ABA3 Is a Molybdenum Cofactor Sulfurase Required for Activation of Aldehyde Oxidase and Xanthine Dehydrogenase in Arabidopsis thaliana*

Received for publication, August 17, 2001, and in revised form, August 30, 2001
Published, JBC Papers in Press, November 1, 2001, DOI 10.1074/jbc.C100472200

Florian Bittner, Mislav Oreb, and Ralf R. Mendel†
From the Botanical Institute, Technical University of Braunschweig, 38023 Braunschweig, Germany

The xanthine oxidase class of molybdenum enzymes requires a terminal sulfur ligand at the active site. It has been proposed that a special sulfurase catalyzes the insertion of this ligand thereby activating the enzymes. Previous analyses of mutants in plants indicated that the genetic locus aba3 is involved in this step leading to activation of the molybdenum enzymes aldehyde oxidase and xanthine dehydrogenase. Here we report the cloning of the aba3 gene from Arabidopsis thaliana and the biochemical characterization of the purified protein. ABA3 is a two-domain protein with a N-terminal NifS-like sulfurase domain and a C-terminal domain that might be involved in recognizing the target enzymes. Molecular analysis of three aba3 mutants identified mutations in both domains. ABA3 contains highly conserved binding motifs for pyridoxal phosphate and for a persulfide. The purified recombinant protein possesses a cysteine desulfurase domain and a C-terminal domain that might be involved in recognizing the target enzymes. Molecular analysis of three aba3 mutants identified mutations in both domains. ABA3 contains highly conserved binding motifs for pyridoxal phosphate and for a persulfide. The purified recombinant protein possesses a cysteine desulfurase domain and a C-terminal domain that might be involved in recognizing the target enzymes. Molecular analysis of three aba3 mutants identified mutations in both domains. ABA3 contains highly conserved binding motifs for pyridoxal phosphate and for a persulfide. The purified recombinant protein possesses a cysteine desulfurase domain and a C-terminal domain that might be involved in recognizing the target enzymes. Molecular analysis of three aba3 mutants identified mutations in both domains. ABA3 contains highly conserved binding motifs for pyridoxal phosphate and for a persulfide. The purified recombinant protein possesses a cysteine desulfurase domain and a C-terminal domain that might be involved in recognizing the target enzymes. Molecular analysis of three aba3 mutants identified mutations in both domains. ABA3 contains highly conserved binding motifs for pyridoxal phosphate and for a persulfide. The purified recombinant protein possesses a cysteine desulfurase domain and a C-terminal domain that might be involved in recognizing the target enzymes. Molecular analysis of three aba3 mutants identified mutations in both domains. ABA3 contains highly conserved binding motifs for pyridoxal phosphate and for a persulfide.

According to additional molybdenum ligands, the enzymes are divided into two subgroups: enzymes with a dioxo-molybdenum center like nitrate reductase and sulfate oxidase carry two oxygen atoms at the molybdenum, while those with a monooxo-molybdenum center possess only one oxygen atom and a terminal sulfur atom instead of the second oxygen atom (1). In the plant Arabidopsis thaliana, aldehyde oxidase (AO; EC 1.2.3.1) and xanthine dehydrogenase (XDH; EC 1.1.1.204) belong to this latter group of molybdenum enzymes (3). During the last decade, plant mutants have been described for Arabidopsis (aba3 (4)), tomato (flacca (5)), and tobacco (aba1 (6)) that completely lack the activities for AO and XDH but show normal activities for nitrate reductase. In crude extracts of these plant mutants, the activities of AO and XDH could be restored in vitro by anaerobic treatment with sulfide/dithionite, and thus it was proposed that these plants are defective in the final sulfuration step for AO and XDH. All of these mutants show reduction or total loss of seed dormancy, have a wilt phenotype, and are impaired in stress response, which is typical for the lack of the phytohormone abscisic acid (ABA). It was described that AO catalyzes the last step of ABA biosynthesis, the conversion of abscisic aldehyde to ABA (7). In Arabidopsis, four AOs are known (8) that catalyze the oxidation of ABA-aldehyde (9), although with varying affinities (10). In other eukaryotes, mutants similar to aba3 are known as well. In the fly Drosophila melanogaster, a mutation in the maroon-like locus (ma-l) impairs the activities of AO and XDH, while the activity of sulfate oxidase is unaffected (11). Recently, the genes for ma-l (12) and for similar loci in humans (hmc5 (13)), cattle (mcsu (14)), and fungi (hxB (12)) were cloned, but no biochemical data for the proteins are available so far. Sequence analyses of Drosophila and Aspergillus genes (12) revealed homologies to bacterial NifS proteins, which are known as L-cysteine desulfurases using pyridoxal phosphate (PLP) for transferring sulfur from L-cysteine via a protein-bound persulfide intermediate to various targets (15).

