Changes in developmental state: demolish the old to construct the new

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Development, along with other cellular changes of state, can be conceived as the sequential adoption of stable, epigenetically determined patterns of gene expression. The article by Laney and Hochstrasser (2003) in this issue is the most recent to exploit the Saccharomyces cerevisiae mating-type system to characterize molecular mechanisms that act when cells change gene expression and cellular state. They examine the relationship between transcriptional regulators of mating type and components of the ubiquitin-mediated protein degradation pathway, and demonstrate that proteolysis is required not only to end one program of gene expression, but also to prevent an erroneous subsequent transcriptional state.

Laney and Hochstrasser (2003) conclude that ubiquitin-mediated proteolysis of the Matα2 transcriptional repressor is fundamentally required for the developmental transition that is the yeast mating-type switch. Their work is dedicated to the memory of Ira Herskowitz, and this is a fitting tribute because he did so much to further our understanding of mating-type switching and the regulation of mating type by regulatory factors. Herskowitz coined the term “master regulatory” gene (Herskowitz 1985) and applied it to the regulators of mating type; this term has also been adopted for critical transcriptional regulators in metazoan development (Weintraub et al. 1989, Baker 2001).

Regulation of mating type

Saccharomyces lives vegetatively in either the haploid or the diploid state. Haploids are found in two mating types, MATa or MATα, which mate with each other to form the preferred diploid cell type. In 1981, Herskowitz and colleagues proposed that the MATα and MATa loci encode regulatory molecules that control the expression of other genes that determine the phenotype of cells [Strathern et al. 1981]. They proposed that in α cells, Mata1 functioned as an activator, whereas Matα2 functioned as a repressor, and that in diploids, Mata1 and Matα2 worked together as the α1/α2 repressor [Fig. 1]. This α1/α2 hypothesis was a remarkably prescient prediction, as it was based solely on genetic analysis. Subsequent cloning and sequencing of the MAT genes showed that they encoded homeodomain transcription factors, and that these proteins recognized sites in the promoters of the relevant target genes.

Phenotypically, α and α cells differ from each other only in the expression of genes controlling the production and sensing of mating pheromones. α-specific genes are expressed only in α cells, but these genes are repressed in α cells by α2, along with the abundant and pleiotropic regulator Mcm1 and the Ssn6 and Tup1 co-repressors (Fig. 1; for review, see Sprague 1990). α-specific genes are expressed only in α cells, because they are activated by α1, also in concert with Mcm1. In diploid cells, haploid-specific genes, required for mating or to block meiosis, are repressed by the heterodimeric α1/α2 transcription factor, composed of one protein expressed from MATα and one from MATa. α1/α2 also recruits Mcm1 and Ssn6-Tup1 in order to repress haploid-specific genes in diploid cells. In α cells, α-specific genes are expressed without any mating-type specific factor, as the α cell type is a default ground state. Thus Mata1 has no role in α haploid cells, but functions only in the diploid heterodimeric context.

Mating-type switching

Genetically, haploid yeast can switch to the opposite mating type by changing the identity of the MAT locus DNA from that encoding the Mata1 transcription factor to one encoding Matα1 and Matα2, or vice versa [Haber 1998]. This switching is very efficient, occurring every generation in the larger mother cells arising from the asymmetric mitosis in yeast. This genetic change is a gene-conversion event initiated by the HO endonuclease. [Diploids do not switch because HO is a haploid-specific gene, one of the targets of α1/α2 diploid repression.] The HO endonuclease cleaves at the transcriptionally active MAT locus, already containing either α- or α-transcription factor-encoding information, and this double-stranded break is repaired by gene conversion using one of two transcriptionally silent loci, HMLα or HMRα, that contain information for the regulatory genes. Note that the vast majority of laboratory yeast strains are heterothallic, containing a mutation in the HO gene [Meiron et al. 1995], or else it would be impossible to maintain stable haploid strain stocks. However,
Figure 1. Control of mating type by MAT. In a MATα haploid cell, the Mata1 protein activates expression of α-specific genes, and the Mata1 protein repression expression of α-specific genes. Expression of haploid-specific genes does not require any MAT-encoded activator. In a MATα haploid cell, the Mata1 protein is expressed, but it is not required to specify the MATα phenotype, as α-specific genes and haploid-specific genes are expressed by constitutive activators, and the α-specific genes are not expressed due to the absence of their activators. In a/α diploids, the Mata1 and Mata2 proteins form the α1/α2 heterodimer that blocks expression of haploid-specific genes. α1/α2 also represses expression of MATα1, and as the Mata1 protein is not expressed, there is no activator for the α-specific genes. Additionally, the Mata2 protein blocks expression of α-specific genes.

Laney and Hochstrasser (2003) used homothallic strains that switch mating type, allowing sensitive in vivo assays for MAT protein function.

