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Uncontrolled Septation in a Cell Division Cycle Mutant of the Fission Yeast *Schizosaccharomyces pombe*

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A temperature-sensitive *Schizosaccharomyces pombe* mutant, *cdc16-116,* has been isolated which undergoes uncontrolled septation during its cell division cycle. The mutant accumulates two types of cells after 3 h of growth at the restrictive temperature: (i) type I cells (85% of the population), which complete nuclear division and then form up to five septa between the divided nuclei; and (ii) type II cells (15% of the population), which form an asymmetrically situated septum in the absence of any nuclear division. *cdc16-116* is a monogenic recessive mutation unlinked to any previously known *cdc* gene of *S. pombe.* It is not affected in a previously reported control by which septation is dependent upon completion of nuclear division. We propose the *cdc16-116* is unable to complete septum formation and proceed to cell separation and is also defective in a control which prevents the manufacture of more than one septum in each cell cycle.

Septation is a well-characterized morphogenetic process which requires a stringent control of both spatial localization within the cell and of timing during the cell division cycle. As such, it has attracted attention in bacteria (16) and in simple eucaryotes, such as the budding yeast *Saccharomyces cerevisiae* (1) and the fission yeast *Schizosaccharomyces pombe* (8). Previously, the septum in *S. pombe* has been called the cell plate (11). We propose to use the term septum, as it is more commonly applied to fungal cells, whereas the term cell plate is usually used to describe a morphogenetically different structure observed during the division of plant cells.

Mutants defective in septation have been described in *S. cerevisiae* (6, 7) and in *S. pombe* (14). In *S. pombe,* a distinction can be made between early and late septation mutants, as the latter accumulate disorganized septal material detectable by its fluorescence after staining with primulin. Another class of mutants which indirectly prevent septation are those defective in earlier cell cycle events such as DNA replication and nuclear division. The phenotype of these mutants indicates the presence of a regulatory control which prevents septation until nuclear division has been completed (6, 7, 14). The nature of this regulatory signal is not understood.

A screening procedure developed for isolating temperature-sensitive conditional mutants blocked in cell division also yielded a mutant with an abnormal pattern of septation. This mutant has revealed a hitherto unknown control which prevents the manufacture of more than one septum in each cell cycle.

MATERIALS AND METHODS

All of the strains used were derived from the wild type 972 (h^-) and 975 (h^+) of Leupold (10). The mutants *ura5-294, lys1-131,* and *ade6-616* were UV induced in 975 (h^+) (U. Leupold, umpublished data). The mating-type mutant *mei1-102* (2) and the cell cycle mutants *cdc2-33* and *cdc15-140* (14) have been described previously. The mutant *cdc16-116* was isolated while screening for *cdc*$^-$ and cellular morphology mutants among about 500 temperature-sensitive mutants obtained after nitrosoguanidine mutagenesis (14). The strain of *cdc16-116* used in the present work was backcrossed twice to wild type. The genetic procedures used for *S. pombe* were described previously (5, 9).

Growth media and conditions for the temperature shifts were those used by Nurse et al. (14). Synchronous cultures were prepared as described by Mitchison (11). Microscopic examinations in vivo were made with a Zeiss photomicroscope, using dark-field optics and a Planapochromatic ×16/0.35 objective or phase-contrast optics and a Neofluar ×25/0.60 objective. Preparations were stained with Giemsa stain by the method of Robinow (15) as modified by Nurse et al. (14) and were examined under bright-field optics with a Planapochromatic ×100/1.30 oil immersion objective. Time-lapse photomicrography was performed at either 25 or 35°C as described by Fantes (3). The estimations of cell number, protein, and DNA were carried out as previously described (11, 14).

RESULTS

Genetic analysis of *cdc16-116.* *cdc16-116* is a temperature-sensitive mutant of 972 (h^-) which divides at 25 but not at 35°C. The temperature sensitivity phenotype segregated 2:2 in 18 tetrads and can therefore be considered mon-

ogenic. Recombination analysis by the "criss-cross" procedure of Gutz et al. (5) showed that it was unlinked to all of the previously described *cdc* genes of *S. pombe* (14), as well as the two genes *wee1* and *wee2* which are involved in the regulation of cell division (13, 17).