Here we report the cloning, purification, and biochemical characterization of Arabidopsis ABA3. ABA3 is a two-domain protein with an N-terminal NifS-like sulfurase domain and a C-terminal domain that might be involved in recognizing the target enzymes. We established a fully defined in vitro system, which demonstrated that the purified recombinant protein is able to activate Arabidopsis AO by using L-cysteine as sulfur donor. We also show that the expression of the aba3 gene is inducible by drought-stress.

**Experimental Procedures**

**Plant Material and Plant Growth**—A. thaliana seeds were grown in pots containing low nutrient soil in an AR-36L Arabidopsis growth chamber (Percival Scientific, Perry, IA) at 16 h light/8 h darkness, 20 °C, 70% relative humidity. For drought stress experiments, soil was completely removed from the roots prior to incubation under normal conditions in the chamber for 4 h (loss of fresh weight about 50%).

**Preparation of RNA**—Total RNA was prepared as described previously (16); mRNA was prepared using the Oligotex mRNA Midi kit (Qiagen, Hilden, Germany).

**Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)**—Arabidopsis RNA was reverse-transcribed withavian myeloblastosis virus-reverse transcriptase (Promega, Madison, WI) and oligo-d(T)15-primers. RT-PCR was performed on a PCR-Express gradient cycler (Hybaid, Heidelberg, Germany) using the ProFocus-Sprinter™ kit (Hybaid). Degenerated primers were ABA3-Deq.1+ (5'-CCT ACT GGC CTG GCC GCT CTG CTT GT-3') and ABA3-Deq.2- (5'-AAA TGC AGC ACA GGA

*This work was supported by the Deutsche Forschungsgemeinschaft (to R. R. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Botanical Inst., Technical University of Braunschweig, 38023 Braunschweig, Germany. Tel.: 49-531-391-5870; Fax: 49-531-391-8128; E-mail: R.Mendel@tu-bs.de.

1 The abbreviations used are: Moco, molybdenum cofactor; AO, aldehyde oxidase; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; PLP, pyridoxal phosphate; XDH, xanthine dehydrogenase; ABA, abscisic acid; RT-PCR, reverse transcriptase-polymerase chain reaction; UTR, untranslated region; bp, base pair(s); NTA, nitrolotriacetic acid.
CTT GAT TGG GTA-3'); specific primers were abaA3-5′-UTR (5′-CGT CGG CTA TTT TTC AGA GAT CAG G-3′) and abaA3-3′-UTR (5′-CAA TGG TAT ACA GGT CCA GTA ACA G-3′). All RT-PCR-generated cDNAs were directly ligated to pGEM-T Easy (Promega).

Construction of Expression Vector—aba3 cDNA was used as template for PCR to remove 5′- and 3′-UTRs and to generate BamHI sites at each end (aba3-start-BamHI/NotI, 5′-TTT CTT GGA TCC ATG GAA GCA GGA TTT CTT AAG GAA GAA TTC-3′; aba3-stop-BamHI, 5′-CAC ACG AGG ATC CTT ATT CAA TAT CTG GAT TAA CTT CTT CCC C-3′). The resulting 2.5-kilobase pair PCR fragment containing the total coding region was subcloned into pQE80 (Qiagen).

