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A novel promoter sequence is involved in the oxidative stress-induced expression of the adult T-cell leukemia-derived factor (ADF)/human thioredoxin (Trx) gene

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ABSTRACT

Adult T cell leukemia-derived factor (ADF) is a human thioredoxin (Trx) and is a disulfide reducing protein with various biological functions. We found that expression of the ADF/Trx gene was increased by oxidative agents such as hydrogen peroxide, diamide and menadione in Jurkat cells. Analysis using a CAT expression vector plasmid under the control of the ADF/Trx gene promoter revealed that CAT gene expression in Jurkat cells was increased after exposure to oxidative agents. A series of deletion analyses showed that a region from –976 to –890 of the 5′ flanking sequence was required for enhancement of ADF/Trx promoter activity against the oxidative agents. Gel mobility shift assay revealed the specific DNA binding activities to the sequences from –953 to –930 in the nuclear extracts from the Jurkat cells. The sequences in this region showed no homology with any known consensus sequences for DNA binding factors. It is suggested that ADF/Trx gene expression is enhanced through a novel *cis*-acting regulatory element responsive for the oxidative stress and a new factor(s) is involved in this oxidative stress responsive element.

INTRODUCTION

Eukaryotic cells continuously produce reactive oxygen species (ROS) as a result of electron transfer reactions and ROS readily react with cellular molecules, either damaging them directly or starting a chain reaction wherein the free radical is passed from one molecule to another, resulting in extensive damage to cellular structures such as membrane (1). This also occurs in cells exposed to UV light, γ rays or hydrogen peroxide, and those stimulated with cytokines (2). Oxidative stress induces defensive reactions dealing with ROS or ROS-induced damages. These involve induction of enzymes with radical scavenging and repair activities. In bacteria, two systems of ROS-responsive transcription factors, oxyR (3) and soxRS (4), have been reported. They control the expression of multiple antioxidative enzymes in response to hydrogen peroxide and superoxide anions, respectively. Analogous reactions have been reported in human cells. For example, heme oxygenase is a major stress protein in human cells treated with oxidants, and oxidative stress as well as heat shock highly induce CL100 which is a Tyr/Thr-protein phosphatase (5). c-*fos* and c-*jun* genes are also induced by hydrogen peroxide (6) and UV irradiation (7). However, in eukaryotic cells the mechanisms and factors regulating oxidative and antioxidative responses have not been well established.

Adult T cell leukemia-derived factor (ADF) has been reported to be produced by many human T cell lymphotropic virus-I-transformed T cells (8,9) and EBV-transformed B cells (10). ADF shows several biological activities, such as the growth promotion of lymphoid cells (11), synergism with IL-1 and IL-2 (12), and augmentation of cysteine transport (13). Cloning of cDNA for ADF (14,15) showed a close homology with a sulfhydryl reducing coenzyme, thioredoxin (Trx), first found in prokaryotes (16). Trx has a redox active disulfide (Cys-Gly-Pro-Cys) and has various activities as a hydrogen donor, such as the degradation of insulin (17), the stabilization of glucocorticoid receptor (18), the enhancement of binding of the Jun–Fos complex to AP-1 site through the reduction of ref-1 protein (19), and the restoration of the DNA binding-activity of the oxidized NF-κB (20–23). Trx is involved in an electron-transfer system common to a variety of organisms and recombinant ADF/Trx shows a reducing activity of hydrogen peroxide (24). Furthermore, rADF has a protective activity against TNFα-dependent cytotoxicity in U937 cells (25), in which the involvement of ROS is reported (26,27). On the other hand, hydrogen peroxide, UV irradiation can induce ADF/Trx expression (28–30). These suggest that ADF/Trx plays an important role in cellular responses against oxidative stress.

Although the human Trx encoding gene has been cloned (31,32), the regulation of the gene expression remains unknown. By examining the ADF/Trx promoter region we show here that oxidative agents such as hydrogen peroxide, diamide and menadione increase ADF/Trx gene expression, and the enhancement of gene expression appears to depend on a promoter region from –951 to –932, which showed no homologous sequence to

