A nitrogen starvation-induced dormant G₀ state in fission yeast: the establishment from uncommitted G₁ state and its delay for return to proliferation

Sophia S. Y. Su¹, Yusuke Tanaka¹*, Itaru Samejima¹‡, Kenji Tanaka² and Mitsuhiro Yanagida¹‡

¹Department of Biophysics, Faculty of Science, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-ku, Kyoto 606, Japan
²Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Tsurumai, Showa-Ku, Nagoya 466, Japan
*Present address: Primate Research Institute, Kanrin, Inuyama, Aichi-ken 484, Japan
†Present address: ICMB, University of Edinburgh, Darwin Building, Mayfield Road, Edinburgh EH9 3JR, UK
‡Author for correspondence

SUMMARY

Fission yeast cells either remain in the mitotic cell cycle or exit to meiotic sporulation from an uncommitted G₁ state dependent on the presence or absence of nitrogen source in the medium (Nurse and Bissett, 1981). We examined how heterothallic haploid cells, which cannot sporulate, behave under nitrogen-starvation for longer than 25 days at 26°C. These cells were shown to enter a stable state (designated the dormant G₀) with nearly full viability. Maintaining the dormant cells required glucose, suggesting that the cells remained metabolically active although cell division had ceased. They differed dramatically from mitotic and uncommitted G₁ cells in heat resistance, and also in cytoplasmic and nuclear morphologies. After nitrogen replenishment, the initial responses of dormant G₀ cells were investigated. The kinetics for reentry into the proliferative state were delayed considerably, and the changes in cell shape were enhanced particularly for those recovering from extended nitrogen starvation. A part of the delay could be accounted for by the duration of nuclear decondensation and cell elongation for the first cell division.

Key words: G₀, G₁ phase, Cell polarity, Cell cycle control, Heat shock

INTRODUCTION

The fission yeast Schizosaccharomyces pombe is an excellent eukaryotic model organism to study the control of cell cycle (Mitchison, 1970; Robinow and Hyams, 1989; Nurse, 1994a). A large number of mutant strains defective in mitotic division cycle and meiotic sporulation have been isolated. In S. pombe, nitrogen source limitation triggers cells into the nonproliferative states. For sexually competent homothallic h⁹⁰ strains or for heterothallic haploid strains in the presence of both h⁺ and h⁻ mating types, cells undergo meiotic conjugation and karyogamy after going through an uncommitted G₁ stage for mitotic or meiotic cell cycle (Nurse and Bissett, 1981). This stage is the key point controlling cell cycle progression, and takes place at the G₁ phase of the mitotic cell cycle prior to START (Hartwell, 1974). Cells in this uncommitted G₁ stage are offered a choice to either proceed through or to exit the mitotic cell cycle. The duration for this uncommitted G₁ stage is not long. In the complete nutrient medium, the G₁ phase of S. pombe is very short. In the absence of nitrogen source, cells divide and stay temporarily in this G₁ stage before committing to meiotic differentiation. Nitrogen-deprived S. pombe cells can return to the proliferative mitotic cell cycle if nitrogen source is resupplied within 4 hours after removal (Nurse and Bissett, 1981). In the budding yeast Saccharomyces cerevisiae, nitrogen starvation also triggers a/α diploids to initiate meiotic differentiation and leads to the formation of spores (Esposito and Klapholz, 1981).

The meiotic program is triggered by the coordinate activity of a number of gene products which relays the mating type heterozygosity and nitrogen starvation signals required for the induction of meiosis (Beach et al., 1985; Egel et al., 1990; Iino and Yamamoto, 1985a,b; McLeod and Beach, 1988; McLeod et al., 1987; Nurse, 1985). In nutrient-rich medium, both heterothallic and homothallic S. pombe strains divide asexually. Homothallic cells undergo frequent mating type switching such that the resulting culture is a mixture of cells with different mating types and are sexually competent. These cells are induced to enter meiosis after conjugation in a nitrogen-deficient environment, but cells of heterothallic strains without their respective sexual partners in the same culture cannot do so (Egel, 1989; Egel et al., 1990).

