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Ubiquitin-dependent control of development in *Saccharomyces cerevisiae*

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In response to external environmental stimuli and intrinsic developmental cues, yeast cells reset their gene expression programs and change phenotype. These switches in cellular state require the dismantling of an initial regulatory program, in addition to the induction of different sets of genes to specify the new cell phenotype. Recent experiments examining the role of protein degradation in these transitions have highlighted the importance of inactivating previously utilized regulators and have led to advances in our understanding of how cells change from one phenotypic state to another.

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Abbreviations

HO	the homothallism gene, which encodes the HO endonuclease
MAPK	mitogen-activated protein kinase
MAT locus	mating-type locus
SCF	Skp1/Cullin/F-box protein complex
Ub	Ubiquitin

Introduction

The differentiation of eukaryotic cells into distinct cell types requires changes in gene transcription as well as alterations in cell-cycle progression. Recently, it has become clear that proteolytic mechanisms play an equally important role in promoting the switches between distinct cellular states that underlie cell differentiation. Although the yeast *Saccharomyces cerevisiae* is a simple single-celled organism, yeast cells undergo several different cell-state transitions that serve as paradigms for more complex conversions in higher eukaryotes [1–3]. Thus, studies in yeast have provided several fundamental insights into the basic molecular mechanisms of

development and differentiation that are common to all eukaryotes.

Here, we review recent progress in characterizing some of the different phenotypic transitions that occur during the yeast life-cycle, focusing on the role of ubiquitin (Ub)-dependent protein degradation in the control of these cellular switches.

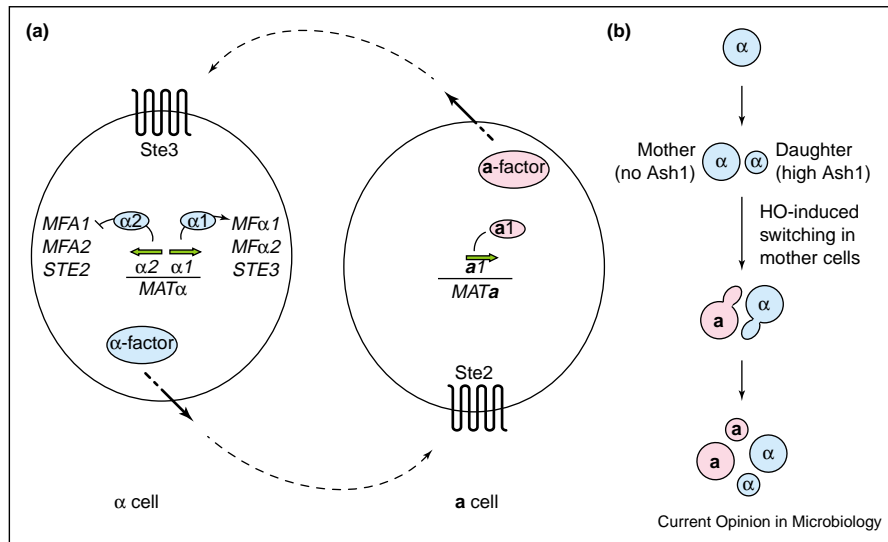
Transition between mating types

Yeast cells normally exist as one of three distinct cell types: two mating-competent haploid variants (**a** and α) and a non-mating **a**/ α diploid, formed by the fusion of the two different kinds of haploid cells. Haploids of one mating-type sense and respond to cells of the opposite mating-type through the action of cell-specific peptide pheromones and pheromone receptors. For example, **a** cells secrete a specific pheromone, called ‘**a**-factor’, that engages the **a**-factor receptor (Ste3), which is expressed exclusively on the surface of α cells; the opposite is true in α cells, which produce α -factor that binds to a receptor (Ste2) that is found only in **a** cells (Figure 1a). These specific pheromone–receptor interactions result in the activation of a common intracellular signaling cascade that leads to an array of cellular responses, including cell-cycle arrest, polarized growth towards the mating partner, and upregulation of genes involved in cell adhesion and fusion [4].

These different mating phenotypes (and their underlying cell type-specific gene expression programs) are ultimately controlled by the master regulatory transcription factors that are encoded at the mating-type (*MAT*) locus [5]. In α cells, the α 1 activator turns on the α -specific genes (such as the **a**-factor receptor gene *STE3* and the *MFA1* and *MFA2* genes encoding α -factor), and α 2 represses the transcription of **a**-specific genes (such as the α -factor receptor gene *STE2* and the **a**-factor-encoding genes *MFA1* and *MFA2*) [6,7]. Because the α 1 and α 2 transcriptional regulators are normally only present in α cells, **a**-specific genes are transcribed exclusively in **a** cells and α -specific genes are expressed only in α cells (Figure 1a).

