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The *Arabidopsis thaliana* apurinic endonuclease Arp reduces human transcription factors Fos and Jun

(redox control/transcriptional regulation)

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ABSTRACT **An *Arabidopsis thaliana* cDNA encoding an analogue, referred to as Arp for *a*purinic endonuclease-*r*edox *p*rotein, of the human redox factor REF has been cloned. Arp stimulates *in vitro* DNA-binding activity of the human transcription factors Jun and Fos by the reduction of a cysteine residue located in the DNA-binding domain. Based on amino acid sequence homology, this redox activity is probably confined to the small internal domain of the Arp protein. In analogy to REF, we show that the *Arabidopsis* Arp protein also functions as an apurinic/apyrimidinic class II endonuclease. This base-free endonuclease activity resides in the carboxyl-terminal domain, and this part of the protein has significant sequence similarity to bacterial (*Escherichia coli* exonuclease III and *Streptococcus pneumoniae* exonuclease A) and animal (*Drosophila* Rrp1 and human REF/HAP) apurinic/apyrimidinic endonucleases. The amino-terminal domain of the Arp protein is highly charged and apparently increases the affinity of the protein for DNA. Therefore, the *Arabidopsis* Arp protein is multifunctional and may be involved both in DNA repair and in the regulation of transcription.**

The formation of reactive oxygen intermediates, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, is an inevitable and potentially dangerous consequence of aerobic metabolism. Reactive oxygen intermediates are highly reactive and cause damage to nucleic acids, proteins, and lipids (1). Generation of abasic sites in DNA is a direct consequence of the radical attack, and such sites are formed during repair of oxidized DNA bases (1–3). Abasic sites are mutagenic at the level of DNA replication and repair, and they are a source of transcription errors (3, 4). The survival of an organism depends on the timely repair of abasic sites by characteristic endonucleases (2, 5). The major class of apurinic/apyrimidinic (AP) endonucleases (class II) introduces DNA strand breaks 5′ to the base-free sites. The sugar-phosphate residue is then removed by a phosphodiesterase, followed by filling in of the nucleotide gap by DNA polymerase and DNA ligase (3).

AP endonucleases are multifunctional enzymes. The best studied is *Escherichia coli* exonuclease III. This enzyme possesses both phosphodiesterase and phosphomonoesterase activities, which are quite different on the basis of reactions catalyzed (6). Eukaryotic AP endonucleases seem to have acquired additional functions. The *Drosophila* AP endonuclease Rrp1 was originally purified as a DNA strand transferase from fly embryos (7), and it also renaturates single-stranded DNA (8). The human AP endonuclease, HAP, which was originally isolated as a DNA repair enzyme (9), has recently been found to have a redox activity (10, 11). Transcription factors such as AP-1, Myb, p53, and NF-κB bind to the respective DNA recognition sites only if cysteine residues in their DNA-binding domains are reduced (12–14). It has been postulated that reduction/oxidation may be a regulatory mechanism of transcription factor activity *in vivo*. This conclusion was strengthened by the fact that in some oncogenic variants of Jun the cysteine in the DNA-binding domain is replaced by other amino acids and therefore escapes the cellular redox control (12). The nuclear factor from HeLa cells, REF, which can reduce transcription factors *in vitro*, was isolated and turned out to be identical to the human AP endonuclease HAP (10, 11).

Here, we describe the isolation of a plant homologue of the human REF/HAP AP endonuclease.§ This *Arabidopsis thaliana* protein, designated Arp for *a*purinic endonuclease-*r*edox *p*rotein, is also shown to have a dual activity. It has an apurinic endonuclease activity and is able to activate the human Fos and Jun transcription factors *in vitro*.

MATERIALS AND METHODS

Plant Material. *A. thaliana* ecotype Columbia was grown in soil under 16-hr-light/8-hr-dark conditions at 22°C.

