J. Cell Sci. **49**, 353–367 (1981)
Printed in Great Britain © Company of Biologists Limited 1981

NUCLEAR CYCLE OF *SAPROLEGNIA FERAX*

I. BRENT HEATH* AND KAREN RETHORET
Biology Department, York University, 4700 Keele Street, Downsview, Ontario M3J 1P3, Canada

SUMMARY

The mitotic nuclear (equivalent to cell) cycle of the oomycete fungus, *Saprolegnia ferax*, was analysed by quantitative serial-section electron microscopy of hyphal nuclear populations synchronized by inhibition of DNA synthesis by fluorodeoxyuridine (FdUrd). Following telophase and karyokinesis, kinetochore mitrotubules persist into G_1 stage as a single group of $\sim$ 42 per nucleus ($2n$ = 42 for this species). During G_1 the centrioles replicate and kinetochore microtubules separate into 2 groups of $\sim$ 21, a configuration they retain through S and G_2. During metaphase a new population of kinetochore microtubules are formed, each one of an amphitelic pair connecting to the opposite pole to that associated with the persistent microtubule from the previous division. Thus, by the end of metaphase, there are $\sim$ 42 kinetochore microtubules per half spindle. FdUrd, applied for 2 h with uracil, completely blocks DNA synthesis yet permits centriole replication and causes nuclei to accumulate with 2 pairs of centrioles, 2 arrays (each of 21) of kinetochore microtubules, and apparently enlarged nucleoli. Removal of FdUrd permits rapid (within 30 min) DNA synthesis followed by successive rounds of decreasingly synchronous nuclear cycles. These post-FdUrd cycles are 2·5 times longer than normal at 2·5 h, with S plus G_2 being more extended than other phases. Calculated durations of a normal nuclear cycle are: G_1, 33 min; S, 7 min; G_2, 10 min; metaphase, 8 min; anaphase, 0·5 min; and telophase, 4 min.

INTRODUCTION

The analysis of the eukaryotic cell cycle in many species is relatively simple because the definitive morphological changes are detectable and the cycle takes a long time for completion. However, among the fungi, the morphological events are often difficult or impossible to detect with the light microscope and the duration of the cycle is much shorter. The most intensively studied fungal cell cycle is that of *Saccharomyces cerevisiae*, but even in this species there is still no clear, detailed description of the correlation between chromatin and spindle behaviour and the classic phases of the cell cycle (e.g. Hartwell, 1974, 1978). This gap is particularly unfortunate because *Saccharomyces*, and a number of other fungi, are increasingly used as eukaryote models for analysis of the control of both the cell cycle and chromosome activity. Apart from the necessity to fill this gap for complete information on the cycle, we must also know how fungal cycles compare with those of other eukaryotes before we can determine the generality of fungal data. For various technical reasons, a full description of the yeast cell cycle remains unavailable. However, recently, other fungi such as members of the Oomycetes have begun to be used to investigate various aspects of cell and molecular biology (e.g. Horgen, 1977; Law, Rozek & Timberlake, 1978). Oomycetes are

* Author to whom correspondence should be sent.

especially favourable for analysis of morphological aspects of the cell cycle (or, more accurately in a coenocyte, the nuclear cycle), because they give clear images following standard electron microscopy preparation techniques. We have recently provided detailed information on the ultrastructure and timing of events in the nuclear cycle of *Saprolegnia ferax* (Heath, 1980*a*, *b*; Heath & Rethoret, 1980), but these studies were incomplete because we did not know the time of DNA synthesis nor were we able to be specific about the details of kinetochore microtubule formation. However, we have recently obtained the first unequivocal chromosome count for a *Saprolegnia* species (Tanaka, Heath & Moens, unpublished data). These results have shown us how we could attempt to undertake a complete analysis of the nuclear cycle. This paper presents the results of such an analysis.

MATERIALS AND METHODS

The organism used is an uncertain species of *Saprolegnia*, probably *ferax* or *monoica* (Holloway & Heath, 1977), which is deposited in the Aquatic Phycomycete Culture Collection of Reading University and the American Type Culture Collection (ATCC 36051). Colonies were grown, prepared for electron microscopy, sectioned and analysed as described previously (Heath, 1980*a*). Colony DNA content was determined by the DAPI procedure (Brunk, Jones & James, 1979). Details of this procedure, and the means of determining the duration of the nuclear cycle and the stages of mitosis are given by Heath & Rethoret (1980). In that paper we noted that the differentiation between stages $P_{2/1}$ and $P_{2/2}$ was not always clear. In the present isolate it was usually clearer so that ambiguity was rare, especially following the treatments with FdUrd. All nuclei recorded as $P_{2/2}$ were unambiguously in that stage. DNA synthesis was inhibited by growing colonies at 25 °C in 9·5 ml of liquid medium (designated OM by Heath & Greenwood, 1968) in Petri dishes for the required time, at which point 0·1 ml of aqueous 5-fluorodeoxyuridine (O'Donovan & Neuhard, 1970) (FdUrd; Sigma Chemical Co., St Louis) and 0·5 ml of aqueous uracil (Sigma Chemical Co.) were added to give final concentrations of 0·5 mg/ml and 0·1 mg/ml, respectively. Following incubation in the inhibitor for the required time, colonies were rinsed (~ 30 s) in ~ 200 ml of OM to remove the FdUrd, then placed in fresh OM for the desired times prior to fixation or homogenization as appropriate. Mithramycin staining of hyphal nuclei was carried out as reported previously (Heath, 1980*b*). Nuclear and nucleolar areas were measured with an electronic planimeter (Heath & Rethoret, 1980).

RESULTS

Outline of mitosis

The stages of the nuclear cycle of the present isolate of *Saprolegnia* are morphologically indistinguishable from those reported earlier for *S. ferax* (Heath, 1980*a*). The terminology of Heath & Rethoret (1980) will be briefly outlined for orientation. Nuclei with a single pair of attendant centrioles and a single array of kinetochore microtubules are termed P_1. The arrangement of these kinetochore microtubule arrays takes the form of a population of short (~ 0·1 μm) divergent microtubules with their convergent ends located adjacent to the pocket region of the nuclear envelope as illustrated by Heath (1980*a*). Centriole replication yields P_2 nuclei, which may be subdivided into $P_{2/1}$ if the kinetochore microtubules form a single array or $P_{2/2}$ if they have separated into 2 arrays. Spindle formation yields metaphase nuclei with anaphase and telophase following in a relatively conventional manner. At the end of telophase, prior to karyokinesis, each spindle pole is very similar to a P_1 nucleus.