The present paper describes the fate of heterothallic cells without sexual partners in the absence of nitrogen source. These ‘bachelor’ cells cease division, but cannot mate. Cells contain an unreplicated complement of DNA characteristic of the G₁ phase of the mitotic cell cycle (Costello et al., 1987; Nurse, 1985). Cells may remain at the uncommitted G₁-like state prior to START for a long time. Not much is known of their properties as they have been scarcely investigated. We will show that these ‘bachelor’ cells arrested in the nitrogen-deprived condition are able to survive very long periods of incubation, and enter a novel cell
state if glucose is present. This stable cell state is perhaps metabolically active but remains dormant for cell growth and division until the nitrogen source is replenished. These cells display unique cellular structures visualized by light and electron microscopy, and resistance to heat shock treatment. Furthermore, the dormant cells show a significant delay for return to the proliferative state when the nitrogen source is replenished. The intermediary cell structures formed after nitrogen replenishment are reminiscent of those of cells germinating from spores. The structures of the dormant cells may mimic spores though they require glucose for their establishment and maintenance. These results lead us to define dormant cells as a third cell fate derived from the uncommitted G1 stage.

**MATERIALS AND METHODS**

**Strains and culture conditions**

The heterothallic haploid strain 972 h– isolate of *S. pombe* was used.
in this study (Gutz et al., 1974; Leupold, 1970). Unless otherwise indicated, all experiments were conducted at 26°C. Liquid yeast cultures were grown in YEPD medium (1% yeast extract, 2% polypeptone, 2% glucose) or in synthetic EMM2 medium (Mitchison, 1970). Nutrient-deficient media EMM2-N, EMM2-NG and EMM2-NGV are identical to EMM2 except that certain nutrients are missing. EMM2-N lacks the nitrogen source NH₄Cl, EMM2-NG lacks both NH₄Cl and the carbon source, glucose, and EMM2-NGV lacks NH₄Cl and glucose, as well as the vitamin component. All plates contain 1.5% agar. For nitrogen starvation, cells grown in EMM2 to a density of 1-5×10⁶ cells/ml were washed four times with sterile distilled water to be free of growth medium, then transferred to an equal volume of EMM2-N medium. Samples were taken for tests after various incubation periods. For nitrogen replenishment, four volumes of fresh EMM2 medium containing ample nitrogen source was added to nitrogen-starved cultures. Samples were taken at various times for assays after readdition of fresh medium.

Cell viability and heat shock sensitivity tests
Cell density of all cultures was estimated by the Microcellcounter (Sysmex). Cell viability was determined by making appropriate dilutions of samples with distilled water, then plating on YEPD agar plates. After three days incubation at 26°C, viable colonies were counted. Viability was expressed as a fraction of survived colonies in the total number of cell bodies plated.

For heat shock sensitivity tests, samples were diluted ten-fold into media prewarmed to 48°C and incubated at the same temperature for 30 minutes. For heat treatment, samples were cooled on ice for 5 minutes, plated on YEPD agar plates following appropriate dilution, and incubated for three days at 26°C. Viability was determined as described above.

Microscopy
S. pombe liquid cultures were collected by low speed centrifugation and fixed with 30% formaldehyde as described by Hagan and Hyams (1988). Alternatively, they were fixed in 2.5% glutaraldehyde as described pre-
viously (Toda et al., 1981). In either case, nuclei were stained with the DNA-specific fluorescent probe 4,6-diamidino-2-phenylindole (DAPI). For thin-section electron microscopy, the procedures described for the freeze-substitution method were followed (Tanaka and Kanbe, 1986).

FACScan analysis
A modified procedure of Costello et al. (1986) was employed: 1-5×10^7 cells were collected, washed twice in sterile distilled water, and resuspended in 1.0 ml sterile water. Ice-cold ethanol was added slowly with mixing to 70% ethanol, and then incubated at 4°C for 12 hours. Cells were subsequently washed in 1.0 ml 50 mM sodium citrate (pH 7.0) and digested with RNase (0.5 mg/ml) (Sigma, St Louis, MO) for 3 hours at 37°C. Cells were stained with propidium iodide (12.5 mg/ml) and analyzed by FACScan (Beckton Dickinson, San Jose, CA).

