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Yap1p, a yeast transcriptional activator that mediates multidrug resistance, regulates the metabolic stress response

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Overexpression of the YAP1 transcriptional activator renders yeast cells resistant to multiple metabolic inhibitors. In an effort to identify other gene products required for this phenotype we have isolated genomic mutations which neutralize this effect. One such mutation was further characterized and the affected gene was shown to be identical to *TPS2* which encodes trehalose phosphate phosphatase, an enzyme catalysing the second step in trehalose biosynthesis. We have analysed the transcriptional regulation of the *TPS2* gene and have shown that its transcription is induced by a variety of stressful conditions caused by metabolic inhibitors, osmotic shock and heat shock. This transcriptional activation is mediated by multiple stress promoter elements (C_4T) and requires the function of Yap1p as well as reduced activity of the cAMP-regulated protein kinase. Using an appropriate reporter gene we have shown that Yap1p is generally required for transcriptional regulation through the C_4T stress element. These results show that the YAP1 protein has a pivotal role in the metabolic stress response and the acquisition of stress tolerance.

Key words: C_4T stress element/stress tolerance/*TPS2* gene/trehalose

Introduction

All living cells have mechanisms which allow survival in adverse and abrupt environmental changes. Among these, the heat shock response is the most widely studied but similar mechanisms exist for other types of stress which cause metabolic disturbances. In most known cases the end result of the response is the transcriptional induction of a set of genes whose products neutralize the hazardous consequences of the imposed stress. The importance of such defences in living cells and organisms has recently been emphasized by the demonstration of the deceleration of the ageing process in flies overexpressing two genes encoding enzymes that combat oxidative stress (Orr and Sohal, 1994).

In the yeast *Saccharomyces cerevisiae*, stress induces the expression of heat shock genes, as well as of genes involved in protein degradation, glycolysis, plasma membrane function, antioxidative defence, metal homeostasis and trehalose biosynthesis (Mager and Moradas Ferreira, 1993). The expression of a subset of these genes is also induced when cells enter stationary phase, which can be considered as a stress response triggered by nutrient limitation (Werner-Washburne *et al.*, 1993). Although the exact roles of all the induced proteins are not well understood it is evident that stress tolerance requires the coordinated activity of a number of gene products involved in diverse cellular functions.

The transcriptional induction of most of the heat- and stress-induced genes in yeast is mediated by the heat shock factor (HSF, Sorger and Pelham, 1988; Gross *et al.*, 1990). This transcriptional activator is constitutively bound as a trimer to a *cis* promoter DNA sequence, the heat shock element (HSE), and its activity is induced upon stress (Sorger and Nelson, 1989; Gross *et al.*, 1990). Recently, a novel element that mediates stress-induced transcription has been identified having the consensus CCCCT (C_4T). It has been shown that this element alone can mediate the stress-induced expression of the *DDR2* (Kobayashi and McEntee, 1993) and *CTT1* (Marchler *et al.*, 1993) genes and is also present in the promoter region of other stress-induced genes such as *UBI4*, *HSP12* and *PTP2*. A protein factor has been shown to interact with this DNA element which has been named stress-regulated element (STRE; Marchler *et al.*, 1993).

The signalling pathway that triggers the stress-induced gene expression is not well understood. In the case of HSF a role for the Hsp70 has been proposed in maintaining this DNA-bound factor inactive (Craig and Gross, 1991) and a role for the cAMP-regulated protein kinase (PKA) has also been suggested for intracellular signalling (Shin *et al.*, 1987; Piper *et al.*, 1990). It has been shown that mutations which inactivate this kinase result in the elevated expression of several heat shock proteins and this correlates with entry into the stationary phase (Brazzell and Ingolia, 1984; Finley *et al.*, 1987; Tuite *et al.*, 1990). A more direct involvement of PKA has been suggested for transcriptional induction through the STRE (Marchler *et al.*, 1993). Although alternative or overlapping pathways have been suggested, PKA should play a significant role in the stress response.