There appears to be poor coordination between the behaviour of the centrioles and karyokinesis, because we have occasionally observed telophase nuclei that are pre-karyokinetic but have a $P_{2/2}$ arrangement at each pole. These nuclei are rare and do not seriously detract from the generality of the normal pattern described above.

Kinetochore microtubules

As noted above, the general behaviour of the kinetochore microtubules (as opposed to the non-kinetochore microtubules, which were also present but not described in the present paper) during mitosis was comparable to that described previously (Heath, 1980*a*). However, the present work has utilized a less diverse population of hyphae and yielded more consistent quantitative data as shown in Fig. 1. At P_1 there were 39·4 ($\pm$ 4·0, n = 11) kinetochore microtubules per group; at $P_{2/1}$, 38·3 ($\pm$ 5·4, n = 8) and at $P_{2/2}$, 17·8 ($\pm$ 3·4, n = 12). During metaphase the number per half spindle increases so that by anaphase there were 34·5 ($\pm$ 3·7, n = 4; this value is low since one of the two spindles lacked one of the serial sections, which would have probably contained 2 or 3 kinetochore microtubules per half spindle). At telophase there were 37·9 ($\pm$ 3·6, n = 13) short kinetochore microtubules at each pole of the spindle. The increase in the number of kinetochores per spindle during metaphase is positively and significantly correlated (r = 0·63, n = 20, $P < 0{\cdot}01$) with spindle length (which is an indicator of temporal progress through mitosis). Similarly there is a comparable correlation between the percentage of kinetochores that were judged to be paired (Heath, 1980*a*) and spindle length (r = 0·59, n = 20, $P < 0{\cdot}01$). If, as considered in the Discussion, one interprets this data to indicate that assembly of each new kinetochore microtubule occurs in a paired configuration relative to each old, $P_{2/2}$-derived kinetochore, then one would predict that throughout metaphase the observed total number of kinetochore microtubules per spindle (i.e. those already formed) *plus* a number equal to the total number of unpaired kinetochore microtubules per spindle (i.e. each unpaired kinetochore microtubule is assumed to be destined to have another kinetochore microtubule formed opposite it by the end of metaphase) would add up to a constant number. As shown in Fig. 1, within the limits of the methodology, this was found to be the case.

Nuclear cycle analysis

In order to describe the relationship between the behaviour of the kinetochore microtubules and the stages of a conventional nuclear cycle, it was necessary to induce mitotic synchrony and then analyse the nuclei with the resolution of the electron microscope. This was achieved by using FdUrd to block DNA synthesis reversibly. Initial experiments, based on linear growth rates, showed that 0·5 mg/ml was the minimal concentration of FdUrd that completely inhibited growth. As shown in Figs. 2–4, this concentration completely inhibits DNA synthesis rapidly and reversibly. Uracil alone either had no effect or was slightly stimulatory (Figs. 2, 3). Following removal of the FdUrd, DNA synthesis (*S* period) was rapidly initiated and ceased again within 30 min after removal of the block (Figs. 2, 4). A succeeding period of synthetic quiescence was followed by further alternating periods of synthesis and