Isolation of *arp* cDNA. The part of the cDNA encoding *arp* was first isolated by PCR amplification using two degenerate oligonucleotides, 5′-AARATITGYIIITGGAAYGTI-GAYGG-3′ and 5′-GTIATIGGRCARCARTGRTC-3′ (R = G or A; Y = T or C), corresponding to the most conserved regions of apurinic endonucleases from bacteria, animals, and *Drosophila* (11). The cDNA template for PCR reactions was synthesized on 5 μg of total leaf RNA using the preamplification kit (GIBCO/BRL). The amplified PCR fragment was used to screen ≈10^6 plaques of an *Arabidopsis* cDNA library made from cell suspension culture mRNA in λZap. Four positive clones were identified, and the *arp* cDNA containing pBluescript SK(+) phagemids was excised *in vivo* from the λ vector using the helper phage VSC-M13 (Stratagene). The sequence of the longest cDNA insert was determined on both strands by the dideoxynucleotide chain-termination method.

Purification of the Recombinant Arp Protein. *Sac* I–*Xho* I or *Nru* I–*Xho* I fragments of the *arp* cDNA were subcloned into the *Bam*HI site of the pQE-8 expression vector (Qiagen, Chatsworth, CA), which had been treated with mung bean nuclease. The in-frame fusions were verified by sequencing, and the recombinant plasmids were transformed into *E. coli* M15. The production of the polyhistidine–Arp fusion proteins was induced in midlogarithmic *E. coli* cultures grown in 2× YT medium (10 g of Bacto yeast/16 g of Bacto tryptone/5 g of NaCl, pH 7) by 2 mM isopropyl β-D-thiogalactopyrano-

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Abbreviations: AP, apurinic/apyrimidinic; DTT, dithiothreitol.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. X76912).

side. After 5 hr of growth, cells were collected by centrifugation and lysed in 6 M guanidine hydrochloride, and proteins were purified on nickel chelate agarose affinity columns as described by the manufacturer (Qiagen). Proteins that were eluted from the column were dialyzed and stored at −70°C. The dialysis buffer [50 mM sodium phosphate, pH 7.3/1 mM EDTA/5% (vol/vol) glycerol] was supplemented with 1 mM dithiothreitol (DTT) to prepare reduced proteins; alternatively, DTT was omitted to prepare oxidized forms of Arp (10, 15). Proteins used for the AP endonuclease assays were additionally purified by denaturing preparative PAGE as described (16).

AP Endonuclease Assay. The AP endonuclease activity of Arp was measured by the formation of nicked forms of supercoiled plasmids containing abasic sites essentially according to Puyet *et al.* (17). Briefly, 5 μg of supercoiled pBluescript KS(+) plasmid was incubated for 5 min in 15 mM sodium citrate at 65°C and placed on ice. Plasmid DNA was desalted on Sephadex G-50 spin columns equilibrated with TE buffer (10 mM Tris·HCl, pH 8.0/1 mM EDTA). Partially apurinated or untreated plasmid (500 ng) was incubated with recombinant Arp in 20-μl assay reaction mixtures in reaction buffer (10 mM Tris·HCl, pH 8.0/5 mM $MgCl_2$/1 mM EDTA/0.01% Nonidet P-40) for 15–20 min at 37°C. Half of the reaction was stopped by chilling on ice. To the other half of the reaction, SDS and EDTA were added to 1% and 7 mM final concentrations, respectively. Samples were electrophoresed on 0.7% agarose gels, subsequently stained with ethidium bromide, and photographed.

AP-1 Reduction Assay. This assay was done according to Xanthoudakis and Curran (10). One nanogram of both Fos-(118–211) and Jun-(224–234) (18) were incubated in the presence of 10 mM DTT or different amounts of recombinant Arp for 15 min at 37°C in binding buffer [50 mM Tris·HCl, pH 7.3/50 mM NaCl/5 mM $MgCl_2$/1 mM EDTA/5% (wt/vol) sucrose/5% glycerol]. Then, 1 μg of poly(dI-dC) and ≈0.2 ng of the ^{32}P-labeled oligonucleotide (≈30,000 cpm) containing two AP-1 binding sites were added to the reaction mixtures. After a 20-min incubation at room temperature, reaction mixtures were loaded on 4.5% PAGE gels and run in 25 mM Tris/195 mM glycine buffer. After electrophoresis, gels were dried and exposed to x-ray film. When oxidized Arp was used in the assays, 100 ng of the *E. coli* thioredoxin (Promega) was added to the reaction mixtures. The AP-1 binding sites (underlined) were reconstructed by the annealing of the following complementary oligonucleotides (19): 5′-TCGACGTGACTCAGCGCGCATCGTGACTCAGCGCGC-3′ and 5′-TCGAGCGCGCTGAGTCACGATGCGCGCTGAGTCACG-3′.