Haploid yeast life history usually consists of rapid homothallic switching of mating type, followed by rapid mating to form a diploid, which is the predominant natural and presumably preferred form. The mechanism of cell-type specification utilizes Mata2 as a potent repressor in two contexts [Fig. 1]. Both cause difficulties for the newly switched MATα cell in preparing to mate and diploidize. Thus, a change in mating cell type must proceed not only by the initiation of a new phenotypic expression program, but also by the inactivation of the determinants of the prior cell type. Herskowitz predicted that many of the components involved in cell-type specification, including cell-surface receptors and regulatory proteins such as Mata2 would, by necessity, be unstable proteins [Herskowitz 1986]. In fact, the half-life of Mata2 is only about 5 min [Hochstrasser and Varshavsky 1990].

Ubiquitin-mediated protein degradation

Conjugation of ubiquitin to a protein usually leads to formation of a mult ubiquitin chain, resulting in degradation of the protein by the proteasome [Hershko and Ciechanover 1998]. Protein ubiquitylation occurs via a multienzyme cascade. First, the 76-amino acid ubiquitin polypeptide is activated by covalent attachment to the E1 ubiquitin-activating enzyme, and then transferred to an E2 ubiquitin-conjugating enzyme. Finally, the E2 ubiquitin conjugate and an E3 ubiquitin-protein ligase transfer the ubiquitin to the substrate protein. Most cells have many different E2 and E3 enzymes, which are responsible for the specificity in substrate recognition. The spectrum of specificity is probably enhanced by combinatorial relationships among the E2s and E3s. In Saccharomyces, there are at least three E1 ubiquitin-activating enzymes, 13 E2 ubiquitin-conjugating enzymes [Ubc], and eight E3 ubiquitin-protein ligases [Hochstrasser 1996]. Protein degradation-mediated cellular processes include mitotic progression, DNA repair, stress response, signal transduction, metabolic regulation, and epigenetic developmental programming and differentiation. Not all of these processes are dependent on the well-characterized final resting place of many ubiquitylated proteins, the 26S proteasome, nor are all protein-degradation processes mediated by ubiquitin.

The unstable Mata2 protein is ubiquitylated

It was shown previously that the Mata2 protein is unstable in vivo, and that it is ubiquitylated prior to destruction [Hochstrasser et al. 1991]. Two distinct ubiquitin-addition pathways act on Mata2, one utilizes the E2s Ubc4 and Ubc5, but the determinants of interaction within Mata2 are unknown. The second system works through the E2s Ubc6 and Ubc7, and recognizes a signal in Mata2 termed Deg1, containing an exposed hydrophobic surface of an amphipathic helix at its N terminus. Using a sensitive genetic selection scheme based on stabilization of the Deg1 domain, the novel E3-encoding DOA10 [degradation of alpha2] gene was isolated as necessary for the turnover of Deg1-containing proteins [Chen et al. 1993]. Comfortingly, mutations in ubc6 and ubc7 were also recovered in this screen. Doa10 activity is specific to the second Deg1-dependent pathway.

Now, Laney and Hochstrasser (2003) investigate the relative contributions of these two degradation pathways to Mata2 turnover. They find that although either the ubc4 or the ubc6 [also doa10] single mutations have minor effects on stabilization of Mata2, the remaining pathway that is still active elicits near-normal degradation rates. Only in the ubc4 ubc6 [or ubc4 doa10] double-mutant strains is Mata2 significantly more prevalent, both at the level of bulk Mata2 protein, as well as that fraction bound to the chromatin of repressed genes in vivo. Most importantly, they show that there are phenotypic consequences in these doubly mutant strains, resulting in a defect in diploidization through mating after a mating-type switch. This phenotype is specific to Mata2 stabilization and not to hyperactivation or stabilization of some other aspect of the switching or mating process.

Mata2 stabilization prevents mating after a mating-type switch

To further analyze these phenotypic consequences of Mata2 stabilization, Laney and Hochstrasser [2003] used...
several assays for mating function. Homothallic strains, with the wild-type HO gene, are competent as haploids to switch mating type in either direction, but in diploids HO is not expressed and switching does not occur [Nasmyth 1993]. Homothallic diploids are induced to undergo meiosis, haploid spores are isolated and germinated, and after sufficient growth to generate a colony, their mating ability can be sensitively assessed with a bioassay. Homothallic haploid spores efficiently switch mating type, then mate with closely apposed siblings during outgrowth of the germinated spore, and thus form diploids. This is evidenced by their virtually complete inability to mate with haploid tester cells, as genes required for mating are repressed in diploids by a1/a2. [Only cells that can mate with the tester strain form colonies in this assay, because the resulting diploids have complementing auxotrophic nutritional markers.] In contrast, ubc4 ubc6 or ubc4 doa10 double-mutant strains form colonies at high efficiency in this mating assay, because they mate with the tester strain, indicating their previous failure to efficiently switch mating type phenotypically, and thus mate with siblings. Thus, the ubc/doa strains containing stabilized Mata2 are defective in diploidization, which requires a rapid change in cellular phenotype.