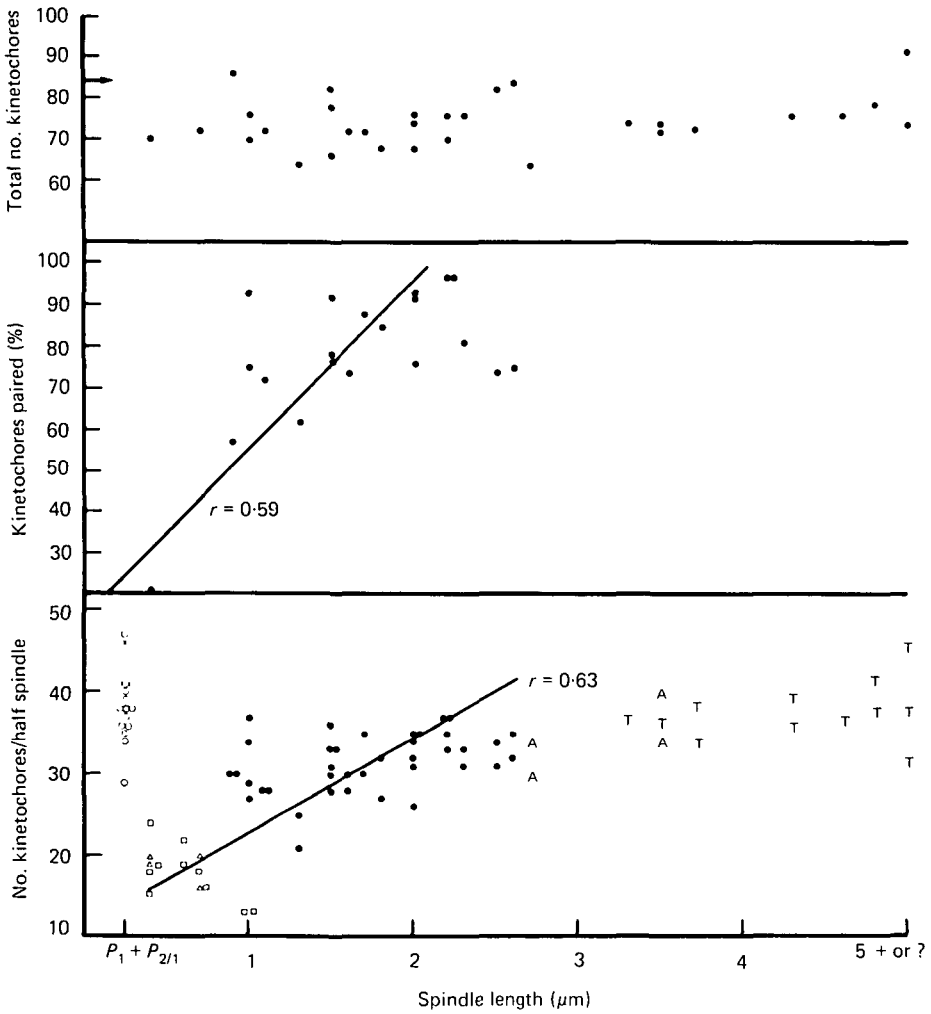

Fig. 1. Quantitative analysis of kinetochore microtubule (abbreviated to 'kinetochore' on the ordinate) behaviour through the nuclear cycle. After P_1 and $P_{2/1}$, progress through mitosis is indicated by the length of the spindle. One end of one telophase nucleus was on an incompletely sectioned nucleus hence the length was unknown. In the lower panel the number of kinetochore microtubules per group at P_1 and $P_{2/1}$ and the number per group or per half spindle at later stages are indicated. The linear regression line and correlation coefficient were calculated only for P_2/M transition and metaphase nuclei. In the centre panel the percentage of kinetochore microtubules judged to be paired is recorded. The 0·7 μm P_2/M transition nucleus was 100% unpaired and was excluded from the linear regression analysis. In the upper panel, the total number of kinetochore microtubules per nucleus was calculated for all P_2/M transitions and metaphase nuclei by summing the observed kinetochore microtubules and adding a number equal to the number of unpaired kinetochore microtubules per spindle (mean = 74·5 ± 6·2). The figures for the anaphase and telophase nuclei are simply the total observed per nucleus. The mean is 11·3 % below the predicted value of 84 (arrow). Lower panel: ○, $P_1+P_{2/1}$; □, $P_{2/2}$; △, P_2/M; ●, M; A, A; T, T.

quiescence, with each synthetic period lasting longer and having a shallower slope than its predecessor (Fig. 2). The periodicity of DNA synthesis, following a FdUrd block, was approximately 2·5 h (Figs. 2, 4). In other experiments, in the absence of FdUrd treatment, the DNA content of colonies increased linearly over the 16–44 h period (Heath & Rethoret, 1980).

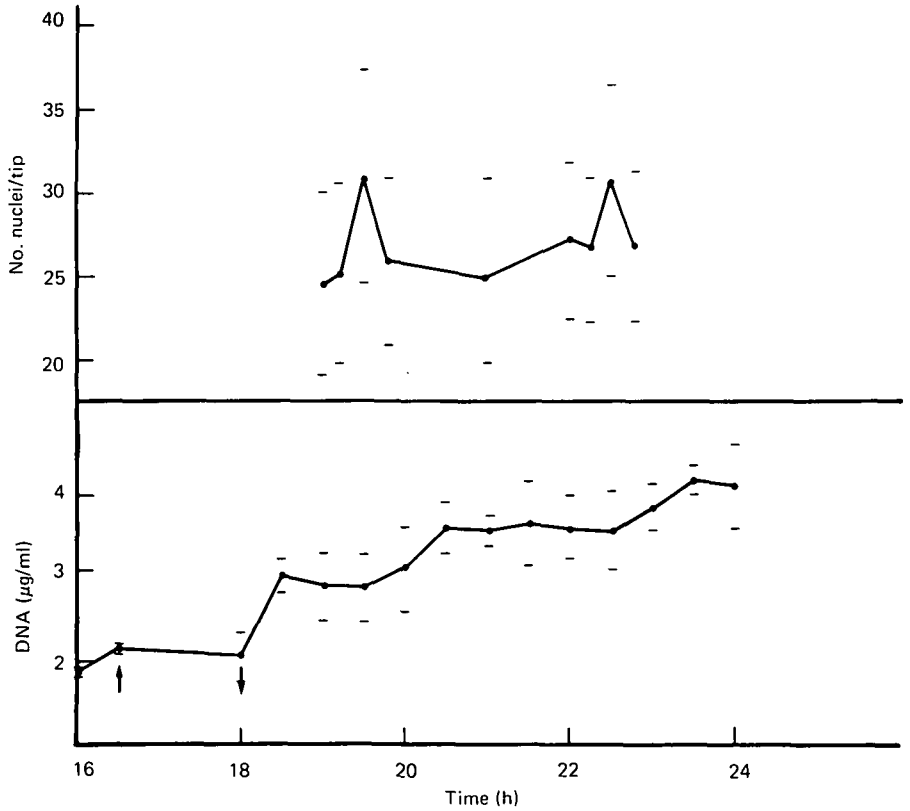

Fig. 2. DNA and nuclear behaviour following treatment with FdUrd. At 16·5 h 0·5 mg/ml of FdUrd and 0·1 mg/ml uracil were added to the colonies (↑). At 18 h colonies were rinsed and placed in fresh medium (↓). DNA content of triplicate colonies (with standard deviations indicated) was measured at 0·5-h intervals after the block. Three steps in the DNA curve are evident. Addition of uracil alone gave a linear increase in DNA (of 16 h to 16·5 h). In the upper panel the number of nuclei per tip was recorded from mithramycin-stained colonies from the same experiment. 'Tip' is defined as a length of hypha 227 μm behind the most apical nucleus. The most apical nucleus is typically ~ 10 μm behind the hyphal tip. Values given are means of 25 hyphae, with standard deviations indicated. Two clear post-karyokinetic peaks are found, the first at ~ 1 h after termination of the first round of DNA synthesis and the second, less sharp, at ~ 2 h after the second burst of synthesis.

Because *Saprolegnia* grows vegetatively in the form of aseptate hyphae, each of which contains a variable population of about 25 nuclei per apex ('apex' being arbitrarily defined here as the most apical ~ 200 μm, a length that includes all of the non-vacuolate cytoplasm and the bulk of the dividing nuclei) and grows by continuous

