

光敏色素分子特性及其信号转导机制

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摘要: 结合生物物理、分子遗传学和细胞生物学的方法已证实, 光敏色素信号转导是一个空间分布的、非线性信号传递链。尤其是最近又发现了不同种类的光敏色素分子及其它们在 Pr、Pfr 光转换中产生的中间体, 不仅说明了光敏色素信号转导链是一个多维的信号网络, 而且这也暗示着光转换中产生的中间体也直接参与了早期的信号转导。在此, 综述了光敏色素分子光转换及其早期信号转导的若干新进展, 讨论光敏色素原初光反应及其信号转导的机制。
关键词: 光敏色素; 光转换; 信号转导; 信号中间组分

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Molecular Properties of Phytochromes and Their Signalling Mechanism

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Abstract: Through a combination of biophysical, molecular genetic and cell biological approaches, it is compelling evidence that phytochrome signal transduction is spatially separated nonlinear chain of events. In particular, the discovery that different phytochrome species and their intermediates during Pr, Pfr phototransformation show multidimensional signal network, which lead to the suggestion that these intermediates are directly involved in these early signal transduction. Here, the primary photorsection of phytochrome and the corresponding signalling mechanism will be discussed by reviewing some of recent progress in phytochrome phototransformation and its signal transduction in early stage.
Key words: Phytochrome; Phototransformation; Signalling transduction; Signalling intermediates

As sessile organisms, plants are unable to move actively towards favorable or away from unfavorable environmental conditions like animals. Therefore, plant have evolved diverse photoreceptor systems for detecting light intensity, quality, and duration to adjust their life in flucting environmental conditions. Phytochrome, as the primary

photoreceptor, exert their photoregulatory effects at all stages of the plant life cycle, including chloroplast movement, construction of the photosynthetic apparatus, seed germination, de-etiolation and seedling establishment, shade avoidance and the induction and timing of flowering, thus determining the strategy of their optimum light de-

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velopment^[1], that is, photomorphogenesis. The photosignal perception and transduction is based on the photoreversible phototransformation of the initial red-light (R) absorbing form (Pr, λ_{\max} 666 nm) into a physiologically active far-red-light (FR) absorbing form (Pfr, λ_{\max} 730 nm), so the primary photoreaction is the start for us to learn the photochrome signalling mechanism.

For that the past decade has seen dramatic advances in our knowledge of phytochromes and their signal transduction pathways that leads to various physiological responses, there are many reviews on the cell biology of phytochrome signalling^[1-4], but few systematical literature on phytochrome phototransformation and the corresponding signalling mechanism. Here, we briefly review the most recent progress on this topic that maybe provide new insights into leaning phytochrome signal transduction.

1 Phytochrome Structure and Function

The major achievement of recent years is the discovery of a small family of genes encoding for several phytochrome apoproteins, e.g., *phyA*–*phyE* in *Arabidopsis thaliana*. (Sharrock and Quail, 1989), *Arabidopsis phyB* and *phyD* polypeptides are approx. 80% identical and are somewhat more related to *phyE* than they are to either *phyA* or *phyC* (approx. 50% identity). Counterparts of *phyA*, *phyB* and other PHY genes are present in most, if not all, higher plants^[5].

All of the higher plant phytochromes share the same basic structure, consisting of two structural domains: a photosensory: globular N-terminal chromophore-binding domain which is sufficient for light absorption and photoreversibility (– 70 kDa), and a regulatory, conformationally more extended C-terminal domain (– 55 kDa) which encompasses two histidine kinase related domains (HRKD) and two motifs with homology to PAS (PER-ARNT-SIM) domains^[6,7]. PAS domains are sensible to environmental signals such as light conditions, oxygen levels, and redox potential. They may also mediate protein-protein inter-

actions^[8,9]. The amino-terminal half of phytochromes can be considered as a light-sensing domain whilst the carboxyl-terminal half can be regarded as the regulatory domain^[10].

