UV-responsive Genes of Arabidopsis Revealed by Similarity to the Gcn4-mediated UV Response in Yeast*

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A UV response that involves the Ras proteins and AP-1 transcription factors has recently been described in mammals and yeast. To test whether an equivalent response exists in plants, we monitored the expression of Arabidopsis histidinol dehydrogenase gene (HDH), a homologue of the yeast HIS4 gene, which is strongly induced by UV light and is a target of the transcriptional activator Gcn4. We show that HDH mRNA levels increase specifically in response to UV-B light. Only small increases were detected upon exposure to other wavelengths. To isolate plant genes involved in this UV response, a gcn4 mutant was transfected with an Arabidopsis thaliana cDNA library. A new type of nucleotide diphosphate kinase (NDPK Ia) with a significant homology to the human tumor suppressor protein Nm23 rescued the gcn4 phenotype. NDPK Ia specifically binds to the HIS4 promoter in vitro and induces HIS4 transcription in yeast. In Arabidopsis, the NDPK Ia protein is located in the nucleus and cytosol. Expression studies in seedlings revealed that the level of NDPK Ia mRNA, like that of HDH, increases in response to UV-B light. It appears that NDPK Ia and HDH are components of a novel UV-responsive pathway in A. thaliana.

 Exposure of cells and organisms to UV light evokes a complex set of protective and repair responses. Because short wavelengths are absorbed mostly by nucleic acids, it is reasonable to assume that the most significant UV-mediated damages occur in cellular DNA. Various DNA repair systems are indeed induced following exposure to UV. This response has been identified in all prokaryotes and eukaryotes studied (1–4). In mammalian cells, this response involves activation of membrane components, such as tyrosine kinase receptors and the proto-oncoprotein Ras (6–9). Activated Ras in turn induces cytoplasmic cascades that lead to activation of transcription factors of the AP-1 family (e.g. c-Jun and ATF2) and NFkB (6, 10, 11). A similar UV response, which is not related to DNA damage, has also been identified in yeast (12). In this organism, the UV response involves activation of Ras2, which leads to activation of a transcription factor of the AP-1 family (Gcn4, a functional homologue of c-Jun) (13). As a result, Gcn4 target genes, such as HIS4 (see below), are strongly activated by UV. Indirect evidence suggests that these conserved Ras2/AP-1 pathways have a protective function (5, 12).

In yeast, the bZIP transcription factor Gcn4 is activated not only by UV but also by amino acid starvation. Under these conditions, Gcn4 activation does not involve the Ras cascade, but rather the activity of the Gcn2 kinase (12, 14, 15). Activation of Gcn4 in response to amino acid starvation seems rational because Gcn4 target genes encode amino acid biosynthetic enzymes. Some of the most prominent Gcn4 targets are HIS3 and HIS4, which encode enzymes of histidine biosynthesis (16). Although starvation and UV induce different signal transduction elements for activation of Gcn4, both stimuli need intact Gcn4 for induction of HIS genes (12, 14).

Plants also respond to UV in the range between 280 nm and 350 nm by a complex set of reactions (17). Beside the activation of DNA repair systems (reviewed in Ref. 3), plants respond with the rapid synthesis of pigments, primarily flavonoids, which requires activation of specific genes and transcription factors (18). Although UV-dependent cis-acting elements were identified in the promoter of chalcone synthase, which encodes a key enzyme in flavonoid synthesis, the biochemical system that senses the UV signal and exerts the relevant genes is still elusive. Indirect evidence points to the involvement of calcium, calmodulin, and kinases in UV-dependent flavonoid synthesis (19–21) and to the involvement of jasmonate in UV-dependent expression of proteinase inhibitor genes (22), suggesting that various UV signaling pathways exist in plants.

It is assumed that amino acid biosynthesis in plants is similar to that in bacteria and yeast (23). This similarity is manifested in the fact that several genes related to this pathway were capable of functionally complementing the respective yeast mutants (24–27). Histidine biosynthesis in Arabidopsis involves several genes with high sequence homology to corresponding genes from yeast. Representative examples are the genes encoding imidazole glycerol phosphate dehydratase (IPGD) with 44% identity to yeast HIS3 (28), and histidinol dehydrogenase (HDH) with 51% identity to yeast HIS4 (29).

