Mutational analysis of Yap1 protein, an AP-1-like transcriptional activator of *Saccharomyces cerevisiae*

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Received 16 September 1997

Abstract To define the essential amino acid residues of Yap1 in stress response, we generated *yap1* mutations by in vitro mutagenesis, which cause defects in mediating resistance to the stress of H_2O_2, but not of $CdCl_2$. Sequence analysis of the mutant *yap1* genes revealed three point mutations and two truncation mutations near the carboxy-terminus. The truncation mutations resulted in hyperresistance to cadmium. Northern blot analysis of stress-induced levels of *TRX2* and *GSH1* mRNAs indicated that the ability of the mutant Yap1 protein to induce transcriptional activation of target genes correlates well with its ability to confer stress resistance. The carboxy-terminal domain of Yap1 appears to act negatively in cadmium resistance.

Key words: AP-1; Oxidative stress; Cadmium stress; *Saccharomyces cerevisiae*

1. Introduction

Human transcription factor AP-1, composed of products of the proto-oncogenes c-*jun* and c-*fos*, is activated by various stimuli such as growth factors, cytokines, T cell activators, neurotransmitters, phorbol esters and UV irradiation [1–3]. The activity of AP-1 has been shown to be regulated at the transcriptional and the posttranscriptional level. However, environmental signal transduction pathways that lead to the regulation of AP-1 activity remain unclear.

In the yeast *Saccharomyces cerevisiae*, the *YAP1* gene was originally identified as a functional homologue to mammalian AP-1 on the basis of its ability to bind to an AP-1 recognition element (ARE) [4]. The same gene was isolated in several laboratories as a gene that confers pleiotropic drug resistance when present in high copy number and has also been named *PDR4* [5], *SNQ3* [6] or *PAR1* [7]. The null mutant of the *YAP1* gene displays hypersensitivity to H_2O_2 [7], cycloheximide [6] and cadmium [8], suggesting that *YAP1* is required for the adaptive responses to a variety of stress signals. In fact, various Yap1 target genes important for cell response have been identified, which include the *TRX2* gene encoding thioredoxin for the H_2O_2 response [9], the *GSH1* gene encoding γ-glutamylcysteine synthetase [10] and the *YCF1* gene encoding a MRP-type ABC superfamily protein [11] for the cadmium response, the *TPS2* gene encoding trehalose phosphate phosphatase for the metabolic stress response [12] and the *PDR5/YDR1/STS1* and *SNQ2* genes encoding multidrug resistance ABC superfamily proteins for the heat-shock response [13]. The mechanism that determines target specificity of Yap1 in response to various extracellular stimuli remains unclear. It was recently demonstrated that a cysteine-rich domain at the carboxy-terminus is responsible for the regulated localization of Yap1 protein to the nucleus [9].

In this report, a functional analysis of Yap1 was performed by in vitro mutagenesis to define the amino acid residues important in the adaptive response to H_2O_2. Two residues (P316 and G633) and the carboxy-terminal domain were found to be important for the H_2O_2 response, but not for the cadmium response. It was also found that Yap1 truncations near the carboxy-terminus lead to increased resistance to cadmium. Northern blot analysis of stress- (H_2O_2- and cadmium-) induced levels of *TRX2* and *GSH1* mRNAs indicated that the ability of the mutant Yap1 protein to induce transcriptional activation of target genes correlates well with its ability to confer stress resistance. The carboxy-terminal domain of Yap1 was shown to act negatively in cadmium resistance.

2. Materials and methods

2.1. Yeast strains

S. cerevisiae strains W303-1A (*MAT***a** *ho his3 leu2 ura3 trp1 ade2 can1-100*) [14] and DHA1-7a (*MAT***a** *W303 yap1::HIS3*) [8] were used.

2.2. In vitro mutagenesis

Plasmid YCp-YAP1 carries the *YAP1* gene [8] in YCp50 [15]. YCp-YAP1 was mutagenized by treatment with hydroxylamine as described by Rose and Fink [16]. DNA (10 μg) was incubated with 0.8 M hydroxylamine hydrochloride at 65°C for 2 h. The efficiency of mutagenesis was estimated by the reduction in the ability of the plasmid DNA to transform an *Escherichia coli* host. The efficiency of transformation by hydroxylamine-treated YCp-YAP1 DNA was about 2% of that of untreated control plasmid.

