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Yeast Cbk1 and Mob2 Activate Daughter-Specific Genetic Programs to Induce Asymmetric Cell Fates

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Summary

In *Saccharomyces cerevisiae*, mothers and daughters have distinct fates. We show that Cbk1 kinase and its interacting protein Mob2 regulate this asymmetry by inducing daughter-specific genetic programs. Daughter-specific expression is due to Cbk1/Mob2-dependent activation and localization of the Ace2 transcription factor to the daughter nucleus. Ectopic localization of active Ace2 to mother nuclei is sufficient to activate daughter-specific genes in mothers. Eight genes are daughter-specific under the tested conditions, while two are daughter-specific only in saturated cultures. Some daughter-specific gene products contribute to cell separation by degrading the cell wall. These experiments define programs of gene expression specific to daughters and describe how those programs are controlled.

Introduction

Multicellular organisms are made up of different cell types organized into tissues and organs. One source of cellular diversity is asymmetric cell division due to differential segregation of mRNAs and proteins that determine cell fate. Numerous mechanisms underlying such asymmetry have been described in bacteria (Jacobs and Shapiro, 1998), worms (Rose and Kemphues, 1998), flies (Lu et al., 1998, 2000), and yeast (below).

Saccharomyces cerevisiae reproduces by budding. Mother cells form buds that separate to become daughters. Daughters and mothers are genetically identical but constitute distinct cell types. Many daughter components, such as the cell wall and plasma membrane, have been newly synthesized during the budding process. In addition, newborn daughters are smaller than their mothers, and thus spend more time in G1 before they are large enough to start budding themselves. Separation of daughters from mothers is asymmetric. During budding, chitin is preferentially deposited at the bud site, where it forms a ring around the base of the emerging bud (Sloat and Pringle, 1978). After the cell membranes separate, a septum made of cell wall material forms between the cells, inside the chitin ring. Degradation of this septum causes cell separation. The mother retains most of its chitin and displays a ring (the "bud scar") visible by staining chitin with calcofluor, while the daughter is left with a circle in which chitin has been degraded (the "birth scar"; Pringle, 1991).

During the 1970s, one mechanism underlying mother-daughter asymmetry began to be revealed. Haploid yeast switch between a and α mating types. Mothers switch mating type, while daughters do not. Once they have budded, the former daughters (now mothers) switch mating type (Strathern and Herskowitz, 1979 and references therein). Switching occurs by replacement of a- or α-specific DNA sequences at the *MAT* locus with sequences of the opposite mating type (Oshima and Takano, 1971), a process initiated when the HO endonuclease, expressed only in mothers (Nasmyth, 1983), makes a double strand break at *MAT* (Strathern et al., 1982). Since the 1980s, study of the molecular mechanisms causing the differences between mothers and daughters has occupied a mainstream place in molecular biology. Yeast mother-daughter asymmetry provides a tractable system to elucidate the means by which cells give rise to progeny whose gene expression differs as a consequence of internal molecular events rather than as a consequence of different environmental conditions.

Continued work during the 1980s revealed the SWI gene products, positive regulators of *HO* transcription (Stern et al., 1984; Breeden and Nasmyth, 1987). By the early 1990s, some lines of evidence led to the view that mother-specific transcription of *HO* was due to Swi5, which was degraded in daughters but a small portion of which was protected in mothers (Tebb et al., 1993). However, failure to detect asymmetrical localization of Swi5 led to the suspicion that this idea might be incorrect (Nasmyth, 1993), and isolation of *ash1* mutants, in which switching occurs in daughters, led to the current model for mother-specific expression of *HO* (Sil and Herskowitz, 1996; Bobola et al., 1996). In this view, daughters do not switch mating type because Ash1 represses transcription of their *HO* genes. Ash1 functions only in daughters because its mRNA, synthesized in early G1, is transported via actin cables to the bud, a process that requires Myo4, She2, She3, She4, and Bni1. In the bud, *ASH1* mRNA is translated at anaphase, whereupon Ash1 protein translocates to the daughter nucleus and represses *HO* (Sil and Herskowitz, 1996; Bobola et al., 1996; Jansen et al., 1996). Aside from this regulation of *HO*, molecular mechanisms for mother-daughter asymmetry have not been described.

ASH1 transcription itself requires *SWI5*. Gene expression monitoring studies have shown that *SWI5* belongs to a cell cycle regulated "expression cluster" induced during the G2 and M phases of the cell cycle—in this case, the "*CLB2* cluster," named for its prototype member, the gene encoding the B-type cyclin Clb2 (Spellman et al., 1998). Phosphorylation of Swi5 by Cdc28/Clb2 causes Swi5 to concentrate in the cytoplasm. At anaphase, Clb2 is degraded, and dephosphorylated Swi5 accumulates and migrates to the nucleus (Nasmyth et al., 1990; Moll et al., 1991), where it induces expression of *ASH1* and other genes in early G1.

ACE2 is another member of the *CLB2* cluster. Ace2 and Swi5 are paralogs that date from a duplication that doubled the ancestral *S. cerevisiae* genome (Wolfe and Shields, 1997). The proteins are 34% identical in se-

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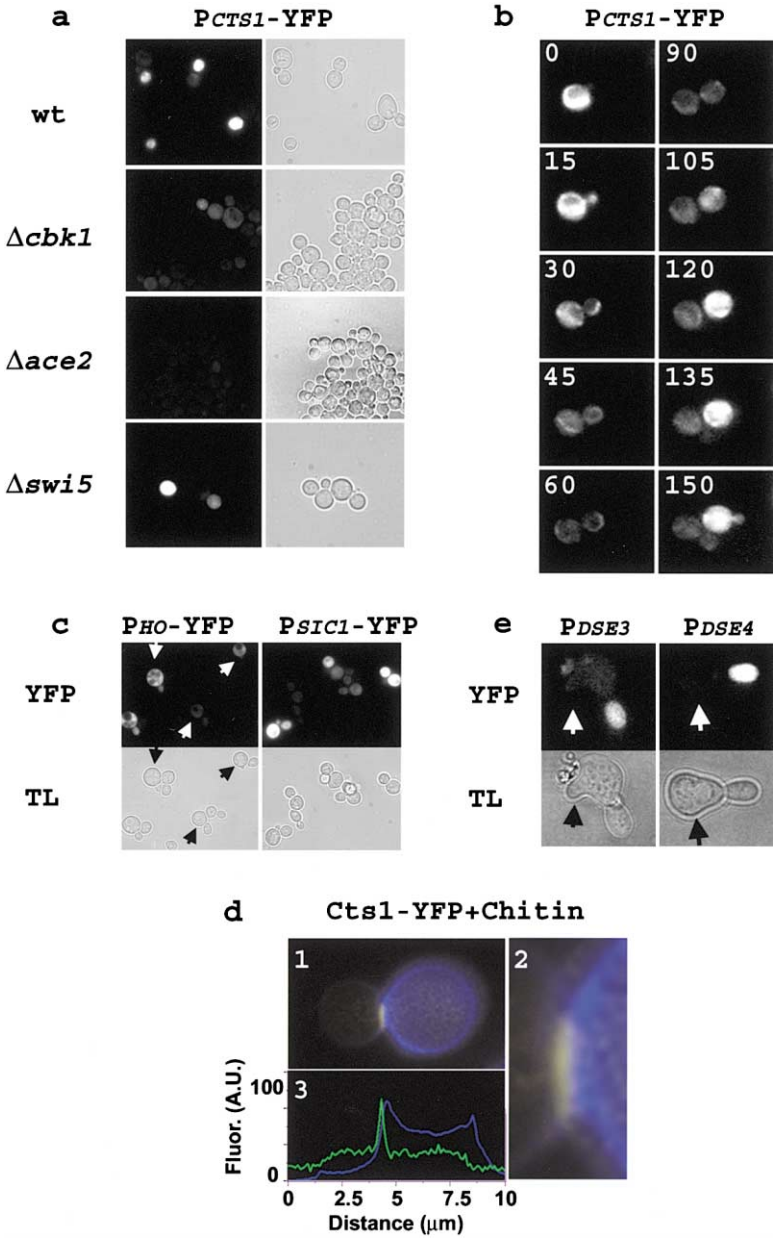

Figure 1. Cts1 Is Expressed Only in Daughters, and Its Expression Depends on Cbk1 and Ace2

(a) Left panels: P_{CTS1}-YFP fluorescence in wt (YAS254-5c), Δ*cbk1* (ACLY245), Δ*ace2* (ACLY-311), and Δ*swi5* (ACLY313) cells. Right panels: transmission light image.

