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Identification of Residues in the Human DNA Repair Enzyme HAP1 (Ref-1) That Are Essential for Redox Regulation of Jun DNA Binding

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The DNA binding activity of the c-*jun* proto-oncogene product is inhibited by oxidation of a specific cysteine residue (Cys-252) in the DNA binding domain. Jun protein inactivated by oxidation of this residue can be efficiently reactivated by a factor from human cell nuclei, recently identified as a DNA repair enzyme (termed HAP1 or Ref-1). The HAP1 protein consists of a core domain, which is highly conserved in a family of prokaryotic and eukaryotic DNA repair enzymes, and a 61-amino-acid N-terminal domain absent from bacterial homologs such as *Escherichia coli* exonuclease III. The eukaryote-specific N-terminal domain was dispensable for the DNA repair functions of the HAP1 protein but was essential for reactivation of the DNA binding activity of oxidized Jun protein. Consistent with this finding, exonuclease III protein could not reactivate Jun. A minimal 26-residue region of the N-terminal domain proximal to the core of the HAP1 enzyme was required for redox activity. By site-directed mutagenesis, cysteine 65 was identified as the redox active site in the HAP1 enzyme. In addition, it is proposed that cysteine 93 interacts with the redox active site, probably via disulfide bridge formation. It is concluded that the HAP1 protein has evolved a novel redox activation domain capable of regulating the DNA binding activity of a proto-oncogene product which is not essential for its DNA repair functions. Identification of a putative active site cysteine residue should facilitate analysis of the mechanism by which the HAP1 protein may alter the redox state of a wide range of transcription factors.

Modulating the level of expression of specific genes is a prerequisite for the control of cellular growth and differentiation. Gene expression is controlled by sequence-specific DNA binding proteins (transcription factors) which in certain cases are targets for signals transduced from cell surface receptors. The importance of this process for growth control is emphasized by the finding that several proto-oncogenes, including c-*myc*, c-*myb*, c-*fos*, and c-*jun*, encode sequence-specific transcription factors (reviewed in reference 20). Although the activity of these factors can be modulated by phosphorylation (4, 11), recent evidence has emerged for an additional and unusual form of regulation of DNA binding activity mediated by changes in reduction-oxidation (redox) status. For example, the binding of Fos-Jun heterodimers (comprising the AP-1 transcription factor) and Jun-Jun homodimers to DNA requires that these proteins be in a reduced state (1, 3, 9). This form of redox regulation may be widespread because the DNA binding activities of several other transcription factors, including Myb, Rel, and NF-κB, are similarly sensitive to changes in oxidation state (10, 12, 16, 17).

The target for redox regulation of Jun DNA binding activity is a conserved cysteine residue (Cys-252 in chicken Jun), which when mutated to serine results in DNA binding activity which is no longer sensitive to inactivation by oxidation (3). This cysteine residue is replaced by serine in the transforming viral oncogene v-*jun* (15), suggesting that the oncogenic potential of this virus may be realized in part through escape from this putative regulatory process.

A factor from human cell nuclei which facilitated binding of the Fos-Jun complex to its DNA recognition sequence under oxidizing conditions has been identified (1, 3, 30). cDNAs encoding this factor (designated Ref-1 by these authors) were isolated (31) and found to encode a previously characterized DNA repair enzyme (also designated HAP1 [21], APE [7], or APEX [26]). The activity of the HAP1 (Ref-1) factor is also sensitive to oxidation, with a gradual decline in ability to reactivate Fos-Jun DNA binding observed during the course of purification (30). Activity can be restored by treatment with thioredoxin (30), suggesting that a redox-sensitive group in the HAP1 (Ref-1) protein is involved in the reductive activation of the Fos-Jun complex.

HAP1 is the human homolog of *Escherichia coli* exonuclease III protein (7, 21) and, like its bacterial counterpart, is involved primarily in the repair of DNA lesions generated by reactive oxygen species (reviewed in reference 8). More specifically, the HAP1 protein participates in the repair of DNA containing apurinic-apyrimidinic (AP) sites via endonucleolytic cleavage 5′ to the baseless site. HAP1 also possesses a 3′ phosphodiesterase activity capable of removing lesions (such as phosphoglycolate) blocking the 3′ side of DNA strand breaks generated by ionizing radiation or certain radiomimetic drugs such as bleomycin (22). These atypical 3′ lesions obstruct the priming of DNA repair synthesis.

