The F-box domain, so called after a conserved domain found in human cyclin F (5), was described in 1996 (6) after first being denoted a conserved N-terminal domain found in a subset of proteins (110). The F-box hypothesis was introduced shortly after (162, 185) and holds that F-box-containing proteins (henceforth F-box proteins) act as scavengers in the cell, collecting “junk” proteins to deliver to a “waste processor,” called the SCF complex, to which they dock through their F-box domain. In the SCF complex, the junk proteins are marked with ubiquitin for “incineration” in the proteasome. F-box proteins do not act indiscriminately but recruit specific, often modified proteins to the SCF complex and in this way regulate the level of certain proteins in a cell. F-box proteins are found in all eukaryotes and display a large variety of functions. In fungi they are, for example, involved in control of the cell division cycle, glucose sensing, mitochondrial connectivity, and control of the circadian clock.

F-box proteins are commonly identified by the presence of a stretch of primary sequence that matches the consensus for an F-box domain (Fig. 1). However, it can be questioned whether just the occurrence of an F-box domain in a protein sequence is sufficient to assume compliance with the F-box hypothesis. The F-box hypothesis is based on the assumption that an F-box domain mediates assembly into an SCF complex through binding to the Skp1 subunit (Fig. 2). The SCF complex consists of Skp1 (suppressor of kinetochore protein mutant) (34), Cul1 (Cullin) (135), Rbx1 (ring-box protein) (86), and an F-box protein and catalyzes (like other E3 ligases), in cooperation with the E1 and E2 enzymes, the transfer of the small protein ubiquitin to the target protein (108, 162). Among fungi, the methods of regulation of the SCF complexes and hence the methods of regulation of the F-box proteins in these complexes appear to differ. SCF complexes are likely activated and regulated through a recycling mechanism (35), which involves three main contributors: the neddylyator protein DCN1, responsible for the transfer of Nedd8 to Cul1 (112, 133, 155, 159, 174, 211); the deneddylyator CSN (COP9 signalosome) (136, 137, 146, 179, 200) (reviewed in references 159, 201, and 208); and the CAND1 protein (127, 215), which binds to deneddylylated Cul1 and competes out the Skp1–F-box complex from the core of the SCF complex. A new round of neddylation removes CAND1 and thereby creates binding space for a new Skp1–F-box complex. In budding yeast (Saccharomyces cerevisiae), deletion mutants for Nedd8 and CSN5, the CSN subunit responsible for the deneddylation reaction, are both viable (36, 115, 124). This means that, although the components of the SCF recycling mechanism are present, this process is not required for survival. A second difference in budding yeast in comparison to other fungi is that is does not have the CAND1 protein, adding to the notion that in budding yeast recycling acts differently. In fission yeast (Schizosaccharomyces pombe), CAND1 is present, and Nedd8 is required for survival (155), but the CSN5 subunit is not (145, 216). Apparently, in fission yeast, the neddylation reaction is required for proper SCF function, but deneddylation is not, suggesting that in fission yeast an alternative deneddylation may be present. In Neurospora crassa, a deletion mutant for subunit 2 of CSN, Δcsn-2, is viable but lacks a normal circadian rhythm and conidiation (64), while in Aspergillus nidulans, four CSN mutants, including one for subunit 5 (Δcsn-i), all lack fruiting body development (25, 26). Together, these data suggest that, in filamentous fungi, proper recycling of the SCF is strictly required only for certain developmental processes, in accordance with the requirements of CSN in development in more-complex, multicellular organisms (reviewed in reference 179).

Some F-box proteins appear to function without binding to Skp1, suggesting that not all F-box proteins take part in an SCF complex. This also means that not all proteins interacting with an F-box protein will be ubiquitinated and proteosomally degraded. In another deviation from the F-box hypothesis, some F-box protein/Skp1 complexes do not seem to be involved in ubiquitination. Furthermore, even when an F-box domain mediates assembly into an SCF complex, the result may be self-ubiquitination rather than fulfillment of a scavenger function.

Since 1996, several review articles covering the emerging theme of ubiquitin-mediated protein degradation and the widespread occurrence of F-box proteins have been published (38, 68, 71, 72, 92, 118, 198, 204, 205). Here, we discuss fungal F-box proteins, including their targets (if identified), and when possible, classify these F-box proteins according to degree of compliance with the F-box hypothesis. Most literature on fungal F-box proteins covers those found in budding yeast and, to a lesser extent, fission yeast, but important findings have also been reported for filamentous ascomycetes. In Table 1, fungal F-box proteins described in the literature are listed according to their main cellular function. The distinctly related budding and fission yeasts share 10 (likely) orthologous F-box proteins. Budding yeast contains an additional 11 F-box proteins and...
fission yeast 7 (89). Cdc4, Grr1, and Met30 from budding yeast and their counterparts in other fungi are the most studied fungal F-box proteins and are conserved throughout the fungal kingdom. In total, 31 F-box proteins are discussed, exclusively from ascomycetes: the “model” fungi *S. cerevisiae*, *S. pombe*, *Kluyveromyces lactis*, *A. nidulans*, *Hypocrea jecorina*, and *N. crassa* and the pathogenic fungi *Candida albicans*, *Fusarium graminearum*, *Fusarium oxysporum*, and *Magnaporthe grisea*.

**F-BOX PROTEINS COMPLYING WITH THE F-BOX HYPOTHESIS**

To date, the best-described fungal F-box proteins comply with the F-box hypothesis; the targets of these F-box proteins are commonly first phosphorylated before being recognized and ubiquitinated by the SCF complex and finally degraded by the proteasome. In a study (113) in which interactors of Skp1 and the SCF complex were identified in budding yeast, 13 F-box proteins were found to bind Skp1, and these 13 could all be copurified with an SCF complex. These are Cdc4, Ctf13, Dia2, Grr1, Hrt3, Mdm30, Rey1, Ufo1, and five uncharacterized F-box proteins. In another study investigating the binding partners of Skp1 and Cul1 (181), Met30 and Saf1 were also found to bind Skp1, and two more uncharacterized F-box proteins were found to bind Skp1 and/or Cul1. In the study mentioned above (113), autoubiquitination of F-box proteins was also investigated using two different E2 enzymes, Cdc34 and Ubc4. Twelve out of the 13 F-box proteins showed self-ubiquitination; only Grr1 was found not to be ubiquitinated, and Dia2 and Mdm30 showed very little ubiquitination. Also, it was demonstrated that these F-box proteins were differentially ubiquitinated by the two different E2 enzymes and that different numbers of ubiquitin molecules were attached to the F-box proteins. In other reports, ubiquitination and degradation of Cdc4, Met30, and Grr1 were demonstrated (55, 217). It is still an open question whether F-box proteins are ubiquitinated and degraded together with their targets in each degradation round. Another possibility is that they are recycled after recruiting their targets and ubiquitinated and degraded only when unbound to a target protein.

In a study with fission yeast, 11 F-box proteins investigated (Pop1/2, Pof1, Pof3, Pof5, Pof7, Pof8, Pof9, Pof10, Pof12, Pof13, and Fbh1/Pof15) (120) could all bind to Skp1. The interactions were further studied with a temperature-sensitive mutant of Skp1 with point mutations in the Skp1–F-box interaction core. Only the binding to Pof1, Pof3, and Pof10 was weaker with this mutant than with the wild-type Skp1. The effect of this weakened binding on the function of the individual F-box protein is not known, and targets of most of these fission F-box proteins remain to be identified.

**C-TERMINAL PROTEIN-PROTEIN INTERACTION DOMAINS**

Most of the fungal F-box proteins that comply with the F-box hypothesis have a recognizable C-terminal protein-protein interaction domain (Table 1). Four F-box proteins carry a WD40 domain: Cdc4 (and its orthologs), Fwd1, Ufo1, and Met30 (and its orthologs). WD40 is a domain of about 40 amino acids often terminating with the two amino acids Trp and Asp (WD) and forms a beta-propeller structure (150). Grr1 and its orthologs carry an LRR (leucine-rich repeat) domain (85), a repeat of about 25 amino acids forming a nonglobular, crescent-shaped structure. Saf1 carries an RCC1 (regulator of chromosome condensation 1) repeat (168), another domain forming a beta-propeller structure involved in protein-protein interactions. Of the F-box proteins that comply with the F-box hypothesis, only Mdm30 does not contain any known protein-protein interaction motif, and it is unknown how it interacts with its targets Fzo1, Mdm34, and Gal4c. The presence of a recognizable protein-protein interaction domain might be an indication that an F-box protein complies with the F-box hypothesis. One of the uncharacterized budding yeast F-box proteins (Ylr352w) that binds Skp1 and Cul1 also contains an LRR domain, suggesting that this F-box protein might also comply with the F-box hypothesis.

For Cdc4, Dia2, Grr1, Met30, Mdm30, Saf1, and Ufo1 of budding yeast, one or more targets are known, and most of these targets are degraded via the SCF complex. This suggests that at least these seven F-box proteins completely fulfill the F-box hypothesis. For Cdc4, Dia2, Grr1, and Met30, homologs in other
fungal species have been found and characterized, in some cases together with their targets. The degree of conservation of the functions of these F-box proteins between the different species can now be assessed by comparing the different phenotypes of deletion mutants and the conservation of targets.

Cdc4: AN F-BOX PROTEIN CONTROLLING THE CELL DIVISION CYCLE, MORPHOGENESIS, NUTRIENT SENSING, AND CALCIUM SIGNALING

In *S. cerevisiae*, Cdc4 (cell division cycle 4) regulates multiple processes in the cell by recruiting various proteins for degradation (Fig. 3), and especially in the cell cycle process, Cdc4 plays an important role by recruiting different cell cycle inhibitors, a transcription factor, a cyclin, and a replication factor for degradation. The *CDC4* gene was first identified from a yeast mutant unable to initiate DNA replication during transition from the G1 to the S phase (213).