2.3. DNA sequencing

Nucleotide sequences were determined by the method of Sanger et al. [17] using an automated DNA sequencer (ALF red DNA sequencer, Pharmacia Biotech.).

2.4. Construction of plasmids

Epitope-tagged Yap1 was constructed as follows. The *YAP1* fragment containing the *Xba*I site after the initiating ATG codon of the open reading frame of the *YAP1* gene was generated by PCR. The 5′ non-coding region of *YAP1* was synthesized using primers 5′-AAAGTCGACGCTCTCCCTTATGCGA-3′ (sense strand) and 5′-AAATCTAGACATGGTTTAAGAAACA-3′ (antisense strand), where the *Sal*I and *Xba*I sites are underlined. The N-terminal region of the *YAP1* gene was synthesized using primers 5′-AAATCTAGAAGTGTGTCTACCGCCA-3′ (sense strand) and 5′-AAAGGATCCAAATCTTGCTTTTTGC-3′ (antisense strand), where the *Xba*I and *Bam*HI sites are underlined. These oligonucleotides were designed in such a manner that the amplified sequence contained the initiating ATG codon before the *Xba*I site. A 0.7-kb amplified

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PII S0014-5793(97)01233-7

fragment of the 5′ non-coding region of the *YAP1* gene was subcloned into the *Sal*I-*Xba*I sites of pUC119, yielding TYH1. A 0.2-kb amplified fragment of the N-terminal region of the *YAP1* gene was subcloned into the *Xba*I-*Bam*HI sites of pUC119, yielding TYH3. Plasmid TYH4 was constructed by integrating the 0.7-kb *Sal*I-*Xba*I fragment from plasmid TYH1 into the *Sal*I-*Xba*I site of TYH3. A 96-bp DNA fragment encoding three copies of an epitope sequence consisting of nine amino acid residues derived from the hemagglutinin of the influenza virus (HA1) [18] was amplified by PCR using primers 5′-AAATCTAGATACCCATACGATGTTC-3′ and 5′-AAATCTAGATCTAGCGTAATCCGGT-3′, where the *Xba*I site is underlined. The amplified fragment was cloned into the *Xba*I site of plasmid TYH4, yielding TYH5. Plasmid TYH6 was constructed by integrating the 1.1-kb *Bam*HI-*Sal*I fragment from plasmid TYH5 into the *Bam*HI-*Sal*I site of YCp-YAP1. Plasmid TYH6 expressing the epitope-tagged Yap1 was confirmed to fully complement the H_2O_2 and cadmium sensitivities when introduced to the *yap1* disruptant. Similarly, plasmids expressing mutant Yap1 tagged with HA epitopes were constructed by integrating the 1.1-kb *Bam*HI-*Sal*I fragment from plasmid TYH5 into the *Bam*HI-*Sal*I site of each mutant *yap1* gene.

2.5. Immunochemical analyses

Yeast cell extracts were prepared according to Matsusaka et al. [19]. TEG buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% glycerol, 30 mM NaCl, 1 mM dithiothreitol and 2 mM phenylmethylsulfonyl fluoride) was used for cell disruption by glass beads. Glass beads and large debris were removed by centrifugation at $5000 \times g$ for 10 min at 4°C. The supernatant of the lysate was solubilized in SDS sample buffer, and separated by SDS-polyacrylamide gels. Proteins were electrically transferred onto nitrocellulose filters. For detection of the tagged Yap1 protein, monoclonal antibody 12CA5 was used [20,21]. Horseradish peroxidase-conjugated sheep anti-mouse IgG (Amersham Corp.) was used as the second antibody. Chemiluminescence (ECL system, Amersham Corp.) was used to detect bound antibody.

2.6. Northern blot analysis

Cells in early exponential growth phase (1×10^7 cells/ml) in SD-Ura medium were treated with 1 mM H_2O_2 or 7.5 μM $CdCl_2$. The cells were harvested in a microcentrifuge at 4°C, and total RNA was isolated by the hot-phenol method [22]. The isolated RNA was separated on 1% agarose gel, transferred to a nylon membrane and then subjected to Northern blot analysis [23]. The probes were generated by random primed labeling of the 2.5-kb *Bam*HI-*Sal*I fragment of the *TRX2* gene [9], the 0.6-kb *Bam*HI-*Xho*I fragment of the *GSH1* gene [24], and the 1.1-kb *Xho*I-*Kpn*I fragment of *ACT1*, with [α-^{32}P]dCTP using a Multiprime DNA labeling kit (Amersham Corp.).

