pinnae are in white light. (b) The regions of both low K⁺ and high K⁺ are broad and separated by only a few cell diameters in white-irradiated pulvini, but are narrow and separated by a broad region of monotonically changing K⁺ values in FR pretreated dark pulvini.

Fig. 8 of (28) shows the distribution of K⁺ ions in a darkened, rhythmically open pulvinus. These plants were exposed to cool white fluorescent light (high $P_r$ level) before darkness. Thus, comparison with Fig. 2 ($P_r$ level is low) reveals differences in the K⁺ distribution attributable to differences in the phytochrome state. It appears that $P_r$ reduces K⁺ in the extensor cells and increases K⁺ in the flexor cells, i.e. $P_r$ inhibits or acts to antagonize the K⁺ fluxes that presumably promote pinna opening.

$P_r$ control of K⁺ flux was also tested in another experiment with excised pinnae (Figs. 3–5). Closed paired pinnae were excised and separated...
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°. 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).

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**Figure 3.**

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° 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.
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
close more rapidly at 24° than at 37°, but \( P_r \) has less of an effect at the lower temperature.

**DISCUSSION**

*Nyctinastic and Rhythmic Movement*

Although leaflet angle increases during rhythmic opening and decreases during nyctinastic closure, there are similarities in the effect of \( P_r \) 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° than at 24°, 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-
nastic closure, the shorter the predark light period. These similarities in \( P_r \) control of leaflet movement imply similarities in \( P_r \) control of \( K^+ \) flux. \textit{Albizzia} resembles \textit{Samanea} in all these respects (24, 26, 29).

**Interaction of Phytochrome and Rhythms**

In earlier papers we proposed that rhythmic leaflet movement in \textit{Albizzia} (24, 26, 27) and \textit{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 \( P_r \) 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 \( P_r \) 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 \textit{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 Albizia (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 Pf₀-dependent pumps that transport K⁺ ions in the opposite direction. We further proposed that the effect of Pf₀ on the K⁺ content of the dorsal cells was an indirect consequence of the phytochrome state in that a high Pf₀ 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 Pf₀ 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 Albizia 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 Pf₀ 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


2. Ventral and dorsal cells have opposite roles in the movement of Albizia pinnules and Samanea pinnæ, since the former move upward and the latter downward during closure.
Satter et al. Potassium Flux and Leaf Movement in Samanea saman