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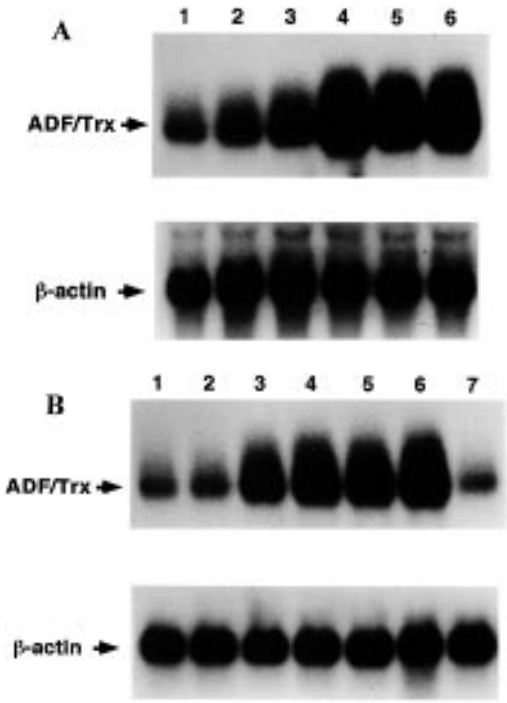

Figure 1. Induction of ADF/Trx mRNA expression in Jurkat cells against oxidative agents. (**A**) Jurkat cells were treated with the oxidative agents and incubated for 6 h. Lane 1, control; lane 2, 1 μM hydrogen peroxide; lane 3, 10 μM hydrogen peroxide; lane 4, 100 μM hydrogen peroxide; lane 5, 5 μM diamide; lane 6, 5 μM menadione. (**B**) Time course of the induction of ADF/Trx mRNA levels during hydrogen peroxide. Jurkat cells were treated with 100 μM hydrogen peroxide for the indicated periods of time. The blots were reprobed with β-actin cDNA as a loading control.

any known motif of DNA–protein interactions. We also show that factor(s) specifically binds to the sequences, and may be involved in the gene expression against oxidative stress.

MATERIALS AND METHODS

Cell culture

Jurkat cells were cultured at 37°C in 5% CO_2 in RPMI1640 medium (Gibco-BRL, Grand Island, NY) supplemented with 10% (v/v) fetal calf serum (MBA laboratories, Tokyo, Japan), 100 μg/ml streptomycin and 100 U/ml penicillin.

Isolation of genomic clones

All molecular biological procedures were performed as described (33,34) unless otherwise noted. A genomic cosmid library derived from lymphocytes of a human male (kindly provided by Dr Sugita, Kyoto University, Kyoto, Japan) was screened by using α-^{32}P-labeled ADF/Trx cDNA probe. A 2.6 kb *Hin*dIII and *Bam*HI restriction fragment that reacted positively was sucloned into pBluescript KS(–) vector (Stratagene, La Jolla, CA) to construct pBTrx(–2629/+28). The subcloned fragment of genomic DNA was sequenced on both strands by using the dideoxynucleotide chain termination procedure.

Northern blot analysis

Jurkat cells were treated with oxidative agents for 30 min at 37°C as described in the figure legend, incubated for 6 h and harvested.

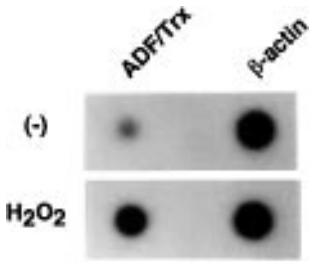

Figure 2. Nuclear run-on analysis of ADF/Trx gene transcription after hydrogen peroxide treatment. Jurkat cells were cultured for 2 h with 100 μM hydrogen peroxide or without hydrogen peroxide. Nuclear run-on transcription was performed with isolated nuclei from untreated (–) or hydrogen peroxide treated (100 μM for 2 h) Jurkat cells. Each ^{32}P-labeled RNA was hybridized to a nylon membrane, which was blotted with denatured ADF/Trx cDNA insert or β-actin insert.

Total cellular RNA was isolated by single-step method (35), and RNA samples (20 μg/lane) were separated on a 1% formaldehyde/agarose gel and transferred to a nylon membrane (Hybond N^+, Amersham International plc., UK), and hybridized with [α-^{32}P]dCTP-labeled ADF/Trx cDNA probe.

Nuclear run-on assay

From untreated or H_2O_2-treated (100 μM for 2 h) Jurkat cells, intact nuclei were isolated and subjected to nuclear run-on transcription. Transcribed ^{32}P-labeled RNA was precipitated with trichloroacetic acid, trapped onto a glass filter, and washed extensively. The human ADF/Trx cDNA insert and the human β-actin gene fragment were alkaline denatured and blotted onto a nylon membrane, to which purified labeled RNA was hybridized.