Interestingly, in most strains found in the wild [strains that carry the *HO* allele (‘homothallic’ strains)], the mating phenotype of haploids is unstable and cells can switch back and forth between the alternative **a** and α states [8,9]. This phenotypic change, termed mating-type switching, is initiated by a gene conversion event at

Figure 1



Mating-type determination and switching in *Saccharomyces cerevisiae*. **(a)** The two haploid mating-types are distinguished by the allele present at the *MAT* locus, which encodes a set of transcription factors ($\alpha 1$, $\alpha 2$, and $a 1$) that regulate the expression of small sets of genes involved in mating. *MAT α* cells express the a -factor receptor Ste3 and secrete the α -factor pheromone that is encoded by the *MFA1* and *MFA2* genes. This mating pheromone engages the Ste2 α -factor receptor expressed specifically on the surface of *MATa* cells. The a cells also produce a -factor, which is the product of the *MFA1* and *MFA2* genes. **(b)** Mating-type switching results in two pairs of closely apposed cells of opposite cell-type. Switching occurs only in the large mother cell, which does not express the repressor Ash1, just before bud emergence. Mother cells and her descendants are along the left-hand side.

the *MAT* locus, in which DNA encoding α information is replaced with a information or vice versa. This genetic swap is triggered by the HO endonuclease, which delivers a double strand break at the active *MAT* locus that is subsequently repaired with sequences copied from transcriptionally silent donor sites found elsewhere on the same chromosome [10,11]. Owing to the complex transcriptional regulation of the *HO* gene, mating-type switching occurs only in certain cells of a mitotic lineage and only at particular stages of the cell cycle. Specifically, the larger of the two mitotic progeny of the asymmetric yeast-cell division, the so-called 'mother' cell, changes *MAT* information just before entering S phase, thereby producing two cells with the same new mating-type. The smaller daughter cell cannot normally switch and therefore divides into two cells with the same mating phenotype as the daughter (Figure 1b) [12]. This mother-daughter asymmetry, with respect to changing cell-type, is a result of the daughter cell-specific accumulation of the Ash1 transcription factor, which functions as a repressor that blocks expression of *HO* and thereby inhibits the mating-type gene exchange [13,14].

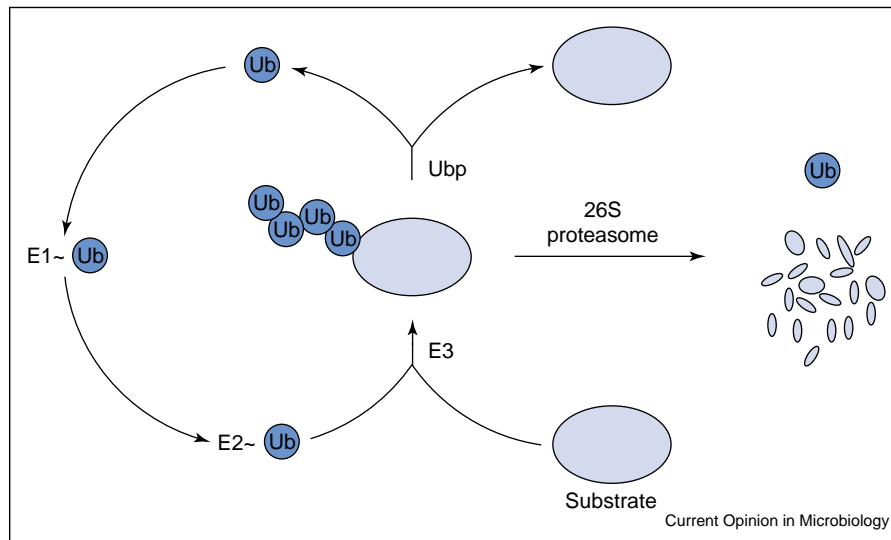
Inactivation of these various cell-type regulators and switching components is also crucial for effective mating-type switching. The genetic change of mating information at the *MAT* locus is tightly coupled to a phenotypic switch in cell type, which is apparent within a single division cycle. Therefore, the previous cell-type deter-

minants must be inactivated somehow because their persistent activity would disrupt the acquisition of the new cellular state. Furthermore, mating-type gene switching occurs during a small temporal window and generates a new *MAT* allele product that is itself a substrate for HO endonuclease-mediated cleavage. This implies that once *HO* gene transcription is initiated, the activity of the *MAT*-specific endonuclease is quickly inactivated. Finally, the daughter cells that were not capable of switching because of Ash1 repressor accumulation become switching-competent mother cells at their next cell division, requiring the inactivation of Ash1 during this single cell-cycle. In all of these cases, proteolysis has been shown to be the mechanism of inactivation of the different regulators, as will be seen in the following sections.

Mat transcription factor degradation

Proteolysis of the three Mat transcription factors occurs by Ub-mediated targeting to the 26S proteasome for degradation [15–17] (Figure 2). All of these proteins are rapidly degraded *in vivo*; the $\alpha 2$ repressor, for example, has a half-life of ~ 5 min and is targeted for ubiquitination by two distinct pathways [18,19] (Table 1). The rapid degradation of $\alpha 2$ by the Ub system is essential for cells to switch effectively from the α to the a mating phenotype, despite the fact that strains with stabilized $\alpha 2$ can undergo the gene conversion process at *MAT* in a completely normal fashion [20^{*}]. This phenotypic

