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REVIEW

EARLY EVENTS OCCURRING DURING LIGHT SIGNAL TRANSDUCTION IN PLANTS AND FUNGI

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Abstract – Light signals constitute major factors in regulating gene expression and morphogenesis in plants and fungi. Phytochrome A and B were well characterized red and far-red light receptors in plants. Red light signals increased the phosphorylation of 18 kDa protein, which was identified to be nucleoside diphosphate (NDP) kinase. The NDP kinase catalyzed autophosphorylation and had a protein kinase activity similar to MAP (mitogen activated protein) kinase. As candidates for blue light photoreceptors, cDNAs for CRY1 and CRY2 were isolated. The N-teminal regions of these proteins showed a high homology to DNA photolyase. The 120 kDa protein first detected in *Pisum sativum*, which showed blue light induced phosphorylation was also detected in *Arabidopsis thaliana*. The 120 kDa protein was encoded by the *nph1* gene, which regulated positive phototropism of the plant. In *Neurospora crassa*, blue light irradiation of the membrane fraction prepared from mycelia stimulated the phosphorylation of the 15 kDa protein, which was also identified to be an NDP kinase. Recent progress in understanding early events in light signal transduction mainly in *Pisum sativum* Alaska, *Arabidopsis thaliana* and *Neurospora crassa* was summarized.

INTRODUCTION

In plants light signals are transduced into the cells, inducing several responses in the organism.[1] These light controlled responses are summarized as follows.[2]

(1) Current of ions such as Ca^{2+} and K^{+} *via* plasma membrane.[2]
(2) Stomatal opening.[2]
(3) Movement and localization of chloroplasts.[2]
(4) Light activated expression of genes including *CAB2*.[3]
(5) Phase shift of circadian expression of the *CAB2* gene.[4]
(6) Development of chloroplasts.[5]
(7) Germination of seeds.[6]
(8) Elongation of stem.[7]
(9) Positive bending of stem.[8]
(10) Transition of apical meristem from vegetative growth to inflorescence and to flower bud formation.[9]

These processes are regulated mainly by light, and light is certainly one of the major factors controlling the development of plants.[1]

In *Neurospora crassa*, light signals control more than 10 processes of the fungal development. However, we will review the role of light in the morphogenesis of the sexual organ from protoperithecia to perithecia in this article.[10]

(1) The formation of protoperithecia in wild type on nitrogen deficient medium is stimulated by blue light.

Such a light effect can be detected neither in *wc-1*, nor in *wc-2* mutant.[11]

(2) The perithecial beak formed at the top of perithecia bends toward the direction of light.[12] However, in *wc-1* or *wc-2* mutants, light induced bending of the perithecial beak is not detected.

(3) The ascospores formed and matured in the asci in the perithecia are ejected by osmotic pressure of the cell sap in the asci.

(4) We detected that the prerithecial beak was formed at random places when wild type perithecia was formed under darkness. However, under light irradiation parallel to the solid medium, the perithecial beak was formed at the top of peritehcia. This effect of light inducing the positioning of the beak on the perithecia was defined as "light induced polarity of perithecia." Light induced polarity of perithecia could be detected neither in *wc-1* mutant, nor in *psp* (phosphorylation of small protein) mutant.[13]

PHOTORECPETORS

Phytochrome, a photoreceptor, has been analyzed in

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Table 1. Ranges of absorption spectra of photoreceptors in a plant, *Arabidopsis thaliana*, and fungi.

Designation of light	Range of wave[a] length (nm)	Photoreceptor[b]	Range of absorption	Mutant gene
Vacuum ultraviolet	~ 180			
Ultraviolet	180 ~ 380			
UV-C	180 ~ 280	UVC receptor (?)	(UV-C ~ Blue)	
UV-B	280 ~ 320	UVC receptor (?)	(UV-B ~ Blue)	
UV-A	320 ~ 380	UVC receptor (?)	(UV-A ~ Blue)	
Violet	380 ~ 430	DNA photolyase (type II)[c]	(Violet ~ Blue)	
Blue	430 ~ 490	Cryptochrome 1 (CRY1) Cryptochrome 2 (CRY2)	(UV-A ~ Green)	*hy4*
Green	490 ~ 550	120 kDa Protein	(UV-A ~ Green)	*hph1*
Yellow	550 ~ 590			
Orange	590 ~ 640			
Red	640 ~ 700	Phytochrome A	(UV-A ~ Far-red)	*phyA (hy8, fre1, fhy2)* *(veA)*[d]
		Phytochrome B Phytochrome C Phytochrome D	(Red ~ Far-red)	*phyB (hy3)* *(wc-1)*[e]
Far-red	700 ~ 780	Phytochrome E		
Infra-red	780 ~			