3. Results

3.1. Isolation of mutant YAP1 alleles defective in adaptive response to H_2O_2 stress

Mutations were induced by in vitro mutagenesis of the wild-type *YAP1* gene integrated into plasmid YCp (YCp-YAP1) by hydroxylamine treatment. Mutagenized plasmid was amplified in *E. coli* and about 10000 bacterial colonies were collected. Plasmid DNA was isolated from this pool and introduced into *S. cerevisiae* strain DHA1-7a, whose chromosomal *YAP1* gene had been replaced with a *yap1::HIS3* null allele [8]. From these transformants, the strains that exhibited decreased levels of resistance to H_2O_2 or $CdCl_2$ in comparison with the wild-type *YAP1* transformant were selected. About 3000 colonies of the transformants were picked up and the sensitivity to H_2O_2 or $CdCl_2$ was examined by spot assay on YPD solid medium containing H_2O_2 (4–5 mM) or $CdCl_2$ (75–150 μM). The wild-type *YAP1* transformant could grow on YPD solid medium in the presence of up to 7 mM H_2O_2 or 200 μM $CdCl_2$. Candidate mutant plasmids were recovered through *E. coli* and introduced into the *yap1* null mutant to verify the sensitivity phenotype. In total, 19 plasmids which failed to confer resistance to H_2O_2 at the wild-type level were obtained. Of these plasmids, seven failed to confer resistance only to H_2O_2 (class I mutants; Fig. 1), whereas eight plasmids also failed to confer resistance to $CdCl_2$ (data not shown). None of the plasmids failed to confer resistance only to $CdCl_2$. Surprisingly, four other transformants grew faster than the strain containing wild-type *YAP1* on the plate containing $CdCl_2$, indicating that these mutations result in increased resistance to cadmium (class II mutants, Fig. 1). Mutant *yap1* alleles that caused increased sensitivity to H_2O_2, but not to $CdCl_2$, (class I and class II mutants) were further investigated.

3.2. Identification of yap1 mutations

First, the mutation site of each *yap1* mutant allele was roughly located by constructing chimeric genes between

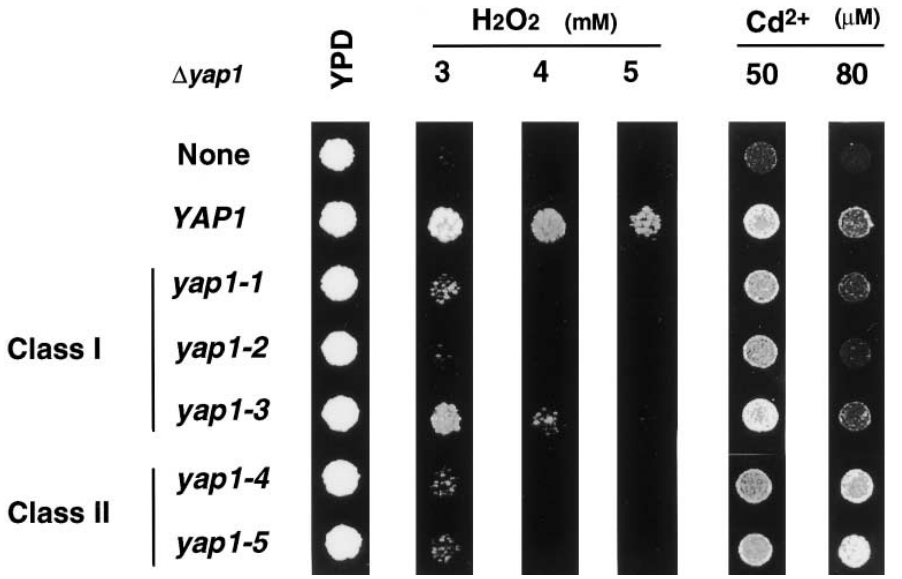

Fig. 1. Classification of H_2O_2-sensitive mutant *yap1* alleles on the basis of their ability to resist $CdCl_2$. Strain DHA1-7a (*yap1::HIS3*) was transformed with plasmids TY10 (*yap1-1*), TY17 (*yap1-2*), TY34 (*yap1-3*), TY3 (*yap1-4*) and TY29 (*yap1-5*). The strains were spotted on YPD solid medium containing various concentrations of H_2O_2 or $CdCl_2$ and incubated at 28°C for 48 h.

