Yap1 Accumulates in the Nucleus in Response to Carbon Stress in *Saccharomyces cerevisiae*

Heather A. Wiatrowski and Marian Carlson*

*Department of Genetics and Development, Columbia University,
New York, New York 10032*

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Yap1 is a transcription factor of the AP-1 family that is required for the adaptive response to oxidative stress in *Saccharomyces cerevisiae*. We recovered Yap1 in a two-hybrid screen for proteins that interact with the Sip2 subunit of the Snf1 protein kinase, which is required for the adaptation of cells to glucose limitation. Yap1 becomes enriched in the nucleus when cells are subjected to oxidative stress. We show that the localization of Yap1 is similarly sensitive to carbon stress. When glucose-grown cells were shifted to medium containing glycerol or no added carbon source, green fluorescent protein (GFP)-Yap1 accumulated in the nucleus. After adaptation to growth in glycerol, GFP-Yap1 was again primarily cytoplasmic. Nuclear accumulation was independent of respiration and of the Snf1, PKA, TOR, and Yak1 pathways, and the mechanism is distinct from that involved in the response to hydrogen peroxide. Addition of glutathione to the medium inhibited nuclear accumulation of GFP-Yap1 in response to carbon stress but did not affect the relocalization of Gal83 or Mig1. Other stresses such as increased temperature, acidic pH, and ionic stress did not cause nuclear enrichment of GFP-Yap1. These findings suggest a role for Yap1 in the response to carbon stress.

The AP-1 transcription factors are bZIP DNA-binding proteins that bind as homo- or heterodimers to sequences termed AP-1 sites (for reviews, see references 33 and 53). The AP-1 family of proteins is highly conserved in eukaryotes, and specific family members function to activate transcription of particular sets of genes in response to distinct signals. In mammalian cells, AP-1 proteins have been implicated in proliferation, differentiation, apoptosis and responses to stress and radiation. In the yeast *Saccharomyces cerevisiae*, the AP-1 factor Gcn4 has the same DNA-binding specificity as the mammalian Jun and Fos factors and activates transcription of numerous genes in response to amino acid starvation (25). Other yeast AP-1 (Yap) factors, Yap1 through Yap8, have slightly different DNA-binding specificity from other AP-1 factors and have overlapping but distinct functions in cellular responses to oxidative stress and to cytotoxic agents, including various drugs and heavy metals (13, 26, 42, 51, 53, 59).

Yap1 is particularly important for the adaptive response to oxidative stress (36, 47, 51; for reviews see references 28 and 54). When exposed to a low dose of an oxidant, cells adapt and are subsequently able to tolerate much higher levels than untreated cells. Yap1 is critical for this response. When cells are treated with various oxidants, Yap1 accumulates in the nucleus (9, 37, 61) and induces transcription of genes that are essential for defense against oxidative stress, including *GSH1*, which encodes γ-glutamylcysteine synthase; *GLR1*, which encodes glutathione (GSH) reductase; *GPX2*, which encodes GSH peroxidase; *TRX2*, which encodes thioredoxin; *TRR1*, which encodes thioredoxin reductase; *SSA1*, which encodes an Hsp70 protein; and many others (11, 16, 20, 22, 27, 36, 39, 47, 51, 60). The mechanism responsible for nuclear accumulation of Yap1 in response to oxidative stress is understood in molecular detail. The association of the nuclear export factor Crm1 (Xpo1) with the C-terminal cysteine-rich domain (CRD) of Yap1 is inhibited by the formation of intramolecular disulfide linkages; in the case of hydrogen peroxide (H₂O₂)-treated cells, these linkages involve cysteine residues in the N- and C-terminal CRDs, whereas treatment with diamide, a thiol oxidant, induces disulfide linkages involving only the C-terminal CRD (9, 10, 35, 37, 38, 58, 61).