RNA Analysis—Sequence analysis was performed with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit on an ABI Prism 310 cycle sequencer (PE Applied Biosystems, Warrington, UK) with a pop 6 polymer.

RNA Analysis—Northern analysis was performed as described previously (17) with 20 μg of total Arabidopsis RNA. For detection the Gene Image CDP-Star detection module kit (Amersham Pharmacia Biotech) was used. Full-length abaA3 cDNA probes were labeled by PCR with fluorescein-11-dUTP (Amersham Pharmacia Biotech) was used. Full-length cDNAs were directly ligated to pGEM-T Easy (Promega).

Expression of ABA3—For overexpression, abaA3 cDNA was cloned into pQE80 (Qiagen) resulting in a N-terminal 6×His tag fusion. Protein expression was performed in E. coli DH5α. Cells carrying the expression construct were grown aerobically at 37 °C up to an A600

Expression of AOs in Yeast—Overexpression of recombinant 6×His-tagged Arabidopsis AOs in the yeast Pichia pastoris (kindly provided by T. Koshiha, Tokyo, Japan) was performed as described previously (18).

Purification of His-tagged Proteins—Purification of recombinant ABA3 and AOA was performed on a nickel-nitrilotriacetic acid (NTA)-superflow matrix (Qiagen) under native conditions at 4 °C. For further purification, ABA3 was subjected to a cation exchange chromatography using an UNO Q-1 column (Bio-Rad, Münster, Germany) equilibrated with 20 mM Tris/HCl, pH 8.0 (buffer A). Protein samples were applied to the column and eluted with buffer A followed by a linear gradient of 0–50 mM NaCl in buffer A.

Enzyme Assays—Plant material was squeezed at 4 °C in 2 volumes of extraction buffer (100 mM potassium phosphate, 2.5 mM EDTA, 5 mM DTT, pH 7.5), sonicated, and centrifuged. AO activity was detected by activity assay (19) with 5 mM L-hypoxanthine as substrate in 250 mM Tris/HCl, pH 8.5. Nitrate reductase activity was determined as described previously (20).

Determination of L-cysteine desulfurase activity of recombinant ABA3 as well as inhibition of ABA3 activity by thiol-specific alkylation with N-ethylmaleimide were performed as described for NifS (15). The in vitro reconstitution of recombinant AOA by ABA3 was performed aerobically in a total volume of 0.4 ml of 20 mM Tris/HCl, pH 8.0. AOA (80 μg) was incubated with ABA3 (160 μg) in the presence of 1 mM L-cysteine for 1 h at 30 °C, followed by activity staining with indole-3-carboxaldehyde as substrate. Inactivation of recombinant AOA by RCN treatment and anaerobic reconstitution of AOA/XDH by sulfide and di-thionite were performed as described previously (21).

RESULTS AND DISCUSSION

Identification and Cloning of the Arabidopsis aba3 Gene—Arabidopsis aba3 mutants were proposed to be affected in molybdenum enzyme activation because of their lack in AO and XDH activities while activity of NR is preserved (4). To clone the aba3 gene, degenerated primers were derived from highly conserved regions in bovine molybdopterin (GenBank™ AB035649), Drosophila melanogaster (abl, GenBank™ AF162861), and Aspergillus niger (GenBank™ AF128114) and yielded an 808-bp RT-PCR fragment from A. thaliana leaf mRNA. Sequence analysis of this fragment showed strong similarities to the previously mentioned genes on peptide level, and it matched to the genomic BAC clone F19K19/AC011808 of Arabidopsis chromosome 1, which then was analyzed by exon/intron prediction software (ORNAL Grail (compbio.ornl.gov/Grail-1.3/) and GenScan (genes.mit.edu/GENSCAN/)). Information about transcription initiation and termination was used to create primers for 5′- and 3′-untranslated regions, and RT-PCR was performed to amplify full-length cDNAs of aba3. The 2460-bp coding sequence of the resulting cDNA (GenBank™ AF325457) consists of 21 exons with an average size of 117 bp. The aba3 gene includes a region of 5737 bp (F19K19 position 71914/ATG to position 73458/TAAT) with an average intron size of 164 bp, and genomic DNA hybridization showed that it represents a single copy locus (data not shown).