In the 1980's, spectrophotometric studies indicated that there are at least two distinct pools of phytochromes, Type I (light labile) and type II (light stable). Type I phytochrome is synthesized as Pr in darkness and decay rapidly in the light as a labile Pfr form. In contrast, type II phytochrome is stable in the Pfr form and is present at relatively constant levels both in the light and in darkness. Now it is accepted that *phyA* corresponds to the major light-labile pigments (type I), whereas the minor light-stable pigments (type II) comprise *phyB* and the other phytochromes. There is certain overlapping between the absorption spectra of Pr and Pfr, which is important for *phyA* activity because *phyA* is light dependent and requires selective recognition and ubiquitination of Pfr^[11,12]. *PhyA* participates in seed germination, responsible in a way of the very low fluence response (VLFR, 10^{-4} to $10^{-1} \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) or the high-irradiance response (HIR, $> 1000 \mu\text{mol} \cdot \text{m}^{-2}$) in seedling de-etiolation, including inhibition of hypocotyl elongation, the expansion of cotyledons, changes in gene expression and the synthesis of anthocyanin, etc. On the other hand, *phyB* and the other phytochromes control the photoreversible effects of the so-called low-fluence responses (LFR, 1 to $10^3 \mu\text{mol} \cdot \text{m}^{-2}$)^[11,12], whose distinguishing feature is its conformity to the Bunsen-Roscoe Reciprocity Law, which states that a response should be dependent only on the total amount of photons received irrespective of the duration of the exposure^[13]. Phytochrome B is also considered to be the main phytochrome responsible for the shade avoidance response (elongated growth habit, reduced leaf area, increased apical dominance and early flowering^[14]). The phytochrome family members play different roles in the photoregulation processes, and phytochromes also show redundancy of function. Clearly, phytochromes also interact and coact with other photoreceptors^[15].

2 Phytochrome Phototransformation and Phytochrome Heterogeneity

The photochemistry of phytochrome activation is based upon the *cis-trans* isomerization of the chromophore. This configurational transition leads to the formation of a signalling state of sufficient stability to communicate the presence of photons to a downstream signal transduction partner. Therefore, phytochrome phototransformation can be defined as the start of the primary photoreaction, which is involved in the start of signal transduction.

The $\text{Pr} \rightleftharpoons \text{Pfr}$ conversion is a low-energy storing photoreaction based on photobiological criteria, of which primary processes are a *Z, E* isomerization around the $\text{C15}=\text{C16}$ -methine bridge of the tetrapyrrolic chromophore proceeding within several tens ps with participation of the singlet excited state^[16, 17], and at almost the same time the structure of apoprotein is conformationally restructured. A number of spectrophotometrically identifiable intermediates have been detected (Fig. 1). Lumi-R ($\tau < 100 \mu\text{s}$, ca. 700 nm) is the first inter-

mediate stable at low temperature accompanying the transformation process, which decays on a microsecond time scale to meta-Ra. Interestingly, picosecond kinetic measurements on phyA reveal a decay of the Pr excited states (Pr^*) within about 15-40 ps to prelumi-R which in its turn decays with a delay (up to 100 ps) to the Lumi-R (state 1, photoequilibrium between Pr and lumi-R) where the Pr

Pfr conversion is transitorily stopped^[17, 18]. It is worthy to note that the decay kinetics of the lumi-R are significantly different between phyA and phyB^[19], and the quantum yield for the formation of lumi-R is higher than Pr \rightarrow Pfr reaction, so the changes in the Pr \rightarrow lumi-R is complex^[20]. However, some scholars concluded that there must be the existence of the heterogeneity of the emitting species produced from the Pr ground state populations, several pools of lumi-R and the excited states of the several pools^[17, 21, 22], because interaction of the chromophore with the apoprotein obviously affects the formation of ground state lumi-R, and whatever physical or chemical process may be involved therein. Furthermore, it is suggested that *Z-E/E-Z* isomerization takes place during the Pr

