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INVITED REVIEW

LIGHT-REGULATED LEAF MOVEMENT AND SIGNAL TRANSDUCTION IN NYCTINASTIC PLANTS

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Abstract – Leaf movements in nyctinastic plants are produced by changes in the turgor of extensor and flexor cells, collectively called motor cells, in opposing regions of the leaf movement organ, the pulvinus. In *Samanea saman*, a tropical tree of the legume family, extensor cells shrink and flexor cells swell to bend the pulvinus and fold the leaf at night, whereas extensor cells swell and flexor cells shrink to straighten the pulvinus and extend the leaf in the daytime. These changes are caused by ion fluxes primarily of potassium and chloride, across the plasma membrane of the motor cells. These ion fluxes are regulated by exogenous light signals and an endogenous biolgical clock. Inward-directed K^+ channels are closed in extensor and open in flexor cells in the dark period, while these channels are open in extensor and closed in flexor cells in the light period. Blue light opens the closed K^+ channels in extensor and closes the open them in flexor cells during darkness. Illumination of red light followed by darkness induces to open the closed K^+ channels in flexor and to close the open K^+ channels in extensor cells in the light. The dynamics of K^+ channels in motor cells that are controlled by light signals are consistent with the behavior of the pulvini in intact plants. Therefore, these cell types are an attractive model system to elucidate regulations of ion transports and their signal transduction pathways in plants. This review is focused on light-controlled ion movements and regulatory mechanisms involved in phosphoinositide signaling in leaf movements in nyctinastic plants.

LEAF MOVEMENT

Plants live in a variable environment. In the wild, light, CO_2, O_2, temperature, humidity, and wind are changed from time to time and appeared frequently in unbalanced mixtures for optimal growth. Plant responses, including leaf movement, growth, and development are affected by these environmental variables. In addition to these external variables, internal cues, for example the biological clock, also regulate leaf movement, growth, and development in plants. While external and internal signals are very important for plants, the mechanisms of signal transduction that lead to plant responses are not well known.

Most plants are usually considered as fixed in time and space. However, some plants show leaves that change their orientation in response to external and internal cues. For examples, leaves of *Dinaea muscipula* close when they are burnished by insects. Leaves of *Mimosa pudica* begin to fold together (close) within a couple of second after they are touched. Leaves in nyctinastic (night closure) plants are generally horizontal in the light, and fold together in the darkness[1]. Studies of leaf movements in nyctinastic plants provide us important clues for understanding and characterizing light- and the biological clock-regulatory mechanisms in plants.

As noted above, leaves of nyctinastic plants, for example *Samanea saman*, are generally open (in a horizontal orientation) in the light and closed (in a vertical orientation) in the darkness. They oscillate between these two positions at approximately 24 hour intervals under constant environmental conditions; such movements are called circadian (circa = about + dien = a day) and are driven by a biological clock[2]. Leaf movements are affected by changes in the pulvinus at the base of each leaf stalk. Movements are controlled by reversible changes in the size and shape of cells in opposing regions of the pulvinus. The pulvinus has a cylindrical shape when leaves are open and a U shape when leaves are closed. A cross section of the *S. saman* pulvinus shows two kinds of functional cortical cells, extensor (lower side) and flexor (upper side) cells, collectively called motor cells, separated by vascular bundle which is not involved in leaf movement.

Leaf movement of the tropical legume *Samanea saman* (a 16-h light; 8-h dark cycle) is an attractive model system to study the regulation of ion movements and its signal transduction pathway in plants, because massive ion fluxes occur across the plasma membrane and the ion fluxes are regulated by both external light

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† *Abbreviations:* IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; BL, blue light; WL, white light; RL, red light

signals and the internal biological clock[1]. Light and the biological clock send signals to the motor organ, the pulvinus, that lead to regulation of the activities of ion transporters at the plasma membrane, regulating in leaf movement of *S. saman*. Increased turgor occurs in extensor cells during leaf opening and decreased turgor occurs during closure, while turgor in flexor cells changes in the opposite manner. These changes in turgor are caused by ion fluxes across the plasma membrane, primarily of potassium and chloride[1]. These fluxes are regulated, as indicated above, by both exogenous light signals and an endogenous circadian clock[1,3].