The function of a number of stress-induced proteins correlates with the acquisition of tolerance. Acquired stress tolerance in yeast has been studied extensively in the case of thermotolerance. Evidence has been presented suggesting the induction of the synthesis of a set of heat shock proteins, in particular of Hsp104, as a prerequisite for the development of thermotolerance (Sanchez *et al.*, 1992). Stress tolerance is also acquired in cells at the stationary phase possibly through the high expression of a subset of heat shock proteins (Boucherie, 1985). On the other hand, thermotolerance can be acquired in the absence of an HSF (Smithe and Yaffe, 1991). Finally, changes in the intracellular levels of trehalose have been also correlated with the acquisition of thermal and osmotic tolerance

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(Wiemken, 1990). It is evident that acquisition of stress tolerance is mediated by the concerted and possibly redundant function of multiple gene products.

In principle, acquired stress tolerance in vegetative cells should result in resistance to multiple metabolic inhibitors. In that respect the pleiotropic drug resistance which is mediated by the overexpression of two related yeast transcriptional activators, YAP1 and CAD1 (Leppert *et al.*, 1990; Bossier *et al.*, 1993; Wu *et al.*, 1993), may be a consequence of such tolerance. In this study, a genetic analysis of the multiple drug resistance conferred by high levels of Yap1p showed a direct involvement of this protein in the stress response pathway. We have demonstrated that the YAP1 protein is required for the stress-regulated expression of genes through the C_4T element. These results strongly support the idea that multiple drug resistance can be intimately linked to changes in the stress response pathway.

Results

Overexpression of the YAP1 gene rescues the gcn4 phenotype

We have isolated the *YAP1* gene in a screen for high copy suppressors of the amino acid analogue sensitivity of a *gcn4*Δ strain (Driscoll-Penn *et al.*, 1984). Although Yap1p has a DNA binding domain similar to that of Gcn4p (Moye-Rowley *et al.*, 1988) its ability to support growth of a *gcn4* strain in the presence of 3-aminotriazole (a competitive inhibitor of the *HIS3* gene product) when overexpressed was not a result of functional substitution. This was previously shown by the inability of Yap1p to activate the expression of a reporter gene harbouring the GCN4 binding site (Moye-Rowley *et al.*, 1989). Thus, the phenotypic suppression of the *gcn4* strain through the increased dosage of the *YAP1* gene was the result of the already reported pleiotropic drug resistance that such overexpression mediates (Leppert *et al.*, 1990; Wu *et al.*, 1993). These results showed that *YAP1* overexpression confers resistance to the toxic effects of inhibitors of amino acid biosynthesis such as 3-aminotriazole.

A tps2 mutation neutralizes the phenotype of YAP1 overexpression

In an effort to understand the basis of *YAP1*-dependent pleiotropic drug resistance and to identify genes which operate in concert with this transcriptional activator we took a genetic approach. We mutagenized a wild-type strain overexpressing *YAP1* and selected for sensitivity to both cycloheximide and cadmium. In order to distinguish mutations in the *YAP1* gene the used strain was co-transformed with a β-galactosidase reporter gene whose transcription was driven by the AP1 element (Georgakopoulos and Thireos, 1992), the target of Yap1p. We have isolated one mutation which satisfied all the imposed criteria. This mutant strain grew normally at 30°C but very poorly at 37°C. This temperature sensitivity formed the basis for cloning by complementation. Following the cloning of the affected gene, limited sequence analysis revealed that it encoded the already known enzyme trehalose phosphate phosphatase (TPS2), catalysing the second and final step in trehalose biosynthesis (DeVirgilio *et al.*, 1993). As shown in Table I, strains

Table I. Drug resistance and transcriptional activity through the AP1 element in relation to *YAP1* overexpression and genetic background

Strain[a]	Growth[b]		β-Galactosidase activity[c]
	Minimal	Minimal + cycloheximide	
WT	+	−	1.2
WT+*HYAP1*	+	+	12.3
tps2	+	−	1.4
tps2+*HYAP1*	+	−	11.9
*yap1*Δ	+	−	0.7

[a]HYAP1 indicates overexpression of the *YAP1* gene.
[b]Growth was monitored on minimal plates (−) or minimal plates supplemented with 2 μg/ml cycloheximide.
[c]Units of β-galactosidase activity of the *AP1−lacZ* reporter gene was measured following growth in minimal medium.

harbouring the *tps2* mutation could not support *YAP1*-dependent drug resistance without affecting its activation potential. Disruption of the *TPS2* gene also neutralized the Yap1p-dependent phenotypes (data not shown) and had a temperature sensitive growth defect as previously reported (DeVirgilio *et al.*, 1993).

