

Mother and Daughter Are Doing Fine: Asymmetric Cell Division in Yeast

Minireview

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The underlying principle of every developmental program is that single cells divide to give rise to daughter cells with different developmental fates. This is true not only for the fertilized egg that develops into an adult, but also for the yeast cell that buds. In general, two mechanisms can account for this phenomenon. Differences between daughter cells may originate from differences in their environment. For example, vulva formation in *Caenorhabditis elegans* requires cell–cell interaction and signaling between equipotent cells (reviewed by Kenyon, 1995). Alternatively, differences between-daughter cells can be generated by unequal segregation of cell fate determinants during cell division. Examples of this process include cell division in the bacterium *Caulobacter crescentus*, whereby a nonmotile stalked cell divides into a motile swarmer cell and another stalked cell, spore formation in *Bacillus subtilis* (reviewed by Shapiro, 1993), and sensory organ formation in *Drosophila melanogaster* (reviewed by Rhyu and Knoblich, 1995).

One of the best studied examples of asymmetric segregation of cell fate is mating-type switching in the budding yeast *S. cerevisiae*. Haploid yeast cells exist in either of two mating types, *a* or α . Mother cells, defined as cells which have budded in the previous cell cycle and have thus given birth to a daughter cell, typically switch their mating type (Figure 1A). Daughter cells, on the other hand, do not switch mating type. This results in a close apposition of cells of opposite mating type and thus promotes mating and the formation of *a*/ α diploid cells (Figure 1B; reviewed by Herskowitz, 1988; Nasmyth, 1993). Mating-type switching is ultimately accomplished by a gene conversion event in which *a*- or α -specific sequences at the mating-type locus (*MAT*) are replaced by information specifying the opposite mating type, which resides in a transcriptionally silent region elsewhere in the genome (reviewed by Nasmyth, 1993; Herskowitz et al., 1992). The Ho endonuclease initiates this mating-type interconversion and, consistent with this role, is expressed only in mother cells (Nasmyth, 1983). Restriction of Ho activity to mother cells is largely if not solely determined by the pattern of *HO* transcription. The gene is transiently transcribed only in mother cells and only late in the G1 phase of the cell cycle. Thus, determining how *HO* gene expression is confined to mother cells seems to be the key to understanding why only mother cells switch mating type.

Two regions in the *HO* promoter are responsible for restricting *HO* expression to the late G1 phase of the cell cycle and to mother cells. *URS2* (upstream regulatory sequence 2) confines *HO* expression to late G1, whereas *URS1* is necessary for mother cell specificity. At least ten regulatory factors, designated Swi1p–Swi10p, have been identified that are required for correct expression

of the *HO* gene. Among these, Swi4p and Swi6p confer cell cycle regulation through *URS2* (reviewed by Koch and Nasmyth, 1994) and the zinc finger transcription factor Swi5p activates *HO* transcription in mother cells via the *URS1* element (Tebb et al., 1993, and references therein). None of these regulatory factors, however, seems to be the primary or sole determinant for the asymmetric expression of the *HO* gene. At first, Swi5p had appeared to be a promising candidate because *SWI5* is required for *HO* expression in mother cells and because either constitutive expression of *SWI5* (Lydall et al., 1991) or the stabilization of Swi5p by mutation (Tebb et al., 1993) causes a certain fraction of daughter cells to switch mating type. However, Swi5p is found in both mother and daughter cells (Figure 2A) and activates transcription of genes other than *HO* equally well in both mother and daughter cells (Koch and Nasmyth, 1994). Thus, some other factor, either a mother cell-specific activator or a daughter cell-specific repressor, must exist to generate the asymmetric expression of *HO*.