(b) Time-lapse images of a YAS245-C cell bearing P_{CTS1}-YFP growing on an agarose pad. Numbers indicate elapsed time (in minutes) after the initial frame.

(c) Top panel: P_{HO}-YFP and P_{SIC1}-YFP fluorescence in wt cells. Bottom panels: Transmission light image.

(d) Cts1-YFP fluorescence in ACLY362 grown at pH 7. (1) Overlay of Cts1-YFP fluorescence (yellow) and calcofluor staining of chitin (blue). (2) Enlarged view of the mother-daughter pair in (1). (3) Linear scan along the long axis of the mother-daughter cell pair in 1. Yellow, YFP; blue, calcofluor.

(e) Top panels: P_{DSE3}- and P_{DSE4}-YFP fluorescence in wt (YAS254-5C) cells 100 min after release from α factor arrest. Bottom panels: Transmission light image of cells in left panels. Arrowheads in (c) and (e) indicate mothers.

quence and recognize the same DNA sites in vitro (Dohrmann et al., 1996). Either is sufficient for expression of several genes (McBride et al., 1999), including *SIC1* (Nugroho and Mendenhall, 1994; Toyn et al., 1997), *EGT2*, which encodes a protein involved in cell separation (Bobola et al., 1996; Kovacech et al., 1996), and *ASH1* (Bobola et al., 1996). However, in some cases, the function of the paralogs has diverged. Ace2, but not Swi5, is needed for the expression of *CTS1*, *SCW11*, *YER124C*, and *YHR143W* (Kuranda and Robbins, 1991; Doolin et al., 2001), while Swi5, but not Ace2, is required for expression of *HO* (Dohrmann et al., 1992).

Expression of *CTS1*, which encodes chitinase, a secreted enzyme required for cell separation, requires the protein kinase Cbk1 (Racki et al., 2000). Δ*cbk1* strains fail to separate after mitosis and grow in clumps (Racki et al., 2000, and this paper). Mutations in *ACE2* suppress the Δ*cbk1* separation defect, and Cbk1 and Ace2 interact in two-hybrid experiments, suggesting that Cbk1 might regulate *CTS1* expression by activating Ace2 (Racki et al., 2000). We isolated Cbk1 independently as a target in a hunt for peptide aptamers that blocked α factor-induced cell cycle arrest (Geyer et al., 1999). We showed that Cbk1 interacts with the pheromone pathway proteins Ste5 and Ste50 (Geyer et al., 1999). Δ*cbk1* strains show partial resistance to α factor and defects in mating projection formation (shmooing), which prompted us to propose that Cbk1 connects the pheromone pathway to pathways that regulate cell morphology (Geyer et al., 1999). Cbk1 interacts with a number of proteins, including Mob1 (Ito et al., 2001), which is needed for exit from mitosis, presumably to activate the Mob1-interacting protein kinase Dbf2 (Lee et al., 2001). Cbk1 also interacts with Mob2, a protein whose se-

quence is 90% identical to that of Mob1 (Racki et al., 2000).

Here, we describe two distinct programs of gene expression in daughter cells. Both programs require Cbk1, Mob2, and Ace2. We first found that the *CTS1* gene is expressed only by daughters. We identified other genes coexpressed under seemingly irrelevant conditions, then determined which of these candidate genes had promoters that drove daughter-specific expression. Daughter-specific expression results from Cbk1- and Mob2-dependent activation and localization of Ace2 to the daughter nucleus. One daughter-specific expression program consists of eight genes induced in daughter cells in early G1. It includes genes of known function, and four relatively uncharacterized genes which we named *DSE*, for *Daughter-Specific Expression*. The other program consists of two genes, *ASH1* and *EGT2*. This program is conditional: in exponentially growing cultures, these genes are expressed in both mothers and daughters, but in saturated cultures, they are expressed only in daughters. Taken together with the enzymatic activities of the encoded proteins, our results indicate that some daughter-specific gene products bring about cell separation by degrading the septum from the daughter side of the mother-daughter pair.

Results

CTS1 Is Expressed in and Localized to Daughter Cells

To characterize Cbk1-dependent *CTS1* regulation, we constructed a single-copy plasmid that carried the YFP gene under the control of the *CTS1* promoter (−762 to −1; here, we refer to the nucleotide upstream of the start codon as −1). In exponentially growing yeast carrying P_{CTS1}-YFP, fluorescence was restricted to daughters in mother-daughter pairs (Figure 1a). In contrast, only mothers expressed $P_{HO\ (-1807\ to\ -1)}$-YFP, and both mothers and daughters expressed $P_{SIC1\ (-1213\ to\ -1)}$-YFP (Figure 1c). To confirm the daughter cell designation, we monitored cells bearing P_{CTS1}-YFP by time-lapse photomicroscopy. We observed that after each cell finished dividing, only the newly budded cell, the daughter, became fluorescent (n = 50 cells). In Figure 1b, we show one example cell. At time 0, the unbudded cell displays fluorescence, consistent with *CTS1* being expressed in early G1. As the cell buds, YFP fluorescence decreases (from 15 to 90 min), and the newly formed daughter becomes fluorescent (from 105 to 150 min). These results are consistent with the conclusion that *CTS1* expression is restricted to daughters. We also observed that in W303 diploid strains carrying P_{CTS1}-*YFP*, fluorescence was restricted to daughters (not shown).

Since *CTS1*, the gene encoding chitinase, was expressed only in daughters, we wondered whether chitinase might be concentrated in the daughter cell wall. We constructed ACLY362, a strain that expresses a Cts1-YFP fusion, and examined the fluorescence localization. In mother-daughter pairs, Cts1-YFP localized to the bud neck (Figure 1d). To determine whether Cts1-YFP was localized to the daughter side of the bud neck cell wall, we stained the cells with calcofluor white. As shown in Figure 1d, YFP fluorescence was seen on one side of the bud neck, while calcofluor staining of chitin was concentrated on the opposite side. These results are consistent with the idea that chitinase, secreted by daughters, is concentrated on the daughter side of the bud neck.

The only previously studied case of asymmetric expression in yeast, mother-specific expression of *HO*, results from daughter-specific accumulation of the *HO* repressor Ash1. Therefore, we tested whether daughter-specific expression of *CTS1* depended on Ash1 or Ash1 localization. We observed the expression of P_{CTS1}-YFP in Δ*ash1*, Δ*myo4*, and Δ*she4* cells (not shown). In all strains, the YFP signal was daughter-specific. Therefore, daughter-specific *CTS1* expression was independent of Ash1 and the machinery involved in *ASH1* mRNA transport.

We investigated the dependence of *CTS1* daughter-specific gene expression on Cbk1 and Ace2. In Δ*ace2* cells, P_{CTS1}-YFP fluorescence was undetectable; in Δ*cbk1* cells, P_{CTS1}-YFP fluorescence was greatly reduced and asymmetric expression was lost. Deletion of *SWI5*, which is not involved in *CTS1* expression, did not affect asymmetric P_{CTS1}-YFP fluorescence (Figure 1a). In contrast, deletion of *cbk1* or *ace2* did not affect mother-specific expression of P_{HO}-YFP, while deletion of *SWI5* abolished it (not shown).

CTS1 Defines a Larger Set of Cbk1- and Ace2-Dependent Daughter-Specific Genes

We set out to identify possible additional daughter-specific genes by whole-genome gene expression monitoring. No mother-daughter differential whole-genome expression data was available to us. However, we tested the idea that other daughter-specific genes might be coregulated with *CTS1* under different, seemingly irrelevant conditions.