A diploid strain with the genetic constitution *meil-102 ade6-616 cdc16-116/h⁻ ura5-294 lys1-131* did not show any temperature sensitivity, indicating that the *cdc16-116* phenotype is recessive to wild type. The heterozygosity for the *cdc16* gene was checked by haploidization by using *m*-fluorophenylalanine (9). In this haploidization experiment, *cdc16* cosegregated with the *lys1* (on chromosome I) but recombined freely with both *ura5* (on chromosome II) and *ade6* (on chromosome III), allowing *cdc16* to be allocated to chromosome I.

Properties of an exponentially growing culture of *cdc16-116* shifted from 25 to 35°C. An exponentially growing culture of *cdc16-116* was shifted to 35°C for 5 h (Fig. 1). The culture was followed for increases in DNA, protein, and cell number and for nuclear division and septum formation. In several respects the response of *cdc16-116* was similar to that previously described for *cdc⁻* mutants defective in a late stage of septation (14). Cell number increased for about 0.5 h, giving a transition point at 0.82 of a cell cycle (14). The rate of cell number increases for the first 0.5 h after the shift is, however, somewhat reduced as compared with wild type (14). Cells elongate only slowly once they have attained a length of about 13 μm (Fig. 2), which is close to 14 μm, the normal length at division of *S. pombe* at 35°C (17). The cells get swollen, indicating that they are blocked at a constant length in the constant-volume phase of the cycle, which in wild type coincides with septation. Protein synthesis began to slow down about 3 h after the shift, and nuclear division and DNA synthesis came to almost a complete halt. As already discussed by Nurse et al. (14), this is presumably due to the combined effect of cells being blocked at a constant length in the constant-volume phase of the cycle, together with the action of a size control which regulates the initiation of nuclear division (13). The cellular morphology of *cdc16-116* cells at 35°C, however, has not been encountered before in the *cdc* mutants previously described. Between 1 and 4 h after shift to 35°C, the population progressively accumulates two different morphological cell types, hereafter called type I cells and type II cells (Fig. 3 and 4). Type I cells are binucleate and contain multiple septa between the two nuclei. They make up about 85% of the total cell population. Type II cells, which make up the remaining 15% of the cell population, are uninucleate and have an asymmetrically located septum. The nucleus is contained in the larger cell compartment.

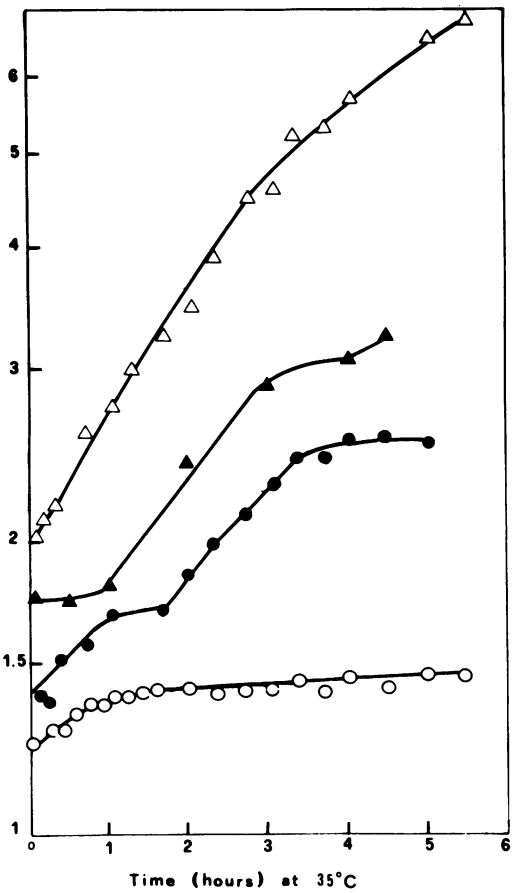

FIG. 1. *Cell number, number of nuclei, DNA, and protein of cdc16-116 mutant on shift from 25 to 35°C. The culture was grown throughout on minimal medium and was shifted from 25 to 35°C at zero time. The experimental parameters are plotted per unit volume of culture on an arbitrary log scale. The real values of the experimental parameters, per milliliter of culture (equivalent to one unit on the arbitrary log scale), are given within parentheses: ○, cell number (2 × 10⁴); ▲, number of nuclei (10⁴); ●, DNA (0.565 ng); △, protein (15 μg).*

