Journal of Bioenergetics and Biomembranes, Vol. 35, No. 1, February 2003 (© 2003)

Putative Functions of Nucleoside Diphosphate Kinase in Plants and Fungi

Kohji Hasunuma,[1,2] Naoto Yabe,[1] Yusuke Yoshida,[1] Yasunobu Ogura,[1] and Tohru Hamada[1]

Received September 1, 2002; accepted October 4, 2002

The putative functions of NDP (nucleoside diaphosphate) kinases from various organisms focusing to fungi and plants are described. The biochemical reactions catalyzed by NDP kinase are as follows. (i) Phosphotransferring activity from mainly ATP to cognate NDPs generating nucleoside triphosphates (NTPs). (ii) Autophosphorylation activity from ATP and GTP. (iii) Protein kinase (phosphotransferring) activity phosphorylating such as myelin basic protein. NDP kinase could function to provide NTPs as a housekeeping enzyme. However, recent works proved possible functions of the NDP kinases in the processes of signal transduction in various organisms, as described below.

1) By use of the extracts of the mycelia of a filamentous fungus *Neurospora crassa* blue-light irradiation could increase the phosphorylation of a 15-kDa protein, which was purified and identified to be NDP kinase (NDK-1). By use of the etiolated seedlings of *Pisum sativum* cv Alaska and *Oryza sativa* red-light irradiation of intact plants increased the phosphorylation of NDP kinase. However, successive irradiation by red–far-red reversed the reaction, indicating that phytochrome-mediated light signals are transduced to the phosphorylation of NDP kinase.
2) NDP kinase localizing in mitochondria is encoded by nuclear genome and different from those localized in cytoplasm. NDP kinase in mitochondria formed a complex with succinyl CoA synthetase.
3) In *Spinicia oleraceae* two different NDP kinases were detected in the chloroplast, and in *Pisum sativum* two forms of NDP kinase originated from single species of mRNA could be detected in the choloroplast. However, the function of NDP kinases in the choloroplast is not yet known.
4) In *Neurospora crassa* a Pro72His mutation in NDP kinase (*ndk-1*^{Pro72His}) deficient in the autophosphorylation and protein kinase activity resulted in lacking the light-induced polarity of perithecia. In wild-type directional light irradiation parallel to the solid medium resulted in the formation of the perithecial beak at the top of perithecia, which was designated as "light-induced polarity of perithecia." In wild-type in darkness the beak was formed at random places on perithecia, and in *ndk*^{Pro72His} mutant the perithecial beak was formed at random places even under directional light illumination. The introduction of genomic DNA and cDNA for *ndk-1* demonstrated that the wild-type DNAs suppressed the mutant phenotype.

With all these results except for the demonstration in *Neurospora*, most of the phenomena are elusive and should be solved in the molecular levels concerning with NDP kinases.

KEY WORDS: *Awd* (*A*bnormal *w*ing *d*isc formation); non-metastatic (nm)23; GTP-binding protein; light signal transduction; phytochrome; protein phosphorylation.

[1] Kihara Institute for Biological Research and Graduate School of Integrated Science, Yokohama City University Maioka-cho, Totsuka-ku, Yokohama, Japan.
[2] To whom correspondence shoud be addressed.

0145-479X/03/0200-0057/0

INTRODUCTION

NDP kinase (EC.2.7.4.6, ATP: nucleoside diphosphate phosphotransferase; nucleoside diphosphate kinase) has been detected independently by Berg and Joklick (1953) and Krebs and Hems (1953). NDP kinase has been considered to be a regulator of nucleotide pools catalyzing following reactions.

$$N_1TP + E \rightarrow N_1DP + E \sim P$$

$$\underline{E \sim P + N_2DP \rightarrow N_2TP + E}$$

$$N_1TP + N_2DP \rightarrow N_1DP + N_2TP$$

Growing evidences have been accumulated that NDP kinases are not only housekeeping enzymes, but also enzymes functioning in the central part of signal transduction in bacteria, fungi, plants, invertebrate, and vertebrate. The major putative functions in the signal transduction are summarized as follows.

1) By the reaction catalyzing $ATP + GDP \rightarrow ADP + GTP$, NDP kinases elevated the concentration of GTP in the vicinity of GTP-binding proteins, and thus activated GTP-binding proteins.
2) NDP kinase includes histidine kinase as an active center and autophosphorylated at Ser residues.
3) NDP kinases localized in the vicinity of cytoskeleton, and could function coupled with small GTP-binding proteins rho/rac, localized in mitochondria and also in chloroplast.
4) NDP kinase could function as a transcription factor for the activation of c-*myc* gene.
5) NDP kinases have protein kinase activity tranferring the γ-phosphate to proteins, such as myelin basic protein (MBP), which is the substrate of MAP (mitogen-activated protein) kinase.

These basic reactions observed in a wide range of living organisms was suggested to be functioning. However, the precise and strict demonstration of the function remained to be analyzed.

THE FUNCTION OF NDP KINASES IN FUNGI

Dictyosterium discoideum

In *Dictyostelium discoideum* two cDNAs encoding for Gip 17 and Guk 7.2 proteins were isolated. The protein Gip 17 was purified from the amoebae and from the extract of *Escherichia coli* expressed with the cDNA, and identified to be NDP kinase. Gip 17 is 77% homologous to human Nm23-H1 protein and 75% homologous to *Drosophila melanogaster* Awd protein. The mRNAs detected by these cDNAs revealed rapid reduction in the abundancy during the focus formation of amoebae caused by nutritional deprivation (Lacombe *et al.*, 1990; Wallet *et al.*, 1990). However, overexpression of *Gip 17* in amoebae did not cause any effect on the differentiation of it. The overexpression of mutant cDNA with His 122 Cys resulted in the normal differentiation of the amoebae. Pro 105 Gly mutation caused alteration in the stability of the protein, and the overexpression of the cDNA in the amoebae resulted in the increase in the overall activity of the NDP kinase.