We unexpectedly recovered Yap1 in a two-hybrid screen for proteins that interact with Sip2, a β subunit of the Snf1 protein kinase. The Snf1 kinase is important for adaptation to glucose depletion and for growth on carbon sources other than glucose; Snf1 regulates the transcription of many glucose-repressed genes and the activity of metabolic enzymes (6, 15, 24). Snf1 has also been implicated in various stress responses, but has no known role in oxidative stress (2, 34, 52). The Snf1 kinase complex comprises the catalytic subunit Snf1, the activating subunit Snf4, and one of three alternate β subunits, Gal83, Sip1, or Sip2 (31, 63). The β subunits, which have divergent N termini and conserved C termini, exhibit distinct subcellular localization patterns and direct Snf1 to specific targets (46, 56, 57, 63). The Sip2 subunit, together with Gal83, is important for growth on nonfermentable carbon sources (46, 56), and Sip2 has also been implicated in life span, aging, and survival during stationary phase (3, 4). Sip2 is localized in the cytoplasm during growth on a variety of carbon sources (57). We carried out a two-hybrid screen (14) using the N terminus of Sip2 as the bait with the expectation of recovering proteins that interact specifically with this β subunit.

The recovery of Yap1 in this screen suggested a functional connection to the Snf1 kinase, which seemed plausible because adaptation to carbon stress is associated with increased resistance to oxidative stress (28, 29). When glucose becomes depleted during fermentative growth, cells enter the diauxic shift...
and then resume growth by respiratory metabolism of the ethanol produced during fermentation until they enter stationary phase. During the growth phases following glucose depletion, cells acquire increased oxidative stress resistance. Respiratory growth on nonfermentable carbon sources also leads to increased resistance. In both cases, Yap1 contributes to oxidative stress tolerance (22, 51).

We have investigated the role of Yap1 in the response to carbon stress. We report that Yap1 becomes nuclear localized in response to carbon starvation by a mechanism that is independent of the Snf1 pathway and distinct from that involved in the response to H₂O₂.

**MATERIALS AND METHODS**

**Strains and genetic methods.** *S. cerevisiae* strains used in this study are listed in Table 1. A DNA fragment carrying the yap1Δ::URA4 sequence (36) was used to introduce this allele into the S288C genetic background. A PCR-based method was used to replace the YAK1 coding region (nucleotides −1 to 2422) in strain MY1402 with the KanMX6 gene. Selective synthetic complete (SC) medium (44) was used to maintain selection for plasmids. For carbon source shift experiments, carbon source (nucleotides 687 to 2452) in strain BY4742 was used to replace the yap1 gene. Selective synthetic complete (SC) medium (44) was used to introduce this allele into the S288C genetic background. A PCR-based method was used to replace the YAK1 coding region (nucleotides −1 to 2422) in strain MY1402 with the KanMX6 gene. Selective synthetic complete (SC) medium (44) was used to maintain selection for plasmids.

**Plasmids.** The fusion of Sip2 residues 3 to 199 [Sip2(3-199)] and LexA used in this study are listed in Table 1. A DNA fragment carrying the yap1Δ::URA4 sequence (36) was used to introduce this allele into the S288C genetic background. A PCR-based method was used to replace the YAK1 coding region (nucleotides −1 to 2422) in strain MY1402 with the KanMX6 gene. Selective synthetic complete (SC) medium (44) was used to maintain selection for plasmids.

**Transformants** were selected on SC medium. Nuclei were stained by addition of 4',6-diamidino-2-phenylindole (DAPI) (0.8 g/ml) for 5 min to 1 h. Cells were collected by centrifugation and resuspended in approximately 20 µl of the residual medium, and 1.4 µl of the suspension was placed on a microscope slide. GFP localization was monitored by direct fluorescence. Cells were viewed using a Nikon Eclipse E800 fluorescence microscope. Images were taken with an Orca100 (Hamamatsu) camera using Open Lab (Improvision) software and were processed in Adobe Photoshop 5.5.

**Determination of GSH content.** Total and oxidized GSH levels were determined by using method I of Aebi and Sies (1). Derivatization of GSH was accomplished by incubating cell suspensions in 5% 2-vinylpyridine at 23°C for 1 h.

**Preparation of protein extracts and immunoblot analysis.** Protein extracts were prepared as described previously (55), except the buffer contained 5 mM sodium pyrophosphate and 2.5 mM sodium fluoride. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% polyacrylamide) (SDS–10% PAGE) and were subjected to immunoblot analysis. Proteins were detected with polyclonal anti-LexA antibody (Invitrogen) or a mixture of two monoclonal antibodies to GFP (Roche Molecular Biochemicals) and enhanced chemiluminescence with ECLPlus reagents (Amersham).