Stress-induced expression of the TPS2 gene is regulated by Yap1p

The involvement of trehalose biosynthesis in YAP1-mediated drug resistance was in concert with the potential role of this metabolite is stress tolerance. It has been reported that trehalose accumulates upon exposure to heat shock and toxic dosage of heavy metals (Wiemken, 1990) and that *TPS2* expression is induced by heat shock (DeVirgilio *et al.*, 1993). We extended these observations by assaying for the expression of the *TPS2* gene under additional stress conditions. Figure 1 shows that *TPS2* mRNA accumulation was increased under conditions of heat or metabolic shock (exposure to cycloheximide). Since according to our genetic screen Yap1p could regulate the expression of *TPS2* directly we assayed for *TPS2* mRNA accumulation in a strain overexpressing *YAP1*. Surprisingly, as shown in Figure 1, such overexpression in fact reduced the extent of *TPS2* mRNA accumulation in response to stress. By contrast, in a *yap1*-disrupted strain (*yap1*Δ) the stress-induced expression of *TPS2* was completely abolished. Similar results were obtained using media supplemented with 0.3 M NaCl which resulted in an osmotic shock (Machler *et al.*, 1993).

In order to quantify our results we introduced a DNA fragment containing the presumptive promoter of the *TPS2* gene (shown in Figure 2) in front of a *lacZ* reporter gene. This fragment drove the expression of the reporter gene in full agreement with the *TPS2* mRNA results. As shown in Figure 3, heat and osmotic shock resulted in a 4-fold increase in β-galactosidase activity in a wild-type strain. This stimulation was decreased when *YAP1* was over-expressed and completely impaired in a *yap1*Δ strain. We concluded that Yap1p is required for stress-induced expression of the *TPS2* gene.

Yap1p acts downstream of protein kinase A in the stress response of the TPS2 gene

It has been reported that PKA negatively regulates the stress-induced expression of certain genes (see Intro-

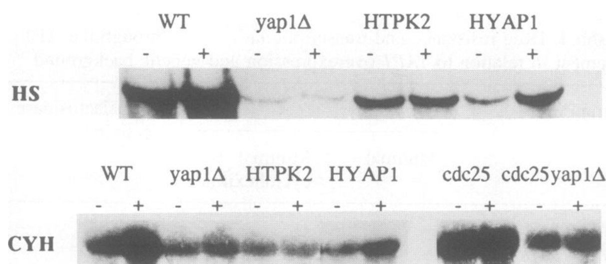

Fig. 1. *TPS2* mRNA accumulation as a function of growth conditions and genetic background. Total mRNA was extracted from a wild-type strain (WT), a *yap1Δ* strain, a *TPK2*-overexpressing strain (HTPK2), a *YAP1*-overexpressing strain (HYAP1), a *cdc25* strain and a *cdc25 yap1Δ* strain. These strains were grown either under heat shock conditions (grown at 38°C for 1 h; top panel, HS+) or in the presence of cycloheximide (grown for 2 h; bottom panel, CYH+). (−) indicates growth in minimal medium at 23°C. The RNAs (10 μg) were electrophoretically separated, transferred onto nylon membranes and hybridized with ^{32}P-labelled *TPS2* DNA probe. The fact that equal amounts of RNAs were transferred onto the membrane was verified by re-probing with a DNA fragment containing the actin gene.

