Nucleo-cytoplasmic partitioning of the plant photoreceptors phytochromes

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Phytochromes in harmony with blue light photoreceptors play a major role in controlling plant growth and development from germination to seed maturation. Light absorption by phytochromes triggers a signaling cascade, phototransduction, which culminates in regulated gene expression. A major regulatory step at the cellular level, which affects specificities of light-induced physiological responses, seems to be the light-quality and light-quantity dependent nuclear import of the phytochromes themselves. The correlations found between the nuclear import of phytochromes (phyA and phyB) and various physiological responses regulated by these photoreceptors provides strong support for this hypothesis.

Key words: photomorphogenesis / phytochrome / phototransduction / nuclear import / transcription

Light as an electromagnetic wave can easily penetrate plant tissues. Red and far-red light can even pass through multiple rows of leaves or compact organs like stems and activate the phytochrome photoreceptors probably in all cells of any tissue exposed to light directly or indirectly. Physiological studies indicated that phytochrome is distributed throughout the plant although certain cell types, depending on the developmental stage, contain different amounts of the photoreceptor. These observations were further corroborated by studies performed to determine tissue and cell specific expression patterns of the PHYA-PHYE genes\textsuperscript{1} in transgenic plants, in a most detailed fashion, for genes encoding phytochrome-A\textsuperscript{2,3} (phyA) and phytochrome-B\textsuperscript{3,4} (phyB). Therefore, it is probably safe to conclude that each plant cell contains at least one type of phytochrome at any stage of development.

During the past 20 years there has been a long and controversial debate about the intracellular localization of phytochrome. This debate strongly influenced the interpretation of experimental results concerning molecular events mediating and models explaining phytochrome-regulated signaling in higher plants. First, red/far-red reversible pelletability of phytochrome,\textsuperscript{5} reflecting sequestered areas of phytochrome,\textsuperscript{6} indicated that phytochrome is localized in the cytosol and/or perhaps associated with the plasma membrane. After the identification of the five different genes encoding phyA–E it became evident that this observation describes only the localization of phyA. Immunohistological studies,\textsuperscript{7} cell fractionation assays describing non-specific association of phytochrome with nuclei isolated from dark-grown pea seedlings\textsuperscript{8} and microinjection/pharmacological experiments\textsuperscript{9,10} all seemed to corroborate the prevailing view that phytochrome, notably phyA, is localized dominantly outside the nucleus. This view was further strengthened by the well-documented action dichroism for phytochrome-controlled-directional growth and chloroplast movement in lower plants since these data clearly showed association of phytochrome with the plasma membrane.\textsuperscript{11} In addition, until recently, nothing was known about the intracellular localization of the other types of phytochromes.

Intracellular localization of phyB in light and darkness

Sakamoto and Nagatani\textsuperscript{12} demonstrated by immunocytochemical methods that nuclear fractions isolated from light-grown \textit{Arabidopsis} plants contain
significantly higher amounts of phyB than do nuclear fractions derived from dark-adapted tissues. Moreover, by analysing the expression patterns of chimeric proteins consisting of truncated phyB and the GUS reporter in plant cells and protoplasts, the same authors showed that phyB contains a functional nuclear translocation signal (NLS). They showed that some of these fusion proteins were imported in the nucleus, albeit in a light-independent fashion.

More recently the same laboratory used the full-length phyB:GFP fusion protein to complement an Arabidopsis phyB-deficient mutant in vivo in transgenic plants. They demonstrated that the fusion protein is a functional photoreceptor and provided evidence that its nuclear translocation is induced by red light and accompanied by the formation of nuclear spots of phyB:GFP. These observations were further extended by results reported by Kircher et al. and Gil et al. In these studies the expression of the tobacco phyB:GFP photoreceptor in transgenic plants resulted in the complementation of a phyB-deficient Nicotiana plumbaginifolia mutant or led to an overexpression phenotype in the wild-type background. In etiolated seedlings and dark-adapted plants the phyB:GFP fusion protein was localized in the cytosol. Figure 1 shows that the nuclear translocation and spot formation of the fusion protein was induced by continuous red light treatment, or multiple red light pulses which were reversible by subsequent far-red treatment, indicating that the nuclear import of phyB is mediated by a low-fluence response (LFR) of phytochrome. It follows that the nuclear import of phyB is regulated by phyB itself, and/or by some other red light absorbing photoreceptor(s).

Detailed photophysiological and kinetic studies provided evidence that the nuclear import of phyB:GFP is relatively slow (maximum accumulation of GFP in the nuclei is detected within 3 h after the onset of light treatment) and that the import process is strongly fluence-rate dependent.