[a] There were small differences in the wavelengths among the references.
[b] Precise molecular characterization was made only on phytochrome A and B.
[c] DNA photolyase could be classified into two types; type I found in bacteria and budding yeast, and type II in cyanobacteria and chlorophyceae. Type I DNA photolyase shows λ_{max} 356 ~ 405 and contains $FADH_2$ and 2,10-methenyl-tetrahydrofolate as prosthetic groups, while type II shows λ_{max} 435 ~ 445 and contains $FADH_2$ and a derivative of 8-hydroxy-5-deazaflavine.
[d] *ve*A gene product in *Aspergillus nidulans* was thought to be a photoreceptor with homology to phytochrome.
[e] WC-1 protein in *Neurospora crassa* includes a homologous region to phytochrome C in *A. thaliana*.

Avena sativa,[14] tomato (*Lycopersicon esculentum*),[7] *Pisum sativum*[2] and *Arabidopsis thaliana*.[15] In *Arabidopsis thaliana*, other groups of photoreceptors were analyzed.[16,17] In a filamentous fungus *Aspergillus nidulance, veA* gene product showed homology to phytochrome.[18] In *Neurospora crassa, wc-1* was reported to have a region homologous to phytochrome C in *Arabidopsis thaliana*.[19] The results of recent research of photoreceptors and the mutants obtained were summarized in Table 1.

Phytochromes

In *Arabidopsis thaliana*, the genes for phytochromes, *PHYA, PHYB, PHYC, PHYD* and *PHYE* were identified by the cloning of cDNAs.[20] As the mutants of *PHYA* gene, *hy8, fre1, fhy2* and *phyA* were isolated by independent groups. Phytochrome A showed responses to a wide range of wavelengths of light from UV-A to far-red. In *Arabidopsis thaliana*, phytochrome A, with this wide range of absorption spectra, could control the germination of seeds. In tomato, phytochrome A functions to stimulate seed germination by red light, but the germination was not stimulated by the sequential irradiation by red and far-red.[6,21] Such a phenomenon was postulated after observing that the *aurea* mutant of tomato, lacking the chromophore of phytochrome, showed altered light responses of gene expression in a wide range of light spectra including blue to far-red.[22] The mutant of phytochrome A showed late flowering in a long day plant, *Arabidopsis thaliana*.[3] We postulate that by lacking of the function of a photoreceptor, the effect of light was reduced and flowering response was delayed.

On phytochrome B, mutants, *hy3, phyB-4* and *phyB-101* were isolated.[23] Upon perception of a red light signal, phytochrome B is converted to Pfr (Pigment absorbing far-red), and upon perception of far-red light, it is converted to Pr (Pigment absorbing red). Pfr elicits signals in the plant cells and stimulates some procesess such as seed germination.[24] However, Pr rather inhibits the stimulated processes. Thus phytochrome B can function as a molecular switch.[15] The mutant of phytochrome B, *hy3*, shows early flowering, but the molecular mechanism is not known yet. One of the possible explanations is that phytochrome B mainly functions under light conditions and chlorophyll a and b in the leaf may shield red light, since both chlorophyll a and b had strong absorption spectra around the red region. However, chlorophyll a and b show almost no absorption in the far-red wave length region. Therefore, phytochrome B may be exposed mainly to far-red in the leaf, and therefore the Pfr of phytochrome B may be eliminated by the effect of far-red light.

The mutants *hy1, hy2* and *hy6* have been detected in the process of the biosynthesis of chromophore of phytochrome or in the process of the attachment of chromophore to the apoprotein of the phytochrome

molecule. These mutants are known to be early flowering.[3]

Phytochrome B was reported to be mobilized to nucleus, when the molecule was converted to Pfr.[25] The protein moleclule of phytochrome C was partly characterized recently, but no mutant was isolated.