ION MOVEMENT IN THE EXTENSOR AND FLEXOR CELLS

Transport of K^+ plays a crucial role in the regulation of volume and turgor of plant cells[4,5]. In legumes such as *S. saman* and *Phaseolus*, leaf movements depend upon differential changes in the volumes of cells in the pulvinus. Leaf movements correlate with massive migrations of K^+ and Cl^- across the plasma membranes of extensor and flexor cells. K^+ and Cl^- change 15 fold and 20 fold when leaves oscillate between the open and closed states, respectively[1]. Extensor cells take up K^+, Cl^-, and water and swell, while flexor cells lose K^+, Cl^-, and water and shrink after exposure to white light (WL) † or blue light (BL) during the dark period. In contrast, flexor cells take up K^+, Cl^-, and water and swell but extensor cells lose K^+, Cl^-, and water and shrink after exposure to red light (RL) followed by darkness during the light period[6]. Similarly, during circadian rhythmic leaf movement, K^+, Cl^-, and water are taken up alternatively and rhythmically by extensor and flexor cells[7].

Potassium channels have been described in cells involved in turgor-mediated movements. Voltage-gated K^+ channels activated by membrane depolarization have been observed in patch-clamp studies of plasma membrane of *S. saman* protoplasts[8,9], and of guard cell protoplasts[10-12]. These channels might serve as the pathway for K^+ efflux from shrinking cells. Hyperpolarization-activated K^+ channels have been reported in guard cell protoplasts[11,12] and in *S. saman* protoplasts[13]. These channels might serve as a pathway for K^+ influx into swelling cells. Passive K^+ diffusion through channels argues for the prior favorable electrochemical gradients for K^+. In stomatal guard cells, H^+ secretion provides the driving force for K^+ uptake[14] as is also proposed for pulvinar cells[15]. Cl^- efflux and the resulting depolarization may provide the driving force for K^+ efflux[16]. Cytoplasmic free Ca^{2+} concentration may regulate channel conductances[17,18].

LIGHT/CLOCK INTERACTION IN LEAF MOVEMENT

In constant darkness or constant light, leaves alternatively open and close with a circadian periodicity. The phase of the biological clock is maintained endogenously but can be reset or shifted by exogenous signals such as light. We can consider two different aspects of light/clock interactions, the biological clock directs responses to light; light also resets or phase-shifts the clock. Light resets the circadian clock through a synchronization pathway, and the circadian clock controls the sensitivity of the photoreceptors. Light and biological clock regulate ion movements through unknown signal transduction pathways and, in turn, regulate leaf movement of *S. saman*[19] (Fig. 1).

BL absorbed by BL receptors promotes the swelling of extensor cells and the shrinking of flexor cells and results in leaf opening[20]. A brief pulse of RL followed