In *Drosophila melanogaster* $Awd^{k\text{-}pn}$ mutation was identified to be a mutation Pro 97 Ser and resulted in the instability of the NDP kinase. Similar examination was performed by use of Gip 17 cDNA. Pro 100 Ser and Pro 100 Gly mutations caused alteration in the process of homohexamer formation and of the folding of each protein molecule. In in vivo homohexamer may be functioning and alteration in the molecule by Pro 97 Ser mutation in $Awd^{k\text{-}pn}$ may cause difficulty in the process of homohexamer formation explaining the mutation to be dominant (Lascu *et al.*, 1993).

The analysis of the active center of NDP kinase has been performed by the replacements of amino acids, Lys16, Tyr 56, Arg 92, Thr 98, Arg 109, Asn 119, Ser 124, and Glu 133. These amino acids are conserved in NDP kinases cloned in the cDNAs from several organisms. All of these mutations resulted in the reduction of the specific activity of the enzyme. Lys 16, Arg 109, and Asn 119 were important in the intermolecular association of the enzyme molecules. Lys 16 and Asn 119 were functioning in the intermolecular association of the enzyme molecules, and in the catalytic function of the enzyme. Arg 109 interacted with the phosphate group of the substrate. All of these amino acids tested were found to be a functional part of the enzyme forming catalytic domains and forming domains necessary to assume homohexamer (Tepper *et al.*, 1994).

On the purified NDP kinase (Gip 17) X-ray analysis of the homohexamer has revealed a large loop at the mutated region of Pro 92 Ser of $Awd^{k\text{-}pn}$. This region functions for the formation of homotrimer. Four α-helixes interlocated with four β-sheets constituted the NDP kinase and a large loop containing *k-pn* mutation in between $\alpha 3$ and $\beta 4$ (Dumas *et al.*, 1992).

The nuclear gene *Gip 17* encodes an NDP kinase locating in the cytosol, and *Guk 7.2* encodes the other NDP kinase to be localized in mitochondria. The leader sequence to mitochondria at the N terminus of nascent Guk 7.2 included 57 amino acids, which are removed after the translocation into mitochondria. The mitochondrial

NDP kinase (Guk 7.2) shears 3% of total amount of NDP kinase. The function of NDP kinase in mitochondria and also in the cytosol is not yet known (Troll *et al.*, 1993).

In *Dictyostelium discoideum* a receptor for cAMP is well characterized. Upon reception of cAMP by the receptor GTP-binding protein is activated with subsequent activation of phospholipase C. The membrane fraction prepared from amoebae was mixed with [γ-^{32}P]ATP and cAMP, and the results identified two proteins of 20 and 36 kDa phosphorylated specifically in the presence of cAMP. The 20-kDa protein is shown to be NDP kinase by immunoprecipitation by use of the specific antibody. The 36-kDa protein was identified to be succinyl CoA synthetase. The membrane fraction mixed with 50 μM GDP and [γ-^{32}P]ATP in the presence of cAMP showed twofold increase in the formation of GTP compared with that in the absence of cAMP. The result indicated that the receptor for cAMP may be activated by the presence of cAMP and NDP kinase may be activated catalyzing ATP + GDP $\rightarrow$ ADP + GTP. However, NDP kinase is known to bind cAMP. Further analysis is needed to clarify this (Bominaar *et al.*, 1993).

The transition state of histidine phosphorylation in the crystal structure of NDP kinase with MgADP was analyzed by use of either AlF3, $AlF4^-$, or $BeF3^-$. AlF3 was found to be suitable to analyze the transition state of phosphoryl transfer to active center histidine to discriminate from the ground state by X-ray crystallographic analysis (Xu *et al.*, 1997a).

The phosphorylation from ATP to nucleoside diphosphates catalyzed by NDP kinase was analyzed by Asn119Ala mutant of *Dictyostelium* NDP kinase by use of AZT (3′-azido-3′deoxythymidine) as substrate, by X-ray crystallographic analysis. The analog AZT binds to the same position as the normal substrate thymidine diphosphate. However, the azido group that replaces the 3′OH of the deoxyribose in AZT caused the displacemant of the lysine side chain working in catalysis. Further 3′OH and oxygen connecting β- and γ-phosphate form internal hydrogen bond, which is required to transfer the γ-phosphate (Xu *et al.*, 1997b).

Schizosaccharomyces pombe

cDNA for NDP kinase was cloned in *Schizosaccharomyces pombe* and the gene *ndk1* was disrupted. The mutant was viable and did not show apparent phenotype. However, when cDNA for NDP kinase with the mutation Cys 116 Tyr just in the neighbor of His 117 to be phosphorylated as histidine kinase was overexpressed in the mutant strain with disrupted *ndk1* gene, the strain showed reduction in the rate entering into the mating process and also in the process of spore formation, possibly by the effect of dominant negativity, since the overexpressed mutant proteins may form complex with wild-type protein and reduce the residual activity of NDP kinase (Izumiya and Yamamoto, 1995). NDP kinase was suggested to activate GTP-binding protein by supplying GTP in the vicinity of it. In the organism mating pheromone is known to stimulate a homologue of Ras, and the spore formation is regulated via Ras protein and via the homologues of protein kinases constituting MAP kinase cascade. As one of the possible explanation, the reduction of the activity of Ras protein because of the reduction of the activity of NDP kinase may be reflected to the reduction of mating process.