### Table 1. Fungal F-box proteins described in the literature and discussed in this review

| Cellular function(s)                                      | F-box protein | Fungal species | Additional motif(s) | Target(s) | Skp1 binding | SCF assembly |
|-----------------------------------------------------------|---------------|----------------|---------------------|-----------|--------------|--------------|
| Cell division cycle, morphological switch, and nutrient and calcium sensing | Cdc4          | *S. cerevisiae* | WD40                | Sic1, Swi5, Far1, Cdc6, Cbl6, b | Yes | Yes |
| DNA replication                                           | Pop1          | *S. pombe*     | WD40                | Rum1, Cdc18, Cig2 | Yes | Yes |
|                                                         | Pop2          | *S. pombe*     | WD40                | Rum1, Cdc18, Cig2 | Yes | Yes |
|                                                         | Cdc4          | *C. albicans*  | WD40                | Sol1, Far1 | ? | ? |
| Cell division cycle, glucose uptake, growth on nonglucose carbon sources, and retrograde signaling | Dia2          | *S. cerevisiae* | TPR + LRR           | Tec1 | Yes | Yes |
|                                                         | Po3           | *S. pombe*     | TPR + LRR           | ? | | |
| Methylmercury resistance                                  | Grr1          | *S. cerevisiae* | LRR                 | Cln1/2, Gic2, Ime2, Hsf1, Std1, Mtb1, Gis4, Pfk27, Tye7, Gala/b, Msk1 | Yes | Yes |
| Mitochondrial function                                    | Hrt3          | *S. cerevisiae* | ?                   | Fzo1, Gal4c, Mdm34 | Yes | Yes |
| Quiescence                                                | Mdm30         | *S. cerevisiae* | ?                   | Fzo1, Gal4c, Mdm34 | Yes | Yes |
| Genome stability                                          | Saf1          | *S. cerevisiae* | RCC1                | Aah1, Ura7? | Yes | Yes |
| Circadian clock                                           | Ufo1          | *S. cerevisiae* | WD40 + UIM          | Ho, Rad30 | Yes | Yes |
| Sulfur metabolism                                         | Fwd1          | *N. crassa*    | WD40                | Frq | Yes | Yes |
| DNA repair                                                | Met30         | *S. cerevisiae* | WD40                | Met4 | Yes | Yes |
| Root infection                                             | Pof1          | *S. pombe*     | WD40                | Zip1 | Yes | ? |
| Peroxide resistance                                       | Scon2         | *N. crassa*    | WD40                | Cys3 | Yes | ? |
| Kinetochoore assembly                                     | SconB         | *A. nidulans*  | WD40                | MetR | Yes | ? |
| Membrane trafficking                                      | Lim1          | *H. jecorina*  | WD40                | ? | | ? |
| DNA repair                                                | Fbh1          | *S. pombe*     | Helicase            | ? | Yes | ? |
| Peroxide resistance                                       | Frl1          | *F. oxysporum* | ?                   | ? | Yes | ? |
| Kinetochoore assembly                                     | Pof14         | *S. pombe*     | ?                   | Erg9 (inhibited, not degraded) | Yes | ? |
| Membrane trafficking                                      | Ctf13         | *S. cerevisiae* | ?                   | ? | Yes | No |
| DNA repair                                                | Rey1          | *S. cerevisiae* | SEC10 + CAAXa       | Snc1, Kex2 (both recycled, not degraded) | Yes | Yes |
| Mitochondrial function                                    | Pof6          | *S. pombe*     | SEC10 + CAAX        | Sip1 (not recycled or degraded) | Yes | No |
| Exit from mitosis                                         | Mtb1          | *S. cerevisiae* | WD40                | ? | | ? |
| Mitochondrial function                                    | Amn1          | *S. cerevisiae* | LRR                 | Not in insect cells | | |

*SEC10, a domain of approximately 650 residues long found in proteins of the eukaryotic exocyst complex, which specifically affects the synthesis and delivery of secretory and basolateral plasma membrane proteins (126); CAAX motif, a C-terminal prenylation motif (where C is the prenylated cysteine, A is usually aliphatic, and X may be many different residues) (3).*

*b, unknown or uncertain.*
target, Cdc28, and another kinase, Pho85 (153, 209). Phosphorylated Sic1 is recognized by Cdc4 and marked for degradation by polyubiquitination (49, 197). Recently, the transcription factor Swi5, which activates transcription of \( \text{SIC1} \) (93), was also found to be degraded through interaction with Cdc4. Degradation of the transcription factor Swi5 via Cdc4 during the early G1 phase allows efficient removal of Sic1 in the late G1 phase (93). This means that Cdc4 is responsible for Sic1 removal by degrading both the protein itself and the activator of its transcription. Through Sic1 degradation, Cdc4 also regulates expression of \( \text{OCH1} \), a gene encoding alpha-1,6-mannosyltransferase, suggesting that Cdc4 is involved in regulation of cell wall composition during the cell cycle (40). Far1 (factor arrest 1) is also phosphorylated by the Cdc28 kinase complex and then recognized by Cdc4 (67). Degradation of Far1 is nucleus specific, suggesting that Cdc4 may act specifically in the nucleus (20). Cdc6 (cell division cycle 6) is a DNA replication initiation factor that is degraded via Cdc4 in the late G1/early S phase as well as in the G2/M phase. Phosphorylation of Cdc6 to ensure recognition by Cdc4 at both time points requires the Cdc28 kinase. Cdc6 degradations at these two time points differ in the degradation rates and in the cyclins that take part in the Cdc28 kinase complex (42). The first difference is probably due to the fact that degradation depends on two different interaction domains in Cdc4 (163). It has also been suggested that Cdc4 has a role in the degradation of Clb6 (79), a cyclin that triggers, together with Clb5, the progression from G1 into S phase. Clb6 is rapidly degraded at the end of the S phase and stabilized in \( cdc4 \) mutants. Moreover, its sequence harbors Cdc4 degron motifs. Direct interaction, however, has not yet been demonstrated. Cdc4 also targets another F-box protein involved in kinetochore assembly and function, Ctf13 (see Ctf13: a Kinetochore Assembly F-Box Protein). \( \text{S. pombe} \) contains two homologs of \( \text{CDC4} \), called \( \text{POP1} \) and \( \text{POP2} \) (polyploidy 1 and 2) (102). \( \text{POP2} \) was also discovered in another study, where it was called \( \text{SUD1} \) (stops unwanted diploidy 1) (80). Pop1 and Pop2 are structurally related but function independently from each other. The phenotypes of both deletion mutants are comparable in that both display polyploidization, but neither protein cannot fully take over the function of the other since overexpression of \( \text{POP1} \) or \( \text{POP2} \) could not suppress the defects caused by loss of the other gene (101). The polyploidization phenotype is caused by the accumulation of the cyclin-dependent kinase (CDK) inhibitor Rum1 (80, 102) (homolog of budding yeast Sic1) and S-phase regulator Cdc18 (80, 207) (homolog of budding yeast Cdc6). These two proteins are normally degraded during defined stages of the cell cycle, but in the \( \text{pop1} \) and \( \text{pop2} \) mutants and the \( \text{pop1 pop2} \) double mutant, the levels of these proteins are high compared to those in the wild type. The accumulation and polyubiquitination of Rum1 and Cdc18 in a proteasome-deficient mutant support the notion that Pop1 and Pop2 recruit these two proteins for degradation. Also, a direct interaction between Pop1 and Cdc18 was found using coimmunoprecipitation. Pop1 and Pop2 can form homodimers and heterodimers, resulting in three alternative SCF complexes, \( \text{SCF}^{\text{POP1/POP1}} \), \( \text{SCF}^{\text{POP1/POP2}} \), and \( \text{SCF}^{\text{POP2/POP2}} \), but the different molecular functions of these three complexes remain unclear (101). The S-phase cyclin Cig2 (homolog of budding yeast cyclin Cln2) is also stabilized in \( \text{pop1} \) and \( \text{pop2} \) deletion mutants, suggesting a
role for both proteins in Cig2 degradation (210). Coimmuno-precipitation revealed that Pop1 and Cig2 interact in the cell independently from Pop2 and that this interaction requires phosphorylation of Cig2 and at least the central 93 amino acid residues (residues 181 to 273). In fungi, Pop1 and Pop2 are currently the only examples of two homologous F-box proteins functioning in the same degradation pathway. The advantage of having two F-box proteins for the same function might be that degradation of certain proteins can be fine-tuned and regulated at an extra level. The methods of degradation of Cig2 are remarkably different between budding and fission yeasts, since Cin2 is degraded by Grr1 in budding yeast (see Grr1 and the Cell Cycle) but its fission yeast homolog Cig2 is degraded by Pop1 (the role of Pop2 in degradation of Cig2 is still unclear).

Cdc4 AND PSEUDOHYPHAL GROWTH

Cdc4 is also involved in degradation of transcription factors that regulate pseudohyphal growth. The transcription factor Tec1 (transposon enhancement control 1) is phosphorylated by mitogen-activated protein kinase Fus3 and then recognized by Cdc4, promoting its degradation (32). Tec1 is responsible for the onset of filamentous growth in S. cerevisiae. This morphological switch is made when nutrient availability is low, but the switch needs to revert after pheromone sensing to allow mating. Whether Cdc4 is solely responsible for degradation of Tec1 is disputable, because it has been shown that another F-box protein, Dia2, is also able to induce degradation of Tec1 after pheromone sensing (9). In the human pathogen C. albicans, Cdc4 plays a role in the switch from hyphal to yeastlike growth, as demonstrated by deletion of CDC4, which results in constitutive hyphal growth (4). This dimorphic switch is important for pathogenicity, as the hyphal form contributes to the cell division cycle. The transcription factor Hac1 (homologous to Atf/Creb1) from poor to rich medium, Gcn4 is degraded via Cdc4 when ER stress is removed (158). Gcn4 is also required for the activation of transcription of amino acid and purin biosynthesis genes during starvation. After the switch from poor to rich medium, Gcn4 is degraded via Cdc4, likely after being phosphorylated by Pho85 (139). The degree of conservation of this Cdc4 function is unclear because the respective targets have not been studied in this respect for other fungi.