The double-stranded oligonucleotide was labeled by a filling-in reaction with Klenow fragment of DNA polymerase I in the presence of [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) and purified on a native 8% PAGE gel.

Miscellaneous Methods. The DNA strand transferase activity was tested according to Lowenhaupt *et al.* (7). Total *Arabidopsis* DNA and RNA were extracted as described (20). DNA and RNA gel blot analyses and construction of recombinant plasmids were performed according to standard procedures (21). PCR amplification fragments were cloned into ddTTP-tailed pBluescript KS(+) (22). The sequence analysis software package by the Genetics Computer Group Inc. was used for the analysis of DNA and protein sequences.

RESULTS

Isolation and Characterization of *arp* cDNA. Degenerate oligonucleotides designed according to two highly conserved regions (underlined in Fig. 1) of human HAP/REF-1 (11), *Drosophila melanogaster* Rrp1 (23), *Streptococcus pneumoniae* exonuclease A (17), and *E. coli* exonuclease III (6) were

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ARP      QSSAIYVAKFLVVPLRSLRVGSSFVGVGVGTRSFNKRLMSNATAFSINNSKRKELKIPGA   60
ARP      AIDQNCHQMGSDTDRDEMGTLQDDRKEIEAMTVQELRSTLRKLGVPVKGRKQELISTLRL  120
ARP      HMDSNLPDQKETSSSTRSDSVTIKRKISNREEPTEDECTNSEAYDIEHGEKRVKQSTEKN  180
ARP      LKAKVSAKAIAKEQKSLMRTGKQQIQS..KEETSSTISSELLKTEEIISSPSQSEPWTVL  238
REF/HAP                              MPKRGKKGAVAED...GDELRTEPEAKKSKT..   28

ARP      AHKKPQKDWKAYNPKTMRPPPLPEG.....TKQVKVMTWNVGLRGLLKFESFSALQLAQ  293
REF/HAP  AAKKNDKEAAGEGPALYEDPPDQKTSPSGKPATLKICSWNVDGLRAWIKKK...GLDWVK   85

ARP      RENFDILCLQETKLQVKDVEEIKKTLIDGYDHSFWSCSVSKLGYSGTAIISRIKPLSVRY  353
REF/HAP  EEAPDILCLQETKCSENKLPAELQEL.PGLSHQYWSAPSDKEGYSGVGLLSRQCPLKVSY  144

ARP      GTGLSGHDTEGRIVTAEFDSFYLINTYVPNSGDGLKRLSYRIEEWDRTLSNHIKELEKSK  413
REF/HAP  GIGDEEHDQEGRVIVAEFDSFVLVTAYVPNAGRGLVRLEYR.QRWDEAFRKFLKGLASRK  203

ARP      PVVLTGDLNCAHEEIDIFNPAGNKRSAGFTIEERQSFGANLLDKGFVDTFRKQHPGVV.G  472
REF/HAP  PLVLCGDLNVAHEEIDLRNPKGNKKNAGFTPQERQGFGELLQAVPLADSFRHLYPNTPYA  263

ARP      YTYWGYRHGGRKTNKGWRLDYFLVSQSIAANVHDSYILPDINGSDHCPIGLILKL       527
REF/HAP  YTFWTYMMNARSKNVGWRLDYFLLSHSLLPALCDSKIRSKALGSDHCPITLYLAL       318
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FIG. 1. The amino acid sequence of the *A. thaliana* Arp protein. The amino acid sequence of an open reading frame encoded by the longest *arp* cDNA is presented in comparison with the human REF/HAP AP endonuclease (10). The underlined sequences were used to design degenerate primers for the PCR amplification of the *arp* cDNA. Similarity between the two proteins is indicated by vertical bars. Diamonds above the Arp sequence indicate the positions at which the Arp protein was fused to the polyhistidine tag for expression in *E. coli*. The cysteine residues important for the REF/HAP redox activity (14) as well as putatively important ones in Arp are shaded.

