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evolutionary adaptation to living in the shade

A combination of experiments and modelling shows how plants can detect far-red light

by Julia Rausenberger, Eberhard Schäfer, Jens Timmer, Andreas Hiltbrunner und Christian Fleck

In order to detect light, humans and animals have light-sensitive proteins in the sensory cells of the retina. Analogously, plants too have light-sensitive proteins known as photoreceptors for detecting changes in their light environment. Phytochromes are photoreceptors that are activated by red light and are therefore optimally able to detect the red part of light. But plants also use phytochromes to detect far-red light, although their photophysical properties make them ill-suited to do so. By combining experimental approaches with mathematical modelling we found an explanation for this paradox of which scientists have long been aware (Rausenberger *et al.*, 2011).

Photoreceptors help in selecting a suitable development strategy

Light influences the life cycle of a plant in a variety of ways. Via the process of photosynthesis, plants derive from light the energy they need for survival. Unlike animals, which in adverse circumstances can simply move away and look for a better place, plants are stationary and have to adapt to the prevailing conditions in the place where they germinate. Various aspects of the light environment, such as the day length, the direction from which the light comes or its spectral composition give plants important information about their environment. As days grow shorter, for example, plants start to prepare for approaching winter, or a change in the light spectrum enables them to recognise rivals before they become a threat to survival.

To detect light, plants use various light-sensitive proteins known as photoreceptors. These phytochromes, cryptochromes and phototropins give plants the ability to record important parameters of their environment. In order to absorb light, all three photoreceptor families depend on chromophores.

Phytochromes contain phytochromobilin, a linear tetrapyrrole, as a chromophore (Fig. 1a), while cryptochromes and phototropins have flavin-based chromophores (Fig. 1b). Already very young seedlings choose between two development strategies, depending on the light (Fig. 1c). After germinating, in the absence of light they use their limited stocks of storage substances for more elongated growth in order to reach the light, thereby enabling photosynthesis and thus photoautotrophic growth for the little plant (Greek *photos* = light, *autotroph* = self-feeding). This strategy is called skotomorphogenesis. As soon as light is available, the plant switches to a second development strategy known as photomorphogenesis, where the main emphasis is on growing more leaves and optimising the photosynthesis process. Thus, seedlings with the same genetic background can develop very differently, depending on the environmental and light conditions. Over the course of the earth's history, as plant growth became increasingly dense, a further characteristic of plants became important – the ability to survive in the shadow of other plants. Beneath a dense cover of vegetation, the ratio of far-red light is strongly increased because the chlorophyll in the leaves of overshadowing plants filters out the blue and red light part of the sunlight. In these conditions, detecting far-red light is indispensable in order to enable the transition from skotomorphogenesis to photomorphogenesis after germination and therefore to photoautotrophic growth.

Phytochromes absorb most light in the red light region, but can achieve maximum effect in far-red light

In the red and far-red regions of the light spectrum (625–740 nm), light quality and quantity is detected by the photoreversible phytochrome system. A phytochrome consists of a protein component and a light-absorbing component, the chromophore. This chromophore, phytochromobilin, and the phytochrome molecules exist in two spectroscopically differentiable forms, the Pr and the Pfr form (Figs. 1a and 1d). The Pfr form is

Researching light detection in plants: from left to right Christian Fleck, Florian Wüst, Eberhard Schäfer, Julia Rausenberger, Andreas Hiltbrunner and Jens Timmer (Photo: Christian Fleck).

considered to be the physiologically active form, while the Pr form is inactive. By absorbing light, the two forms can be converted into each other. While the rate of conversion from the Pr to the Pfr form is greatest in red light, the rate of conversion from the Pfr to the Pr form is highest in far-red light (Fig. 1d). As a result, in red light 85% of phytochrome molecules are in the physiologically active Pfr form, while the proportion of Pfr in far-red light is only 3%. Correspondingly, one would expect phytochrome-dependent responses to be activated by red light and inactivated by far-red light, i.e. that phytochromes would act as "light switches" switched on by red light and switched off by far-red light.