Plasmid construction

Deletion constructs were generated by the following two ways. One was by cloning relevant fragments into a pBluescript vector after DNA had been digested by specific restriction enzymes. Alternatively, the constructs were linearized by restriction enzymes, digested with exonuclease III (Toyobo Corp., Tokyo, Japan) and mung bean nuclease (Toyobo Corp., Tokyo, Japan), filled in and self-ligated. Those fragments upstream from the ADF/Trx gene were cloned into the pCAT-Basic vector (Promega Corp., Madison, WI). m-pTrxCAT(–1017) was constructed by PCR-mutagenesis (36).

Chloramphenicol acetyltransferase assay

Transfections were performed by electroporation method by using Gene Pulser (Bio-Rad, Richmond, CA). Jurkat cells (1×10^7)were incubated in RPMI 1640 with 10% FCS at 0°C with 5 μg plasmid DNA, and subjected to a single pulse at 1000 V/cm and 960 μF. Pact β-gal (5 μg), which contains the cDNA β-galactosidase under the control of chicken β-actin promoter (37), was co-transfected to assess the amount of DNA incorporation. After 24 h the cells were treated with various oxidative agents as indicated in the figure legends. Twelve hours after the treatment cell extracts were prepared by freeze–thaw for three cycles, and CAT activity was determined.

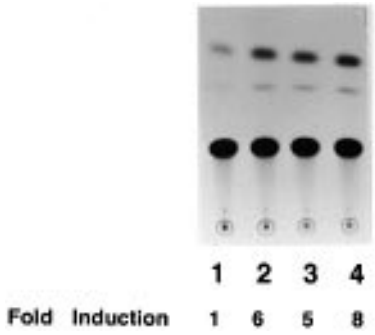

Figure 3. Induction of ADF/Trx promoter activity after the treatment of oxidative agents. Jurkat cells were transfected with CAT expression, pTrxCAT(–2629), using the electroporation method. Twenty-four hours after transfection, cells were treated with the oxidative agents, and CAT activity was assayed 12 h thereafter. Two other independent assays showed almost the same data as presented in this figure. Lane 1, control; lane 2, 100 μM hydrogen peroxide; lane 3, 5 μM diamide; lane 4, 5 μM menadione. The promoter activity in Jurkat cells is indicated as CAT activity relative to that of pTrxCAT(–2629), which is normalized by each β-galactosidase activity (the mean of three independent experiments).

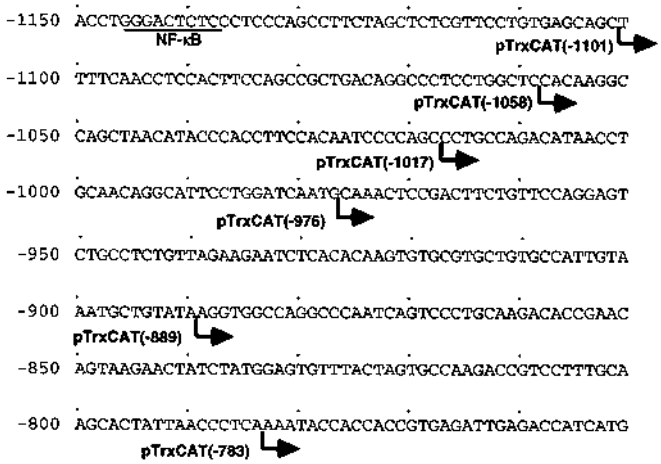

Figure 4. Nucleotide sequence of the 5′ flanking region of the human ADF/Trx gene. Nucleotide 1 corresponds to the T of the transcription initiation site (32), and residues preceding it are represented by negative numbers. The 5′ endpoints of the various deletion mutants are indicated by arrows.

Gel mobility shift assay

The double-stranded oligonucleotide was labeled with a [γ-^{32}P]ATP using T4 polynucleotide kinase (Toyobo Corp., Tokyo, Japan). The binding reaction was performed at 0°C for 30 min in 80 mM KCl, 14% glycerol, 0.02 mM EDTA, 20 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 1.2 mM DTT, 1 μg poly(dI–dC), and 10 000 c.p.m. probe. Electrophoresis was done with 4% polyacrylamide gel with 0.5× TBE buffer. The total extracts were used for gel mobility shift assay without further purification.

