Skp1-Cullin-F-box-dependent Degradation of Aah1p Requires Its Interaction with the F-box Protein Saf1p*

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When yeast cells enter into quiescence in response to nutrient limitation, the adenine deaminase Aah1p is specifically degraded via a process requiring the F-box protein Saf1p and components of the Skp1-Cullin-F-box complex. In this paper, we show that Saf1p interacts with both Aah1p and Skp1p. Interaction with Skp1p, but not with Aah1p, requires the F-box domain of Saf1p. Based on deletion and point mutations, we further demonstrate that the F-box domain of Saf1p is critical for degradation of Aah1p. We also establish that overexpression of Saf1p in proliferating cells is sufficient to trigger the degradation of Aah1p. Using this property and a two-dimensional protein gel approach, we found that Saf1p has a small number of direct targets. Finally, we isolated and characterized several point mutations in Aah1p, which increase its stability during quiescence. The majority of the mutated residues are located in two distinct exposed regions in the Aah1p three-dimensional model structure. Two hybrid experiments strongly suggest that these domains are directly involved in interaction with Saf1p. Importantly, we obtained a mutation in Aah1p that does not affect the protein interaction with Saf1p but abolishes Aah1p degradation. Because this mutated residue is an exposed lysine in the Aah1p three-dimensional model, we propose that it is likely to be a major ubiquitylation site. All together, our data strongly argue for Saf1p being a bona fide Skp1-Cullin-F-box subunit.

Proteolysis is critical for proper regulation of many cellular functions. Degradation of soluble proteins is mainly achieved by the ubiquitin proteasome system (1). Ubiquitin proteasome system catalyzes polyubiquitylation of target proteins, resulting in their subsequent degradation by a specific protease complex named proteasome. Polyubiquitylation of the substrate typically requires three distinct enzymatic activities, named E1, E2, and E3 (2, 3). First, a ubiquitin molecule is bound to the E1 ubiquitin-activating enzyme and then transferred to the E2 conjugating enzyme and is finally attached to the target protein by an E3 ubiquitin ligase. Repetition of this enzymatic sequence leads to polyubiquitylation of the target, which is then specifically recognized and degraded by the 26 S proteasome complex.

In the polyubiquitylation reaction, the E3 enzymes are critical to provide the substrate specificity. Several families of E3 enzymes have been described, and among them the Skp1-Cullin-F-box (SCF) complexes have been widely studied (4, 5). These complexes contain three core constant subunits (cullin, Skp1, and Rbx1/Roc1) and one variable F-box subunit (for reviews, see Refs. 6 and 7). In each SCF complex, a specific F-box protein interacts with the Skp1 core subunit through a conserved F-box motif and with the substrate via a specific protein-protein interaction domain. The latter is typically a leucine-rich repeat or a WD repeat. In yeast, 21 proteins containing an F-box domain have been identified on the basis of sequence alignments (8); two of them carry a WD40 repeat, and 11 contain a leucine-rich repeat domain. We have recently characterized a new F-box protein named Saf1p (9), which does not contain a leucine-rich repeat or WD40 domain but carries RCC repeats and is therefore different from previously described yeast F-box SCF subunits.

Because several yeast F-box proteins have been shown to exert their function independently of the SCF (10–12), the question arises as to whether Saf1p participates in a SCF complex dedicated to the degradation of specific substrates. Several lines of argument support this idea. First, Saf1p is involved in the degradation of adenine deaminase (Aah1p), which occurs upon transition from proliferation to quiescence and which also depends on the E2 Cdc34p and core subunits of the SCF (Skp1p and Hrt1p) (9). Second, Saf1p was copurified with Cdc34p, Skp1p, and Cdc53p in three independent interactome analyses (13–15). To confirm the involvement of Saf1p in a SCF complex, we have undertaken to characterize Saf1p interaction with its partners both at the structural and functional levels. In this paper, we show that Saf1p interacts with both Aah1p and Skp1p and that this interaction is critical for proper degradation of Aah1p. Using deletion and point mutations, we functionally delineate the domains of Saf1p and propose structural models for these domains. Finally, we identified several point
mutations in Aah1p that increase its stability. These data strongly argue for Saf1p being a bona fide SCF subunit.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—The following yeast strains were used in this study: HY (MATa/MATα; ura3 his3 ade2 trp1 leu2 gal4 gal80 URA3-GAL1acZ LYS2-GAL1HIS3); Y1624 (MATα; saf1::Kanr; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0); Y1544 (MATα; saf1::Kanr; his3Δ1; leu2Δ0; ura3Δ0; lys2Δ0); BY4742 (MATα; his3Δ1; leu2Δ0; ura3Δ0; lys2Δ0); Y2972 (MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ARG1-GFP-HIS3); Y2970 (MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; URA7-GFP-HIS3); Y1731 (MATα; saf1::Kanr; ura3) MJ159 (skp1-11; ade2; trp1; can1; leu2; his3; ura3); Y2446 (MATα; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; trp1::HIS3); Y2447 (MATα; saf1::Kanr; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; trp1::HIS3). Yeast cells were grown in YPD (1% yeast extract, 2% glucose) or in synthetic medium (0.17% nitrogen base, 0.5% ammonium, and 2% of either glucose (SD), raffinose or galactose). SDCasa is SD medium supplemented with 0.2% (w/v) casaminoacids (Difco), and SDCasaWA is SDCasa supplemented with tryptophan (40 mg liter⁻¹) and adenine (40 mg liter⁻¹). Histidine (10 mg liter⁻¹), lysine (10 mg liter⁻¹), uracil (20 mg liter⁻¹), and methionine (20 mg liter⁻¹) were added when necessary.