Figure 2



The ubiquitin (Ub)–proteasome pathway for protein degradation. Before it can be conjugated to other proteins, the small polypeptide Ub must be activated by the Ub-activating enzyme E1, generating an E1~Ub intermediate. Activated Ub is then transferred to one of several different Ub-conjugating enzymes (E2), which, in concert with a Ub–protein ligase (E3), catalyzes the formation of a multi-ubiquitin chain on the substrate. These ubiquitinated substrates are targeted to the 26S proteasome for degradation. Substrate degradation is accompanied by the regeneration of free Ub. The multi-ubiquitin chain on a substrate can also be disassembled by Ub-specific processing proteases (Ubp), sparing the substrate from degradation.

switching defect occurs because the persistence of the $\alpha 2$ protein after a *MAT* genetic exchange causes two different failures in the cell type-specific gene expression program of the new differentiated state. First, *a*-specific gene expression is blocked because $\alpha 2$ -containing transcriptional repression complexes are not removed. Second, genes required for mating are repressed because of the inappropriate formation of another $\alpha 2$ -containing regulatory complex. These miscues lead to the ectopic repression of two different sets of genes, both of which need to be expressed for the genetically switched cells to achieve the *a*-cell phenotype [20^{*}]. It is worth noting that homologous mating-type regulatory proteins are produced in the related pathogenic yeast *Candida albicans*, where they are important for mating and virulence [21]. It would be interesting to determine if the regulation of the

stability of these factors plays a role in mating, phenotypic switching and/or pathogenesis.

Pheromone receptor degradation

Similar to the master regulators of mating-type encoded by *MAT*, other cellular factors that specify cell-type must be inactivated if cells are to adopt a new differentiated state after a switch at the *MAT* locus. The pheromone receptors are a primary determinant of mating specificity because they specifically recognize ligands secreted by cells of the opposite mating-type. If the pheromone receptors remained at the cell-surface following mating-type switching, two different responses could occur: co-expression of both pheromone receptors in the same cell would lead to receptor interference that inhibits all pheromone signaling, whereas co-expression of a cognate pheromone receptor–mating pheromone pair in the same cell would cause an autocrine-type response [22–24]. Both of these actions are inappropriate for mating-competent haploid cells. For cells to undergo an effective α -to-*a* switch in mating phenotype, for example, the Ste3 *a*-factor receptor must be removed from the cell surface and replaced with its counterpart, the Ste2 α -factor receptor. Elimination of the receptors, like that of $\alpha 2$ and the other Mat regulators, occurs in a Ub-dependent manner [25,26] (Table 1). However, instead of targeting these factors to the 26S proteasome for destruction, Ub functions in an alternative way, as a signaling molecule that promotes the internalization and trafficking of these

Table 1

E2/E3 enzyme requirements for the Ub-dependent degradation of substrates involved in mating-type switching.

Substrate	E2	E3
$\alpha 2$	Ubc6, Ubc7, Ubc4, Ubc5	Doa10 ?
Ste2	Ubc1, Ubc4, Ubc5	Rsp5
Ste3	Ubc4, Ubc5	?
HO	Rad6, Cdc34	SCF ^{Ufo1}
Ash1	?	SCF ^{Cdc4}

cell-surface proteins to the vacuole for degradation by vacuolar proteases [27].

HO endonuclease degradation

The Ub–proteasome pathway also destroys the HO endonuclease, the enzyme that starts the mating-type switching process. HO is targeted for ubiquitination by a Skp1/Cullin/F-box (SCF)-type E3 Ub–protein ligase [28*,29] (Table 1). The F-box subunits of SCF complexes recruit specific substrates for ubiquitin addition. Before recruitment by F-box factors, targeted proteins are usually modified post-translationally. Often, this modification is phosphorylation. Indeed, the degradation of HO requires the function of two different protein kinases, Mec1 and Chk1 [28*]. Interestingly, these kinases, as well as another protein (Rad9) required for HO proteolysis, are key mediators of a cellular surveillance mechanism known as the DNA-damage checkpoint response. These observations suggest that the endonuclease activity of HO is monitored and directly coupled to its degradation; as soon as the double strand break at *MAT* (created by HO) is detected by the DNA-damage checkpoint proteins, HO is rapidly destroyed. This tight link between the function of HO, which is synthesized only in a narrow window of the G₁ cell-cycle phase, and its turnover might serve to protect the newly switched *MAT* allele from re-cleavage and to ensure that mating-type switching occurs only once per generation, providing a tidy explanation for why both progeny of a switched cell always have the same new mating phenotype [12].