The finding that the HAP1 protein not only possesses DNA repair activity but also has a possible role in redox activation of transcription factors raised the possibility that the enzyme may be bifunctional with two distinct active centers. To study this, we have generated both a series of N-terminally truncated forms of the HAP1 protein and a set

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of site-directed mutant forms of HAP1 and have shown that the DNA repair and redox activation functions of the enzyme can be separated. Crucial residues for redox activity, but not for DNA repair functions, lie in a 61-residue N-terminal domain absent from the bacterial homologs of HAP1. A cysteine residue (Cys-65), located immediately adjacent to the N-terminal domain of HAP1, was identified as essential for redox activity and is proposed to be the active site for regulation of Jun protein DNA binding.

MATERIALS AND METHODS

Subcloning of the HAP1 cDNA. The HAP1 protein coding region was amplified by the polymerase chain reaction (PCR). For cloning into pT7-7 (28) the following primers were used. The 5′ primer incorporated an *Eco*RI site, and the 3′ primer incorporated a *Hin*dIII site (5′ primer, 5′-CTGAGAATTCGCCCGAAGCGTGGGAAA-3′; 3′ primer, 5′-GATCAAGCTTTCACAGTGCTAGGTATAGGGT-3′). For cloning into pKK223 (Pharmacia) the same 3′ primer was used, together with the 5′ primer CTGAGAATTCAT GCCGAAGCGTGGGAAA. PCR was performed with 1 μg of HAP1 plasmid template with five rounds of amplification under the following conditions: 94°C, 0.5 min; 55°C, 0.33 min; and 72°C, 1 min. The initial cycle included a denaturation step for 3 min, and the final cycle had an elongation step of 5 min. The PCR products were incubated with 0.5 U of Klenow polymerase for 15 min at 37°C prior to electrophoresis on a 1% agarose gel. The amplified product was excised from the gel and purified with Geneclean (Stratech Scientific). The product was then restricted with *Eco*RI and *Hin*dIII and was subcloned between the *Eco*RI and *Hin*dIII sites of either pT7-7 or pKK223. This placed the HAP1 protein coding region under the control of the T7 or *tac* promoters, respectively.

Generation of N-terminally truncated forms of the HAP1 protein. N-terminally truncated forms of the HAP1 protein were generated by PCR amplification with oligonucleotides complementary to the appropriate region of the HAP1 protein coding region. Restriction enzyme sites were included in the oligonucleotides to allow directional cloning into pT7-7.

Site-directed mutagenesis. Mutagenesis was performed by a PCR-based technique derived from that of Landt et al. (14) which uses two separate PCR reactions involving a single mutagenic primer and two vector-specific primers. Modifications to the method included use of 1 μg of template DNA in each reaction and use of 5 and 10 cycles of amplification in the first and second rounds, respectively. Denaturation, annealing, and elongation steps were similar to those outlined above.

Mutant forms of the HAP1 cDNA either were cloned into pKK223 (Pharmacia) for low-level inducible expression in *E. coli* or were cloned into pT7-7 for high-level expression and subsequent protein purification, as described below. All site-specific mutant cDNAs were confirmed by DNA sequencing.

The pKK223 derivatives were introduced into a *dut xth*(Ts) strain as previously described (21). Site-directed mutant forms of the HAP1 cDNA in pT7-7 were transformed into BL21 (DE3), an *E. coli* strain incorporating a chromosomally integrated T7 polymerase gene under the control of the *lac* promoter (27). Expression of the T7 polymerase gene could be induced by addition of IPTG (isopropyl-β-D-thiogalactopyranoside) to the culture medium.

Purification of recombinant HAP1 and the site-specific mutant HAP1 proteins. HAP1 protein was purified from *E. coli* BL21 (DE3) cells treated for 2 h with 1 mM IPTG to induce expression from the T7 promoter in pT7-7. The procedure for purification will be described in detail elsewhere (unpublished data). Briefly, lysozyme was used to lyse bacteria and proteins precipitated with 70% $(NH_4)_2SO_4$. The protein pellet was subjected to chromatography on phosphocellulose P11 and fast-performance liquid chromatography phenyl-Superose columns, with active fractions eluting from these columns at approximately 450 mM NaCl and 50 mM $(NH_4)_2SO_4$, respectively. All recombinant proteins were more than 90% pure as judged by electrophoresis on 12% polyacrylamide gels containing sodium dodecyl sulfate (13).