RESULTS
High cell viability after prolonged nitrogen starvation
We first examined cell division growth of wild-type h− 972 haploids after long-term nitrogen starvation. Log phase cells cultured in the synthetic EMM2 medium at 26°C were transferred to the same medium except that the nitrogen source NH_4Cl is absent. The cell number was estimated at various times after nitrogen starvation by a Sysmex microcellcounter

Fig. 4. Recovery of cells after 24 days of nitrogen starvation. (A) A nitrogen-starved EMM2-N culture incubated for 24 days at 26°C was supplemented with fresh EMM2 medium and continued incubation at 26°C. At the indicated times during incubation, cell samples were taken, fixed with aldehyde, and stained with DAPI. Nomarski and DAPI photo micrographs were taken for the same field of representative cells at each time point. (B) Recovery of cells after one day of nitrogen starvation. A nitrogen-starved EMM2-N culture incubated for one day at 26°C was supplemented with fresh EMM2 medium and continued incubation at 26°C. Cell samples were taken at the indicated times during incubation, fixed with aldehyde, and stained with DAPI. Nomarski and DAPI photo micrographs were taken for the same field of representative cells at each time point.
A dormant state in fission yeast

Following nitrogen starvation at 26°C, cells divided twice, and then the cell number (open circles) maintained at a constant level throughout 35 days of starvation at 26°C.

The viability of nitrogen-starved cells was determined to assess whether the cell bodies had died after being deprived of nitrogen source for such a long time. Plating efficiency was scored at various days after transferring growing cells to nitrogen-deficient medium. Surprisingly, extended nitrogen starvation did not significantly affect the viability of wild-type h− strains after 35 days. Cell viability was almost fully maintained at about 85-90% (filled circles; Fig. 1). These cells appeared to stay at a resting state by nitrogen starvation, and could fully resume vegetative growth in nutritionally complete medium.

Lag in cell regeneration depends on the starvation period
To determine whether the starvation period affects the return to proliferative cell growth, the kinetics of cell number increase were studied after adding fresh EMM2 medium which contained NH4Cl as the nitrogen source to cells that had been starved in the EMM2-N medium deficient of NH4Cl for one day or 24 days at 26°C. We found that cell number started to increase logarithmically after a defined lag period in each case (Fig. 1B). The lag period (16 hours) was twice as long for cells starved for 24 days (filled circles) than for cells starved for one day (lag period, 8 hours; open circles). The longer the starvation period, the slower it seems to take for cells to begin vegetative growth.

Onset of S-phase is delayed
To assess when DNA synthesis first began in one day- or 24 day-starved cells, we examined the DNA contents of living cells at various times after transfer to nitrogen-enriched medium. DNA of individual cells was stained with propidium iodide and analyzed by flow cytometry analysis (FACScan, Beckton-Dickinson) as shown in Fig. 1C. In one day-starved cells, a G2 peak containing a 2C DNA content became more prominent after 2-4 hours incubation in EMM2+N, indicating that cells had begun to replicate their DNA (left). In the 24 day-starved cells, the 2C DNA peak increased after 6-8 hours incubation in EMM2+N (right). A small 1C peak remained visible until 10-12 hours indicating that all cells had completed DNA synthesis. Not only was the lag period to the onset of S-phase longer for long-term starved cells, but the duration of all cells to complete DNA replication seems longer as well.

Changes in cell size and shape
The cell size of h− strain 972 was affected by prolonged nitrogen starvation. Vegetatively growing cells appear as hemispherically capped rods with a diameter of approximately 3.5 μm and a length ranging from 7-15 μm (average 10.5 μm; Fig. 2A) (Johnson et al., 1989). Nitrogen depletion caused cells to shorten in size and become round. After six and 24 hours incubation in EMM2-N at 26°C, the cell length shortened from 10.5 μm to 6.4 μm and 5.8 μm, respectively (Fig. 2B,C). Cells appeared shorter and more round after 24 days incubation (4.3 μm, Fig. 2D).

Conversely, nitrogen-starved cells regenerated the cylindrical cell shape when nitrogen source was replenished to the medium. About two hours after transfer to EMM2, cells that had been starved for one day began to grow lengthwise (open circles; Fig. 3A), while cells that were starved for 24 days did not start to grow until after a two to four hour delay. One day-starved cells reached an average size of 9 μm at approximately 5.5 hours, whereas 24 day-starved cells required at least 10 hours before reaching the same size (Fig. 3A). Therefore, the time at which cells resumed their vegetative cell size depended upon the duration of nitrogen starvation.