Properties of *cdc16-116* cells shifted to 35°C as a function of their age in the cell cycle. As shown above, a culture of *cdc16-116* shifted to 35°C produces two types of cells with distinct cellular morphology. The two morphologies could be explained if *cdc16-116* cells respond differently according to their age in the cell cycle at the time of the shift. This was tested by investigating the phenotype of cells shifted to 35°C at various times during the cycle. Two

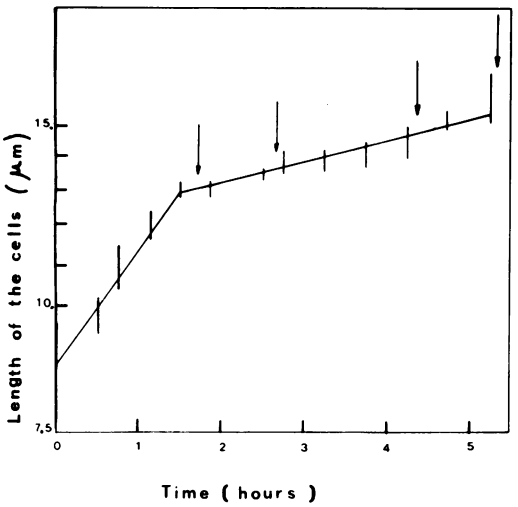

FIG. 2. *Elongation and septation pattern of cdc16-116 upon a temperature shift occurring when cells are at any early stage of the division cycle. A total of six freshly divided cells with lengths between 8.5 and 9.0 µm were shifted to 35°C and followed for cell elongation and occurrence of septation by time-lapse photomicrography. Average lengths (in logarithmic scale) are given with standard errors as a function of incubation time at 35°C. The arrows correspond to the successive average times of septation.*

experimental approaches were used and are described in turn.

(i) Shift-up from a synchronous culture. Cells of *cdc16-116* were shifted to 35°C for 3 h at intervals throughout a synchronous culture and were scored for type I and type II cells. The proportion of the cell types formed was strongly dependent upon the position of the cells in the cell cycle at the time of the shift. Type II cells were formed predominantly from cells shifted at about 0.85 of the way through their cell cycle (Fig. 5). This corresponds to the time at which septum formation is taking place. It is also close to the transition point of 0.82, calculated for *cdc16-116* from the cell number increase seen in Fig. 1 after shift to 35°C. A transition point of 0.82 means that all cells after this point in the cell cycle can go on to divide. Therefore, it seems likely that type II cells are derived from cells that have divided at 35°C. This point was confirmed by the following experiment.

(ii) Analysis of individual cells from an exponential culture shifted to 35°C by time-lapse microphotography. A culture of *cdc16-116* growing exponentially at 25°C was shifted to 35°C and followed by time-lapse microphotography as described above. A total of 75 individual cells were analyzed for a 6-h growth period at 35°C. The cell age at the time of the shift was determined by measuring cell length at that time, as previously described (3, 11). Again either type I or type II cells were formed, depending upon whether they had been shifted to 35°C before or after 0.85 of a cell cycle when the septum was made. The formation of the two cell types will be described in turn.

(a) *Cells shifted before the formation of a septum.* A total of 56 belonged to this group. Their phenotypic response to the shift was homogenous and is schematically represented in Fig. 3A. They elongated at a rate identical to the wild-type rate until they reached a length of about 13 µm (Fig. 2). From then on, their rate of elongation was considerably reduced, being less than 10% of the former rate. They became swollen and accumulated several septa, producing type I cells, as shown in Fig. 3A. The septa were formed one at a time every 45 to 90 min. No cell separation occurred among these cells.