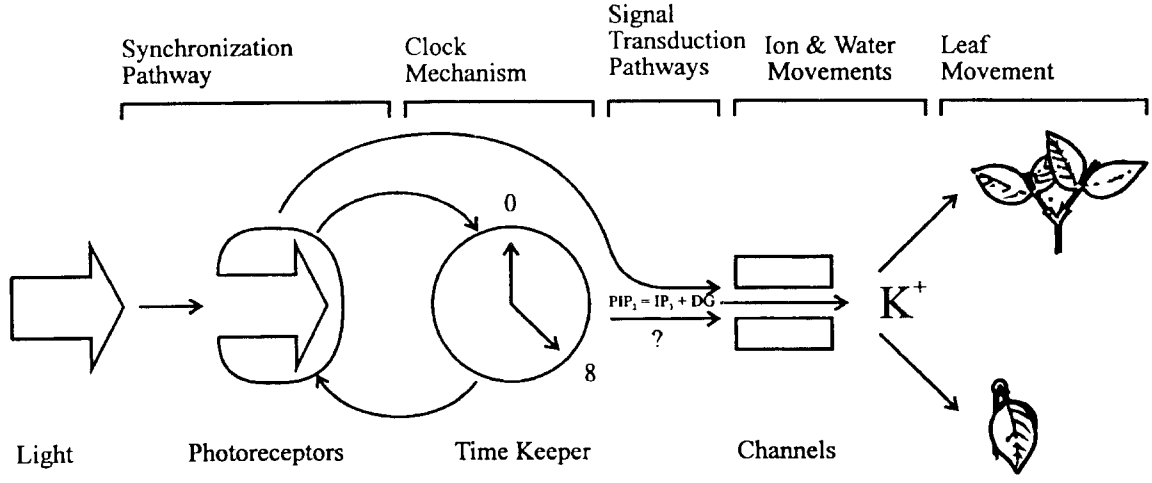

Figure 1. A hypothetical pathway of light/biological clock regulated leaf movement of *S. saman*. The clock protein (Time keeper) is phased to the light through a synchronization pathway, and it controls the state of ion channels by a signal transduction pathway, leading to opening or closing of leaves[19].

by darkness promotes swelling of flexor cells and shrinking of extensor cells, leading to enhanced, dark-induced leaf closure[21]. The enhancement of dark-induced closure potentiated by RL is reversible by immediate treatment with far-red light, implicating phytochrome as a RL receptor involved in the leaf closure[21]. As described earlier, leaf movement is correlated with K^+ redistribution. BL promotes K^+ uptake by extensor cells, while RL followed by darkness promotes K^+ uptake by flexor cells[1,3]. The activities of the K^+ fluxes in extensor and flexor cells are modulated differently by light and biological clock.

If the biological clock regulates leaf movements, it might be expected to affect the activity of K^+ transport in extensor and flexor cells of *S. saman*. Kim *et al.*[22] recently reported that rhythms in extensor and flexor cells were one-half of a cycle out of phase with each other; at hours 4 to 8 after transfer to darkness, K^+ channels were open in flexor but closed in extensor cells, whereas at hours 16 to 21 after transfer to darkness channels were open in extensor but closed in flexor. The results are consistent with the K^+ fluxes that are expected on the basis of leaf movement of intact plants in constant darkness. Changes of K^+ channel states are involved in the biological clock-regulated leaf movement of *S. saman*. How does the clock regulate the states of K^+ channels in both extensor and flexor cells? the regulatory mechanism in leaf movements still remains unknown.

LIGHT- AND DARK-REGULATED K^+ CHANNELS

During darkness pulvinus straighten when irradiated with BL or WL, although RL has no effect. Pulvinus in the light bend when treated RL followed by transfer to darkness. Extensor cells take up K^+, Cl^-, and water and swell but flexor cells lose them and shrink after exposure to WL or BL, whereas flexor cells take up K^+, Cl^-, and water and swell but extensor cells lose them and shrink after exposure to RL followed by darkness[4,6].

Initially, inward-directed K^+ channels were open in flexor protoplasts and closed in extensor protoplasts in the dark period (at hours 4 to 8 after transfer to darkness). BL or WL caused K^+ channels to close in flexor and to open in extensor protoplasts, but RL had no effect[23]. Changes of the K^+ channels states in both cells are consistent with the K^+ fluxes that are expected on leaf movement of intact plants; BL or WL evokes that extensor cells are swollen from the uptake of K^+ and flexor cells are shrunken from the release of K^+, resulting leaf opening.

In the light period (at hours 10 to 12 after transfer to light), K^+ channels were open in extensor and closed in flexor protoplasts. RL followed by transfer to darkness induced K^+ channel opening in flexor and closure in extensor protoplasts; BL had no effect[22]. Interestingly, transfer to darkness without previous irradiation with RL was sufficient to close the channels in extensor protoplasts but did not open the channels in flexor protoplasts[22]. These are also consistent with the K^+ fluxes that are expected on the basis of leaf movement of intact plants[21,24] and the finding that apoplastic K^+ is decreased in flexor region and increased in extensor region of the pulvinus after RL followed by darkness[6].