used in a PCR reaction with *A. thaliana* cDNA as a template (see *Materials and Methods*). The 800-bp PCR fragment was used to screen an *Arabidopsis* oligo(dT)-primed cDNA library. Four positive cDNA clones were isolated, and the complete nucleotide sequence of the longest cDNA clone was determined. This cDNA is 1753 bp in length and contains one contiguous open reading frame, which encodes a putative protein of 527 amino acids. Because no in-frame stop codons are present upstream from the first methionine of this open reading frame, it is likely that the putative protein is longer. The region of Arp that has homology to DNA repair enzymes known to cleave DNA phosphodiester bonds 5′ from AP sites is situated in the carboxyl-terminal part of the Arp protein (amino acids 270–527). The highly charged amino-terminal part (≈250 amino acids) of the Arp does not show significant sequence similarity to any protein present in the Swiss-Prot data bank sequences (Release 77) (Fig. 1).

To determine the copy number of the *arp* gene, Southern blot analysis under low stringency conditions was performed on genomic DNA isolated from *A. thaliana* ecotype Columbia. As was expected from the *arp* cDNA physical map, single hybridizing fragments were detected when DNA was digested with *Eco*RI, *Bam*HI, and *Sac* I. Three and two fragments were detected with *Pst* I and *Kpn* I, respectively (Fig. 2*A*). We can conclude that *arp* is a single-copy gene and that no other closely related sequences are present in the *Arabidopsis* genome. Using the *arp* cDNA as a probe, a single 1.8- to 1.9-kb mRNA was detected by Northern analysis. The *arp* mRNA is present in all tested *Arabidopsis* organs, with the highest levels in leaves (Fig. 2*B*).

To further analyze the function of Arp, we have expressed nearly full-length (amino acids 1–527; referred to as Arp) and amino-terminally truncated (amino acids 191–527; referred to as ΔArp) versions of the protein as hexahistidine fusion proteins in *E. coli*. The recombinant Arp proteins were purified to near homogeneity by nickel chelate chromatography from bacterial extracts. The purified Arp and ΔArp

