COLCEMID SENSITIVITY OF FISSION YEAST

II. Sensitivity of Stages of the Cell Cycle

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INTRODUCTION

In an attempt to understand the regulation of chromosome segregation and cell division in eukaryotic cells, we are examining the inhibition of cell division of the fission yeast *Schizosaccharomyces pombe* by the antimitotic drug Colcemid (N-deacetyl-N-methylcolchicine, Ciba Pharmaceutical Co , Summit, N. J.) (1). Our approaches include a study of the physiological parameters accompanying inhibition by Colcemid, a genetic study of mutants resistant to the drug, and an analysis of binding of the drug to protein fractions from sensitive and resistant strains of this yeast.

In the present paper, we report on the sensitivity to Colcemid of different stages in the cell cycle of synchronous cultures of *S. pombe*.

MATERIALS AND METHODS

Yeast Strains

A haploid strain of *S. pombe* h^- and a Colcemid-resistant mutant, h^- *cid5*, (1) were used.

Growth Conditions

Nutrient medium (YEG) of 0.5% yeast extract (Difco Laboratories, Inc , Detroit, Mich.) and 3% glucose and YEG medium containing 5.4 mM (0 2%) Colcemid (YEG-Colcemid) were used at 30°C (1).

Fixation and Staining

In a modification of the technique of Ganesan and Swaminathan (2, personal communication), cells were washed with distilled water and resuspended in 2 mM 8-hydroxyquinoline for 1–2 h at 30°C. Cells were then fixed in Carnoy's fluid (ethanol:chloroform:acetic acid, 6:3:1, vol:vol:vol) for 10 min at 22°C, washed in 70% ethanol and in distilled water, and affixed to cover slips. These were then incubated in 1% sodium chloride at 60°C for 1 hr, washed in 0.05 M sodium phosphate buffer, pH 7, and hydrolyzed in 1 N hydrochloric acid at 60°C for 7 min. They were then washed with buffer and immersed in 2% Giemsa stain (Allied Chemical Corp., New York) for 30 min at 22°C.

Synchronization

Exponentially growing cells were synchronized according to Mitchison (3) using a 16–60% gradient of glycerol in 0.5% yeast extract. The frequency of cells which coincide in their formation of a cell plate (cell plate peak), ranged from 40–60% in the first cell cycle.

RESULTS

Synchronous cultures revealed the stages within a cell division cycle of 140 min in YEG medium (Fig. 1) (3). By 85 min, the frequency of mononucleate cells decreased to 30% and a peak of binucleate cells appeared. After 100 min, the cell plate peak was reached. The number of cells separating normally was maximal at 115 min, and a new peak of mononucleate daughter cells appeared at 140 min. These results are similar to those of Mitchison (3). Three of the cell types (mononucleate, binucleate, and cell plate) are shown (Fig 2 *a*).

When 5.4 mM Colcemid was added at zero time to synchronized cells, nuclear division to form binucleate cells and the appearance of cells with cell plates were delayed by about 45 min as compared to the control culture without Colcemid (Figs 1 and 2 *b*). By 170 min, approximately 75% of the cells failed to separate and collected as V doublets, a cell type infrequent in control cultures After overnight incubation, the entire population was converted to V doublets Thus, at least two stages of the cell cycle are sensitive to Colcemid, nuclear division whose process is slowed, and cell separation which is more rigorously inhibited

In another experiment, a sample of a synchronized culture of *S pombe* in YEG was trans-