Ash1 degradation

Much like the destruction of HO, rapid degradation of the switching repressor Ash1 allows the activity of the protein to persist for only a short period of time. In wild-type cells, the Ash1 protein is not found in mother cells, but instead localizes to daughter-cell nuclei to inhibit the transcription of *HO*. Ash1 is present in daughter cells only for a brief window and soon after the cell-cycle stage in which it is synthesized, the Ash1 protein is rapidly destroyed [13,14]. An SCF-type E3 Ub–protein ligase complex containing the F-box protein Cdc4 (SCF^{Cdc4}) appears to target Ash1 for Ub-dependent degradation (Q Liu and M Tyers, unpublished) (Table 1). All known substrates of SCF^{Cdc4} are targeted in a phosphorylation-dependent manner; Ash1 is no exception — turnover of this repressor requires the cyclin-dependent kinase Pho85 and Ash1 is directly phosphorylated by Pho85. Strains lacking this kinase no longer exhibit asymmetric localization of Ash1 and the protein is found not only in daughter cells but also in mother cells, where it ectopically inhibits *HO*-mediated mating-type switching. In *pho85* mutants, the Ash1 protein is initially localized to daughter-cell nuclei, but it fails to disappear as these daughter cells proceed through their division cycle and mature into mother cells [30*]. This persistence of Ash1 activity impairs the ability of cells to shed their switching-

incompetent daughter cell phenotype after becoming a mother cell.

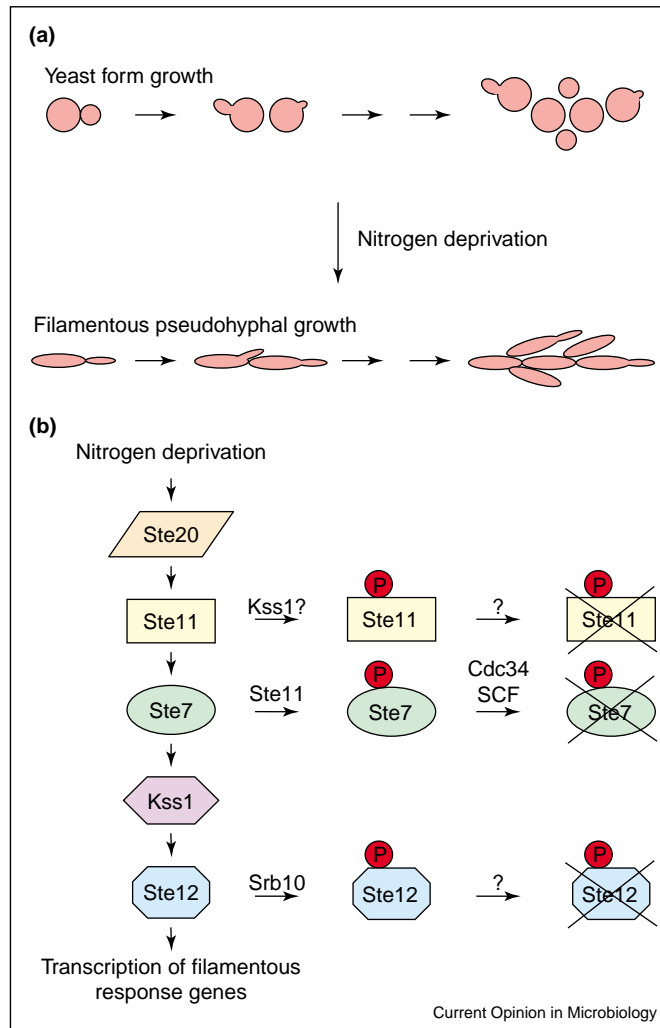
Transition to pseudohyphal growth

Beyond its role in promoting transitions between mating-types, regulated proteolysis is also required for the control of other differentiation events in yeast. For example, in response to nitrogen starvation, yeast cells change their pattern of growth and form filamentous pseudohyphal colonies that are able to forage for nutrients (Figure 3a). Nitrogen-limiting conditions activate a complex signal-transduction pathway that promotes a coordinated response involving cell elongation, lengthening of the G₂/M phase of the cell cycle, a switch in budding pattern and persistent mother–daughter cell adhesion, as well as invasion of the growth substrate [31,32]. Of the many gene products that are implicated in the switch to pseudohyphal growth are proteolytic factors, in particular, components of the E3 Ub–protein ligase SCF^{Grr1}.

Mutations in genes encoding the SCF cullin Cdc53 and the F-box protein Grr1 lead to enhanced filamentous growth [33,34], suggesting that the degradation of one or more SCF^{Grr1} substrates normally inhibits the switch to the pseudohyphal growth state. Several targets of SCF^{Grr1}-mediated proteolysis have been identified, but it remains unclear whether the destruction of any of these factors mediates the change to filamentous growth. For example, Grr1 targets the G₁ cyclins Cln1 and Cln2 for SCF-dependent degradation [35,36] and although these two cyclins are required for pseudohyphal development [37], the hyperfilamentous phenotype of *grr1* mutants is not altered by the inactivation of *CLN1* and *CLN2* [38]. Similarly, the degradation of the cell-polarity factor Gic2 requires SCF^{Grr1} [39], but a role for Gic2 in pseudohyphal differentiation has not been reported.

The yeast Forkhead transcription factors Fkh1 and Fkh2 are also important negative regulators of the transition to a pseudohyphal state: cells lacking both Fkh1 and Fkh2 are constitutively filamentous [40–42]. The Fkh proteins form a transcription factor complex with the Ndd1 co-activator. This complex is required for the expression of the mitotic cyclin gene *CLB2* and a group of co-regulated genes, called the *CLB2* cluster [40–44]. Loss of Fkh–Ndd1 activity alters cell-cycle progression, causing the accumulation of cells in G₂/M, much like *clb*-deficient strains. As mentioned previously, pseudohyphal differentiation is associated with such a G₂/M delay; accordingly, the filamentous morphology of *fkh1Δ fkh2Δ* mutants is suppressed by the overexpression of *CLB2* [40]. Given that many transcriptional regulators are targets of rapid proteolysis, it is tempting to speculate that the Fkh transcription factors are also inactivated by Ub-dependent protein degradation, and that the proteolytic downregulation of these key regulators controls the switch from vegetative to pseudohyphal growth.