It was therefore decided to test whether HDH, the plant homologue of HIS4, is UV responsive, as is the case in yeast. We demonstrate here that this is indeed the case. We show that UV-B irradiation of Arabidopsis seedlings specifically stimulates HDH mRNA accumulation. This result suggests that a
Arabidopsis NDPK Ia Complements UV-insensitive Yeast Mutant

UV-dependent Ras/AP-1 cascade might exist in plants, which is similar to the mammalian and yeast cascade. This plant pathway could be revealed via systematic functional complementation of the relevant yeast mutants. In a first functional complementation screen, we searched for Arabidopsis thaliana cDNAs that would rescue the gcn4 phenotype. We have identified a new nuclease diaphosphate kinase (termed NDPK Ia) from Arabidopsis with remarkable homology to the mammalian tumor suppressor protein Nm23. Nm23-H2 was reported to bind DNA and is hypothesized to be a transcriptional regulator (30). We show that Arabidopsis NDPK Ia binds the promoter of HIS4 and induces HIS4 transcription in yeast. Its transcription in plants is strongly induced by UV-B light in a pattern very similar to that of IHHD expression. NDPK Ia might play a role as a transcriptional regulator in the UV-B light-mediated expression of histidine biosynthesis in plants and yeast. Our results also imply that aspects of the (non-DNA damage) UV response are common to plants and yeast.

**Experimental Procedures**

**Escherichia coli and Yeast Strains—**E. coli strain XL1 blue (Stratagene) was used for propagation of a cDNA library. Expression of glutathione S-transferase (GST) fusion proteins was carried out in the strain M15 [pREP4] (Qiagen, Hilden, Germany). Nearly isogenic Saccharomyces cerevisiae strains SP1 (MATa, his3, can, ade8, leu2, trp1, ura3), SP1 harboring HIS3 (isogenic to SP1 but with integrated HIS3), and gcn4Δ (MATa, ura3, leu2, gcn4IΔ) were used in this study (12).

**Plant Material, Cell and Protoplast Formation Culture, and Transformation—**A. thaliana (L.) ecotype Landsberg erecta were grown on soil with 16 h light/8 h dark cycles at 20 °C, and leaf, stem, and floral tissues were isolated from 6-week-old plants. Etiolated seedlings were grown on filter paper for 3 or 5 days in darkness and then transferred to different light programs. An isogenic, auxotrophic gcn4 mutant, cells were grown on plates containing synthetic complete medium CM lacking uracil (S) and were transformed with the cDNA library and grown at 30 °C for 5–6 days on plates containing synthetic complete medium CM lacking uracil and histidine and supplemented with 20 μM 3-amino triazole (CMUH + 3-AT). Plasmids from growing colonies were isolated and re-transformed into gcn4 to confirm complementation and exclude false positives.

**Plasmid Constructions—**Arabidopsis cDNA clones from the complementation screen were excised from pFL61 by NotI digestion and integrated into pKS for sequence analysis. The N terminus of NDPK Ia was subsequently cloned by rapid amplification of 5′ cDNA ends from an Arabidopsis cDNA library. For construction of fusion proteins, BamHI-compatible restriction sites were introduced at the 5′-ends and SalI sites at the 3′-ends of NDPK Ia and of Δ1–79 NDPK Ia ORF by PCR using the gene-specific primers (5′-end, CCAGCCCTGGTGTAGGCATCAGGTTTCCACC; Δ1–79 5′-end, GGA TCCAAATGGAGGACGCTTG; 3′-end, GTGACGCTCTTCACTAGC) and subcloned into pKS. The GST fusion cassette pGEX-5x-1 (Amersham Pharmacia Biotech) was transformed into S. cerevisiae, and the NDPK Ia cDNA was integrated in the frame at the C terminus of the glutathione S-transferase cDNA. The GFP expression cassette was removed from the pBI121-mGFP4 construct (34) by HindIII/EcoRI digestion and transformed into pUC18 (mGFP4-pUC18). Afterward a 5′ BamHI followed by an in-frame Smal restriction site was introduced into mGFP4 coding region by PCR using the primers following primers: 5′-end, CCGGATCCCGCGCGGTGAGTAAAGGAAGACATTTTAC; 3′-end, GCGGACGTTCCTGTTGGTTTTCCAATCACCCGCCATGCCC. The resulting plasmid (mAV) was used for the construction of the Δ1–79 NDPK Ia coding regions fused to the C terminus of GFP in mGFP4 by BamHI/SalI digestion and in-frame integration into mAV4. The NLS-mGFP4 and PHYA-mGFP4 constructs will be described elsewhere. All new DNA sequences were verified by sequencing both strands.