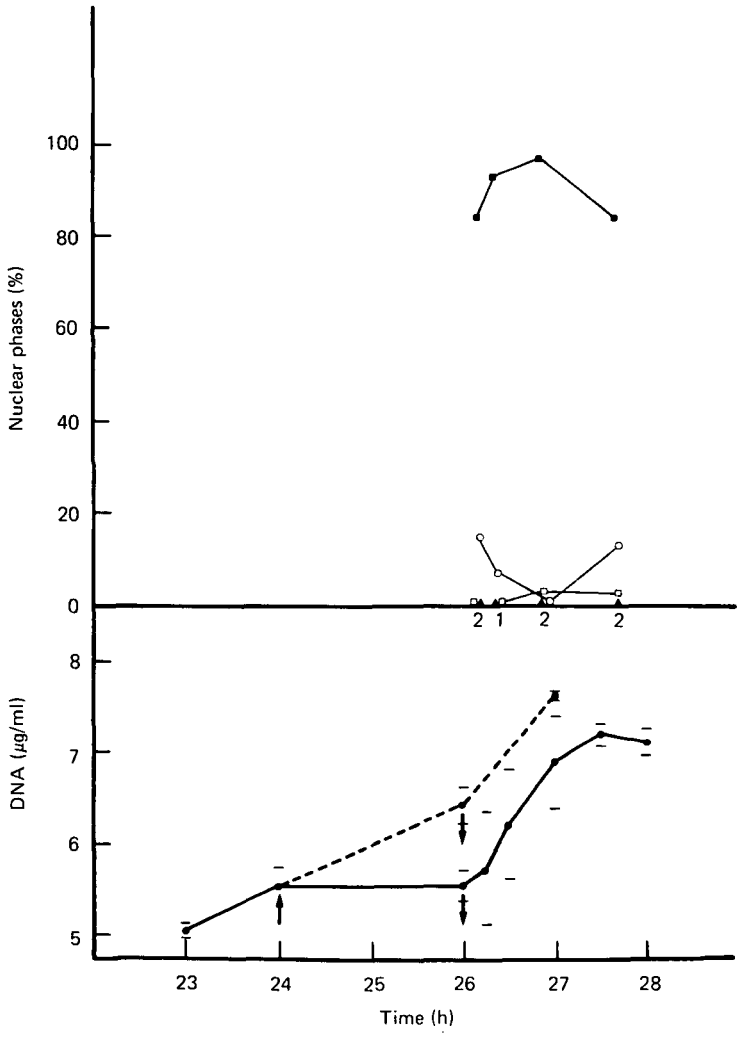

Fig. 3. Correlated DNA and nuclear behaviour following treatment with FdUrd. In the lower panel the DNA content of the colonies is indicated. Uracil alone gave a linear increase in DNA (broken line), whereas addition of FdUrd (↑) blocked DNA synthesis and its removal (↓) permitted a rapid increase. The rate of increase in DNA content was probably not as rapid as in Figs. 2 and 4 because of the use of larger colonies, which caused inadequate removal of the FdUrd. In the upper panel are shown the stages of the nuclei in hyphae taken from treated colonies at the indicated times. Numbers under the abscissa indicate the number of hyphae sampled at each time point. ▲, P_1; □, $P_{2/1}$; ■, $P_{2/2}+P_{2/2}/M$ transition; ○, D.

linear extension, it is difficult to determine directly the time of mitosis relative to the S period. However, one might expect a transient increase in the number of nuclei per tip immediately following karyokinesis, prior to re-establishment of the normal number by dilution following tip growth. As shown in Figs. 2 and 4, such increases were detected. Their peaks occurred 1–2 h after the end of the preceding S period (Figs. 2, 4).

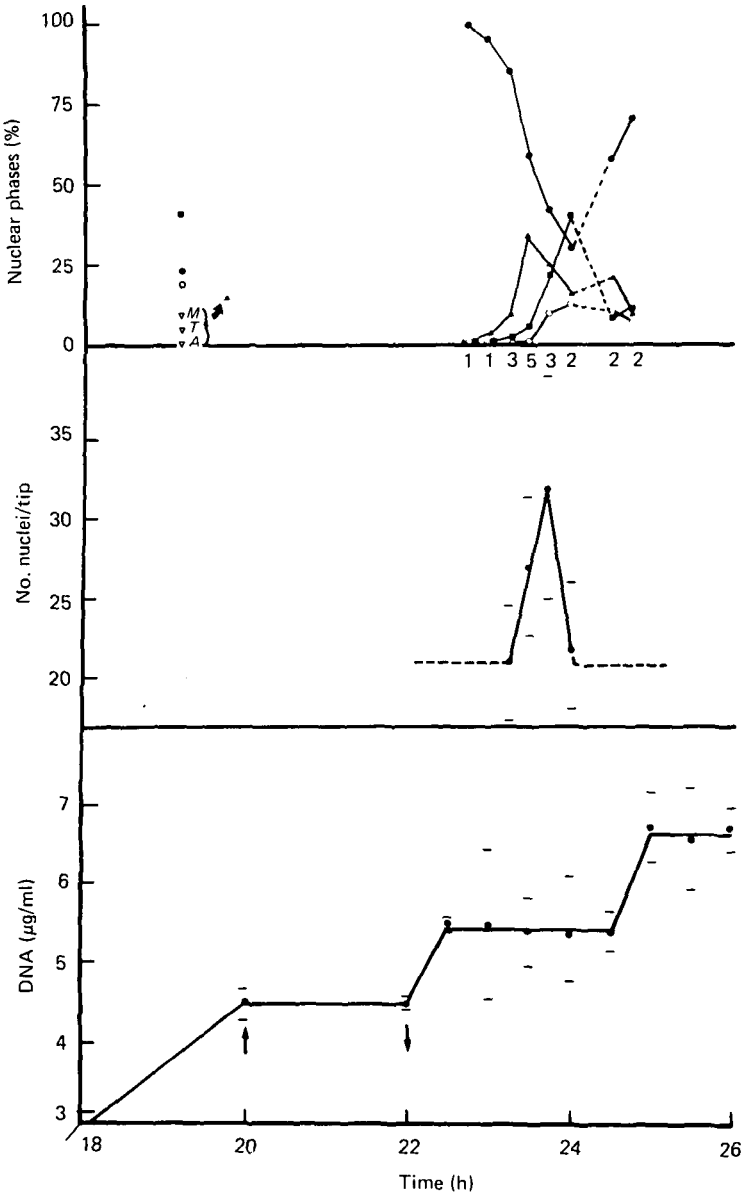

Fig. 4. Correlated DNA, nuclear number and nuclear phase analysis following treatment with FdUrd. The protocol was comparable to that for Figs. 2 and 3 but FdUrd and uracil were added at 20 h (↑) and removed at 22 h (↓). The nuclear population was measured in a 185-μm zone rather than the 227-μm zone of Fig. 3. The values in the upper panel are given in more detail in Table 2. ■, P_1; ○, $P_{2/1}$; ●, $P_{2/2}$; ▲, D. The values on the left of that panel are those obtained from untreated hyphae. $M+A+T=D$. The numbers under the abscissa of the upper panel denote the number of hyphae sampled at each time point.