**Analysis of the redox state of LexA-Yap1 protein.** Yeast cells were lysed with trichloroacetic acid, and proteins were resolubilized in the presence of iodoacetamide to alkylate free thiols and were subjected to SDS–10% PAGE under nonreducing conditions, as described previously (10) except that the gel contained 37.5% acrylamide-bisacrylamide. In control experiments, proteins were treated with 200 mM dithiothreitol (DTT) prior to addition of iodoacetamide (10). Proteins were detected by immunoblot analysis as described above.

**Invertase assays.** Cells were grown to exponential phase in SC medium and assayed for secreted invertase activity (30).

**RESULTS**

**Isolation of Yap1 in a two-hybrid screen for interaction with the N terminus of Sip2.** To identify proteins that interact with the Sip2 β subunit of the Snf1 kinase, we screened a library of Gal4 activation domain (GAD) fusions to yeast cDNAs for two-hybrid interaction with the N terminus of Sip2 (residues 3-199) fused to LexA. Of 31 positive clones, five contained YAP1 sequences, one contained the homoserine kinase gene *THRI*, and 25 contained the Hsp70 genes *SSB1* and *SSB2*. The largest GAD-Yap1 clone contained YAP1 sequence from the β-galactosidase activity in a filter lift assay (62), and 31 positive clones were recovered. Library plasmids were isolated from these transformants and subjected to restriction site analysis. Representative plasmids were used to retransform the reporter strain to confirm that they conferred a His+ phenotype and expressed β-galactosidase when present with Sip2(3-199)-LexA but not with LexA or LexA-lamin. Plasmids were then subjected to sequence analysis.

**Microscopy.** Yeast cells expressing a GFP fusion protein were grown to mid-log phase in SC medium. Nuclei were stained by treatment with 200 mM dithiothreitol (DTT) prior to addition of iodoacetamide (SDS–10% PAGE) and were subjected to immunoblot analysis. Proteins were detected with a polyclonal anti-LexA antibody (Invitrogen) or a mixture of two monoclonal antibodies to GFP (Roche Molecular Biochemicals) and enhanced chemiluminescence with ECLPlus reagents (Amersham).

**TABLE 1. S. cerevisiae strains used in this study**

| Strain   | Genotype                                      | Source or reference |
|----------|-----------------------------------------------|---------------------|
| MCY4062  | MATα snf1Δ10 his3Δ200 lex2Δ112 trplΔ1 ura3Δ53 lys2Δ801 | This study          |
| MCY4063  | MATα yap1Δ::URA4 his3Δ200 lex2Δ112 trplΔ1 ura3Δ53 lys2Δ801 | This study          |
| MCY4068  | MATα his3Δ200 lex2Δ112 trplΔ1 ura3Δ53 lys2Δ801          | This study          |
| MCY4455  | MATα his3Δ200 lex2Δ112 trplΔ1 ura3Δ53 lys2Δ801          | This study          |
| MCY4457  | MATα sip2Δ::LEU2 his3Δ200 lex2Δ112 trplΔ1 ura3Δ53 lys2Δ801 | This study          |
| ASY62    | MATα trp1Δ::ADE8 pk2:HIS3 trp3::TRP1 msn2Δ::HIS3 msn4Δ::LEU2 ura3Δ53 his3Δ2 lys2Δ801 trplΔ ade8 | Research Genetics   |
| W303 trp1Δ ade8 | MATα trp1::URA3 pk2::HIS3 trp3::TRP1 | 19                 |
| BY4741   | MATα his3Δ1 lex2Δ0 met15Δ0 ura3Δ0                  | Research Genetics   |
| BY4742   | MATα his3Δ1 lex2Δ0 met15Δ0 ura3Δ0                  | Research Genetics   |
| BY569    | MATα yap1Δ::KanMX4 his3Δ1 lex2Δ0 met15Δ0 ura3Δ0     | Research Genetics   |
| BY10569  | MATα yap1Δ::KanMX4 his3Δ1 lex2Δ0 met15Δ0 ura3Δ0     | Research Genetics   |
| MY1402   | MATα his3Δ1 lex2Δ0 trplΔ1 ura3Δ0                   | Microbia           |
| YR18     | MY1402 yap1Δ::KanMX6                                | This study          |
| TAT7(L40-ura3) | MATα ura3Δ53 ade2 his3Δ200 lex2Δ112 trplΔ1 ura3Δ53::lexAop-lacZ LYS2::lexAop-HIS3 | 48                 |