(b) *Cells shifted at the time of septum formation or after.* A total of 17 cells belonged to this group. Again, they gave a homogeneous response to the shift, which is schematically represented in Fig. 3B. These cells underwent cell separation along the septum which they had formed under the permissive growth conditions, giving two daughter cells. One of the daughter cells immediately started to elongate at a wild-type rate until it became a type I cell containing several septa. The other daughter cell became a type II cell. It did not start elongation until it had formed a septum, which was located asymmetrically in the cell and was always nearest the end with the birth scar. From the nucleus-stained cells it could be seen that the enucleate compartment was always smaller than the nucleate one. Because the smaller compartment was always nearest the birth scar, it follows that the compartment located next to the birth scar was the one that was enucleate. A longer period of time-lapse photomicrography showed that the enucleate compartment failed to elongate any further. The nucleate compartment began to elongate after about 1 h, and after growth to a length of 15 to 16 µm it produced multiple septa located in its center, like a type I cell.

Genetic evidence that expression of *cdc16-116* is dependent upon nuclear division and early septation. Previous work has shown that septation is usually dependent upon the completion of nuclear division (6, 7, 14). *cdc16-116,* which accumulates several septa without nuclear division taking place, might be defective in that control. To check this, we constructed a double mutant, *cdc2-33 cdc16-116. cdc2-33* is a temperature-sensitive mutant which prevents nuclear division at 35°C and has a transition point close to nuclear division at 0.78

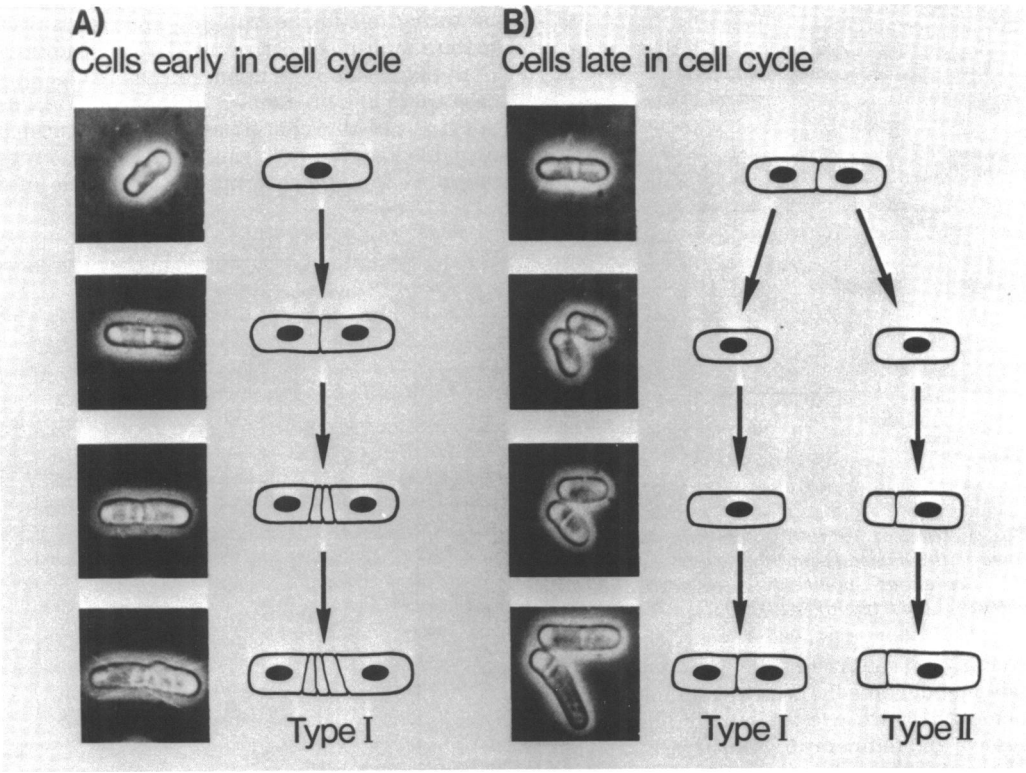

FIG. 3. *Cellular morphologies displayed by cdc16-116 cells within 4 h at 35°C. (A) Cells shifted when in an early phase of their cycle (i.e., until completion of nuclear division), failing to divide, producing a type I cell. (B) Cells shifted at a later stage of the cycle, undergoing cell separation and producing one type I and one type II daughter cell. The type I cell reproduced here has only one septum, but multiseptate cells were observed upon further incubation (data not shown). Septa show up on the phase-contrast pictures as a strongly contrasting structure and were redrawn for more clarity in the accompanying sketches. Nuclei cannot be detected by phase-contrast microscopy under the conditions used, but were incorporated in the drawings on the basis of a Giemsa-staining analysis, as described in the text.*