In the absence of gross changes detectable by light microscopy (e.g. chromatin condensation, loss of nuclear envelope, etc.), one must utilize quantitative electron microscopy to correlate the *S* period with details of the mitotic system. In untreated vegetative hyphae the nuclear population is distributed among the various stages of division as shown in Fig. 4 and Table 1. As reported previously (Heath, 1980*a*;

Table 1. *Nuclear stages following FdUrd block*

Time after removal of FdUrd (min)	No. nuclei/hypha	Nuclear stages (% of total) P_2/M transition	M	A	T
Untreated	52	2	6	2	6
	60	1·7 } 1	17 } 10	0 } 0·5	5 } 5
	19	0	5	0	5
	25	0	12	0	4
45	26	0	0	0	0
60	31	36	0	3	0
75	30	27	10·3	3·5	0
	24	21 } 16	4 } 8·4	0 } 3·5	0 } 0
	27	0	11	7	0
90	30	7	13	3	3
	35	6	17	14	0
	32	0 } 2·6	22 } 25·4	19 } 9	0 } 0·6
	21	0	29	0	0
	22	0	46	9	0
105	33	0	12	0	3
	24	0 } 0	17 } 19	8 } 2·7	4 } 3·7
	25	0	28	0	4
120	37	0	19	3	3
		} 0	} 11·5	} 1·5	} 3·5
	23	0	4	0	4
150	38	0	10·5	0	0
		} 1·9	} 18·2	} 1·9	} 3·7
	27	3·7	25·9	3·7	7·4
145	27	0	7·4	0	0
		} 0	} 8·3	} 0	} 4·6
	11	0	9·1	0	9·1

This Table illustrates some of the expanded data from the experiment shown in Fig. 4.

Heath & Rethoret, 1980), all nuclei contained some array of kinetochore microtubules. Note that ~ 40% are in $P_{2/1}$ plus $P_{2/2}$. This value rises dramatically following 2 h incubation in FdUrd and has been recorded in 3 separate experiments. In the first (not illustrated), using the previously used *S. ferax* (Heath, 1980*a*), 3 hyphal tips containing a total of 81 nuclei had 91% in an undetermined mixture of $P_{2/1}$ plus $P_{2/2}$. The results of the second, using the present isolate of *Saprolegnia*, are shown in Fig. 3, where $P_{2/2}$ alone accounted for an average of 89% of the nuclei during the 100-min period following the removal of FdUrd. In both of these experiments the

remaining nuclei were mainly in metaphase, anaphase or telophase, P_1 was totally absent and $P_{2/1}$ was rare. In the third experiment, shown in general in Fig. 4 and in detail in Table 1, 100% were in $P_{2/2}$ 45 min after removal of FdUrd .These data show that inhibition of DNA synthesis causes nuclei to arrest in $P_{2/2}$, at which stage they remain until the completion of DNA synthesis. The configuration of the post-block

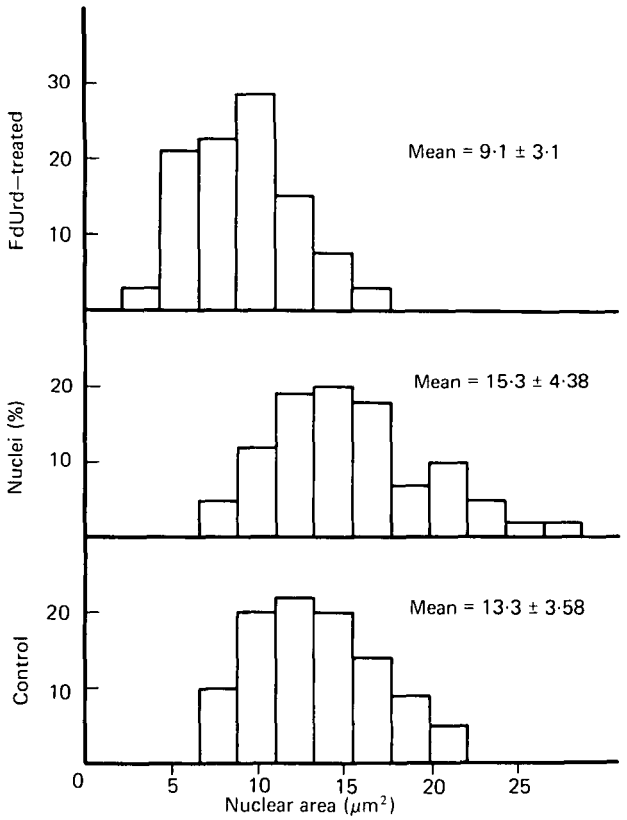

Fig. 5. Increase in nuclear size following treatment with FdUrd. Nuclear areas were measured from prints of mithramycin-stained nuclei magnified to 3818 × life following 3 h in 0·5 mg/ml FdUrd and 0·1 mg/ml uracil. 100 nuclei were measured in each population except for the upper panel where only 67 of the 100 FdUrd-treated nuclei shown in the centre panel contained sufficiently clear nucleoli to permit measurement of nucleolar area. A *t*-test comparison of FdUrd and control populations showed that they were different at the $P < 0{\cdot}001$ level.

P_2 nuclei is not greatly different from normal although the inter-centriole pair distance was significantly ($P < 0{\cdot}001$) greater, increasing from 0·51 μm (±0·16, $n = 77$) to 0·61 μm (±0·20, $n = 74$) in one experiment and to 0·93 μm (±0·19, $n = 45$) in the experiment shown in Fig. 3. The nuclei were also significantly ($P < 0{\cdot}001$) larger (Fig. 5). The increase in nuclear size was probably due to larger nucleoli since these were typically much more visible than in the control nuclei. However, it was not possible to prove this point because the nucleoli are only occasionally clearly visible in control nuclei, so that amassing sufficient quantitative data was impractical. The

number of kinetochore microtubules per group in the post-block P_2 nuclei was indistinguishable from normal P_2 arrays (mean $17{\cdot}7 \pm 2{\cdot}8$, $n = 12$ *versus* $17{\cdot}8 \pm 3{\cdot}36$, $n = 12$ from controls in Fig. 1). Following the FdUrd-induced accumulation of $P_{2/2}$ nuclei, the nuclear population began to enter division phases at about 30 min after the completion of DNA synthesis (i.e. 1 h after removal of FdUrd) (Fig. 4 and Table 1). Initially there was a remarkable increase in the normally relatively rare $P_{2/2}/M$ transition stage (defined essentially as a long $P_{2/2}$ configuration with one or two non-kinetochore microtubules running between the spindle poles), which was soon followed by an abundance of metaphases and anaphases, and finally by telophases (Table 1 and Fig. 4). The peak number of dividing nuclei (metaphase + anaphase + telophase) was observed 15 min before the peak number of nuclei per tip was recorded in the mithramycin-stained hyphae. Following the first round of DNA synthesis and mitosis after removal of FdUrd, the nuclei enter a second cycle of synthesis. By the beginning of this second round of DNA synthesis, the nuclear population again accumulated a high percentage of $P_{2/2}$ stages, which became more abundant by the middle of the synthetic period (Fig. 4).

