HEREDITY AND ENVIRONMENT; LIGHT SIGNAL TRANSDUCTION IN PLANTS AND FUNGI.

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Contents

Summary

Plants are sessile organisms that respond to environmental stimuli such as light, temperature, gravity, ions, draught, wounding and infections by viruses and fungi. Plants respond to daily changes of sunlight, recognizing the length of duration of day and night. Plants determine the transition from vegetative growth to flowering under the control of day length, (although length of dark period (night) is critically important). Under nitrogen-limited conditions *Neurospora crassa* enters a sexual cycle from mycelia (vegetative growth) to protoperithecium formation. These processes are under the control of light.

In *Arabidopsis thaliana* light signals are perceived by several photoreceptors including phytochrome A, B, C, D and E, cryptochrome 1 (CRY1) and 2 (CRY2), phototropin 1 (Phot1) and 2 (Phot2), and zeaxanthin. The candidates of signal transducer of light immediately downstream of phytochrome are nucleoside diphosphate kinase, NDK, as well as PIF3 (phytochrome interacting factor 3). The downstream of Phot1 is followed

by NPH 2 (non phototropic hypocotyl 2), NPH3 and NPH4. Phot1 forms a complex with NPH3.

In *Neurospora crassa* putative photoreceptors are WC-1 and WC-2 proteins. The former includes LOV (light, oxygen and voltage) domain, and the latter has PAS domain with putative binding domain of chromophore (cumaric acid) of photoactive yellow protein (PYP), although there is no strict evidence that WC-1 and WC-2 are photoreceptors. Both proteins are also suggested to function as transcription factors.

From *in vitro* analysis the nucleoside diphosphate kinase (NDK-1) was rapidly phosphorylated in response to blue light irradiation of the reaction mixture. NDK-1^{Pro72His} protein from a mutant *ndk-1*^{Pro72His} showed neither autophosphorylation nor protein kinase (phosphate transferring) activity. *ndk-1*^{Pro72His} lacked to cause light induced polarity of perithecia. Purified NDK-1 showed: i) nucleoside diphosphate kinase activity of ATP+GDP ⇄ ADP+GTP, ii) autophosphorylation activity, and iii) protein kinase (phosphate transferring) activity to phosphorylate myelin basic protein. The *ndk-1*^{Pro72His} mutant lacked the latter two activities. By the nucleoside diphosphate kinase activity of i) activity, NDK-1 is suggested to provide GTP in the vicinity of GTP-binding protein. A new signal transduction pathway designated as NDK cascade via the activities of ii) and iii) are suggested. New opsin-1 (NOP-1) bound retinal and generated proton by light illumination. The *nop-1* mutant showed deficiencies in the light induced expression of *nop-1* transcript, and also deficiencies in the light induced production of aerial hyphae and conidium. In *Neurospora crassa* circadian rhythm of conidium formation are well known. By use of *band* (*bd*) strain several mutants in circadian rhythm including *frq* mutants were isolated and analyzed.

1. Introduction

Plants and fungi are sessile organisms which are exposed to severe changes in environmental circumstances such as daily changes of sunlight, temperature, gravity, nutritional ions, draught, wounding, waving by wind, and infections by viruses, bacteria and fungi. Those organisms have developed elaborate genetic systems to respond to these environmental stimuli.

In complete darkness seeds of the pea (*Pisum sativum*) geminate if they have enough moisture (water) and appropriate temperature, elongating long white stems. The leaves have not expanded, and are white or yellow because of the lack of chlorophyll. This type of morphogenesis is designated as scotomorphogenesis, and is shown in Figure 1. Seeds that are relatively large in size show this pattern of germination, enabling the shoot to 'search' for light in deep places in the soil. Seeds, such as those of *Arabidopsis thaliana* and of lettuce (*Lactuca sativa*), which are small in size usually have the ability to geminate under light, in addition to the appropriate conditions of moisture and temperature. This pattern of control of seed germination is derived from natural selection, since small seeds do not have enough nutritional resource to permit a search for light in deep soil. As shown in Figure 1, peas show completely different morphogenesis under light—a phenomenon called photomorphogenesis.