of a cell cycle (14). If a population of *cdc2-33 cdc16-116* cells is shifted from 25 to 35°C, cells which are past 0.78 of their cycle would be able to complete nuclear division or would have already completed it. However, most cells will be before 0.78 of their cycle when shifted and will not undergo nuclear division. If septation in a *cdc16-116* background is dependent upon nuclear division, one would predict that the large fraction of cells which are between 0 and 0.78 of their cycle would fail to undergo septation and would accumulate the elongated and mononucleate cells typical of *cdc2-33* alone. This is indeed what was observed, as 78% of the double-mutant cells had the phenotype of the *cdc2-33* parent at 35°C, whereas 10 and 12% formed the type I and type II phenotypes of the *cdc16-116* parent, respectively. A similar experiment done with a synchronous culture of *cdc2-33 cdc16-116* transferred to 35°C at various stages of the cell cycle confirmed this observation. This experiment also showed that the only cells which eventually displayed the *cdc16-116* phenotype were those shifted late in the cycle.

Similarly, a double-mutant *cdc15-127 cdc16-116* was constructed; *cdc15* is a gene controlling an early function of septation (14). On shift to 35°C, most cells of this double mutant had the phenotype of *cdc15-127* alone, forming elongated cells with several nuclei but no septum. This indicates that the multiple septa of *cdc16* are produced by the normal morphogenetic process leading to septation in wild type, rather than by an alternative way of cross-wall formation in the cell. Unlike *cdc16-116* alone, the cells of the double mutant also became very elongated. This indicates that *cdc16-116* is not defective in cell elongation, ruling out a possible interpretation that *cdc16-116* was primarily defective in elongation and that this led to the preferential formation of cross-wall material.

DISCUSSION

The *S. pombe* mutant *cdc16-116* is affected in

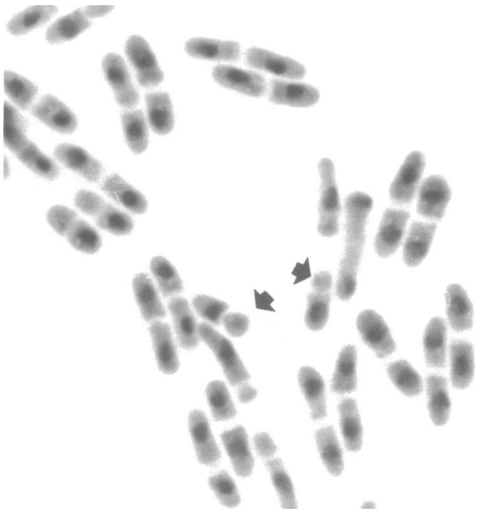

FIG. 4. *Nuclear staining of cdc16-116. Cells stained with Giemsa stain after 2 h of incubation at 35°C. The arrows point to type II cells (with an enucleate compartment).* ×1,000.

the control of septation. The mutation defines a single mendelian gene which is unlinked to any previously known *cdc*$^-$ genes. Its phenotype is recessive and temperature sensitive, and on shift to the restrictive temperature the mutant shows the following behavior. (i) Cells shifted early in their cell cycle (before a transition point of 0.82) proceed normally through the cell cycle until they have completed mitosis. At this point they cease elongation and undergo successive rounds of septation, making one septum at a time and forming enucleate compartments. Cell separation does not take place. These successive rounds resemble the situation in the *cdc4* mutants of *S. cerevisiae*, in which there are cycles of bud emergence from mononucleate cells (6, 7). However, the timing of the repeated morphological events in *S. pombe* is less than the usual cell cycle time, whereas it is equal to it in *S. cerevisiae*. (ii) Cells shifted later in their cell cycle do proceed through cell separation, but one of the daughter cells then produces an extra septum which is assymetrically located in the cell. The septum is produced before the daughter cell has undergone mitosis, forming an enucleate cytoplasmic compartment at one end of the cell. Enucleate cytoplasmic compartments are only rarely formed in eucaryotes and can usually only be obtained by physical manipulation, such as microdissection or centrifugation.