Whilst the data in Table 1 and Fig. 4 are clearly indicative of some synchrony in the nuclear population, it is evident that the degree of synchrony is poor even within a single hypha. This point is emphasized by the appearance of a few P_1 nuclei by 45 min after completion of DNA synthesis (Fig. 4), well before the majority of nuclei have entered metaphase.

From the stepped DNA curves (Figs. 2, 4) one can calculate that the nuclear cycle time following the FdUrd block is approximately 2·5 h. Because of the nature of hyphal colony growth one cannot directly determine the normal cycle time from the linear increase in DNA content of vegetative colonies (Heath & Rethoret, 1980). However, one can obtain a close estimate from spore germination studies as outlined by Heath & Rethoret (1980). Using that approach we obtained a mean nuclear cycle time of 62·4 min. Table 2 presents the calculated duration of each part of the nuclear cycle based on various parameters.

DISCUSSION

The present results confirm those obtained previously on another isolate of *Saprolegnia* (Heath, 1980*a*; Heath & Rethoret, 1980). However, more importantly, they extend that work to permit a more complete understanding of the events of the nuclear cycle. The numerical data in Fig. 1 can most simply be interpreted as showing that, during P_2 the parental kinetochore microtubules segregate into 2 adjacent groups, and that during metaphase daughter kinetochore microtubules are synthesized from (or to) the newly replicated kinetochores, thereby forming the typical amphitelic pairs characteristic of most metaphase nuclei. The scatter in the kinetochore microtubule and pairing data during metaphase suggests that the coordination between spindle elongation and daughter kinetochore microtubule formation is not very tight. The variability is greatest in shorter spindles (1–1·5 μm), where the rapid rise in numbers suggests that the bulk of new kinetochore microtubule synthesis occurs. However, the

occurrence of sub-telophase numbers and less than 100% pairing even in late (e.g. ~ 2·5 μm long) metaphase spindles suggests that kinetochore microtubule formation continues throughout metaphase. This point is difficult to resolve unambiguously due to the difficulty in achieving 100% certainty in identifying the rather poorly resolved kinetochores. Fortunately, in the isolate of *Saprolegnia* used in this work we can now predict the number of kinetochore microtubules that should be present because we know that the haploid chromosome number is 21 (Tanaka, Heath & Moens, unpublished data). Because the vegetative hyphae of the oomycetes are diploid (see

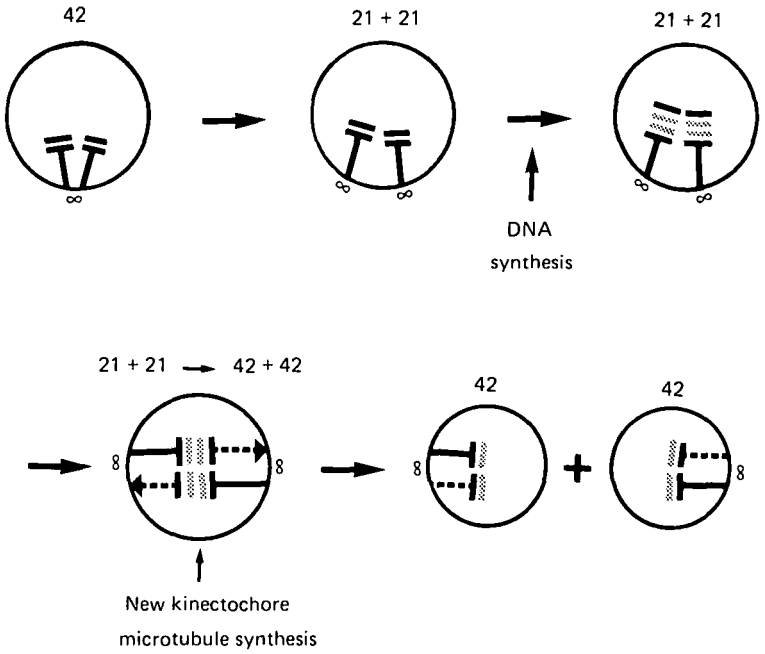

Fig. 6. Diagrammatic interpretation of the main features of the nuclear cycle of *Saprolegnia*. Adjoined extranuclear circles indicate centrioles and numbers indicate predicted kinetochore microtubule numbers. Stippling indicates newly synthesized DNA.

reviews by Dick & Win Tin, 1973;Win Tin & Dick, 1975), one would expect to find 42 kinetochores per half spindle in metaphase mitotic nuclei. Assuming that there is one kinetochore microtubule per kinetochore, as is common for many fungi (Heath, 1978), one would also predict 42 kinetochore microtubules in each half of a metaphase mitotic spindle. Similarly, if the postulated kinetochore microtubule behaviour pattern is true, one can predict the numbers that should be present at each stage of the nuclear cycle. These numbers are shown in Fig. 6. A comparison between the data in Fig. 1 and the predicted numbers shows that at all stages of the nuclear cycle one is failing to identify ~ 10% of the kinetochore microtubules (6% at P_1, 9% at $P_{2/1}$, 15% at $P_{2/2}$, 18% at A and 10% at T. The mean total number from Fig. 1 is 11·3% below prediction). This level of 'experimental' error is far from optimal but not sufficient to

invalidate the general conclusions that may be drawn from the numerical data. These are (1) that P_1 nuclei contain the diploid number of kinetochore microtubules. (2) During P_2 these microtubules segregate into 2 groups, each containing only the haploid number of kinetochore microtubules. (3) During metaphase, a new daughter kinetochore microtubule is formed to connect the daughter kinetochore to the pole opposite to that to which the parent was attached during $P_{2/2}$. This is the only known mitotic system where half of the kinetochore microtubules used during mitosis persist from the previous division and half are *de novo*. Whether or not this system is widespread among the fungi and, if so, has anything to do with the non-random segregation of mitotic chromosomes (Rosenberger & Kessel, 1968; Williamson & Fennell, 1980) awaits further work. Unfortunately, because the chromatin does not condense sufficiently to become detectable in thin sections (Heath, 1980*b*), it is still not possible to determine whether or not the chromosomes are permanently connected to the kinetochore microtubules throughout the nuclear cycle; a problem discussed in more detail previously (Heath, 1980*a*).