In addition to the 2460-bp open reading frame we also identified various mRNA splice forms, which all follow the g/vag splice rules, leading to truncated forms of the protein if translated (data not shown). We found that in the case of alternative splicing at least two splice events occur in each affected mRNA, mainly in the 3′-region.

The deduced ABA3 protein consists of 819 amino acids and shows a two-domain structure (Fig. 1A). The N-terminal domain has homologies to bacterial NifS proteins that are known from ABA response proteins. The C-terminal domain shows a two-domain structure (Fig. 1B), two-domain structure of ABA3. The NifS-like domain are shown.

FIG. 1. Domain structure of ABA3 protein and molecular characteriziation of aba3 mutants. A, two-domain structure of ABA3. The signatures for binding of RNA, PLP, and persulfide (PS) within the NifS-like domain are shown. Arrows indicate the mutant loci. B, comparison of Arabidopsis wild type Landsberg erecta (Le) and aba3.2 mutant sequences. In aba3.2, the splice acceptor site of intron 10 is destroyed by a G→A substitution (framed), resulting in the use of an alternative splice site 19 bp downstream within the following exon 11. This 19-bp truncation of exon 11 leads to a frameshift and translation termination 22 bp downstream of the alternative splice site. In case of translation, a resulting protein would have a size of just 393 amino acids. Wild type and new aba3.2 splice sites are shown as white letters in black boxes. Two further point mutations within the following four base pairs were found but do not affect the coding region. C, comparison of Arabidopsis wild type Wassilewskija (WS) and 13.5 mutant sequences. Within the aba3 gene of the 13.5 mutant a deletion of 39 bp removes the splice donor site of intron 4, resulting in the use of an alternative splice site 61 bp upstream of that of the wild type within the preceding exon 4. Possible translation would be terminated 52 bp downstream of the alternative splice site. Wild type and new 13.5 splice sites are shown as white letters in black boxes.
cholerae, GenBank™ AAF96821). Conclusions derived from this observation will be discussed below.

**Molecular Characterization of aba3 Mutants**—We analyzed the Arabidopsis mutants aba3.1, aba3.2, and a novel T-DNA-tagged mutant line 13.5 (provided by A. Marion-Poll, INRA, Versailles, France), presenting a similar phenotype. In all three mutants, AO and XDH activities could be restored by anaerobic treatment with sulfide and dithionite (data not shown), indicating that the same locus is affected.

For molecular analysis of the mutants we cloned the aba3 mutant alleles by RT-PCR and genomic PCR. We found that the mutation in aba3.1 resides in a substitution of G to A on cDNA position 1406, resulting in the exchange of a glycine by glutamic acid within a highly conserved region located in the C-terminal domain at amino acid position 469 (Fig. 1A). For aba3.2 (Fig. 1B) and 13.5 (Fig. 1C), more complex mutations were found. In both cases splice sites are affected within the NifS-like domain, leading to truncated open reading frames of the aba3 transcript. Sequence analyses of aba3-homologous mutants in humans (13) and cattle (14) localized the mutations in the NifS-like domain, while in fly mutants intragenic deletions were found. In both cases splice sites are affected within the NifS-like domain, showing that both domains are essential for the sulfuration function of ABA3.