DNase I foot printing analysis

A deleted mutant of ADF promoter pTrx(–1058/+28) was digested with *Hin*dIII, labeled with Klenow enzyme (Toyobo Corp., Tokyo, Japan) and [α-^{32}P]dATP, and digested with *Xho*I, yielding the 710 bp fragment labeled at one end. The labeled fragments were isolated with agarose gel electrophoresis. Nuclear extracts (20 μg) were preincubated with poly(dI–dC) (1 μg) for 15 min at 0°C and then incubated with the end-labeled DNA probe (10 000 c.p.m.) in 50 μl for 10 min at 20°C. The reaction mixture was treated with DNase I and analyzed by electrophoresis in a sequencing gel.

RESULTS

Induction of the expression of ADF/Trx gene by oxidative agents

To study the mechanism of the ADF/Trx gene induction, we examined T lymphoma cell line Jurkat cells for the expression of ADF/Trx gene. We first examined the levels of mRNA of ADF/Trx of Jurkat cells after treatment with oxidative agents. As shown in Figure 1A, ADF/Trx mRNA was induced 6-, 5- and 7-fold by hydrogen peroxide (100 μM), diamide (5 μM) and menadione (5 μM), respectively, while the levels of β-actin mRNA showed no significant change. Figure 1B shows that ADF/Trx mRNA was induced by 100 μM hydrogen peroxide within 2 h and was maintained at higher levels for at least 12 h. The mRNA decreased to the basal level after 24 h.

We next performed nuclear run-on experiments to learn about the increase in the transcription of the ADF/Trx gene after hydrogen peroxide treatment. As shown in Figure 2, ADF/Trx transcription was activated at 2 h of treatment, while the level of β-actin transcription showed no significant change.

We then studied the function of the 5′-flanking region of the human ADF/Trx gene to assess the transcriptional regulation of hydrogen peroxide induction using transient expression assay. Jurkat cells were transfected with pTrxCAT(–2629), in which the CAT gene was under the control of the ADF/Trx gene promoter region ranging from –2629 to +28, and treated with the oxidative agents 12 h after transfection. Addition of hydrogen peroxide, diamide and menadione to the culture medium resulted in 6-, 5- and 8-fold induction of CAT activities, respectively (Fig. 3). These data suggested the presence of a site(s) responsible for the gene induction against the oxidative agents in the ADF/Trx promoter region.

Identification of ADF/Trx promoter regions required for the induction

To delineate the sequences required for the induction by the oxidative agents, we made a series of 5′ deletion mutants of the ADF/Trx gene promoter linked to the CAT gene. Figure 4 shows the endpoint of the deletions and Figure 5 shows the CAT activities in Jurkat cells after the addition of hydrogen peroxide to the culture medium, compared with the basal level. The CAT expression of pTrxCAT(–2629), pTrxCAT(–1101), pTrxCAT (–1058), pTrxCAT(–1017) and pTrxCAT(–976) plasmids was augmented by treatment with hydrogen peroxide, whereas constructs pTrxCAT(–889) and pTrxCAT(–783) showed no increase in CAT activity. Moreover, a construct pTrxCAT[–2629, Δ(–968,–884)] which was constructed from pBTrx(–2629/+28) by deleting a region from –968 to –884, showed no increase in CAT activity. These data suggest that the induction by hydrogen peroxide is mediated mainly by upstream sequences between positions –976 and –890 of the ADF/Trx gene promoter.

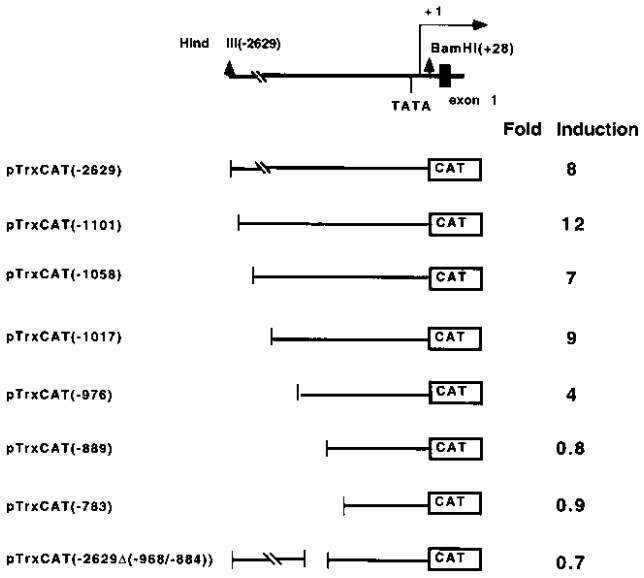

Figure 5. Expression of CAT activity in Jurkat cells transfected with various deletion constructs of ADF/Trx gene promoter (see Fig. 4). After 24 h post-transfection, cells were treated with 100 µM hydrogen peroxide, and CAT activity was assayed 12 h thereafter. Two other independent assays were performed, and showed almost the same data as indicated in this figure. The relative inducibility by hydrogen peroxide is shown for each construct transfected into Jurkat cells (the mean of three independent experiments).