Saccharomyces cerevisiae

In *Saccharomyces cerevisiae* NDP kinase was purified and the biochemical characteristics were analyzed. The gene *YNK* was cloned and the gene was disrupted or overexpressed. However, no apparent phenotype concerning growth rate, spore formation, mating reaction, or morphogenesis could be detected. By the disruption of *YNK* about 10% of remaining activity of NDP kinase could be detected (Fukuchi *et al.*, 1993).

In *Saccharomyce cerevisiae* and also *Schizosaccharomyces pombe*, there may be the activity of mitochondrial NDP kinase as well as in *Dictyostelium discoideum*. Such gene was not yet cloned in these organisms and the extent of the contribution of the mitochondrial NDP kinase in the other site of cell is not yet known.

Further subunit V of cytochrome *c* oxidases in *Saccharomyces cerevisiae*, *Neurospora crassa*, and heart muscle of bovine were known to show high homologies to NDP kinase (Capaldi *et al.*, 1986; Cumsky *et al.*, 1987; Power *et al.*, 1984). Since NDP kinase is known to form protein complex, the NDP kinase in mitochondria may form complex with cytochrome *c* oxidase. Such a possibility remained to be clarified.

Neurospora crassa

By use of the membrane fraction of the mycelia of *Neurospora crassa band* (*bd*) strain, an *in vitro* system to explore light signal transduction was developed. Blue-light irradiation of the extract mixed with 4×10^{-8}M [γ-^{32}P]ATP caused an increase in the phosphorylation of a 15-kDa protein (Fig. 1). Membrane fraction from neither *wc-1* nor *wc-2* showed an increase in the phosphorylation of the 15-kDa protein. However, the mixing of *wc-1* and *wc-2* membranes recovered the ability to stimulate the phosphorylation of the 15-kDa protein

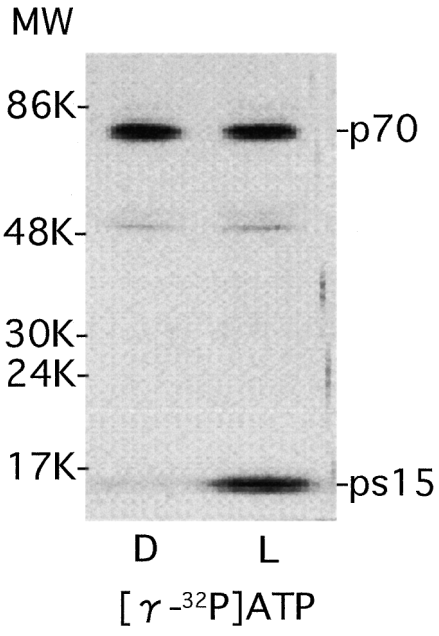


Fig. 1. Blue-light-induced phosphorylation of 15-kDa protein (ps 15; phosphorylated small 15-kDa protein). Crude membrane fraction from *band* mycelia was mixed with 4×10^{-8}M[γ-^{32}P]ATP and with [α-^{32}P]ATP, and the mixture was irradiated by blue light for 1 and 5 s after blue-light irradiation the reactions were stopped by adding SDS-sample buffer. The proteins were separated by SDS-polyacryl amide gel electrophoresis (PAGE). The radioactivity of proteins was detected by X-ray film. D, dark control; L, blue light; ps 15, 15-kDa protein to be identified as NDK, and designated NDK-1. 70 kDa, 70-kDa protien (Oda and Hasunuma, 1994).

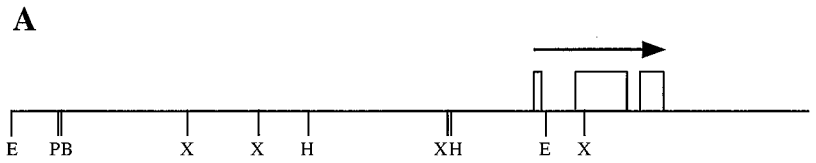
(Oda and Hasunuma, 1994). WC-1 and WC-2 are known to be putative photoreceptors, and they are suggested to function as transcription factors.

The protein purified showed characteristics of NDP kinase and designated to be NDK-1. The purified protein showed K_m values from 0.04 to 0.77 mM for CDP, GDP, UDP, dCDP, dGDP, and dTDP by using the pyruvate kinase-lactate dehydrogenase assay. The purified NDK-1 also showed protein kinase activity for myelin basic protein with the K_m value of 0.36 mM. cDNA for the NDP kinase, *ndk-1* (No. D88148), was cloned (Fig. 2) (Ogura *et al.*, 1999a,b). A mutant *psp* (phosphorylation of small protein) with no phosphorylation of the 15-kDa protein was isolated, and the gene was mapped on LGVR between *al-3* and *his-6*. From the *psp* mutant a cDNA for NDP kinase was isolated by use of RT-PCR, which included an amino acid replacement Pro 72 His (Oda and Hasunuma, 1997; Ogura *et al.*, 1999a,b).

psp mutant showed blindness in the process of the morphogenesis of perithecial beak formed for the ejection of ascospores. Wild-type strain formed the perithecial beak at the random places of it in darkness, as though the perithecia were rolling down on the solid medium. When the culture was illuminated by a light parallel to the solid medium, the perithecia form the beak at the top of perithecia. The reaction was defined as "light-induced ordering of the polarity of perithecia," which was completely missing in the *psp* mutant. *psp* mutant showed temperature-dependent morphogenesis of aerial hyphae when it was grown in a small tube with liquid medium. At 25°C the aerial hyphae of wild type and *psp* grow up to the middle part of small tubes with liquid Fries minimal medium. At 37°C wild type showed enhancement in the growth of aerial hyphae to the top of the small tube. However, *psp* showed very reduced growth at the temperature. Complementary DNAs and genomic DNAs from wild type had the capacity to complement these two defects shown in *psp* mutants (Ogura, Yoshida, Yabe, and Hasunuma, unpublished).