**Protein Expression and Electrophoretic Mobility Shift Assays—**The Δ1–79 NDPK Ia-GST fusion protein was expressed in M15[pREP4], and single step purification of the fusion protein was carried out as described (34). The N-terminal GST protein was removed from the mixture of the fusion protein with 10 μg of factor Xa (in 20 μl Tris (pH 8), 100 μM NaCl, 2 mM CaCl2) for 12 h at 20 °C. All purification steps were monitored by protein staining of each fraction after SDS-PAGE. In vitro transcription and subsequent in vitro translation reactions were carried out as described in (36).

DNA binding of NDPK Ia was assessed in gel electrophoretic mobility shift assays. Standard reactions were carried out with 32P-labeled yeast HIS4 promoter fragments containing the Gen4 sites (−235 to −67). Each binding reaction was performed with 1 ng of 32P-labeled probe, 100 ng of protein, 100 ng of poly(dI-dC) in Gen4-binding buffer (38). The mixture was incubated for 30 min on ice, and NDPK Ia/DNA complexes resolved on 6% native polyacrylamide gels.

**Determination of Specific Enzymatic Activity—**NDPK activity was measured spectrophotometrically in a coupled pyruvate kinase/lactate dehydrogenase assay using 100–400 ng of recombinant protein (39). To determine the effect of DNA binding on the enzyme, activity fractions containing NDPK (400 ng) and DNA from the HIS4 promoter (1 ng) were preincubated on ice for 30 min before determination of the enzymatic analysis.

**In Vivo Localization Studies in Arabidopsis Protoplasts—**GFP fluorescence in transiently transformed Arabidopsis protoplasts was studied by epifluorescence and Nomarski interference contrast microscopy and analyzed in an Axiovert microscope (Zeiss, Oberkochem, Germany). Excitation of GFP was performed with standard fluorescein isothiocyanate filters, and a clear distinction between GFP fluorescence and autofluorescence of plant cells could be detected (see Fig. 6, A and B). For each construct, 10–20 protoplasts were documented using an automatic Contax 167 MT camera and Eastman Kodak Co. 64T film.

**RNA Preparation, Northern Blot Analysis, and Primer Extension—**RNA from Arabidopsis was isolated as described (40). Gene-specific cDNA probes of HHD (29) and IPG (28) were kindly provided by Dr. E. Ward (Ciba, Research Triangle Park, NC), and these probes, as well as a BamHI/SalI fragment of NDPK Ia and 18SrRNA, were labeled by random priming (Roche Molecular Biochemicals) and poly (dI-dC) used for hybridization. Aliquots of 20 μg of total RNA were used for Northern blot hybridization as described previously (40). Membranes were washed twice in 2× SSC/0.2% SDS at 42 °C and in 1× SSC/0.2% SDS at 64 °C. X-ray films were exposed at −80 °C using intensifying screens.

Total RNA from yeast was prepared from cultures grown to logarithmic phase on synthetic complete medium (lacking uracil) and was assayed by primer extension analysis (12). RNA (20 μg) was hybridized with 32P-labeled oligonucleotides (HIS4, GCTGTTTTACGATC3′; HIS3, 5′-CGCAAATCTGAATTTGGTTTC-3′; actin, 5′-GGTATCAAATACCAAAACGACGACAC-3′). Avian myeloblastosis virus reverse transcriptase was used for the extension reaction, and the products were separated on 6% acrylamide/7 M urea gels.