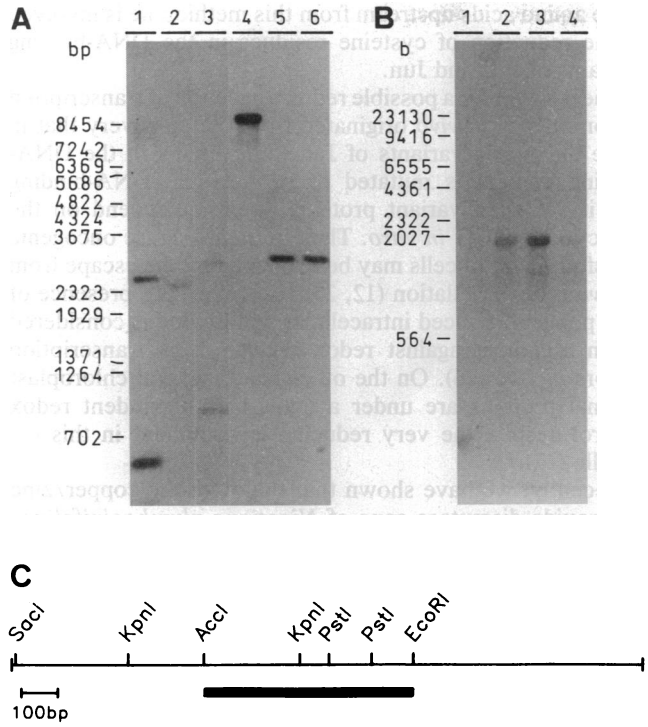

FIG. 2. Determination of the *arp* gene copy number and mRNA size. (*A*) *Arabidopsis* genomic DNA gel blot analysis. Two micrograms of genomic DNA was digested with *Pst* I (lane 1), *Sac* I (lane 2), *Kpn* I (lane 3), *Bam*HI (lane 4), *Eco*RI (lane 5), and *Eco*RI plus *Bam*HI (lane 6) and electrophoresed on a 0.7% agarose gel. After blotting, the nylon membrane was hybridized with a probe indicated by the black bar in *C*. The number of hybridizing bands fits with the physical map of the *arp* cDNA (*C*) and indicates the presence of a single *arp* gene in the *Arabidopsis* genome. (*B*) RNA gel blot analysis of the *arp* mRNA. Total RNA (15 μg) and *Hin*dIII-digested ^{35}S-labeled λ DNA were denatured with glyoxal, electrophoresed on a 1.5% agarose gel, blotted on nylon membranes, and hybridized with a ^{32}P-labeled *arp* probe. The hybridizing band of ≈1.9 kb was detected in RNA extracted from siliques (lane 1), flowers (lane 2), leaves (lane 3), and stems (lane 4). (*C*) Physical map of the cloned *arp* cDNA. Sites of restriction endonucleases used for DNA gel blot analysis and present in the cDNA are indicated. The bar under the map corresponds to the DNA fragment used to prepare ^{32}P-labeled probes for hybridizations.

proteins had the expected masses of 76 kDa and 44 kDa, respectively (Fig. 3).

Arp Has an AP Endonuclease Activity. Class II AP endonucleases make DNA strand breaks 5′ to abasic sites in DNA, and a sensitive assay based on the conversion of apurinated supercoiled plasmid to an open circle has been developed to detect AP nuclease activity (17). When the purified recombinant Arp and ΔArp are incubated with supercoiled plasmids containing apurinic sites, they efficiently convert them to the open circle (Fig. 4, lanes 2–6). These proteins do not affect untreated supercoiled plasmids (Fig. 4, lanes 8 and 9). In the presence of the Arp protein, small amounts of both apurinated and untreated plasmid DNA are immobilized in the slots of the agarose gel (Fig. 4*C*, lanes 2–4, and 9). These DNA–protein aggregates disappear when SDS is included in the loading buffer (compare lanes 2–4, and 9 in Fig. 4 *B* and *C*). The ΔArp protein does not cause such DNA aggregation.

Arp Is a Redox Factor. A number of animal transcription factors only bind to their recognition DNA sequences when cysteine residues in their DNA-binding domains are reduced (ref. 11 and references therein). Usually DTT is added as a reducing agent in *in vitro* studies of DNA-binding proteins. However, recently it has been shown that the human REF/

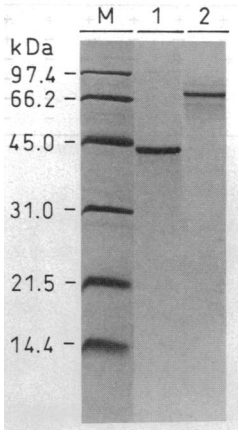

FIG. 3. Purification of the recombinant Arp proteins. After purification on a nickel chelate column, ≈1 μg of *E. coli*-produced ΔArp (lane 1; amino acids 191–527) and Arp (lane 2; amino acids 1–527) were separated on a denaturing SDS/12% PAGE gel. Proteins were stained with Coomassie blue. Protein molecular size markers are shown in lane M.

HAP AP endonuclease can reduce cysteine residues in the DNA-binding domains of AP-1 and some other transcription factors *in vitro* (10, 11). We have analyzed whether the *Arabidopsis* Arp protein can stimulate DNA-binding activity of the recombinant Jun-(118–221) and Fos-(224–234) heterodimers to the AP-1 DNA recognition site. DNA binding was followed by an electrophoretic mobility shift assay. As shown in Fig. 5, DTT stimulates the formation of four specific DNA–protein complexes. In the binding reaction, when DTT