His-tagged NDK-1 and NDK-1 Pro72His were expressed in *E. coli* and these proteins were purified by Ni^{2+}-chelating column. His-tagged NDK-1 showed three activities of (i) γ-phosphate-transferring activity of ATP + GDP $\rightarrow$ ADP + GTP, (ii) autophosphorylation activity, and (iii) protein kinase activity phosphorylating myeline basic protein. Immediately after the preparation of His-tagged NDK-1 Pro72His, the mutated enzyme showed these three enzyme activities. However, after standing for 1 day the enzyme lost rapidly the activities described in (ii) and (iii). The Km's of γ-phosphate transferring activity in both enzymes were roughly unaffected by the mutation. However, the V_{max} of protein kinase activities of His-tagged NDK-1 Pro72His was one order lower compared with His-tagged NDK-1. Wild-type cDNA and genomic DNA transformed into *psp* mutant strain complemented the defects caused by the inability of autophosphorylation and protein kinase activities. These results indicate that NDK-1 not only elicit the light signal by the γ-phosphate-transferring activity of ADP + GDP $\rightarrow$ ADP + GTP, but also elicit light signals through autophosphorylation and protein kinase activities (Hasunuma, 2000).

To the *psp* mutant, cDNA of genomic DNA for *ndk-1* was introduced and the transformants, which showed wild-type amounts of NDK-1 as assayed by Western blotting and also showed wild-type level of phosphorylation activity of NDK-1. These transformants showed complete recovery of "light-induced ordering of perithecial polarity" as shown in Fig. 3. From this experiment *psp* mutation was identified to be *ndk-1*^{Pro72His}. The *ndk-1*^{Pro72His} showed about 25% in the amount of NDK-1 protein compared with that of wild type. GST-NDK-1^{Pro72His} protein purified from *Schizosaccharomyces pombe* showed about 5% of autophosphorylation activity and 2% of V_{max} of protein



B

```
NNK-1        MSNQEQTFIAVKPDGVQRGLVGNIISRFENRGFKLVAMKL   40
S.c.       MS.QT.R..............SQ.L....KK.Y....I..   41
Sc.p.       ..-T.........A.....I.Y...K..LK.Y..R.L.F   39
HUMAN       .A.C.R....I..........E..K...QK..R..GL.F   40
RAT         .A.L.R....I..........E..K...QK..R.....F   40
D.m.       MAA.K.R...MV..........K..E...QK......L.F   41
D.d.    MSTNKV.K.R..L.V.....A.....E..A.Y.KK..V..GL.Q   44
M.x.         A-I.R.LSII....LEK.VI.K......EK.L.P..IR.   38
E.c.        .A-I.R..SII..NA.AKNVI...FA...AA...I.GT.M   39
                       *

NNK-1        TQPGQAHLEKHYEDLNTKPFFAGLIKYMNSGPICAMVWEG   80
S.c.         VKADDKL..Q..AEHVG....PKMVSF.K....L.T....   81
Sc.p.        LV.SRDLV.E..AEHKG...YEK.VGF.A...V...I...   79
HUMAN        M.ASEDL.KE..V..KDR......V...H...VV......   80
RAT          LRASEE..KQ..I..KDR...P..V.......VV......   80
D.m.         .WASKEL.....A..SAR...P..VN......VVP.....   81
D.d.         LV.TKDLA.S..AEHKER...G..VSFIT...VV...F..   84
M.x.         QHLS..QA.GF.AVHKAR...KD.VQF.I...VVL..L..   78
E.c.         LHLTVEQARGF.AEHDGK...D..VEF.T....VVS.L..   79
                        *       *

NNK-1        KDAVKTGRTILGATNPLASAPGTIRGDFALDMGRNVCHGS   120
S.c.         ..V.RQ.......G.........GI.L.........       121
Sc.p.        .Q......LM...S...D.........YGI.L.........   119
HUMAN        LNV.....VM..E...AD.K........CIQV...II...   120
RAT          LNV.....VM..E...AD.K........CIQV...II...   120
D.m.         LNV.....QM.....AD.L........CIQV...II...    121
D.d.         .GV.ASA.LMI.V.........S.....GVDV...II...   124
M.x.         EN..LAN.D.M.....AQA.E....K...TSIDK.TV...   118
E.c.         EN..QRH.DL.....ANALA..L.A.Y.DSLTE.GT...    119
                    *                 *          *   *

NNK-1        DSVENAKKEIALWFKPEELNQWNHHSAAWIFE           152
S.c.         ...DS.ER..N....K...VD.ESNQ.K..Y.    64.9%  153
Sc.p.        ..I.S.NR..K...Q.S.IQVYDRTIEP..Y.    59.9%  151
HUMAN        ....S.E...G...H....VDYTSCAQN..Y.    62.5%  152
RAT          ....S.E...G........IDYKSCAHD.VY.    63.8%  152
D.m.         .A..S.E.......NEK..VT.TPAAKD..Y.    63.4%  153
D.d.         ....S.NR...........-LTEVKPNPNLY.    55.1%  155
M.x.         ..L....I...YF.RET.IHSYPYQK          45.4%  144
E.c.         ....S.AR...YF.GEG.V--CPRTR          40.1%  143
```