Plants have various types of phytochromes that differ hardly at all in terms of photophysical characteristics. The two most important phytochromes are phytochrome A and phytochrome B. Phytochrome B behaves as one would expect a phytochrome to do: It has the strongest effect in red light, where the Pfr proportion is highest. In contrast, the effect of phytochrome A has been found to be greatest in far-red light. Phytochrome A is the photoreceptor that enables plants to survive beneath a dense cover of vegetation by stimulating the transition to photoautotrophic growth in far-red light. The paradox that the effect of phytochrome A is greatest in far-red light despite the fact that in that part of the spectrum only 3% of all phytochrome A is in

Figure 1:
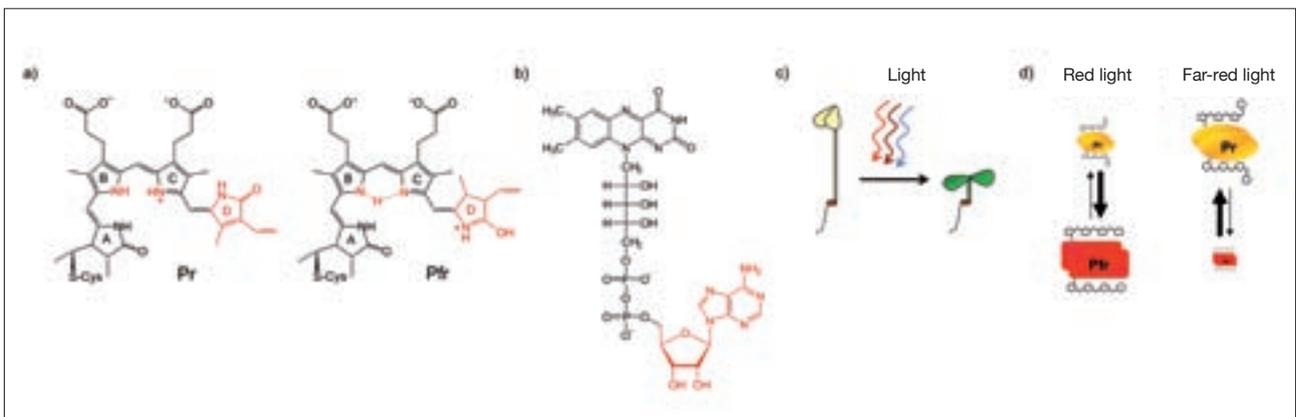
a) Structure of phytochromobilin, a linear tetrapyrrole, in the Pr and Pfr form (differences in red). In phytochromes, phytochromobilin is covalently bonded to a cysteine (Cys) (Source: http://de.wikipedia.org/w/index.php?title=Phytochrom&oldid=92061197).

b) Cryptochromes and phototropins, both blue-light receptors in plants, unlike phytochromes, have a flavin-based chromophore. Cryptochromes have a FAD (overall structure), while phototropins have a FMN (black) (Source: http://de.wikipedia.org/w/index.php?title=Flavin-Adenin-Dinukleotid&oldid=92551360).

c) Skotomorphogenesis vs. photomorphogenesis: Seedlings that grew in the dark (left) use their reserves to grow more elongated, whereas seedlings that grew in the light (right) strive to optimise photosynthesis by growing more leaves (Source: J. Rausenberger, A. Hiltbrunner).

d) The photoreversible phytochrome system: Under the influence of red light the Pr form is converted into the physiologically active Pfr form, which reverts through far-red light back to the Pr form. When exposed to red light, around 85% of phytochromes are in the Pfr form, as opposed to only around 3% in far-red light (Source: J. Rausenberger, A. Hiltbrunner).

Wild type   **Less FHY1 protein than wild type**   **More FHY1 protein than wild type**

Figure 2: *Arabidopsis thaliana* seedlings that have grown for four days in far-red light. The seedlings in the middle have lower and those on the far right higher quantities of the protein FHY1 than is present in wild type seedlings (left) (Photo: A. Hiltbrunner).

the active Pfr form, has preoccupied plant researchers for more than 50 years. In all that time, no convincing explanation of this phenomenon, otherwise known as the high-irradiance response (HIR), was found.

Initial mathematical analyses of this problem led Schäfer in 1975 to a cyclical pattern of reaction as an interpretation of what was known at the time about the kinetics of the phytochrome. Although this model was an important step towards understanding HIR, the actual mechanism at the cellular and molecular level was still not understood.