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| MCY4068        | MATα his3Δ200 lex2Δ112 trplΔ1 ura3Δ53 lys2Δ801          | This study          |
| MCY4455        | MATα his3Δ200 lex2Δ112 trplΔ1 ura3Δ53 lys2Δ801          | This study          |
| MCY4457        | MATα sip2Δ::LEU2 his3Δ200 lex2Δ112 trplΔ1 ura3Δ53 lys2Δ801 | This study          |
| ASY62          | MATα trp1Δ::ADE8 pk2:HIS3 trp3::TRP1 msn2Δ::HIS3 msn4Δ::LEU2 ura3Δ53 his3Δ2 lys2Δ801 trplΔ ade8 | Research Genetics   |
| W303 trp1Δ ade8 | MATα trp1::URA3 pk2::HIS3 trp3::TRP1 | 19                 |
| BY4741         | MATα his3Δ1 lex2Δ0 met15Δ0 ura3Δ0                  | Research Genetics   |
| BY4742         | MATα his3Δ1 lex2Δ0 met15Δ0 ura3Δ0                  | Research Genetics   |
| BY569          | MATα yap1Δ::KanMX4 his3Δ1 lex2Δ0 met15Δ0 ura3Δ0     | Research Genetics   |
| BY10569        | MATα yap1Δ::KanMX4 his3Δ1 lex2Δ0 met15Δ0 ura3Δ0     | Research Genetics   |
| MY1402         | MATα his3Δ1 lex2Δ0 trplΔ1 ura3Δ0                   | Microbia           |
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codon 268 to the C terminus. In other two-hybrid assays, Yap1 did not interact significantly with full-length Sip2 or with other components of the Snf1 kinase pathway, including Snf1, Snf4, Sip1, Gal83, Sip5, and Reg1. Because the C-terminal sequence of Yap2 is similar to that of Yap1 (26), we considered the possibility that GAD-Yap2 would interact strongly with Sip2 (3-199)-LexA, but that was not the case. Although these two-hybrid assays did not provide compelling evidence for genuine interaction between Yap1 and the Snf1 kinase, Yap1 has a role in oxidative stress resistance under conditions when the Snf1 kinase is active, in particular when glucose becomes depleted during fermentative growth and during growth on nonfermentable carbon sources. We therefore investigated the behavior of Yap1 in response to carbon stress.

Nuclear localization of Yap1 in response to carbon stress. Previous studies showed that the localization of Yap1 fused to GFP changes in response to oxidative challenge (9, 37, 61). Yap1 is predominantly cytoplasmic in exponentially growing cells, but treatment with oxidants causes Yap1 to accumulate in the nucleus. To determine if carbon stress affects the localization of Yap1, we examined cells expressing GFP-Yap1 from the YAP1 promoter on a centromeric plasmid (9). Cells were grown to exponential phase in SC mid-log phase in YEP/Dextrose (YEP/D) and shifted to 3% glycerol in both YEP and SC medium. Comparison of the growth curves provided no evidence that Yap1 confers any significant advantage under these particular laboratory conditions.

Yap1 localization is unaffected by increased temperature, acidic pH, or ionic stress. We next examined whether nuclear accumulation of GFP-Yap1 is specific to carbon and oxidative stress or occurs as part of a general stress response. Exponentially growing cells were shifted to 37°C, shifted to medium containing high salt (1 M NaCl), or shifted to acidic medium (pH 4). Each of these environmental changes causes a global change in transcription (7), but none of these changes caused nuclear accumulation of GFP-Yap1 (Fig. 2).

Snf1 kinase does not regulate nuclear localization of Yap1 in response to carbon or oxidative stress. Because GAD-Yap1 exhibits two-hybrid interaction with the Sip2 subunit of the Snf1 kinase, we assessed the role of Snf1 in regulating the nuclear enrichment of Yap1 in response to carbon and oxidative stress. In a snf1Δ mutant, as in the wild type, GFP-Yap1 was largely cytoplasmic in glucose-grown cells and became nuclear enriched after a shift to glycerol (Fig. 3A). Treatment with H2O2 also caused nuclear accumulation of GFP-Yap1 in the snf1Δ mutant (data not shown). Mutation of other components of the Snf1 signaling pathway (Sip2 and Gal83, singly and in combination; the activating subunit Snf4; and Reg1, a negative regulator of kinase activity) did not perturb the regulation of Yap1 localization in either assay (data not shown). An analysis of the kinetics of nuclear accumulation in response to carbon stress was carried out for the sip2Δ mutant, which grows well in glycerol (unlike the snf1Δ mutant); however, no significant difference from the wild type was detected (Fig. 1D). Thus, the Snf1 pathway has no apparent role in regulating the subcellular localization of Yap1 in response to either carbon deprivation or oxidative stress.