Fig. 2. Isolation of cDNA and genomic DNA for NDK-1 in *Neurospora crassa*. (A) The structure of restriction sites around *ndk-1* gene. The three exones of *ndk-1* were indicated by boxes. The direction of the transcript is indicated by an arrow. The restriction sites are as follows: B, *Bam*H1; E, *Eco*RI; H, *Hind*III, P, *Pst*I; X, *Xho*I. (B) Comparison of amino acid sequences between NDK-1 and NDKs in other organisms. S.c., *Saccharomyces cerevisiae* YNK (Fukuchi *et al.*, 1993); Sc. p., *Shizosaccharomyces pombe* Ndk1 (Izumiya and Yamamoto, 1995); Human, human Nm23-H1 (Rosengard *et al.*, 1989); Rat, rat NDK-α (Kimura *et al.*, 1990); D.d., *Dictyostelium discoideum* NDK (Lacombe *et al.*, 1990); M.x., *Myxococcus xanthus* NDK (Muñoz-Dorado *et al.*, 1990); and E.c., *Escherichia coli* NDK (Hama *et al.*, 1991). Identical amino acids are shown with periods. The identity percentage with NDK-1 is given at the end of each sequence. The amino acids of NDK-1 which are identical to those of polypeptides from the 15-kDa protein are indicated with lines on the top of the sequence. The amino acids with asterisks are the NDP-binding sites. The histidine which is phosphorylated in the reaction is shown in a bold letter. The K-pn loop is enclosed by a box.

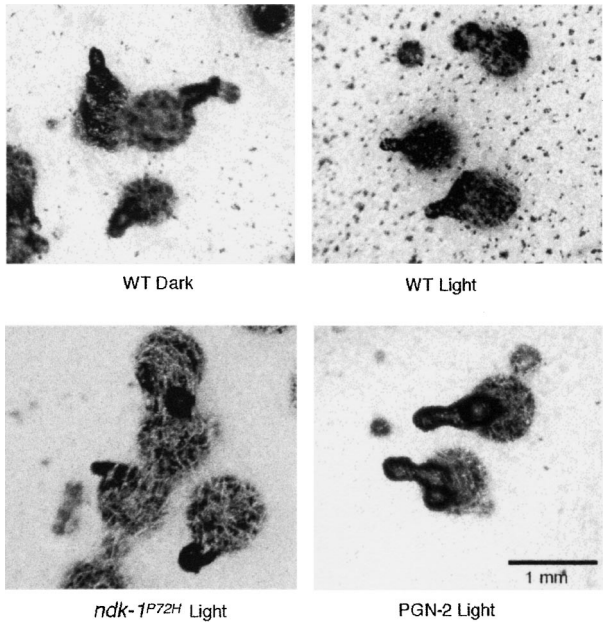


Fig. 3. Light-Induced perithecial polarity of wild-type (FGSC#987A), psp (*ndk-1*^{Pro72His}), and the *ndk-1* transformants. White light was provided parallel to the solid medium from the left in the photograph. Perithecia were observed by dissecting microscopy and photographed. WT Dark, Wild-type perithecia formed in darkness; WT Light, Wild-type perithecia formed under directional light; *ndk-1*^{P72H}, *ndk-1*^{Pro72His} perithecia formed under directional light; PGN-2 Light, *ndk-1*^{Pro72His} transformed with wild-type genomic *ndk-1*.

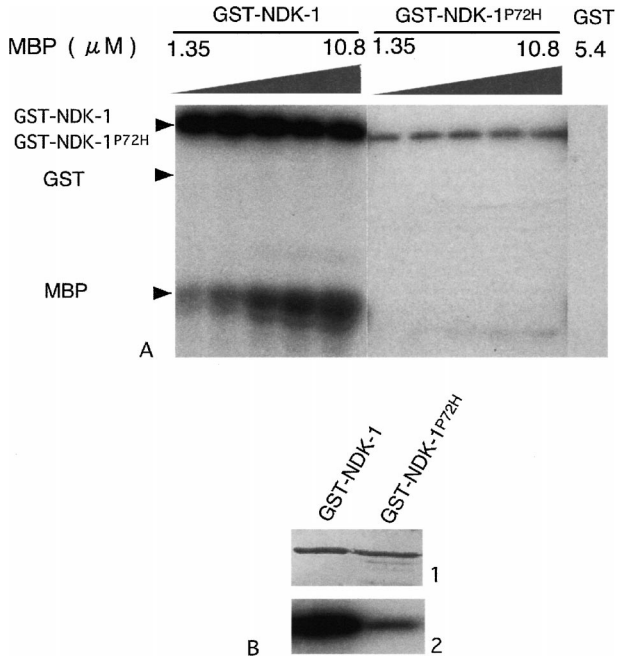


Fig. 4. Characteristics of protein phosphorylation activities of recombinant proteins. (A) By use of various concentration of MBP (1.35, 2.7, 5.4, 8.1, and 10.8 μM), 10 ng of GST-NDK-1 or GST-NDK-1^{Pro72His} was incubated for 1 min at 25. The reaction mixtures were separated by SDS-PAGE, and the gel dried after staining with CBB R-250 was exposed to X-ray film. (B) The autophosphorylation of GST-NDK-1 and GST-NDK-1^{Pro72His} was performed as above described, except that 100 ng of purified enzymes was used. Western blotting with anti-NDK-1 antiserum (upper panel), and autophosphorylation activity by autoradiography on X-ray film (lower panel) are shown.

kinase activity as compared with those of wild-type GST-NDK-1. The results are shown in Fig. 4. The results indicate that there is a new signal transduction pathway via (ii) the autophosphorylation and (iii) protein kinase activities. The newly detected signal transduction pathway was designated as NDK cascade (Hasunuma, 2000; Ogura *et al.*, 2001). The model of signal transduction of light is presented in Fig. 5.