Since *CTS1* is cell cycle regulated, we first examined other genes that were cell cycle regulated in a *CTS1*-like manner. From gene expression monitoring experiments, *CTS1* has been grouped in a set of genes designated as the "*SIC1* cluster". This cluster is transcribed in late M and/or early G1 (Spellman et al., 1998; Figure 2a). Some of the *SIC1* cluster genes are known to require either Swi5 or Ace2 for expression, and most contain putative binding sites for these activators in their regulatory regions (Spellman et al., 1998). We used YFP reporters to examine expression from the promoters (from −1200 to −1 for each gene) for the genes in the *SIC1* cluster and from other genes whose expression is controlled by Swi5 and Ace2 (see Tables 1 and 2). Seven of the 26 tested promoters (*CST13*, *PRY3*, *SCW11*, *YER124C*, *YHR143W*, *YOR264W*, and *YNR067C*) directed daughter-specific fluorescence in exponentially growing cells. 16 promoters displayed approximately equal fluorescence in mothers and daughters, and three showed no detectable YFP fluorescence (Figure 1e and Tables 1 and 2). Six of the eight daughter-specific promoters (including *CTS1*) belonged to a smaller, eight-gene subnode of the *SIC1* cluster (Figure 2a). Four of the genes (*YER124C*, *YHR143W*, *YOR264W*, and *YNR067C*) were previously only partly characterized (Doolin et al., 2001, Drees et al., 2001); we named these *DSE1*, *DSE2*, *DSE3*, and *DSE4* (for *Daughter-Specific Expression*). As

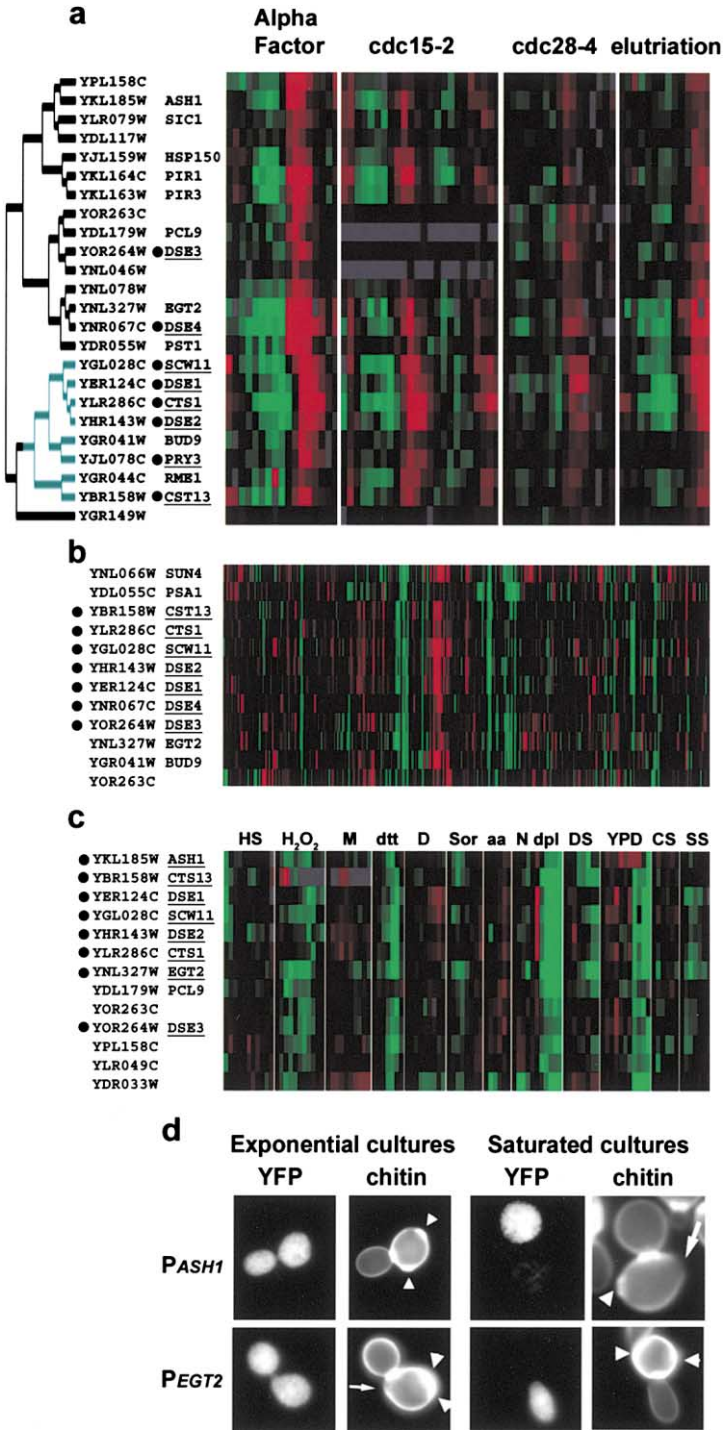

Figure 2. Coregulation of Daughter-Specific Genes and Analysis of Existing Whole-Genome Expression Data

(a) Expression of the "*SIC1* cluster" genes as yeast progress through the cell cycle after release from α factor-, cdc15-2-, and cdc28-4-arrest and after collection from an elutriation chamber (Spellman et al., 1998). Members of the first group of daughter-specific genes defined here (*CTS1*, *CST13*, *DSE1*, *DSE2*, *SCW11*, *DSE3*, *DSE4* and *PRY3*) are underlined and marked with a circle. Note that *CTS1*, *CST13*, *DSE1*, *DSE2*, and *SCW11* belong to one branch of this cluster (in cyan) while *DSE3* and *DSE4* belong to another. These two genes differ in their regulation from the other daughter-specific genes in response to some perturbations; in particular, *DSE3* and *DSE4* are not induced by α factor.

(b) Daughter-specific genes (marked with circles) are coregulated in response to deletion of different genes (Hughes et al., 2000). Each column corresponds to a different deletion or drug treatment.

(c) Unconditional and conditional daughter-specific genes (marked with circles) cluster together in the environmental stress response data set (Gasch et al., 2000). Stress conditions are heat shock (HS), increasing concentrations of hydrogen peroxide (H_2O_2), menadione (M), dithiothreitol (dtt), diamide (D) and sorbitol (Sor), time after amino acid deprivation (aa), nitrogen depletion (N dpl) or growth on YPD (YPD), time course of diauxic shift (DS) or steady state (SS), and growth in different carbon sources (CS). In (a) and (b), we clustered the original raw data using the Cluster software (Eisen et al., 1998); in (c), the clustering was performed by Gasch et al. (2000). Red indicates upregulation and green indicates downregulation with respect to reference mRNA from cells grown asynchronously (a), from wild-type cells (b), and from cells that were not perturbed by stressful treatment (c). Gray boxes indicate data missing from the published datasets.

(d) *ASH1* and *EGT2* expression in saturated cultures is daughter-specific. Expression of P_{ASH1}-YFP and P_{EGT2}-YFP in cells from exponentially growing (left) or saturated cultures ($OD_{600}\sim5$, right) of YAS245-5C. We identified cells as mothers or daughters by staining chitin with calcofluor (arrows indicate birth scars; arrowheads, bud scars). Figure shows representative mother-daughter pairs.

for *CTS1*, there was no detectable YFP expression from these seven promoters in Δ*cbk1* or Δ*ace2* strains, and YFP expression in Δ*swi5* strains was indistinguishable from that in wild-type (not shown).