Intracellular localization of phyA in light and darkness

A similar experimental approach was used to gain information about the nucleocytoplasmic distribution of phyA in transgenic plants grown under various conditions. Studies on the intracellular localization of rice phyA:GFP in transgenic tobacco and Arabidopsis phyA:GFP in a phyA-deficient Arabidopsis mutant provided the following observations. In etiolated transgenic tobacco or Arabidopsis seedlings and in dark-adapted plants the phyA:GFP fusion protein was localized in the cytosol. Brief irradiations with red, far-red- or blue-light-induced rapid nuclear import and intranuclear spot formation of phyA:GFP (one magnitude of order faster than that of phyB) that was preceded by an even faster cytosolic spot formation of the fusion protein, a phenomenon reminiscent of SAP formation (Figure 2). Finally, nuclear translocation of phyA was induced by continuous far-red but not by continuous red light treatment. Thus, it was concluded that: (i) the nuclear translocation of phyA is mediated, in contrast to phyB, by the very low fluence response (VLFR) and (ii) by the so-called far-red high irradiance response (HIR) of phytochrome indicating that phyA regulates its own nuclear import independent of any other types of phytochromes. Extension of these studies showed that the nuclear import of phyA is indeed a highly

Figure 1. Localization of tobacco phyB:GFP expressed in the phyB mutant hlg2 of N. plumbaginifolia. Adult plants were re-etiolated in darkness for 2 days and leaf discs were subjected to different light treatments before analysis of trichome cells by epifluorescence microscopy. Leaf discs were kept in darkness (cD) or irradiated either with 3 h of continuous red light (3 h R) or three consecutive hourly pulses of 5 min of red light (pR) or 5 min of red light followed by 5 min of far-red light (pR/FR). The arrows are pointing to the nuclei (nu), the red fluorescence is due to excitation of plastidic chlorophyll.
regulated, complex process. Nuclear import of the same Arabidopsis phyA:GFP fusion protein exhibited characteristically different features in transgenic Arabidopsis as compared with tobacco. The unexpected differences for such a basic cellular process between two closely related species suggest a careful approach for the interpretation and extrapolation of data obtained in heterologous systems.

Nucleocytoplasmic distribution of phyC, phyD and phyE

Up until now no information has been available about the subcellular localization of these phytochrome species. To characterize and compare the intracellular distribution of phyC, phyD and phyE to that of phyA and phyB we generated chimeric proteins containing the full-length phyC–E fused to GFP and expressed these constructs in transgenic Arabidopsis. Surprisingly, the analysis of these transgenic plants indicates that the nucleocytoplasmic partitioning of these phytochrome species itself seems not to be regulated by light. The phyC–phyE:GFP fusion proteins can be detected in the cytosol and the nucleus in etiolated seedlings. But, phyC–phyE:GFP fusion proteins similar to phyA or phyB:GFP are forming nuclear spots in a light-dependent manner (Kircher et al., submitted). Figure 3 shows white-light-induced nuclear spot formation of phyA–phyE. The white light induction of the translocation of phyA is fast but only transient and the kinetics of nuclear spot formation show subtle differences between phyB, phyC, phyD and phyE. It is noticed that among these phytochrome species phyB and phyE seem to follow the fastest kinetics.

Factors modulating nucleocytoplasmic partitioning of phytochromes

The nuclear import of proteins is a complex cellular process that can be divided into the following operational steps. Nuclear translocation is initiated by the substrate–receptor recognition, followed by nuclear envelope docking and translocation through the nuclear membrane and subsequent dissociation of the transport complex. This scheme offers numerous possibilities for regulation by environmental factors and in fact, in plant cells, a large variety of proteins is imported into the nuclei in a regulated manner. At least the phytochromes A and B represent one class of these proteins and in this case nuclear import is regulated by light quality and quantity. At present we have limited general information about the molecular machinery that mediates protein import in higher plants. Yet it is tempting to speculate about a few factors that might modulate the import process mediating phytochrome translocation into the nuclei, for example the substrate–receptor recognition.
Are phytochromes retained in the cytosol in the dark?