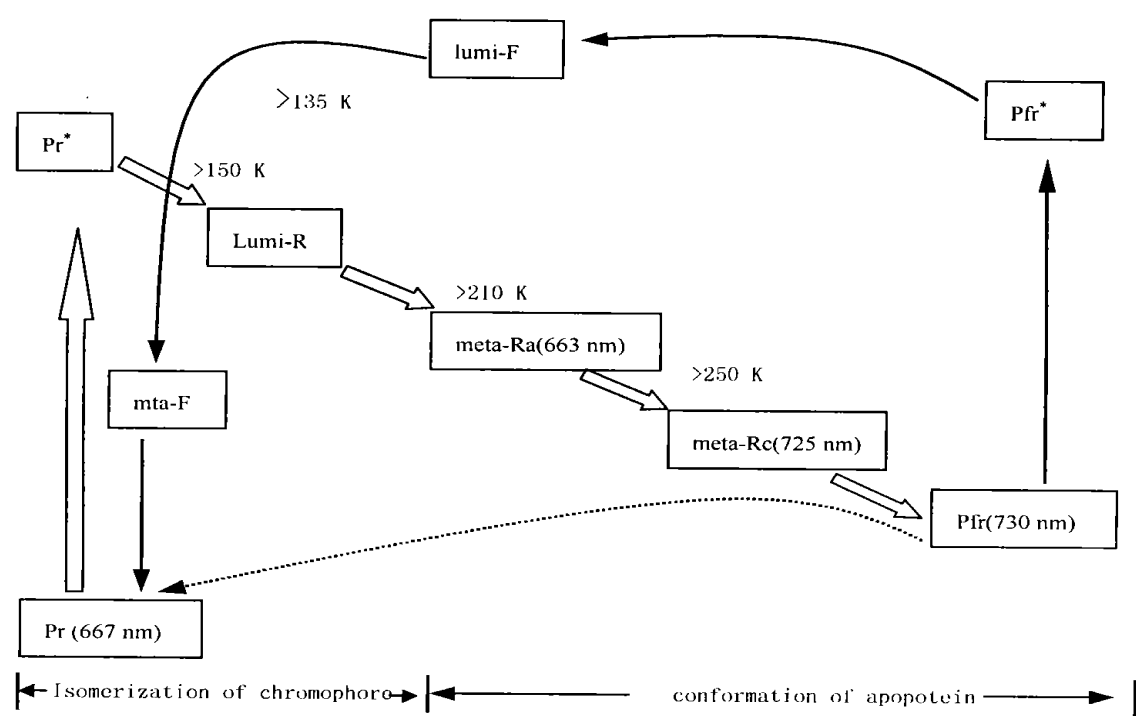


Fig. 1 Simplified energy level diagram of the phytochrome photocycle reflecting

lumi-R, and lumi-R may act physiologically as functional intermediate, whereas C15 single bond rotation occurs in one of the subsequent steps. For instance, Pr (one of the two phenomenological Pr types) is more efficient in the Pr → lumi-R photo-conversion. So, it is possible that the short-lived lumi-R state could affect the concentration of the physiologically active Pfr by changing the photocycle of the Pr → Pfr, or possibly, the following protein conformational changes^[23].

As we all know, the physiological effect of Pfr is related to the absolute or relative concentration of Pfr or the stabilization of Pfr in plant tissues, and Pfr should be subject to rapid changes due to its degradation and dark reversion into Pr^[24]. The Pfr photoreversion reaction including the thermal reversion in the dark is very important in maintaining a physiological balance in plant development. Recently, the reverse Pfr photoreversion is reported to be sequentially followed by several other meta intermediates such as the first intermediate, lumi-F (τ 320 ns, ca. 673 nm), and the second, meta-F (τ 265 μ s, ca. 660 nm)^[22], and is described as a bi-exponential process with two time constants in the range between 150–650 fs and 2–5 ps^[25, 26]. Notably, Pr phototransformation pathway does not share any intermediate with the Pfr photoreversion pathway (Fig. 1), which may be also seen from the different spectra of the interconversion. It seems that all these intermediates have no obvious physiological activity, but they are necessary for the interconversion between Pfr and Pr upon irradiation and passing photons to a downstream signal transduction particle. Comparing with photosynthetic photons transfer pathway, We little know how phytochrome percept photons and the primary photosignal transduction pathways, but these intermediates may be just the implication. Recent studies from Furuya's lab suggest that the FR-HIR requires a short-lived intermediate(Pr⁺) generated during Pfr to Pr photoreversion. Most interestingly, this response is FR/R reversible (not R/FR), which is opposite to what is seen in the LFRs such as FR inhibition of light-

regulated gene expression or lettuce seed germination^[27].