In summary, BL or WL closes the open K^+ channels in flexor and opens the closed K^+ channels in extensor cells and then elicits leaf opening during dark period. RL followed by darkness opens the closed K^+ channels in flexor and closes the open K^+ channels in extensor cells and then induces leaf closing during light period. Therefore, the dynamics of K^+ channel states in extensor and flexor cells that are light-sensitive are consistent with the behavior of pulvini in intact plants.

How does the same light signal control the K^+ channel states in opposite ways? Kim *et al.*[22] found that K^+ channels in extensor protoplasts were regulated by BL receptor rather than by phytochrome, wheras K^+ channels in flexor protoplasts were alternately response to the BL receptor and to phytochrome. As indicated above, the circadian clock may determine which photoreceptor is coupled to the K^+ channels in flexor cells (Table 1).

LIGHT PERCEPTION : PHYTOCHROME AND BLUE LIGHT RECEPTORS

The red light receptor, phytochrome, is a photochromic pigment that consists of a linear tetrapyrrole chromophore covalently attached via a thioether linkage to cysteine-321 on the apoprotein[25]. Phytochrome exists in two forms: Pr, RL absorbing form and Pfr, the far-red light absorbing form; these forms are photorevesible with RL or far-red light, respectively. Pfr is considered the physiologically active form of phytochrome leading to a wide variety of molecular, cellular, and developmental responses in plants[26, 27]. The photoconversions of phytochrome, Pr $\rightarrow$ Pfr and Pfr $\rightarrow$ Pr, are accomplished through a series of photochemical intermediates[27-29].

Phytochrome is associated with chloroplasts, mitochondria, nuclei, the plasma membrane, and the endoplasmic reticulum[30]. Phytochrome in the Pr form is distributed uniformly through out the cytosol as a soluble protein. Following photoconversion to Pfr by RL, some of the phytochrome within the cell associates with membrane, but the nature of this association has not yet identified. Determining the localization of phytochrome is important for understanding to biological functions, which play roles in many different subcellular compartments.

The most important unsolved question about phytochrome functions is how signal is transduced from Pfr form of phytochrome. Partner compounds that receive

Table 1. The effects of light and pharmacological agents on the states of K^+ channels in extensor and flexor cells.[a] K^+ channel states were measured at the two different time points(*, in the dark period; $^+$, in the light period). Abbreviations: D, darkness; BL, blue light; RL red light; ND, not done.

Treatment	Extensor Cells		Flexor Cells	
	K^+ channel	cell state or drug effect	K^+ channel	cell state or drug effect
*D	closed	shrink	open	swell
*D → BL	opening	swelling	**closing**	**shrinking**[b]
*D + mastoparan	opening	activate	**closing**	**activate**
*D → BL+neomycine	opening	no effect	**open**	**inhibit**
$^+$WL	open	swell	closed	shrink
$^+$WL → D	**closing**	**shrinking**	closed	no effect
$^+$WL → RL → D	closing	shrinking	openng	swelling
$^+$WL + mastoparan	**closing**	**activate**	opening	activate
$^+$WL → D + neomycine	**open**	**inhibit**	closed	no effect
$^+$WL → RL → D + neomycine	ND		opening	no effect

[a] These data were modified from Tables 1 and 2 of Ref. 43.

[b] Bolds indicate that K^+ channel closing and cell shrinking triggered by shrinking signals via activation of phospholipase C and IP_3 production.

the signal from Pfr and transmit it to one of mediators in the signal transduction pathway might exist. In 1973 Quail *et al.*[31] first found evidence for a RL-induced association of soluble phytochrome to membrane fractions or particulate components in etiolated maize and pumpkin tissues. Interaction of Pfr of phytochrome with membranes was repeatable in various plants. Pfr in pea and oat bound well to liposome and protoplasts[30]. These results suggest that phytochrome might interact with membrane or membrane components, for example G protein, resulting in the signal activation fo a signal transduction pathway by RL[32].