Cdc4 AND GROWTH RESPONSES AFTER NUTRIENT SENSING

The transcription factors Hac1 (homologous to Atf/Creb1) and Gcn4 (general control non-repressible 4) are both involved in the activation of unfolded-protein-responsive genes whose products assist in the folding of proteins in the endoplasmic reticulum (ER) lumen upon ER stress. Hac1, a basic leucine zipper transcription factor, is degraded in the nucleus via Cdc4 when ER stress is removed (158). Gcn4 is also required for the activation of transcription of amino acid and purin biosynthesis genes during starvation. Cdc4 AND CALCIUM SENSING

Cdc4 acts in calcium homeostasis by targeting Rcn1 for destruction upon calcium availability (94). Rcn1 (regulator of calcineurin 1) inhibits calcineurin, a phosphatase that mediates cellular responses after stress and Ca2+ uptake (41). Calcineurin mediates its own inhibition by a negative-feedback loop: it stimulates the expression of Rcn1 and stabilizes Rcn1 by dephosphorylation. Phosphorylation of Rcn1 makes it recognizable for Cdc4 and marks it for degradation, allowing calcineurin to break out of its negative-feedback loop and increase its activity.

From the details described above, it is clear that between yeasts, Cdc4 is conserved in some functions, like the degradation of the CDK inhibitors Sic1 and Far1. Whether the function of Cdc4 in regulation of pseudohyphal growth is conserved between budding yeast and C. albicans is uncertain, since the target protein in this pathway in C. albicans has not yet been found. The involvement of Cdc4 in nutrient sensing and calcium signaling in filamentous fungi is unlikely, since deletion of CDC4 in these fungi has not been reported to lead to defects in these processes. A main difference between budding yeast and the other fungi is that in budding yeast the search for targets has been more intensive, for example, by use of yeast two-hybrid screens (93). Application of such screens to other fungi would be helpful to more fully evaluate the conservation of targets between the different fungi.

Although no genetic studies of the Cdc4 homolog in N. crassa have been reported, this homolog was found to be targeted by a plant-defensive peptide. By use of a yeast two-hybrid screen, defensin 1 from Pisum sativum was shown to interact specifically with Cdc4 (131). Defensins are plant peptides exhibiting an antifungal activity as part of the plant innate immune system. Interaction with Cdc4 can explain how defensin 1 inhibits fungal growth, namely, by interference with the fungal cell cycle. By use of microscopy, it was observed that defensin 1, tagged with a fluorophore, was localized in the nuclei of N. crassa and Fusarium solani, suggesting that defensin 1 can enter the fungal cell and interfere in the nucleus with the cell division cycle.
Dia2: AN F-BOX PROTEIN INVOLVED IN DNA REPLICATION

The F-box protein Dia2 (digs into agar 2) plays a role in DNA replication in S. cerevisiae and is thereby also involved in cell growth and division. As mentioned earlier, Tec1, a transcription factor regulating filamentation genes, is degraded via Dia2 (9), probably in joint action with Cdc4 (32). These two F-box proteins are also both capable of degrading ectopically expressed human cyclin E (99), even though they bear different protein-protein interaction domains (LRR and WD40, respectively). Deletion of DIA2 in budding yeast causes a defect in invasive and pseudohyphal growth, slower growth at low temperatures, early entry into the S phase, and accumulation of DNA damage (98). These defects were also observed in DIA2Δ F-box mutants, suggesting that binding of Dia2 to Skp1 is necessary for these functions. Dia2 binds both early- and late-firing origins and is thereby involved in resetting the origin. It recruits the SCF complex to the replication origins, suggesting that a possible target becomes ubiquitinated there. Yra1, pre-recruits the SCF complex to the replication origins, firing origins and is thereby involved in resetting the origin. It is possible that binding of Dia2 to Skp1 is required for this action. In another study (18), deletion of DIA2 resulted in accumulation of DNA damage after the collapse of replication forks. This suggests that a possible target of Dia2 may be found among proteins that interfere with replication fork stability in certain genomic regions. Also, genetic interactions of Dia2 were found with DNA replication, repair, and checkpoint pathways (18). A role for Dia2 in DNA repair was suggested as well by the requirement of Dia2 for resistance to certain DNA-damaging compounds. These observations indicate that there are likely more targets or functions of Dia2 than only targeting Tec1 for degradation.

Pof3 from S. pombe is the ortholog of Dia2 from budding yeast. Deletion of POF3 results in multiple phenotypes: G2-phase delay (probably due to activation of the DNA damage checkpoints), hypersensitivity to UV radiation, telomere dysfunction, and chromosome instability and segregation defects (89). Targets of Pof3 are not yet known but may be found among proteins playing a role in chromatin structure and/or function. Fission yeast does not have a Tec1 ortholog, so targets different from Tec1 must be responsible for the phenotype of the deletion mutant. A protein that was found to interact with Pof3 is Mc1, ortholog of the budding yeast S-phase regulator Ctf4 (134). Mc1 is a protein essential for chromosome maintenance and contains WD40 repeats and SepB boxes (100, 206). A Δmc1 strain shows phenotypes similar to those of the Δpof3 mutant. Normally, Mc1 is not rapidly degraded in wild-type cells, and no ubiquitination of Mc1 could be demonstrated, suggesting that Mc1 is not a target of Pof3. This is also in accordance with the fact that the two deletion mutants share the same phenotype, something that would not be expected if Mc1 were a target of Pof3.

Dia2 is conserved not only in fission yeast but also in filamentous fungi, suggesting a well-conserved function (BLAST searches and our observations). It would be worthwhile to investigate whether and how Dia2 regulates DNA replication in filamentous fungi.

Grr1: AN F-BOX PROTEIN INVOLVED IN GLUCOSE AND AMINO ACID SENSING, CELL DIVISION CYCLE, MEIOSIS, AND RETROGRADE SIGNALING

Grr1 (glucose repression resistant 1) in S. cerevisiae plays a role in a large number of cellular processes: retrograde signaling, pheromone sensitivity and cell cycle regulation, nutritionally controlled transcription, glucose sensing, and cytokinesis (122) (Fig. 4). Grr1 was initially found in budding yeast through a mutation causing resistance to glucose repression, with a deletion mutant additionally showing growth defects (7, 52).

Grr1 AND THE CELL CYCLE

That Grr1 is involved in cell cycle control was shown by the accumulation of the cyclins Cln1 and Cln2 in a GRR1 deletion mutant (12, 95, 116, 177, 180, 186). Degradation of the cyclins Cln1 and Cln2 via Grr1 is required after completion of the G1 phase or when cells have to arrest in G1, for instance, after pheromone sensing. Binding of Grr1 to Cln2 has been established in multiple ways, but binding to Cln1 has never been detected, suggesting that Grr1 might target Cln1 indirectly. Another target of Grr1 is Gic2, a protein that accumulates throughout the G1 phase and reaches its peak just before bud emergence. At that time, Cdc42, a Rho-related GTP-binding protein required for polarized growth of the cytoskeleton during bud emergence, is activated and binds Gic2. When the bud has emerged, polarized growth ceases and Gic2 is degraded to avoid morphological defects. Only Gic2 bound to Cdc42 can be phosphorylated and eventually recognized by Grr1 (81). During cytokinesis, the process of cell separation, Grr1 is responsible for the degradation of Hof1. Hof1 first forms a ring around the bud neck of the mother cell and then forms another ring in the daughter cell. Just after septum formation and separation, Hof1 normally disappears (196). Grr1 is recruited to the mother bud neck and binds to Hof1 after the activation of the mitotic exit network (19). This suggests that Grr1 not only is active in the nucleus and cytoplasm but also can be recruited to specific cellular structures. The Grr1 protein of C. albicans is 46% identical to Grr1 from budding yeast, and C. albicans GRR1 can fully complement a yeast Δgrr1 strain. A Δgrr1 strain of C. albicans exhibits pseudohyphal growth under yeastlike growth-inducing conditions and does not grow on glucose (123). The constitutive pseudohyphal growth phenotype (27) of the Δgrr1 deletion strain could be explained by the stabilization of the two G1 cyclins Ccn1 and Cln3. That Grr1 mediates degradation of Ccn1 and Cln3 was demonstrated by the fact that both cyclins are stabilized; additionally, Cln3 was found as a hyperphosphorylated protein in the Δgrr1 strain. Elevated levels of Hof1 were also detected in the Δgrr1 strain. These data suggest that Grr1 function in degradation of cyclins and Hof1, as well as glucose uptake, is conserved between the two yeast species. The fact that the genes from C. albicans can functionally replace budding yeast GRR1 suggests that interactions of Grr1 with its targets are conserved between the two yeasts.
Grr1 AND MEIOSIS