**Nucleotide Sequence Accession Number—**The nucleotide sequence for the full-length NDPK Ia has been deposited in the GenBank database under accession number AJ012758. The corresponding Δ1–79 NDPK Ia sequence has been deposited under GenBank accession number AF058391.
RESULTS

Expression of HDH, the Arabidopsis Homologue of Yeast HIS4, Is Induced by UV-B Light—To test whether a UV response similar to the yeast Ras/Gcn4-mediated response (which results in induction of *HIS4* transcription) exists in plants, we studied the effect of different wavelengths on transcription of the Arabidopsis HDH gene. RNAs were prepared from seedlings that were grown in darkness for 3 days and then transferred to different wavelengths. Northern blot analyses of these RNAs (Fig. 1) show that HDH mRNA levels are strongly expressed in the presence of UV-B light, whereas exposure to either white light, UV-A, blue light, or red light irradiation did not result in significant expression. Remarkably, the difference between UV-A and UV-B irradiation consisted only in additional wavelengths from 305 to 340 nm (2 μmol·m⁻²·s⁻¹), underscoring the high degree of specificity of the response to UV-B light. Our results also demonstrate that in plants, like in yeast, expression of a gene related to histidine biosynthesis is induced by UV light. The light regime (UV-B supplemented by UV-A) stimulates photoreactivation in *Arabidopsis*, leading to the reversion of possible damage to DNA (3).

Isolation of Arabidopsis cDNA, Which Rescues Starvation Sensitivity of a Yeast gcn4 Mutant—The high degree of conservation of the UV response between yeast and plants suggests the use of yeast mutants for functional cloning of plant genes involved in this response. To test this approach, we introduced an *Arabidopsis* cDNA library (33) into a yeast gcn4 mutant. Transformants were grown under amino acid limitation (20 mM DAF058391, also referred to as NDPK Ia), and the corresponding protein had an apparent size of approximately 25 kDa (data not shown). The protein contains domains, which are described in other NDPKs as a ATP/GTP binding site motif A (P-loop, amino acids 166–173), and the crucial site for the catalytic mechanism (His-197). In addition, two out of the three basic amino acids (Lys-113 and Lys-214), which are crucial for DNA binding of the human homologue Nm23-H2 (41), were found in the NDPK Ia sequence. The cDNA contains an N-terminal extension, which is common in plant type II NDPKs. Although this region is suggested to mediate chloroplast targeting of some plant NDPKs, no similarity to known chloroplast leader sequences was found for NDPK Ia by the use of data base PSORT. Further data base searches revealed three more NDPK sequences from *A. thaliana* (ecotype Columbia). Compared with NDPK Ia, NDPK 2 differs in only 3 amino acids (98.7% identity), whereas NDPK 3 shows only 39% identity. NDPK 1 lacks an N-terminal extension and has a 53.9% homology to NDPK Ia (Fig. 3). The high similarity to NDPK 2 may reflect the cloning of an identical gene from a different ecotype (*Landsberg erecta versus* Columbia). The highest level of identity from other plants including the N-terminal extension was found for spinach and pea NDPK (62.8%). Surprisingly, a remarkable sequence identity was also found with the *Drosophila* Aud (abnormal wing disc) gene (57%) and with the human tumor-suppressor protein Nm23-H1 (54%) and Nm23-H2 (51%).

The unexpected isolation of NDPK Ia as a suppressor of cells lacking Gcn4 activity raises the question of specificity. Does NDPK Ia induce transcription of Gcn4 target genes, or does it rescue the *gcn4* phenotype via an indirect mechanism, as described (42)? To address this question, we determined NDPK Ia DNA binding activity and its ability to induce *HIS4* and *HIS3* transcription in yeast. We also monitored its intracellular localization and light-dependent transcription in *Arabidopsis*.

*Arabidopsis* NDPK Ia Binds to the Yeast HIS4 Promoter in Vitro—The finding that NDPK Ia is able to complement the *gcn4* mutation implies its function as a transcriptional regulator. The yeast *HIS4* promoter contains three Gcn4 binding sites (43) and represents a target of the complementation screening.

![Fig. 1. HDH mRNA levels in Arabidopsis seedlings are stimulated by UV-B light.](image1)

![Fig. 2. Complementation of yeast gcn4 phenotype by Arabidopsis NDPK Ia.](image2)
Arabidopsis NDPK Ia

| Name               | Accession Numbers | Description                                      | Identity (%) |
|--------------------|-------------------|-------------------------------------------------|--------------|
| Arabidopsis NDPK Ia | 1                 | Transcription activation in yeast               | 70           |
| Arabidopsis NDPK 2 | 2                 | Transcription activation in yeast               | 70           |
| Plasmid NDPK II precursor | 3          | Expression in yeast                            | 70           |
| Arabidopsis NDPK 3 | 4                 | Transcription activation in yeast               | 70           |

**Multiple alignment of NDPK Ia and comparison with sequences from other NDPKs.**

Fig. 3. Multiple alignment of NDPK Ia and comparison with sequences from other NDPKs. Numbers referring to the amino acid residues. The percentage of identity between NDPK Ia and related proteins is calculated either including the N-terminal region (−L) or excluding the N terminus (+L). Residues identical to NDPK Ia amino acid residues are indicated by asterisks (*). The accession numbers for *A. thaliana* (Columbia) proteins were AF017641 (NDPK 1), AC017640 (NDPK 2), and AF044265 (NDPK 3).