In etiolated seedlings, when phytochrome exists as a Pr conformer, the phyA:GFP and phyB:GFP fusion proteins are localized outside the nucleus. A phyB mutant that is unable to bind its chromophore is localized constitutively in the cytosol. These data indicate that the Pr to Pfr conformation change is required for translocation. However, in a few cases, we noted that strong overexpression of phyA:GFP and phyB:GFP resulted in diffuse GFP fluorescence in the nuclei of dark-grown seedlings. Moreover, it was shown that a fusion protein containing the C-terminal part of phyB fused to GFP, illustrated by Figure 4, is constitutively localized in the nuclei. In addition we found that the insertion of one or more SV-40 NLS domains into the phyB:GFP protein at various positions did not change the translocation pattern. The interpretation of these observations can be that: (i) the Pr to Pfr conformation change is not absolutely required for initiation of nuclear import; (ii) NLS motifs mediating phytochrome binding to the nuclear import receptor importin α are not masked even in the Pr conformer and that (iii) the C-terminal portion of phyB is not able to interact, whereas overexpressed phyB escapes interaction with a factor that ensures cytosolic localization in darkness. This could be also an explanation of the nuclear localization of phyC–E:GFP in darkness, especially if the very low endogenous amounts of these phytochromes are considered. This interpretation leads to a model which assumes that Pr conformers of phytochromes are anchored/retained in the cytosol, Pr (when it escapes retention, for example by saturating the retention mechanism) and Pfr (which does not interact with the anchoring protein) are both subject of nuclear import and that the import process is mainly regulated at the retention level. It follows that the physiological function of the Pr to Pfr conformation change is to provide ‘retention-free’ phytochrome molecules for the nuclear import.

What is the role of the kinase activity of phytochrome?

It has been demonstrated that phyB is an atypical serine/threonine kinase which autophosphorylates and phosphorylates other proteins in vitro and in vivo. Autophosphorylation of phytochrome occurs in darkness and light but there is circumstantial evidence indicating that some amino-acid residues are specifically phosphorylated in light. It has been hypothesized that (auto)phosphorylation of phytochromes may, at least partly, mediate the Pr to Pfr conformation change. It follows that changes in the autophosphorylation pattern of phytochromes may be essential for mediating retention/release of the photoreceptor, whereas phosphorylation of other proteins, such as PKS1 (phytochrome kinase substrate 1), could either play a role in modulating retention or result in modifications of other proteins, for example the b-ZIP transcription factor CPRF2 imported in the nuclei in a red-/far-red reversible fashion.

Does cross-talking to other signaling cascades modulate phototransduction at the level of nucleocytoplasmic partitioning of phytochromes?

Phytochrome-controlled inhibition of hypocotyl growth has been extensively studied. In red light phyB is the dominating photoreceptor and its responsiveness is modulated by phyA, CRY1 and by phyB itself. Studies performed on transgenic tobacco seedlings demonstrated similar fluence rate and wavelength dependence for light-controlled nuclear import of phyB:GFP and inhibition of hypocotyl growth. Furthermore it was shown that nuclear import of phyB:GFP also displays responsiveness amplification by activation of phyB and by blue light absorbing (CRY1) photoreceptors. Surprisingly, no responsiveness amplification of the nuclear transport of phyB:GFP by far-red light was detected although inhibition of hypocotyl growth was, as expected,
strongly affected. Thus, responsiveness amplification of phyB responses mediated by phyB or blue light receptors is already manifested at the level of nuclear import whereas the phyA-driven process must be acting at a different level.

There are numerous Arabidopsis mutants that exhibit enhanced or reduced sensitivity to light (see the article by Hudson, this volume). Theoretically any of these mutants, even mutations within the phytochrome molecule itself, can affect intracellular distribution of phyA–phyE photoreceptors directly or indirectly, unless otherwise proven. Regulation of phototransduction by other signaling cascades (stress, hormones etc.) may take place in the cytosol or in the nuclei. It is plausible to assume that if cross-talking between different signaling cascades takes place in the cytosol, it results in modulating nuclear import of phytochromes and thereby regulates the amount of photoreceptors available for interaction with other components required for light-regulated transcription.22,23 This hypothesis can be verified and it is expected that a detailed characterization of some of these mutants with respect to nucleocytoplasmic partitioning of photoreceptors will help to solve confusion surrounding some of the earlier physiological observations.

Conclusion

In etiolated seedlings and dark-adapted plants phytochromes A and B are localized in the cytosol. Nuclear transport of these phytochromes is light-dependent and each phytochrome species shows a specific light quality and quantity dependence of either the transport or nuclear spot formation. Thus, most of the specificity of phytochrome-regulated responses is already manifested at the level of light regulation of the intracellular localization. This is most obvious for a comparison of phyA and phyB, since far-red light is the most effective wavelength to induce nuclear import of phyA whereas it is completely ineffective for phyB. The amount of phytochromes localized in the nucleus is not constant during extended dark or light periods but exhibits characteristic, dynamically changing patterns. Thus, it is likely that protein export and/or degradation, probably mediated by the COP/DET repressory proteins also play an important role in regulating the availability of phytochrome in the nuclei and thereby modulating recruitment/activation of transcription complexes/factors required for light-induced gene transcription.22,23

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