Figure 3



(a) Pseudohyphal differentiation in response to nitrogen limitation. The growth pattern of yeast cells changes under nitrogen-limiting conditions. The oval, yeast form of cells divides asymmetrically and exhibits bi-polar budding that switches to an elongated pseudohyphal pattern of growth, which is characterized by synchronous, unipolar budding and persistent mother–daughter adhesion. This results in the formation of filamentous chains of cells (pseudohyphae). **(b)** The MAPK signaling pathway involved in filamentous growth. Degradation of the components Ste11, Ste7 and Ste12 (indicated by 'X') occurs in a phosphorylation-dependent manner by the indicated enzymes.

Interestingly, pseudohyphal differentiation requires many of the same elements of the MAPK signaling cascade that operates during the mating pheromone-response of haploid cells [45,46] (Figure 3b). Two kinases in this signaling module that are shared by these pathways, Ste7 and Ste11, appear to be ubiquitinated and destroyed in response to mating pheromone stimulation [47,48,49] (Figure 3b). Degradation of these signaling proteins serves to limit their activity: upon removal of the upstream stimulus, the signaling pathway quickly returns to its basal, non-stimulated state. Perhaps a similar proteolytic mechanism operates as cells switch back from the filamentous pattern to a yeast form of growth, enabling the rapid resumption of normal vegetative growth after starvation signals are removed. Another component that is

shared by the mating pheromone-response and pseudohyphal growth pathways is the transcription factor Ste12, which functions downstream of the MAPK cascade to regulate both pheromone-inducible and filamentous response genes. The activity of this transcription factor is also dependent upon the cyclin-dependent kinase Srb10, which directly phosphorylates Ste12 and promotes its degradation (Figure 3b). Under the nitrogen-limiting conditions that promote pseudohyphal growth, the Srb10 protein itself appears to be degraded, leading to an increase in the Ste12-dependent transcription of filamentous response genes that presumably reflects the stabilization of Ste12 [50]. Although the precise mechanisms of these proteolytic events remain to be determined, modulation of the stability of both Ste12 and Srb10 appears to

play a key role in the transition between vegetative and pseudohyphal states of growth.

Transition to quiescence

As yeast cultures are grown to saturation and carbon sources are depleted from the growth medium, cells stop proliferating and become quiescent. During this switch to the quiescent state, a coordinated downregulation in the synthesis of many proteins occurs, due to a global shut-down in the transcription of genes encoding ribosomal proteins and RNA [3]. However, examination of the phenotypes of mutants lacking vacuolar-resident proteases or components of the Ub-proteasome pathway indicates that proteolysis is also a necessary element of this cell-state transition.

Cells lacking either of the main vacuolar proteases, Pep4 or Prb1, cannot maintain viability when grown into stationary phase [51], a phenotype similar to *ubi4*, *ubc1* or *ubc5* mutants, which bear defects in genes encoding polyubiquitin or different E2 Ub-conjugating enzymes, respectively [52–54]. Furthermore, during the growth into stationary phase of cells lacking Doa4, Ub is depleted; this is closely followed by cell death [55]. Doa4 is a deubiquitinating enzyme that recycles ubiquitin from ubiquitinated proteins. Thus, robust protein degradation by both the vacuole and the proteasome appears to be essential to maintain the viability of cells as they progress into a quiescent state.

Although proteolysis is necessary for entry into quiescence, prolonged starvation leads to a decrease in Ub-dependent protein degradation [56^{*}]. This reduction in activity correlates with the disassembly of 26S proteasomes into their substituent components, the 20S catalytic and 19S regulatory particles. Although no significant change in the expression levels of individual proteasomal proteins was observed, a time-dependent decrease in 26S proteasome levels, accompanied by a corresponding increase in 20S catalytic particles occurred during incubations in stationary phase. Interestingly, an ‘open channel’ proteasomal mutant, which lacks the N-terminal tails of particular subunits that function to block the channel leading into the catalytic chamber, does not survive extended quiescence and exhibits delayed outgrowth from stationary phase [56^{*}]. Because this mutant exhibits increased basal proteasomal activity that persists abnormally during stationary phase, it was suggested that this sustained degradation eventually targets proteins that are essential for cell viability.