ASH1 and *EGT2* Define Another, Conditional, Set of Cbk1- and Ace2-Dependent Daughter-Specific Genes

If the eight daughter-specific genes defined a transcriptional regulon, then they might be expected to show similar patterns of expression in circumstances other than cell cycle progression. To test this idea, we examined whole-genome expression data from cells perturbed by environmental stresses and carbon source changes (Gasch et al., 2000) and by deletion of different genes and various drug treatments (Hughes et al., 2000). In both datasets, most of the daughter-specific genes were coregulated (Table 1 and Figure 2c and 2c). *PRY3* did not cluster with the rest of the daughter-specific genes in any of the other datasets, while *DSE4* only

Table 1. Genes that Show Daughter-Specific Expression

Gene	Function	Loc	EG	SC	Env Stress Data Set	Deletion Data Set
CTS1	Endochitinase	CW (E)	D	D	Yes	Yes
CST13	Stress Response?	Nuc (P)	D	D	Yes	Yes
SCW11	Glucanase?	CW (P)	D	D	Yes	Yes
PRY3	Mating Response?	CW (E)	D	D	No	No
DSE1/YER124C	Signal Transduction?	Cyt (P)	D	D	Yes	Yes
DSE2/YHR143W	Glucanase?	CW (P)	D	D	Yes	Yes
DSE3/YOR264W	Glucanase?	Nuc (P)	D	D	No	Yes
DES4/YNR067C	Unknown	CW (P)	D	D	Yes	Yes
ASH1	Transcription Factor	Nuc (E)	M/D	D	Yes	No
EGT2	Endoglucanase?	CW (E)	M/D	D	Yes	Yes

Daughter-specific and conditional daughter-specific expression groups. Possible functions of the encoded proteins (detailed in the text). Abbreviations: "Loc," localization; "CW," cell wall; "Nuc," nuclear; "Cyt," cytoplasmic; "E," experimentally determined; "P," predicted from sequence; "EG," Exponential Growth, pattern of expression in exponentially growing cultures; "SC," saturated cultures ($OD_{600}\sim5$); "D," daughter-specific; "M/D," mother and daughter; "M," mother-specific; "ND," not detected; "Env Stress Data Set," coexpressed in environmental stress response data set (Gasch et al., 2000); "Deletion Data Set," coexpressed in the deletion data set (Hughes et al., 2000).

clustered with the other daughter-specific genes in the deletion data set.

ASH1 was coregulated with the daughter-specific genes in the stress-response dataset but not in the deletion dataset. These results suggested that under environmental stress conditions, expression of *ASH1* (and perhaps of other genes) might be restricted to daughters. To test this idea, we examined YFP fluorescence driven by P_{ASH1} and the other previously tested promoters in saturated cultures (see Experimental Procedures). Here, for daughter cell designation, we stained cells with calcofluor white, which labels bud scars (Pringle, 1991), present only in mothers. Under this stress condition, P_{ASH1}-YFP and P_{EGT2}-YFP-derived fluorescence were only visible in cells lacking bud scars, i.e., daughters, as were the eight previously identified daughter-specific promoters (Figure 2d). The remaining 16 promoters either did not direct YFP expression or directed equal expression in mothers and daughters (see Table 2). In saturated cultures, daughter-specific expression of *ASH1* and *EGT2* required Ace2 and Cbk1 but was independent of Swi5 (not shown). This observation suggested that *EGT2* and *ASH1* define a separate, conditional program of daughter-specific gene expression active in saturated cultures.

Ace2 Localizes to the Daughter Nucleus

Expression of ACE2-dependent genes was restricted to daughters. We hypothesized that this might be the result of asymmetric localization of Ace2. To test this idea, we used a plasmid containing a YFP-Ace2 fusion protein under the control of P_{ACE2}. This plasmid complemented the separation defect in Δ*ace2* cells (not shown), indicating that the fusion protein was functional. We observed the localization of YFP-Ace2 at the end of the first mitosis after α factor arrest (approximately 80 min). Arrest induces formation of mating projections that are not resorbed, allowing mothers to be distinguished from daughters born after release. At the end of mitosis, YFP-Ace2 expression was confined to daughter nuclei (Figure 3a), except for a fraction of cells (~10%) that showed both mother and daughter nuclear YFP signal.

Table 2. Genes that Do Not Show Daughter-Specific Expression

Gene	EG	SC	Gene	EG	SC
BUD9	M/D	ND	REM1	M/D	ND
CDC6	M/D	ND	SIC1	M/D	ND
CHS1	M/D	ND	TEC1	M/D	ND
CYK3	M/D	ND	YMR316C-A	ND	ND
FAA3	ND	ND	YNL046W	M/D	ND
HO	M	ND	YNL078W	M/D	ND
PCL2	M/D	ND	YGR149W	M/D	M/D
PCL9	M/D	ND	YGR086C	M/D	M/D
PIR3	M/D	M/D	YOR263C	ND	ND
PST1	M/D	M/D			

Abbreviations as in Table 1.

Ace2 Transiently Translocates to the Mother Nucleus

We realized that localization of Ace2 to the daughter nucleus could be due to at least three mechanisms: (1) preferential accumulation of Ace2 in the cytoplasm of the future daughter followed by import into the nearest nucleus, (2) export/degradation of Ace2 from/within the mother nucleus, or (3) preferential import of Ace2 into the daughter nucleus. To distinguish among these possibilities, we monitored the localization of YFP-Ace2 in cells released from α factor arrest by time-lapse photomicroscopy. Before its nuclear transport, YFP-Ace2 was evenly distributed in the cytoplasm of the mother and the nascent daughter. Initially, YFP-Ace2 translocated to both mother and daughter nuclei. Whereas YFP fluorescence in the mother nucleus disappeared rapidly, it continued to increase in the daughter nucleus up to a maximum at 22 min, after which it decreased (Figures 3b and 4c). Since YFP-Ace2 was degraded in late G1 in both cell types, it seemed possible that YFP-Ace2 disappeared faster from mother nuclei because mothers complete G1 more rapidly. To test this possibility, we made a strain that carried the cdc15-2 allele and used this temperature-sensitive mutation to arrest cells at telophase. Mothers and daughters released from such arrest cycle nearly synchronously during the first cell division (Futcher, 1999). After release from cdc15 block, YFP-Ace2 still accumulated in daughter nuclei after a transient state in which it localized to both nuclei (not

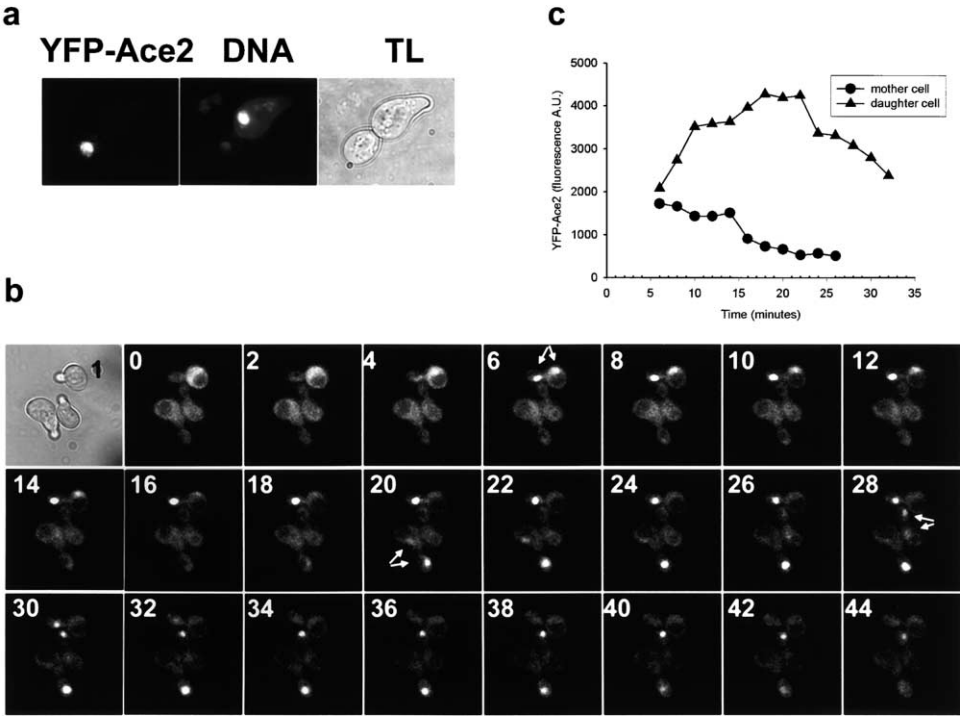

Figure 3. Ace2 Accumulates in the Daughter Nucleus

(a) YFP-Ace2 fluorescence in wt (YAS245-5C) cells. Left: YFP-Ace2 fluorescence 80 minutes after release from α factor arrest. Middle: DNA staining (DAPI). Right: transmitted light image. All three panels show the same cells.