DNA Manipulations—Details of constructs and sequence of primers are available upon request. For two-hybrid experiments, SAF1, SKP1, and AAH1 open reading frames were fused with the GAL4 activation domain (AD) and GAL4 DNA-binding domain (DB) in pACTII (TRP1, 2μ) (16), and pODD80 (LEU2, 2μ) (17) plasmids, respectively. Wild type and various alleles of AAH1 were amplified by PCR with primers 1076 and 1082; SAF1 (wild-type and mutant alleles) was amplified with primers 1075 and 1074; SAF1ΔF-box fragment was amplified with primers 1184 and 1075; and SKP1 was amplified with primers 1072 and 1073. These fragments were digested with Smal and Xhol and cloned in pODD80 at Smal/Xhol sites and in pACTII at Smal/Sall sites. Plasmids obtained were named p2745 (Gal4DB-AAH1), p2747 (Gal4DB-SAF1), p2874 (Gal4DB-SAF1ΔF-box), p3220 (Gal4DB-saf1-1), p3222 (Gal4DB-saf1-6), p3270 (Gal4AD-AAH1), p3202 (Gal4AD-aaah-1-2), p3138 (Gal4AD-aaah-1-3), p3145 (Gal4AD-aaah-1-4), and p3214 (Gal4AD-aaah-1-6), p3203 (Gal4AD-aaah-1-7), and p2741 (Gal4AD-SKP1).

The tet-ycSacF1 plasmids were constructed as follows: SAF1 full length (wild-type and mutants) was amplified by PCR with oligonucleotides 1137 and 681. Resulting BglII/NsiI fragments were cloned in BamHI/PstI p2717 derived from the tet plasmid pCM189 (see above). The SAF1ΔF-box fragment was amplified by PCR using oligonucleotides 1229 and 681 and was cloned in p2717 similarly to SAF1 full length.

In order to express SAF1 driven by the GAL promoter, full-length SAF1 was amplified by PCR with oligonucleotides 680 and 681, digested by BglII/NsiI, and cloned at the BglII/PstI sites of a derivative of pRS305 2μ (18) carrying the GAL1 promoter. The resulting GAL-SAF1 plasmid was named p2356.

Screen for AAH1 Mutants Deregulated in Stationary Phase—This screen consisted in the selection of Aah1p alleles, allowing a high Aah1p β-galactosidase activity in stationary phase. A PCR mutagenesis was carried out on the AAH1 gene with oligonucleotides 102 and 450. The PCR fragment was then digested with Stul enzyme to eliminate the AAH1 promoter. Meanwhile, the p3093 plasmid carrying the AAH1-lacZ fusion was digested with XbaI and Sall in order to eliminate most of the AAH1 coding region. The PCR fragment and the digested plasmid were co-transformed in a wild type strain, and yeast cells carrying a recombined plasmid were selected in a medium selective for the plasmid (SDcasaWA). Transformants were then replicated in duplicate on SDCasaWA solid medium. One plate was conserved, and the other was tested for β-galactosidase activity by “agar overlay” as described previously (9). Plasmids from yeast colonies showing a high β-galactosidase activity were extracted and further characterized. The mutations were finally identified by sequencing AAH1 open reading frame.