RNA blotting of samples from different organs of Arabidopsis plants showed that aba3 was expressed in all organs tested with highest levels in leaves and roots of adult plants (data not shown). After exposing whole plants to dehydration for 4 h, increased amounts of aba3-mRNA were observed in the leaves (Fig. 2A). Additionally, increased expression levels of AOIs were detectable (Fig. 2B), which underlines the physiological importance of ABA3 for the plant to cope with drought stress. Because significant amounts of desulfo forms of AO and XDH are present in plants at each time, one might suggest that increased expression of ABA3 and subsequent sulfuration of desulfo-AO/XDH under stress is a fast way of adaptation to new environmental conditions.

**Identification of the Reaction Catalyzed by ABA3**—ABA3 was recombinantly expressed in E. coli and purified by nickel-NTA chromatography, exchange chromatography and gel filtration to more than 90% homogeneity (data not shown). The purified protein was yellow in color, indicating the presence of a bound chromophore, which might be PLP as known for NifS and NifS-like enzymes (15). SDS-PAGE analysis gave a molecular mass of 95 kDa for recombinant ABA3, which corresponds to the calculated molecular mass of 92.6 kDa.

ABA3 is proposed to transfer a sulfur from a yet unknown source to AO and XDH. To test whether ABA3 uses in vitro one of the sulfur-containing amino acids as substrate, we added these amino acids to separate samples of purified ABA3 and looked for changes in the visible spectra of the protein. When using l-cysteine, we found a shift of 14 nm in the major absorbance as it was published for NifS (15). These results were confirmed by a coupled assay (15) in which the formation of L-cysteine desulfurase activity of ABA3 and activation of recombinant Arabidopsis AOs by ABA3. A, detection of L-alanine enzymatically produced from L-cysteine. In this coupled assay, L-alanine, enzymatically produced from L-cysteine, is deaminated by glutamate-pyruvate transaminase yielding pyruvate, which then is reduced to lactate in the presence of lactate dehydrogenase. Oxidation of NADH in the last step was monitored by following the decrease in absorbance at 340 nm (15). Columns C and D represent the amount of L-alanine produced from L-cysteine by 200 µg of ABA3 within 1 h at 30 °C in 0.3 mL of 20 mM Tris/HCl, pH 8.0. Under these conditions, ABA3 activities (expressed as moles of l-cysteine converted per molar of ABA3/min) were 0.065 ± 0.05 in reaction C and 1.5 ± 0.14 mol in reaction D. Column A and B are controls without ABA3 (n.d. = not detectable); column E represents the L-alanine control that was used to calibrate the assay and that was set to 100%. B, effect of N-ethylmaleimide on ABA3 activity. Two nanomoles of ABA3 were pretreated with increasing amounts of N-ethylmaleimide prior to determination of remaining l-cysteine desulfurase activity. * indicates the remaining ABA3 activity, expressed as percentage of a control without inhibitor. C, reconstitution of AO activity by ABA3 as visualized by in situ staining for AO activity after native PAGE. Lanes 1 and 4, controls with active and KCN-inactivated AO alone, respectively. Lanes 2 and 5, the same as lanes 1 and 4, but in the presence of l-cysteine. It can be seen that recombinant AO has a basal activity that could be enhanced in the presence of ABA3 and 1 mM l-cysteine (lanes 3 and 6). Lane 7, chemical reconstitution of KCN-inactivated AO in the presence of sulfide and dithionite under anaerobic conditions.
L-alanine is measured. By using this assay we showed that in the presence of DTT, ABA3 converted up to 65% of the given L-cysteine to L-alanine, while only 3% was converted without DTT (Fig. 3A). Obviously the reductant DTT has to release the persulfide from ABA3 for maintaining L-cysteine degradation, so that it can be assumed that in the absence of desulfo-AO/XDH, DTT serves as a substitute for an appropriate acceptor. In general, the enzymatic activity of ABA3 in the absence of its native acceptors appears to be very slow (conversion of 1.5 mol of L-cysteine/mol of ABA3/min, Fig. 3A).