In this issue of *Cell*, the Herskowitz (Sil and Herskowitz, 1996) and Nasmyth (Bobola et al., 1996) groups report an important advance in understanding how *HO* asymmetry is generated: they have identified a negative regulator of *HO* expression that is daughter cell specific. This factor, termed Ash1p (for asymmetric synthesis of *HO*), preferentially accumulates in the daughter cell nucleus. Findings by Jansen et al. (1996 [this issue of *Cell*]) may provide a clue as to how Ash1p becomes localized in this way: an unconventional myosin is required for the preferential accumulation of Ash1p in daughter cells,

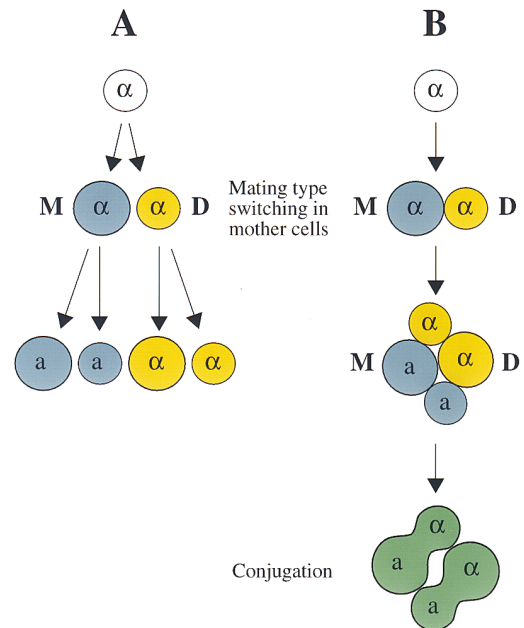


Figure 1. The Pattern of Mating-Type Switching in Budding Yeast (A) Pattern of mating-type switching. Mother (M) but not daughter (D) cells generate two descendant cells with switched mating types. (B) Mating-type switching facilitates zygote formation.

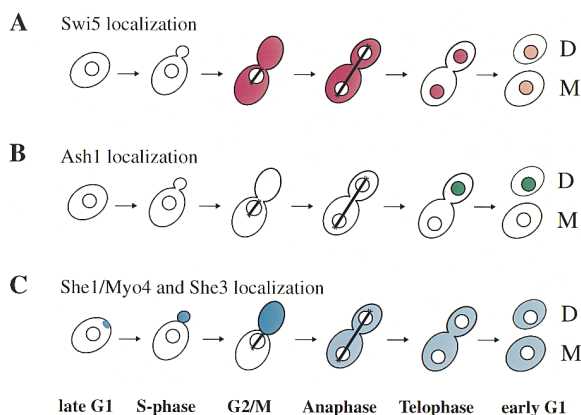


Figure 2. Temporal and Spatial Localization of Swi5p, Ash1p, She1p/Myo4p, and She3p

(A) During G2, *SWI5* RNA synthesis commences and the protein accumulates in the cytoplasm. Shortly after completion of anaphase, the protein enters the nucleus, where it is unstable. By late G1, most but not all Swi5p has been degraded (indicated by light red; Tebb et al., 1993).

(B) Ash1p is absent during early stages of the cell cycle, but shortly after completion of anaphase it preferentially accumulates in the nucleus located in the bud. The protein remains present in G1 daughter (D) cells, but rapidly disappears as daughter cells enter the cell cycle.

(C) She1p/Myo4p and She3p are evenly distributed in early G1, but shortly before entry into the cell cycle they accumulate in a patch, perhaps marking the presumptive bud site. Until late anaphase, the proteins are predominantly localized to the growing bud. Shortly prior to completion of anaphase, the protein becomes evenly distributed between mother (M) and daughter (D) cell.

suggesting that actin-based transport may play a role in generation of *HO* asymmetry.

Three Mutant Hunts Converge on *ASH1*

Three mutant hunts led to the identification of *ASH1*. Although conceptually different, all three were based on the same hypothesis: a daughter cell-specific repressor might be responsible for asymmetric *HO* expression, so that inactivation of such a repressor would lead to expression of *HO* in daughter cells, allowing them to switch mating type.

The screen employed by Sil and Herskowitz (1996) took advantage of the fact that cells exhibit a visible morphological change when exposed to mating pheromone secreted by cells of opposite mating type. In response to α factor pheromone, a cells stop dividing and differentiate into gametes that form a mating projection (known as a shmoo). α Cells respond to a factor pheromone in the same way. Sil and Herskowitz (1996) carried out a direct screen for daughter cells that can switch mating type. Under conditions in which wild-type cells form microcolonies containing both shmoos and budded cells, these mutants were identified because they form distinctive microcolonies containing four shmoos.