DNA repair assays. The native HAP1 and mutant HAP1 proteins were assayed for both AP endonuclease and phosphodiesterase activities, as described by Robson et al. (22). The AP endonuclease assay measured the rate of formation of relaxed (form II) DNA from supercoiled (form I) DNA containing AP sites. These sites were induced in plasmid DNA by treatment at 70°C for 15 min in 0.1 M sodium acetate (pH 5.5) as described previously (22). This treatment induced on average one to two baseless sites per molecule. The phosphodiesterase assay measured the restoration of bleomycin-damaged DNA to serve as a substrate for Klenow polymerase, as described previously (22). This reflected the conversion of the 3′ phosphoglycolate termini of DNA strand breaks induced by bleomycin to 3′ OH groups which can prime DNA synthesis.

Reactivation of oxidized Jun protein gel retardation assays. The expression and purification of chicken c-Jun protein has been described previously (18). Jun protein was oxidized by dialysis for 16 h in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH (pH 7.9) as described by Xanthoudakis et al. (31). HAP1 and mutant HAP1 proteins were dialyzed into the same buffer for 16 h in the presence or absence of 0.25 mM dithiothreitol (DTT). After dialysis, the protein concentration was determined (5) to ensure that equal numbers of moles of protein were used in each assay.

The assay for reactivation of Jun DNA binding was based on the methods of Frame et al. (9) and Abate et al. (2). The reaction was performed in a total volume of 20 μl and contained 10× reaction buffer (100 mM HEPES [pH 7.9], 2 mM EDTA, 940 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 mg of bovine serum albumin per ml, 40% glycerol), 0.1 μM Jun protein, and 0.5 μM HAP1 protein. This mixture was incubated at 37°C for 15 min, after which 1 μg of poly(dI-dC) was added, and the incubation continued for a further 5 min at 20°C. A ^{32}P-end-labelled 30-mer oligonucleotide (containing a consensus Jun binding site) was then added, and the mixture was incubated on ice for 15 min. The sample was then loaded directly onto a 4% polyacrylamide gel (prepared in 0.5× Tris-borate-EDTA buffer) and run at 60 V at 4°C until a control sample of electrophoresis dye (in an adjacent lane) reached the bottom of the gel. The gel was then dried down and exposed to X-ray film.

RESULTS

HAP1, but not *E. coli* exonuclease III, can reactivate oxidized Jun protein. The primary structure of the major AP endonuclease enzyme in prokaryotic and eukaryotic cells has been highly conserved throughout evolution. Approximately 30% identity and 50% similarity in amino acid sequence are apparent between the *E. coli* exonuclease III and human HAP1 proteins (7, 21). However, the HAP1 protein