A fragment of the promoter (from −238 to −58) was therefore chosen as a probe for DNA binding assay. The N-terminal deletion of NDPK Ia (Δ1–79 NDPK Ia) was expressed as GST fusion protein, followed by proteolytical treatment to remove the GST tag before subjected to electrophoretic mobility shift assay. The NDPK Ia full-length protein was expressed by coupled in vitro transcription/translation reactions and subjected to electrophoretic mobility shift assay. Fig. 4 shows that both proteins strongly bind to the HIS4 promoter (Fig. 4, A, lane 1, and C, lane 1). Surprisingly, the Δ1–79 NDPK Ia-GST fusion protein was unable to bind to the promoter under identical conditions as used for the cleaved NDPK Ia protein (Fig. 4A, lane 2 compared with lane 1). Under our conditions, the human homologous protein Nm23-H2 (which was kindly provided by Dr. M. Veron, Institut Pasteur, Paris, France) also interacts with the HIS4 promoter (Fig. 4A, lane 4). As expected, DNA binding of Nm23-H2 was strongly inhibited by excess of poly(dI-dC) (not shown); poly-pyrimidine-rich sequences were shown to be putative binding sites of Nm23-H2 (44). For *Arabidopsis* NDPK Ia and its N-terminal deletion, 100 ng of poly(dI-dC) (Fig. 4) or 500 ng of salmon sperm DNA (Fig. 4C, lanes 1 and 3) did not interfere with DNA binding. The binding specificity of NDPK Ia, Δ1–79 NDPK Ia, and Nm23-H2 to the HIS4 promoter was further demonstrated by efficient competition of the binding with excess of unlabeled probe (Fig. 4, B, lanes 1–7, and C, lanes 3–7).

**HIS4 Transcription Is Activated by NDPK Ia in Yeast.**—The direct involvement of NDPK Ia in transcriptional activation of HIS4 genes was demonstrated in yeast. *Gcn4* cells harboring either the control plasmid pFL61, the NDPK Ia expression plasmid, or Δ1–79 NDPK Ia were grown under nonlimiting conditions. Cells of the RAS2<sup>Vαl-19</sup> strain in which HIS4 expression is constitutively induced (12) were used as a positive control. Primer extension analysis revealed, that HIS3 and HIS4 expression was marginal in *gcn4* (Fig. 5, lanes 1 and 4), whereas HIS4 transcripts were strongly increased in three independent experiments if Δ1–79 NDPK Ia (lane 2) or the full-length protein (lane 5) was expressed in this mutant. HIS4 transcript levels, determined in strains expressing NDPK Ia, were comparable to levels measured in RAS2<sup>Vαl-19</sup> (lane 3), indicating the constitutive activation of the signaling cascade. NDPK Ia was also able to stimulate *HIS3* transcripts in the absence of UV light or starvation, but the induction was significantly lower than that of HIS4 (Fig. 5, lanes 1–3).

**The Enzymatic Activity of NDPK Ia Is Not Altered in the Presence of DNA in Vitro.**—The results presented above suggest that NDPK Ia was able to rescue *gcn4* cells by virtue of its function as a transcriptional activator that binds DNA. This result is not entirely unexpected because mammalian NDPKs have already been suggested as DNA binding proteins and transcriptional regulators (30, 44). Yet it raises the question of whether this activity of the protein is separated from its enzymatic activity as a kinase of nucleoside diphosphates. The enzymatic activity of NDPK Ia was determined and showed efficient transfer of the terminal phosphate group from ATP to either GDP, UDP, or CDP. The corresponding k<sub>cat</sub> values for these substrates were 0.34 m<sup>−1</sup> for CDP, 0.44 m<sup>−1</sup> for UDP, and 0.38 m<sup>−1</sup> for GDP and are similar to values determined for NDPKs from other plants (45). Addition of HIS4 promoter DNA (1–100 ng) to the reaction mixture did not significantly influence the enzymatic activity (data not shown). It seems, therefore, that DNA binding of NDPK Ia to HIS4 promoter does not affect its kinase activity.