These observations indicate that, as in wild-type cells, the initiation of septation is dependent upon the completion of mitosis (14). However, once septation has been initiated, it cannot be turned off in the mutant. Thus, cells accumulate multiple septa or an extra septum early in the next cell cycle upon mitosis. In the latter case there is a breakdown in the usual dependency of cell division. These properties can be explained by the following model for the regulation of septation during the cell cycle of *S.*

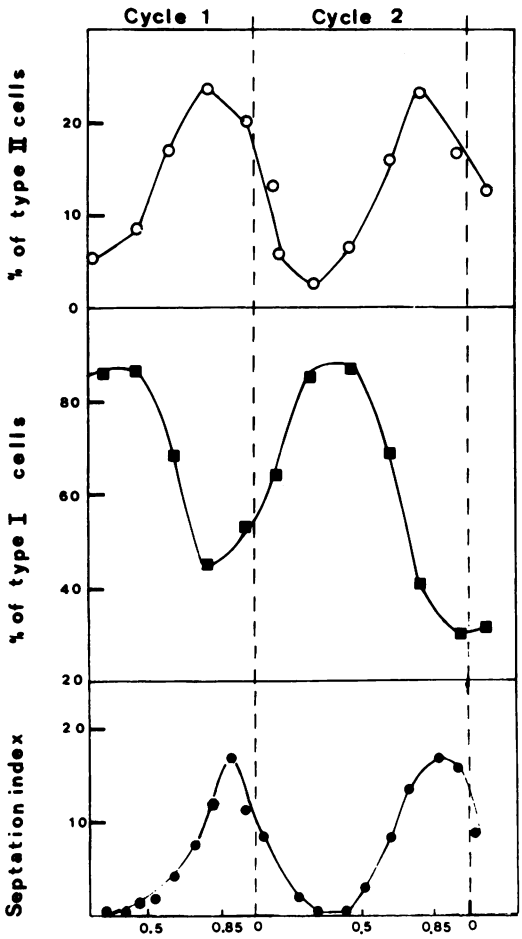

FIG. 5. *Proportions of type I and type II cells in a synchronous culture of cdc16-116 after a 3-h shift-up at 35°C. Cells were grown synchronously at 25°C (generation time, 225 min). The extent of synchrony was determined by following the septation index (percentage of septated cells). This also gives the time at which successive cell cycles are initiated, knowing that cell separation occurs 30 min after septation. Samples were transferred every 45 min from 25 to 35°C and further incubated for 3 h. The proportion of type I and type II cells in each sample was determined by microscopic examination after Giemsa staining. After 3 h at 35°C, not all cells had reached their terminal phenotypes, particularly those destined to become type II cells. For this reason the sum of type I and II cells does not attain 100%.*

pombe (Fig. 6). The model also explains why septation always follows mitosis and why it takes place only once during each cell cycle. The model contains the following elements. (i) Upon completion of mitosis one or several compartments of the machinery required for initiation of septation are synthesized de novo. Alternatively, the components may be activated from an inactive precursor, as suggested for chitin synthetase, which is involved in septation in *S. cerevisiae* (1). (ii) At least one of the components of the septation machinery can support the formation of only one septum at a time. One of the components is also destroyed or inactivated by an inhibitor which is only produced when the cell has completed the first septum and has progressed to cell separation. *cdc16-116* is defective in cell separation and so does not produce this inhibitor.

This model can be regarded as opening and shutting a "competence window" for septation. Only while the window is open is a cell competent to make a septum. The window is opened by the completion of mitosis and is closed by the inhibitory signal generated at cell separation. Cells of *cdc16-116* become blocked at cell separation with the window open and so continue to make further septa.