Although BL regulates growth, movement, and development in higher plants, a BL receptor has not yet charcterized in higher plants[33]. However, many photobiological data show flavins or carotenoids as possible chromophoric groups of BL receptors[34]. Galland and Senger have recently suggested pterins as candidates for chromophores of the BL receptors[35]. Flavoproteins are presently considered as the most likely candidates, although carotenoids are also still being discussed as possible BL receptor pigments[34, 35]. Most research results for a chromophore of the BL receptror lack evi-dence for a direct interaction between the putative chromophore and a photobiological response. Although there is evidence for a flavin-containing BL receptor associated with the plasma membrane[36], there is no direct evidence that this system plays any physiological role in BL perception. Moreover, the conversion of the BL signal into a cellular signal and the transduction into a biological response is only under investigation for several of these responses; *e.g.* the BL-induced binding of GTP to an α-subunit of a G protein in the plasma membrane[37].

How does phytochrome or BL receptor carry information from external light to the downstream mediator in signal transdution cascade in leaf movements? The regulatory mechnism remains speculative. Evidence for G proteins, signal transduction pathway components, and protein phosphorylation in plants suggests that the signal transduction paradigms established in animal systems will give clues for identification of RL or BL signaling pathway[38].

SIGNAL TRANSDUCTION IN LEAF MOVEMENT

Activation of cell-surface receptors by extracellular signals causes the generation of second messengers that are released within the cell. A more recently recognized signal transduction system is the cascade that regulates intracellular Ca^{2+} and protein phosphorylation catalyzed by a diacylglycerol (DAG)-stimulated protein kinase C. In this system, hydrolysis of PIP_2 generates two second messengers, IP_3 and DAG, resulting in the regulation of cell growth, differentiation, development, and photo-transduction[39-42]. The inositol signaling pathway is a major central system in cell biology.

Ion fluxes are controlled by the endogenous biological clock and exogenous light signals through signal transduction pathways. K^+ fluxes controlled by the second messenger Ca^{2+} may be regulated by phytochrome and BL receptors in *Samanea* and *Albizzia*[20]. Inositol trisphosphate levels have been shown to increase with light treatment of *S. saman* pulvini[9,19,40-42]. Intracellular free Ca^{2+} may be liberated from internal sequestration upon inositol trisphosphate stimulation or

it may enter via gated channels in the plasma membrane[40-42]. Ca^{2+} and DAG may act as second messengers for the effects of BL and/or RL on K^+ fluxes in the extensor and flexor cells in *S. saman.*

The signal transduction events coupling BL receptor or phytochrome to the opening or closing of K^+ channels in *S. saman* are greatly complex since we might consider the regulation of inward-directed K^+ channels alone, two different light signals and darkness, two different time period, and two different cell types. Recently, Kim *et al.*[43] provided evidence for the involvement of inositol phospholipid turnover in mediating the effects of light on the K^+ channel states in the two different cell types at the two different time points.

PHOSPHOINOSITIDE SIGNALING IN REGULATION OF K^+ CHANNELS

IP_3 levels increased in flexor protoplasts with illumination of BL, whereas IP_3 levels decreased in extensor protoplasts 30 s after irradiation with BL in the darkness, indicating that IP_3 might be a second messenger regulating K^+ channels in both extensor and flexor protoplasts. BL-induced IP_3 production could trigger K^+ channel closure in flexor protoplasts. Similarly elevated basal IP_3 might help maintain closed channels in the extensor protoplasts, these channels might then be opened by a BL-induced reduction in IP_3 levels[43].

K^+ channels are open in extensor and closed in flexor cells in the light period. At this time Illumination with RL followed by transfer to darkness opens K^+ channels in flexor and closes them in extensor cells[22]. IP_3 levels in extensor protoplasts decreased about 1.8 fold during the 30 s RL treatment; subsequently in darkness, IP_3 levels recovered to near basal levels. IP_3 levels in flexor protoplasts were inactivated by this treatment. Illumination with RL for 5 min decreased IP_3 levels about 2 fold in extensor but did not change them in flexor protoplasts[43].