Generally, the cell cycle is closely connected to the availability of nutrients. In low-glucose medium, diploid cells tend to undergo meiosis and sporulation rather than grow and divide. A role for Grr1 in preventing untimely meiosis and sporulation was demonstrated in a study of the degradation of Ime2, a protein kinase required for multiple steps throughout the sporulation process (166). In a Δgrr1 mutant, accumulation of (nonubiquitinated) Ime2 was found and meiosis still occurred, even under high-glucose conditions. In A. nidulans, GRRA, the ortholog of budding yeast GRR1, was found in a subtraction hybridization screen aimed at identification of genes that are specifically expressed during fruiting body development (107). GRRA is able to complement the Δgrr1 phenotype in yeast partially or, when the gene is overexpressed, almost fully. Complemented phenotypes of the yeast deletion mutant include the morphological abnormalities and changes in gene expression upon a carbon source shift (107). This demonstrated that GrrA from A. nidulans is probably able to bind endogenous targets in budding yeast, suggesting that these interactions are still conserved within GrrA. However, the phenotype resulting from deletion of GRR4 in A. nidulans is quite different from that of the yeast mutants. A. nidulans ΔgrrA mutants showed impaired ascosporogenesis, asexual conidiation, and sexual development, while displaying a normal vegetative growth. A similar phenotype was observed with CSN subunit mutants of A. nidulans (25, 26). This suggests that CSN may be involved in the functioning of GrrA in A. nidulans. From further cytological examination, it was concluded that meiosis, giving rise to crozierlike structures that contain diploid nuclei, does not take place in the grrA mutant. In striking contrast, meiosis does occur in the budding yeast grg1 mutant, even under meiosis-suppressing conditions. In light of this, it would be interesting to investigate whether and how the Ime2 ortholog in A. nidulans is involved in GrrA-controlled sexual development. In the plant-pathogenic fungus F. graminearum (Gibberella zeae), the ortholog of budding yeast Grr1, denoted Fbp1, was found in a restriction enzyme-mediated insertion screen for nonpathogenic mutants (60). The virulence of fbp1 mutants on barley heads was severely reduced compared to that of the wild type, and growth on potato dextrose agar and carrot agar produced less mycelium. Furthermore, Fbp1 plays a role in sexual reproduction. FBP1 deletion caused a loss of perithecium formation as females in self-crosses and smaller and fewer perithecia as a male in the outcross. The asci contained incomplete octads of abnormal spores and did not segregate in a one-to-one manner. Deletion constructs lacking the F box, the LRR, or both domains were nonfunctional both in the interaction with F. graminearum Skp1 and yeast Skp1 and in the ability to complement the sexual reproduction deficiency. Clearly, also in F. graminearum, protein turnover is required for sexual reproduction, but whether the ortholog of budding yeast Ime2 is involved is not known. Grr1 from budding yeast was unable to complement the knockout phenotype. Conversely, FBP1 from F. graminearum was able to partially complement the yeast grg1 mutant. This suggests that during evolution, Grr1 has retained the ability to bind at least some heterologous targets, despite their diversification. Grr1 is likely conserved as a pathogenicity
factor in plant-pathogenic fungi. In a screen for pathogenicity genes of *M. grisea* using insertional mutagenesis, one mutant had a disruption in a *GRR1* ortholog, *PTH1* (pathogenicity 1), and a subsequent deletion of this gene resulted in reduced disease symptoms toward barley (192). Why Grr1 is required for full pathogenicity in this fungus is not known.

**Grr1 IN GROWTH ON GLUCOSE AND NONGLUCOSE CARBON SOURCES**

In addition to regulating meiosis, Grr1 from *S. cerevisiae* conducts other glucose availability-related functions. When high levels of glucose are sensed, Grr1 not only initiates the degradation of Ime2 but also activates hexose permeases (*HXT*) that allow the rapid import of glucose. Activation of *HXT* expression is achieved by the degradation of Std1 and Mth1, which promote the repression of *HXT* genes by binding to the repressor Rtg1. Upon glucose sensing, Std1 and Mth1 are phosphorylated, recognized by Grr1, and degraded. Free, unbound Rtg1 can then be phosphorylated, promoting an intramolecular interaction in Rtg1 that prevents DNA binding (165), thereby releasing repression of the *HXT*genes (91). Although not yet fully understood, the process of Snf1 protein kinase inactivation is also required for degradation of Std1 and Mth1 (160). In *K. lactis*, Grr1 was characterized as an F-box protein required for glucose signaling, just as described for budding yeast (70). Complementation of *S. cerevisiae Δgrr1* with *K. lactis GRR1* showed full restoration of the growth and morphological defects of the deletion strain, demonstrating that *GRR1* from *K. lactis* is a functional homolog of budding yeast *GRR1*. It was also shown that *K. lactis* GRR1 controls the levels of Sm1, the single ortholog of Mth1 and Std1, the budding yeast Grr1 targets. The Sm1 level decreased dramatically after glucose addition, suggesting rapid degradation of Sm1 to allow expression of the hexose transporter genes. Other targets of Grr1 in *K. lactis* have not yet been found, but as the complementation of *S. cerevisiae Δgrr1* with *K. lactis GRR1* shows, Grr1 from *K. lactis* is probably able to bind targets in budding yeast. These targets also include Mth1 and Std1, suggesting that these interactions are still conserved, even though in *K. lactis* only Sm1 is present.

In addition to activating genes required for glucose uptake, Grr1 from *S. cerevisiae* is required for the assimilation of alternative carbon sources (52). Grr1 mediates this process by recruiting Gis4, a target that is ubiquitinated but not degraded (117). The ubiquitinated form of Gis4 binds and activates phosphorylated forms of Snf1, which results in derepression of several genes required for the assimilation of alternative carbon sources. Gis4 is a rare example of a target that is not degraded after ubiquitination but is instead activated. This shows that although Grr1 function generally complies with the F-box hypothesis, ubiquitination of Gis4 is an exception to this rule. Whether and how Gis4 is phosphorylated before recognition by Grr1 and how it is rescued from degradation after addition of ubiquitin is not known.

Grr1 also regulates other metabolic processes in the cell through its involvement in the degradation of Tye7 (131a) and Pfk27 (15). Tye7 is a transcription factor that activates several glycolytic genes (152), and Pfk27 synthesizes the second messenger fructose-2,6-bisphosphate (154). After glucose depletion, the removal of these proteins via Grr1 probably facilitates the switch from glycolysis to gluconeogenesis. This shows that Grr1 is active not only during glucose availability but also during glucose depletion.

Furthermore, together with Mdm30, another F-box protein, Grr1 regulates the activation of the Gal4 transcription activation complex. This complex regulates the transcription of genes involved in galactose assimilation. Degradation of the Gal4 isoforms Gal4a and Gal4b via Grr1 is required when glucose becomes available and galactose assimilation is shut down (147). This was demonstrated by deletion of *GRR1*, which results in stabilization of Gal4a/b and increased activation of Gal4 targets.

**Grr1 AND AMINO ACID SENSING**

Grr1 also plays a role in amino acid sensing by promoting the expression of several amino acid permease genes upon amino acid availability (17, 77). The activation of amino acid permease genes is mediated by the transcription factors Stp1 and Stp2. These two proteins are cleaved after activation of the Ptr3/Ssy5 amino acid sensing pathway and transported to the nucleus (130). Stp1 cleavage depends on Grr1, suggesting that Grr1 targets a protein that normally inhibits cleavage. A candidate might be Ssy5, which is involved in the amino acid permease expression pathway and which is normally degraded upon amino acid availability. On the other hand, higher protein levels of Stp2 were found in Δgrr1 cells than in wild-type *GRR1* cells (15).

**Grr1 AND RETROGRADE SIGNALING**

Mitochondrial retrograde signaling (RTG) is a pathway connecting mitochondria to the nucleus, allowing cells to react to changes in the functional state of mitochondria. The RTG pathway targets two transcription factors, Rtg1 and Rtg3. These two proteins form heterodimers and activate RTG-responsive genes (128). Grr1 functions in this pathway by degradation of Mks1, a negative regulator that inhibits localization of Rtg1 and Rtg3 to the nucleus (129). Grr1 targets Mks1 only when it is unbound to either Rtg2 or Bmh1, a 14-3-3 protein. When the RTG pathway is off, Bmh1 protects Mks1 and allows it to inhibit Rtg1 and Rtg3. When the pathway is on, Mks1 instead binds to Rtg2 and is thereby inactivated. Degradation of free Mks1 via Grr1 ensures that the switch is quick and under tight control.

Grr1 seems to be conserved among yeasts, considering the cell cycle and glucose uptake. Regarding meiosis, however, the role of Grr1 in budding yeast and the role of Grr1 in filamentous fungi seem opposite of each other. Another difference between Grr1 in yeasts and Grr1 in filamentous fungi is involvement in glucose uptake, since, for example, Mig1 and Snf1 repression in filamentous fungi is different from that in budding yeast (28, 172). Regarding other functions, like amino acid sensing and retrograde signaling, hardly anything is known about Grr1 involvement in other fungi, partly because of a lack of knowledge but probably also partly because these processes are differently regulated. Once again, fewer studies to find Grr1 targets have been carried out for other fungi than for budding yeast (15).
Hrt3: An F-box Protein Enhancing Methylmercury Resistance

Hrt3 (high-level expression reduces Ty3 transposition) and Ylr224w in *S. cerevisiae* both promote resistance to methylmercury, a highly toxic compound (75). Overproduction of these two F-box proteins elevated resistance to the toxic compound, in contrast to 15 other F-box proteins studied. This resistance required the F-box domain of these two proteins and also the proteasome, suggesting that degradation of a target protein is involved. Targets of Hrt3 or Ylr224w that could explain the roles of these F-box proteins in methylmercury resistance have not yet been identified. Interactions of Hrt3 other than with ubiquitin conjugation proteins were with alcohol dehydrogenase (Adh2) and Idh1, a subunit of mitochondrial NAD+-dependent isocitrate dehydrogenase, which catalyzes the oxidation of isocitrate to alpha-ketoglutarate in the tricarboxylic acid cycle (73, 109). The biological relevance of the interaction with these two catabolism-related proteins is not clear, but they might be involved in sensitivity to methylmercury. Other interactions with ribosomal proteins Rpl12A and Guf1 and a phosphatase functioning in the G1/S-phase transition were found.

Hrt3 is conserved in the entire fungal kingdom (BLAST searches and our observations), suggesting that it serves a fundamental function in fungi. Still, its characterization is limited; only the overexpressing phenotype was investigated for *S. cerevisiae*. Investigation of a (conditional) deletion mutant of *S. cerevisiae* or other fungi will be crucial to further explore the functions of Hrt3.

