CLN3 Functions in Both Daughter and Mother Cells of S. cerevisiae

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CLN3 Functions in Both Daughter and Mother Cells of S. cerevisiae

CLN3 is a Saccharomyces cerevisiae G1 cyclin that helps activate START and helps control the critical cell size required for START (Nash et al., 1988; Cross, 1988). Mutant cells lacking CLN3 have a modal cell volume roughly 50% greater than wild-type. Lew et al. (1992) suggested that CLN3 regulates START in mother cells but not in daughter cells. However, in our experience *CLN3* functions in both daughters and mothers.

Lew et al. (1992) used elutriation to obtain small G1 phase daughter cells from a *CLN3* wild-type strain and a *cln3* deletion strain, reinoculated these cells, and followed size and budding index with time. The size at budding was an indication of the critical size for START. It was found that the critical size for budding was identical in the *CLN3* and the *cln3* strains (Figure 2B of Lew et al., 1992), and it was concluded that the *CLN3* gene was not functionally important in daughter cells (even though *CLN3* was expressed). This implied that the difference in cell size between the wild-type and mutant starting cultures (Figures 1 and 2A of Lew et al., 1992) must have been due to a difference between the *CLN3* and *cln3* mother cells.

We repeated the experiment with an isogenic pair of strains that differed only by a deletion of *CLN3* in the BF305-15d background. The Coulter Channelyzer plots showed that the cells of the *cln3* strain were larger than the cells of the *CLN3* strain, as expected (Figure 1). Small G1 daughter cells were obtained by elutriation. Calcofluor staining showed that 95% or more of these cells were indeed daughters. Cells were reinoculated, and samples were taken at increasing times as the cells grew. The graph of cell volume versus percentage of budded cells is shown in Figure 2. In contrast with the results of Lew et al., the *cln3* daughter cells grew to a much larger volume than the *CLN3* cells before budding. With the same samples, we assayed START directly by asking whether α factor did or did not prevent budding at each time point. By this execution point assay, the wild-type daughters passed START at a modal size of 30 μm^3, while the *cln3* deletion daughters passed START at a modal size of 64 μm^3. Finally, we also followed the cells through the cycle using fluorescence-activated cell sorting analysis; this also showed that the *cln3* daughters passed through DNA synthesis at a much larger size than did the *CLN3* daughters (data not shown).

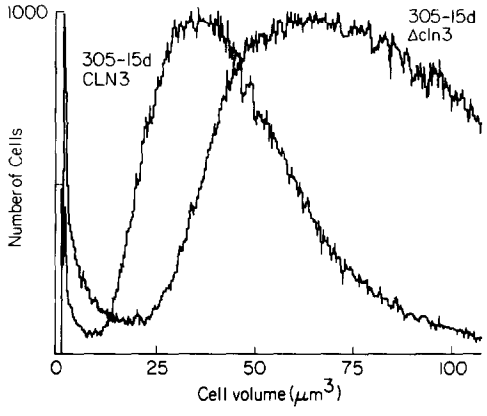

Figure 1. Cell Size Distributions of BF305-15d *CLN3* and Its Isogenic *cln3* Derivative

Cells of strain BF305-15d (*MATa leu2 ura3 his3 trp1 ade1 met14 arg5,6*) and an isogenic *cln3::LEU2* derivative were grown in YNB–sucrose medium at 23°C to early exponential phase. The cultures were sonicated, and cell size distributions were obtained using a Coulter Channelyzer.

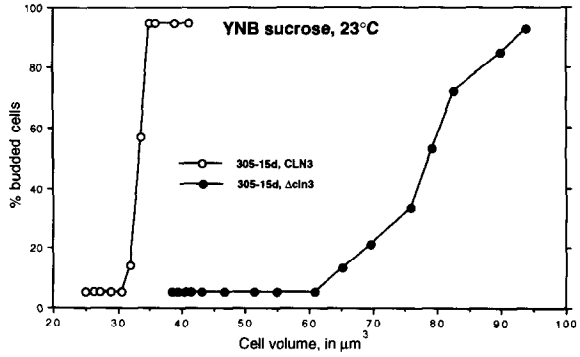

Figure 2. Critical Size for Daughter Cell Budding in Isogenic *CLN3* and *cln3* Strains

The strains shown in Figure 1 were grown to about 10^7 cells per ml in YNB medium plus 2% filter-sterilized sucrose at 30°C. Analysis with a Coulter Channelyzer showed that in this culture the *CLN3* cells had a modal cell volume of 39 μm^3, while the *cln3* cells had a modal cell volume of 57 μm^3. Cells were harvested by centrifugation at room temperature, resuspended in a small volume of the same medium, sonicated, and fractionated for cell size by centrifugal elutriation at 23°C in YNB–sucrose. For each strain, a fraction containing small unbudded cells (and 3% to 5% budded cells) was reinoculated into the same medium at 23°C. Bud scar counts confirmed that the cells were daughters. Samples were taken every 15 min. Cell size and budding index were followed with time. The distribution of DNA content was assayed by flow cytometry, and passage through START was assayed as the α factor execution point (data not shown).

We obtained from Lew et al. the two strains compared in their experiments. When using comparable clonal cultures of these cells (see Lew et al., 1993, below), we obtained the same results as in the 305-15d background: *cln3* deletion daughters passed through START at a much larger size than the wild-type daughters (data not shown).

These results show that *CLN3* functions in both daughter and mother cells, not only in the 305-15d background but also in the BF264-15Dau background, and we believe that *CLN3* generally functions equivalently in daughter and mother cells.