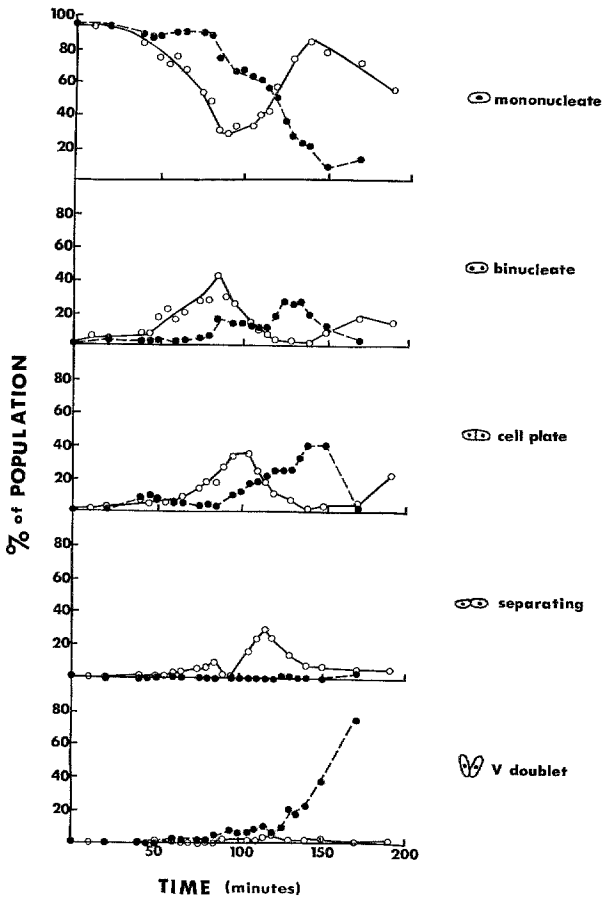

FIGURE 1 Synchronous growth of *S. pombe h⁻*. For each time point 400 Giemsa-stained cells from synchronous cultures were analyzed for the frequency of the various cell types: mononucleate cells, binucleate cells, cells with cell plates, normally separating cells, and V doublets. Cells from cultures in YEG, ○. Cells from cultures in YEG-Colcemid, ●.

ferred to YEG-Colcemid medium after 100 min of growth, or just before the time of the cell plate peak. A delay of about 20 min in passing through the cell plate peak was followed by the accumulation in the population of V doublets. Since in this experiment cells were exposed to the drug after nuclear division had occurred, an additional transient sensitivity to Colcemid at the time of cell plate formation was demonstrated. Moreover, the accumulation of V doublets was found not to require an interference with the previous nuclear division stage.

Asychronous cultures exposed to a fivefold range of drug concentration behaved in accord with this view of multiple sensitive stages. In concentrations of Colcemid of 5.4 mM or greater, the accumulation of V doublets was accompanied by a cessation of growth after about 3 h as measured by turbidity or protein content of the culture (1). At 2.7 mM Colcemid, V doublets accumulated during the first generation to a maximum of 95% of the population without a depression of growth rate of the culture, and then decreased in frequency as single and normally dividing cells reappeared. Above 5.4 mM Colcemid, the time of appearance of V doublets was progressively delayed with increasing concentrations of the drug as though nuclear division was increasingly depressed before the onset of inhibition of cell separation.

One mutant resistant to Colcemid (1) was examined in synchronous culture to see if sensitivity of nuclear division and of cell separation were both

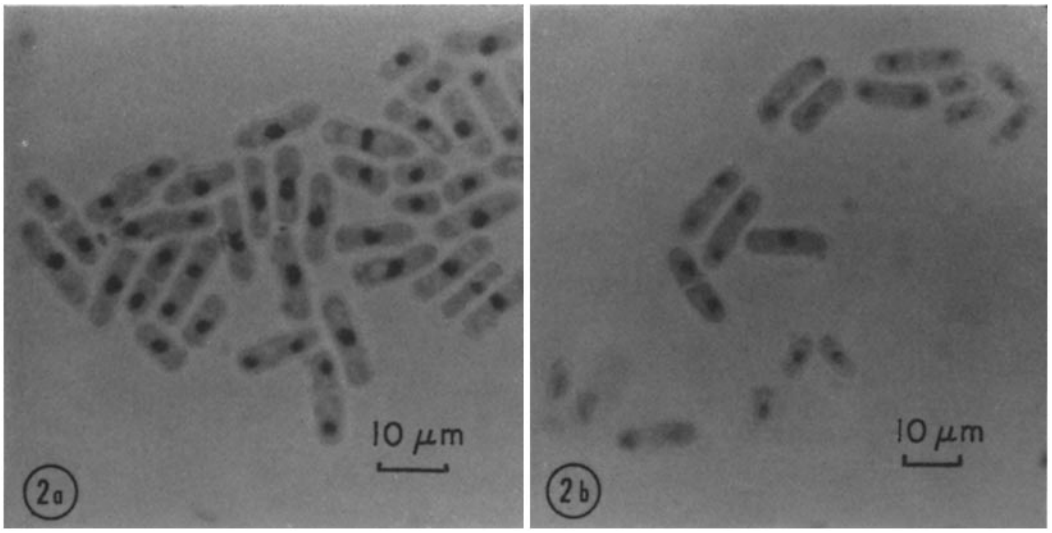

FIGURE 2 *S. pombe h⁻*. (*a*) Giemsa-stained cells from a synchronized culture after 60 min in YEG. (*b*) Giemsa-stained cells from a synchronized culture after 135 min in YEG-Colcemid. Total magnification is × 800.