FUNCTIONAL ANALYSIS OF NDP KINASES IN PLANTS

Spinacia oleracia

From leaves of spinach (*Spinacia oleracia*), NDP kinases, I, II, and III were purified and the cDNAs for NDP kinase I and II were isolated. The purified protein of NDP kinase I, II, and III showed the molecular masses of 16, 18, and 17 kDa, respectively, and constituted homohexamers with molecular masses of 92, 110, and 102 kDa for NDP kinase I, II, and III, respectively. NDP kinase I localized at cytosol and II and III localized in the chloroplast. From the cDNA analysis NDP kinase II included a signal peptide directing either to chloroplast or to mitochondria. From the chloroplast NDP kinase III was purified, and the amino acid sequence was determined. The K_m values for ATP, GTP, CTP, UTP, and dTTP were determined in each NDP kinase (Nomura *et al.*, 1991, 1992; Zhang *et al.*, 1995).

Avena sativa

From etiolated seedlings of *Avena sativa* L. (*cv* Garry oats) NDP kinase was purified. The protein showed 18 kDa by SDS gel electrophoresis, and as a native protein it constituted 100-kDa protein, suggesting that the protein form homohexamer. The amino acid sequence of N-terminal 23 amino acids was 87% homologous to human NDP kinase, Nm23-H1 (Sommer and Song, 1994).

The research group of Belarus (Minsk) isolated plasma membrane from etiolated seedling of *Avena sativa*. Red-light irradiation of the preparation stimulated phosphorylation of 14–18-kDa protein, which is presumed to be NDP kinase.

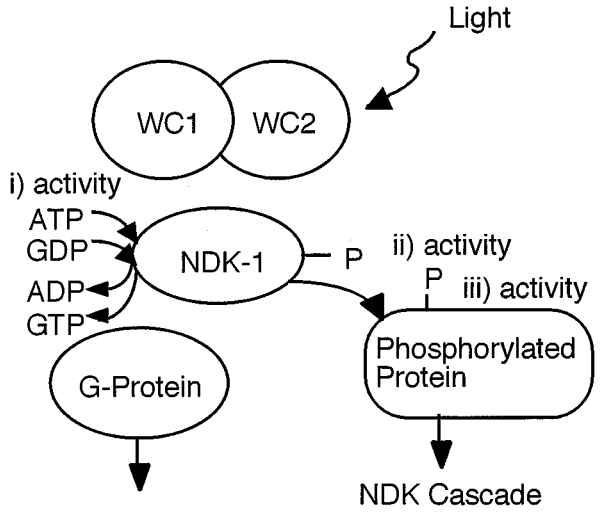


Fig. 5. A model for light signal perception. Putative photoreceptor of WC1/WC2 protein complex perceive light and may activate NDK-1 enabling it to exhibit the activities of (i) NDK activity of ATP + GDP → ADP + GTP, (ii) autophosphorylation activity, and (iii) protein kinase (phosphate transferring) activity to phosphorylate proteins constituting NDK cascade.

Oryza sativa

From *Oryza sativa* cDNA for NDP kinase was isolated. The deduced amino acid sequence revealed 69.8% homologous to that of spinach NDP kinase I, and 73.6% homologous to that of *Arabidopsis thaliana*. The cDNA does not include leader sequence to choloroplast or mitochondria as observed in the NDP kinase II of spinach. The cDNA fused with GST (glutathione S-transferase) was expressed in *E. coli*. The purified protein showed the activity of NDP kinase, and the antibody specific to the enzyme was produced. In the germinating seeds, the amount of the NDP kinase in the embryo could be observed. However, in albumen the amount was reduced. In the root the amount of NDP kinase was constant during 7 days after germination. However, in the stem the NDP kinase showed rapid reduction in the amount (Yano *et al.*, 1993, 1995). By use of dark grown seedlings of rice, the increase in the phosphorylation of NDP kinase by the red-light irradiation of the intact seedlings was detected (Hamada *et al.*, 1999).

Saccharum officinarum L.

In *Saccharum officinarum* NDP kinase was purified from the cultured cells of the plant. The protein is autophosphorylated at Ser residue and phosphorylates histone H1. The NDP kinase increased the activity by fourfold by the heat shock treatment of the cultured cells for 30 min at 36°C (Moisyadi *et al.*, 1994).

Lycopersicon esculentum Mill

cDNA for NDP kinase was cloned in *Lycopersicon esculentum*. By the wounding of the surface of the fruit, the increase in the abundance of mRNA for the NDP kinase was detected (Harris *et al.*, 1994).

Pisum sativum

NDP kinase was purified from the seeds of *Pisum sativum*. cDNA for the NDP kinase, *NDK-P1*, was cloned. The cDNA was expressed in *E. coli* and the purified protein showed the activity of NDP kinase (Finan *et al.*, 1994). From chloroplast of *Pisum sativum* L. cv. Golf, NDP kinase was purified, and the cDNA was also cloned. There are two protein species of 17.4 and 18.5 kDa, both of which are considered to be originated from single mRNA, and the protein may be processed by different proteolysis. The cDNA included leader sequence to direct the protein to chloroplast or to mitochondria. The leader sequence seemed to be hydrolyzed by a protease in the storoma (Lubeck and Soll, 1995).

In *Pisum sativum* cv. Alaska cDNA for NDK kinase was isolated. The cDNA, *PNDK1* (No. D86052), differed in the base sequences from *NDK-P1* at 13 bases. One of which caused amino acid replacement, Glu 140 Gln. PNDK1 had deduced amino acid of Gln 140. From the hypocotyls of dark grown seedlings, NDP kinase was purified, and the protein with pI 6.0 was hydrolyzed with trypsin and the polypeptides fractionated by HPLC were sequenced. The partial amino acid sequences of the polypeptides were completely matched with the amino acid sequence deduced form cDNA, *PNDK1*. The purified preparation of NDP kinase showed (i) γ-phosphate-transferring activity, (ii) autophosphorylation activity, and (iii) protein kinase activity phosphorylating MBP, which is the substrate of MAP kinase. The amino acid sequences of NDP kinases in plants were summarized in Fig. 6 (Tanaka *et al.*, 1998).