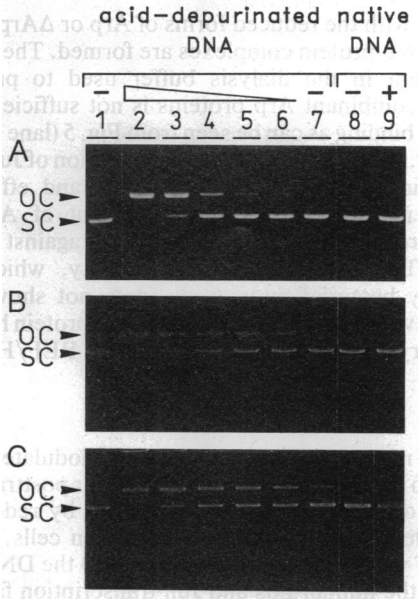

FIG. 4. The AP endonuclease activity of recombinant ΔArp and Arp. Supercoiled plasmid DNA was depurinated and incubated for 15 min at 37°C without additions (lanes 7), with 100 ng of bovine serum albumin (lanes 1), or with 10, 5, 2, 1, and 0.5 ng (lanes 2–6, respectively) of either recombinant ΔArp (*A*) or Arp (*B* and *C*). Untreated supercoiled plasmid DNA was incubated under the same conditions either alone (lanes 8) or in the presence of 10 ng (lanes 9) of either ΔArp (*A*) or Arp (*B* and *C*). After incubation, the reactions were stopped by chilling on ice (*A* and *C*) or by the addition of SDS (*B*). DNA products were resolved on 0.7% agarose gels, stained with ethidium bromide, and visualized under a UV transilluminator. SC and OC correspond to supercoiled and open circular DNA, respectively. Note that in the presence of Arp both depurinated and untreated DNA are trapped in the gel slots (*C*) and that this is eliminated when the reactions are stopped with SDS (*B*).

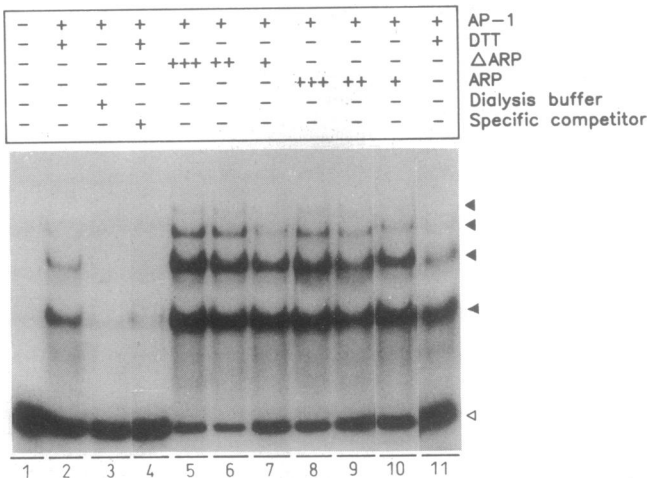

FIG. 5. Arp activation of human Jun and Fos. The radioactively labeled oligonucleotide containing two AP-1 binding sites was incubated with truncated forms of Jun-(224–234) and Fos-(118–211), referred to as AP-1. Each reaction was supplemented with the various compounds as indicated above each lane. Lane 1, oligonucleotide (0.1 μM) alone; lanes 2 and 11, supplemented with 10 mM DTT; lane 5, with 600 ng of ΔArp; lane 6, with 300 ng of ΔArp; lane 7, with 60 ng of ΔArp; lane 8, with 600 ng of Arp; lane 9, with 300 ng of Arp; lane 10, with 60 ng of Arp. DNA–protein complexes were resolved by 4.5% native PAGE and visualized by autoradiography of dried gels. As controls, the DNA binding was assayed in the presence of the dialysis buffer used to prepare reduced Arp proteins (lane 3) and in the presence of an 100-fold excess of the unlabeled oligonucleotide (specific competitor) with labeled probe (lane 4). Free probe is indicated by an open arrowhead, and specific DNA–protein complexes are marked with solid arrowheads.