The process of photomorphogenesis includes following steps in the development: 1)

Regulation of a current of ions such as Ca^{2+}, and K^{+} via membrane systems such as plasma membrane. 2) Regulation of stomatal opening. 3) Regulation of chloroplast relocation. 4) Regulation of gene expression including the expression of chalachone synthetase (CHS), etc. 5) Phase shift of the expression of catalase gene, which shows clear circadian rhythm. 6) Developmental regulation of chloroplast formation from proplastid. 7) Regulation of seed germination. 8) Suppression of elongation of stem hypocotyls and epicotyls. 9) Positive phototropism of stem elongation. 10) Transition from vegetative growth to flowering. These processes are precisely under the control of gene regulation.

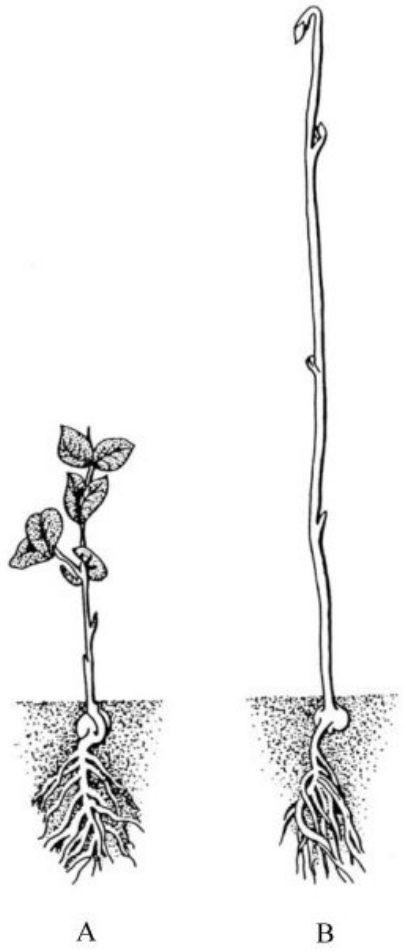

Figure 1. Pea seedlings grown (A) in the light and (B) in complete darkness (etiolated)

In the case of filamentous fungus, *Neurospora crassa* similar light (blue light) regulated morphogenesis can be observed. The life cycle of *Neurospora crassa* is shown in Figure 2. During several processes in the life cycle light has an indispensable role. The major processes controlled by blue light are as follows. 1) Input resistance caused by light in the mycelia indicating light-controlled changes in the current of ions via the plasma membrane. 2) Light-induced accumulation of carotenoids in the mycelia. 3) Light-induced formation of aerial hypha and conida. 4) Under nitrogen-limited conditions blue light induced the formation of protoperithecium. 5) Light-induced perithecial polarity forming a perithecial beak (including the ostiole, a hole through which ascospores shoot out) pointing upward. In darkness it forms at random places on

the perithecia. 6) Positive phototropism of perithecial beak. 7) Light can induce phase shift of circadian rhythm of conidium formation produced by *band* strain. 8) Suppression of circadian production of conidia to constant formation of conidia by strong light.

These processes are precisely controlled by the genetical systems. The description will be focused on the results of research using *Arabidopsis thaliana* and *Neurospora crassa*.

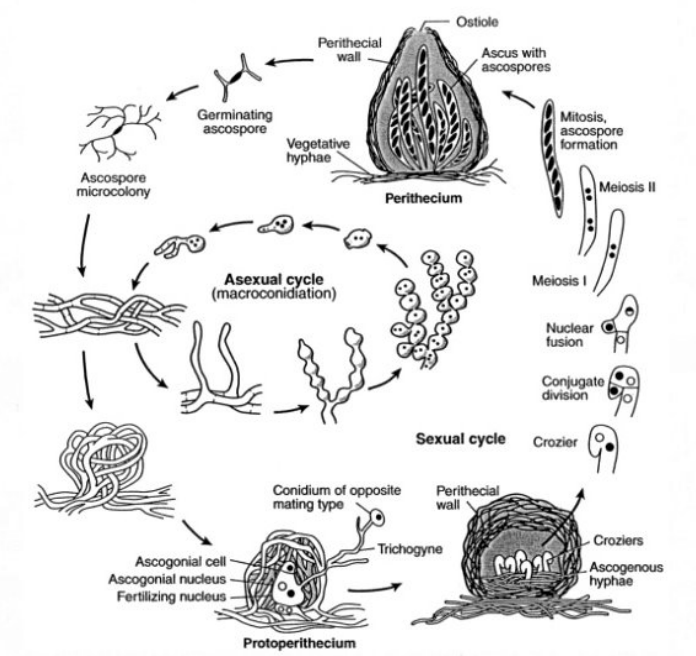

Figure 2. Life cycle of *Neurospora*. The asexual cycle, the inner sequence, depicts the formation of macroconidia from aerial hyphae and their germination to form a new mycelium. Microconidial formation is not shown here. The outer sequence depicts the sexual cycle, originating with a protoperithecium, its fertilization via its trichogyne by a conidium of the opposite mating type, and later events that culminate in the formation of asci, containing ascospores. On the light, nuclear fusion and meiosis are shown in an individual ascus as it develops.