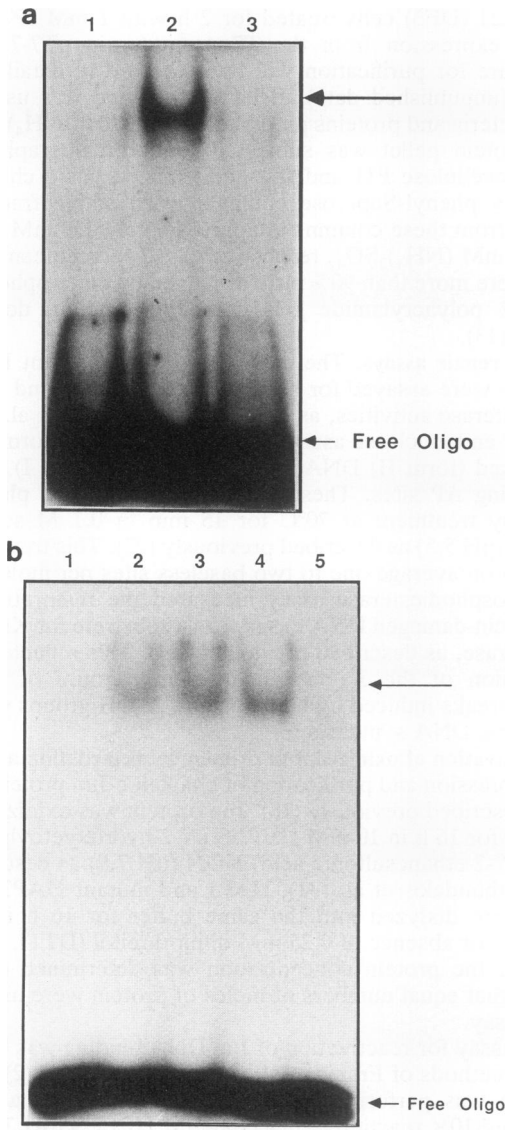


FIG. 1. (a) HAP1, but not exonuclease III, stimulates the DNA binding activity of Jun protein. Oxidized Jun protein was incubated alone (lane 1), with HAP1 protein (lane 2), or with exonuclease III protein (lane 3), and a gel retardation assay was performed with a ^{32}P-labelled oligonucleotide which includes a consensus Jun binding site. The positions of the free oligonucleotide (oligo) and the retarded protein-DNA complex (large arrow) are indicated. (b) HAP1 protein lacking the N-terminal 61 residues cannot stimulate the DNA binding activity of oxidized Jun protein. Lanes: 1, oxidized Jun protein alone; 2, reduced Jun after dialysis into buffer containing 10 mM DTT; 3, oxidized Jun protein plus 100 mM DTT; 4, oxidized Jun protein plus HAP1 protein; 5, oxidized Jun protein plus HAP1$^{(62-318)}$ protein. The positions of the free oligonucleotide and the protein-DNA complex are indicated. The assay was performed as outlined for panel a.

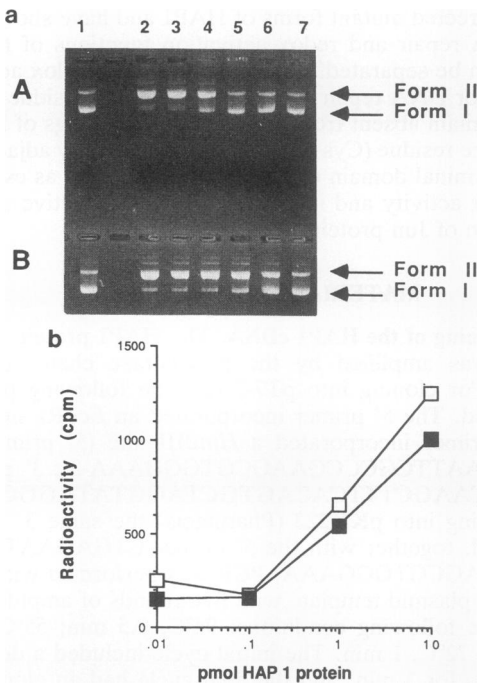


FIG. 2. (a) HAP1$^{(62-318)}$ protein is proficient as an endonuclease on DNA containing baseless sites. Supercoiled DNA containing baseless sites was incubated with HAP1 protein (A) or HAP1$^{(62-318)}$ protein (B); the DNA was electrophoresed on a 1% agarose gel and stained with ethidium bromide. Lanes: 1, control DNA containing baseless sites; 2 to 7, respectively, DNA plus the following amounts of HAP1 or HAP1$^{(62-318)}$ protein—14 pmol, 1.4 pmol, 0.7 pmol, 0.14 pmol, 0.07 pmol, 0.014 pmol. The positions of the form I (supercoiled) and form II (relaxed) DNAs are shown on the right. (b) HAP1$^{(62-318)}$ protein is proficient as a phosphodiesterase as measured by an ability to restore bleomycin-damaged DNA to a substrate for Klenow polymerase. Incorporation of ^{32}P-labelled dCTP into double-stranded calf thymus DNA as a function of the number of moles of HAP1 (□) or HAP1$^{(62-318)}$ (■) protein is shown. The data are from a single representative experiment.