(b) Time-lapse photomicroscopy of YFP-Ace fluorescence in wt cells after release from α factor arrest. Top left: transmitted light image after mounting cells on an agarose pad 30 minutes after release from α factor arrest. Remaining panels show time-lapse pictures starting 40 minutes after mounting (70–114 min after α factor arrest). Numbers correspond to elapsed time in min after the first frame. Double arrows indicate nuclei.

(c) Quantitation of YFP-Ace2 nuclear fluorescence of the cell labeled 1 in (b). Values correspond to the computed YFP fluorescence over time, starting at 6 minutes after the first frame.

shown). These findings are thus consistent with a combination of mechanisms 2 and 3, in which asymmetric localization of Ace2 is the result of its rapid removal from the mother nucleus, either by degradation or export, together with its preferential import into the daughter nucleus.

The Cbk1 Interacting Protein Mob2 Is Required for Daughter-Specific Expression

In order to gain further insight into the mechanism responsible for daughter-specific expression, we analyzed the cell separation phenotype in strains which lacked the genes coding for proteins known to interact with Cbk1: Yol036, Lre1, Ssd1, Not3, and Mob2 (Racki et al., 2000). Among these mutants, the Δ*mob2* strain, ACLY363, but no others, showed a cell separation defect (below). In ACLY363, expression of constitutive daughter-specific genes was undetectable. In saturated cultures, expression of EGT2 and ASH1 was greatly reduced and asymmetric expression was lost (data not shown). This fact suggested that the separation defect in Δ*mob2* cells arises because of defects in the expression of chitinase and other daughter-specific gene products. The function of Mob2 is unknown, but its homolog, Mob1, interacts with the mitotic exit network kinase Dbf2 and is required for its activation (Mah et al., 2001). Dbf1 shares 31% sequence identity and 52% sequence similarity with Cbk1. We suggest that Mob2 may be required to activate Cbk1.

Cbk1 and Mob2 Localize to the Growing Bud Tip, Then to the Bud Neck and the Daughter Nucleus

To understand the mechanism of Cbk1/Mob2/Ace2 activation of daughter-specific genes, we determined the subcellular localization of Cbk1 and Mob2 using protein fusions to GFP (Cbk1) or YFP (Mob2) and tracked fluorescence as cells progressed through the cell cycle after release from α factor arrest. Localization of Cbk1-GFP and Mob2-YFP was similar (Figures 4a and 5d, top). Before release from arrest, Cbk1-GFP and Mob2-YFP were localized to the schmoo tip. In budding cells (50 min after release), both proteins were present in the growing bud membrane. In large budded cells (80 min after release, in which mother and daughter DNA have separated), both proteins were localized to the bud neck and the daughter nucleus. We performed time-lapse photomicroscopy of exponentially growing cultures to determine if, as for Ace2, Cbk1 and Mob2 transiently localize to the mother nucleus. We observed a small amount of Cbk1-GFP and Mob2-YFP in the mother nucleus (Figure 4b and not shown) that lasted for about

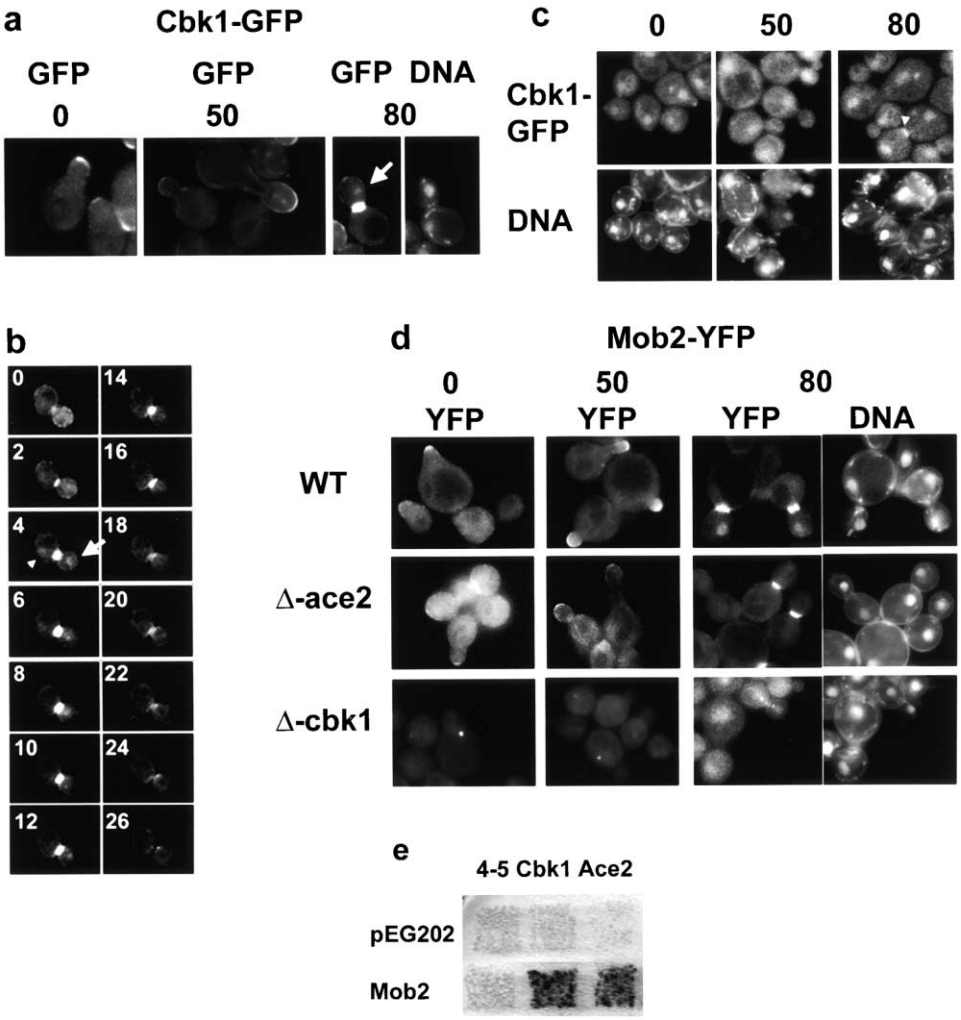

Figure 4. Colocalization of Cbk1 and Mob2

(a) Cbk1-GFP fluorescence in wt (ACLY315) cells after release from α factor arrest.

(b) Time lapse photomicroscopy of ACLY315 cells growing on an agarose pad. Numbers indicate the time in min after the first frame. Arrowhead indicates the mother nucleus.

(c) Top: Cbk1-GFP fluorescence in Δ*mob2* cells (ACLY360). Bottom: DNA staining (DAPI) of cells shown in the top panels. Arrowhead indicates the bud neck.

(d) Mob2-YFP fluorescence in wt (ACLY364), Δ*ace2* (ACLY369), and Δcbk1 (ACLY368) cells.

(e) Two-hybrid mating analysis of Mob2-bait and Cbk1 and $Ace2_{1-449}$ preys. Panel shows a galactose/raffinose Xgal plate after 1 day at 30°. pEG202 and pJG4-5 are the empty bait and prey vectors. Dark patches indicate activation of the *lacZ* reporter due to protein interaction. In (a) and (b), arrows indicate daughter nuclei. In (a), (c), and (d), numbers represent time after release from α factor arrest. Note that in live cells, DAPI also stains mitochondria.

4 min, indicating that Cbk1 and Mob2 also transiently localized to the mother nucleus.