Bobola and coworkers (1996) performed two separate genetic selections to identify genes involved in establishing *HO* asymmetry. In the first selection, they generated a fusion between the *HO* promoter and a gene essential for cell division, the *CDC6* gene. The *HO* promoter restricts expression of *CDC6* to mother cells. Thus, cells whose endogenous *CDC6* gene has been

replaced by an *HO-CDC6* fusion cannot form colonies because daughter cells lack this essential gene product. However, mutant cells that express *HO* inappropriately in daughter cells should divide and form a colony.

The rationale for their second mutant hunt was based on findings by Jansen et al. (1996), who identified five new genes, *SHE1-SHE5* (for Swi5p-dependent *HO* expression), essential for *HO* expression in mother cells. The expectation was that the She proteins might localize to the mother cell. However, *SHE1* encodes an unconventional myosin, which surprisingly preferentially accumulates in the growing bud—the future daughter cell—and not in the mother cell. To explain this paradoxical finding, Bobola et al. (1996) postulated that *she1* mutants are defective in restricting a negative regulator of *HO* expression to daughter cells. According to this model, inactivation of such a negative regulator should allow *she1* mutants to express *HO*. Bobola et al. (1996) therefore looked for mutations that restore *HO* expression in a *she1* mutant background.

ASH1 Is Required for Restricting *HO* Expression to Mother Cells, and Ash1p Preferentially Accumulates in Daughter Cells

The three screens described above identified alleles of a single gene, *ASH1*. Excitingly, *ASH1* fulfills all of the criteria for being the key determinant of asymmetric *HO* expression. First, *ASH1* is necessary for preventing daughter cell switching. Cells deleted for *ASH1* express *HO* in daughter cells, which leads to an increase in the frequency of mating-type switching from less than 1% to approximately 90% (Bobola et al., 1996; Sil and Herskowitz, 1996). Furthermore, overexpression of *ASH1* is sufficient to reduce mating-type switching in mother cells (Sil and Herskowitz, 1996). Most importantly, Ash1p activity is restricted to daughter cells. Bobola et al. (1996) and Sil and Herskowitz (1996) show that the protein preferentially accumulates in daughter cells (Figure 2B). After completion of anaphase, Ash1p accumulates in the nucleus located in the bud—the future daughter cell—where it remains present during G1. The protein rapidly disappears as daughter cells enter the cell cycle and appears to be absent during S phase, G2, and mitosis. This asymmetric accumulation of Ash1p is exhibited by 75% of cells (Sil and Herskowitz, 1996) or more (Bobola et al., 1996). Interestingly, low levels of Ash1p are also observed in 25% of mother cells. This observation might explain why in wild-type cells only 70% of mother cells switch mating type, whereas in *ash1* mutant mother cells the frequency of switching is increased to 100%. Residual Ash1p in some wild-type mother cells might prevent *HO* expression.

Mechanisms of *HO* Repression

Ash1p contains a domain with homology to the zinc finger domain of the GATA-like transcription factor family. It is thus conceivable that Ash1p binds to sequences within the *HO* promoter, thereby inhibiting transcriptional activation. Genetic data support the view that Ash1p antagonizes Swi5p: first, when *SWI5* is deleted in *ash1* mutants, daughter cell switching drops to minimal levels, showing that this switching is dependent on *SWI5*. Second, the phenotypes associated with modulating the levels of *SWI5* are exactly opposite those of *ASH1*. Deletion of *SWI5* causes loss of *HO* expression

in mother cells, whereas loss of *ASH1* function causes both mothers and daughters to express *HO*. Moreover, constitutive expression of *SWI5* allows daughter cells to switch (Lydall et al., 1991), whereas overexpression of *ASH1* blocks *HO* expression (Bobola et al., 1996) and mating-type switching in mother cells (Sil and Herskowitz, 1996). Finally, the accumulation of Ash1p in daughter cell nuclei temporally coincides with that of Swi5p (compare Figures 2A and 2B). Interestingly, deletion of a particular domain of Swi5p allows daughter cells to switch mating type (Tebb et al., 1993). Ash1p may interact with this region of Swi5p and thereby inhibit Swi5p-mediated activation of the *HO* promoter.

