Creating memories of transcription

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Two genetically identical cells grown under the same conditions can exhibit stochastic fluctuations in gene expression. Such cell-to-cell variation in expression of key genes is hypothesized to help drive developmental fate decisions and cell specialization during differentiation. However, to date, most analyses dissecting steps involved in the activation of cell cycle or developmentally regulated promoters have been conducted using cell population-based assays, thereby precluding a detailed appreciation of how transcription factors, cofactors, and chromatin remodelers contribute to the stochastic nature of transcriptional activation in individual cells. In PNAS, Zhang et al. (1) conduct an elegant series of single cell pedigree analyses to explore molecular mechanisms influencing stochastic gene expression from the cell cycle-regulated *HO* promoter that drives cell fate decisions in *Saccharomyces cerevisiae*. Through the use of an unstable GFP reporter to visualize transcription indirectly, Zhang et al. demonstrate that different factors have independent modes by which they can regulate the *HO* promoter. Some factors primarily influence the frequency of transcriptional events or the fraction of cell cycles in which transcription from *HO* occurs, whereas others affect the amplitude or the level of expression from the *HO* promoter achieved within a given cell cycle. Assessing *HO* promoter activation at the single cell level has also permitted the authors to uncover evidence for a role of histone acetylation in conferring short-term *cis*-based memory of transcriptional states.

Mating Types, *HO*, and Asymmetric Gene Expression

In *S. cerevisiae*, mating type is determined by the expression of ***a*** or α mating-type information from the *MAT* locus. On mating of two haploid cells, ***a***/α diploids are formed. When an ***a***/α diploid is exposed to adverse growth conditions, the cell can enter meiosis and undergo sporulation to generate four haploid spores consisting of two *MAT****a*** and two *MAT*α cells. During their first mitotic cycle, each spore divides to create a mother cell and a smaller daughter cell. Both progeny originating from this first mitotic division will retain their original mating type. However, in homothallic strains, on the second mitotic division, asymmetric expression of the HO endonuclease will occur in which *HO* is expressed in G1 phase in the mother cell but not in the daughter cell (Fig. 1*A*). This leads to HO-mediated cleavage at the *MAT* locus and repair of the double-stranded DNA break by gene conversion using one of the silent mating-type loci as a source of homologous sequence information. Ultimately, switching of mating-type information at *MAT* will occur in ∼70% of mother cells per generation. In contrast, *HO* is not expressed in the daughter cell, and therefore, its mating type does not switch (2). This programmed creation of haploid cells with mating types different from their neighbors enables new mating events to occur.

Asymmetric expression of *HO* between mother and daughter cells is ensured through several levels of regulation. Daughter cell-specific repression of *HO* is mediated by the repressor Ash1p. Although *ASH1* is transcribed in both mother and daughter cells during M phase, expression of Ash1p is restricted to daughter cells through transport of *ASH1* mRNA along actin cables to the bud tip of the daughter cell. There, *ASH1* mRNA becomes anchored and translated (3). Ash1p accumulates in the nucleus of daughter cells (Fig. 1*A*) in late anaphase, where it binds within the URS1 region of the *HO* promoter shortly after localization of the transcription activator Swi5p to URS1 (Fig. 1*B*). Ash1p acts as a cell fate-determining factor by repressing transcription in daughter cells and preventing events downstream of Swi5p binding that are necessary for activation of the *HO* promoter. Such events include recruitment of Swi/Snf, SAGA, and SBF to the *HO* promoter (3–5). Ash1p is an integral component of the Rpd3L histone deacetylase complex and has also been proposed to control cell fate by maintaining low levels of acetylation at the *HO* promoter in daughter cells (6).

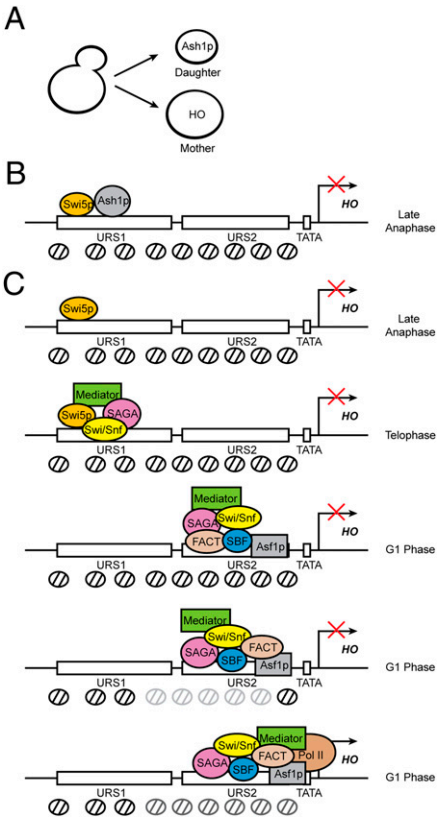

Fig. 1. HO regulation. (*A*) Expression of Ash1p and HO in mother and daughter cells. (*B*) Transcriptional repression of *HO* by Ash1p in daughter cells (Rpd3L not shown). (*C*) Transcriptional activation of *HO* in mother cells. In late M phase, Swi5p is recruited to URS1 and facilitates binding of Swi2/Snf2, SAGA, and Mediator before dissociating from *HO*. SAGA localizes to URS2 to promote binding of SBF and Mediator at the time of Swi2/Snf2 localization to URS2. Swi2/Snf2 transiently remodels several nucleosomes (gray) overlapping URS1 and URS2 and is aided by Asf1p. FACT binds at URS2 before nucleosome displacement. Asf1p similarly associates with URS2 and then with the TATA region along with FACT and Mediator, enabling recruitment of RNA Pol II and transcription initiation.