is replaced with the reduced forms of Arp or ΔArp, the same specific DNA–protein complexes are formed. The amount of DTT present in the dialysis buffer used to prepare the reduced recombinant Arp proteins is not sufficient to stimulate DNA binding as can be seen from Fig. 5 (lane 3). As with REF/HAP, Arp is more efficient in activation of Jun–Fos and Jun–Jun dimers as compared to DTT, and efficiency of activation is proportional to the amount of Arp added. Furthermore, if Arp or ΔArp is dialyzed against the buffer without DTT, it loses its redox activity, which can be restored by bacterial thioredoxin (data not shown). From these data, we conclude that the plant Arp protein has a redox activity very similar to that of the human REF/HAP.

DISCUSSION

Activity of many transcription factors is modulated by phosphorylation (24). Recently, a mechanism for posttranslational regulation of transcription factor activity by redox control has been described (10, 11, 15). In human cells, the redox factor REF was shown to stimulate *in vitro* the DNA-binding activity of the human Fos and Jun transcription factors (10, 11). We have cloned an *Arabidopsis* cDNA encoding a REF-homologous protein, which we call Arp. This protein has a redox activity very similar to that of the human REF/HAP. Arp more efficiently stimulates the DNA-binding activity of the human Fos/Jun transcription factors than DTT, and the redox activity depends on the reduced state of the protein (11, 15). The amino-terminal domain of Arp does not interfere with redox properties; therefore, the same activity may be present in the *Drosophila* Rrp1. By mutational analysis, it has been shown that Cys-65 is critical for the reducing activity of REF/HAP, and Cys-93 may play a regulatory role (15). In Arp, a cysteine in a position homologous to Cys-93 is present, but there is a methionine in the position congruent to Cys-65. Possibly, the cysteine located three amino acids upstream from this methionine is involved in the reduction of cysteine residues in the DNA-binding domains of Fos and Jun.

The concept for a possible redox regulation of transcription factor activity *in vivo* originated from the discovery that in some oncogenic variants of Jun the cysteine in the DNA-binding domain is mutated to serine. The DNA-binding activity of such variant proteins does not depend on the reduction by DTT *in vitro*. Their ability to cause oncogenic transformation of cells may be explained by the escape from *in vivo* redox regulation (12, 25). However, the presence of high pools of reduced intracellular glutathione is considered as an argument against redox regulation of transcription factors *in vivo* (26). On the other hand, several chloroplast stromal proteins are under a tight, light-dependent redox control despite the very reducing environment in this organelle (27).

Recently, we have shown that the cytosolic copper/zinc superoxide dismutase gene of *Nicotiana plumbaginifolia* is inducible *in vivo* by a range of sulfhydryl-containing molecules, such as glutathione, DTT, and cysteine (28). This redox control could be mediated by DNA-binding proteins of the Myb family, which all contain a conserved KSCR motif in their DNA-binding domain (29). The cysteine present in this motif has to be reduced for efficient binding of animal c-Myb to its DNA-binding sites (14). Furthermore, REF/HAP stimulates c-Myb DNA-binding activity *in vitro* (11).

The Arp protein has, in analogy to the human REF factor, an AP endonuclease activity. This activity has been shown to reside in the carboxyl-terminal end of the protein, which is similar to AP endonucleases of bacteria [43% homology with *E. coli* exonuclease III (6) and 44% homology with *S. pneumoniae* exonuclease A (17)] and of animals [56% homology with *D. melanogaster* Rrp1 (23) and 57% homology with human REF/HAP (11)]. Like the *Drosophila* Rrp1 (23), Arp has an extended and highly charged (16.8% positively and 12% negatively charged amino acids) amino-terminal domain, which is not present in bacterial and mammalian AP endonucleases. There is no significant similarity on the amino acid level between the amino-terminal domains of Rrp1 and Arp. In the *Drosophila* Rrp1, the amino-terminal domain is indispensable, although not sufficient for strand transferase and single-stranded DNA renaturation activities (7, 8). The increased affinity to DNA was the only function found in this study for the amino-terminal domain of the Arp. At this point, we cannot exclude that Arp does not have strand transferase activity. The amino-terminal hexahistidine stretch of the recombinant Arp protein may interfere with strand transferase activity.