Cell Polarity May Translate into Developmental Asymmetry

Confining Ash1p activity to daughter cells is clearly important for restricting *HO* expression to mother cells. But how is Ash1p distribution regulated? The *SHE* genes identified by Jansen et al. (1996) in a selection for potential mother-specific activators of *HO* expression provide an important clue: *SHE1*, *SHE2*, *SHE3*, and *SHE5* (*SHE4* has not been tested) are required for the preferential accumulation of Ash1p in daughter cell nuclei at the end of anaphase. Deletion of any of these genes results in a nearly symmetric distribution of Ash1p in mother and daughter cells (Bobola et al., 1996) as well as the failure of mother cells to switch mating type (Jansen et al., 1996). *SHE* genes are therefore essential for preventing accumulation of Ash1p in mother cells, which is crucial for *HO* expression. *SHE2*, *SHE3*, and *SHE4* encode novel proteins, and *SHE5* is allelic to *BNI1*, a gene thought to be involved in cytokinesis. *SHE1* is allelic to *MYO4*, a member of the class V unconventional myosin family (reviewed by Mooseker and Cheney, 1995).

Owing to its mode of cell division (budding), the *S. cerevisiae* cell is structurally asymmetric. The actin cytoskeleton has been implicated in generation of this inherent asymmetry (reviewed by Lew and Reed, 1995). The localization pattern of She1p/Myo4p and She3p is remarkably similar to that of actin and proteins involved in budding and cell polarization: both proteins are cytoplasmic and preferentially accumulate in the bud, the future daughter cell (Figure 2C). The actin-based motor Myo2p, a close relative of She1p/Myo4p, transports vesicles on asymmetric actin filaments into the growing bud (Mooseker and Cheney, 1995). She1p/Myo4p, and perhaps other She proteins, could facilitate accumulation of Ash1p in daughter cells in a similar way. Thus, they might convert the structural asymmetry of the actin cytoskeleton into a developmental asymmetry, the asymmetric accumulation of the mating-type switching determinant Ash1p.

Possible Mechanisms for the Generation of Ash1p Asymmetry

Restriction of *HO* expression to mother cells depends largely, if not solely, on asymmetric accumulation of Ash1p in the daughter cell nucleus. Thus, the key to understanding *HO* asymmetry lies in understanding how Ash1p asymmetry is generated. Although we do not know the answer, several mechanisms can be envisioned that bring about this asymmetry. In a process similar to that used for asymmetric localization of morphogens in the *Drosophila* embryo (St Johnston, 1995),

ASH1 RNA or protein might be localized to the daughter cell nucleus. In this model, the potential actin-based motor She1p/Myo4p would facilitate transport of *ASH1* RNA or protein to the future daughter cell via polar actin filaments.

Alternatively, as yet unidentified factors promoting accumulation of Ash1p in daughter cells might be transported into the bud by She1p/Myo4p and perhaps other She proteins. Such factors might be required to stabilize Ash1p in daughter cells or to promote *ASH1* RNA translation. Indeed, translational control is an important mechanism for spatial restriction of cell fate determinants in many organisms (reviewed by Curtis et al., 1995). Probably the best example of such a mechanism is the regulation of the posterior cell fate determinants *nanos* and *oskar* in *D. melanogaster*. Both RNAs are translated only when localized to the posterior pole of the oocyte (Curtis et al., 1995). Analogously, *ASH1* RNA could be translated only in daughter cells because factors required for its translation are localized specifically to daughter cells by She proteins.

Another possibility is that *ASH1* is transcribed only in daughter cells and that She proteins are required to restrict transcription of *ASH1* to daughter cells. Interestingly, *ASH1* transcription requires *SWI5* (Bobola et al., 1996), which is also required for mother cell-specific expression of *HO*. This finding explains why Ash1p accumulates only after completion of anaphase, at the time when Swi5p enters the nucleus (Figure 2A). Swi5p activity, however, is not asymmetric. Thus, if *ASH1* transcription is daughter cell-specific, other factors must exist that confer asymmetric expression of *ASH1*. She proteins could facilitate transport of such factors into daughter cells.