Selection of Point Mutations in the SAF1 Gene—The BY4742 wild-type strain transformed with a plasmid expressing an AAH1-lacZ fusion (9) was treated with UV (70% survival). Colonies were then grown for 2 days on SDcasaWA medium, and β-galactosidase activity was revealed by agar overlay as described in Ref. 9. Putative mutants showing a blue color, revealing a high expression of AAH1-lacZ in stationary phase, were subcloned and assayed for β-galactosidase activity. Among 15,000 colonies, 20 deregulated mutants were selected and mated to a Δsaf1 strain. Expression of the AAH1-lacZ fusion was measured in the resulting diploids and found to be high in 16 of 20 diploids, indicating that these mutations do not complement the saf1 deletion and are therefore likely to affect the SAF1 gene.

Western Blot Analyses—Western blots were done as previously reported (9).

Adenine Deaminase Activity—Adenine deaminase assays were done as previously reported (9).

Protein Two-dimensional Gel Analysis—Yeast cells expressing either a control vector or the tet-ycSacF1 plasmid were grown to OD 1. Radiolabeled [35S]methionine (1000 Ci/mmol; MP Biomedicals) was added to a final concentration of 0.6 μCi (600 μCi/ml); after 4 min, unlabeled methionine was added to 6 μCi (final concentration); and then cells were harvested at time points 0, 15, 30, and 45 min. For each time point, the same volume of culture, corresponding to ~3 × 10⁷ cells, was removed. Protein extraction and two-dimensional gel analysis were performed as described (19). Gel images were processed using the ImageMaster 2D Elite version 4.01 software (Amersham Biosciences). Drawings of the proteins were generated with Bopscript (20).

RESULTS

The F-box Protein Saf1p Interacts with both Skp1p and Aah1p—As mentioned above, the Saf1p protein carries in its N-terminal part a potential F-box domain. F-box proteins, when they participate to a SCF complex, serve as a link between Skp1p, a constant subunit of SCF, and the target protein. Clearly, Saf1p interacted with both Skp1p and Aah1p in a two-hybrid assay (Fig. 1). As expected, interaction with Skp1p was specifically abolished when the F-box domain was removed from the construct, whereas the Saf1pΔF-box construct was still able to
interact with Aah1p (Fig. 1). Finally, we did not detect any direct interaction between Aah1p and Skp1p (Fig. 1). We conclude that the F-box domain of Saf1p is required for proper interaction with Skp1p but not with Aah1p and that Saf1p serves as a link between Aah1p and Skp1p.

The F-box Domain of Saf1p Is Required for Degradation of Aah1p—To establish the functional role of the F-box domain of Saf1p, we expressed wild-type and F-box-deleted forms of SAF1 on a plasmid driven by a heterologous strong promoter. As expected, the Saf1pΔF-box mutant was unable to destabilize Aah1p in early stationary phase (Fig. 2A). Interestingly, overexpression of Saf1pΔF-box in a wild-type strain resulted in slight but reproducible stabilization of Aah1p in early stationary phase (PD lane in Fig. 2B). This result suggests that the Saf1pΔF-box, which cannot interact with the SCF subunit Skp1p but still interacts with Aah1p (Fig. 1), can somehow protect Aah1p from degradation. Such a dominant negative effect of the F-box subunit lacking its F-box domain has been reported previously (21) and argues for Saf1p being a bona fide F-box subunit. In these experiments, we noticed that overexpression of Saf1p resulted in destabilization of Aah1p in exponential phase (Fig. 2, A and B), whereas deletion of SAF1 had no effect on Aah1p during exponential growth (Fig. 2A) (9). This effect was confirmed by expressing Saf1p under control of a strong galactose-inducible promoter. Shifting exponentially growing cells from glucose to galactose resulted in a significant decrease of Aah1p levels and adenine deaminase activity (Fig. 2C). Finally, by treating the cells with the protein synthesis inhibitor cycloheximide, we could establish that overexpression of Saf1p indeed resulted in a shorter half-life for Aah1p, whereas overexpression of the truncated version had no effect (Fig. 2D). From these experiments, we conclude that Saf1p,
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A