We also examined GFP-Yap1 for Snf1-dependent phosphorylation. Protein extracts were prepared from wild-type and snf1Δ mutant cells after growth in glucose and after a shift to glycerol for 60 min. Immunoblot analysis revealed no Snf1-dependent differences in mobility (data not shown), but we cannot exclude the possibility that some phosphorylation events went undetected.

Other nutrient signaling pathways do not affect carbon source-dependent localization of Yap1. We also tested the possible role of other major nutrient response pathways in regulating localization of Yap1. Protein kinase A (PKA) inhibits the nuclear localization of the transcription factors Msn2 and Msn4 in response to glucose (18). In two different strains that have all three genes for the PKA catalytic subunit mutated (W303 tpk123 msn2 msn4 and ASY62), GFP-Yap1 was predominantly cytoplasmic in glucose-grown cells and accumulated in the nucleus after a 1 h shift to 1% glycerol (Fig. 3D and data not shown). The TOR pathway, which is inactivated by nutrient starvation or the drug rapamycin, regulates nuclear localization of various transcription factors (5). Treatment of glucose-grown wild-type cells with rapamycin had no effect on the localization of GFP-Yap1 (Fig. 3C). Finally, the Yak1 kinase controls carbon source-regulated nuclear localization of

of yap1Δ::KanMX4 mutant and wild-type cultures (BY4741, BY4742, BY569, and BY10569) after a shift from 2% glucose to 3% glycerol in both YEP and SC medium. Comparison of the growth curves provided no evidence that Yap1 confers any significant advantage under these particular laboratory conditions.

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FIG. 1. Nuclear localization of GFP-Yap1 in response to carbon stress. Cultures of strain MCY4455 expressing GFP-Yap1 were grown in selective SC medium. DNA was stained with DAPI, and the subcellular localization of GFP-Yap1 was examined by fluorescence microscopy.

(A) Cells were grown to exponential phase in SC +2% glucose (Glu) and then were shifted for 1 h to SC +1% glycerol (Gly). (B) Cells were grown for many generations in SC +1% glycerol and shifted for 1 h to SC medium with no added carbon source (None). (C) Cells were grown in SC +2% glucose and shifted to SC +1% glycerol for 1 h. Both media contained antimycin A (aa) (1 \mu g/ml). (D) Cultures of wild-type strain MCY4455 (circles) and sip2Δ mutant MCY4457 (squares) expressing GFP-Yap1 were grown in SC +2% glucose and shifted to SC +1% glycerol. Cells (n = 100) were examined for fluorescence at the indicated times and scored for nuclear enrichment of GFP-Yap1. After a 1-h shift to glycerol, GFP-Yap1 returned to the cytoplasm somewhat more rapidly (within 2 h) if the cells were transferred to SC +2% glucose (data not shown).
Cells were examined by fluorescence microscopy.

The localization of the Gal83 β subunit is regulated by an unidentified signaling pathway different from the Snf1, PKA, TOR, and Yak1 pathways (57; H. A. Wiatrowski, unpublished results). Gal83-GFP is excluded from the nucleus during growth in glucose and rapidly localizes to the nucleus when cells are shifted to glycerol. Genetic evidence suggests that Gal83 localization is regulated by glucose-6-phosphate, and the glucose analog 2-deoxyglucose, which can be phosphorylated but not metabolized, causes nuclear export of Gal83-GFP (57). To test whether GFP-Yap1 localization is similarly regulated, we shifted cells from glucose to glycerol in the presence of 2-deoxyglucose. GFP-Yap1 accumulated in the nucleus within 1 h (Fig. 4B), whereas Gal83-GFP remained nuclear excluded as expected (data not shown), indicating that Yap1 and Gal83 respond to different signals.