Coupling Temporal and Spatial Control at the HO Promoter

Unlike other *SWI5*-dependent transcripts, which are transcribed as cells exit mitosis, activation of *HO* expression is delayed until the late G1 phase of the next cell cycle. Thus, temporal as well as spatial regulation might be important for restricting *HO* expression to mother cells. Sil and Herskowitz (1996) and Bobola et al. (1996) propose a model that incorporates both notions: as cells exit mitosis, Swi5p enters the nucleus of both mother and daughter cells, where it activates transcription of *ASH1*. Asymmetric accumulation of Ash1p in the daughter cell nucleus brought about by She proteins therefore coincides with entry of Swi5p into the nucleus (compare Figures 2A and 2B). In daughter cells, Ash1p inhibits Swi5p function or interferes with activation of *HO* transcription (both possibilities are indicated by a question mark in Figure 3). Although Ash1p is absent in mother cells, Swi5p cannot activate *HO* transcription because it must await Swi4p and Swi6p function, which confines *HO* transcription to the late G1 phase of the cell cycle. This temporal delay in activation of *HO* employed by Swi4p and Swi6p might provide a time window in which Ash1p can accumulate in the daughter cell nucleus to levels sufficient to inhibit all Swi5p function or to prevent binding of transcriptional activators to the *HO* promoter (Sil and Herskowitz, 1996). This could lead to repression of *HO* expression in daughter cells (Figure 3).

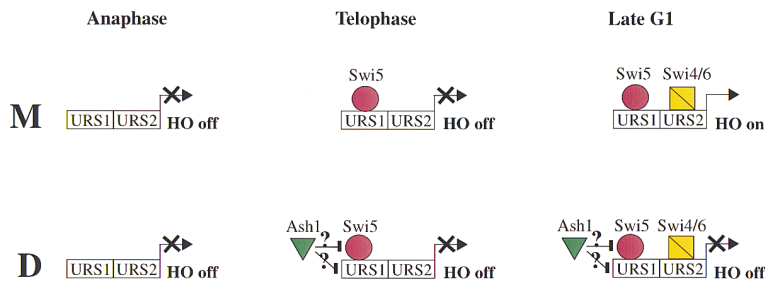


Figure 3. A Model for Differential Regulation of *HO* Gene Expression in Mother and Daughter Cells

Accumulation of Ash1p in the daughter cell nucleus coincides with the entry of Swi5p into the nucleus in mother (M) and daughter (D) cells. Ash1p either inhibits Swi5p function or interferes with activation of *HO* transcription (both possibilities are indicated by question marks) in daughter cells. In mother cells, Ash1p is absent, but Swi5p is not sufficient to activate *HO* transcription. Swi4p and Swi6p, which form a complex, delay *HO* transcription to the late G1 phase of the cell cycle. This delay might provide a time window for Ash1p to inactivate the *HO* promoter in daughter cells.

In 1979, Strathern and Herskowitz showed that mother and daughter cells differ in their competence to switch mating type. The first breakthrough in understanding mother–daughter specificity of mating-type switching was the finding that the *HO* gene is expressed only in mother cells (Nasmyth, 1983). Now, the identification of a daughter cell–specific repressor of *HO* expression might well have solved the puzzle of how *HO* expression, and thus mating-type switching, is confined to mother cells. It seems, however, that *ASH1* is not all that different from *HO*: it too accumulates preferentially in one of the two descendent cells—this time in the daughter cells. Although the origin of this asymmetry is not understood in detail, actin-dependent transport may be responsible. In the *Drosophila* oocyte, polarized microtubules and the actin cytoskeleton are thought to provide a structural basis for localizing cell fate determinants (reviewed by Lehmann, 1995; Erdélyi et al., 1995). Perhaps the polarized actin cytoskeleton plays a similar role in facilitating localization of Ash1p. Utilizing cytoskeletal asymmetry to generate developmental asymmetry may prove to be a common mechanism by which a cell generates two daughter cells of different fates.

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