Based on primary sequence comparison to NiS enzymes and to eukaryotic Moco sulfurases, an active site cysteine appears to be involved in catalysis and therefore should be sensitive to alkylating reagents. When we pretreated ABA3 with the thiol-specific alkylating reagent N-ethylmaleimide in a molar ratio of 1:4 (ABA3:N-ethylmaleimide; Fig. 3B), we found an inhibition of L-cysteine desulfurase activity of about 71%, indicating that an active site SH group actually must be involved in catalysis.

In summary, the following observations lead us to suggest that ABA3 catalyzes a reaction similar to bacterial NiS: (i) ABA3 contains a PLP binding motif highly conserved among eukaryotes and bacteria, (ii) ABA3 contains a conserved sequence motif including an invariant cysteine that in the case of NiS was shown to bind the intermediate persulfide responsible for sulfurtransfer from cysteine to the target; thiol-specific alkylation of ABA3 inhibited this sulfurtransfer, (iii) purified ABA3 has a yellow color and showed a NiS-like change in absorbance in the presence of L-cysteine, and (iv) ABA3 has a cysteine desulfurase activity.

To finally prove that ABA3 has a Moco sulfurase function and is able to transfer sulfur from cysteine to AO and XDH, we developed a fully defined in vitro assay with Arabidopsis AO as target. For this assay, isoform AOα encoded by AAO1 cDNA was recombinantly expressed and purified from P. pastoris (18). In the assay, 80 μg of purified recombinant AOα and 160 μg of recombinant ABA3 were incubated for 1 h at 30 °C under aerobic conditions in the presence of 1 mM L-cysteine. Variations of this assay were performed with partially KCN-inactivated AOα protein. For subsequent determination of AO activity we did not use a spectrophotometric assay but chose the more sensitive in situ staining after native PAGE, using indole-3-carboxaldehyde as substrate. The results shown in Fig. 3C depict that AOα as prepared from Ficha has a basal activity that could be enhanced by ABA3 in the presence of L-cysteine and that ABA3 reconstituted the AO activity of KCN-treated AOα protein in the presence of L-cysteine. This ABA3-mediated activation of AOα was as efficient as the chemical activation by anaerobic treatment of inactivated AOα with sulfide and diithionite (Fig. 3C).

Previous analyses of aba3-like mutants in Arabidopsis (4), tomato (5), tobacco (6), Aspergillus (12), Drosophila (11), humans (13), and cattle (14) proposed a defect in the final sulfuration step activating the molybdenum center in AO and XDH. Our experiments show that purified ABA3 is able to activate AO in a defined in vitro system using L-cysteine as sulfur donor. The data obtained provide a first hint that the reaction type of ABA3 is similar to that of bacterial NiS involving an active site (most probably the invariant Cys360) that is sensitive to alkyl-ation. Whether or not, also under in vivo conditions, L-cysteine is the native sulfur donor remains to be determined. Finally we have to discuss how ABA3 can distinguish between its correct targets (=enzymes of the xanthine oxidase family) and its incorrect targets (=enzymes of the sulfite oxidase family). Here the 2Fe/2S centers may be an important distinction, because the former family of enzymes possesses 2Fe/2S centers, while the latter group of enzymes lacks them. It could well be that ABA3 recognizes its correct group of target enzymes on their FeS center binding fold. Keeping in mind that the C-terminal domain of ABA3 shows homologies to bacterial proteins that carry 2Fe/2S centers, one might speculate that the C-terminal domain of ABA3 is involved in recognizing this important property of its target molybdenum enzymes. Further experiments will show whether this assumption is correct.

Acknowledgments—We thank A. Marion-Poll (Versailles, France) for providing aba3 mutants, T. Koshiba (Tokyo, Japan) for providing the AAO-overexpressing Ficha strain, and J. Zeenova (East Lansing, MI) for kindly donating ABA-aldehyde. We are grateful to V. Finnerty and G. Schwarz for helpful discussions. We thank P. Hanzelmann and S. Leimkühler for critical reading of the manuscript.