Further research in the model plant *Arabidopsis thaliana* fundamentally changed the simple picture of the phytochrome as a "light switch". This started with the discovery that in the dark phytochromes are localised in the cell cytosol and are transported to the nucleus only after activation by light, i.e. after conversion into the active Pfr form (Kircher *et al.*, 1999, 2002; Yamaguchi *et al.*, 1999). This was followed by the discovery that two helper proteins, FHY1 and FHL, are needed to transport phytochrome A to the nucleus (Hiltbrunner *et al.*, 2006; Rösler *et al.*, 2007; Genoud *et al.*, 2008). The FHY1 and FHL proteins interact specifically with the Pfr form of phytochrome A, but detach themselves from it after it converts into the Pr form. Surprisingly, the quantity of FHY1 and FHL is much lower than the quantity of phytochrome A they transport into the nucleus. It was therefore postulated that once their work is done, the proteins are transported back out of the nucleus into the cytosol so as to be available for multiple transport cycles. Plants that contain higher quantities of FHY1 show greater inhibition of elongated growth in far-red light and less when the quantity of FHY1 is reduced (Fig. 2). This finding supports the assumption that the quantity of FHY1/FHL has a limiting effect on nuclear transport and on the action of phytochrome A.

One problem of many previous approaches to solving HIR was that they tried to explain the phytochrome system using a simple "light switch" model and did not take the actual dynamics of the photoreceptor and its nuclear transport and interaction with

other proteins into account. A successful theoretical approach had to take account of both the light-dependent nuclear transport and the particular dynamics of FHY1 and FHL, in addition to the specific phytochrome dynamics. Precisely this approach provided the possibility of decoding the unsolved problem of HIR and of being able to explain it at the molecular level (Fig. 3a).

Close combination of experiment and theory decodes counterintuitive high-irradiance response

In laboratory experiments with transgenic *Arabidopsis* plants we were able to show that the helper proteins FHY1 and FHL needed for the nuclear transport of phytochrome A detach themselves from it in the nucleus and migrate back into the cytosol, where they are available for further transports. We were also able to identify a mutated form of phytochrome A that was constitutively present in the physiologically active Pfr form and therefore binds to FHY1 and FHL permanently. Surprisingly, the effect of this mutated form was not greater than the wild type, but rather noticeably reduced. Further experiments showed that nuclear transport of the mutated form was less efficient than of the wild type. Based on the findings of these experiments, we developed a mathematical reaction model for the effect of phytochrome A. The goal was to find out whether this model reflected HIR and what reactions in this network were fundamental to phytochrome A's effectiveness in far-red light.

Due to the high number of open parameters that could not be defined experimentally, we chose a qualitative approach that could be guided by the following question: Are there combinations of parameters for which the reaction model set up (Fig. 3b) meets all the conditions that were previously defined on the basis of experimental observations? Systematic testing of 1,000,000 parameter combinations resulted in approximately 6,000 combinations that met all the predefined conditions. Although maximum effect in far-red light was not one of the criteria for choosing the 6,000 parameter combinations, nearly

all these combinations resulted in a maximum effect in far-red light – and not in the red region of the light spectrum as one would expect, given the photophysical characteristics of phytochromes.

Maximum effect in far-red light, the crucial characteristic of HIR, is therefore an intrinsic characteristic of the reaction model shown in Fig. 3b. However, these computer simulations did not mean that HIR had been understood. Although the mathematical reaction network showed the desired characteristics, it was unclear which were the key components, and how they must be interlinked in order to push the maximum effect from the red light into the far-red light. To find this out, we switched to an abstract way of looking at the problem that was inspired by synthetic biology and designed theoretical networks with phytochromes. If we were to start with the smallest possible network and augment it by systematically adding more and more components, at some point we must find the simplest network that showed the necessary maximum effect

in far-red light. It must then be possible to find this smallest network, which we called the HIR module, again as a sub-unit of the phytochrome A reaction network in Fig. 3b. Interestingly, a linear network with just three phases showed the required maximum effect of HIR at a wavelength of 720 nm, i.e. in far-red light. Mathematical analysis of this network yielded a sur prisingly simple insight: Two photoconversion cycles operating in opposite directions (i.e. Pr→Pfr and Pfr→Pr), combined with a system in which continuous synthesis and breakdown takes place, which is therefore not in balance, are the long sought-after essential network elements. Furthermore, these key components could also be identified as a structural element in the more extensive phytochrome A reaction network shown in Fig. 3b. In laboratory experiments we were also able to establish that in the plant the proteins responsible for transporting phytochrome A to the nucleus, FHY1 and FHL, link the two opposite phytochrome A photoconversion cycles with each other (Fig. 3b).

