Cell Cycle Specificity of Certain Antimicrotubular Drugs in Schizosaccharomyces pombe

By GRAEME M. WALKER†
The Biological Institute of the Carlsberg Foundation, 16 Tagensvej, Copenhagen N, DK-2200 Denmark

(Received 24 February 1981; revised 8 May 1981)

Of the seven antimicrotubular drugs tested, nocodazole, mebendazole and trifluralin at saturable concentrations failed to inhibit cell division in Schizosaccharomyces pombe, while carbenazim, thiabendazole and chloropropham each at 50 μg ml⁻¹ and amiprophos methyl at 200 μg ml⁻¹ completely arrested cell division. This inhibition was associated with striking morphological changes in which carbenazim- and thiabendazole-treated cells became elongated and pseudohyphal, whereas chloropropham- and amiprophos methyl-treated cells appeared small and rounded with occasional V-shaped pairs. Lomofungin staining revealed that nuclear division was also arrested by these drugs. Suspected blockage of defined cell cycle stages was confirmed by pulse-induction experiments which revealed that cells could be synchronized into division using exposure to a drug for one generation. Further experiments with synchronous cultures prepared by size selection showed that different drugs possessed different transition points; for example, carbenazim and thiabendazole were effective in blocking a late stage of the cell cycle just prior to division, whereas amiprophos methyl affected a very early stage. The results suggest that some of the drugs used exert cell cycle specificity in S. pombe either by impairing microtubule assembly mechanisms (as with carbenazim and thiabendazole) or by inhibiting synthesis of tubulin subunits (as with amiprophos methyl). These drugs could prove useful in studies of microtubule biogenesis during the cell cycle in yeast.

INTRODUCTION

Microtubules are elongated, tubular organelles found in all nucleated cells which participate in many diverse intracellular activities (Dustin, 1978). They are composed principally of protein subunit dimers, α and β tubulins, each with a molecular weight of about 55,000. Studies of the regulation of tubulin synthesis, its polymerization and assembly into microtubules, and the subsequent orientation of microtubules into a functional mitotic apparatus, are central to our understanding of nuclear and cell division. Yeast cells possess great appeal in such studies since, in spite of lacking the highly ordered centriole of higher eukaryotes, they perform a mitosis which is regarded as being essentially orthodox (Petersen & Ris, 1976). The fission yeast Schizosaccharomyces pombe is amenable to biochemical, physiological and genetic analyses, and is therefore a particularly useful model eukaryote, especially with regard to the cell cycle (see Mitchison, 1971). During mitosis in S. pombe, electron-dense spindle pole bodies (also called ‘kinetochore-equivalents’ or ‘microtubule organizing centres’ or ‘centriolar plaques’) duplicate within the nuclear membrane and eventually migrate to opposite poles with a longitudinal array of microtubules appearing between them (McCully & Robinow, 1971). In addition to these intranuclear spindle microtubules, S. pombe also contains cytoplasmic microtubules which appear to be directly opposed to the spindle pole bodies on the nuclear envelope (Hereward, 1974).

† Present address: Department of Biochemistry, University of Otago, Dunedin, PO Box 56, New Zealand.

0022-1287/82/0000-9806 $02.00 © 1982 SGM
Fungal cells in general are insensitive to the classical spindle poison, colchicine, and fungal tubulin has little affinity for this drug (Lederberg & Stetten, 1970; Haber et al., 1972; Davidse & Flach, 1977; Baum et al., 1978). Burns (1973) has shown that soluble proteins of S. pombe fail to bind [3H]colchicine and that the colchicine derivative, colcemid, affects S. pombe cell division only when present at concentrations in the millimolar range (Stetten & Lederberg, 1973). Furthermore, the specific binding of colcemid to yeast tubulin has been questioned (Baum et al., 1978). Although the presence of tubulin in yeast has been verified by a number of workers (Water & Kleinsmith, 1976; Baum et al., 1978; Clayton et al., 1979), it occurs in very low amounts (Haber et al., 1972; R. G. Burns, personal communication). This, together with the fact that yeasts are resistant to antimitotic plant alkaloids like colchicine, has somewhat hindered research into tubulin synthesis and assembly during the yeast cell cycle.