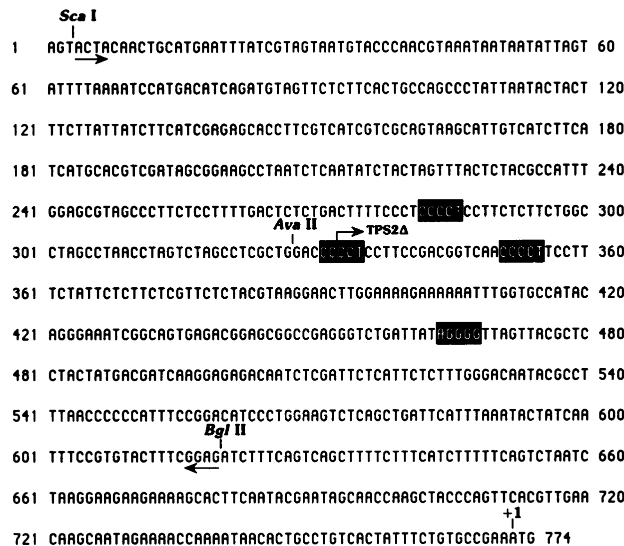

Fig. 2. Nucleotide sequence of the promoter region of the *TPS2* gene as determined by DeVirgilio *et al.* (1993). The *Sca*I−*Bgl*II and the TPS2Δ−*Bgl*II DNA fragments were used to drive the transcription of the *HIS3*−*lacZ* reporter gene. The four STRE sequences within this promoter are highlighted in black boxes. +1 indicates the position of the initiator ATG.

duction). In order to substantiate the role of Yap1p in the regulation of the *TPS2* gene, we examined the effects of active and inactive forms of PKA. Three relevant strains were used: one strain with an activated PKA, a *cdc25* strain in which the RAS-cAMP signalling pathway is impaired and thus PKA is constitutively inactivated (Broek *et al.*, 1987), and a *cdc25 yap1Δ* double mutant in order to determine their epistatic relationships. Activation of PKA was accomplished through the overexpression of *TPK2*, one of the three yeast genes encoding the catalytic subunit of PKA (Toda *et al.*, 1987). Stress induction was measured by both *TPS2* mRNA accumulation and β-galactosidase activities from the *TPS2*−*lacZ* reporter gene. As shown in Figure 1 (RNA analysis) and Figure 3 (β-galactosidase activity), activated PKA impaired the stress-induced expression of the *TPS2* gene, whereas

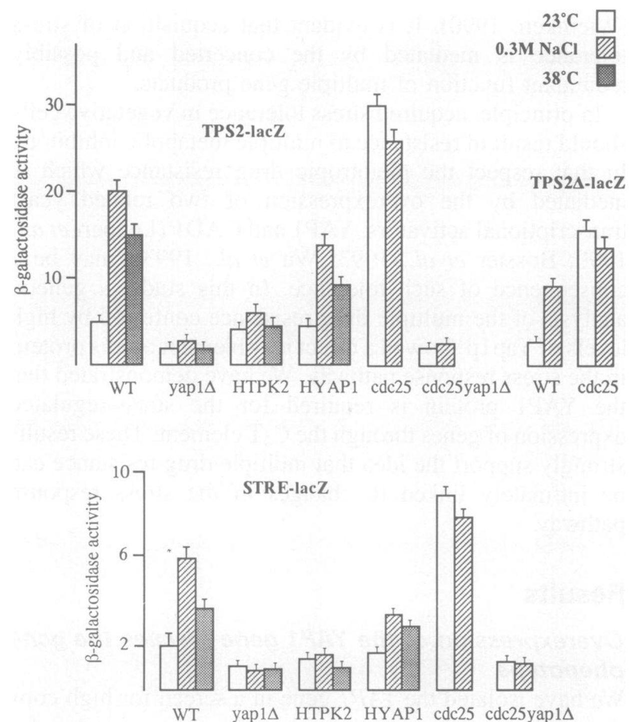

Fig. 3. Graphic representation of the β-galactosidase activities produced by the *TPS2Δ*−*lacZ* reporter gene (top) or by the STRE−*lacZ* (bottom) reporter gene as a function of growth conditions and genetic background. Cells were grown in minimal medium at 23°C and growth was continued for either 2 h in the presence of 0.3 M NaCl or for 1 h at 38°C. The genetic backgrounds used were the same as in Figure 1.

inactivated PKA *(cdc25)* resulted in the constitutive expression of the *TPS2* gene. Finally, *YAP1* was epistatic to the RAS-PKA pathway since in a *cdc25 yap1* strain the *cdc25*-dependent constitutive expression of the *TPS2* gene was completely abolished. This latter observation substantiated the involvement of *YAP1* in the transcriptional regulation of the *TPS2* gene.