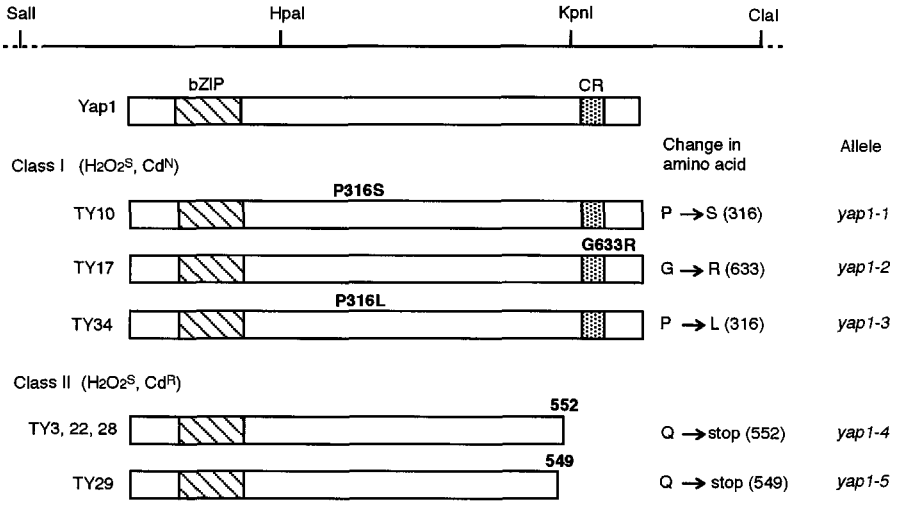

Fig. 2. Schematic representation of the mutant Yap1 proteins. The conserved basic leucine zipper region (b-ZIP) and the carboxy-terminal cysteine-rich region are indicated. The restriction sites used for constructing the chimeric genes between wild-type and mutant alleles are indicated at the top.

wild-type *YAP1* and mutant *yap1* alleles. The 1.2-kb *Hpa*I-*Kpn*I or the 0.6-kb *Kpn*I-*Cla*I fragment of wild-type gene was replaced by the corresponding segment excised from the mutant gene (Fig. 2). Of the three restriction fragments, the *Sal*I-*Hpa*I fragment of the mutant was omitted from the chimera construction, because the sequences for the b-ZIP motif which is known to be essential for normal AP-1 function in the sequence-specific DNA binding is contained in this fragment, and many mutations in this motif were expected to yield nonfunctional Yap1 [25,26]. Nucleotide sequencing of the fragment which was suggested to contain the mutation site was performed. The nucleotide and the deduced amino acid changes found in the *yap1* mutant alleles are summarized in Fig. 2. By analysis of class I mutant alleles, three point mutations that resulted in a single amino acid substitution were identified. The *yap1-1* mutant allele in plasmid TY10 carried a C to T transition at nucleotide position 946 that resulted in replacement of Pro^{316} with Ser, the *yap1-2* allele in plasmid TY17 carried a G to A transition of the nucleotide at position 1897 that resulted in replacement of Gly^{633} with Arg and the *yap1-3* allele in plasmid TY34 carried a C to T transition at nucleotide position 947 that resulted in replacement of Pro^{316} with Leu. Class II mutant alleles contained a termination codon at residue 552 in plasmids TY5 and TY52 (*yap1-4*) or at residue 549 in plasmids TY24 and TY31 (*yap1-5*), generating truncated Yap1 proteins that lack the conserved region (CR) near the carboxy-terminus.