By use of the third internodes of the etiolated seedlings of *P. sativum cv*. Alaska, light signal transduction through phytochrome was analyzed. After irradiation of the third internodes by red light or sequential irradiation by red and far-red light, the tissues were homogenized. To the crude extract 4×10^{-8}M[γ-^{32}P]ATP was added and after 15 s at 0°C the reaction was stopped. The phosphorylated proteins were separated by two-dimensional electrophoresis. The phosphorylated 18-kDa proteins with

pI 5.2 and pI 5.4 showed clear increase in the phosphorylation of the protein by red-light irradiation of the third internodes. However, the sequential irradiation of the tissue by red and far-red light rather suppressed the phosphorylation of these two proteins (Hamada *et al.*, 1996; Hamada and Hasunuma, 1994). The proteins phosphorylated were separated by two-dimensional electrophoresis, excised from the gel, and analyzed for their phosphorylated amino acids. The pI 5.2 and 5.4 phosphorylated proteins included P-Ser. The protein pI 6.0 was autophosphorylated and separated by two-dimensional electrophoresis. The phosphorylated and pI 5.4 protein appeared (Tanaka *et al.*, 1998). Blue light also increased the phosphorylation of the NDP kinase (Ito *et al.*, 1995). By the cutting of the third internodes rapid increase in the amount of mRNA for PNDK1 for 1 h at 25°C could be detected (Hasunuma, unpublished). Intact dark grown seedlings of *P. sativum* Alaska were also irradiated with red light. The crude extract prepared from the third internodes of irradiated plant was used for the *in vitro* analysis of the phosphorylation of proteins. With this procedure the phosphorylation of NDP kinase was also stimulated by the red-light irradiation of the intact plant (Ogura *et al.*, 1999a,b).

Etiolated seedlings of intact pea plant were irradiated by red light, and the third internodes were cut off into liquid nitrogen. The crude extract was labeled by [γ-^{32}P]ATP at 0°C for 15 s. The increase in the phosphorylation of 18-kDa protein (NDP kinase) could be detected. The red-light irradiation followed by far-red light diminished the increase in the phosphorylation of 18-kDa protein. The 18-kDa protein identified as NDP kinase formed different protein complexes in the membrane fraction, and also in the soluble fraction.

Arabidopsis thaliana

In *Arabidopsis thaliana* cDNAs and genomic DNAs for NDP kinases were cloned. The four different clones of genomic DNAs and corresponding cDNA were isolated by several groups including our group (Yabe, unpublished). They are designated as *AtNDK1 (NDPK1), AtNDK2 (NDPK2), AtNDK3 (NDPK3), AtNDK4 (NDPK4)*. *NDPK1a* was reported to be the same with that of *NDPK2*. *NDPK3* was reported to localize in the intermembrane space of mitochondria (Sweetlove *et al.*, 2001).

The research group of P. S. Song reported that carboxy terminal part of NDPK2 in *Arabidopsis thaliana* formed complex with phytochrome A and also phytochrome B by use of two hybrid method in Yeast, *Saccharomyces cerevisiae*. However, NDPK1 or NDKPK3 did not show to form complex with in method. Active form of phytochrome A Pfr from *Avena sativa* stimulated the NDP kinase activity of ATP + GDP → ADP + GTP by NDPK2, but not by NDPK1 or by NDPK3 (Choi *et al.*, 1999).

From our research results major part of red-light-induced increase in the phosphorylation of NDP kinase in *Pisum sativum* was PNDK1, which is closest homologue of NDPK1 in *Arabidopsis thaliana*, and therefore there may be fundamental discrepancy between their results and our results. A more precise and detailed experiment seems to be needed to settle these problems.

Triticum aestivum

To analyze molecular component of light signal transduction putative GTP-binding proteins, arrestin and NDP kinase in the tissue culture of embryonic cells of *Triticum aestivum* were analyzed by use of the antibodies of these proteins. An NDP kinase with 16 kDa could be detected in the soluble fraction of embryogenic cells and also nonembryogenic cells (Nato *et al.*, 1997).

CONCLUDING REMARKS

1. NDP kinases contribute to the regulation of ATP, GTP, CTP and UTP pools by the γ-phosphate-transferring activity of $N_1TP + N_2DP \rightarrow N_1DP + N_2TP$.
2. NDP kinase was strongly suggested to provide GTP in the vicinity of GTP-binding protein by the activity descrived previously.
3. NDP kinase has the autophosphorylation and protein kinase activities phosphorylating myelin basic protein. The mutation of *ndk-1* in *Neurospora crassa*, of *ndk-1*^{Pro72His} lacked these activities. Since *ndk-1*^{Pro72His} mutant shows deficiencies in the light-induced polarity of perithecia, there is a new signal transduction pathway, which is designated as NDK cascade.
4. Upon reception of light signal by phytochrome and/or putative photoreceptor, WC-1/WC-2, NDP kinase may be activated by unknown means. The activated NDP kinase may elicit the signal either by activating GTP-binding protein or by activating NDK cascade or both. Thus immediately after the reception of light signal the signal was suggested to bifurcate to the above two pathways.

REFERENCES

Berg, P., and Joklik, W. K. (1953). *Nature* **172**, 1008–1009.
Bominaar, A. A., Molijn, A. C., Pestel, M., Veron, M., and Van Hastert, P. J. M. (1993). *EMBO J.* **12**, 2275–2279.