Conclusions

Clearly, selective proteolysis of key regulatory proteins is an effective and commonly used strategy for inactivating a regulatory apparatus during cell-state changes, whether programmed by developmental cues or engendered by environmental stimuli. However, in many of the exam-

ples where protein degradation has been implicated in these transitions, the specific proteolytic substrates are not yet known. Furthermore, the mechanistic reasons why these factors need to be degraded for cells to make successful phenotypic switches are only just becoming clear. An array of experimental methods, from single-cell assays to large-scale proteomic analyses, can be exploited with yeast, so rapid progress in addressing these questions should be made in the coming years. The results will also undoubtedly continue to inform and guide studies on the dynamics of cell differentiation in metazoans.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Haber JE: **Mating-type gene switching in *Saccharomyces cerevisiae***. *Annu Rev Genet* 1998, **32**:561-599.
 2. Mosch HU: **Pseudohyphal development of *Saccharomyces cerevisiae***. *Contrib Microbiol* 2000, **5**:185-200.
 3. Gray JV, Petsko GA, Johnston GC, Ringe D, Singer RA, Werner-Washburne M: **‘Sleeping beauty’: quiescence in *Saccharomyces cerevisiae***. *Microbiol Mol Biol Rev* 2004, **68**:187-206.
 4. Sprague GF, Thorner JW: **Pheromone response and signal transduction during the mating process of *Saccharomyces cerevisiae***. In *The Molecular and Cellular Biology of the Yeast *Saccharomyces**. Edited by Jones EW, Pringle JR, Broach JR. Cold Spring Harbor Laboratory Press; 1992, 2:657-744.
 5. Strathern J, Hicks J, Herskowitz I: **Control of cell type in yeast by the mating type locus. The alpha 1-alpha 2 hypothesis**. *J Mol Biol* 1981, **147**:357-372.
 6. Sprague GF Jr, Jensen R, Herskowitz I: **Control of yeast cell type by the mating type locus: positive regulation of the alpha-specific STE3 gene by the MAT alpha 1 product**. *Cell* 1983, **32**:409-415.
 7. Johnson AD, Herskowitz I: **A repressor (MAT alpha 2 Product) and its operator control expression of a set of cell type specific genes in yeast**. *Cell* 1985, **42**:237-247.
 8. Oshima Y, Takano I: **Mating types in *Saccharomyces*: their convertibility and homothallism**. *Genetics* 1971, **67**:327-335.
 9. Hicks JB, Herskowitz I: **Interconversion of yeast mating types. I. Direct observations of the action of the homothallism (HO) gene**. *Genetics* 1976, **83**:245-258.
 10. Strathern JN, Klar AJ, Hicks JB, Abraham JA, Ivy JM, Nasmyth KA, McGill C: **Homothallic switching of yeast mating type cassettes is initiated by a double-stranded cut in the MAT locus**. *Cell* 1982, **31**:183-192.
 11. Hicks JB, Strathern JN, Herskowitz I: **The cassette model of mating-type interconversion**. In *DNA Insertion Elements, Plasmids, and Episomes*. Edited by Bukhari AI, Shapiro JA, Adhya SL. Cold Spring Harbor Laboratory; 1977:457-462.
 12. Strathern JN, Herskowitz I: **Asymmetry and directionality in production of new cell types during clonal growth: the switching pattern of homothallic yeast**. *Cell* 1979, **17**:371-381.
 13. Bobola N, Jansen RP, Shin TH, Nasmyth K: **Asymmetric accumulation of Ash1p in postanaphase nuclei depends on a myosin and restricts yeast mating-type switching to mother cells**. *Cell* 1996, **84**:699-709.
 14. Sil A, Herskowitz I: **Identification of asymmetrically localized determinant, Ash1p, required for lineage-specific transcription of the yeast HO gene**. *Cell* 1996, **84**:711-722.