3.3. Immunoblot analysis of the mutant Yap1 proteins

To compare the levels of Yap1 proteins in various *yap1* mutants, immunoblot analysis of the cell extract prepared from the cells carrying mutant *yap1* alleles was performed. At the same time, to see if the stability of the mutant Yap1 proteins is altered during incubation with H_2O_2 or $CdCl_2$, extracts were prepared from cells cultivated for 1 h in the presence of H_2O_2 or $CdCl_2$. For this purpose, wild-type *YAP1* and mutant *yap1* genes encoding epitope-tagged Yap1 proteins at the amino-terminus were constructed [20]. The modified version of the wild-type *YAP1* could fully complement the H_2O_2 and cadmium hypersensitivity of the *yap1* null mutant, indicating that HA-tagging had no significant effect on the ability of Yap1 to resist these stresses. Similarly, modified versions of mutant *yap1* genes exhibited a similar phenotype as the respective original mutants (data not shown). Immunoblotting with monoclonal antibody 12CA5 against HA detected a specific band in the transformant with the plasmids (Fig. 3). The observed molecular mass of HA-tagged wild-type Yap1 (90 kDa) was higher than that deduced from the gene (75 kDa), in agreement with the observation that Yap1 protein behaves anomalously on SDS-PAGE [4]. A band with similar intensity as the HA-tagged Yap1 (wild-type) was detected in cells expressing Yap1-2 and Yap1-3 mutant proteins, suggesting that expression level and stability were not significantly altered (Fig. 3). The amount of Yap1-1 and Yap1-5 protein detected was less than that of wild-type Yap1 (40% and 50%, respectively), indicating that the *yap1-1* point mutation and the carboxy-terminal truncation significantly reduce protein levels in vivo (Fig. 3). Similarly, a decrease in the level of the truncated protein Yap1-4 was observed (data not shown). Similar observations were made previously with the carboxy-terminal truncations of Yap1, and it was suggested that the carboxy-terminal sequences are important for the stability of Yap1 in vivo [27,28]. The amount of the mutant Yap1 proteins in the cells incubated with H_2O_2 or $CdCl_2$ was similar to that of control, indicating that the stability of the

Fig. 3. Immunoblot analysis of wild-type and mutant Yap1 proteins. The *yap1* null mutant strain (DHA1-7a) harboring YCp plasmids containing HA-tagged wild-type *YAP1*, *yap1-1*, *yap1-2*, *yap1-3* and *yap1-5* grown in the presence of 1 mM H_2O_2 (H), 7.5 μM $CdCl_2$ (C) or without an additive (−) for 1 h. Supernatant (60 μg protein) of total cell extract was subjected to SDS-PAGE (10% polyacrylamide gel) followed by immunoblotting.

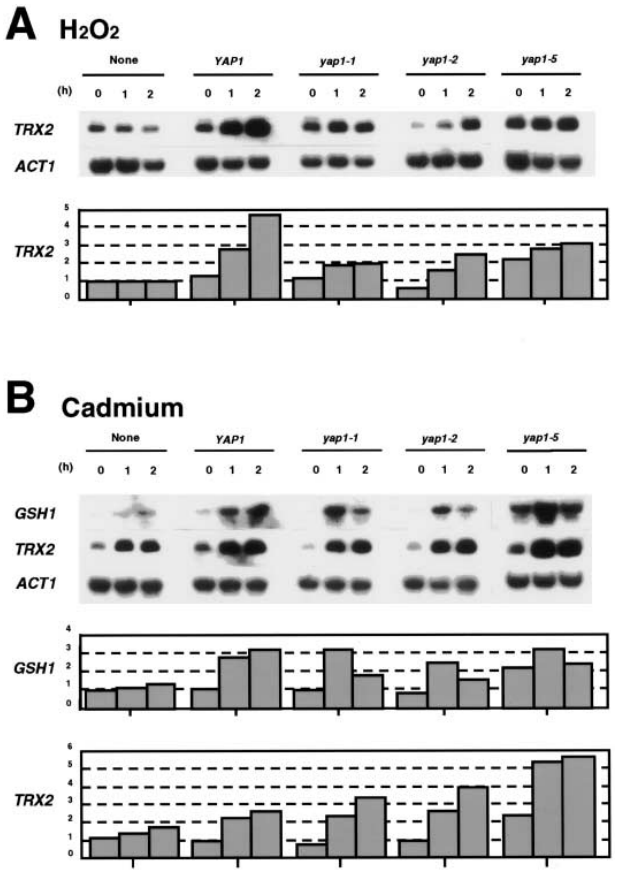

Fig. 4. Transcriptional induction of *TRX2* and *GSH1* genes in various *yap1* mutants. Total cellular RNA was prepared from the *yap1* disruptant (DHA1-7a) transformed with the indicated YCp plasmids at various times (h) after addition of 1 mM H_2O_2 or 7.5 μM $CdCl_2$. RNA (20 μg per slot) was applied to the gel. The filters were hybridized with radioactive probes containing *TRX2*, *GSH1* or *ACT1*. The intensity of mRNA was measured using a BAS-2000 Bioimaging analyzer (Fuji Photo Film Co.), and the *TRX2* and *GSH1* mRNA levels were normalized to the individual *ACT1* mRNA level.

mutant protein is not significantly altered under the stress conditions (Fig. 3).