To determine when mitosis occurs for cells recovering from nitrogen starvation, the number of cells with two nuclei was monitored by examining DAPI-stained cells. The frequency of binucleate cells in a one day-starved culture reached half its maximum level at 7 hours after the addition of EMM2, which was 4.5 hours earlier than that of 24 day-starved cells (Fig. 3B). Nuclear division in recovering cells occurred at the maximum cell size of 11 μm, consistent with a cell size requirement for nuclear division (Fantes and Nurse, 1977, 1978; Fantes, 1977; Nurse and Thuriaux, 1977).
As depicted in the regeneration of 24 day-starved cells (Fig. 4A), the transformation of round cells to rod-like cells goes through intermediate cell shapes. Cell growth started from one end, resulting in a pear-like structure which first appeared at 6 hours (Nishi et al., 1978; Padilla et al., 1975). Growth extension continued unidirectionally which made the cell appear asymmetrical, and in some cases looks like a long-neck vase (10-13 hours). By 15 hours, cell shape was essentially indistinguishable from vegetative rod-like cells. Thus, the presence of nitrogen source may first stimulate the increase of cell length through unidirectional growth. The intermediate pear-like cells, however, were not clearly observed during the regeneration of one day-starved cells (Fig. 4B).

**Shrinkage of chromatin structure**

The interphase nuclear chromatin domain of an exponentially growing cell appeared as a hemisphere (Fig. 2A; Toda et al., 1981). In contrast, incubation in EMM2-N for 28 days dramatically altered the nuclear chromatin region to appear flat and adhere close to the cell periphery (Fig. 5A, also see 2D). These flat sheets of chromatin domain seemed to appear only when cells were nitrogen starved for a long period of time. Cells that were starved for 6 hours or one day did not display flat nuclear chromatin (Fig. 2B, C). Cells that are nitrogen starved for an extended amount of time may undergo some kind of nuclear morphological change different from briefly nitrogen-starved cells.

Fission yeast cells were subjected to thin-sectioned electron microscopy in order to observe fine structures unique to nitrogen-starved cells. Electron microscopy of cells deprived of nitrogen source for 30 days (Fig. 6A) revealed that these cells were filled with two kinds of organelles: electron-dense round organelles (V) reminiscent of vacuoles, and white lipid globules (L) (Tanaka and Hirata, 1982). The cytoplasm was greatly reduced in volume. The nucleus (N) appeared squashed (Fig. 6A). It appeared to associate closely with the cell membrane. The small nucleolar region (Nu) was seen. The nuclear volume was also reduced to approximately half the volume of growing cells (Tanaka and Kanbe, 1986; Fig. 6B). The outer cell surface was entirely covered with a fibrous structure as well (Fig. 6A, F). The morphological characteristics of long-term nitrogen-starved cells resembled those of spores (Tanaka and Hirata, 1982).

**Vegetative chromatin is restored upon nitrogen replenishment**

The hemispherical nuclear shape could be restored after nitrogen source was resupplied to the medium. During recovery from nitrogen starvation, the chromatin sheet in long-term nitrogen starved cells was transformed into a comet-like structure (Figs 5B and 4A, 8 hours). The frequency of these comet-like structures increased soon after pear-shaped cells began to form and continued to be observed until normal hemispherical nuclei became more prominent after 10 hours. In Fig. 6B, the electron micrograph for a cell after 15 hours in nitrogen rich medium is shown. The nucleus is still somewhat asymmetric but the cytoplasm appears nearly normal for the vegetative state.

Hemispherical nuclei were observed prior to the appearance of binucleates for cells recovering from long-term nitrogen starvation, suggesting that the restoration of nuclear shape may be necessary before nuclear division occurs (Fig. 3B). For one day-starved cells, most nuclei remained hemispherical at all stages during recovery to vegetative cell division (Fig. 4B). The flat-sheet and comet-like nuclear chromatin could be unique for the recovery of long-term starved cells. The regeneration processes from the nitrogen-deprived state may depend on the reformation of the hemispherical chromatin region.