Transfer to darkness without previous illumination with RL is sufficient to close channels in extensor cells but not open them in flexor cells[22]. Upon transfer to darkness without RL illumination, IP_3 levels increased about 1.7 fold in extensor but did not change in flexor protoplasts, suggesting that a dark-induced increase IP_3 levels might mediate closure of K^+ channels in the light period. However, changes in IP_3 levels cannot be the only regulatory mechanism since the absolute levels do not always correlate with the state of the K^+ channels. These results also indicate that IP_3 levels play no regulatory role in K^+ channel openning and closing in flexor cells during the light period. In other words, shrinking signals, BL in flexor cells (dark period) and darkness in extensor cells (light period), only elicit increase of IP_3 levels and cell shrinking (Table 1).

In animal cells, G proteins carry information from the signal-activated receptor to activate phospholipase C. The effects of mastoparan, a wasp vernom tetradecapeptide toxin which is an activator of G proteins, on extensor and flexor cells suggest that G proteins might similarly mediate BL- and darkness-induced changes in IP_3 levels in pulvinar cells[43] (Table 1).

In the dark period, 10 μM mastoparan mimicked BL illumination, increasing IP_3 levels 2.7 fold and closing K^+ channels in flexor protoplasts. Mastoparan at 10 μM increased IP_3 levels about 2 fold in extensor protoplasts in the light period similar to the effect of darkness at this time. Mastoparan also mimicked the effect of RL followed by darkness, colsing open K^+ channels in extensor and opening closed K^+ channels in flexor protoplasts[43].

G protein may mediate BL- and dark-induce accumulation of IP_3 and subsequent K^+ channels closure in ether cell type at the appropriate time. Interestingly, 10 μM mastoparan mimicked the effect of irradiation with RL followed by darkness on the K^+ channels of flexor protoplasts, opening them, even through this treatment did not affect IP_3 levels, indicating that other G proteins mediated signal transduction pathway(s), regulating K^+ channel opening in flexor protoplasts on the light period[43].

The antibiotic neomycin is a potent inhibitor of hydrolysis of PIP_3 by phospholipase C. In the dark period, 10 μM neomycin inhibited BL-induced IP_3 production and K^+ channel closure in flexor protoplasts but had no effect on K^+ channel opening in extensor protoplasts. In the light period, neomycin blocked dark-induced IP_3 production and K^+ channel closure in extensor protoplasts. In contrast, neomycin had no effect on the K^+ channels opening induced by RL followed by darkness in flexor protoplasts[43]. Taken together, these results suggest that phospholipase C activation causes the IP_3 accumulation which correlates with K^+ channel closure in flexor cells in the darkness and extensor cells in the light period (Table 1).

In flexor protoplasts, RL followed by darkness opened the closed K^+ channels in the light period but had no effect in IP_3 levels, indicating that IP_3 was not a second messenger in the opening K^+ channels in flexor protoplasts. Mastoparan mimicked the effect of RL followed by darkness, opening K^+ channels in flexor protoplasts during light period[43]. This suggests that a G protein, not involved in activation or inactivation of phospholipase C, may be involved in channel opening in flexor cells.

CALCIUM SIGNALING

Ca^{2+} signaling is a crucial role in the regulation of cell responses in animal and plant cells. In animal cells multiple second messengers, IP_3, CDP-glucose, cADP-ribose, and calcium influx factor (CIF) contribute to regulation of intracellular Ca^{2+} levels[39,44-46]. In contrast

to animals, Ca^{2+} signaling mechanisms are poorly understand in plants. Notably, IP_3 is recognized as the key second messenger regulating Ca^{2+} mobilization from intracellular stores in plant cells as well as animal cells.

How might cytosolic Ca^{2+} regulate the state of K^+ channels, lead to cell shrinking in pulvinar cells? We may hypothesize that intracellular Ca^{2+} inhibits K^+ influx and activates Cl^- efflux, leading to cell shrinking and leaf movement in *S. saman*. Like leaflet movement, opening and closing of leaf stomata also depends on changes in turgor. Micromolar Ca^{2+} levels inhibit the inward-directed K^+ channels of guard cells and stimulate Cl^- channels[18,47]. The IP_3, released from a caged derivative previously microinjection into guard cells, triggers a rapid and transient increase in cytosolic Ca^{2+} and subseguently loss of turgor and closure of the stomata[48]. These data in stomata guard cells are consistent with results from *S. saman*, as described above, indicating an identical role for IP_3 in *S. saman* motor cells.