saf1-1

saf1-2

saf1-3

saf1-4

saf1-5

saf1-6

α-BGal

α-Aah1p

α-Ade13p

B

saf1-1

saf1-2

saf1-3

saf1-4

saf1-5

saf1-6

α-Myc

α-Aah1p

α-Ade13p

C

2D prediction

Saf1p

Cdc4p

3D structure

consensus

MSKSERKQKKFIG...LKLNRDIDIT

LxEIPFLXKLXISxLYDLXXLLX

2D prediction

Saf1p

Cdc4p

3D structure

consensus

xVCKRWYxLIDd

dxLWRxL

D

GalaAD fusion

GalaAD fusion

empty

empty

empty

empty

empty

empty

AAH1

SKP1

AAH1

SKP1

AAH1

SKP1

AAH1

SKP1

empty

empty

empty

empty

E

saf1-1

saf1-2

saf1-3

saf1-4

saf1-5

saf1-6

α-Myc

α-Aah1p

α-Ade13p

FIGURE 3. Characterization of SAF1 mutants. A, SAF1 mutants stabilize Aah1p during postdiauxic growth. The saf1-1 to saf1-6 mutants were transformed with a plasmid expressing an AAH1-lacZ fusion and grown for 2 days in SCasawa medium. Proteins were extracted and analyzed by Western blot using anti-β-gal, anti-Aah1p, or anti-Ade13p polyclonal antibodies. B, stability of SAF1 mutants. The indicated saf1 alleles placed under control of the constitutive tet promoter were expressed in the Y1624 δsaf1 strain. Samples were collected after 2 days (postdiauxic). Proteins were extracted and analyzed by Western blot using anti-Myc, anti-Aah1p, and anti-Ade13p antibodies. C, alignment of Saf1p (accession number P38352) and Cdc4p F-box (P07834) sequences. Secondary structure prediction of Saf1p (Fig. 3A) was modeled from the yeast N-terminal F-box domain of Saf1p (residues 10–65) and Cdc4p (PDB code 1nex). The Leu14 and Leu15 side chains drawn in black ribbons belong to the interface.

dashed line

dotted line

when overexpressed, can interact with Aah1p during exponential growth and is sufficient to trigger its degradation.

Saf1 Mutants Define Two Domains—To further delineate the important functional regions in the Saf1p protein, we searched for point mutations in SAF1 that abolish degradation of Aah1p in the early stationary phase. Among 20 yeast mutants selected for their ability to express an AAH1-lacZ fusion in early stationary phase (for details, see “Experimental Procedures”), functional complementation analysis revealed that 16 mutants were unable to complement the deletion of SAF1 and were therefore likely to be saf1 alleles. Sequencing of the SAF1 gene in these mutants revealed six premature STOP codons, four frameshifts, and six missense mutations. The six missense alleles were named saf1-1 to saf1-6. Stabilization of the fusion protein and the endogenous Aah1p in the original missense mutants is shown in Fig. 3A. From these data, it is clear that all six alleles strongly affect Aah1p degradation. However, when these mutant forms of SAF1 were expressed under control of the tet promoter, only two of them showed wild-type levels of Saf1p, whereas the four others appeared unstable (Fig. 3B).

Of note, Saf1p is naturally very poorly expressed, and to be detected it has to be placed under the control of a heterologous promoter. Under these conditions, Saf1p migrates as a double band for unknown reasons. Intriguingly, the four unstable mutants (saf1-1 (G372V), saf1-2 (S387T), saf1-3 (L580S), and saf1-4 (R263K/R264K)) affect residues in the C-terminal part of Saf1p. The instability of these mutant proteins was independent of SCF and proteasome (data not shown).

The two other mutants (saf1-5 (L24P) and saf1-6 (L35P)) affected conserved residues in the F-box domain of Saf1p (Fig. 3C) and, as expected, were unable to interact with Skp1p while they were still interacting with Aah1p (Fig. 3D).

Using both sequence and secondary structure prediction alignments (Fig. 3C), the yeast N-terminal F-box domain of Saf1p in complex with Skp1p was modeled from the yeast Cdc4p-Skp1p-CPD complex structure (22) (Fig. 3E). In our structural model, the L24P and L35P mutations affect two interacting conserved residues involved in the hydrophobic interface with Skp1p and are therefore likely to affect the F-box domain structure.