In this paper, the mode of action of selected antimicrotubular drugs on nuclear and cell division in S. pombe has been investigated. The compounds chosen possess either herbicidal, fungicidal, antihelminthic or antitumour properties and have been shown to exert strong antimitotic activity in various cell types by impairing microtubule function. Four of the drugs tested — nocodazole, carbendazim, mebendazole and thiabendazole — are benzimidazole derivatives, some of which are known to be highly toxic fungicides. For example, carbendazim, the stable breakdown product of benomyl, has been shown to bind to fungal tubulin (Davidse & Flach, 1977; Baum et al., 1978) and also to disrupt fungal microtubules (Howard & Aist, 1980). The other three drugs tested — chloropropham, trifluralin and amiprophos methyl — are herbicides which were deemed useful in this study because of their specific effects on microtubule biogenesis in algae (Brown & Bouck, 1974; Collis & Weeks, 1978), protozoa (Banerjee et al., 1975), higher plants (Hepler & Jackson, 1969; Bartels & Hilton, 1973; Kiermayer & Fedtke, 1977) and mammalian cells (Oliver et al., 1978). Amiprophos methyl is of special interest because it has been shown to be a selective inhibitor of tubulin synthesis as opposed to tubulin assembly (Collis & Weeks, 1978).

**METHODS**

**Organism and culture conditions.** Schizosaccharomyces pombe haploid strain 972h− was originally obtained from Dr Jørgen Friis, University of Odense, Denmark, and was grown at 32 °C in Edinburgh Minimal Medium no. 2 (EMM2) (Mitchison, 1970). Viable stock cultures were maintained on EMM2 agar slopes at 4 °C and were subcultured monthly by growing cells for 3–4 d at 32 °C prior to cold storage. Weekly subculturing was carried out by inoculating stationary phase cells into 10 ml EMM2, and 1 ml samples of actively dividing precultures were used to initiate experimental cultures which were aerobically propagated in Erlenmeyer flasks in a shaking water bath at 32 °C.

Cell numbers (the mean of three or four counts of 150–200 cells) were determined using a Burker-Türk haemocytometer employing the criterion of Mitchison (1970), in that a dividing cell was scored as two only when the constriction between daughter cells appeared. The cell plate index was calculated as the percentage of the total population in which a complete cell plate or septum was visible.

**Nuclear staining and photomicroscopy.** Nuclei of S. pombe were stained with the antibiotic lomofungin (5-formyl-1-methoxycarbonyl-4,6,8-trihydroxyphenazine, a generous gift of Dr G. B. Whitfield, Upjohn Co., Kalamazoo, Michigan, U.S.A.) using the following modification of the method described by Kopecka (1976). Portions from a stock solution of lomofungin (2 mg ml−1, freshly prepared in analytical grade dimethyl sulphoxide) were added directly to S. pombe cultures to a final concentration of 50 μg ml−1 and left for at least 15 min with shaking at 32 °C. Cells were quickly pelleted by microcentrifugation and resuspended in one drop of distilled water. Slides were prepared and cells were photographed under phase contrast using a Reichert Photo-Automatic microscope.

**Establishment of synchronous cultures by size selection.** Cells were synchronized by selection using the following modification of the density-gradient sedimentation procedure described by Mitchison & Vincent (1965). Cells from an exponentially growing culture were harvested by filtration and concentrated to about 5 × 106 cells ml−1 by resuspension in 5 ml EMM2. This suspension was carefully layered on top of a linear lactose gradient (15–30%, w/v, prepared in EMM2) and centrifuged at 1000 rev. min−1 in an MSE 6L centrifuge for 6 min. Using a long, thin syringe needle, about 2 ml of cell suspension (containing small cells at an early stage of the cell cycle)
was removed from the uppermost layer of the gradient and re-inoculated into fresh EMM2 to initiate synchrony. As far as possible, all steps were performed at 32 °C.