Mdm30: A Mitochondrion-associated F-box Protein

Mdm30 (mitochondrial distribution and morphology) and Mfb1 (mitochondrion-associated F-box protein) in *S. cerevisiae* both control membrane fusion dynamics of mitochondria. The membranes of mitochondria continuously undergo fusion and fission to maintain a dynamic morphology. A target of Mdm30 is Fzo1 (mitofusin), a membrane-bound GTPase involved in membrane fusion. Fzo1 is ubiquitinated and targeted to the proteasome in an Mdm30-controlled manner (33). Another target of Mdm30 is Mdm34, a mitochondrial outer membrane protein (157). Interaction between Mdm30 and Mdm34 is essential for growth on nonfermentable carbon sources and for normal mitochondrial morphology. If and how ubiquitination of Mdm34 contributes to these functions are not yet understood.

Additionally, Mdm30 (alternatively called Dsg1 [does something to Gal4]) is required for destruction of Gal4c, the inhibitory isoform of Gal4, and thereby plays a role in carbon assimilation (147) together with Grr1, which is required for the degradation of Gal4a/b, the Gal4 active isoforms. A Δdsg1 strain shows elevated levels of Gal4c, which results in the inability to use galactose as a carbon source.

Mdm30 binds to Skp1 via its F-box domain, and these two proteins together with other components of the SCF complex participate in Fzo1 degradation (33). This means that Mdm30, in contrast to earlier views (68, 71), can be part of an SCF complex and conforms to the F-box hypothesis. Mdm30 is not conserved in other fungi, but insight into how mitochondrial morphology is regulated by F-box proteins in *S. cerevisiae* is valuable, as in other fungi alternative F-box proteins or at least ubiquitination and protein turnover could also be involved in this intriguing process.

Safl: An F-box Protein Involved in Entry into Quiescence

Safl (SCF-associated factor 1) is an F-box protein required for the degradation of adenine deaminase 1 (Aah1) in *S. cerevisiae*. A microarray study showed clear AAH1 downregulation during the shift from proliferation to quiescence (47, 48). Quiescence, also known as the stationary phase, is a state that yeast cells enter when nutrients are limiting. A SAF1 deletion mutant showed no downregulation of AAH1 expression, and stabilized protein levels of Aah1 were detected upon entry into quiescence. Degradation of Aah1 relies on Safl, Skp1, and the proteasome and is dependent on the interaction between Safl and Skp1 via the F-box domain of Safl. Safl interacts in a yeast two-hybrid experiment with both Aah1 and Skp1. Loss or mutation of the F-box domain of Safl abolished the interaction with Skp1 but not with Aah1, although the latter interaction was slightly weakened. Mutation of the lysine at position 329 of Aah1 did not affect the interaction with Safl but increased the stability of Aah1, suggesting that this lysine might be the ubiquitination site. Other targets of Safl are not yet known, but a candidate might be Ura7, a protein that is present at reduced levels in *safl*-overexpressing strains and is stabilized in *safl* strains (36). Curiously, although the known target(s) of Safl is conserved among fungi, Safl itself is not. In fungi other then budding yeast, degradation of these Safl-targeted proteins might not be required upon quiescence or the proteins might be turned over in a different manner.

Ufo1: an F-box Protein Involved in DNA Damage Response

In *S. cerevisiae*, Ufo1 (UV-F box-HO target) targets the endonuclease Ho for proteasomal degradation and functions in genome stability and in response to DNA damage (88). After DNA damage, the MEC1/RAD9/CHK1 pathway phosphorylates Ho, stimulating its recognition and degradation. Ufo1 itself is also degraded via self-ubiquitination. This ubiquitination reaction is mediated by the ubiquitin interaction motifs (UIMs) in the C terminus of Ufo1 that bind during assembly in the SCF complex to Ddi1, a protein containing ubiquitinlike (UBL) and ubiquitin-associated (UBA) domains (78). Removal of the UIM domain in Ufo1 (Ufo1Δuim) stabilizes the protein and inhibits the degradation of other proteins normally degraded by SCF complexes. It therefore seems that Ufo1Δuim may prevent assembly of other F-box proteins into an SCF complex.

Ufo1 also appears to regulate the degradation of Rad30, since that protein is stabilized in proteasome mutants and in cells lacking Skp1 or Ufo1. Direct interaction between Ufo1 and Rad30 has, however, not yet been demonstrated. Rad30 is a polymerase *eta* necessary for DNA replication near damaged DNA (184) and is removed again after replication because of its high error frequency.

Recently, a study of the interactome of green fluorescent
protein (GFP)-labeled Ufo1 identified new proteins taking part in Ufo1 function (9a). The proteins interacting specifically with GFP-Ufo1 and bearing PEST degrons—potential phosphorylation sites that are often found in proteins targeted for degradation (167)—are Rpb2, Spf5, Fas2, and Gip2. Rpb2 is an RNA polymerase II (Pol II) subunit (39), and Spf5 is a protein that mediates both activation and inhibition of transcription elongation (125). Fas2 is a fatty acid synthetase component (142), and Gip2 is a putative regulatory subunit of the protein phosphatase Glc7p, involved in glycogen metabolism (195). Whether these proteins are targets of Ufo1 is not known. Ufo1 is not conserved in other fungi, suggesting that this type of regulation of the DNA damage response is restricted to (close relatives of) budding yeast.

**Fwd1: AN F-BOX PROTEIN CONTROLLING THE CIRCADIAN CLOCK**

In *N. crassa*, Fwd1 (F-box protein containing a WD40 repeat) was found to be involved in controlling the circadian clock via degradation of Frequency (Frq) (65, 66). Circadian clocks regulate a wide variety of physiological and molecular processes during oscillation between day and night. Besides being regulated by Frq, the circadian clock in *Neurospora* is further regulated by light and controlled by the transcription factors Wc-1 and Wc-2 (44). Frq inhibits its own transcription by inhibiting Wc-1 and Wc-2 (1, 2). When Frq is hyperphosphorylated by CK1 and CKII (63), it is recognized by Fwd1 and degraded. This releases Wc-1 and Wc-2 activity, leading to the production of new Frq.

The function of Fwd1 in the SCF complex is regulated by the COP9 signalosome CSN. Disruption of a subunit of CSN impaired the degradation of Frq, probably because reduced amounts of Fwd1 were present in the *csn* mutant: the half-life of Fwd1 is reduced from 6 to 9 h to 45 min, and other components of the SCF complex proved to be unstable. In a *Δcsn*-2 mutant, SCF is constitutively neddylated, which enhances the degradation rate of Fwd1. This degradation is probably independent of binding of Frq to Fwd1, resulting in reduced amounts of Fwd1 and impaired degradation of Frq1 (64). The *CSN*-2 deletion mutant also exhibits slow growth and reduced production of aerial hyphae compared to levels for the wild-type *CSN*-2, suggesting that other F-box proteins might also be affected. *N. crassa* is the main model organism for the investigation of circadian rhythms in fungi, and only for this fungus has Fwd1 been studied intensively. Nevertheless, this protein, as well as circadian rhythms, is present in other filamentous fungi (14, 114), as are homologs of *N. crassa* clock components, like Frq, WC-1, and WC-2 (132). However, in *A. nidulans* no homolog of *FRQ* is present (57), even though an *FWD1* homolog is present (our observations). This suggests that at least in some fungi, Fwd1 has other targets.

Interestingly, in plants, involvement in rhythmic processes has also been demonstrated for several F-box proteins, like ZEITLUPE, FKF1, and AFR (61, 151, 187), which play a role in photocontrol of the circadian period, the circadian clock, and phytochrome A-mediated light signaling, respectively.

**Met30: AN F-BOX PROTEIN INVOLVED IN SULFUR METABOLISM**

As described above, the F-box hypothesis states that the targets of F-box protein are degraded after ubiquitination, but ubiquitination of the Grr1 target Gis4 does not lead to degradation. Met30 is another example of an F-box protein whose target is not necessarily degraded. Met30 is an F-box protein from *S. cerevisiae* that can recruit its target to the SCF for degradation, but it can also activate its target by ubiquitination when the target is assembled into a transcription activation complex.

A major Met30 target is Met4, a transcriptional activator of the sulfate assimilation pathway controlling *MET* and *SAM* genes for uptake and biosynthesis of sulfur-containing compounds (194). Met4 is also required for cadmium tolerance by activating the expression of genes involved in glutathione biosynthesis (10, 21, 212).

The transcription of Met30 is regulated through a feedback loop, as Met4 controls the activation of Met30 (171). The way Met4 is regulated by Met30 has been under discussion for several years (23, 24, 83, 140, 141). A picture in which Met30 regulates Met4 in multiple ways has emerged (30). First, Met30 activates Met4 when low levels of methionine are available, resulting in the expression of *MET* and *SAM* genes. When, through *MET* and *SAM* activation, higher intracellular levels of methionine are obtained, the intracellular concentration of cysteine also increases through the S-adenosyl-methionine and cysteine biosynthesis pathways. High levels of cysteine again lead to the inactivation of Met4 by Met30. The alternative activation and inactivation of Met4 by Met30 are explained in a two-step model. In the inactive state, dimerization of Met4 causes low interaction with cofactors, leading to intermediate expression of *MET* and *SAM* genes. Met30 relieves this dimerization through degradation of one of the dimerized Met4 proteins, leaving the other Met4 subunit free to assemble into an activation complex, thus triggering expression of the *MET* and *SAM* genes. Met30 then activates Met4 when low levels of sulfur-containing amino acids are present, Met30 binds to Met4 in the assembled promoter complex, leading to Met4 ubiquitination (step 2); this ubiquitinated promoter complex represses transcription of the *MET* and *SAM* genes. Eventually, Met4 is degraded and the complex disassembles, making space for new complexes to form on the promoter when levels of sulfur-containing amino acids are low again.