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A Suppressor of *cln3* for Size Control

Prompted by the results of Linskens et al. (1993, above), we have reexamined the role of CLN3 in daughter cells of S. cerevisiae. Contrary to our conclusions based on experiments with the *cln3* strain DL4 (BF264-15Dau background; Lew et al., 1992), we found that CLN3 was essential for the correct timing of START in daughter cells of a *cln3* strain provided by Dr. Futcher (W303a background). Further experiments revealed that DL4 contained two classes of cells (both *cln3*) that differed in their size: DL4-b ("big") cells were comparable in size to those of other *cln3* strains, while DL4-l ("little") cells were similar to wild-type cells in their size distribution (Figure 1A). Colonies derived from single DL4-l cells were found to produce cells of both types upon culture in YNB–sucrose medium (e.g., Figure 1B), suggesting that the size phenotype was unstable. The switching frequency was quite high, so that clear detection of the different size classes required analysis of recently colony-purified populations, and even these often consisted of quite mixed populations (e.g., Figure 1B). Genetic analysis showed that the ability to produce little cells was due to a single nuclear mutation unlinked to *CLN3* (data not shown) that we have named *scs1* (suppressor of *cln3* size defect). Repetition of our experiments with a DL4 (*cln3*) population containing >95% big cells showed that CLN3 was essential for the correct timing of START in daughter cells. Based on this, we conclude that CLN3 is necessary for daughter cell size control and that the results we obtained previously were in fact due to an unsuspected suppressor mutation (*scs1*) present in the DL4 strain. Presumably, our experiments were performed on a mixed cell population containing both little and big *cln3* cells, which we erroneously interpreted as a homogeneous population containing small daughter and large mother cells.

We examined the expression of *CLN1* and *CLN2* in *cln3 scs1* cells to determine whether the suppression of the *cln3* size defect was due to a misregulation of the other CLNs. Indeed, elutriated (early G1) cells from this strain (>95% little cells) contained significantly higher levels of *CLN1* and *CLN2* mRNAs compared with similarly sized elutriated wild-type cells (Figure 2). This was not general, since expression of *CDC9*, a gene transcribed with similar cell cycle periodicity to *CLN1* and *CLN2*, was not elevated in the mutant cells (Figure 2). Thus, it may be that *scs1* specifically affects expression of *CLN1* and *CLN2*, which results in a return to approximately wild-type size in cells lacking CLN3. The basis for the instability of the sup-

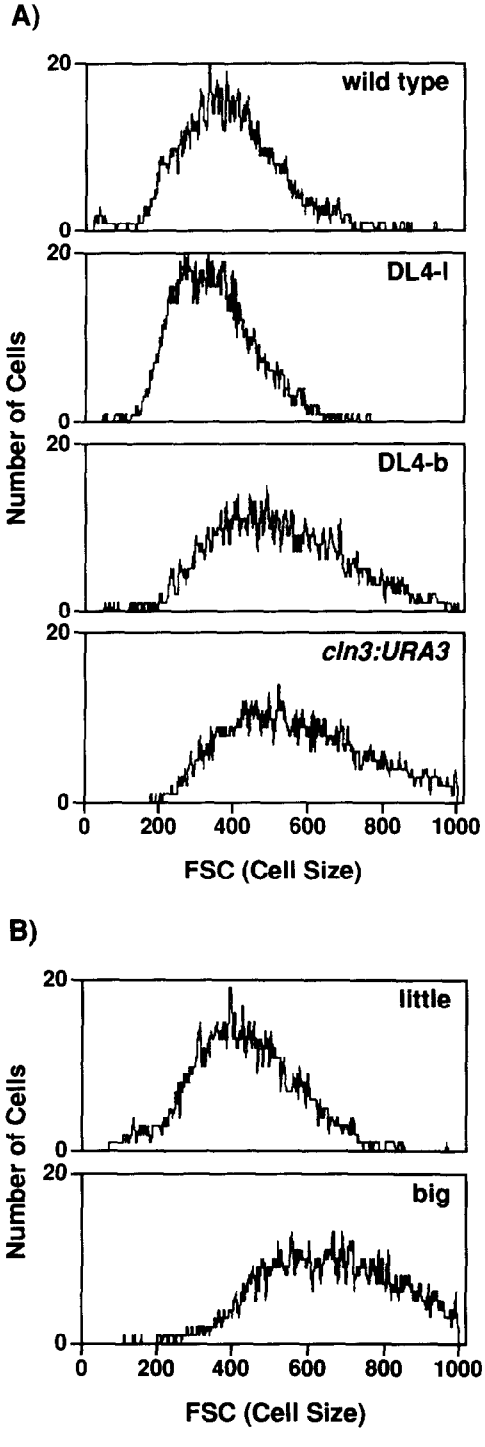

Figure 1. DL4 Cells Display Two Distinct Size Phenotypes

(A) Colony-purified exponentially growing populations of wild-type (BF264-15Dau), DL4 (*cln3 scs1*) little and big, and *cln3::URA3* (DLY206) cells were analyzed by forward angle light scattering (FSC: a measure of cell size) as described (Lew et al., 1992).

(B) Cells from a single little *cln3 scs1* colony were inoculated into YNB–sucrose, grown to stationary phase at 30°C, and streaked out for single colonies on YEP–sucrose plates. Twenty colonies were analyzed as above, and in this instance 16 produced the little distribution (upper) while 4 produced the big distribution (lower). One example of each is shown.