Cbk1 Kinase Activity and Mob2 Are Required for Transcriptionally Active Ace2 to Accumulate in the Daughter Nucleus

Since Cbk1 and Mob2 were necessary for the induction of the daughter-specific Ace2-dependent genes, we examined whether asymmetric nuclear localization of Ace2 required Cbk1 and Mob2. We monitored YFP-Ace2 in Δ*cbk1* and Δ*mob2* cells. In these cells, YFP-Ace2 was present in both mother and daughter nuclei (Figure 5a and data not shown), suggesting that Cbk1 and Mob2 might be required for accumulation of Ace2 in the daughter nucleus but not for nuclear translocation per se. In the absence of Cbk1 or Mob2, daughter-specific genes were not expressed (Figure 1 and data not shown), even though Ace2 was in the nucleus. This result suggested that Cbk1 and Mob2 have an additional function(s) needed for Ace2 to activate transcription.

To test whether these functions required Cbk1 protein kinase activity, we constructed a strain that expresses a mutant Cbk1 (Cbk1-M429A) with a lesion in its ATP binding pocket. This lesion makes the ATP binding site larger, allowing it to interact with the inhibitors NaPP1 and 1NM-PP1 (Bishop et al., 2000). In the absence of

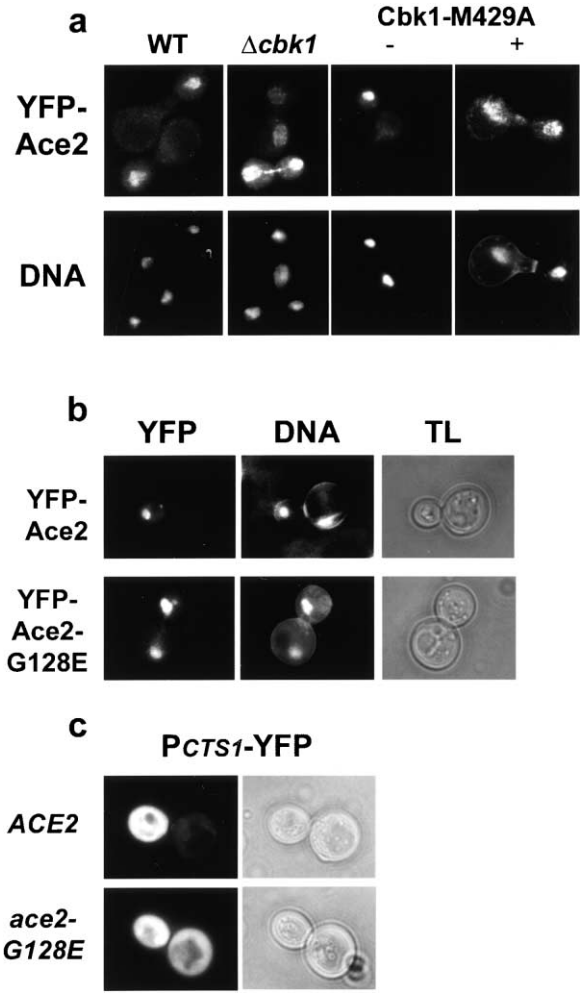

Figure 5. Cbk1 Targets Ace2 to the Daughter Nucleus and Activates It, Resulting in Daughter-Specific Gene Expression

(a) Top: YFP-Ace2 expression under the control of the *ACE2* promoter in YAS245-5C (wt), ACLY245 (Δ*cbk1*), and ACLY330 (expressing Cbk1-M429A). Fixed cells were imaged 80 minutes after release from α factor arrest. In the case of ACLY330 cells, we released cultures from arrest and grew them in the presence or absence of 2 μM NaPP1. Bottom: DAPI staining of the images on top.

(b) Left: YFP-Ace2 (top) or YFP-Ace2-G128E (bottom) fluorescence in wt (YAS245-5C) cells transformed with either pACE2-YFP-Ace2 or pACE2-YFP-Ace2-G128E. Middle: DAPI staining of the left panels. Right: transmitted light image of the left panel.

(c) Left: P_{CTS1}-YFP fluorescence in YAS254-5c (wt) or ACLY382 (expressing Ace2-G128E). Right: transmitted light image of the left panel.

inhibitors, as judged by cell morphology and expression of daughter-specific reporter constructs, the mutant kinase had normal function. However, in the presence of 100 nM NaPP1 or 1 μM 1NM-PP1, cells carrying Cbk1-M429A showed the characteristic Δ*cbk1* separation defect (not shown). Under these conditions, a Cbk1-M429A-GFP fusion exhibited a normal pattern of localization (not shown).

In untreated Cbk1-M429A cells, YFP-Ace2 was localized only to daughter nuclei. In mutants treated with inhibitors, YFP-Ace2 was localized to both mother and daughter nuclei (Figure 5a). Similarly, Cbk1-M429A cells treated with inhibitors showed no YFP fluorescence from P_{CTS1} and other daughter-specific reporters (not shown). These experiments indicate that Cbk1 kinase activity is required for specific accumulation of Ace2 in the daughter nucleus, and also for an additional function needed to activate transcription of daughter-specific genes.

Active Ectopic Ace2 Activates Daughter-Specific Genes in Mothers

To determine if translocation of active Ace2 to the daughter nucleus was sufficient to bring about daughter-specific gene expression, we delivered active Ace2 to the mother nucleus and determined whether active ectopic Ace2 could induce daughter-specific genes in mothers. Activation and proper localization of wild-type Ace2 requires Cbk1 and Mob2. We reasoned that an Ace2 gain-of-function mutant such as Ace2-G128E, which is active in Δ*cbk1* mutants (Racki et al., 2000), might accumulate in both nuclei. To test this idea, we used a reporter plasmid expressing YFP-Ace2-G128E controlled by P_{ACE2} in wt cells. This Ace2 mutant accumulated in mother and daughter nuclei at the end of mitosis (Figure 5b). We then constructed a strain which expressed Ace2-G128E instead of Ace2 and examined expression of P_{CTS1}-YFP. In this strain, P_{CTS1}-YFP expression was visible in both mothers and daughters (Figure 5c). These results are consistent with the idea that localization of Ace2 to the daughter nucleus and its activation by Cbk1/Mob2 are sufficient for daughter-specific gene expression.

Cbk1, Mob2, and Ace2 Are Required to Localize One Another

Cbk1 and Mob2 were required to localize Ace2 to the daughter nucleus. Since Cbk1 and Mob2 interacted and colocalized to the growing bud tip, the bud neck, and the daughter nucleus, we examined the relevant deletion strains to determine whether they were required for each other's localization. In Δ*mob2* cells, Cbk1-GFP was not visible at the bud tip, although it still concentrated at the bud neck at the end of mitosis (Figure 4c). In this mutant, a fraction of Cbk1-GFP localized constitutively to the nucleus. In Δ*cbk1* yeast, Mob2-YFP fluorescence showed a related pattern; it was not localized to the bud tip or the bud neck during budding, but at the end of mitosis it concentrated in both mother and daughter nuclei (Figure 4d, bottom). These results are consistent with the conclusion that Cbk1 and Mob2 are required for the correct localization of one another in time and space.

The pattern of nuclear localization of Mob2 was similar to that of Ace2. Therefore, we asked if Ace2 was required for Mob2 nuclear transport. To test this idea, we used Δ*ace2* cells that expressed Mob2-YFP. In these cells, Mob2-YFP fluorescence localized to the bud tip and the bud neck but did not accumulate in the nucleus (Figure 4d, middle). This finding suggested that Ace2 was required for Mob2 nuclear transport. In further support of this conclusion, in yeast bearing the gain-of-function mutant Ace2-G128E, Mob2-YFP fluorescence localized to both mother and daughter nuclei at the end of mitosis

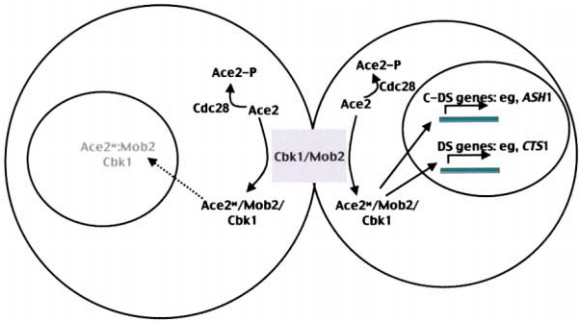

Figure 6. Summary Model for Induction of Daughter-Specific Genetic Programs

Ace2 is localized to the cytoplasm during mitosis due to phosphorylation (Ace2-P) by Cdc28/Cbl1 and Cdc28/Clb2. At the end of mitosis, Clb1 and Clb2 are degraded, increasing the amount of nonphosphorylated, noncytoplasmically retained Ace2. At this time, Cbk1 and Mob2 are localized to the bud neck. Modification of Ace2 by Cbk1 (Ace2*), probably by phosphorylation, both targets Ace2 to the nucleus of the daughter cell and activates the Ace2 transcriptional function. Ace2 translocates to the nucleus, perhaps in a complex with Cbk1 and Mob2. In the mother nucleus (left), the Ace2 complex (gray letters) disappears, due to export or degradation. In the daughter nucleus (right), Ace2 accumulates and induces expression of daughter-specific genes, such as *CTS1*, and of mother-daughter genes, such as *SIC1*. In saturated cultures, Ace2 also induces expression of a second set of conditional daughter-specific genes, including *ASH1* and *EGT2*.