In *S. pombe*, the Met30 homolog Pof1 is an essential protein that targets the Met4 homolog Zip1 (a basic leucine zipper) (62). Like in *S. cerevisiae*, Zip1 mediates cadmium tolerance by activation of cadmium response genes. Regulation of Met4 by Met30 and regulation of Zip1 by Pof1 show similar patterns. However, one difference between the two systems is that Zip1 is required for the biosynthesis of sulfur-containing amino acids only under low levels of sulfur and is not required during normal growth conditions, as is Met4 (13). In *N. crassa*, the Met30 homolog Scon2 (sulfur controller) is also required for sulfur uptake and assimilation (110, 111). Cys3, the Met4 ortholog of *N. crassa*, is degraded via Scon2 and regulates the entire set of sulfur uptake and assimilation genes. Interaction between Scon2 and Scon3 (*N. crassa* Skp1) was observed using a yeast two-hybrid screen and coimmunoprecipitation and was
dependent on the F-box motif in Scon2 (183). Cys3 activates not only sulfur utilization genes but also the transcription of CYS3 itself and of the SCON2 gene. Therefore, when Scon2 targets Cys3 for proteolysis, its own activation is also reduced, ensuring the possibility of rapidly activating Cys3 again. Mutational analysis of Scon2 showed that the F-box domain plays an important role in the regulation of Cys3. Eleven out of 14 mutations in the F-box domain gave rise to a constitutively repressed phenotype corresponding to the Δcys3 phenotype. This is at first sight surprising, since mutation of the F box is expected to impair Skp1 binding (although loss of binding to Skp1 was not verified) and thereby decrease the ability to degrade Cys3. This would in turn be expected to lead to a constitutive activation phenotype of CYS3. It is possible that Scon2 is required not only for the degradation of Cys3 but also for its activation, as demonstrated for Met30 and Met4 in budding yeast. The role of the Met30 homolog SconB (148) in A. nidulans has proven to be similar to that of Scon2 in N. crassa, including binding to Skp1, called SconC in A. nidulans (164). Still, differences have also been found: SCONB is not transcriptionally activated by the Cys3 counterpart of A. nidulans, MetR (149), and although MetR and Cys3 both recognize the same DNA sequences, full-length CYS3 cannot fully complement the ΔmetR phenotype.

In addition to its role in sulfur metabolism, Met30 is essential for cell cycle progression. It regulates multiple aspects of the cell cycle, including the expression of cyclins required for G1-phase progression and the accumulation of proteins involved in replication and progression through the M phase (161, 190). A target of Met30 was believed to be Swe1 (34), a Weel family kinase that inhibits Cdc28 by phosphorylation, since high activity of Swe1 and nonubiquitinated forms of Swe1 were found in Δmet30 cells. An in vivo interaction between Met30 and Swe1 was also demonstrated (34). A later study concluded, however, that Met30 is not responsible for degradation of Swe1 but that degradation is a result of the interaction between Swe1 and Hsl7 (84, 138). This interaction with Hsl7 mediates the translocation of Swe1 out of the nucleus to the mother bud neck, where its degradation takes place in an unknown manner. The involvement of Met30 in Swe1 degradation is therefore disputable but cannot be ruled out entirely. Regardless of the exact mechanisms, it is now clear that the activation and degradation of proteins involved in the cell cycle are under the control of multiple F-box proteins (Cdc4, Grr1, and Met30). These F-box proteins either target cell cycle proteins directly or regulate their levels indirectly.

Finally, recently a Met30 homolog, Lim1, was found in H. jecorina through a yeast one-hybrid screen, and it was demonstrated that Lim1 can bind promoter sequences of the cellobiohydrolase gene CBH2 (58). Clarification of the role in transcription of Lim1 and the involvement of possible targets awaits further investigation.

**F-BOX PROTEINS WITHOUT IDENTIFIED TARGETS**

F-box proteins from which targets are known to be ubiquitinated through binding to Skp1 and assembly into an SCF complex are described above. For several other fungal F-box proteins, binding to Skp1 does not appear to be required for function or target inactivation. The functions of these proteins, then, seem to fall outside the F-box hypothesis, as probably no targets are recruited to an SCF complex. The F-box domain in some of these proteins could interact with proteins other than Skp1. Alternatively, interaction with Skp1 is required only for self-ubiquitination of the F-box protein to control protein levels.

**Fbh1: A DNA REPAIR F-BOX PROTEIN**

Fbh1 (F-box DNA helicase) is an F-box protein from S. pombe involved in the regulation of recombination levels and DNA repair (144, 156, 175). Like its human homolog, Fbh1 contains a helicase domain to unwind DNA. Human Fbh1 functions downstream of the recombinase enzyme Rhp51 (the ortholog of S. cerevisiae Rad51). Although Fbh1 binds Skp1, it appears that the F box is not necessary for Fbh1 to promote DNA repair, since two mutations in the F-box domain did not alter growth or genotoxin resistance (120). A mutation in the helicase domain, however, did affect the DNA repair function. The human homolog assembles into an SCF complex, but its targets, if any, are also still unknown. Possibly, Skp1 binding mediates only self-ubiquitination of Fbh1. Remarkably, Fbh1 is the only fungal protein that contains an F box combined with a helicase domain, and it is found only in fission yeast.

**Frp1: AN F-BOX PROTEIN REQUIRED FOR ROOT INVASION**

In F. oxysporum f. sp. lycopersici, a vascular wilt pathogen of tomato, Frp1 (F-box protein required for pathogenicity 1) was found using an insertional mutagenesis screen for pathogenicity genes (46). Frp1 is required for assimilation of various (nonsugar) carbon sources as well as induction of genes for cell wall-degrading enzymes, which would explain the deficient plant root colonization and penetration by the Δfrp1 mutant (W. Jonkers et al., in press). Frp1 binds to Skp1 in yeast two-hybrid and pull-down assays, but mutations in the F-box domain of Frp1 that impair binding to Skp1 do not affect the phenotype, suggesting that the main function of Frp1 does not depend on ubiquitination of targets (W. Jonkers and M. Rep, unpublished results). Because FRP1 orthologs are present in other plant-pathogenic fungi, it will be interesting to study their role in pathogenicity in these fungi. The deletion of the FRP1 ortholog in F. graminearum has been reported previously, but an initial characterization revealed no obvious differences from the wild type (60).

**Pof14: AN F-BOX PROTEIN THAT INHIBITS ERGOSTEROL SYNTHESIS**

Pof14 is an F-box protein in S. pombe required for survival upon hydrogen peroxide stress (193). In response to such stress, Pof14 binds and inhibits Erg9, a squalene synthase involved in ergosterol synthesis. Ergosterol enhances the permeability of the membrane and thereby the uptake of hydrogen peroxide. Pof14 and Erg9 bind to each other in a membrane-bound complex, as was demonstrated by tagging both proteins with fluorescent tags. Binding of Pof14 to Erg9 inhibits the activity of Erg9, and overexpression of POF14 leads to decreased levels of squalene synthase activity and ergosterol.
Transcription of \textit{POF14} is induced after treatment with hydrogen peroxide, and deletion of \textit{POF14} decreases viability after hydrogen peroxide treatment (193). Decreased viability was not observed upon deletion of the F-box domain, suggesting that binding of Pof14 to Skp1 is not required for peroxide resistance. However, binding of Pof14 to Skp1 may promote the degradation of Pof14 itself. In wild-type cells, Pof14 has a half-life of 20 to 40 min, but in temperature-sensitive mutants of Skp1, Pof14 is stable for at least 60 min (193). Whether this stabilization is due to defective assembly of Skp1 and Pof14 into an SCF complex for self-ubiquitination is not known.

**NON-SCF F-BOX PROTEINS**

Ctf13 and Rey1 are two F-box proteins that were found to bind Skp1 but nevertheless function independently of an SCF complex and probably also do not have targets to be ubiquitinated; therefore, they are unlikely to be involved in protein inactivation. The binding of these proteins to Skp1 may be evolutionarily conserved but may have acquired an alternative function.

**Ctf13: A KINETOCHORE ASSEMBLY F-BOX PROTEIN**

In \textit{S. cerevisiae}, Ctf13 (chromosome transmission fidelity 13) is part of the CBF3 complex, which in turn is part of the centromere-bound scaffold, where the microtubule binding components of kinetochores assemble. The CBF3 complex consists of four components: Skp1, Ctf13, p64 (encoded by \textit{CEP3} and containing a zinc finger centromere binding domain), and p110 (a protein complex encoded by three genes, \textit{Cbf2}, \textit{Ndc10}, and \textit{Ctf14}) (105, 214). The binding of Ctf13 to Skp1 requires the phosphorylation of Ctf13 (87, 173) and the interaction with Sg1 and Hsp90 (8, 188) for the assembly and function of the kinetochore complex. When mutations that prevent binding of Ctf13 to Skp1 were introduced, severely impaired cell growth was observed. Interestingly, Ctf13 is targeted by another F-box protein, Cdc4, for degradation, which is in accordance with Ctf13 not being part of an SCF complex itself. Binding of Ctf13 to p64 rescues Ctf13 from degradation. Probably only free Ctf13 is degraded via Cdc4, and Ctf13 degradation might be required to tightly regulate kinetochore assembly.