15. Hochstrasser M, Varshavsky A: **In vivo degradation of a transcriptional regulator: the yeast $\alpha 2$ repressor.** *Cell* 1990, **61**:697-708.
16. Johnson PR, Swanson R, Rakhilina L, Hochstrasser M: **Degradation signal masking by heterodimerization of MAT α 2 and MAT α 1 blocks their mutual destruction by the ubiquitin-proteasome pathway.** *Cell* 1998, **94**:217-227.
17. Johnson PR: **Regulated turnover of the *Saccharomyces cerevisiae* MAT transcription factors [Ph.D. Thesis].** Chicago, IL: University of Chicago: 1997.
18. Chen P, Johnson P, Sommer T, Jentsch S, Hochstrasser M: **Multiple ubiquitin-conjugating enzymes participate in the in vivo degradation of the yeast MAT α 2 repressor.** *Cell* 1993, **74**:357-369.
19. Swanson R, Locher M, Hochstrasser M: **A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matalpha2 repressor degradation.** *Genes Dev* 2001, **15**:2660-2674.
20. Laney JD, Hochstrasser M: **Ubiquitin-dependent degradation of the yeast Mat α 2 repressor enables a switch in developmental state.** *Genes Dev* 2003, **17**:2259-2270.
- This paper examines the biological consequences of stabilizing $\alpha 2$, a key determinant of yeast cell-type, during mating-type switching. Mutants defective in $\alpha 2$ degradation switch the allele present at the MAT locus, but the acquisition of the new a-cell phenotype is strongly impaired. The detailed knowledge of mating-type regulatory circuitry allowed a precise mechanistic explanation for why the elimination of a previously used regulatory protein is needed to effect a change in cellular state.
21. Soll DR: **Mating-type locus homozygosity, phenotypic switching and mating: a unique sequence of dependencies in *Candida albicans*.** *Bioessays* 2004, **26**:10-20.
22. Bender A, Sprague GF Jr: **Yeast peptide pheromones, a-factor and alpha-factor, activate a common response mechanism in their target cells.** *Cell* 1986, **47**:929-937.
23. Bender A, Sprague GF Jr: **Pheromones and pheromone receptors are the primary determinants of mating specificity in the yeast *Saccharomyces cerevisiae*.** *Genetics* 1989, **121**:463-476.
24. Nakayama N, Miyajima A, Arai K: **Common signal transduction system shared by STE2 and STE3 in haploid cells of *Saccharomyces cerevisiae*: autocrine cell-cycle arrest results from forced expression of STE2.** *EMBO J* 1987, **6**:249-254.
25. Hicke L, Riezman R: **Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis.** *Cell* 1996, **84**:277-287.
26. Roth AF, Davis NG: **Ubiquitination of the yeast a-factor receptor.** *J Cell Biol* 1996, **134**:661-674.
27. Hicke L: **Gettin' down with ubiquitin: turning off cell-surface receptors, transporters and channels.** *Trends Cell Biol* 1999, **9**:107-112.
28. Kaplun L, Ivantsiy Y, Kornitzer D, Raveh D: **Functions of the DNA damage response pathway target Ho endonuclease of yeast for degradation via the ubiquitin-26S proteasome system.** *Proc Natl Acad Sci USA* 2000, **97**:10077-10082.
- Systematic testing of mutations in the Ub-proteasome pathway reveals that the Ub-conjugating enzymes Rad6 and Cdc34 and an SCF-type Ub-protein ligase are necessary for the rapid degradation of the HO endonuclease. In addition, protein kinases with a role in the DNA-damage checkpoint response are implicated in HO turnover, suggesting a link between the degradation of HO and its enzymatic activity.
29. Kaplun L, Ivantsiy Y, Bakhrat A, Raveh D: **DNA damage response-mediated degradation of Ho endonuclease via the ubiquitin system involves its nuclear export.** *J Biol Chem* 2003, **278**:48727-48734.
30. McBride HJ, Sil A, Measday V, Yu Y, Moffat J, Maxon ME, Herskowitz I, Andrews B, Stillman DJ: **The protein kinase Pho85 is required for asymmetric accumulation of the Ash1 protein in *Saccharomyces cerevisiae*.** *Mol Microbiol* 2001, **42**:345-353.
- Beginning with the observation that *pho85* mutants show an Ash1-dependent decrease in HO mRNA levels, this paper demonstrates that Ash1 is improperly localized to both mother and daughter cells in *pho85* mutants. Ash1 initially localizes to daughters, as in wild-type cells, but then persists as the mutant cells mature into mother cells. Ash1 is phosphorylated directly by Pho85 and appears to be degraded in a phosphorylation-dependent manner. This clears Ash1 from the cell so that a new phenotypic state can be adopted.
31. Pan X, Harashima T, Heitman J: **Signal transduction cascades regulating pseudohyphal differentiation of *Saccharomyces cerevisiae*.** *Curr Opin Microbiol* 2000, **3**:567-572.
32. Gancedo JM: **Control of pseudohyphae formation in *Saccharomyces cerevisiae*.** *FEMS Microbiol Rev* 2001, **25**:107-123.
33. Blacketer MJ, Madaule P, Myers AM: **Mutational analysis of morphologic differentiation in *Saccharomyces cerevisiae*.** *Genetics* 1995, **140**:1259-1275.
34. Palecek SP, Parikh AS, Kron SJ: **Genetic analysis reveals that FLO11 upregulation and cell polarization independently regulate invasive growth in *Saccharomyces cerevisiae*.** *Genetics* 2000, **156**:1005-1023.
35. Barral Y, Jentsch S, Mann C: **G1 cyclin turnover and nutrient uptake are controlled by a common pathway in yeast.** *Genes Dev* 1995, **9**:399-409.
36. Patton EE, Willems AR, Sa D, Kuras L, Thomas D, Craig KL, Tyers M: **Cdc53 is a scaffold protein for multiple Cdc34/Skp1/F-box protein complexes that regulate cell division and methionine biosynthesis in yeast.** *Genes Dev* 1998, **12**:692-705.
37. Loeb JD, Kerentseva TA, Pan T, Sepulveda-Becerra M, Liu H: ***Saccharomyces cerevisiae* G1 cyclins are differentially involved in invasive and pseudohyphal growth independent of the filamentation mitogen-activated protein kinase pathway.** *Genetics* 1999, **153**:1535-1546.
38. Hsiung YG, Chang HC, Pellequer JL, La Valle R, Lanker S, Wittenberg C: **F-box protein Grr1 interacts with phosphorylated targets via the cationic surface of its leucine-rich repeat.** *Mol Cell Biol* 2001, **21**:2506-2520.
39. Jaquenoud M, Gulli MP, Peter K, Peter M: **The Cdc42p effector Gic2p is targeted for ubiquitin-dependent degradation by the SCFGrr1 complex.** *EMBO J* 1998, **17**:5360-5373.
40. Hollenhorst PC, Bose ME, Mielke MR, Muller U, Fox CA: **Forkhead genes in transcriptional silencing, cell morphology and the cell cycle. Overlapping and distinct functions for FKH1 and FKH2 in *Saccharomyces cerevisiae*.** *Genetics* 2000, **154**:1533-1548.
41. Zhu G, Spellman PT, Volpe T, Brown PO, Botstein D, Davis TN, Futcher B: **Two yeast forkhead genes regulate the cell cycle and pseudohyphal growth.** *Nature* 2000, **406**:90-94.
42. Kumar R, Reynolds DM, Shevchenko A, Goldstone SD, Dalton S: **Forkhead transcription factors, Fkh1p and Fkh2p, collaborate with Mcm1p to control transcription required for M-phase.** *Curr Biol* 2000, **10**:896-906.
43. Koranda M, Schleiffer A, Endler L, Ammerer G: **Forkhead-like transcription factors recruit Ndd1 to the chromatin of G2/M-specific promoters.** *Nature* 2000, **406**:94-98.
44. Pic A, Lim FL, Ross SJ, Veal EA, Johnson AL, Sultan MR, West AG, Johnston LH, Sharrocks AD, Morgan BA: **The forkhead protein Fkh2 is a component of the yeast cell cycle transcription factor SFF.** *EMBO J* 2000, **19**:3750-3761.
45. Liu H, Styles CA, Fink GR: **Elements of the yeast pheromone response pathway required for filamentous growth of diploids.** *Science* 1993, **262**:1741-1744.
46. Roberts RL, Fink GR: **Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth.** *Genes Dev* 1994, **8**:2974-2985.
47. Wang Y, Dohlman HG: **Pheromone-dependent ubiquitination of the mitogen-activated protein kinase kinase Ste7.** *J Biol Chem* 2002, **277**:15766-15772.
48. Wang Y, Ge Q, Houston D, Thorner J, Errede B, Dohlman HG: **Regulation of Ste7 ubiquitination by Ste11 phosphorylation and the Skp1-Cullin-F-box complex.** *J Biol Chem* 2003, **278**:22284-22289.