Table 1. Sequence alignment of the synthetic oligonucleotides used as competitors in the cross-competition experiments

Oligonucleotide	Sequence alignment[a]
C(–956,–927)	5′-AGGAGTCTGCCTCTGTTAGAAGAATCTCACAC-3′ TCCTCAGACGGAGACAATCTTCTTAGAGTGTG
C(–948,–935)	5′-GCCTCTGTTAGAAGAA-3′ CGGAGACAATCTTCTT
C(–951,–932)	5′-TCTGCCTCTGTTAGAAGAATCT-3′ AGACGGAGACAATCTTCTTAGA
C(–954,–929)	5′-GAGTCTGCCTCTGTTAGAAGAATCTCAC-3′ CTCAGACGGAGACAATCTTCTTAGAGTG
M1(–954,–929)	5′-GAGTATGAATATGTTAGAAGAATATAAA-3′ CTCATACTTATACAATCTTCTTATATTT
M2(–954,–929)	5′-GCGTCTGCCTCTGTTCGCCGCCTCTCCC-3′ CGCAGACGGAGACAAGCGGCGGAGAGGG
M3(–954,–929)	5′-GAGGCGGCCGCGGGGAGAAGAAGCGCAC-3′ CTCCGCCGGCGCCCCTCTTCTTCGCGTG
M4(–954,–929)	5′-GATTCTTCCTCTTTTATAATAATCTCAC-3′ CTAAGAAGGAGAAAATATTATTAGAGTG
M5(–954,–929)	5′-GAGTCTGCACAGAATTCCCTAATCTCAC-3′ CTCAGACGTGTCTTAAGGGATTAGAGTG

[a]Dots indicate substituted bases.

Trans-acting factor(s) binding to the sequences

The transfection experiments using CAT expression plasmids suggested that the sequence between –976 and –890 was important in enhancing the expression of the ADF/Trx gene against oxidative stress. We next assessed whether any *trans*-acting factor is involved, and what sequence is required for the induction of the ADF/Trx gene by using gel mobility shift assay. A double strand oligonucleotide (–976, –890) was used as probe (Fig. 6A). Proteins extracted from nuclei of Jurkat cells pretreated with hydrogen peroxide retarded the movement of the probe through the gel, and we found one DNA–protein complex binding to labeled fragment (Fig. 6B). To identify the sequence to which a nuclear protein binds, we attempted to compete with a 100-fold molar excess amount of oligonucleotides containing partial sequences of the probe (Fig. 6A). Among these oligonuclotides, only C(–956,–927) efficiently competed with the complex formation of the probe (Fig. 6B). Nuclear extract from untreated Jurkat cells also showed one DNA–protein complex binding to labeled oligonucleotide (–976, –890), and only C(–956,–927) efficiently competed with the complex formation of the probe (data not shown).

Next, we examined in detail the sequence required for the nuclear proteins binding to the DNA. C(–956,–927) showed one retarded band. C(–954,–929) and C(–951,–932) efficiently competed with the complex formation of the labeled C(–956,–927), but C(–948,–935) did not (Fig. 6C). The artificial oligonucleotides M1(–954,–929), M2(–954,–929), M3(–954,–929), M4(–954,–929) and M5(–954,–929) made by substituting bases shown in Table 1, did not compete with the complex formation of the labeled C(–956, –927) (Fig. 6C). In addition, the CAT expression of m-pTrxCAT(–1017) plasmid, whose sequence from –954 to –929 was replaced with the sequence M5(–954,–929) (Table 1), showed only a slight increase in CAT activity upon treatment with hydrogen peroxide (Fig. 6D).

To examine the precise sequence, we performed DNase I footprinting assays using nuclear extracts from Jurkat cells treated with hydrogen peroxide. DNase I protection was observed in the region from –951 to –936 in the minus strand (Fig. 7). Enhanced protection was observed in the nuclear extract from hydrogen peroxide treated Jurkat cells. Moreover, enhanced binding of the specific complex was observed in the nuclear extracts prepared after the treatment of the cells with the oxidative agents (Fig. 8).