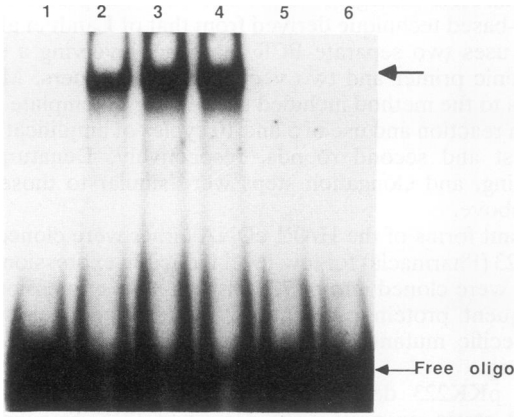
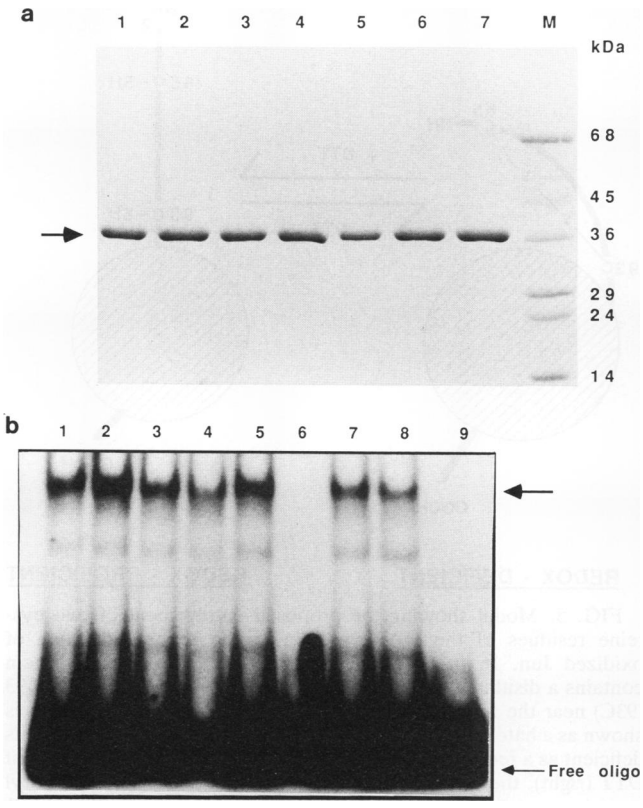


FIG. 3. Identification of a minimal region of the HAP1 N-terminal domain essential for the stimulation of Jun DNA binding. Oxidized Jun protein was incubated with full-length HAP1 protein or various truncated derivatives, and a gel retardation assay was performed as outlined in the legend to Fig. 1. Lanes: 1, oxidized Jun protein alone; 2, oxidized Jun plus HAP1 protein; 3, oxidized Jun plus HAP1$^{(29-318)}$; 4, oxidized Jun plus HAP1$^{(36-318)}$; 5, oxidized Jun plus HAP1$^{(51-318)}$; 6, oxidized Jun plus HAP1$^{(62-318)}$. The positions of the free oligonucleotide (oligo) and the retarded protein-DNA complex are shown.

includes 61 additional residues at the N terminus. To ascertain whether HAP1 and exonuclease III share redox activity in addition to their common DNA repair functions, the abilities of the HAP1 and exonuclease III proteins to reactivate oxidized Jun protein were compared. As shown in Fig. 1a, while the HAP1 protein could greatly enhance the DNA