Cryptochrome

A hypocotyl elongation mutant, *hy4*, exhibits blindness to the blue light region. The cDNA *HY4* encoded a 75 kDa protein, and was designated as cryptochrome 1 (CRY1). The N-terminal region of the deduced amino acid sequence included a domain homologous to DNA photolyase[16]. The overexpression of CRY1 in *Arabidopsis thaliana* and in tabacco (*Nicotiana tabacum*) resulted in hypersensitivity to blue light and caused the shortening of hypocotyl. CRY1 was suggested to bind FAD and the activated intermediate, FADH ·(flavosemiquinone). Furthermore, the other homologue of CRY1, CRY2 was characterized by the isolation of the cDNA. The overexpression of CRY2, resulted in the abnormal enlargement of the cotyledons.[26] The *hy4* mutant showed late flowering, suggesting that the decrease in the light effect resulted in the slowing of the flowering response. The N-terminal domains of CRY1 and CRY2 had high homology to the DNA photolyase (type I), from bacteria and fungus. Neither CRY1 nor CRY2 exhibited the activity of DNA photolyase. These photoreceptors were localized in the cytosol. In *Arabidopsis thaliana* DNA photolyase (type II) with high homology to those in cyanobacteria and Chlorophyceae was cloned. The *hy4* mutation affected the elongation of hypocotyls under blue light but showed no effect on the bending of hypocotyls under directional light.

A 120 kDa protein phosphorylated in response to blue light

The group of W. R. Briggs identified a 120 kDa protein localized in plasma membrane in the third internodes of etiolated *Pisum sativum*, that was heavily phosphorylated in response to blue light.[17] The third internodes of etiolated seedlings were homogenized immediately after the blue light irradiation. After two successive centrifugations, a crude membrane fraction (microsomal fraction) was prepared. The proteins in the microsomal fraction were phosphorylated by 1 m*M*[γ-^{32}P]ATP at 37 ℃ for 30 sec, and the proteins were separated by SDS gel electrophoresis. A protein with a molecular mass of 120 kDa, which showed an increase in phosphorylation by an increase in blue light irradiation, was detected. The phosphorylation of the 120 kDa protein could also be detected when the membrane fraction was irradiated by blue light.[27] The dose dependency of the blue light irradiation of the third internodes and the intensity of the phosphorylation of the 120 kDa protein was well correlated.

In *Arabidopsis thaliana*, the 120 kDa protein was also detected in the hypocotyls of the seedlings grown under darkness. Blue light enhanced phosphorylation of the 120 kDa protein was very clear. In a mutant, JK224, showing nonphototropic bending of the hypocotyls, the increase in the phosphorylation of the protein was severely reduced. Further, in *nph1* (nonphototropic1) mutant, the 120 kDa protein was missing. The *nph1* mutation was shown to have a deletion of the gene for 120 kDa protein. The cDNA for NPH1 was cloned and the deduced amino acid sequence included a presumed binding domain of chromophore for blue light. At the C-terminal region, amino acid sequences homologous to Ser, Thr kinase were detected. The 120 kDa protein was thus identified as controlling the bending of hypocotyls and the protein was suggested to be a blue light photoreceptor. The *nph2*, *nph3* and *nph4* mutants, showing blindness to blue light, which induces bending of hypocotyl, had the ability to respond normally in the phosphorylation of the 120 kDa protein. Among them, *nph4* lost the capacity to respond to gravity.[28]

Table 2. Summary of the results of *in vitro* systems to analyze the protein moleclules, showing an increase in the binding of ATP, GTP or UTP and in phosphorylation in response to red light and also to blue light.

Molecular size (kDa)	Red	Red and far-red reversible	Blue	Nucleotide binding or phosphorylation
18(15)	+	+	+	phosphorylation
16	+			ATP, GTP
21	+	(±)		GTP, UTP
32	+			GTP
37	+	(±)		GTP
40			+	GTP
50	+			phosphorylation
52				ATP, GTP
55	+			GTP
70	+			phosphorylation
79	+			UTP
83			+	phosphorylation
92	+	+	+	ATP
120	+		+	phosphorylation

Putative photoreceptors for UV-A, UV-B and UV-C

There are a lot of evidences suggesting the existence of the photoreceptors UV-A, UV-B and UV-C, mainly controlling the expression of genes including chalchon synthetase. However, the existence of these photoreceptors was only presumed with no direct evidence[29].