In conclusion, the Arp protein is multifunctional, and in view of its strong homology to the REF/HAP, it is probably involved in DNA repair and in redox regulation of transcription factors. Such a protein may be involved in processes sensing DNA damage.

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1. Halliwell, B. & Gutteridge, J. M. C. (1989) *Free Radicals in Biology and Medicine* (Clarendon, Oxford).
2. Imlay, J. A. & Linn, S. (1988) *Science* **240,** 1302–1309.
3. Lindahl, T. (1993) *Nature (London)* **362,** 709–715.
4. Zhou, W. & Doetsch, P. W. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6601–6605.
5. Demple, B., Halbrook, J. & Linn, S. (1983) *J. Bacteriol.* **153,** 1079–1082.
6. Saporito, S. M., Smith-White, B. J. & Cunningham, R. P. (1988) *J. Bacteriol.* **170,** 4542–4547.
7. Lowenhaupt, K., Sander, M., Hauser, C. & Rich, A. (1989) *J. Biol. Chem.* **264,** 20568–20575.
8. Gu, L., Huang, S.-M. & Sander, M. (1993) *Nucleic Acids Res.* **21,** 4788–4795.
9. Robson, C. N. & Hickson, I. D. (1991) *Nucleic Acids Res.* **19,** 5519–5523.
10. Xanthoudakis, S. & Curran, T. (1992) *EMBO J.* **11,** 653–665.
11. Xanthoudakis, S., Miao, G., Wang, F., Pan, Y.-C. E. & Curran, T. (1992) *EMBO J.* **11,** 3323–3335.
12. Abate, C., Patel, L., Rauscher, F. J., III, & Curran, T. (1990) *Science* **249,** 1157–1161.
13. Matthews, J. R., Kaszubska, W., Turcatti, G., Wells, T. N. C. & Hay, R. T. (1993) *Nucleic Acids Res.* **21,** 1727–1734.
14. Mirset, A. H., Bostad, A., Jamin, N., Lirsac, P.-N., Toma, F. & Gabrielsen, O. S. (1993) *EMBO J.* **12,** 4625–4633.
15. Walker, L. J., Robson, C. R., Black, E., Gillespie, D. & Hickson, I. D. (1993) *Mol. Cell. Biol.* **13,** 5370–5376.
16. Hager, D. A. & Burgess, R. R. (1980) *Anal. Biochem.* **109,** 76–86.
17. Puyet, A., Greenberg, B. & Lacks, S. A. (1989) *J. Bacteriol.* **171,** 2278–2286.
18. Abate, C., Luk, D., Gentz, R., Rauscher, F. J., III, & Curran, T. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1032–1036.
19. Rauscher, F. J., III, Voulalas, P. J., Franza, B. R., Jr., & Curran, T. (1988) *Genes Dev.* **2,** 1687–1699.
20. Shirzadegan, M., Christie, P. & Seemann, J. R. (1991) *Nucleic Acids Res.* **19,** 6055.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
22. Marchuk, D., Drumm, M., Saulino, A. & Collins, F. S. (1991) *Nucleic Acids Res.* **19,** 1154.
23. Sander, M., Lowenhaupt, K., Lane, W. S. & Rich, A. (1991) *Nucleic Acids Res.* **19,** 4523–4529.
24. Hunter, T. & Karin, M. (1992) *Cell* **70,** 375–387.
25. Okuno, H., Akahori, A., Sato, H., Xanthoudakis, S., Curran, T. & Iba, H. (1993) *Oncogene* **8,** 695–701.
26. Meyer, M., Schreck, R. & Baeuerle, P. A. (1993) *EMBO J.* **12,** 2005–2015.
27. Holmgren, A. (1985) *Annu. Rev. Biochem.* **54,** 237–271.
28. Hérouart, D., Van Montagu, M. & Inzé, D. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3108–3112.
29. Shinozaki, K., Yamaguchi-Shinozaki, K., Urao, T. & Koizumi, M. (1992) *Plant Mol. Biol.* **19,** 493–499.