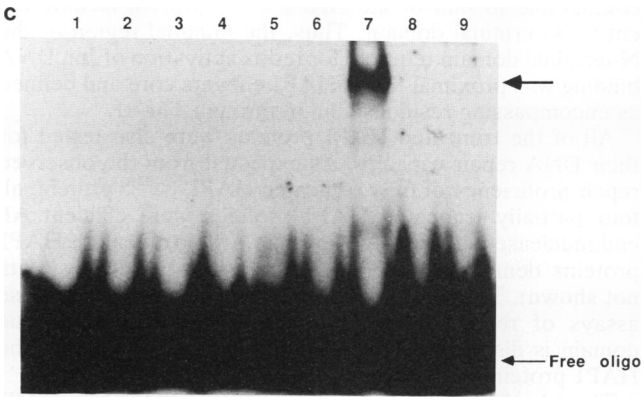


FIG. 4. (a) Purification of site-specific HAP1 mutant proteins containing a cysteine-to-alanine substitution at one of the seven cysteine residues of the native HAP1 protein. A Coomassie blue-stained SDS polyacrylamide gel is shown. Lanes: 1, HAP1:C65A; 2, HAP1:C93A; 3, HAP1:C99A; 4, HAP1:C138A; 5, HAP1:C208A; 6, HAP1:C296A; 7, HAP1:C310A. The position of the purified proteins is shown by an arrow on the left. The sizes of molecular mass standards run in parallel (lane M) are shown on the right. (b) HAP1:C65A is unable to stimulate Jun DNA binding. HAP1 and the seven cysteine-to-alanine substitution mutants were dialyzed under reducing conditions (with 0.25 mM DTT) and incubated with oxidized Jun protein, and a gel retardation assay was performed. All reactions contained oxidized Jun protein. Lanes: 1, HAP1:C296A; 2, HAP1:C138A; 3, HAP1:C310A; 4, HAP1:C208A; 5, HAP1:C99A; 6, HAP1:C65A; 7, HAP1:C93A; 8, wild-type HAP1; 9, oxidized Jun protein alone. The positions of the free oligonucleotide (oligo) and the retarded protein-DNA complex (large arrow) are shown on the right. (c) HAP1:C93A can activate Jun DNA binding in the absence of DTT. HAP1 and the seven cysteine-to-alanine substitution mutants were dialyzed under oxidizing conditions (absence of DTT) and incubated with oxidized Jun protein, and a gel retardation assay was performed. The lanes are as indicated in panel b.

binding activity of oxidized Jun protein (as determined by a gel retardation assay), exonuclease III protein could not.

The N-terminal domain of the HAP1 protein is required for redox activity but is not essential for DNA repair functions. The failure of exonuclease III to reactivate Jun protein suggested the possibility that the eukaryote-specific 61-residue N-terminal domain present in the HAP1 protein was required for redox activity. To test this, cDNA constructs encoding N-terminally truncated HAP1 protein were expressed in *E. coli*, and the protein lacking the first 61 residues of the 318-residue native HAP1 protein was purified [designated HAP1$^{(62-318)}$]. Figure 1b shows that the HAP1$^{(62-318)}$ protein failed to efficiently reactivate oxidized Jun protein, confirming that the N-terminal 61 residues of the HAP1 protein are required for redox activity.

The DNA repair activities of the truncated HAP1 protein were also assayed. HAP1$^{(62-318)}$ protein retained near wild-type AP endonuclease activity, as judged by two criteria.

TABLE 1. Temperature sensitivity of strains

Strain	Relevant genotype	Relative plating efficiency at 42 or 30°C[a]
KL16	Wild type	1.00
BW287	*dut-1 xthA3*(Ts)	1.6×10^{-4}
BW287(pKK223/HAP1)	Same as BW 287 but pHAP1^{+}	0.87
BW287[pKK223/HAP1$^{(62-318)}$]	Same as BW287 but pHAP1$^{(62-318)}$	0.88

[a] Determined by counting colonies on plates incubated at either 30 or 42°C.

First, the purified protein cleaved supercoiled plasmid DNA containing AP sites with an efficiency approximately equal to that of full-length HAP1 protein (Fig. 2a). Second, the HAP1$^{(62-318)}$ cDNA was able to rescue a *dut xth*(Ts) double mutant of *E. coli* at the nonpermissive temperature of 42°C (Table 1). This bacterial strain is normally nonviable at 42°C because of an increase in the size of the intracellular dUTP pool and subsequent incorporation of dUTP into DNA. After excision of the incorporated uracil by the repair enzyme uracil DNA glycosylase, the failure to remove the AP sites (due to lack of exonuclease III) thus generated leads to cell death (6).

The HAP1$^{(62-318)}$ protein also restored the ability of bleomycin-damaged DNA to serve as a substrate for Klenow polymerase. This phosphodiesterase activity primarily involves removal of phosphoglycolate residues from the 3′ end of DNA strand breaks induced by this drug (29). The phosphodiesterase activities of the HAP1 and HAP1$^{(62-318)}$ proteins were indistinguishable (Fig. 2b).

Identification of a minimal region of the HAP1 N-terminal domain essential for redox activity. Four additional truncated HAP1 proteins were overexpressed in *E. coli* and purified. These proteins were truncated from their N termini by 7, 28, 35, or 50 residues and were designated HAP1$^{(8-318)}$, HAP1$^{(29-318)}$, HAP1$^{(36-318)}$, and HAP1$^{(51-318)}$, respectively. While the HAP1$^{(8-318)}$, HAP1$^{(29-318)}$, and HAP1$^{(36-318)}$ proteins were as efficient as full-length HAP1 in reactivating oxidized Jun protein (Fig. 3 and data not shown), the HAP1$^{(51-318)}$ protein showed much reduced activity (Fig. 3),

comparable to that of the HAP1$^{(62-318)}$ protein lacking the entire N-terminal domain. Thus, the minimal region of the N-terminal domain required for redox activation of Jun DNA binding was proximal to the HAP1 enzyme core and defined as encompassing residues Glu-36 through Thr-61.