Mutations Stabilizing Aah1p Are Clustered in Distinct Domains—To identify the residues required for Saf1p-dependent degradation of Aah1p, we searched for mutations in the AAH1 gene that result in stabilization of Aah1p during postdiauxic growth. For this purpose, we generated a collection of plasmids carrying an AAH1-lacZ fusion in the original missense mutations. The six missense alleles were obtained (substitutions were as follows: aah1-1, I69T; aah1-3, N72K; aah1-4, D219G; aah1-6, E237V; aah1-7,
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When these mutants were tested for interaction with Saf1p in a two-hybrid assay, one mutant (K329E) was clearly still interacting with Saf1p, whereas the remaining four mutants showed very weak interaction with Saf1p (Fig. 4B). Strikingly, the aah1-7 mutant, while still interacting with Saf1p, was not affected by overexpression of Saf1p (Fig. 4C). On the contrary, aah1-3, aah1-6 (Fig. 4C), and aah1-2, aah1-4 (data not shown), were destabilized by overexpression of Saf1p.

To get a better understanding of how these mutations might affect Aah1p, a modeling of Aah1p was done from *Mus musculus* (23) and *Plasmodium yoelii* (24) adenosine deaminase (ADA) x-ray structures. This was possible due to high conservation in the adenine and adenosine deaminase superfamily (25). The model is a (β/α)8 barrel from which the Lys329 residue is highly solvent accessible and protrudes from a loop at the bottom of the barrel (Fig. 4D). Clearly, the four mutations affecting Aah1p interactions with Saf1p are located on two distinct areas on the surface of the protein. Ile69 and Asn72 are together on the top, whereas the two acid residues Asp219 and Glu237 belong to the side of the barrel (Fig. 4D).

We propose that in these four mutants, although interaction with Saf1p is diminished (Fig. 4C), overexpression of Saf1p can somehow compensate for this lower interaction and restore some degradation (Fig. 4B). In the case of the K329E mutant, interaction with Saf1p (Fig. 4C) is not sufficient to trigger degradation even when Saf1p is overexpressed (Fig. 4B). Thus, the mutated lysine residue appears crucial for degradation of Aah1p independently of its interaction with Saf1p. Since lysine are the most frequently ubiquitylated residues, it is tempting to speculate that lysine at position 329 could be a major ubiquitylation site in Aah1p.

How Many Targets for Saf1p?—

At this point, it is not clear whether Saf1p interacts with a limited number of specific target proteins or participates in a more general quality control process leading to degradation of multiple proteins. To address the issue of Saf1p target specificity, we took advantage of the K329E. Western blot analysis confirmed that the Aah1p-β-galactosidase fusion was indeed stabilized in these mutants (Fig. 4A). Importantly, these aah1 mutants were still able to complement an aah1 deletion, indicating that they still have adenine deaminase activity and are therefore likely to be correctly folded (data not shown).

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these results, we conclude that overexpression of Saf1p does not lead to major changes in the yeast proteome but rather specifically affects a small number of target proteins.

**DISCUSSION**

In this paper, we have characterized a new F-box protein named Saf1p. Among 21 yeast F-box proteins, only a few of them were clearly found to participate to a SCF complex. Our data and other results indicate that Saf1p is a *bona fide* SCF subunit. Indeed, interactome studies have shown that Saf1p copurifies with SCF core subunits (Skp1p (14) and Cdc53p (15)) and with the SCF associated-E2 Cdc34p (13). Additionally, Saf1p contains a consensual F-box domain (8), which is critical for its function. We have shown that deletion of this domain or point mutations in its conserved residues abolish interaction of Saf1p with Skp1p and result in stabilization of Aah1p. Finally, we observed that the F-box-deleted mutant shows a dominant negative effect on Aah1p degradation. Since Saf1pΔF-box can still interact with its target (Fig. 1), it could be that this interaction might protect it from degradation by the endogenous Saf1p. Such an observation has been reported for several other F-box subunits, such as βTrCP (21). All together, our data strongly argue for Saf1p being the F-box subunit of a new yeast SCF complex.