REFERENCES

1. Hille, R. (1996) Chem. Rev. 96, 2757–2816
2. Kisker, C., Schindelin, H., and Rees, D. C. (1997) Annu. Rev. Biochem. 66, 233–267
3. Mendel, R. R., and Schwarz, G. (1999) Crit. Rev. Plant. Sci. 18, 33–69
4. Schwartz, S. H., Leon-Kloosterziel, K. M., Koornneef, M., and Zeenova, J. A. (1997) Plant Physiol. 114, 161–166
5. Marin, E., and Marion-Poll, A. (1997) Plant Physiol. Biochem. 35, 369–372
6. Leydecker, M.-T., Moureaux, T., Kraegel, Y., Schmer, K., and Cabsche, M. (1995) Plant Physiol. 107, 1427–1431
7. Zeenova, J. A. D. (1999) in Biochemistry and Molecular Biology of Plant Hormones (Hooykaas, P., Hall, M. A., and Libbenga, K. R., eds) pp. 189–207, Elsevier Science Publishers B. V., Amsterdam
8. Sekimoto, H., Seo, M., Kawakami, N., Komano, T., Deslairre, S., Liotenberg, S., Marion-Poll, A., Cabsche, M., Kamiya, Y., and Koshiba, T. (1998) Plant Cell Physiol. 39, 433–442
9. Seo, M., Koiwai, H., Akaba, S., Komano, T., Oritani, T., Kamiya, Y., and Koshiba, T. (2000) Plant J. 23, 481–488
10. Seo, M., Peeters, A. J., Koiwai, H., Oritani, T., Marion-Poll, A., Zeenova, J. A., Koornneef, M., Kamiya, Y., and Koshiba, T. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 12908–12913
11. Wahl, R. C., Warner, C. K., Finnerty, V., and Rajagopalan, K. V. (1982) J. Biol. Chem. 257, 3951–3962
12. Amrani, L., Primus, J., Glatigny, A., Arcangeli, L., Scatuzzo-Ch, C., and Finnerty, V. (2000) Mol. Microbiol. 38, 114–125
13. Ichida, K., Matsumura, T., Sakuma, R., Huooya, T., and Nishino, T. (2001) Biochem. Biophys. Res. Commun. 282, 1194–1200
14. Watanabe, T., Ibara, N., Itoh, T., Fujita, T., and Sugimoto, Y. (2000) J. Biol. Chem. 275, 21789–21792
15. Zheng, L., White, R. H., Cash, V. L., Jack, R. F., and Dean, D. R. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 2754–2758
16. Logemann, J., Schell, J., and Willmitzer, L. (1987) Anal. Biochem. 163, 16–20
17. Zambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed. Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Koiwai, H., Akaba, S., Seo, M., Komano, T., and Koshiba, T. (2000) J. Biochem. (Tokyo) 127, 659–664
19. Koshiba, T., Saito, E., Ono, N., Yamamoto, N., and Sato, M. (1996) Plant Physiol. 110, 781–789
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed. Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Koiwai, H., Akaba, S., Seo, M., Komano, T., and Koshiba, T. (2000) J. Biochem. (Tokyo) 127, 659–664
22. Finnerty, V. (1976) in The Genetics and Biology of Drosophila (Ashburner, M., and Novitski, E., eds) pp. 721–807, Academic Press, New York
ABA3 Is a Molybdenum Cofactor Sulfurase Required for Activation of Aldehyde Oxidase and Xanthine Dehydrogenase in Arabidopsis thaliana
Florian Bittner, Mislav Oreb and Ralf R. Mendel

J. Biol. Chem. 2001, 276:40381-40384. doi: 10.1074/jbc.C100472200 originally published online September 11, 2001

Access the most updated version of this article at doi: 10.1074/jbc.C100472200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 19 references, 7 of which can be accessed free at http://www.jbc.org/content/276/44/40381.full.html#ref-list-1