The model is supported by a number of experimental observations. Inhibition of mitosis by an inhibitor or by *cdc*$^-$ mutants prevents further septation in both budding and fission yeasts (6, 7, 12, 14). This can be explained if a signal produced on completion of mitosis initiates the synthesis or activation of components of the septation machinery. Such a control would explain why enucleate cells are not normally found and why septation is coordinated with cell growth. This is because mitosis itself is coordinated with cell growth by a mass titrating mechanism (4, 13, 14).

Mutants which have initiated septation but cannot proceed to cell separation continue to undergo the septation process. Such mutants are those blocked in late septation, which accumulate large quantities of disorganized septal material (14), and *cdc16-116,* which is blocked in cell separation and accumulates multiple septa. These observations can be explained if an inhibitor preventing further septation was produced just before or during cell separation. If the inhibitor destroyed or inactivated one or more of the initiation components of the septation machinery dependent upon mitosis, then in wild-type cells no more septa could be made until the next mitosis. Cells of *cdc16-116* produce only one septum at a time, as shown by our time-lapse observations. This can be explained if a component of the septation machinery cannot support the formation of more than one septum simultaneously.

cdc16-116 cells shifted late in their cell cycle produce a daughter cell which forms an extra septum in the absence of mitosis. A possible interpretation of this observation is the following. On shift to the restrictive temperature the *cdc16-116* gene product begins to be thermally inactivated. The partial inactivation of the *cdc16-116* gene product slows down cell separation in those cells shifted late in the cell cycle, as suggested by the reduced rate in cell number increase immediately after a temperature shift of *cdc16-116* (Fig. 1). Because of the delay in cell separation, the inhibitor may not be produced soon enough to prevent the initiation of an additional septum. The latter will be completed in one of the daughter cells, leading to the formation of an enucleate cytoplasmic compartment. By this time cell separation will have produced the inhibitor preventing the formation of any further septa until the next mitosis in each of the two daughter cells. The final result will be the formation of a type II cell in the one daughter cell in which the additional septum was

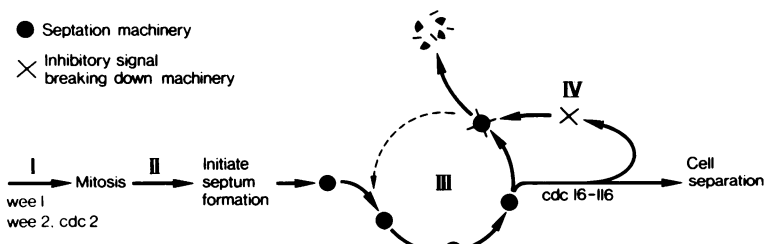

FIG. 6. *Our present view of the controls programming septation during the cell cycle of S. pombe. (I) Size control over mitosis (4, 13, 17), ensuring that mitosis only takes place when the cell has reached a given mass or size. (II) Dependency of septum initiation on mitosis (14) ensuring that septation cannot take place before nuclear division. (III) Septation machinery activated upon mitosis and producing one septum at a time. (IV) Inactivation of the septation machinery after completion of a first septum (in cdc16-116 cells fail to undergo this inactivation at the restrictive temperature).*

initiated, whereas the other daughter cell produces a type I phenotype.

Although the details of this model are speculative, we can conclude that the temporal control of septation during the cell cycle is determined by two antagonistic controls. The first initiates septation as a consequence of mitosis, and the second prevents the initiation of any further septa once one has been completed. These controls would mark the opening and shutting of the competence window for septation. Such a view of temporal control during the cell cycle is more complex than that suggested by Hartwell (6) for *S. cerevisiae*. He speculates that there is a "cascading system of positive regulation where the function of early gene products turns on the synthesis of later gene products." This control system would initiate periodic cell cycle events but would not turn them off. Two antagonistic controls opening and shutting a competence window explains the dependence of septation on mitosis and also why the event only takes place once. An analogous control may also operate over other cell cycle events, such as DNA replication and mitosis.

ACKNOWLEDGMENTS

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