**Inhibitor additions and pulse-induction synchronization.** The antimicrotubular drugs used in this study were: nocodazole (NOCO; methyl 5-(2-thiencarbonyl)-1H benzimidazol-2-yl-carbamate, obtained from EGA-Chemie, Steinheim am Albuch, F.R.G.), carbenazim (MBC; methyl benzimidazol-2-yl-carbamate, obtained from Riedel-DeHaen, Seelze-Hannover, F.R.G.), mebendazole (MBZ; methyl 5-benzoylbenzimidazol-2-yl-carbamate, obtained from Janssen Pharmaceutica, Beerse, Belgium), thiabendazole (TBZ; 2-(thiazol-4-yl)benzimidazol, obtained from Riedel-DeHaen), chloropropham (CIPC; isopropyl N-(3-chlorophenyl)-carbamate, obtained from Sigma), trifluralin (TRIF; trifluro-2,6-dinitro-N,N-dipropyl-p-toluidine, obtained from Riedel-DeHaen), and amiprophos methyl (APM; O-methyl-O-(4-methyl-6-nitrophenyl)-N-isopropyl-phosphorothioimide, obtained from Bayer, Leverkusen, F.R.G.). Stock solutions of these compounds were prepared in analytical grade dimethyl sulphoxide (DMSO) and portions were added to experimental cultures to obtain the desired final concentrations of the drugs. The concentration of DMSO in cultures was always less than 0.5% (v/v); this concentration of DMSO had no effect on cell division in control cultures.

The minimum concentrations of the above reagents required to inhibit cell division rapidly and completely in early-exponential phase cultures of *S. pombe* (1–3 × 10⁴ cells ml⁻¹) were estimated by comparing the increase in cell numbers in treated cultures with that in untreated controls. Minimum inhibitory drug concentrations were used to synchronize cell division using the following modification of the pulse-induction method employed by Walker & Duffus (1979) for synchronization of *S. pombe* using chelating agents. Exponentially growing cells (10 ml cultures) were exposed to inhibitors for a normal generation time of 2.5 h, then quickly harvested by filtration through a Millipore membrane (0.45 μm pore size), washed twice with distilled water and finally resuspended in fresh EMM2, which lacked inhibitors. This harvesting, washing and resuspension procedure, which took only a few minutes to complete, was carried out at room temperature and it did not affect cell division in identically treated control cultures.

**RESULTS**

In initial experiments the minimum concentrations of the selected antimicrotubular drugs necessary to arrest cell division in *S. pombe* were determined (Table 1). MBZ, NOCO and TRIF at concentrations of 1000, 100 and 200 μg ml⁻¹, respectively, all failed to arrest cell division over a period of six cell generations; at higher concentrations, these drugs precipitated when added to the growth medium. MBC, TBZ and CIPC (all 50 μg ml⁻¹) and APM (200 μg ml⁻¹), however, all arrested cell division over this period. Figure 1 shows the time course of inhibition by short-term exposure to the drugs. It is evident that saturable concentrations of MBZ, NOCO and TRIF only very slightly affected the division rate during the first few cell generations. In the presence of MBC or TBZ, cells completed about one doubling then ceased dividing; CIPC- or APM-treated cells were immediately and completely inhibited. The inhibition caused by MBC, TBZ, CIPC and APM was associated with striking morphological changes (Figs 2 and 3). For example, after the first few hours (Fig. 2), MBC- and TBZ-treated cells appeared abnormally elongated and few possessed cell plates, whereas CIPC- and APM-treated cells were smaller with many of the latter possessing cell plates. This contrasted with untreated control cells which possessed normal asynchronous division morphology in which cells were of variable length and around 10% of the population had cell plates. Lomofungin, which quickly and intensely stains *S. pombe* nuclei (Kopecka, 1976; Walker & Duffus, 1979), revealed that drug-treated cells failed to continue nuclear division after residual cell division had ceased. This was apparent from the discrete, spherical morphology of the nuclei which were located centrally in the cytoplasm. The nuclei of untreated, exponentially growing cells, on the other hand, displayed characteristic cell cycle stages, including mitosis (see Fig. 2a). After more prolonged exposure to the drugs (Fig. 3), the morphological changes described above became noticeably more pronounced. Cells exposed to MBC and TBZ, for example, were very elongated – as much as eight times the length of control cells which appeared small and round, characteristic of normal stationary phase *S. pombe* cultures (Mitchison, 1970). In addition, many cells took on a ‘pseudohyphal’ appearance with long, branched structures protruding from the cells and with occasional aberrant cell plate formation. Lomofungin staining again failed to provide evidence of nuclear division, either in these cells or in cells exposed to CIPC or APM for extended periods. The
Table 1. Inhibition of cell division in *S. pombe* by long-term treatment with antimicrotubular drugs