DISCUSSION

Low levels of ROS are produced as a normal part of cellular metabolism of eukaryotes, and cells contain several enzymes, such as catalase, glutathione peroxidase and superoxide dismutases. As a stress response, oxidative stress induces these defensive mechanisms. ADF is a human thioredoxin (Trx) with a sulfhydryl reducing activity (14), and the ADF/Trx system is one of the endogenous reducing systems (24) in addition to the GSH system.

In this paper, we demonstrated that the expression of the ADF/Trx gene was enhanced by oxidative agents such as hydrogen peroxide, diamide and menadione in Jurkat cells. The induction of the ADF/Trx gene by hydrogen peroxide was shown to be mainly dependent on the sequences between positions –976 and –890 by CAT assay. Gel mobility shift assay showed one retarded band binding to a double strand oligonucleotide (–976,–890). Oligonucleotide C(–956,–927) efficiently competed with the complex formation, whereas C(–966,–937) and C(–946,–917) did not. In addition, 20 bp sequences C(–951,–932) efficiently competed with the complex formation, while 14 bp sequences C(–948,–935) did not. These indicated that at least the region of the sequences from –949 to –934 is necessary to form the complex and the complex formation was lost by the deletion

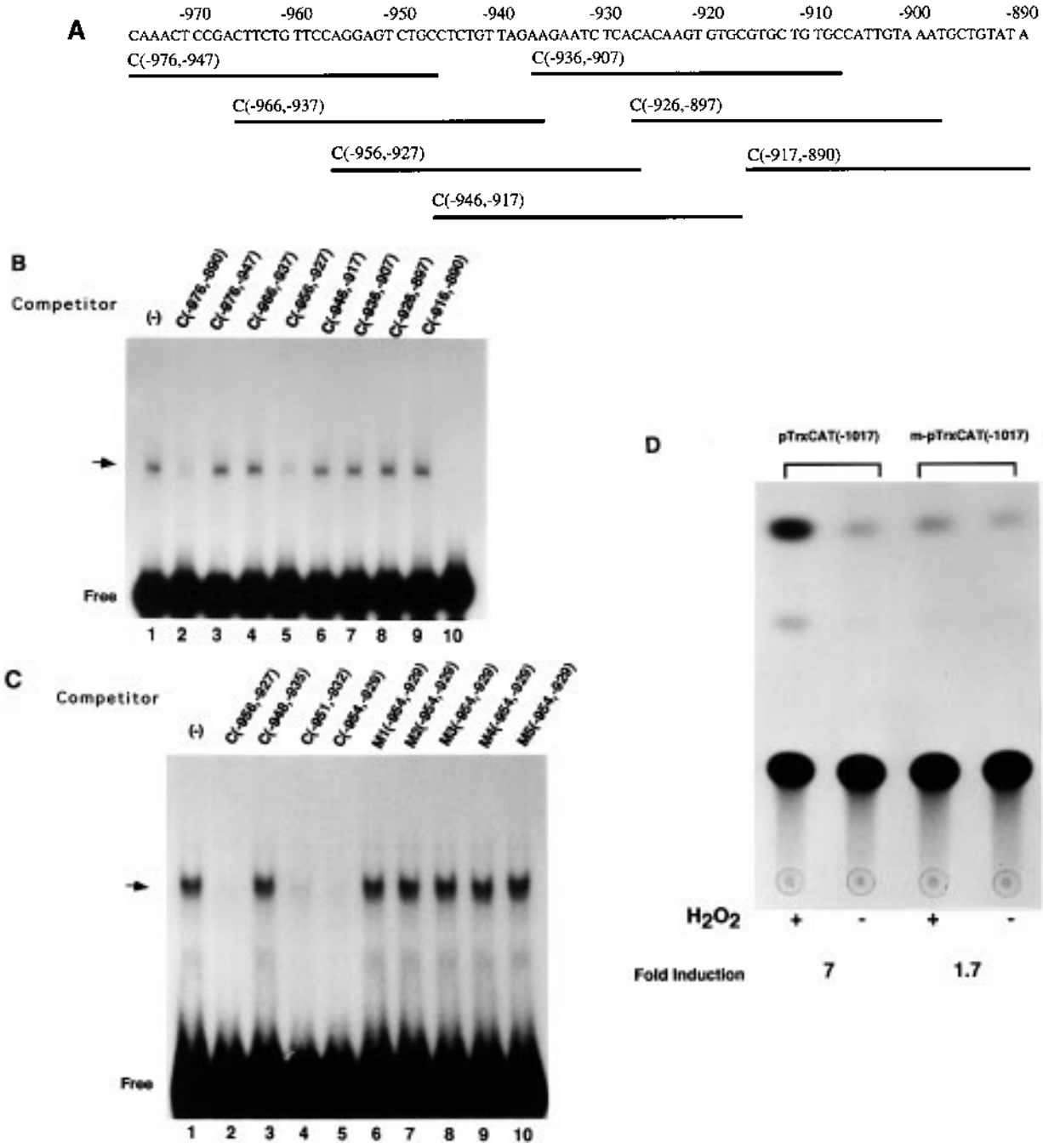

Figure 6. Identification of the sequence required for the nuclear proteins binding to the DNA. (**A**) Nucleotide sequences of the probe and competitors used for gel mobility retardation assays. (**B**) Gel mobility retardation assay with nuclear extract prepared from the hydrogen peroxide treated Jurkat cells. Oligonucleotide (–976,–890) labeled with [γ-^{32}P]ATP at the 5′-end was used as a probe. Unlabeled competitors were added at a 100-fold molar excess of the labeled probe to the reaction mixture. (**C**) Competition for complex formation between nuclear protein and labeled C(–956,–927). Gel mobility retardation assay with nuclear extract prepared from the hydrogen peroxide treated Jurkat cells. C(–956,–927) labeled