A second mating assay used to investigate the phenotypic effect of Mata2 stabilization is the single-cell bioassay known as the α-factor pheromone confrontation assay [Hicks and Herskowitz 1976]. Homothallic diploids are sporulated and germinated in the presence of α-factor. One half of the spores will be of the MATα mating type, and these cells will respond to α-factor by arresting in G1 of the cell cycle and forming a microscopically observable change in cell shape known as shmoos. In this assay, we are interested in what happens to the MATα spores. MATα cells are insensitive to α-factor, and after germination, they divide into two cells. After each mitosis, in the next G1 phase, one of the two cells will undergo a mating-type switch. The switch always occurs in the mother cell, the larger of the two mitotic progeny [Strathern and Herskowitz 1979]. A mating-type switching event in an α cell can be scored, because after this cell divides, it gives rise to two α cells that arrest in response to the α-factor and form shmoos. Laney and Hochstrasser [2003] found that the increased Mata2 stability in the ubc4 ubc6 double-mutant blocks mating-type switching. Importantly, whereas phenotypic switches from α to a mating type are inhibited in the ubc4 ubc6 mutant, the reverse switches from a to α mating type occur normally. Additionally, switching was normal in the ubc4 and ubc6 single mutants.

Mata2 stabilization affects switching in two ways

The authors investigate the mechanism of the switching defect caused by the long-lived Mata2 protein. Mata2 regulates gene expression in two ways, by repressing a-specific genes in α cells, and by repressing haploid-specific genes by the a1/a2 heterodimer [Fig. 1]. Thus, a defect in degradation of Mata2 could result in two types of misprogramming in the new MATα state that would block mating competence. A continued repression of a-specific genes or an adoption of a pseudo-diploid state due to a1/a2-based repression of haploid-specific genes.

One a-specific gene is STE2, encoding the α-pheromone receptor, whose expression is critically required for mating in MATα cells. To address whether a failure to express STE2 underlies the mating defect in the strains with stabilized Mata2, STE2 was ectopically expressed from a different promoter and found to provide modest rescue of the phenotypic switching defect. To test the role of haploid-specific repression by inappropriately stabilized Mata2 present in a1/a2 heterodimers, the MATa1 gene was deleted from the transcriptionally silent HMRα locus. As HMRα is the donor for the mating-type transposition, gene conversion will produce a switched cell in which there is no Mata1 protein. This experiment also allows modest rescue of the ubc4 doa10 switching defect. Importantly, combining both the ectopically expressed STE2 and the MATa1 deletion in the same ubc4 doa10 strain results in essentially wild-type levels of switching. This is an incisive observation, indicating that both types of inappropriate repression by the remaining Mata2 must be relieved for the new developmental program to be manifested. Thus, in strains with stabilized Mata2, repression of a-specific genes by Mata2 and haploid-specific genes by a1/a2 both contribute to the mating defect.

Ubiquitin-mediated destruction of transcription factors

The majority of transcription factors are unstable, and for many of these factors, it has been shown that degradation occurs following ubiquitination [Conaway et al. 2002; Muratani and Tansey 2003]. It may be that tran-
scription factors with an intrinsically short half life are important to allow cells to transition from one developmental state to another, as observed for yeast cells switching mating type. Additionally, recent work suggests that ubiquitylation of transcription factors plays an important positive role in transcriptional activation per se, and there is a correspondence between transcriptional activation domains and degrons, sequences that promote degradation (Salghetti et al. 2000, 2001). The Rsp5/hPRF1 E3 ubiquitin ligase function as coactivators for the steroid-hormone receptor family of transcription factors (Imhof and McDonnell 1996). Salghetti et al. [2001] showed that a met30 mutation that prevents ubiquitylation of the artificial LexA-VP16 activator results in protein stabilization, but this mutation also eliminates the ability of the protein to function as an activator. Moreover, the Gal4 activator recruits the 19S regulatory particle of the proteosome to the promoter (Gonzalez et al. 2002). Taken together, these results have generated significant new details of the roles of ubiquitination in transcription regulation.

However, the situation with Mata2 is different, as this protein functions as a repressor, and thus does not have an activation domain. Further work will be needed to understand the difference between unstable activators and unstable repressors, as both are ubiquitylated before destruction, but ubiquitylation apparently contributes to the activation potential for activators. Nonetheless, it seems likely for both activators and repressors that inherent instability is important for cells to be able to change programs of gene expression during development.

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