(not shown). Taken together, these results suggested that Mob2 might physically interact with Ace2. Consistent with this idea, we observed that a Mob2 bait and an $Ace2_{1-449}$ prey interact in two-hybrid experiments (Figure 4e). Thus, our results indicate that these three proteins interact and are needed for the correct localization of one another.

Discussion

We have identified two distinct programs of daughter-specific gene expression in *S. cerevisiae*. Both programs require Cbk1, Mob2, and Ace2. One consists of genes that are constitutively expressed in daughters. The other consists of genes that are expressed in daughters conditionally, under the stress of growth to saturation. Genes in both programs are required for cell separation. Daughter-specific expression is the result of Cbk1- and Mob2-dependent localization of Ace2 to the daughter nucleus. Cbk1 and Mob2 associate in two-hybrid experiments and likely form a complex, much like the Dbf2 kinase pairs with Mob1 (Mah et al., 2001). Moreover, the fact that in two-hybrid experiments Cbk1 and Mob2 each interact with Ace2 and that all three proteins accumulate in the daughter nucleus at the end of mitosis suggests that they might form a three protein complex (Finley and Brent, 1994).

Cbk1/Mob2 Control of Daughter-Specific Gene Expression

Cbk1/Mob2 control of daughter-specific gene expression is summarized in Figure 6. Ace2 is expressed during G2 (Spellman et al., 1998), before new daughters are born. At that time, phosphorylation by Cdc28 causes accumulation of Ace2 in the cytoplasm (O'Conallain et al., 1999). At the end of anaphase, when Cdc28 kinase activity drops, Ace2 specifically accumulates in the daughter nucleus. Our data suggest that initially, Cbk1/Mob2-activated Ace2 translocates to both mother and daughter nuclei, perhaps in a complex with Mob2 and Cbk1. Later, import into the mother nucleus stops, and Ace2 in the mother nucleus is either degraded or exported. Meanwhile, import of activated Ace2 into the daughter nucleus triggers expression of daughter-specific genes.

The Unconditional Program

Our experiments defined a set of daughter-specific genes. One of these genes, *CTS1*, is rather well studied, and three, *SCW11*, *PRY3*, and *CTS13*, have been at least partially characterized (Cappellaro et al., 1998; Entian et al., 1999; Ouspenski et al., 1999). The other four genes, *YER124C*, *YHR143W*, *YOR264W*, and *YNR067C*, are relatively uncharacterized. We named these genes *DSE1*, *DSE2*, *DSE3*, and *DSE4*, for *Daughter-Specific Expression*. Induction of these eight genes required Cbk1, Mob2, and Ace2. Cbk1-dependent (but not daughter-specific) expression of *CTS1*, *SCW11*, *PRY3*, and *DSE4/YNR067C* has been noted recently by Bidlingmaier et al. (2001). *DSE1* might participate in pathways regulating cell wall metabolism, since yeast bearing deletions of *dse1* have increased sensitivity to drugs that affect cell wall (Doolin et al., 2001), and Dse1 interacts by two-hybrid analysis with the bud formation proteins Boi1 and Boi2 (Drees et al., 2001). Consideration of existing sequence data suggested that the *SCW11*, *DSE2*, and *DSE4* products might help degrade the cell wall and separate daughter cells from mother cells (see below). By contrast, the sequence of the other daughter-specific genes (*CST13*, *PRY3*, and *DSE3*) did not immediately suggest their functions, but their daughter-specific expression suggests that they may also help establish daughter fate.

The Conditional Program

Our results also uncovered a second program. This program causes daughter-specific expression of genes under particular environmental conditions. In exponentially growing cultures, *ASH1* and *EGT2* are expressed in mothers and daughters, and either Swi5 or Ace2 can activate their expression. However, in saturated cultures, transcription of these genes is restricted to daughter cells and shifts from dual control by Swi5 and Ace2 to exclusive control by Ace2 (Figure 2d and Table 1). In saturated cultures, expression of the Swi5-dependent *HO* promoter is greatly diminished (Table 2), suggesting that the shift in control may be due to a reduction in Swi5 activity.

ASH1 protein product is expressed in daughters due to daughter-specific localization of its mRNA. Our finding that, in saturated cultures, activity of the *ASH1* promoter is also confined to daughters identifies a second mechanism of daughter-specific *ASH1* expression. It is possible that in saturated cultures (and perhaps in colonies), both mechanisms are active. If so, the effect would be to localize Ash1 (and perhaps other proteins)

to daughters of daughters and thus to bring about cell fates specific to granddaughters. Such localization of Ash1 would mean that, in saturated cultures, mothers and daughters, but not granddaughters, could switch mating types.

Cell Separation

Cells lacking *CBK1* or its target genes *CTS1* and *SCW11* show defects in mother-daughter separation (Kuranda and Robbins, 1991, Cappellaro et al., 1998). *CTS1* encodes chitinase. *SCW11* encodes a protein with sequence similarity to glucanases. *DSE2* and *DSE4* also encode secreted proteins with sequence similarity to glucanases. It seems likely, then, that at least these four daughter-specific genes encode proteins that degrade the cell wall from the daughter side and cause daughters to separate from mothers. Cells lacking the conditional daughter-specific gene *EGT2* also show separation defects. *EGT2* encodes a cell wall protein, and cells lacking *EGT2* show overall reductions in glucanase levels (Kovacech et al., 1996; Smits et al., 1999). Our results provide an explanation for the bud-scar/birth-scar asymmetry after cell division: upon cell separation, daughter-specific expression of Cts1 and these other proteins degrades material on the daughter-proximal side of the septum and causes the birth scar. The remaining material on the mother-proximal side of the septum is undegraded, and forms the bud scar.

A Possible Cytokinesis Checkpoint

Why does Ace2 need to be activated by Cbk1/Mob2? Our findings indicate that daughter-specific gene expression leads to the degradation of the connecting septum, followed by cell separation (see below). We found that Cbk1 and Mob2 localized to the bud neck just when Ace2 began to accumulate in the daughter nucleus, suggesting that Cbk1/Mob2 movement to the bud neck might trigger Ace2 movement to the nucleus. The Cbk1 and Mob2 proteins would thus appear suited to transduce a signal indicating the completion of membrane fission in the bud neck. We propose that this Cbk1/Mob2 signal is part of a "cytokinesis checkpoint" that ensures that cell wall degradation does not commence until membrane fission is completed. In this model, at the end of mitosis, Ace2 would integrate a cell cycle and a morphogenic signal.

Systematizable Methods to Identify Programs of Gene Expression

We have identified programs of gene expression specific to daughter cells. Some of the methods that enabled these findings deserve mention. First, we made extensive use of fluorescent protein reporters to look among many candidate promoters to find those which were in fact daughter-specific. Second, in two cases, we identified sets of candidate daughter-specific genes by the fact that they were coregulated under other, seemingly irrelevant, circumstances, and in response to other, seemingly irrelevant, perturbations. This ability to reexamine preexisting expression data is potentially quite useful. We suspect that enough published gene expression data now exists to identify other genetic programs involved in other processes by coregulation under seemingly unrelated circumstances. Moreover, as the quantity of expression data collected for other purposes, or even collected without reference to any hypothesis, increases, the utility of irrelevant coregulation to identify new programs and pathways should also increase.