GSH inhibits nuclear accumulation of Yap1 in response to carbon stress. It remained possible that rapid carbon deprivation generates transient oxidative stress, independent of respiration. GSH is required for protection against oxidative stress (20, 21, 50), and oxidative stress results in Yap1-dependent upregulation of enzymes of the GSH pathway (20, 60). We therefore tested whether addition of GSH to the media would prevent nuclear enrichment of GFP-Yap1. Cells were shifted from glucose to glycerol in the presence of GSH (50 μg/ml), and GFP-Yap1 failed to accumulate in the nucleus (Fig. 4A). This concentration of GSH did not alter the growth rate in glucose (data not shown), and the presence of GSH (200 μg/ml) also did not compromise glucose regulation of SUC2 (<1 U of invertase activity during growth in 2% glucose and 80 U after a 1.5 h shift to 1% glycerol). Other control experiments addressed the possibility that GSH inhibits GFP-Yap1 nuclear accumulation by nonspecifically disrupting nuclear trafficking. In the presence of GSH (50 μg/ml), Gal83-GFP remained excluded from the nucleus in glucose and rapidly accumulated in the nucleus upon a shift to glycerol (Fig. 4B); this finding further supports the conclusion that distinct mechanisms control localization of Yap1 and Gal83. In addition, localization of GFP-Mig1, which is nuclear during growth on glucose and becomes excluded from the nucleus upon a shift to glycerol by an Snf1-dependent mechanism, was unaffected by GSH (50 μg/ml; data not shown).

To investigate the possibility that shifting the cells from glucose to glycerol changes the redox status of GSH, we assayed total and oxidized GSH (GSSG) in cultures that were grown in SC + 2% glucose and shifted for 1 h to SC + 1% glycerol. Total GSH remained the same, and the percentage of GSSG was not significantly different (1 and 1.8%, respectively). These findings are consistent with evidence that when cells are exposed to an oxidant, Yap1 does not sense the GSH/GSSG ratio but rather senses a signal directly from the oxidant (10, 35).

The mechanisms by which oxidative stress cause nuclear enrichment of Yap1 are understood in some detail: oxidants such as H2O2 and diamide induce formation of disulfide bonds in Yap1 that prevent interaction with the nuclear export factor Crm1 (9, 10, 35, 37, 38, 58, 61). In the case of H2O2, the disulfide linkage also causes a conformational change that can be detected by SDS-PAGE under nonreducing conditions (10). We used this method to assess whether carbon stress affects Yap1 by the same mechanism. We transformed cells with a centromeric plasmid expressing LexA-Yap1 from the YAP1 promoter. Proteins were prepared from glucose-grown cells and from cells shifted to 1% glycerol. The mobility of LexA-

![FIG. 2. GFP-Yap1 does not localize to the nucleus in response to heat, acidic pH, or ionic stress. Wild-type cells (MCY4455) expressing GFP-Yap1 were grown to exponential phase in selective SC + 2% glucose at 30°C and then incubated for 1 h at 37°C (A), shifted for 1 h to SC + 2% glucose containing 1 M NaCl (B), and shifted for 90 min to SC + 2% glucose containing 50 mM sodium succinate (pH 4) (C). Cells were examined by fluorescence microscopy.](Image)

![FIG. 3. Nuclear accumulation of Yap1 is not affected by Snf1, PKA, Yak1, TOR, or 2-deoxyglucose. All cells expressed GFP-Yap1. (A, D, and E) Cells were grown in SC + 2% glucose (Glu) and were shifted for 1 h to SC + 1% glycerol (Gly). Strains used were snf1Δ mutant MCY4062 (A), W303 tpk123 msn2 msn4 (D), and yak1Δ mutant YRH8 (E). (B) Wild-type cells (MCY4455) were grown in SC + 2% glucose and shifted for 1 h to SC + 1% glycerol containing 0.05% 2-deoxyglucose (2DG). (C) Wild-type cells were grown in SC + 2% glucose, and rapamycin (1 μg/ml) was added for 30 min. Cells were examined by fluorescence microscopy. DNA was stained with DAPI.](Image)
Yap1 was the same in all samples (Fig. 5, lanes b to f) and was not altered by addition of DTT (data not shown), whereas LexA-Yap1 from H2O2-treated cells had increased mobility (Fig. 5, lane a), as previously reported (10). Thus, the mechanisms are distinct; however, it remains possible that carbon stress induces formation of different disulfide bonds that do not cause a mobility change in this assay, as is the case for diamide (10).