Recent success in investigation is connected with the discovery of heterogeneity of phytochrome in the cell observed using the low-temperature (85 K) fluorescence spectroscopy as well other experimental approaches^[26, 28, 29]. Two Pr types were distinguished physiologically, Pr-major longer wavelength (687/673 nm, emission/absorption maxima) and Pr-minor, shorter wavelength (682/668 nm). It was show that Pr and Pr correspond to type I and type II, respectively^[30, 31]. Subsequently, Sineshchekov obtained the experimental evidence that phyA is heterogeneous and comprises two spectroscopically distinct species, namely, the light-labile phyA (belongs to Pr) and the light-stable phyA (belongs to Pr)^[28, 32]. PhyB was later shown to have characteristics close to those of phyA and belongs to the same phenomenological Pr type. Schmidt also found that the existence of Pr isoforms by investigation of thermochromy temperature-induced absorption spectra changes^[33]. Although the exact structural difference between the two Pr types is not known, it is assumed that they could mediate different photoresponses. Based on his long-run investigation, Sineshchekov concluded that phyA should be responsible for de-etiolation while the invariable phyB and phyA could functional through the plant life cycle^[31]. Nonetheless, the results was just achieved by spectroscopy analysis, not biochemical and genetical method, little is currently known regarding Pr or Pr signalling pathways, still less the downstream components. So to say, transduction of the light-generated signal and identification of the various phytochrome signal transduction pathways are still a major field in the coming future.

3 The Cell Biology of Phytochrome Signalling Transduction

The central question of the biochemical transduction that constitutes signal transfer from the photoactivated phytochrome molecule to its primary signalling intermediates has intrigued re-

searchers in the field for many years^[34].

3.1 Phytochromes as light-regulated kinase

The carboxy terminal domain of polypeptides contains a region with sequence similarity to prokaryotic two component histidine kinases, which suggested a biochemical mechanism for phytochrome signaling. The Lagarias laboratory recently has provided convincing evidence that phytochrome functions as a photoreceptor kinase (an unusual Ser/Thr kinase with two His kinase-like domains)^[35]. The data showed that PKS1, as differential transphosphorylation of a protein substrate, was phosphorylated on serine and threonine residues, and that conversion of phyA to the active Pfr form enhanced the level of PKS1 phosphorylation by two more fold relative to that observed for Pr. Park *et al.* (2000) has speculated that phosphorylation of phytochrome may contribute to the retention and release of phytochromes^[36]. The protein substrate, PKS1, may play a role in this retention mechanism resulting in signal transduc-

tion.

3.2 The primary signaling intermediates

Early pharmacological studies using microinjection of a tomato phytochrome mutant have identified heterotrimeric G proteins, cGMP and Ca, as second messengers in phytochrome signaling^[37,38]. Genetic screens have identified two classes of signaling components, those acting down-stream of a single photoreceptor (e.g. PIF3) and those acting downstream of multiple photoreceptors (see Fig. 2). This presumably reflects the fact that light signals perceived by different photoreceptors must be integrated. The latter class includes both positively acting factors (i.e. HY5) and a large group of negative regulators of photomorphogenesis (DET/COP/FUS). How do these various components implicated in photosignal transduction function? The answer remain unknown although many components have now been cloned. However, it is notable that the cloned factors localised to nucleus, which suggest that early light signalling events are nuclear-localised.