EARLY PROCESSES OF LIGHT SIGNAL TRANSDUCTION THROUGH NDP KINASE

We developed an *in vitro* system for the light signal transduction that used the membrane and soluble

Table 3. Putative functions of NDP kinases

1. NDP kinase are known to catalyze following enzymatic reactions

$$N_1TP + E \rightleftharpoons N_1DP + E\text{-}P$$
$$E\text{-}P + N_2DP \rightleftharpoons N_2TP + E$$
$$N_1TP + N_2DP \rightleftharpoons N_1DP + N_2TP$$

   NDP kinase has His 177, which is phosphorylated and functions as histidine kinase. NDP kinase is autophosphorylated at Ser 112 and Ser 115. NDP kinase phosphorylated histone H1, ovalvumin and myelin basic protein.[37, 38, 39]
2. By use of the extract of *Pisum sativum* Alaska, *Arabidopsis thaliana, Oryza sativa*, and *Neurospora crassa* light irradiation could increase the phosphorylation of NDP kinase.[13, 32]
3. The effect of NDP kinase to the heterotrimeric GTP-binding protein.
   i) NDP kinase activated the GTP-binding proteins, Gs, Gi and Gt by supplying GTP in the vicinity of GTP-binding protein.[40]
   ii) K^+ channel controlled by GTP-binding protein could be activated by the process descrived in i).[41]
4. The effect of NDP kinase to small GTP-binding protein.
   i) NDP kinase could interact with Ras protein. The mutation in *Drosophila melanogaster* of *pn(prune)* encodes a protein with high homology to GAP(GTPase activating protein) interacting with Ras caused an additional mutation, *k-pn* (killer of prune) leading to the lethality of the fly. The *k-pn* mutation was determined to be the mutation of NDP kinase (Awd; Abnormal wing disc), *Awd k-pn*.[42, 43, 44, 45]
   ii) Small GTP-binding protein, rho/rac was reported to interact with NDP kinase. The rho/rac localized to the cytoskeleton.[42, 45, 46]
   iii) Small GTP-binding protein localizing in the membrane of golgi apparatus could be activated *via* NDP kinase.
5. NDP kinase was suggested to localize to cytoskeleton. The phenomenon coinsides with the association with rho/rac protein.[42, 45, 46]
6. NDP kinase (Nm23-H2) could function as a transcription factor for *c-myc*.[47] GT-1 is known to be a transcription factor in plants. GT-1 is one of the major transcription factors activating light activated *CAB1* and *RBCS* genes.
7. NDP kinase localizing in mitochondria is encoded by nuclear genome and different in the molecule from those localized in the cytosol. NDP kinase in mitochondria formed a complex with succinyl CoA synthetase and phosphorylate, its α-subunit.[48, 49]
8. In *Spinacia oleracea*, 2 different NDP kinases were detected, and the cDNA for one of them was isolated. The function of these NDP kinases was not determined.[50]
9. In human, rat and mouse, one of the NDP kinases (Nm23-H1) could suppress, or in some cell types promote, the metastasis of the tumor.[48, 51]
10. In human, a new NDP kinase, Dr-nm23 was detected, controlled the differentiation of cells and regulates the apoptosis. The polypeptide of N-terminal 80 amino acids of NDP kinase could induce differentiation of blood stem cells.[52]

fractions of the third internodes of etiolated *Pisum sativum* Alaska, seedlings of *Arabidopsis thaliana*.[30] The analysis of the binding of ATP, GTP and UTP to the plasma membrane was summarized in Table 2. About 10 species of proteins ranging from 16 kDa to 92 kDa were detected to change the ability to bind ATP, GTP or UTP at 0 ℃ for 5 min in response to red light. Among them the 92 kDa protein was shown to bind ATP in response to red light. Sequential irradiation by red and far-red light diminished the effect of red light. Among the other proteins, 32 kDa and 37 kDa may be GTP-binding proteins, and they showed an increase in the binding of GTP in response to red light irradiation of the third internodes.[31]

The phosphorylation of proteins in the membrane fraction and the soluble fraction was also examined by using of 4×10^{-8} M[γ-^{32}P]ATP at 0 ℃ for 15 sec. The proteins were separated by 2 dimensional gel electrophoresis. As shown in Table 2, 18 kDa proteins (formerly 15 kDa proteins) were phosphorylated in response to red light irradiation. The results showed an increase in the phosphorylation of the 18 kDa proteins in response to red and also to blue light irradiation. The sequential irradiation of the third internodes by red and far-red reduced the stimulation of the phosphorylation of the 18 kDa proteins.[32,33] Similar results were obtained by use of etiolated seedlings of *Arabidopsis thaliana*. The 18 kDa proteins were purified to the homogeneity and identified to be NDP kinase. The purified NDP kinase showed not only γ-phosphate transferring activity to NDP but also autophosphorylation activity and protein kinase activity phosphorylating myelin basic protein (MBP), which is the substrate of MAP (mitogen activated protein) kinase. From *Pisum sativum* Alaska cDNA for NDP kinase (No.D86052) was isolated. From the deduced amino acid sequence it contained His (118) showing histidine kinase activity, which may control three activities of the NDP kinase above described (submitted). Possible role of NDP kinase to signal transduction was reported by using of *Avena sativa*.[34]

The putative functions of NDP kinases were summarized in Table 3. One of the major functions of NDP kinase may be the activation of the GTP-binding protein by supplying GTP in the vicinity of the GTP-binding protein. Such a hypothesis is consistent with the results reported by the group of N. H. Chua, who showed the involvement of GTP-binding protein using tomato *aurea* mutant. An administration of GTP to the stem cells resulted in an increase in the gene expression of *CAB1*.