Trehalose accumulates in response to stress in a YAP1- and PKA-dependent manner

TPS2 encodes one of the enzymatic activities which are required for the biosynthesis of trehalose. In order to examine whether the observed regulation of the *TPS2* gene involves the whole biosynthetic pathway we measured the extent of trehalose accumulation under stress conditions and for the strains used in this study. As shown in Table II, trehalose accumulated in a wild-type strain upon heat shock or exposure to cycloheximide. These measurements were in agreement with those previously reported (Attfield, 1987; Hottiger *et al.*, 1987). Such stress-induced accumulation was dependent on the presence of the *YAP1* gene; it occurred constitutively in the *cdc25* strain and was completely impaired when the *TPK2* gene was overexpressed and in a *cdc25 yap1* strain. These results raised the possibility that the expression of the other genes involved in trehalose biosynthesis, such as *TSL1* and *TSS1* (Vuorio *et al.*, 1993), were also regulated by the *YAP1* gene.

YAP1 is a general regulator of the stress response

Since our results suggested that Yap1p is involved in the stress-induced expression of genes involved in trehalose

Table II. Trehalose accumulation as a function of stress and genetic background

Strain[a]	Trehalose (mg/g dry weight)[b]		
	23°C	38°C	Cycloheximide
WT	0.73	3.20	2.50
yap1Δ	0.60	0.70	0.72
HTPK2	0.72	0.82	0.93
HYAP1	0.72	2.10	1.80
cdc25	5.20	ND	4.90
cdc25 yap1Δ	0.71	ND	0.82

[a]*HTPK2* indicates overexpression of the *TPK2* gene
[b]Cells were grown exponentially in minimal medium at 23°C and were shifted either to 38°C for 1 h or to minimal medium supplemented with 2 μg/ml cycloheximide for 2 h.

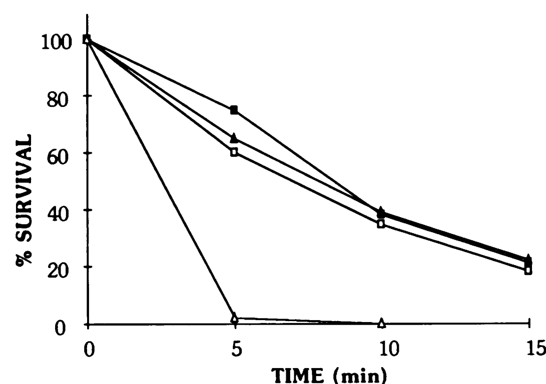

Fig. 4. Acquired thermotolerance following exposure to heat or metabolic stress. A wild-type (closed symbols) or a *yap1Δ* (open symbols) strain grown exponentially at 23°C was either shifted to 38°C for 10 min (squares) or grown for an additional 2 h in the presence of cycloheximide (triangles). Following such an exposure cultures were shifted to 52°C for the indicated time periods. Survival at this temperature was measured by counting cells following appropriate dilutions and platings. The values represent the average of three independent experiments.

biosynthesis, we examined the possibility that this transcriptional activator might be more globally involved in the stress response. This idea was further supported by examining the promoter of the *TPS2* gene. Such sequence inspection revealed no potential Yap1p binding sites but instead four stress response elements of the consensus CCCCT (Figure 2). This element is found within the promoter of a number of genes which are induced by a variety of stresses including heat and osmotic shock (see Introduction). The involvement of these elements in the regulation of the *TPS2* gene was further supported by the fact that deletion of the two distal elements resulted in quantitative but not qualitative differences in the stress induction (Figure 3, *TPS2ΔlacZ*). Such synergism among these stress elements has been reported for the *DDR2* promoter (Kobayashi and McEntee, 1993). Finally, such elements also exist within the promoter regions of *TSL1* and *TSS1* which encode components of the trehalose synthetase complex (Vuorio *et al.*, 1993).