**NDPK Ia Is Localized in the Cytosol and Nucleus of Arabidopsis Protoplasts.**—The capability of NDPK Ia to bind DNA in vitro and its effect on gene expression in yeast point to a nuclear localization of the protein as has been shown for NDPKs in human cells (46, 47). To study the intracellular localization of NDPK Ia in plants, the full-length cDNA and its N-terminal deletion were expressed as GFP fusion in *Arabi-
dopsis protoplasts. GFP fluorescence in protoplasts was analyzed by epifluorescence and Nomarski interference contrast microscopy (Fig. 6). For comparison, GFP fusion constructs targeted to different compartments of Arabidopsis cells were chosen: a cytosol localized phytochrome A-GFP (Fig. 6C), a nuclear targeted NLS-GFP (Fig. 6D) (consisting of the nuclear leading sequence of the bZIP protein CPRF4) (48), and a chloroplast-located GGP-GFP (Fig. 6E) (Geranyl-Geranyl pyrophosphate synthase) (49). The detection of GFP fluorescence indicated that both the Δ1–79 NDPK Ia-GFP (Fig. 6F) and the full-length protein (Fig. 6G) are distributed between the nucleus and the cytosol. Comparison with GGP-GFP excludes their localization in chloroplasts. Irradiation of the protoplasts did not affect the intracellular distribution of NDPK Ia-GFP within the cell (data not shown).

**NDPK Ia mRNA Is Induced by UV-B Light in Arabidopsis Seedlings**—Light-dependent expression of NDPK Ia mRNA in etiolated Arabidopsis seedlings was determined by Northern blot analysis (Fig. 7). Seedlings were grown in darkness and then transferred to different wavelengths for 72 h. NDPK Ia cDNA hybridized to a mRNA of about 800 base pairs. No cross-hybridization to other NDPKs was detectable. Seedlings irradiated with UV-B light strongly expressed NDPK Ia mRNA, whereas white light, UV-A, blue light, and red light irradiation caused only low mRNA accumulation (Fig. 7A).

**NDPK Ia binds to the yeast HIS4 promoter.** DNA binding activity was determined by electrophoretic mobility shift assay using radiolabeled HIS4 promoter (−238 to −58) as a probe. The DNA fragment was incubated with recombinant Δ1–79 NDPK Ia and Nm23-H2 (A and B) or with reticulocyte lysates programmed with NDPK Ia mRNA (C). Δ1–79 NDPK Ia interacts with DNA (A, lanes 1 and 4), whereas the fusion protein and GST do not bind (A, lanes 2 and 3). Unlabeled probe was added in the indicated molar excess over probe (B, lanes 2–4, 6, and 7, and C, lanes 4–7). 500 ng of salmon sperm DNA (ss) was added to the reticulocyte lysate as a competitor of unspecific DNA binding (C, lane 2). FP, free probe.

**Fig. 5. NDPK Ia activates HIS3 and HIS4 expression in gcn4.** The levels of HIS3 and HIS4 mRNA were monitored by primer extension using radiolabeled gene-specific oligonucleotides. RNAs were prepared from gcn4 strains containing either the control plasmid pFL61 (lanes 1 and 4), pFL61 with cDNA of NDPK Ia (lane 5), or the N-terminal deletion (Δ1–79 NDPK Ia) (lane 2). Ras2Δ1-13 (lane 3) was used as a control exhibiting constitutive HIS expression. 20 μg of total RNA was used in each reaction.
Three independent experiments revealed that this expression pattern is strikingly identical to that of HDH (Fig. 1).

Irradiation of seedlings for 2–24 h with UV-B light revealed that NDPK Ia and the genes related to histidine synthesis IPGD and HDH were transiently stimulated 6 h after start of irradiation, and mRNAs of all three genes reaccumulated after longer irradiation (Fig. 7B). The expression of IPGD mRNA was considerably lower but correlated with HDH and NDPK Ia mRNA accumulation.

The spatial expression pattern of NDPK Ia was determined in tissues from adult plants and revealed mRNA accumulation in green leaves and flowers, but no expression in green stems (Fig. 7C).