3.4. Stress-induced transcriptional activation of target genes by mutant yap1 alleles

To investigate the relationship between the abilities of mutant Yap1 to confer stress resistance and to activate the expression of target genes, stress-induced *TRX2* and *GSH1* mRNA levels were determined by Northern blot analysis (Fig. 4). Basal transcript levels of *TRX2* and *GSH1* genes in the *yap1* disruptant carrying control plasmid (YCp50) were similar to those of the wild-type strain, indicating that Yap1 is not responsible for basal transcription of these genes. In contrast, a weak activation of *TRX2* and *GSH1* transcription still occurred in the *yap1* disruptant in response to cadmium stress, suggesting that additional transcription factor(s) are also involved in cadmium-induced activation of these genes. The H_2O_2-induced *TRX2* expression was abolished in the *yap1* disruptant, indicating that Yap1 plays a crucial role in H_2O_2-induced activation of *TRX2* transcription. The function of the *YCF1* gene which encodes an ABC superfamily protein important for cadmium tolerance is dependent on *YAP1* [11]. However, the cadmium-induced level of *YCF1* transcript was not significantly dependent on *YAP1* at least in our genetic background (data not shown).

The wild-type *YAP1* transformant exhibited a 2–4-fold increase in induced levels of *TRX2* transcription in response to H_2O_2 and cadmium stresses, and a 3-fold elevation of *GSH1* transcript level in response to cadmium stress. The level of transcriptional activation of the *TRX2* gene in response to H_2O_2 was significantly reduced (10–37% of wild type) in all H_2O_2-sensitive *yap1* transformants (*yap1-1*, *yap1-2* and *yap1-4*) (Fig. 4). In contrast, induced levels of *GSH1* and *TRX2* genes in response to cadmium stress were not significantly altered. The *yap1-5* allele which caused hyperresistance to cadmium exhibited increased levels of transcription of the *GSH1* and *TRX2* genes in response to cadmium stress (Fig. 4).

4. Discussion

To identify the amino acid residues important for Yap1 function in adaptive response to H_2O_2 stress, *yap1* mutant alleles that exhibit a decreased ability to resist the stress of H_2O_2, but not cadmium, were isolated and characterized.

The defect in the ability of the mutant Yap1 protein to confer H_2O_2 resistance was caused by the single amino acid substitutions at Pro^{316} in *yap1-1* (to Ser) and *yap1-3* (to Leu), and at Gly^{633} in *yap1-2* (to Arg), and truncation mutations at Asn^{552} in *yap1-4* and Asn^{549} in *yap1-5*. The mutant *yap1* alleles lead to decreased levels of H_2O_2-induced *TRX2* transcription, indicating that these residues and the carboxy-terminal domains are essential for the adaptive response to H_2O_2 stress. Thus, the ability of the mutant *yap1* alleles in the adaptive response to H_2O_2 stress appears to correlate well with the ability to activate transcription of the target gene in response to the stress. It was recently shown that the conserved cysteine residues in the carboxy-terminal domain are important for regulating nuclear localization of Yap1 in response to oxidative stress, blocking nuclear transport of Yap1 protein under unstressed conditions [28]. Whether the H_2O_2-sensitive *yap1* point mutations cause the defect of stress-induced Yap1 activation or Yap1 nuclear transport remains to be determined.

Yap1 and Yap2 proteins share two highly homologous regions, a region containing a b-ZIP motif and a region containing the carboxyl-terminal cysteine-rich sequences. Yap1 and Yap2 proteins have an overlapping function in the resistance to cadmium [8,29]. The carboxy-terminal cysteine-rich sequences of Yap1 and Yap2 may perform an analogous function in the response to cadmium stress. The class II H_2O_2-sensitive alleles (*yap1-4* and *yap1-5*) exhibited increased resistance to cadmium in comparison with the wild-type allele even though cellular Yap1 proteins were less abundant than that of wild-type cells. Thus, the carboxy-terminal region of Yap1 appears to be responsible for regulating cadmium response negatively. Since the carboxy-terminal cysteine-rich domain of Yap1 is important for regulated nuclear translocation in response to oxidative stress [28], Yap1-5 protein may lead to the increased level of *TRX2* basal expression (Fig. 4). However, the mutant allele (*yap1-5*) is unable to stimulate *TRX2* transcription by H_2O_2 and to confer H_2O_2 resistance to the level of the wild-type, indicating that the elevation of the basal level alone is not sufficient for the resistance to H_2O_2. An additional unknown factor seems to be required for the in-

duction of *TRX2* expression by H_2O_2 and this factor may be important for the adaptive response to H_2O_2. In contrast, the same mutant allele is able to induce *TRX2* expression in response to cadmium stress and confers hyperresistance to this toxic metal. These results demonstrate that Yap1 responds to the stresses caused by H_2O_2 and cadmium differently by specifically activating the respective target genes.