In order to investigate whether Yap1p is involved in regulation through this element, we synthesized a double-stranded oligonucleotide derived from the sequence of one of these elements contained within the promoter region of the heat-induced gene *DDR2* gene (oligo 31/32 of Kobayashi and McEntee, 1993) and placed it in front of the *lacZ* reporter gene as the sole element providing a UAS function. As shown in Figure 3, transcription through this element was induced when wild-type cells were exposed to heat and osmotic stress. This induction was completely impaired in a *yap1* strain or in a strain with an activated PKA. In a *cdc25* strain transcription from this promoter was constitutively elevated but this effect was neutralized in a *cdc25 yap1* double mutant strain. We concluded that the *yap1* function is required for transcriptional induction through this element. *In vitro* binding assays showed that this effect was indirect since Yap1p could not bind to this element (data not shown).

YAP1 is not involved in the regulation through the heat shock element

Since *YAP1* was required for the proper function of the stress element we wanted to investigate whether it was required for activation through the HSE. A first indication that this was not the case came from the observation that although *yap1* strains could not acquire thermotolerance after exposure to cycloheximide as wild-type strains did, they could tolerate high temperatures when briefly heat shocked (Figure 4). A more direct demonstration is shown in Figure 5. Analysis of the induction of the major heat shock proteins following a temperature shift from 23°C to 38°C showed an identical pattern for the wild-type and the *yap1* strain. We concluded that Yap1p was not involved in transcriptional regulation through the HSE.

Discussion

High levels of Yap1p confer pleiotropic drug resistance. The typical determinants of multiple drug resistance in eukaryotic cells are transport proteins responsible for the efflux of toxic compounds. In this context the P-glycoprotein family of transporters accounts for the resistance of tumour cells to anticancer drugs (Endicott and Ling, 1989). Membrane proteins with similar structural and functional characteristics have been recently described in yeast, such as the PDR5 protein whose expression is regulated by the PDR1 transcriptional activator (Balzi *et al.*, 1994; Dexter *et al.*, 1994). Overexpression of the *PDR1* gene confers multiple drug resistance. Based on such paradigms the expectation was that the YAP1 transcriptional activator might similarly regulate the expression of a membrane transporter. Although we cannot exclude such a possibility we present strong evidence that *YAP1* is involved in the stress response pathway in both a general and a specific way. It is this involvement that allows *YAP1*-overexpressing cells to proliferate under conditions of metabolic toxicity simply a reflection of an acquired stress-tolerant state.

The generalized role of Yap1p in stress tolerance is supported by our data demonstrating that it is required for transcriptional induction through the recently described STRE (Kobayashi and McEntee, 1993; Marchler *et al.*, 1993). We have shown this directly using a reporter gene whose transcription is mediated through a STRE sequence derived from the promoter of the *DDR2* gene. By contrast, Yap1p is not required for transcriptional induction through the HSE. We have shown that the induction of the major

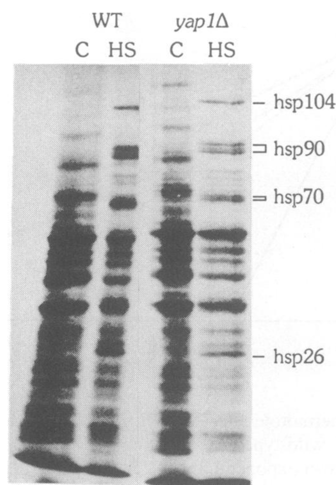

Fig. 5. Induction of the synthesis of the major heat shock proteins in a wild-type (WT) and a *yap1Δ* strain. Exponentially grown cells were incubated for 5 min with [^{35}S]methionine either at 23°C (C) or following a 10 min exposure at 38°C (HS). Total proteins were visualized by fluorography following electrophoretic separation on a 10% SDS–polyacrylamide gel. The positions of the major familites of heat shock proteins are indicated.

heat shock proteins is normal in *yap1* strains. In agreement with this, we have shown that such strains can acquire thermotolerance only when exposed to a brief heat shock but not when given a toxic but not lethal dose of cycloheximide. These results restrict the function of Yap1p to only one set of stress-inducing conditions, possibly ones that cause direct metabolic disturbances.