2. Historical aspects of analysis of response to light

The effects of sunlight on the development of plants had been described in the nineteenth century (Henfrey 1852; Kjellman 1885), although Garner and Alland (1920) initiated a period of further rapid development of the research. Flowering response induced by long nights, in the case of short-day plants, could be prevented by the interruption of the dark period by a short pulse of light, called a ‘night break’. Parker and Hendricks (1946) reported that red light was most efficient as a night break, and the effect of red light could be cancelled in some case by a subsequent illumination by far-red light.

The very small seeds of some plant species that can lie for a long period in the soil, can be activated by light, and most efficiently by red light (Flint and McAlister. 1937;

Borthwick *et al*. 1952). Whithrow *et al*. (1957) determined the action spectra for both the red light effect and the reversal effect by using the straightening of the plumular hook of etiolated bean plants by quantification of the efficiency of different wavelengths of light. Borthwick *et al* (1952) and Shropshire Jr. *et al* (1961) determined the action spectra for seed germination, and for photoperiodic induction of flowering (Borthwick *et al* 1948), leading to the discovery of photomorphogenic pigment to phytochrome. Borthwick *et al* (1952) suggested that the chromophore of phytochrome was an open chain tetrapyrrole. The action spectrum of a red/far red response in the induction and reversed reaction of hook opening in bean, and the absorption spectrum of purified oat phytochrome are presented in Figure 3.

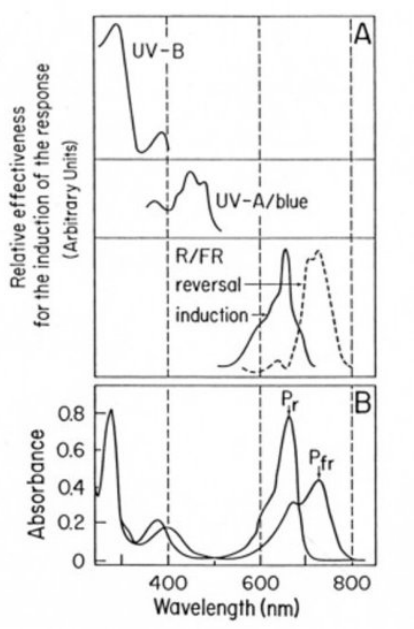

Figure 3. Action spectra for UV-A, UV-B and red/far red response and absorption spectra of purified oat phytochrome. **A** Action spectra for: a UV-B response (anthocyanin synthesis in *Sorghum*), a UV-A/blue response (phototropism in oat), and a red/far red response (induction of an reversal of hook opening in bean). **B** Absorption spectra of purified oat phytochrome in the red-absorbing P_r form and the far red-absorbing P_{fr} form. (Vierstra and Quail 1983)

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Biographical Sketches

Kohji Hasunuma graduated from Tokyo University, Faculty of Science, School of Biology (Plant Science) in 1966, and Graduate School of Biology (Plant Science) in 1971. He was a Research Associate of Tokyo University, Faculty of Arts and Culture, from 1971 to 1979, and Associate Professor of National Institute for Basic Biology, 1979 to 1990. Visiting researcher at Carnegie Institution of Washington at Stanford at 1990. Professor of Yokohama City University, Kihara Institute for Biological Research, The Hirase Prize was awarded to Prof. Hasunuma by Japan Society of Plant Morphology in 2000 for his success in proving the molecular mechanism of light signal transduction in *Neurospora crassa* and *Pisum sativum*.

Naoto Yabe graduated from the Faculty of Science, Department of Biology (Plant Science), Tokyo University, in 1989, and from the Graduate School of Biology (Plant Science) at 1995. Since 1995 he has been a Research Associate of the Kihara Institute for Biological Research, Yokohama City University.