FIG. 4. GSH inhibits nuclear localization of GFP-YAP1 in response to carbon stress. Wild-type cells (strain MCY4455) expressing GFP-Yap1 (A) or Gal83-GFP (B) were grown to mid-log phase in SC + 2% glucose, shifted to SC + 2% glucose supplemented with reduced GSH (50 μg/ml) for 90 min, and then shifted to SC + 1% glycerol containing GSH (50 μg/ml) for 1 h. DNA was stained with DAPI. Cells were examined by fluorescence microscopy.

Carbon metabolism is linked to cellular reducing power. The pentose phosphate pathway is a primary source of NADPH, and mutants lacking glucose-6-phosphate dehydrogenase (encoded by ZWF1) and other enzymes of the pentose phosphate pathway show increased sensitivity to oxidizing agents (32, 43). Exposure to H2O2 has been shown to alter carbohydrate metabolism so as to favor generation of NADPH (17). Moreover, GSSG is recycled to GSH in an NADPH-dependent reaction catalyzed by GSH reductase.

The connection between Yap1 and the Snf1 signaling pathway rests on the two-hybrid interaction of Yap1 with the N terminus of Sip2. The N terminus is the region of greatest divergence between the three Snf1/H9252 subunits and might be expected to have roles in mediating specific interactions between the kinase and particular targets. Although we detected no defect in the ability of yap1 mutants to adapt to carbon limitation, we tested only a few particular laboratory conditions. It is possible that interaction with the Snf1 kinase affects the

Snf1 kinase, and we present evidence that Yap1 accumulates transiently in the nucleus in response to carbon stress. This nuclear enrichment occurs in the absence of respiration and does not depend on the Snf1, PKA, TOR, or Yak1 nutrient signaling pathways. The addition of GSH to the medium eliminated the effect of carbon stress on the localization of Yap1; in contrast, GSH had no effect on the carbon source-regulated localization of Gal83 or Mig1, and the GSH redox balance has been shown not to be involved in the response of Yap1 to oxidants (10, 35). There are several mechanisms by which GSH could potentially affect the localization of Yap1. GSH may act as a free-radical scavenger to directly detoxify reactive oxygen species that accumulate as a result of carbon stress, or GSH may act through GSH peroxidase or other antioxidant enzymes. It is also possible that GSH reduces disulfide bonds in Yap1 that interfere with nuclear export under conditions of carbon stress; although thioredoxin is responsible for reduction of Yap1 following treatment with H2O2, it is not involved in reversing the effects of diamide (54).

Discussion

Yap1 has previously been characterized as a transcriptional regulator of the oxidative stress response. Here, we recovered Yap1 as a protein that interacts with the Sip2 subunit of the

FIG. 5. Analysis of LexA-Yap1 by electrophoresis under nonreducing conditions. Wild-type cells (strain MCY4455) expressing LexA-Yap1 from pHW28 were grown to mid-log phase in SC + 2% glucose (lane b) and were then treated with 0.3 mM H2O2 for 15 min (lane a) or shifted to SC + 1% glycerol for the indicated times (lanes c to f). Cells were lysed with trichloroacetic acid, and proteins were resolubilized in the presence of iodoacetamide and subjected to SDS–10% PAGE under nonreducing conditions (10). Proteins were detected by immunoblot analysis with anti-LexA. Samples were also taken at hourly intervals up to 6 h after the shift to glycerol, but no change in mobility was observed; for samples taken at 90 and 120 min, proteins were treated with 200 mM DTT prior to addition of iodoacetamide, but this treatment did not alter the mobility of LexA-Yap1 (not shown).
ability of Yap1 to stimulate transcription of a specific subset of genes that are important under other circumstances. There is precedent for the idea that Yap1 could function by a Snf1-dependent mechanism at certain promoters but not others. Mutational analysis suggests that transcriptional activation by Yap1 occurs by different mechanisms at the promoters of genes involved in \( \text{H}_2\text{O}_2 \) tolerance and those involved in diamide tolerance (9, 58). In addition, induction of some genes by \( \text{H}_2\text{O}_2 \) requires cooperative action by Yap1 and the transcription factor Skn7, whereas induction of other genes requires only Yap1 (36, 39, 41). Yap1 may cooperate with a variety of other transcription factors, perhaps in some cases in a Snf1-dependent manner.

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