**DISCUSSION**

This report provides the first evidence for a novel plant response that is similar to the yeast Ras/Gen4-mediated UV response. Transcription of a plant gene, HDH, was found to respond to UV just like the transcription of its yeast homologue HIS4. Also, a new Arabidopsis nucleoside diphosphate kinase (NDPK Ia), which was identified by functional complementation of the yeast gen4 mutant, was shown to be UV-responsive.
and to induce expression of genes related to histidine synthesis in yeast. NDPK Ia binds to the HIS4 promoter in vitro, and HIS4 transcription is stimulated by NDPK Ia in the absence of Gcn4 activity. These results imply that NDPK Ia acts as a transcriptional activator in yeast. Partial complementation of gcn4 is also achieved by the maize transcription factor Opaque-2 (50, 51), but other functional homologues of Gcn4 have so far not been isolated from plants. Under our selective conditions (20 mM 3-AT), bZIP proteins from Arabidopsis and parsley with considerable sequence homology to Opaque-2 (GFB1 and GFB2 (52); CPRF1, CPRF2, and CPRF4 (36, 48)) failed to complement the gcn4 phenotype (data not shown).

Sequence comparison of NDPK Ia with cDNAs from other plants revealed a high degree of homology to chloroplast-located type II NDPKs (39). Three Arabidopsis NDPK sequences are described in the data bases, of which two contain putative organellar leader sequences. However, several facts make it unlikely that NDPK Ia is located in the chloroplast: (i) there is no homologous pattern of its N terminus with chloroplast leader sequences from other plants, (ii) the NDPK Ia/GFP fusion and the leader-free deletion (31–79) are localized in the cytosol and nucleus but not in Arabidopsis chloroplasts (Fig. 5), and (iii) the expressed protein is not transported into isolated chloroplasts in vitro (data not shown). Our findings indicate that NDPK Ia is expressed as a 25-kDa protein without further posttranslational modification in Arabidopsis. In mammalian cells, the NDPK Nm23-H2 was shown to function as a DNA-binding protein and is proposed to act directly as a transcriptional regulator (30, 44). Amino acids that are crucial for DNA binding activity (41) are conserved in the Arabidopsis NDPK Ia sequence. Nm23-H2, as well as NDPKs from other organisms, are distributed in the nucleus and cytosol (47, 53, 54), as was found for NDPK Ia. These NDPKs may make up a subfamily, which might be involved in transcriptional processes. Complementation of gcn4 by NDPK Ia and the direct stimulation of HIS4 transcription strongly suggest a direct involvement of NDPK Ia in trans-activation. However, the protein has only weak trans-activation potential in a yeast one-hybrid system (data not shown) and is more likely to function as a co-factor modifying Bas1, Bas2, or other activators. These factors also bind to the HIS4 promoter and are responsible for expression of HIS4 under limiting conditions (55). Similarly, the transcriptional activation capacity of Nm23-H2 is still a matter of controversy, and its putative function as a cofactor modifying trans-activating factors during tumor suppression has been discussed (56).

Which genes could be the target of NDPK Ia in plants? Given its effect on the yeast HIS4 gene together with the UV-dependent expression of HDH, it is tempting to speculate that NDPK Ia target genes encode enzymes of amino acid biosynthesis pathways, in particular of the histidine synthesis pathway. Analysis of the HDH promoter should reveal whether it serves as a NDPK Ia target. NDPK Ia mRNA is strongly stimulated after UV-B light irradiation of Arabidopsis seedlings, and there is an obvious correlation to the expression of HDH, the Arabidopsis homologue of yeast HIS4. Similarly to yeast, induction of histidine biosynthetic genes in Arabidopsis occurs not only following exposure to UV light but also during starvation (57), providing additional proof of the similarity between the systems.

Although the mammalian and yeast Ras/AP-1 cascades were shown to play important roles in protecting against UV irradiation (6, 12, 58), this process is not well understood. In addition, it is difficult to interpret the relevance of the UV-dependent expression of HDH and NDPK Ia in Arabidopsis. However, using the UV responsive genes identified in this study as a starting point, it should be possible to reveal the plant signal transduction pathway and to investigate its biological significance. Considering the similarity to the corresponding system in yeast, it is suggested that the use of yeast genetics could considerably facilitate such studies.

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