**Rey1: AN F-BOX PROTEIN INVOLVED IN VESICLE TRAFFICKING**

Rey1 (recycling 1) was found in a genetic screen for \textit{S. cerevisiae} mutants defective in membrane trafficking through the endocytic pathway. Deletion of \textit{RCY1} results in an arrest of the endocytic pathway and leads to accumulation of enlarged compartments close to areas of cell expansion (202). For Rey1 to function, it needs to bind Skp1, but other components of the SCF are not required for recycling or for degradation of Rey1 itself. Rey1 contains two SEC10 domains and a CAAX box, implicated in mediating interaction with membranes and also needed for recycling. Rey1 is required for the recycling of the v-SNARE Snclp, a membrane protein that fuses exocytic vesicles with the plasma membrane (56). During vegetative growth, Snclp is localized at the plasma membrane and continually recycles through the Golgi body (121, 143). Rey1 binds via its C-terminal domain to two GTPases, proteins that regulate vesicle transport during exo- and endocytosis and are required for Golgi body function in yeast (16). Rey1 interacts specifically with the active forms of the two GTPases, and together they colocalize to the Golgi body and endosomes.

A second recycled protein by Rey1 is Kex2, a calcium-dependent serine protease involved in preprotein processing. Kex2p is a membrane-bound protein cycling between trans-Golgi vesicles and late endosomal compartments (54, 203). These studies suggest that the involvement of Rey1 in vesicle transport is not related to protein degradation. Indeed, ubiquitination of the interacting proteins seems unlikely, since assembly into an SCF complex is not required for Rey1 function. The F box of Rey1 is required for binding to Skp1, but the biochemical function of this small complex during vesicle trafficking remains unclear. Perhaps surprisingly, Rey1 has been found in an SCF complex (113), but it remains unknown whether this is a functional complex. The \textit{S. pombe} homolog of Rey1, Pof6, also forms a complex with Skp1 and does not function in an SCF complex (69). Pof6 is required for septum processing and sporulation. Deletion of \textit{POF6} results in the formation of a thick septum and the absence of viable spores, which differs from the deletion phenotype of Rey1, which is not lethal. Recently, a specific Pof6 interactor, Sip1, was found using TAP (tandem affinity purification) purification and MudPIT (multidimensional protein identification technology) analysis (82). It was shown that Pof6 and Sip1 form a non-SCF complex with Skp1 and that both proteins require interaction with Skp1 for stability. Sip1 is a widely conserved protein in eukaryotes and consists of HEAT (Huntington, elongation factor 3, the regulatory A subunit or protein phosphatase 2fA and Tor1) repeats required for interaction with other proteins. Like Pof6, Sip1 is essential and plays a role in endocytosis and cytokinesis. The budding yeast ortholog of Sip1, Lal1, has not yet been identified as an interactor of Rey1 but might also be part of the Rey1-Skp1 complex, as it too mediates protein transport between the trans-Golgi network and endosomes (50).

Clearly, the role of Rey1 is conserved between budding yeast and fission yeast. In fact, Rey1 seems to play a fundamental role in vesicle trafficking in fungi, since it is conserved throughout the fungal kingdom (BLAST searches and our observations).

**NON-Skp1 BINDING F-BOX PROTEINS**

Some proteins with an F-box domain do not bind Skp1 but instead bind another E3 ligase subunit. For other F-box proteins, binding to Skp1 could not be demonstrated or has not been investigated. The F-box domain in some of the latter proteins may also mediate assembly into different complexes.

**Ela1: AN ELONGIN COMPLEX F-BOX PROTEIN**

Ela1 (elongin A 1) and Elc1 (a Skp1 homolog) in \textit{S. cerevisiae} were identified as the homologs of mammalian elongin complex components (106). Also, in yeast, Ela1 and Elc1 are present in the same complex. Probably, Ela1 does not act in an SCF complex, since it binds Elc1 instead of Skp1 and since Elc1 does not bind Cul1. Ela1 and Elc1 likely bind to Cul3.
instead. Cul3 is a Cullin that is normally part of an E3 ligase complex called BC3·B, consisting of Cul3, a BTB domain-containing protein, and Rbx1. Apparently, Ela1 exists in a complex (Ela1/Elc1/Cul3/Rbx) that is a combination of the human E3 ligase Von Hippel-Lindau (VHL) and the BC3·B complexes. This new combinatory complex was not reported earlier, and it shows the possibility that subunits from different complexes can interchange to form new complexes, potentially broadening the arsenal of ubiquitin ligase superfamilies.

Ela1 and Cul3 were found to be required for cell survival after treatment with UV or the mutagen 4-nitroquinoline 1-oxide. Both proteins are also required for degradation and poly-ubiquitination of subunit Rpb1 of RNA Pol II. Pol II is normally removed from damaged DNA to make room for the nuclear excision repair machinery to assemble at that site and repair damaged DNA strands (169).

**Mfb1: A MITOCHONDRION-ASSOCIATED F-BOX PROTEIN**

Mfb1 (mitochondrion-associated F-box protein) in *S. cerevisiae* controls membrane fusion dynamics of mitochondria, like Mdm30, described above. Deletion of *MFB1* results in abnormal mitochondrial morphologies, including short tubules, aggregates, and fragments in different combinations (103). Binding to Tom71 localizes Mfb1 to mitochondria, and binding to Tom70 ensures stable association with these organelles (104). The paralogous TPR (tetratricopeptide repeat) proteins Tom70 and Tom71 are both associated with mitochondrial membrane (120). Loss of *MDM30* also results in short tubules, aggregates, and fragments but in a different distribution than in *Δmfb1* mutants (53). A double knockout of *MFB1* and *MDM30* results in a decreased number of short tubules but more aggregates and fragments. On rich dextrose and glycerol plates, an *mfb1* mutant grows like the wild type, an *mdm30* mutant grows more slowly, and a double mutant displays a severe growth problem, probably due to mitochondrial DNA instability (45). Possible targets of Mfb1 are proteins involved in mitochondrial morphogenesis, but for none of the candidate proteins were larger amounts seen in the *Δmfb1* mutant than in the wild-type *MFB1*. This observation and the lack of demonstration that Mfb1 binds Skp1 suggest that Mfb1 may not function as part of an SCF complex.

**Amn1: A MITOSIS EXIT STATE F-BOX PROTEIN**

Amn1 (antagonist of mitotic exit network 1) from *S. cerevisiae* is listed as one of the 21 budding yeast F-box proteins in an earlier review (205). The protein shares homology with another F-box/LRR protein, Pof2 of fission yeast, with little conservation of the F-box domain, in part because of an interspersed region of 56 amino acids in the motif. Although a genetic interaction has been found, a physical interaction between Amn1 and Skp1 could not be demonstrated (in insect cells), perhaps because of the interspersed region in the F-box domain. Amn1 itself might be targeted for SCF-mediated proteolysis, since stabilized forms of Amn1 were found in *cull1* and *skp1* mutant strains. AMN1 expression peaks at the M/G1 phase, and Amn1 is normally degraded when cells enter the S phase, showing an accumulation pattern similar to that of the Cdc4 target Sic1. Amn1 is required to turn off the mitotic exit pathway after it is completed, and it inhibits the function of Tem1, a small GTPase that activates the mitotic exit network, which causes spindle breakdown, degradation of mitotic cyclins, cytokinesis, and cell separation (11). It was shown that Amn1 binds to Tem1 and inhibits its function by obstructing the binding of Tem1 to Cdc15. This ensures that the cell can exit from mitosis and enter the G1 phase (199). Tem1 levels are elevated in a Δamn1 mutant (153), suggesting that Amn1 may regulate Tem1 levels. Since Amn1 apparently does not bind Skp1, this regulation may not involve ubiquitination.

**OTHER FUNGAL F-BOX PROTEINS**

Broad, genomics-based interaction and localization studies have provided some information on F-box proteins that have not been investigated individually (Table 2). Most of these proteins bind Skp1 and can assemble into an SCF complex (113, 181), and some also interact with ribosomes (Ynl311c) (51) or other proteins, like Sgt1 (Ynl311c and Ydr306c) (43). Sgt1 binds to Skp1 and can assemble into an SCF complex (51, 181), and some also interact with ribosomes (Ynl311c) (43). Sgt1 binds to Skp1 and other SCF components (96) and acts as a “client adaptor,” linking the chaperone Hsp90 to SCF and CBF3 complexes containing Skp1 (29). For one F-box protein (Ymr258c), it was determined that it localizes to the cytoplasm and nucleus using a GFP fusion (74), and for another (Ylr224w), it was demonstrated that it is readily monoubiquitinated in vitro by SCF-Ubc4 complexes (113).

Finally, two other F-box proteins, Cos111 and Pof10, cannot be classified into one of the above-mentioned categories but have been studied and can be related to specific cellular process. In *S. cerevisiae*, the F-box protein Cos111 was identified from a mutant that showed increased sensitivity to ciclopirox olamine, an antifungal agent that chelates iron and other ions and thereby inhibits metal-dependent enzymes (119). The *cos111* mutant is sensitive to ciclopirox olamine at 36°C and is also sensitive to hydroxyurea. In addition, the *cos111* deletion

| TABLE 2. Additional F-box proteins in *S. cerevisiae* and *S. pombe* without targets and ascribed cellular function or Skp1/SCF binding data |

| F-box protein | Yeast | Additional motif | Skp1 binding | SCF binding |
|---------------|-------|-----------------|--------------|-------------|
| Cos111        | *S. cerevisiae* | ? | ? | |
| Das1<sup>a</sup> | *S. cerevisiae* | yes | yes | yes |
| Ydr306c       | *S. cerevisiae* | RNI-like<sup>b</sup> | yes | yes |
| Pof5          | *S. pombe* | RNI-like | yes | yes |
| Ynl311c       | *S. cerevisiae* | yes | yes | yes |
| Ylr224c       | *S. cerevisiae* | yes | yes | yes |
| Ymr258c       | *S. cerevisiae* | yes | yes | yes |
| Ydr131c       | *S. cerevisiae* | yes | yes | yes |
| Ylr352w       | *S. cerevisiae* | LRR | yes | yes |
| Pof10         | *S. pombe* | WD40 | yes | yes |
| Pof11         | *S. pombe* | ? | ? | ? |
| Pof12         | *S. pombe* | yes | yes | yes |
| Pof13         | *S. pombe* | yes | yes | yes |
| Pof16         | *S. pombe* | ? | ? | ? |

<sup>a</sup>Das1 (Das1Δ 6-azauracil sensitivity) from *S. cerevisiae* is a putative SCF ubiquitin ligase F-box protein of which a null mutant suppresses Δstl sensitivity to 6-azauracil (http://db.yeastgenome.org/cgi-bin/locus.pl?locus=DAS1) (S. Chavez, personal communication).