The mitotic synchrony induced by FdUrd permits correlation of the morphological stages of the nuclear cycle with the familiar cell cycle phases. Because nuclei accumulate at $P_{2/2}$ in FdUrd and re-enter that stage at the onset of the second round of post-block DNA synthesis (Fig. 4) it seems certain that $P_{2/2}$ and S are coincident. Since no morphological change occurs between the end of S and the $P_{2/2}/M$ transition, G_2 must also occur whilst the nuclei are in $P_{2/2}$. Alternatively, one could conclude that this period is equivalent to mitotic prophase and thus the cycle would lack a G_2 phase. The differentiation between G_2 and prophase is not always clear (Prescott, 1976), and since the key prophase event of pre-mitotic chromatin condensation does not occur in *Saprolegnia* (Heath, 1980*b*) we cannot differentiate between these alternatives. However, because the period in question is fairly long (*ca.* 16% of the nuclear cycle) and the primary event of prophase (chromosome condensation) does not occur we believe that it should be considered the equivalent of G_2. Hence we conclude that $P_{2/2}$ is equivalent to S plus G_2. Clearly M plus A plus T are equivalent to the normal mitotic phase and equally the period between karyokinesis and the onset of S must be G_1. G_1 is therefore equivalent to P_1 plus $P_{2/2}$.

Direct comparison between the normal nuclear cycle and that following treatment with FdUrd is complicated by the 2·5-fold elongation of the latter. It is entirely possible that some parts of the nuclear cycle are more extended than others. For example, one might expect S to be more extended because of the time needed to synthesize replacement thymidylate synthetase molecules (following the inactivation of the original population by being bound to FdUMP) and to build up a fresh pool of dTMP (O'Donovan & Neuhard, 1970). If there were no differential effects, the percentage of time occupied by a phase in the elongated cycle should indicate the duration of that phase in the normal cycle. Applying this principle to the mitotic stage ($M+A+T$) gives a very similar result to that observed in the normal population (i.e. $30/150 \times 62{\cdot}4$ from line 4, Table 2, $= 12{\cdot}5$ min compared with 12·9 min from line 3, Table 2). A comparable calculation suggests that, since G_1 equals P_1 plus $P_{2/1}$, these 2 stages should last for $45/150 \times 62{\cdot}4 = 18{\cdot}7$ min in the normal cycle. The fact

that they occupy 32·6 min (Table 2) means that FdUrd causes less elongation of G_1 than of other phases. Conversely, since S plus G_2 are equivalent to $P_{2/2}$, one would expect this stage to last for $75/150 \times 62{\cdot}5 = 31{\cdot}2$ min instead of the observed normal cycle time of 16·9 min. This preferential extension of G_2 plus S is in agreement with the above prediction based on the site of action of FdUrd. However, because the extended time amounts to about 35 min, which is longer than all of the post-FdUrd S,

Table 2. *Duration of nuclear cycle phases*

	Phases					
				M	*A*	*T*
	P_1	$P_{2/1}$	$P_{2/2}$		*D*	
Stages of nuclei in untreated hyphae (%)*	41·4	19·3	23·8	9·9	0·5	5·0
Nuclear % corrected for age gradient†	33·5	18·8	27·1	13·0	0·7	7·1
Time based on mean spore line cycle of 62·4 min (min)	20·9	11·7	16·9	8·1	0·4	4·4
					12·9	
	G_1		*S*	G_2	*D*	
Time derived from stage curve in Fig. 4 (min)‡	~ 45	< 30	~ 45		~ 30	

* Data from the 4 untreated hyphae in Table 1.

† The observed percentages of nuclei at various stages were corrected for the age gradient of the population according to the equation in Frankel (1960).

‡ These figures were derived from Fig. 4 in the following manner. P_1 is the interval between the peak of the nuclei/tip curve (i.e. immediately after the bulk of the nuclei underwent karyokinesis) and the onset of the second round of DNA synthesis at 24·5 h. $P_{2/1}$ is equated with the duration of each DNA synthetic period (see discussion on this point). $P_{2/2}$ is the interval between the end of the first DNA synthetic period after the block and the onset of the appearance of a substantial number of dividing nuclei in the nuclear phase panel (*ca.* 23·25 h). Division was judged to last from the 23·25-h point until the peak of the nuclei/tip curve, as discussed above for P_1.

G_2 must also be extended. This makes it difficult to determine accurately the relative durations of S and G_2 in the normal cycle. An approximation of the maximum duration of S can be made since S lasts for 30/75 of S plus G_2 following treatment with FdUrd. Therefore S must occupy a maximum of $30/75 \times 16{\cdot}9$ min = 7 min (time of normal $P_{2/2}$) with a corresponding value of 10 min for G_2. These values are summarized in Fig. 7.

Apart from the obviously rapid rates, the nuclear cycle is essentially comparable to that of many higher eukaryotes, with all the normal phases and approximately equivalent relative durations. This point could not have been established without the combined electron microscopy–nuclear synchrony approach used here. Now that it is established it reinforces the idea that these fungi may indeed be useful model eukaryotes for the study of diverse cellular and nuclear phenomena.