All of the truncated HAP1 proteins were also tested for their DNA repair capacity. As expected from the observed repair proficiency of fully truncated HAP1$^{(62-318)}$ protein, all four partially truncated HAP1 proteins were efficient AP endonucleases in vitro. Moreover, the truncated HAP1 proteins demonstrated 3′ phosphodiesterase activity (data not shown). Thus, as far as could be determined with these assays of repair competence, the N-terminal 61-residue domain is dispensable for the DNA repair activities of the HAP1 protein in vitro.

The role of cysteine residues in the redox action of the HAP1 protein. The possibility that one or more cysteine residues in the HAP1 protein could be directly involved in the reductive activation of Jun protein was investigated by substituting the cysteine residues at positions 65, 93, 99, 138, 208, 296, and 310 with alanine by using site-directed mutagenesis. The mutant proteins were designated HAP1:C65A, HAP1:C93A, and so on. These site-specific mutant proteins were overexpressed in *E. coli* and purified (Fig. 4a). It was considered possible that the mutant proteins could show altered Jun reactivation capacity either because one or more cysteine residues might participate directly in the redox reaction via electron transfer or, alternatively, might influence higher-order protein structure. In the latter case, if a putative active site cysteine residue was normally capable of forming a disulfide bridge with a second cysteine in the HAP1 protein, redox activity might be masked under oxidizing conditions, thereby explaining both the apparent requirement that the HAP1 protein be in a reduced state to exhibit redox activity, and the stimulatory effect of thioredoxin on this activity (30). For this reason, the cysteine mutants were tested for redox activity after dialysis either into HEPES buffer alone (oxidizing conditions) or into buffer containing 0.25 mM DTT (reducing conditions).

All of the mutant HAP1 proteins were active when assayed under reducing conditions, with the exception of HAP1:C65A, which completely lacked the ability to stimulate Jun DNA binding (Fig. 4b). In contrast, mutant HAP1:C93A was proficient as a redox activator of Jun DNA binding even after oxidation by dialysis in buffer lacking DTT (Fig. 4b and c).

The possibility that the HAP1:C65A protein failed to reactivate Jun for trivial reasons, such as incorrect folding or denaturation, was discounted, because HAP1:C65A protein was as efficient as native HAP1 protein as a DNA repair enzyme, either when assayed for phosphodiesterase activity or when assayed for AP endonuclease activity (data not shown).

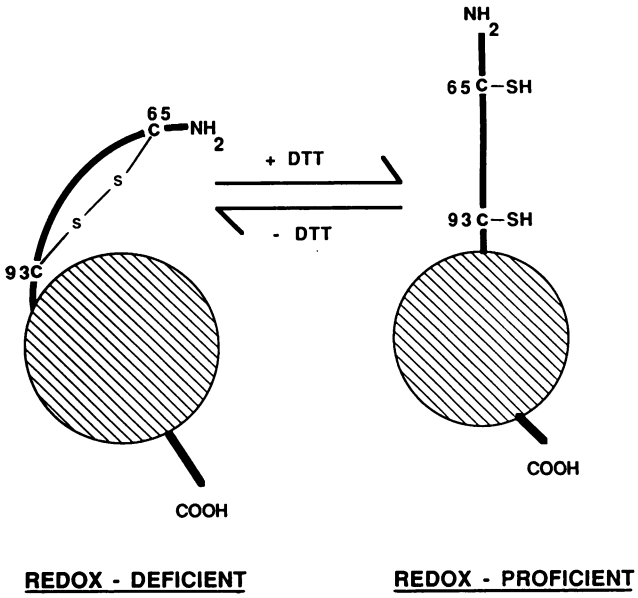


FIG. 5. Model showing the proposed involvement of two cysteine residues of the HAP1 protein in the redox regulation of oxidized Jun. In the absence of DTT (left), the HAP1 protein contains a disulfide bridge (S-S) between cysteines 65 (65C) and 93 (93C) near the N terminus. The remainder of the HAP1 protein is shown as a hatched circle. The HAP1 protein in this conformation is deficient as a redox regulator of Jun DNA binding. Upon addition of DTT (right), the disulfide bridge is broken, regenerating sulfhydryl groups on cysteine 65 and cysteine 93. In this form, the HAP1 protein is proficient as a regulator of Jun DNA binding activity in vitro.