References

[1] Angel, P. and Karin, M. (1991) Biochim. Biophys. Acta 1072, 129–157.
[2] Hunter, T. and Karin, M. (1992) Cell 70, 375–387.
[3] Karin, M. (1995) J. Biol. Chem. 270, 16483–16486.
[4] Moye-Rowley, W.S., Horseman, K.D. and Parker, C.S. (1989) Genes Dev. 3, 283–292.
[5] Hussain, M. and Lenard, J. (1991) Gene 101, 149–152.
[6] Hertle, K., Haase, E. and Brendel, M. (1991) Curr. Genet. 19, 429–433.
[7] Schnell, N. and Entian, K.D. (1991) Eur. J. Biochem. 200, 487–493.
[8] Hirata, D., Yano, K. and Miyakawa, T. (1994) Mol. Gen. Genet. 242, 250–256.
[9] Kuge, S. and Jones, N. (1994) EMBO J. 13, 655–664.
[10] Wu, A. and Moye-Rowley, W.S. (1994) Mol. Cell. Biol. 14, 5832–5839.
[11] Wemmie, J.A., Szczypka, M.S., Thieles, D.J. and Moye-Rowley, W.S. (1994) J. Biol. Chem. 269, 32592–32597.
[12] Gounalaki, N. and Thireos, G. (1994) EMBO J. 13, 4036–4041.
[13] Miyahara, K., Hirata, D. and Miyakawa, T. (1996) Curr. Genet. 29, 103–105.
[14] Rothstein, R. (1983) Methods Enzymol. 101, 202–211.
[15] Johnston, M. and Davis, R.W. (1984) Mol. Cell. Biol. 4, 1440–1445.
[16] Rose, M.D. and Fink, G.R. (1987) Cell 48, 1047–1060.
[17] Sanger, F., Nicklen, S. and Coulson, A. (1977) Proc. Natl. Acad. Sci. USA 87, 5463–5467.
[18] Wilson, I.A., Niman, H.L., Houghten, R.A., Cherenson, A.R., Connolly, M.L. and Lerner, R.A. (1984) Cell 37, 767–778.
[19] Matsusaka, T., Hirata, D., Yanagida, M. and Toda, T. (1995) EMBO J. 14, 3325–3338.
[20] Niman, H.L., Houghten, R.A., Walker, L.E., Reisfeld, R.A., Wilson, I.A., Holge, J.M. and Lerner, R.A. (1983) Proc. Natl. Acad. Sci. USA 80, 4949–4953.
[21] Fields, J., Nikawa, J., Broek, D., MacDonald, B., Rogers, L., Wilson, I.A., Lerner, R.A. and Wigler, M. (1988) Mol. Cell. Biol. 8, 2159–2165.
[22] Schmitt, M.E., Brown, T.A. and Trumpower, B.L. (1990) Nucleic Acids Res. 18, 3091–3092.
[23] Maniatis, T., Fritsch, E.F., Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[24] Ohtake, Y. and Yabuuchi, S. (1991) Yeast 7, 953–961.
[25] Angel, P., Allegretto, E.A., Okino, S.T., Hattori, K., Boyle, W.J., Hunter, T. and Karin, M. (1988) Nature 332, 166–171.
[26] Kouzarides, T. and Ziff, E. (1988) Nature 336, 646–651.
[27] Wemmie, J.A., Wu, A.L., Harshman, K.D., Parker, C.S. and Moye-Rowley, W.S. (1994) J. Biol. Chem. 269, 14690–14697.
[28] Kuge, S., Jones, N. and Nomoto, A. (1997) EMBO J. 16, 1710–1720.
[29] Wu, A., Wemmie, J.A., Edgington, N.P., Goebl, M., Guevara, J.L. and Moye-Rowley, W.S. (1993) J. Biol. Chem. 268, 18850–18858.