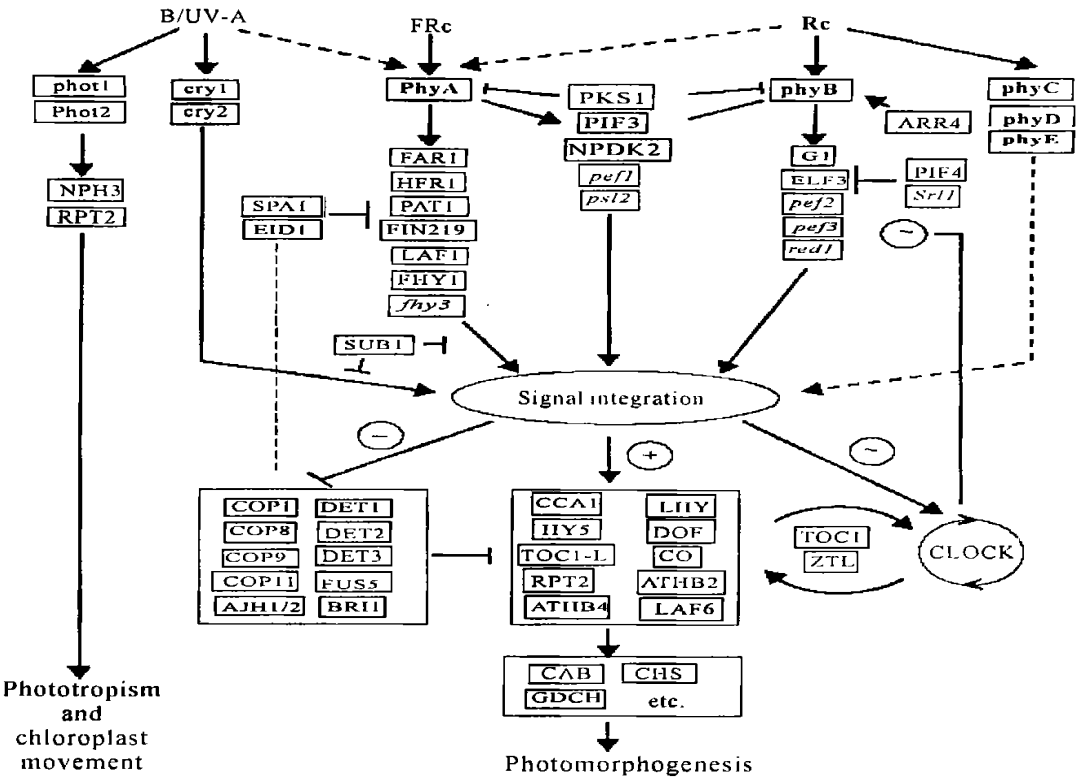


Fig. 2 A simplified model for phytochrome-mediated light signaling(Quail, 2002a)

4 Discussion and Perspectives

Investigations with *Arabidopsis* mutants null for the individual phytochrome and signalling component since 1994, significant progress has been made in unraveling these components in the signal transduction pathways. Coupled the phytochrome dynamic phototransformation with following signal transduction, it is known that phytochrome molecule function as unique binary optical storage devices whose biochemical output is controlled by the stored information. Although a detailed kinetic scheme has not yet been elucidated, these data may provide some information for phytochrome multidimensional signal pathways. For instance, Pr or Pfr* can be also an another signalling intermediate and exert a significant role through the development. To further study the signal pathways.

Based on all these existing literatures, it is tempting to anticipate that the intermediates have the effects on the later signal transduction or that the bioactivity of Pfr (or Pfr-x) is light-dependent because of light-induced nuclear translocation. Conformational changes of the intermediates influence the subcellular localization, stability, as well as the protein kinase activity of phytochrome. It is also proposed that the intermediates and the absorption of photons by photoreceptors change their conformations, resulting in phosphorylation of the receptors, which eventually triggers signal transduction and physiological responses. Therefore, it seems that the urgent task is to find the factors affecting the stabilization of Pfr or Pfr-x form by inhibition of the dark reversion of Pfr (or Pfr-x) to Pr, and determine whether this step is light-dependent or light-independent. Meanwhile, some sophisticated experimental approaches may be applied to determine which step during phototransformation from Pr to Pfr a signal is transduced from the photoreceptor in the cell. However, the difficulty is that there are different phytochromes and their corresponding heterogeneous subpopulations including the intermediates during the primary phototransformation. Luckily, various phy-

tochrome mutants and signal component null mutants are available as model plant to exploit the mechanism coupling with devising light conditions. So tomorrow is light!

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