Swi5p and Activation of the *HO* Promoter

Cell cycle-regulated localization and turnover of Swi5p also limits expression of *HO* to G1 phase. *SWI5* is transcribed in G2 phase, but Swi5p is initially restricted to the cytoplasm until anaphase via phosphorylation that blocks its nuclear localization signal

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(7). In late anaphase, Swi5p is dephosphorylated by Cdc14p, allowing Swi5p to enter the nucleus and transiently bind to nucleosome-depleted regions within URS1 of the *HO* promoter (8). Swi5p binding initiates a series of steps that ultimately result in transcriptional activation of the *HO* promoter in mother cells during late G1 phase (Fig. 1*C*). Swi5p recruits the chromatin remodeling factor Swi2/Snf2, the SAGA acetyltransferase complex, and Mediator and promotes nucleosome loss at URS1 (5, 9) Swi5p's interaction with the *HO* promoter is temporary, and Swi5p is targeted for ubiquitin-mediated degradation by Cdc4p shortly thereafter (5, 10). However, expression from the *HO* promoter does not occur until after most nuclear Swi5p has been degraded and after additional activation steps at the *HO* promoter have occurred (5). After being recruited to URS1, Swi2/Snf2, SAGA, and Mediator then associate with URS2 and promote binding of the activator SBF (Swi4/Swi6) across URS2 (5, 9, 11). Transient Swi2/Snf2-dependent remodeling of several nucleosomes across URS2 occurs at this time and is facilitated by the histone chaperones FACT and Asf1p (12). FACT initially binds at URS2 just before nucleosome displacement and later localizes to the TATA region. Asf1p also initially localizes to URS2 and then associates with the TATA region during gene activation. Enhanced H3 K14 and H4 acetylation at the *HO* promoter follows a similar pattern as nucleosome disassembly. Acetylation is initially enriched at URS1 at the time of Swi5p localization, then at URS2, and finally at TATA, at which time RNA Pol II loads onto the promoter and initiates transcription at *HO* during late G1 phase (5, 9).

Stochastic Events and Chromatin

Through single cell pedigree analyses, Zhang et al. identify several distinct types transcriptional defects that can occur when different regulatory steps in the *HO* activation pathway outlined above are perturbed. Defects in the earliest regulatory events at the *HO* promoter, including disrupting Swi5p function, alter the frequency with which transcription occurs at *HO* across cell cycles. In contrast, defects in executing late steps in *HO* activation, such as those observed in mutants of Mediator, result in a uniform decrease in expression from *HO* in all cells, consistent with inefficient recruitment of Pol II. Finally, defects in intermediate steps during *HO* activation, including those requiring Swi2/Snf2, led to the discovery that some mutants can affect the frequency with which transcription from *HO* occurs across cell cycles, as well as the level of expression achieved during transcription (1). The varying nature of these fluctuations corresponds to molecular differences at the *HO* promoter in the mutants. For numerous organisms, expression of individual genes fluctuates significantly among different cells in a population over time and leads to cell-to-cell variations in copy number of protein products. Such variation has the potential to contribute phenotypic diversity across the population and may provide some individuals with a selective advantage and others with a disadvantage for certain environmental conditions or developmental fates. Thus, stochastic variation itself may reflect a trait, influenced by *cis*- or *trans*-acting factors, that can be selected for or against during evolution (13) to give rise to complex regulatory systems such as that of the *HO* promoter.

Analysis of bimodal transcription from the *HO* promoter has allowed Zhang et al. to uncover evidence for individual cells having a shortterm memory of previous expression from *HO*.

Some genes exhibit continual expression, and cell-to-cell variation can reflect a Poisson-like accumulation of transcripts. However, when a gene exhibits episodic transcription, variation can arise from cell-to-cell differences in the burst size, or the number of mRNAs transcribed from a given gene per burst of activity. The burst frequency from an episodic gene, or the number of bursts of transcription that occur at that gene per cell cycle, reflects the promoter activation rate and may also vary from cell to cell. Recent studies have demonstrated both constitutive and episodic gene expression exist across organisms (14–17). One emerging theme is that chromosomal context matters. For instance, the site of integration of reporter genes across chromosome III in budding yeast influences the burst size of their transcriptional activity, and this may be modulated by histone deacetylation (16). Less widely explored, however, is how chromatin context and stochastic gene expression intersect to influence cell fate decisions (18). Analysis of bimodal transcription from the *HO* promoter has allowed Zhang et al. to uncover evidence for individual cells having a short-term memory of previous expression from *HO*. This memory is *cis*-acting, can enhance SBF binding, and may reflect changes in chromatin states linked to histone acetylation (1). Further studies will be needed to assess how widespread such forms of transcriptional memory are and to define how short-term memory functions. *HO* is anticipated to continue to provide additional surprising insights into the molecular underpinnings driving cell fate decisions.

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