Figure 3:
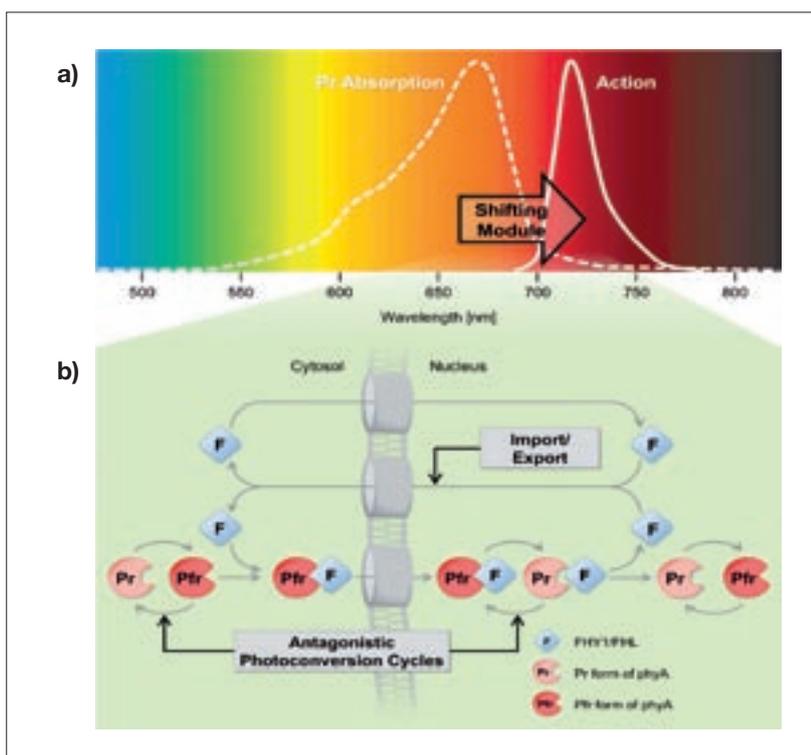
a) A previously unexplained phenomenon: How can a photoreceptor, whose maximum absorption is in the red light region, achieve a maximum effect in far-red light?

b) Main components of the HIR module: Two photoconversion cycles operating in opposite directions and nuclear transport

(Source: A. Hiltbrunner, modified after Rausenberger *et al.*, Cell 2011).

Model calculations show that several concatenated HIR modules lead to a narrowing of the phytochrome A spectrum of activity and that with four HIR modules one obtains a spectrum of activity that best accords with an experimentally defined spectrum of activity for phytochrome A. From an ecological viewpoint, narrowing the spectrum of activity makes good sense, since it allows the plant to separate responses to far-red light much more precisely from those to red light that are mediated by phytochrome B. Laboratory experiments confirm that several HIR modules have to be present in the plant. In future studies we aim to identify by means of experiments the HIR modules predicted by model calculation.

Earlier attempts to explain HIR reached the conclusion that neither the Pr nor the Pfr form was responsible for phytochrome A's detection of far-red light, but an "unknown, intermediary form". However, our approach shows that the Pfr form is indeed sufficient for signal transduction, provided that two conversion cycles operate beforehand in opposite directions. This approach delivers a mechanical explanation of how HIR, and therefore the detection of far-red light, can function at the molecular and cellular level. Therefore, in the course of evolution, plants have not developed a completely new photoreceptor for detecting far-red light. Rather, they use a photoreceptor that is actually optimal for detecting red light and integrate it into a network. This network as a whole achieves a maximum effect in far-red light and thus enables plants to survive beneath a dense cover of vegetation.

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