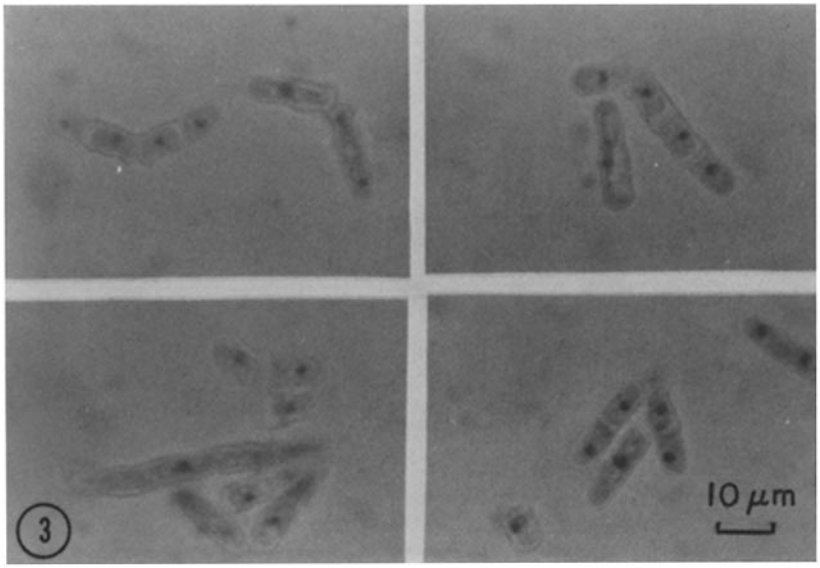

FIGURE 3 *S. pombe h⁻ cid5* in YEG. Giemsa-stained cells from an asynchronous culture in YEG. Total magnification is × 800.

affected by the mutation. Cultures of this strain, as with several other mutants, tended to form clumps of cells which often contained elongated cells with multiple cell plates (Fig 3).

Sonication (Branson Sonic Power, Co , Danbury, Conn., Sonifier model W185) for 3 s at 25 W dispersed the clumps into individual cells which could be processed for synchronous cultures by standard procedure The transition from mononucleate cells to binucleate cells and the time for appearance of the cell plate peak in Colcemid-treated synchronous cultures of this mutant, as

with its wild-type parent, was delayed by about 40 min. Consequently, the genetic resistance to Colcemid in this mutant was not manifested in changes in the sensitivity of nuclear division and cell plate formation. However, untreated synchronous cultures of this mutant differed from the wild type in their mode of cell separation They first formed V doublets and then continued to grow and form new septa in each arm of the doublet. Thus, the clumps observed in random cultures appear to be derived from single cells rather than by aggregation of independent cells. Colcemid treatment did not prevent the formation of clumps of multiseptate cells in the mutant nor interfere with increases in the turbidity of the mutant culture.

DISCUSSION

In previous work on *S. pombe* (1), our observations focused on the inhibition of cell separation by Colcemid and the selection of drug-resistant mutants. The present analysis of synchronous cultures indicates that nuclear division, cell plate formation, and cell separation are independently sensitive to Colcemid. Interference with nuclear division of *S. pombe* by Colcemid may be analogous with the classical model of colchicine-blocked mitosis in higher plant and animal cells (4, 5).

The mechanism for chromosomal segregation in *S. pombe* is still hypothetical. A microtubular bundle within the dividing nucleus of *S. pombe* (6) may be the homologue of the mitotic apparatus of higher cells. Although alternative complex explanations may be proposed, it suffices as a reasonable working hypothesis to consider microtubules (7) to be the intracellular targets for the inhibition by Colcemid of nuclear division and cell separation.

The demonstration that some Colcemid-resistant mutants are pleiotropically affected in cell division suggests that at least this category of resistance affects a required component of cell division for which microtubules are a likely candidate. The abnormal growth pattern of this mutant coupled with its resistance to Colcemid may be interpreted as arising from a change in either the quality or quantity of tubulin, rather than from those other possible causes of drug resistance (e.g., drug permeability or detoxification) which would not be expected to affect cell division.

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