Actively dividing cells of *S. pombe* in the early-exponential phase of growth (about 1 × 10^6 cells ml^-1) were exposed to the drugs for 15 h (about six cell generations), and the cell number increase over this period was expressed as a percentage of that in the untreated control culture.

<table>
<thead>
<tr>
<th>Drug concn (µg ml^-1)</th>
<th>NOCO</th>
<th>MBC</th>
<th>MBZ</th>
<th>TBZ</th>
<th>CIPC</th>
<th>TRIF</th>
<th>APM</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>43.5</td>
<td></td>
<td>82.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>81.4</td>
<td>82.3</td>
<td>81.4</td>
<td>82.3</td>
<td>82.3</td>
<td>82.3</td>
<td>82.3</td>
</tr>
<tr>
<td>15</td>
<td>10-3</td>
<td>10-3</td>
<td>10-3</td>
<td>10-3</td>
<td>10-3</td>
<td>10-3</td>
<td>10-3</td>
</tr>
<tr>
<td>20</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
<td>25</td>
<td>7.7</td>
<td>7.7</td>
<td>7.7</td>
<td>7.7</td>
<td>7.7</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>30</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
</tr>
<tr>
<td>40</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td>45</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td>50</td>
<td>84.3</td>
<td>84.3</td>
<td>84.3</td>
<td>84.3</td>
<td>84.3</td>
<td>84.3</td>
<td>84.3</td>
</tr>
<tr>
<td>100</td>
<td>79.7</td>
<td>79.7</td>
<td>79.7</td>
<td>79.7</td>
<td>79.7</td>
<td>79.7</td>
<td>79.7</td>
</tr>
<tr>
<td>200</td>
<td>78.0</td>
<td>78.0</td>
<td>78.0</td>
<td>78.0</td>
<td>78.0</td>
<td>78.0</td>
<td>78.0</td>
</tr>
<tr>
<td>400</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
</tr>
<tr>
<td>1000</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
<td>66.3</td>
</tr>
</tbody>
</table>

Fig. 1. Time course of inhibition of cell division in *S. pombe* by short-term treatment with antimicrotubular drugs. Actively dividing cells were exposed to the following drugs (at the time indicated by the arrow): ●, none (control culture); ○, TRIF (200 µg ml^-1); ■, NOCO (100 µg ml^-1); □, MBZ (1000 µg ml^-1); △, TBZ (50 µg ml^-1); ▲, MBC (50 µg ml^-1); ▽, CIPC (50 µg ml^-1); ◀, APM (200 µg ml^-1).