Presumably, IP_3 generated by shrinking signals mobilizes intracellular Ca^{2+} from the vacuole and/or from other intracellular Ca^{2+} stores and that this Ca^{2+} might inhibit inward-directed K^+ channels and activate outward-directed Cl^- channels in shrinking cells(Fig. 2).

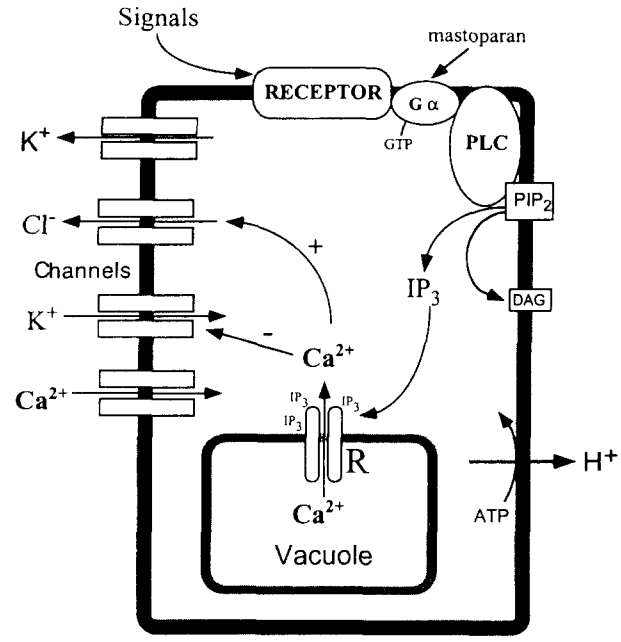

Figure 2. A model for shrinking signals induced shrinking of extensor or flexor cells of *S. saman*. Activation of phospholipase C (PLC) is triggered by shrinking signals (*e.g.* BL or darkness, see text for more detail) via G protein. Hydrolysis PIP_2 by activation generates two second messengers, IP_3 and DAG, lead to regulation of cellular physiology by regulating intracellular Ca^{2+} levels and the activity of protein kinase C, respectively. Intracellular Ca^{2+} inhibits K^+ influx and activates Cl^- efflux. R indicates IP_3 receptor[19].

CONCLUSIONS

Leaf movements in nyctinastic plants show attractive features for study of light-controlled ion transports and their regulatory mechanisms; the pulvinar motor cells are rapidly responsive to external light signals and their distinct signal transduction cascades coupling these light signals to regulation of ion movements. Because these movements are largely massive and probably widespread, the signal trasduction pathways of ion transports in leaf movements of *Samanea saman* may provide us clues for understanding and characterizing regulatory mechanisms involved in ion movements of other plant systems.

Although IP_3 production, as described above, evokes closure of inward-directed K^+ channel in shrinking cells, we do not know how outward-directed K^+ channels, which are a critical role to cell shrinking, are operated. The detailed sequence of signal transduction cascade, that is, mechanism of photoperception, the role of Ca^{2+}, and phosphorylation of proteins in regulation of K^+ channels have not yet studied in shrinking cells.

The signal transduction cascades by which external light signals trigger opening of the inward-directed K^+ channels that lead to cell swelling are still unknown. Although the effects of mastoparan on K^+ channel opening suggest that G protein is one of components in the signal transduction pathway, we do not know how G protein carries swelling signals to the downstream mediators in swelling cells.

Future research will lead to elucidation of downstream events of IP_3 accumulation in K^+ channel closure, leading to cell shrinking. We also need to identify the G proteins involved in both K^+ channel opening and K^+ channel closure and to illustrate the regulatory mechanism that opens inward-directed K^+ channels in swelling cells.

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