Three observations of the FdUrd-treated nuclei deserve mention. The increase in nuclear, probably nucleolar (see Results), volume following inhibition of DNA synthesis suggests that the coordination between the chromosome cycle and synthesis of nucleolus or nucleoplasm is not tight. It seems likely that transcription to produce nucleolar material continues independently of the chromosome cycle, although the determination of the ultimate level of nucleolus volume awaits more extensive treatments with FdUrd. In contrast, centriole migration and spindle formation is very

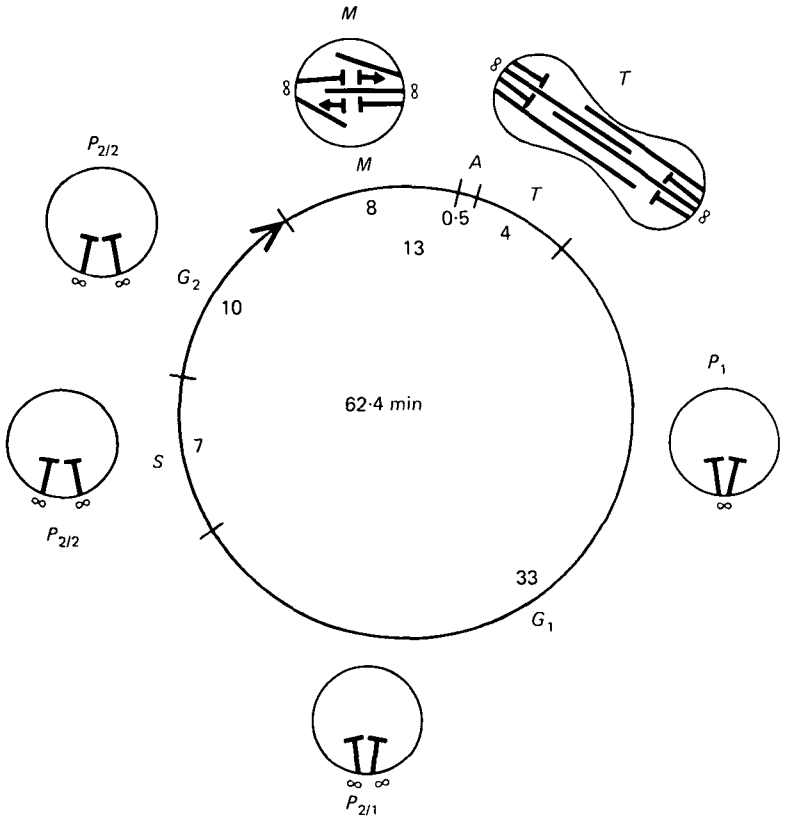

Fig. 7. Interpretive diagram of the correlated morphological and activity cycles of nuclei of *Saprolegnia*. Times indicated are those calculated for untreated hyphal nuclei growing in OM at 25 °C (see Discussion for details of calculations).

tightly coordinated with the chromosome cycle. Whilst the centriole pairs were 0·1–0·4 μm further apart following the treatment with FdUrd, this amount of movement is small relative to the normal degree of separation that would have occurred in this time if the spindle had developed. Again, longer treatment with FdUrd may be instructive, but at present the movement of the extranuclear centrioles appears to be tightly coordinated with the intranuclear chromosome cycle. Finally, centriole replication appears to be independent of nuclear DNA synthesis.

The present data have provided the means for accurately describing the salient events of the nuclear cycle in *Saprolegnia*. This should form a useful basis for sub-

sequent analysis of various aspects of the regulation of cellular activities. Most importantly, it has shown which parts of the nuclear cycle are 'normal' and which 'abnormal' relative to other eukaryotes.

We are pleased to acknowledge the excellent secretarial assistance of Dorothy Gunning and financial support from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

BRUNK, C. F., JONES, K. C. & JAMES, T. W. (1979). Assay for nanogram quantities of DNA in cellular homogenates. *Analyt. Biochem.* **92**, 497–500.

DICK, M. W. & WIN TIN (1973). The development of cytological theory in the Oomycetes. *Biol. Rev.* **48**, 133–158.

FRANKEL, J. (1960). Morphogenesis in *Glaucoma chattoni*. *J. Protozool.* **7**, 362–376.

HARTWELL, L. H. (1974). *Saccharomyces cerevisiae* cell cycle. *Bact. Rev.* **38**, 164–198.

HARTWELL, L. H. (1978). Cell division from a genetic perspective. *J. Cell Biol.* **77**, 627–637.

HEATH, I. B. (1978). Experimental studies of fungal mitotic systems: a review. In *Nuclear Division in the Fungi* (ed. I. B. Heath), pp. 89–176. New York: Academic Press.

HEATH, I. B. (1980*a*). Behavior of kinetochores during mitosis in the fungus *Saprolegnia ferax*. *J. Cell Biol.* **84**, 531–546.

HEATH, I. B. (1980*b*). Apparent absence of chromatin condensation in metaphase nuclei of *Saprolegnia* as revealed by mithramycin staining. *Expl Mycol.* **4**, 105–115.

HEATH, I. B. & GREENWOOD, A. D. (1968). Electron microscopic observations of dividing somatic nuclei in *Saprolegnia*. *J. gen. Microbiol.* **53**, 287–289.

HEATH, I. B. & RETHORET, K. (1980). Temporal analysis of the nuclear cycle by serial section electron microscopy of the fungus, *Saprolegnia ferax*. *Eur. J. Cell Biol.* **21**, 208–213.

HOLLOWAY, S. A. & HEATH, I. B. (1977). An ultrastructural analysis of the changes in organelle arrangement and structure between the various spore stages of *Saprolegnia*. *Can. J. Bot.* **55**, 1328–1339.

HORGEN, P. A. (1977). Steroid induction of differentiation: *Achlya* as a model system. In *Eukaryotic Microbes as Model Developmental Systems* (ed. D. H. O'Day & P. A. Horgen), pp. 272–294. New York: Marcel Dekker.

LAW, D. J., ROZEK, C. E. & TIMBERLAKE, W. E. (1978). Polyadenylate metabolism in *Achlya ambisexualis*. *Exptl Mycol.* **2**, 198–210.

O'DONOVAN, G. A. & NEUHARD, J. (1970). Pyrimidine metabolism in microorganisms. *Bact. Rev.* **34**, 279–343.

PRESCOTT, D. M. (1976). *Reproduction of Eukaryotic Cells*. New York: Academic Press.

ROSENBERGER, R. F. & KESSEL, M. (1968). Nonrandom sister chromatid segregation and nuclear migration in hyphae of *Aspergillus nidulans*. *J. Bact.* **96**, 1208–1213.

WILLIAMSON, D. H. & FENNELL, D. J. (1980). Non-random assortment of sister chromatids in yeast mitosis. In *Molecular Genetics in Yeast; Alfred Benzon Symposium* 16 (ed. D. von Wettstein, J. Friis, M. Kielland-Brandt & A. Stenderup). Copenhagen: Munksgaard. (In Press.)

WIN TIN & DICK, M. W. (1975). Cytology of Oomycetes. *Arch. Mikrobiol.* **105**, 283–293.

(*Received* 30 *September* 1980)