The above observations suggested that MBC, TBZ, CIPC and APM blocked *S. pombe* at defined stages in the cell division cycle. The following experiments were carried out in an attempt to confirm this. These were based on the same rationale as used by Mitchison & Creanor (1971) for DNA synthesis inhibitors in *S. pombe*, whereby asynchronously dividing cells were exposed to the drugs for one complete generation time, followed by re-inoculation into inhibitor-free medium after filtration and washing. Figure 4 shows that the patterns of cell number increase after such a treatment with MBC, TBZ, CIPC and APM were synchronous. Table 2 shows that this synchrony was associated with high synchronization indices.
Fig. 2. Effect of short-term treatment with antimicrotubular drugs on cell and nuclear morphology in S. pombe. (a) Untreated, exponentially growing control culture. (b) Cells treated with CIPC (50 μg ml⁻¹) for 150 min. (c, d) Cells treated with MBC (50 μg ml⁻¹) for 90 and 150 min, respectively. (e, f) Cells treated with APM (200 μg ml⁻¹) for 90 and 150 min, respectively. (g, h) Cells treated with TBZ (50 μg ml⁻¹) for 90 and 150 min, respectively. Cells were stained with lomofungin. The bar marker in (a), which applies to the whole figure, represents 10 μm.

(Engelberg, 1961) and cell plate indices of around 20% for the two synchronous cell divisions. The interdivision times based on cell number increase were in each case approximately equal to the mean generation time of 2.5 h for the control. Table 2 also shows that the time taken for cells to complete their first synchronous division after drug treatment was dependent on the particular drug being used. The implication here is that different drugs
Fig. 3. Effect of long-term treatment (24 h) with antimicrotubular drugs on cell and nuclear morphology in S. pombe. (a) Untreated, stationary phase control culture. (b) Cells treated with CIPC (50 μg ml⁻¹). (c) Cells treated with APM (200 μg ml⁻¹). (d, e, f) Cells treated with MBC (50 μg ml⁻¹). (g, h, i) Cells treated with TBZ (50 μg ml⁻¹). Cells were stained with lomofungin. The bar markers represent 10 μm.

affect different cell cycle stages; that is, they possess different ‘transition points’ (see Mitchison, 1971). However, as it may take some time both for washing the drugs out and for cells to recover after the block such experiments do not allow accurate measurements of transition points. This was overcome by adding the inhibitors at various times during the cell cycle in cultures synchronized by size selection (see Methods) and examining the resultant changes in cell number and cell plate index. By finding the time in the cycle before which the drugs inhibited synchronous cell division and after which they did not, transition points for the different drugs were estimated (Fig. 5). TBZ, CIPC and APM treatment gave approximate transition points of 0·6, 0·75, 0·1, respectively, using the criterion of Nurse et al. (1976), by which the complete cell cycle to division is taken as unity. Using MBC, the results were almost identical to those shown for TBZ.
Cell cycle specificity of antimicrotubular drugs

Fig. 4. Pulse-induction synchronization of cell division in S. pombe using antimicrotubular drugs. Cells were exposed to the drugs for 2.5 h (first arrow), followed by resuspension into fresh medium after filtration and washing (second arrow). Drug concentrations: MBC, TBZ and CIPC, 50 μg ml⁻¹; APM, 200 μg ml⁻¹. The control culture was treated identically except that no drug was added. ●, log₁₀(cell number ml⁻¹); ○, cell plate index.

DISCUSSION

The aim of this work was to define the inhibitory effects of certain established antimicrotubular drugs on mitotic timing in the model lower eukaryote, S. pombe. It was hoped that the study would highlight the potential use of inhibitors other than colchicine or its derivatives in research concerned with microtubule biogenesis and cell cycle control. The drugs selected for investigation are compounds known to possess potent antimitotic properties which are expressed either at the level of tubulin synthesis or tubulin polymerization and assembly.

Three of the drugs, NOCO, MBZ and TRIF, did not affect S. pombe cell division using either short- or long-term exposure. The anticancer drug NOCO, and the structurally related
Table 2. Synchrony parameters of *S. pombe* cultures pulse-induced with antimicrotubular drugs

The data were calculated from Fig. 4, in which the untreated control culture grew asynchronously with a constant cell plate index of around 8% and a mean generation time of around 150 min.