Capaldi, R. A., Gonzalez-Halphen, D., and Takamiya, S. (1986). *FEBS Lett.* **207**, 11–17.
Choi, G., Yi, H., Lee, J., Kwon, Y. K., Soh, M. S., Shin, B., Luka, Z., Hahn, T.-R., and Song, P. S. (1999). *Nature* **401**, 610–613.
Cumsky, M. G., Trueblood, C. E., Ko, C., and Poyton, R. O. (1987). *Mol. Cell. Biol.* **7**: 3511–3519.
Dumas, C., Lascu, I., Moréra, S., Glaser, P., Fourme, R., Wallet, V., Lacombe, M.-L., Véron, M., and Janin, J. (1992). *EMBO J.* **11**, 3203–3208.
Finan, P. M., White, I. R., Redpath, S. H., Findlay, J. B. C., and Millner, P. A. (1994). *Plant Mol. Biol.* **25**, 59–67.
Fukuchi, T., Nikawa, J., Kimura, N., and Watanabe, K. (1993). *Gene* **129**, 141–146.
Hamada, T., and Hasunuma, K. (1994). *J. Photochem. Photobiol. B* **24**, 163–167.
Hamada, T., Hasunuma, K., and Komatsu, S. (1999). *Biol. Pharm. Bull.* **22**, 122–126.
Hamada, T., Tanaka, N., Noguchi, T., Kimura, N., and Hasunuma, K. (1996). *J. Photochem. Photobiol. B* **33**: 143–151.
Harris, N., Taylor, J. E., and Roberts, J. A. (1994). *Plant Mol. Biol.* **25**, 739–742.
Hasunuma, K. (2000). *Plant Morphol.* **12**, 39–51.
Ito, K., Hamada, T., and Hasunuma, K. (1995). *J. Photochem. Photobiol. B:* **28**, 223–227.
Izumiya, H., and Yamamoto, M. (1995). *J. Biol. Chem.* **270**, 27589–27864.
Krebs, H. A., and Hems, R. (1953). *Biochim. Biophys. Acta* **12**, 172–180.
Lacombe, M. L., Wallet, V., Troll, H., and Véron, M. (1990). *J. Biol. Chem.* **265**, 10012–10018.
Lascu, I., Deville-Bonne, D., Glaser, P., and Véron, M. (1993). *J. Biol. Chem.* **268**, 20268–20275.
Lübeck, J., and Soll, J. (1995). *Planta* **196**: 668–673.
Moisyadi, S., Dharmasiri, S., Harrington, H. M., and Lukas, T. J. (1994). *Plant Physiol.* **104**, 1401–1409.
Nato, A., Mirshahi, A., Tichtinsky, G., Mirshahi, M., Faure, J. P., Lavergne, D., De Buyser, J., Ducreux, G., and Henry, Y. (1997). *Plant Physiol.* **113**, 801–807.
Nomura, T., Fukui, T., and Ichikawa, A. (1991). *Biochim. Biophys. Acta* **1077**, 47–55.
Nomura, T., Yatsunami, K., Honda, A., Sugimoto, Y., Fukui, T., Zhang, J., Yamamoto, J., and Ichikawa, A. (1992). *Arch. Biochem. Biophys.* **297**, 42–45.
Oda, K., and Hasunuma, K. (1994). *FEBS Lett.* **345**, 162–166.
Oda, K., and Hasunuma, K. (1997). *Mol. Gen Genet.* **256**, 593–601.
Ogura, T., Tanaka, N., Yabe, N., Komatsu, S., and Hasunuma, K. (1999a). *Photochem. Photobiol.* **69**: 397–403.
Ogura, Y., Yoshida, Y., Ichimura, K., Aoyagi, C., Yabe, N., and Hasunuma, K. (1999b). *Eur. J. Biochem.* **266**, 709–714.
Ogura, Y., Yoshida, Y., Yabe, N., and Hasunuma, K. (2001). *J. Biol. Chem.* **276**, 21228–21234.
Power, S. D., Lochrie, M. A., and Poyton, R. O. (1984). *J. Biol. Chem.* **259**, 6575–6578.
Sellam, O., Veron, M., and Hildebrandt, M. (1995). *Mol. Microbiol.* **6**, 79–85.
Sommer, D., and Song, P. S. (1994). *Biochim. Biophys. Acta* **1222**, 464–470.
Sweetlove, L. J., Mowday, B., Hebestreit, H. F., Leaver, C. J., and Millar, A. H. (2001). *FEBS Lett.* **508**, 272–276.
Tanaka, N., Ogura, T., Noguchi, T., Hirano, H., Yabe, N., and Hasunuma, K. (1998). *J. Photochem. Photobiol. B* **45**, 113–121.
Tepper, A. D., Dammann, H., Bominaa, A. A., and Véron, M. (1994). *J. Biol. Chem.* **269**, 32175–32180.
Troll, H., Winckler, T., Lascu, I., Müller, N., Saurin, W., Véron, M., and Mutzel, R. (1993). *J. Biol. Chem.* **268**, 25469–25475.
Wallet, V., Mutzel, R., Troll, H., Barzu, O., Wurster, B., Véron, M., and Lacombe, M. L. (1990). *J. Natl. Cancer Inst.* **82**, 1199–1202.
Xu, Y., Moréra, S., Janin, J., and Cherfils, J. (1997a). *Proc. Natl. Acad. Sci. U.S.A.* **94**, 3579–3583.
Xu, Y., Sellam, O., Morera, S., Sarfati, S., Biondi, R., Véron, M., and Janin, J. (1997b). *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7162–7165.
Yano, A., Shimazaki, T., Kato, A., Umeda, M., and Uchimiya, H. (1993). *Plant Mol. Biol.* **23**, 1087–1093.
Yano, A., Umeda, M., and Uchimiya, H. (1995). *Plant Mol. Biol.* **27**, 1053–1058.
Zhang, J., Fukui, T., and Ichikawa, A. (1995). *Biochim. Biophys. Acta* **1248**, 19–26.