An interesting particularity of Saf1p is that it does not contain a leucine-rich repeat or a WD40 domain but instead carries a RCC1 repeat. RCC repeats were first characterized in RCC1, a Ran-interacting protein (28). A search for an RCC1-like domain based on the consensus sequence suggests that the C-terminal domain of Saf1p (residues 95–637) probably adopts a seven-blade β-propeller fold similar to the previously described RCC1-like domain structure (Fig. 6). Interestingly, an insert of about 50 residues (residues 240–292) with four predicted strands and a weak similarity with the consensus could suggest the presence of an eighth blade. When placed on the putative structure, the G372V and S387F mutations appear buried and belong to strands A and C, respectively. These mutations could therefore affect the correct fold of the protein. The mutant L580S belongs to a conserved loop connecting C and D strands. Depending on the actual blade number, the double mutant R263K/R264K could be located either in an equivalent loop or in a small peripheral loop. Strikingly, all of the point mutations in the RCC1-like domain of Saf1p resulted in strong instability, although the reason for such an instability remains unclear. Only a few proteins associating with an RCC1-like domain folded as a seven-bladed β-propeller (29) with an F-box domain have been found, and Saf1p is the first example of such a protein shown to interact with Skp1p and contribute to ubiquitin proteasome system-dependent protein degradation. Although it is likely that Saf1p interacts with Aah1p through the RCC1 like domain, the precise mode of interaction is not known. Interestingly, it has been found that bovine ADA, which shares homology with Aah1p, and human dipeptidyl peptidase IV ectodomain, which adopts an eight-bladed β-propeller fold, bind laterally through interaction between two helices of ADA and loops of two blades in the β-propeller (30).

To understand how Aah1p and Saf1p interact, we have isolated nondegradable Aah1p mutants. Five such mutants were characterized that affect residues lying on the surface of the predicted structure (Fig. 4D). It should be noticed that none of them affect phosphorylable residues. Four of the five mutants displayed weak interaction with Saf1p. Surprisingly, these four residues fall into two regions, which are about 40 Å apart on the three-dimensional structure (Fig. 4D). It is tempting to speculate that these domains directly contact Saf1p. The fifth mutant behaved very differently. First, it did not abolish interaction with Saf1p. Second, it was not degraded when Saf1p was overexpressed. Finally, it protected endogenous Aah1p from degradation (Fig. 4A). Together, these data argue for the mutated

**FIGURE 5.** Effect of SAF1 overexpression on the yeast proteome, A, the Y1731 Δsaf1 yeast strain containing either the empty vector or the tet-SAF1 plasmid was grown in minimal medium (SD) to exponential phase and labeled with 35S methionine for 4 min. After a 45-min chase, proteins were extracted and analyzed by Western blot using anti-GFP, anti-Aah1p, and anti-Ade13p antibodies. B, strains expressing ARG1 or URA7 tagged with GFP were transformed with the tet vector or the tet-SAF1 plasmid. Samples were collected during exponential phase. Proteins were extracted and analyzed by Western blot using anti-Myc and anti-Ade13p antibodies. WT, wild type.

**FIGURE 6.** SAF1 RCC1-like domain repeats alignment. The upper line symbolizes the structure of such a repeat, where arrows represent β-strands commonly named from A to D. The mutations are boxed, and the residues predicted to be in strand are shown in *boldface italic* type.
residue to affect Aah1p degradation but not its ability to interact with the SCF. Strikingly, this mutation affects a highly accessible lysine residue, which could thus be a major ubiquiti

An important specificity of the SCF-dependent degradation of Aah1p is that it takes place during entry into quiescence. The transition from proliferation to quiescence is associated with multiple physiological changes allowing yeast cells to adapt to starvation. Among those changes, degradation of specific proteins might be critical, and Saf1p could contribute to this adaptation. This important issue is technically difficult to assess on quiescent cells. To address the question of the role of Saf1p on the yeast proteome, we took advantage of Saf1p overexpression during exponential growth. Under those conditions, degradation of Aah1p by Saf1p efficiently takes place, suggesting that this could be the case for other Saf1p targets. Our protein two-dimensional gel analysis revealed that Saf1p overexpression affects a very small number of yeast proteins, and for most of them, such as Arg1p, the effect of Saf1p is likely to be indirect. From this experiment, Saf1p appears to be very specific for Aah1p.

Since overexpression of Saf1p is sufficient to trigger degradation of Aah1p in exponential growth, we believe that no quiescence-specific modification of Aah1p is required prior to its Saf1p-dependent degradation. Although in most cases in yeast, SCF substrates are phosphorylated before being degraded, approaches such as two-dimensional gel analysis or mass spectrometry did not reveal any post-translational modification of Aah1p. This led us to think that the signal responsible for the degradation of Aah1p by Saf1p is not a phosphorylation. Our results rather suggest that the Saf1p/Aah1p ratio is critical for regulation of Aah1p stability.

All together, our data establish that Saf1p participates in active degradation of Aah1p during postdiauxic growth. Very little is known about protein degradation during entry, maintenance, and exit from quiescence. Considering the major role played by protein degradation during proliferation, it might be expected that proteolysis will also play important roles during quiescence.

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