<table>
<thead>
<tr>
<th>Drug treatment (concen., exposure time)</th>
<th>Synchronization indices* (%): S1 S2</th>
<th>Cell plate indices (%): 1st 2nd</th>
<th>Cell division timing† (min): To 1st division Between divisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBC (50 μg ml⁻¹, 2-5 h)</td>
<td>70 43</td>
<td>17 15</td>
<td>30 150</td>
</tr>
<tr>
<td>TBZ (50 μg ml⁻¹, 2-5 h)</td>
<td>67 47</td>
<td>20 20</td>
<td>38 138</td>
</tr>
<tr>
<td>CIPC (50 μg ml⁻¹, 2-5 h)</td>
<td>77 64</td>
<td>21 21</td>
<td>102 159</td>
</tr>
<tr>
<td>APM (200 μg ml⁻¹, 2-5 h)</td>
<td>79 ca 70</td>
<td>23 18</td>
<td>168 ca 140</td>
</tr>
</tbody>
</table>

* S1 and S2 represent Engelberg synchronization indices (Engelberg, 1961) for the first and second synchronous divisions, respectively. The index was calculated by the method of Bakke & Pettersen (1976).
† Times taken to complete the first cell division and the intermitotic time between the first and second synchronous divisions were calculated from the mid-points of cell number doublings.

Antihelminthic compound MBZ, are both benzimidazole derivatives which have been shown to affect polymerization of mammalian microtubules, either by directly binding to tubulin (Hoebeke *et al.*, 1976; Laclette *et al.*, 1980) or by reducing the level of translatable tubulin mRNA (Ben-Ze'ev *et al.*, 1979). NOCO has also been shown to induce mitotic arrest in *Dictyostelium* (Welker & Williams, 1980) and to inhibit growth of *Aspergillus* (Davidse, 1975). Recently, Künkel (1980) found that low concentrations of NOCO (around 2 μg ml⁻¹) caused specific division arrest during the budding cycle of *Saccharomyces cerevisiae*, thus contrasting with the present results which show *S. pombe* to be relatively insensitive to NOCO at concentrations as high as 100 μg ml⁻¹. The differential sensitivity of *S. pombe* cells to benzimidazoles MBC and TBZ (which effectively arrest cell division) and NOCO and MBZ (which are ineffective) may be due to differential uptake or metabolism of these drugs. However, the molecular basis of such selectivity in fungal cells to benzimidazoles is not known (Davidse & Flach, 1977).

High concentrations of the chemically unrelated herbicide TRIF, which has previously been shown to affect microtubule assembly in protozoa (Banerjee *et al.*, 1975) and plants (Bartels & Hilton, 1973), failed to affect division of *S. pombe* cells and is therefore not regarded as a potentially useful antimicrotubular agent in this organism.

Inhibition of cell division by MBC, TBZ, CIPC and APM was associated with pronounced morphological changes in *S. pombe* and in all cases nuclear division, as well as cell division, was prevented. With the benzimidazoles MBC and TBZ, cell growth continued in the absence of nuclear division suggesting a dissociation of the DNA-division cycle from the growth cycle (Mitchison, 1971). The appearance of elongated and pseudohyphal forms of *S. pombe* after prolonged exposure to MBC and TBZ may be related to the role of microtubules in cell wall deposition and consequential maintenance of cell shape (Dustin, 1978). Similarly, Howard & Aist (1980) found that MBC induces irregular hyphal tip growth in *Fusarium* and Quinlan *et al.* (1980) showed that *Saccharomyces cerevisiae* accumulates as large cell doublets in the presence of MBC. Cell plate formation is also affected in *S. pombe* by MBC and TBZ, indicating a possible involvement of microtubules in cross-wall formation and a dependence of this event on preceding nuclear divisions. It remains to be seen if the effect on cell plate formation is a pleiotropic effect of blocking nuclear division. It would be of value in this respect to study the effect of benzimidazoles on certain cell division mutants of *S. pombe* that
Cell cycle specificity of antimicrotubular drugs