Table 3. Strain List

Strain Name	Relevant Genotype
YAS245-5C	MATa
ACLY245	MATa Δ*cbk1*::*kanMX6*
ACLY311	MATa Δ*ace2*::*his5*+
ACLY313	MATa Δ*swi5*::*his5*+
ACLY315	MATa *cbk1*::*CBK1-GFP*::*his5*+
ACLY330	MATa *cbk1*::*CBK1*-M429A
ACLY360	MATa *cbk1*::*CBK1-GFP*::*his5*+ Δ*mob2*::*TRP1*
ACLY362	MATa *cts1*::*CTS1-YFP*::*his5*+
ACLY363	MATa Δ*mob2*::*TRP1*
ACLY364	MATa *mob2*::*MOB2-YFP*::*his5*+
ACLY368	MATa *mob2*::*MOB2-YFP*::*his5*+ Δ*cbk1*::*kanMX6*
ACLY369	MATa *mob2*::*MOB2-YFP*::*his5*+ Δ*ace2*::*kanMX6*
ACLY382	MATa Δ*ace2*::his5+::p*ACE2*-G128E

Strains used during this work. All were derived from W303a as described in the Experimental Procedures and the Supplemental Experimental Procedures at http://www.cell.com/cgi/content/full/107/6/739/DC1.

Relevance to Other Organisms

Cell fate asymmetry in budding yeast is regulated by mother-specific transcription of *HO* and by daughter-specific programs described here. These results cannot exclude the idea that there may be still other asymmetric processes that regulate events specific to mothers, daughters, and, perhaps (in light of our findings with Ash1), granddaughters. The proteins that govern the daughter-specific proteins, the kinase Cbk1, Mob2, and the transcription factor Ace2, have homologs in other organisms. It would not be surprising to find that these proteins regulate asymmetric cell division and lineage determination in higher eukaryotes.

Experimental Procedures

Genetic and Molecular Biological Methods

Nucleic acid and yeast manipulations were performed as described (Ausubel et al., 1987; Guthrie and Fink, 1991).

Yeast Strains and Plasmids

Strains are detailed in Table 3. YAS245-5C (*can1*::*HO-CAN1 ho*::*HO-ADE2 ura3 ade2 leu2 trp1 his3*) is a W303a derivative (Anita Sil, personal communication). Other strains in Table 3 derive from YAS245-5C. Construction, by standard techniques, is detailed in the Supplemental Experimental Procedures at http://www.cell.com/cgi/content/full/107/6/739/DC1.

We constructed YFP reporter plasmids as follows. We isolated a KpnI-BamHI fragment containing P_{GAL1}, the EcoRI and XhoI sites, and the ADH1 terminator from pJG4-4 (Geyer et al., 1999) and introduced it into pRS316 (Sikorski and Hieter, 1989) to generate pACL7. We made YFP by PCR-mediated site-directed mutagenesis of the GFP sequence from *A. victoria* and introduced the gene into pACL7 to make pACL7-YFP. We removed the P_{GAL1} fragment to make a plasmid backbone into which we introduced fragments containing other promoters. Details of these constructions are given in the Supplemental Experimental Procedures.

Growth Conditions and Cell Cycle Arrest

To test promoter expression patterns, we first grew colonies bearing the appropriate plasmids or integrated constructs overnight in BSM (QBiogene, Inc., Carlsbad, CA) 2% glucose. We then diluted these cultures to OD_{600} 0.1 in BSM 2% raffinose and allowed them to grow to OD_{600} 0.5 at 30°C, then prepared the cells for microscopy. The slow growth of cells on raffinose allows YFP synthesized during late M/early G1 to be degraded before the next round of cell division (in our hands, the half-life of YFP is approximately 2 hr; see Supplemental Experimental Procedures). To determine the pattern of expression in saturated cultures, we diluted overnight cultures to OD_{600} 0.1 in BSM 2% (w/v) glucose and allowed the cultures to grow to OD_{600} 5 ($\sim 10^8$ cells/ml) at 30°C before examination. Since YFP fluorescence is very low on standard (pH 5.0) synthetic medium, to image secreted Cts1-YFP in ACLY362 cells, we shifted exponentially growing cells to synthetic medium equilibrated to pH 7 for 2 hr before observation.

To synchronize cells, we arrested them with α factor as described (Futcher, 1999). Briefly, we diluted overnight cultures to OD_{600} 0.08 in BSM 2% glucose and grew them to OD_{600} 0.3 at 30°C. At this point we added 3 μM α factor. As judged by microscopic observation, cells were arrested and shmooing 90 min later. We removed α factor by filtration and rinsing, resuspended the cells in fresh BSM 2% glucose medium, and took samples every 10 min.

Imaging

We fixed cells for fluorescent microscopy by adding paraformaldehyde (pH 7.2) to 2% for 1 hr on ice. We washed the cells in PBS twice before examination. To image living cells, we directly mounted samples in growing medium. To stain DNA or chitin, we added DAPI (100 ng/ml) or calcofluor (100 ng/ml), respectively, just before mounting.

We determined GFP and YFP fluorescence using the Piston GFP and the HQ YFP filter set (sets 41025 and 41028 from Chroma Technologies Corp, Brattleboro, VT), respectively. We imaged DAPI and calcofluor using the DAPI filter set (set 31000 from Chroma). We captured and analyzed images using a SPOT2e CCD camera (Diagnostic Instruments, Inc., Sterling Heights, MI) coupled to MetaMorph imaging software (Universal Imaging Corporation, Downingtown, PA).

For time-lapse photomicroscopy, we mounted living cells on 2% agarose pads prepared in BSM with 2% glucose and took 2 s exposures at 2 min intervals over a period of 2 hr. We quantified the fluorescence from the resulting images and computed YFP-based fluorescence at each time as follows. First, we measured YFP photobleaching by imaging wt cells containing but not expressing new YFP for 2 s out of 4 s, over 240 s. These cells contained YFP under the control of the *GAL1* promoter; we regulated the initial YFP level by shifting exponentially growing cells from 2% glucose to 2% raffinose, 1% galactose for three hours, followed by a return to 2% glucose for 30 min before imaging. We observed autofluorescence photobleaching by imaging cells lacking YFP for 2 s, every 4 s, over 240 s as above. We obtained the photobleaching function, $\eta = 0.071 + 0.977e^{-0.06t}$, where t is the single image exposure time in seconds, by fitting the net fluorescence data (YFP minus autofluorescence) from these experiments to an offset exponential function ($R^2 = 0.9978$). We then quantified the amount of YFP in the nucleus of individual cells in a visual field using the formula

$$YFP_i = YFP_{i-1} + [(Obs_i - Auto_i) - \eta(Obs_{i-1} - Auto_{i-1})],$$

where YFP_i and YFP_{i-1} are the computed YFP-based fluorescence at times i and i−1, respectively, Obs_i and Obs_{i-1} are the total observed fluorescence at times i and i−1, respectively, $Auto_i$ and $Auto_{i-1}$ are the observed autofluorescence (fluorescence from cells in the field that do not contain YFP) at times i and i−1, respectively, and η is the fraction of YFP fluorescence remaining after photobleaching.

Interaction Mating

We tested the interaction between LexA-Mob2 and $Ace2_{1-449}$ and Cbk1 in interaction mating two-hybrid experiments using the LexAop-*lacZ* reporter pSH18-34 essentially as described (Finley and Brent, 1994).

Analysis of Gene Expression Data

We obtained cell cycle (Spellman et al., 1998) and response to gene deletion (Hughes et al., 2000) gene expression data directly from http://genome-www.stanford.edu/cellcycle/ and http://www.rii.com/publications/cell_hughes.htm. We clustered the raw data using the Cluster software (Eisen et al., 1998). We obtained data already clustered by Gasch et al. (2000) for the response of yeast to different environmental conditions from http://genome-www.stanford.edu/yeast_stress/

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