<sup>b</sup> RNI-like, RNase inhibitor-like.

<sup>c</sup> ?, unknown or uncertain.
mutant showed sensitivity to caffeine, which might indicate a defect in the cyclic AMP signal transduction pathway. Deletion of \textit{COS111} does not lead to any growth defects under normal or stress conditions, like temperature shifts, high salt, or osmotic or oxidative stress, and no alterations in morphology, sporulation, or mating were observed (170). It is unknown whether Cos111 binds Skp1 and regulates protein degradation via an SCF complex.

Pof10 from \textit{S. pombe} is an F-box/WD40 protein that binds Skp1 via its F-box domain (76). Deletion of POF10 does not result in an obvious phenotype, which is remarkable since POF10 is conserved between fission yeast and filamentous fungi (BLAST search and our observations). On the other hand, overexpression of POF10 results in lethality, probably due to sequestration of Skp1, thereby preventing the formation of other SCF complexes. Viability was restored by coconcomitant overexpression of SKP1, presumably by making more Skp1 available for formation of other SCF complexes. Binding to Skp1 may not lead to self-ubiquitination, because Pof10 is highly stable in contrast to other F-box proteins. Although Pof10 bears a protein-protein interaction domain (a WD40 motif), targets of Pof10 have not been identified.

**CONCLUDING REMARKS**

Fungal F-box proteins take part in highly diverse cellular processes, but most share the same molecular function: removal or inactivation of specific proteins. Loss of a fungal F-box protein often results in a pleiotropic phenotype, especially when the F-box protein has multiple targets. For Cdc4, which has 10 known targets, a null mutation is lethal. Conversely, when a gene deletion shows no or little effect, the F-box protein may target only one or a few proteins. For example, the original deletion mutant of \textit{COS111} did not show any phenotype, but it was later demonstrated that \textit{COS111} may have a function in tolerance to an antifungal agent. Targets of F-box proteins can vary from transcription factors, enzymes, DNA repair proteins, structural proteins, and cyclins to inhibitors and/or activators of various other processes. These targets can operate at an intermediate level of a signaling pathway, for example, Rcn1 and Sic1, which are degraded via Cdc4, and Msk1 and Ime2, which are degraded via Grr1. Other targets function at the end of a pathway, examples of which are the transcription factors Tec1 and Gcn1, degraded via Cdc4, and Fq, degraded via Fwd1.

Targets of F-box proteins are recognized mostly when phosphorylated. Such phosphorylation can be performed by many different protein kinases, like CDKs, mitogen-activated protein kinases, Pho kinases, and casein kinases, depending on the pathway or process in which the target protein functions. Different forms of phosphorylation can be required for recognition. For instance, a requirement for hyperphosphorylation of a target causes a threshold before a target is being degraded and ensures that multiple phosphorylation steps control degradation, like for Cdc4-mediated degradation of Sic1 and Fwd1-mediated degradation of Frq. An exceptional case of an unphosphorylated target is Msk1, which is degraded by Grr1 when it is unbound to either Rtg2 or Bmh1. Apparently, the site on Msk1 recognized by Grr1 is masked by these interacting proteins.

In addition to these well-studied F-box proteins, several other F-box proteins interact with proteins without targeting them for disposal, examples being Ctf13, Rcy1, and Pof14. For still other F-box proteins, no targets or interacting proteins have been found, and these are often referred to as “orphan F-box proteins.” It might be that the targets are yet to be found or that no targets exist for these orphan F-box proteins. Especially for those of which mutation of the F-box domain does not (greatly) affect function (like Frp1 and Fbh1), the F-box domains may serve as degradation motifs required solely for self-ubiquitination. Instead of targeting other proteins, they may, for instance, function as DNA binding proteins or perform an enzymatic reaction. Another variation on the F-box hypothesis is seen for Ela1, an F-box protein that does recruit targets for degradation but assembles in a complex different from the SCF complex.

The levels of free and SCF-bound F-box proteins in the fungal cell are regulated by recycling of Skp1–F-box complexes within the SCF core via Nedd8 and CAND1 and by self-ubiquitination. It seems that in unicellular fungi, like budding and fission yeasts, recycling is less important and F-box proteins are regulated mostly by autoubiquitination, as shown for several budding yeast F-box proteins. The difference in regulation could be related to the small number of F-box proteins in these two yeasts (21 and 16, respectively) relative to the number in filamentous fungi, which can be 100 or more (BLAST searches and our observations).

F-box proteins are probably also regulated by recycling and autoubiquitination to remove “free” F-box proteins (i.e., unbound to a target) from SCF complexes, so that these complexes become available for other F-box proteins. Such a scenario was supported by overexpression of POF10, probably leading to constitutive occupation of Skp1, which results in lethality (76), and for the Ufo1Δuim mutant lacking the UIMs. Normally, the UIMs are required for self-ubiquitination of Ufo1; a mutant lacking the UIMs cannot be ubiquitinated anymore and therefore remains in the SCF complex (78). In a variation on self-ubiquitination, Ctf13 is targeted by another F-box protein, Cdc4, when unassembled into a CBF complex.

Besides being regulated on the protein level by ubiquitination and recycling, F-box proteins can be regulated at the transcriptional level. An example is Met30, which creates a negative-feedback loop by degrading the transcription factor Met4, inactivating its own transcription. Another potential mechanism of regulation is localization. Some F-box proteins function specifically at certain sites in the cell or at certain regions on chromosomal DNA. To be transported to these sites, interacting partners can play an important role, as demonstrated for Mfb1 and Dia2/Pof3.

In fungi, regulation of the activity of F-box proteins themselves is usually not an integral part of a signal transduction pathway, in contrast to some cases with plants, where it has been demonstrated that F-box proteins could be activated by direct binding to a small molecule. These F-box proteins act as receptors, with direct hormone binding triggering their activation (reviewed in reference 214a). Such a mechanism remains a possibility also with fungi, for instance, for Met30, which is activated when high levels of methionine, S-adenosyl-methionine, or cysteine are present. Binding studies of these sulfur-
containing amino acids or derivatives to Met30 could confirm this possibility.

Perhaps less sophisticated, many fungal F-box proteins appear to function simply as garbage collectors, removing waste proteins that have been marked for degradation. However, several variations on this theme have emerged. For instance, Met30 regulates the transcription factor Met4 in complex ways and does not simply follow the standard F-box hypothesis. Further in-depth investigations of F-box proteins and their potential targets or other functions may reveal more such variations.

Most F-box proteins discussed in this review are from *S. cerevisiae*, providing a fairly comprehensive overview of the variety of functions that F-box proteins perform in a eukaryotic cell. The additional results obtained with orthologs and other F-box proteins from fission yeast and filamentous fungi give an impression of the degree of functional conservation of F-box proteins between fungal species. For example, functional conservation of Grr1 is, not unexpectedly, less when species are more distantly related—in contrast to the GRR1 ortholog of *C. albicans*, the orthologs from two filamentous fungi could not fully complement the Δgrr1 mutant of yeast. Since Skp1 is highly conserved between species, this is probably due to differences in target recognition. Evolution of an F-box protein is constrained by the requirement to recognize diverse targets. When an F-box protein encounters orthologs of its natural target in another fungus or a “novel” target (i.e., not present in its natural environment), recognition might be less efficient, despite overall sequence conservation in the target recognition domain of the F-box protein. Conservation of F-box protein function between different fungal species can also be assessed by the conservation of targets and the pathways leading to the phosphorylation of these targets. Fission yeast and *C. albicans* harbor orthologs of targets of budding yeast Cdc4 and Grr1, but these have not yet been found in other fungal species (Table 1). For Met4, a target of Met30, homologs are present in fission yeast and in the filamentous fungi *A. nidulans* and *N. crassa*. Apparently, the Met30-Met4 interaction system has remained relatively stable during fungal evolution.

Searches of fungal genome sequences allow an estimation of the numbers of genes encoding F-box proteins in different fungal species. *A. nidulans*, for example, contains about 50 genes encoding F-box proteins, and in different *Fusarium* species 60 to 95 genes encoding F-box proteins are present (Jonkers and Rep, unpublished). In comparing these numbers to the smaller numbers in yeast (21 in budding yeast and 16 in fission yeast), it becomes clear that the potential variation of processes regulated by F-box proteins in filamentous fungi is much more extensive than that for yeasts. Examples of this are regulation of the circadian clock with *N. crassa* and plant infection with *F. oxysporum*.

With fungi, it is relatively easy to investigate F-box proteins, due to the availability of knockout strains and the accessibility to molecular manipulations. Sophisticated screens for target identification and deletion studies of all genes encoding F-box proteins present in a fungal genome, combined with detailed investigations of protein-protein interactions and posttranslational modifications, will promote a deeper and broader insight into the diverse functions of F-box proteins in eukaryotic cells.

Among the general lessons already learned from investigation of the fungal F-box arsenal are that these proteins function in a very broad array of cellular functions and can target many different proteins for degradation. Furthermore, clearly not all F-box proteins comply with the F-box hypothesis, and the regulation of at least some of these proteins is more complex than expected. Concerning the conserved F-box proteins found in budding yeast and filamentous fungi, we learned that they can have both conserved and diversified targets and that accumulation of conserved targets in deletion mutants of F-box proteins can sometimes result in different phenotypes. Fungi remain a rich source for the discovery and understanding of a great variety of intricate cellular processes with which F-box proteins are involved.

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