49. Esch RK, Errede B: **Pheromone induction promotes Ste11 degradation through a MAPK feedback and ubiquitin-dependent mechanism.** *Proc Natl Acad Sci USA* 2002, **99**:9160-9165.

Induction of the mating signal-transduction pathway by pheromone leads to the Ub-dependent turnover of Ste11, a MEK kinase. Ste11 degradation depends on downstream signaling components, suggesting a negative feedback loop where the output of the signaling pathway blocks further signaling. The authors also show that the characteristic desensitization of the signaling pathway in response to continued stimulation is also a Ub-dependent process, possibly occurring through the ubiquitination and degradation of Ste11.

50. Nelson C, Goto S, Lund K, Hung W, Sadowski I: **Srb10/Cdk8 regulates yeast filamentous growth by phosphorylating the transcription factor Ste12.** *Nature* 2003, **421**:187-190.

The data in this paper demonstrate that the transcription factor Ste12, a key regulator of both mating pheromone-response and filamentous growth pathways, is destabilized by Srb10-mediated phosphorylation. Although the phosphorylation of Ste12 by Srb10 inhibits filamentous growth, it has no effect on the mating pheromone-response pathway. Furthermore, the kinase activity of Srb10 is not affected by mating pheromone, but is dramatically inhibited by nitrogen limitation because the Srb10 protein is depleted under these conditions.

51. Teichert U, Mechler B, Muller H, Wolf DH: **Lysosomal (vacuolar) proteinases of yeast are essential catalysts for protein degradation, differentiation, and cell survival.** *J Biol Chem* 1989, **264**:16037-16045.

52. Finley D, Özkaynak E, Varshavsky A: **The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses.** *Cell* 1987, **48**:1035-1046.

53. Seufert W, McGrath JP, Jentsch S: **UBC1 encodes a novel member of an essential subfamily of yeast ubiquitin-conjugating enzymes involved in protein degradation.** *EMBO J* 1990, **9**:4535-4541.

54. Seufert W, Jentsch S: **Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of short-lived and abnormal proteins.** *EMBO J* 1990, **9**:543-550.

55. Swaminathan S, Amerik AY, Hochstrasser M: **The Doa4 deubiquitinating enzyme is required for ubiquitin homeostasis in yeast.** *Mol Biol Cell* 1999, **10**:2583-2594.

56. Bajorek M, Finley D, Glickman MH: **Proteasome disassembly and downregulation is correlated with viability during stationary phase.** *Curr Biol* 2003, **13**:1140-1144.

In this paper, the authors observe a decrease in proteasomal proteolysis after the prolonged starvation of cells. They correlate this to the disassembly of the 26S proteasome holoenzyme. The paper also shows that constitutively 'open' mutants of the proteasome exhibit enhanced peptidase activity and Ub-dependent proteolysis, even after growth into stationary phase. These mutants do not survive prolonged starvation, implying an important role for down-regulating the proteasome during quiescence.