with [γ-^{32}P]ATP at the 5′-end was used as a probe. Unlabeled competitors were added at a 100-fold molar excess of the labeled probe to the reaction mixture. (**D**) Induction of mutated ADF/Trx promoter activity after the treatment of oxidative agents. Jurkat cells were transfected with CAT expression, pTrxCAT(–1017) and m-pTrxCAT(–1017). 24 h after transfection, cells were treated with the oxidative agents, and CAT activity was assayed 12 h thereafter. Two other independent assays were performed, and showed almost the same data as indicated in this figure. The relative inducibility by hydrogen peroxide is shown for each construct transfected into Jurkat cells (the mean of three independent experiments).

of the sequences 5′ to –947 or 3′ to –936. Moreover, the CAT expression of m-pTrxCAT(–1017) plasmid, whose sequence from –954 to –929 was replaced with the mutated sequence M5(–954,–929) (Table 1), showed only a slight increase in CAT activity upon treatment with hydrogen peroxide. DNase I foot printing assay showed the protected 16 nt from –951 to –936 in the minus strand. However, we failed to show a significantly protected region in the plus strand, probably because DNase I could not sufficiently cut the predicted region in the plus strand (data not shown). These results indicated that specific DNA-binding

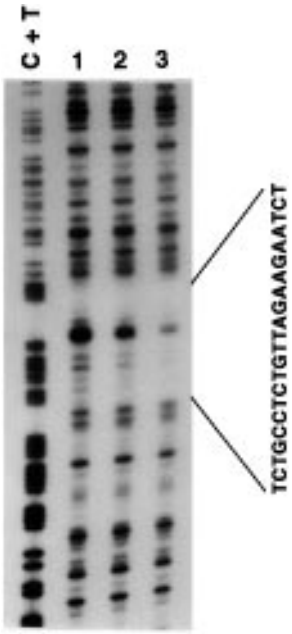

Figure 7. Detection of the factor(s) acting in *trans* by DNase I footprint analysis. (**A**) DNA fragment from –1058 to –348 of the ADF/Trx gene was used for DNase I footprint analysis, as described in Material and Methods. Nuclear extracts from untreated and hydrogen peroxide treated Jurkat cells (~20 µg on a protein basis) were incubated with the end-labeled probe DNA (10 000 c.p.m.) of the minus strands. The reaction mixture were digested by DNase I. Lane 1, digestion by 2 U DNase I without nuclear extract; lane 2, digestion in the presence of nuclear extract from untreated Jurkat cells; lane 3, digestion in the presence of nuclear extract from hydrogen peroxide treated cells.