Fig. 5. Demonstration of cell cycle transition points of antimicrotubular drugs in S. pombe. Cells were synchronized by lactose-gradient size selection and drugs were added at the times indicated by the arrows. Drug concentrations: TBZ and CIPC, 50 μg ml⁻¹; APM, 200 μg ml⁻¹. ○, log₁₀(cell number ml⁻¹); ○, cell plate index.

are defective in cell plate formation. MBC also prevents septation in Saccharomyces cerevisiae (Quinlan et al., 1980) and in Ustilago maydis (Hammerschlag & Sisler, 1973). The above observations are consistent with the findings of Byers & Goetsch (1975) in that inhibition of microtubular function in yeast affects morphogenetic events other than those associated with mitosis. Cells treated with CIPC and APM appear somewhat different from those treated with benzimidazoles in that they fail to continue growth and as a consequence appear small and round after prolonged exposure to the drugs. This might imply that both the growth cycle and the DNA-division cycle have been affected, presumably around the point in the cell cycle at which the two are linked. This contrasts with other cell cycle agents such as the DNA synthesis inhibitors 2'-deoxyadenosine and hydroxyurea which in S. pombe produce oversized cells by dissociating the two cycles.

These morphological observations introduced the possibility that antimicrotubular drugs possess differential cell cycle specificity in S. pombe, and as a first step to establish this, a
series of pulse-induction synchronization experiments were carried out. Cell division in S. pombe can be successfully synchronized using pulse treatments lasting one generation time (Fig. 4). During exposure to the drug, cells may become blocked at set stages during their cell cycle which results in a synchronous progression of accumulated cells on removal of the block. The synchrony patterns revealed intermitotic times approximately equal to the normal generation time, indicating that the cell cycle was relatively unperturbed by the drugs. Recovery times after pulse-treatment, however, depended on the drug used; for example, cells divided quickly after MBC or TBZ treatment, but slowly after APM treatment. If one assumes that the drug is completely removed following pulse-treatment and that no appreciable lag phase has been induced, it is conceivable from what is known of the effects of these drugs on other cells that times for recovery to the first synchronous divisions reflect timing of different stages in microtubule biogenesis. For example, the short recovery time following MBC or TBZ treatment may correspond to a rapid re-assembly of dissociated spindle microtubules; whereas the long recovery time following APM treatment may correspond to de novo synthesis of tubulin subunits. However, further work is needed to establish the effects of APM on yeast tubulin.

Such experiments provide evidence of specific blockage of the cell cycle but they fail to locate the position of the block. Experiments with synchronous cultures prepared by size selection, on the other hand, which are assumed to represent unperturbed cells, were intended to pinpoint these blocks or ‘transition points’ more accurately. In a similar vein, Herbert et al. (1980) have employed pulses of the antimitic drug griseofulvin to naturally synchronous macroplasmodia of Physarum polycephalum in order to expose certain cell cycle-dependent events. Results of the present study (Fig. 5) support the above suggestions that MBC and TBZ affect a late stage in the cell cycle just prior to division (approximate transition point of 0.6 cell cycle units), and APM affects a very early stage in the cell cycle (approximate transition point of 0.1). It should be noted that a much later transition point for APM is estimated if the data from Fig. 4 are used, indicating in this case either incomplete removal of APM or an extended recovery time of the cells, or both. CIPC appears to affect a very late stage in the cell cycle (approximate transition point of 0.75), and this observation may be related to an effect of this drug on spindle pole bodies (Oliver et al., 1978).

In summary, it has been shown by several lines of evidence, including morphological examination and synchronous culture analyses, that MBC, TBZ, CIPC and APM specifically affect the cell cycle in S. pombe. It is very likely that the inhibitory effects are expressed either through microtubule assembly or tubulin synthesis and, therefore, these drugs should be of value in studies concerned with cell cycle control and microtubule biogenesis.

The author was supported by a Royal Society/Science Research Council Postdoctoral Fellowship, funded by the N.A.T.O. Science Fellowship Programme.

REFERENCES


Cell cycle specificity of antimicrotubular drugs