For the expression of *CAB1* gene, *HY5, COP1, COP9, DET1, DET2* and FUS6 were suggested to be involved in the process of light signal transduction.[20] The NDP kinase is considered to be functioning upstream of these proteins. However, an actual demonstration has not yet been done. COP1 had a homologous domain as that of

Table 4. Summary of the results of *in vitro* systems using a crude membrane fraction of *Neurospora crassa* to analyze protein moleclules, showing an increase in the binding of ATP or GTP, in the ADP-ribosylation and in the phosphorylation.

Molecular mass (kDa)	fraction	UV-A(U) or Blue(B)	ATP or GTP-binding ADP-ribosylation phosphorylation	WT	*wc-1*	*wc-2*	*wc-1* + *wc-2*	*psp*
58	Memb.	U > B	ATP, GTP	+	-	-	-	ND
77	Memb.	U > B	ATP, GTP	+	-	-	-	ND
83	Memb.	U > B	ATP, GTP	+	-	-	-	ND
129	Memb.	U > B	ATP, GTP	+	-	-	-	ND
38	Sol.	U < B	ADP-ribosylation	+	+	+	ND	+
56	Sol.	U < B	ADP-ribosylation	+	+	+	ND	+
16	Memb.	U < B	phosphorylation	+	-	-	+	ND

ND : not determined, Memb. : membrane fraction. Sol. : soluble fraction.

Gβ subunit of GTP-binding protein and thus may interact with GTP-binding protein.[35] Also COP1 included Zn^{2+} ring finger domain characteristic of a transcription factor. COP1, further more, had a coiled coil domain, which had the capacity to bind CIP1 (COP1 interacting protein). *COP9* was cloned and the protein formed a complex with COP8 and COP11.[36] *DET1* was also cloned and *DET1* protein was suggested to function to regulate the promoter region of the light activated genes. The cDNA, *FUS6* showed to have a homologous sequence accepting phosphorylation by protein kinase C.

In vitro system to analyze light signal transduction in Neurospora crassa

In vitro systems to analyze light signal transduction that used the membrane and soluble fractions of the mycelia of *Neurospora crassa bd* strain was established. To the membrane fraction, [α-^{32}P]ATP and [α-^{32}P]GTP were mixed. After 1 sec irradiation of UV-A light to the mixture, it was incubated at 25 ℃ for 1 hr. After the cross linking of the nucleotides to the proteins, proteins were separated by SDS gel electrophoresis. As summarized in Table 4, 58 kDa, 77 kDa, 83 kDa, 129 kDa proteins were identified as showing an increase in the binding of ATP/GTP to the proteins.[53] At 25 ℃, 77 kDa protein exhibited an increase in the binding of ATP in 2 min.

[^{32}P]NAD was mixed to the soluble fraction and the mixture was irradiated by blue light for 15 to 100 sec at 0 ℃. The increase in the ADP-ribosylation of 38 kDa protein was detected. Further irradiation of the mixture by blue light for 100-400 sec increased ADP-ribosylation to 56 kDa protein.[54]

To observe the blue light effect on the phosphorylation of proteins, membrane fraction was mixed with 4×10^{-8} M[γ-^{32}P]ATP and the mixture was irradiated with blue light for 1 sec. 5 sec after the irradiation the reaction was stopped by adding SDS-sample buffer. The protein phosphorylated was separated by SDS gel electrophoresis. 15 kDa protein showed an increase in the phosphorylation when wild type mycelia was used as a source of the crude membrane fraction. When those prepared from *wc-1* and *wc-2* mutants were used, no increase in the phosphorylation of the 15kDa protein could be detected. However, when the membrane fractions from *wc-1* and *wc-2* were well mixed, an increase in the phosphorylation of the 15 kDa protein by blue light was detected.[55]

Analysis of psp mutant in Neurospora crassa

We detected a mutant, *psp* (phosphorylation of small protein), with no capacity to phosphorylate the 15 kDa protein. The *psp* mutant was detected among *wc-1* mutant strains as a double mutant (FGSC#3628). By genetic analysis, the *psp* was localized on LGVR between *al-3* and *his-6*. Since *wc-1* is mapped on LGVIIR, *psp* was unambiguously separated from *wc-1*. In *Neurospora crassa* the trait of perithecia is known to be solely dependent on the nature of the protoperithecial mother. By use of wild type (74A and *bd* strain), *psp* strain, *wc-1* strain and the double mutant *psp*; *wc-1*, we checked the light induced morphogenesis of perithecia.