STRE-mediated transcriptional activation is under the control of PKA (Marchler *et al.*, 1993). In agreement with this we have shown that the reporter gene whose transcription is mediated through the STRE is subject to the same regulation by PKA: its transcriptional induction is impaired in strains with activated PKA whereas it is constitutively derepressed in strains in which this kinase cannot be activated, as in a *cdc25* strain. The finding which substantiates the role of the Yap1p in the stress response is that the *yap1* disruption is epistatic to the *cdc25* mutation, a fact suggesting that Yap1p functions downstream of the RAS-PKA pathway.

How does Yap1p, known to bind the AP1 DNA sequence (Moye-Rowley *et al.*, 1989), regulate transcription through STRE? It is reasonable to assume that its requirement for this transcriptional activation is not mediated through direct DNA binding. In fact, Yap1p does not bind STRE (our unpublished data); instead, a protein factor of 104 kDa has been shown to interact with the C_4T element (Marchler *et al.*, 1993). Our preliminary results (N.Gounalaki and G.Thireos, unpublished) indicate that in *yap1*-disrupted strains as well as in strains with an activated PKA this interaction is still evident, suggesting that the binding ability of this factor is not impaired in such genetic regimens. One possibility is that Yap1p is required for the transcription of another factor which collaborates with the STRE binding protein to promote transcription. Alternatively, we can hypothesize that Yap1p interacts with the STRE binding factor(s) offering its transcriptional activation domain in a situation analogous to the DNA-independent function of the Stat1 transcriptional activator in response to α- and β-interferons (Pellegrini and Schindler, 1993). Finally, Yap1p may function further upstream in the signalling pathway that induces transcription through STRE. It has been shown recently that the *MAC1* gene encodes a putative transcriptional activator which is required for the stress-induced transcription of the *CTT1* gene but does not bind STRE (Jungman *et al.*, 1994). Possible direct or indirect interactions between Yap1p and Mac1p may be relevant to the stress response pathway.

The generalized involvement of Yap1p in the stress response pathway does not account for the multiple drug resistance that results when it is overexpressed. In fact, we have observed that transcriptional activation of both the *TPS2* gene and the STRE-regulated reporter is reduced in strains overexpressing the *YAP1* gene. This indicates that these drug-tolerant strains are partly desensitized to the imposed stress. Yap1p could regulate the transcription of a number of genes whose products are directly involved in cellular detoxification. Accordingly, Yap1p-overexpressing strains will become relatively insensitive to the consequences of a battery of toxic compounds and metabolic drugs. Support for this idea was provided in a recent report showing that thioredoxin, a protein which scavenges reactive oxygen species, is essential for *YAP1*-mediated resistance to oxidative stress (Kuge and Jones, 1994). It has been shown that the promoter of the thioredoxin-encoding gene, *TRX2*, is directly regulated by Yap1p.

The suggested direct function of Yap1p in the expression of detoxifying genes is not sufficient to account for its involvement in multidrug resistance. Our genetic analysis shows that this requires biosynthesis of trehalose, a metabolite implicated in tolerance (Wiemken, 1990). Although increased amounts of trehalose are not sufficient for stress tolerance, our results show that accumulation of trehalose is absolutely required for Yap1p-mediated pleiotropic drug resistance. Our results strongly suggest that this accumulation is regulated transcriptionally through STRE. It requires inactive PKA and Yap1p, and both these requirements are reflected on the stress-induced transcription of the *TPS2* gene. STRE is present four times within the *TPS2* promoter and is also found within the promoter region of the *TSL1* and *TSS1* genes which encode the other two subunits of the trehalose synthetase complex (Vuorio *et al.*, 1993). Further support for the involvement of STRE in the regulation of trehalose biosynthesis is provided by our preliminary deletion analysis of the *TPS2* promoter. A promoter sequence in which two such elements are deleted results in reduced levels of stress-induced transcription in agreement with the synergistic function of multiple STREs (Kobayashi and McEntee, 1993).

The totality of the evidence points to a dual role of Yap1p in the acquisition of stress tolerance. One is its indirect and general requirement for transcriptional activation of STRE-regulated tolerance genes and the other is its direct involvement in the synthesis of detoxifying gene products. We believe that the coordinated function of all these gene products is essential for the acquisition of a generalized stress-tolerant state. Further knowledge of the details of Yap1p function and the modes of its activation will enable the manipulation of the stress

response pathway in such ways as to increase the resistance of cells to the toxic compound.