DISCUSSION

We have demonstrated that the human DNA repair enzyme HAP1 (Ref-1) is a bifunctional protein which can regulate the DNA binding activity of the c-*jun* proto-oncogene product in vitro. Using site-directed mutagenesis, we have identified two key cysteine residues in the HAP1 protein which modulate the efficiency of this redox function.

The HAP1 protein is highly homologous to DNA repair enzymes from other mammals and *Drosophila* and bacterial species (7, 21–25). This sequence conservation extends throughout the length of the proteins with the exception of a eukaryote-specific N-terminal domain which comprises 61 residues in the human protein. We have shown that this N-terminal domain is essential for redox activity but not for DNA repair functions. It has been noted previously that this domain contains two short sequence motifs closely matching the consensus for nuclear localization signals (7, 21). This suggests that this short N-terminal domain may direct two functions in vivo: the control of subcellular localization and an involvement in the redox activation of transcription factors.

Our data provide no direct evidence that the N-terminal domain contains residues which participate in the redox active site, although clearly this might be the case. It is also possible that the region of the N terminus proximal to the core enzyme, which was identified as important for redox activity, is required simply for maintenance of the correct tertiary structure of the enzyme, particularly because this N-terminal region is located immediately adjacent to cysteine 65, the proposed redox active site in the HAP1 protein. Alternatively, the N-terminal domain of HAP1 may be required to mediate a direct interaction with the Jun protein that is necessary to bring the redox active site cysteines of the two proteins into close proximity. Further work will be required to distinguish between these possibilities. Ultimately, only after X-ray crystallographic analysis will the three-dimensional array of active site residues in the HAP1 protein be known.

We have shown that *E. coli* exonuclease III protein lacks detectable redox activity under the assay conditions described. This is consistent with the absence of both the N-terminal 61-residue domain and the proposed active site

cysteine 65 residue in this bacterial homolog of HAP1 (7, 21, 24).

The properties of the HAP1:C93A mutant, which was equally active under oxidizing or reducing conditions, were unexpected. The requirement for the HAP1 protein to be in a reduced state to enable it to reactivate Jun protein, together with the previously reported stimulatory effects of thioredoxin on HAP1-mediated redox activity (30), implies the possible presence of a disulfide bridge in the HAP1 protein which must be disrupted before significant redox activity can be observed in vitro. Cysteine 93 is a strong candidate for participating in this disulfide bridge, and we propose that this residue could serve to regulate the redox activity of HAP1 by this mechanism.

We have incorporated the data presented here into a model which is presented in Fig. 5. It is proposed that the oxidized HAP1 protein contains a disulfide bridge between cysteine residues 65 and 93. In this form, the active site residue, cysteine 65, cannot participate in the redox activation of Jun protein. Upon reduction by low concentrations of DTT, the disulfide bridge in HAP1 is broken, exposing the sulfydryl group of the active site cysteine 65 residue and thus permitting a productive interaction between this residue and cysteine 252 in the Jun protein. Conversely, in the HAP1 mutant lacking cysteine 93, no disulfide bridge can be formed and consequently the enzyme is constitutively active in the absence of a chemical reducing agent.

It seems unlikely that the ability of the HAP1 protein to modulate the redox state of other proteins is related to the normal interactions between HAP1 and its nucleic acid substrates, because the data presented here, and other unpublished results with site-directed mutants of HAP1, indicate that DNA repair activity can be compromised without losing redox activity and vice versa.

It will be important to show that redox regulation of Jun DNA binding occurs in vivo after oxidative stress. Evidence that this novel form of regulation exists in eukaryotic cells has been provided by the observation that a mutant Fos protein lacking the critical redox-sensitive cysteine residue in the DNA binding domain was resistant to inactivation by the oxidizing agent diamide and had enhanced transforming potential (19).

In summary, we have identified a putative active site cysteine residue in the HAP1 protein required for the redox regulation of the DNA binding activity of Jun protein and have shown that the DNA repair and redox activities of the HAP1 protein are separable. Further evidence for our model will require both proof of a specific interaction between the active site cysteine residues of the HAP1 and Jun proteins and detailed structural information on the HAP1 protein.

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