Under darkness, wild type formed a perithecial beak at a random place on each perithecium, as though the perithecium was rolling down on the solid media. The beak is a pore through which ascospores in the asci are ejected. However, when the wild type perithecia were grown under the light parallel to the solid media, the perithecial beak was formed at the top of the perithecia. The reaction detected by us was defined as "light induced ordering of polarity of perithecia". In case of the *psp* mutant the effect of light to induce the polarity of perithecia was completely missing. However, the *psp*

mutant showed positive bending of the beaks to the direction of light, similar to those of wild type. The mutant strains, *wc-1* and *psp*; *wc-1* showed blindness in both polarity of perithecia and positive bending of perithecial beaks. From these results, *wc-1* was determined to be epistatic to *psp*. PSP protein is considered to be functioning down stream of the WC-1 protein.[13, 56] The cloned *wc-1* revealed that the protein includes a homologous region to phytochrome C of *Arabidopsis thaliana*.[19]

Characterization of psp mutation

The gene product of the *psp* mutant should be characterized further. The 15 kDa protein was purified and identified to be NDP kinase, and the cDNA for NDP kinase from wild type and *psp* mutant were cloned. The cDNA from the *psp* mutant included the mutation Pro72His.[57] The assumption is that the alteration of the function of NDP kinase in the *psp* mutant caused the alteration of the phenotype of *psp*, and further research is now in progress to confirm this.

NDP kinase had phosphate transferring activity from NTP to NDP, could be autophosphorylated and had protein kinase activity phosphorylating myelin basic protein, and may function similar to MAP kinase. These three activities of NDP kinase may elicit signals downstream, finally to the light induced ordering of perithecial polarity.

FUTURE PROSPECTS AND CONCLUDING REMARKS

The flowering time of long day and short day plants depends on the length of the period of darkness. The night break effect of a short day plant, that is, light irradiation during the night, caused the plant to start a new night period. This phenomenon may be similar to the reset of the end of the day in the research of circadian rhythms.

Under darkness in cyanobacteria[58] and in *Neurospora crassa*, several genes were expressed depending on the circadian time. *CAB1* gene expression in *Avena sativa* was also known to be expressed under the control of circadian rhythms.[59] In *Arabidopsis thaliana*, *CAB2* gene fused with GFP (Green fluorescent protein) was expressed. The abundance of mRNAs of *CAB1* and *CAB2* in these organisms were under the control of circadian rhythms and also the gene expression was under the control of phytochromes.[4] In each plant, under darkness, several other genes may also be expressed which are under the control of circadian rhythms. The expression of those genes may be controlled by the second messengers including cAMP, cGMP, Ca^{2+}, inositoltrisphosphate (InsP3), and diacylglycerol (DG), by the activation of protein kinases depending on these second messengers,[60] and further by the activation of protein kinase cascade including MAP kinase. MAP kinase cascade itself is up-regulated by protein kinase C and down-regulated by Protein kinase A.[61] Although the function of cAMP in the green leaf of a plant is not so promising, in the embryo of wheat the gene activation of amylase was promoted by cAMP. In plants, except for Ca^{2+}, the function of other second messengers is not yet established. However, research to establish the role of these factors are also of considerable interest. In plants, the existence of GTP-binding proteins were established.[62] GTP-binding proteins are known to control the concentration of second messengers. Therefore, light stimulated phosphorylation of NDP kinase may change the intracellular concentration of second messengers via NDP kinase and GTP-binding proteins. In *Lemna paucicostata* 381, circadian oscillations of the concentrations of cAMP and cGMP have been reported.[63] In *Neurospora crassa*,[64] Paramecium micronucleatum, and pineal of a bird, circadian oscillations (changes) of the concentrations of cAMP and cGMP have also been reported. These results suggest that second messengers such as cAMP and cGMP may be one of the components of the circadian rhythm. Such a model was presented by Hasunuma (1987)[60] at the Symposium held at Cheju National University.

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