Materials and methods

Strains, media and genomic libraries

All yeast strains used in this study were derivatives of S288C. Those were a **a** *ura3*-52 *leu2*-112 and a *cdc25*-1 *ura3*-52 *leu2*-112 (obtained from G.Fink). The *yap1*Δ strain was constructed using the two-step replacement method (Rothstein, 1991): a 3750 bp *Sal*I−*Eco*RI DNA fragment containing the *YAP1* gene and flanking regions (Leppert *et al.*, 1990) was subcloned as a blunt fragment into the *Bam*HI site of the Yip5 vector and the 386 bp *Afl*II−*Bam*HI DNA fragment containing the 5′ UTR and part of the coding sequence of the *YAP1* gene was deleted. This plasmid was integrated into the *YAP1* genomic locus and deleted strains were obtained following selection for excision events on plates containing 5-fluoroorotic acid and confirmation through genomic DNA transfer analysis. Standard minimal media were used, and stress was imposed by the addition of 2 μg/ml cycloheximide or NaCl to 0.3 M. The *YAP1* gene was isolated from a high copy number library in the YEp13 vector (constructed by Kim Nasmyth). The yeast genomic library used to clone the *TPS2* gene was carried on a single copy YCp50 vector (Driscoll-Penn *et al.*, 1984).

Mutagenesis

A wild-type strain transformed with the *YAP1* gene on a high copy vector along with the *AP1*−*lacZ* reporter gene (Georgakopoulos and Thireos, 1992) was mutagenized using EMS (Lawrence, 1991) to 70% lethality. Ten thousand mutagenized colonies were replica plated on minimal medium supplemented with 2 μg/ml cycloheximide and onto plates containing 0.125 mM cadmium. Ten colonies unable to grow in both these regimens were selected and were assayed for β-galactosidase activity. Only one strain expressed the *AP1*−*lacZ* gene to wild-type levels. In addition to the lost multiple drug resistance this strain was temperature sensitive for growth. Following two rounds of backcrosses the temperature sensitivity and drug sensitivity co-segregated. The temperature sensitivity was used for cloning by complementation and positive transformants were further assayed for multiple drug resistance when co-transformed with multiple copies of the *YAP1* gene.

Plasmid constructions

A *HIS3*−*lacZ* fusion minimal reporter plasmid was used for the introduction of promoter elements. This minimal reporter has the regulated TATA element of the *HIS3* gene and two cloning sites (*Bam*HI and *Eco*RI) 80 bases upstream where UASs can be introduced and is carried on a YCp88 vector (Georgakopoulos and Thireos, 1992). Into these sites a *Sca*I−*Bgl*II (Figure 2, *TPS2*−*lacZ*) as well as an *Ava*II−*Bgl*II (*TPS2*Δ−*lacZ*) *TPS2* promoter DNA fragment were introduced. In the latter construction the S1 nuclease treatment used to generate blunt ends resulted in the removal of two additional bases and thus the 5′ end of this deleted derivative was at the base indicated in Figure 2. The STRE−*lacZ* reporter was constructed similarly by inserting into the above vector a synthetic double-stranded oligonucleotide whose design was based on the sequence of part of the oligo 31/32 of Kobayashi and McEntee (1993). The sequence of this oligonucleotide was 5′-GGATCCACCCTTATGGGGAAGCTTGAATTC-3′.

Other methods

Trehalose content was measured following extraction with ice-cold 5% trichloroacetic acid using the anthrone procedure (Lilie and Pringle, 1980). RNA extractions were performed using the hot acid/phenol method (Koehrer and Domdey, 1991). RNA was electrophoresed on formaldehyde-containing agarose gels, and transfer and hybridization analysis were performed using standard methodologies. *In vivo* protein labelling was achieved by incubating cells for 5 min in minimal medium supplemented with [^{35}S]methionine. Total pulse-labelled proteins were analysed on a 10% SDS−polyacrylamide and visualized through fluorography. β-Galactosidase assays were performed according to Thireos *et al.* (1984).

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