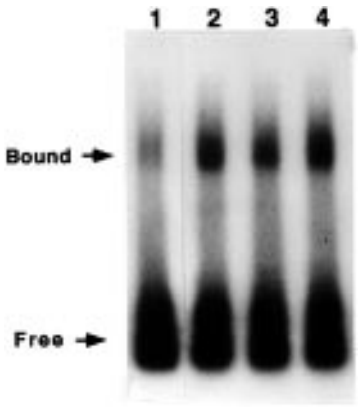

Figure 8. Enhanced DNA binding activity of nuclear extracts from Jurkat cells following the oxidative agent treatment. Jurkat cells were treated with the oxidative agents and 4 h later nuclear extracts were prepared and the gel shift assay was carried out. Lane 1, control; lane 2, 100 µM hydrogen peroxide; lane 3, 5 µM diamide; lane 4, 5 µM menadione.

factor(s) may participate in a signal transduction pathway which activates the ADF/Trx promoter in response to oxidative stress.

Oxidative agents have been shown to induce a number of genes in mammalian cells. Among them, the expression of c-*fos*, early growth response 1 genes and glutathione *S*-transferase Ya are induced through SRE (6), CArG sequence (38) and EpRE sequences (39), respectively, by hydrogen peroxide. On the other hand, hydrogen peroxide activates the bindings of NF-κB (40) and HSF (41) to each DNA regulatory element. In the 5′-flanking region of the ADF/Trx gene, we found an NF-κB consensus sequence from –1146 to –1137. However, there was no significant difference in the inducibility of the CAT gene expression by hydrogen peroxide between pTxrCAT(–1305) and pTxrCAT(–1101) (data not shown). Thus, the NF-κB sequence does not seem to play a major regulatory role in the induction of the ADF/Trx gene against oxidative stress in Jurkat cells.

The AP-1 family has been reported to participate in the redox regulation. YAP1 belongs to the AP-1 family in the yeast, *Saccharomyces cerevisiae*, and can recognize the AP-1 element of simian virus 40. The expression of the TRX2 gene, one of two genes of *S.cerevisiae* that encode thioredoxin protein, is dependent on YAP1 and is enhanced against oxidative stress (42). In human HeLa cells, however, the oxidant hydrogen peroxide is only a weak inducer of AP-1 and rather suppresses phorbol ester activation of the AP-1. In contrast, the DNA binding of AP-1 is enhanced with the antioxidants including thioredoxin (43,44). In the 5′-flanking region of the ADF/Trx gene, there are AP-1 consensus sequences at positions from –1673 to –1667 and from –2332 to –2325. Although the role of the AP-1 sequences in the expression of the ADF/Trx gene is to be determined, they are not likely to play a major role in the regulation of ADF/Trx gene against oxidative agents because the enhancement of CAT activity against hydrogen peroxide showed no significant difference between pTrxCAT(–2629) and pTrxCAT(–1668) (data not shown).

We could not find any homologous sequences in the region from –957 to –928 with the known sequences for DNA binding proteins by computer analysis using the TFD database (45). Thus, these sequences may involve a novel *cis*-acting element which responded against the oxidative stress.

ADF/Trx has two redox-active half-cystine residues in an active center, and shows various activities as a hydrogen donor (46,47). Although the precise *in vitro* role of ADF/Trx in maintaining the cellular redox status remains to be determined, two activities of ADF/Trx seem to be important with respect to the cellular response to oxidative stress. ADF/Trx has been shown to scavenge certain reactive oxygen species (24). Thus, ADF/Trx can protect cells from oxidative stress by scavenging a certain species of the harmful ROS. Secondly, ADF/Trx can act catalytically as a protein oxidoreductase and regenerate enzymes whose critical cysteine residues have been oxidized *in vitro* (24,48). The ADF/Trx gene expression through a factor(s) which binds to the sequences ranging from –957 to –928 may be regulated by ROS *per se* such as OxyR (3), or by the decrease of the reducing form of ADF/Trx. Furthermore, it remains to be determined what kind of species of ROS can induce the expression of ADF/Trx gene and whether these ROS induce expression through the same pathway. In addition to the oxidative agents, mitogen, prostaglandins and estrogen induce the expression of the ADF/Trx gene in sensitive tissues. Whether the ROS-responsive sequences are involved in the ADF/Trx induction by these variety of stimuli remains to be determined.

We have demonstrated here the unique sequences which were necessary to enhance the expression of the ADF/Trx gene upon oxidative stress, and the *trans*-activating factor(s) which bound to the sequences. The transcriptional activation through the sequences may account for most, if not all, of human Trx gene induction against oxidative stress. Further studies are needed to more

precisely identify specific nucleotides and the binding factor(s) that are required for induction by oxidative stress.

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