**Nutrient requirement for entry into a nitrogen-starved dormant state**

The above results strongly suggest that haploid heterothallic S. S. Y. Su and others

Fig. 5. Cell shape changes in cells recovering from nitrogen starvation are accompanied by changes in nuclear morphology. After 28 days incubation in EMM2-N, nitrogen-enriched EMM2 was added to a nitrogen-starved culture to stimulate cells to resume proliferative growth at 26°C. Nomarski (top) and DAPI stained (bottom) photo micrographs are shown for cells deprived of nitrogen source for 28 days (A), cells recovering from nitrogen starvation after 18 hours incubation in EMM2 (B), and log phase growing cells that had fully recovered from nitrogen starvation after 26 hours in EMM2 (C). Bar, 10 µm.
pombe can enter a particular cell state (designated dormant G0 state hereafter) after an extended period of nitrogen starvation. These cells were characterized by distinct cell and nuclear morphologies, and by a retarded response to nitrogen-enriched medium. We addressed whether the dormant state can be established in other culture conditions to determine the minimum nutrient requirement. We assayed the viability of cells at various times after vegetative \textit{h\textsuperscript{+}} 972 cells were transferred to four different culture media: (1) EMM2-N, (2) medium lacking both nitrogen and glucose (EMM2-NG), (3) medium lacking nitrogen, glucose and vitamins (EMM2-NGV), and (4) distilled water. As shown in Fig. 7A, cells were viable up to 28 days at 26°C in EMM2-N medium only. Although the cell number remained constant throughout the incubation period, cells could not survive in EMM2-NG, EMM2-NGV or distilled water.

Since nitrogen starvation causes cells to accumulate at G1 of the mitotic cell cycle, we tested whether cells could establish dormancy in the nutrient-deficient media after being synchronized at a common resting point. Log phase \textit{h\textsuperscript{+}} 972 cells were first transferred to EMM2-N and incubated for one day at 26°C. Then, the \textit{G1} arrested cultures were transferred to EMM-N, EMM-NG, EMM-NGV and distilled water. Viability was determined at various times following incubation at 26°C. We found that EMM2-NG, EMM2-NGV and distilled water still cannot sustain cell viability as high as that of EMM2-N after an extended period of incubation time (Fig. 7B). All cells were virtually dead after 28 days. Therefore, nitrogen deprivation from the culture medium promotes haploid cells to enter into dormancy, but the carbon source, glucose, is required to maintain the dormant state. Thus, there is a parallel relationship between this dormant yeast cell and the G0 arrested cell.

Spores are highly resistant to adverse environmental treatment such as heat shock. We examined whether dormant cells also become heat shock resistant as well. Yeast cells were subjected to heat treatment for 30 minutes at 48°C at various days after nitrogen starvation. We found that cells become more heat resistant as the duration of nitrogen starvation extends (Fig. 7C; filled circles, heat treated; open circles, heat untreated). Vegetative cells were highly heat sensitive as expected (Costello et al., 1986; Egel, 1989). Cells deprived of nitrogen source for two days were almost as sensitive to heat shock treatment as vegetative cells; only 60% of the cells survived heat treatment. However, cells that were starved for 7 days or longer became extremely resistant to heat shock (about 100% survived). Thus, nitrogen starvation for at least 7 days is necessary for cells to acquire heat resistance. Note that the dormant cells are similar to spores in heat resistance but differed from spores in the requirement of glucose for its viability (Egel, 1989).
DISCUSSION

Fission yeast cells either remain in the mitotic cell cycle or exit to meiotic differentiation from an uncommitted G₁ point dependent on the presence or absence of a nitrogen source (Nurse and Bissett, 1981; Egel, 1989). In this report, we propose that cells enter the dormant G₀ phase from this uncommitted G₁ state after long-term nitrogen starvation in the absence of sexual partners (Fig. 8A). This G₀ dormant state defines a third cell state derived from the uncommitted G₁ point. The other two states represent mitotic and meiotic cells. Maintaining the dormant cells requires glucose, but not nitrogen source. The dormant G₀ cells can survive heat treatment, but the uncommitted G₁ cells cannot (Costello et al., 1986; Egel, 1989). Dormant cells display a distinct cell structure which is not present in the uncommitted G₁ cells, and are resistant to metabolic as well as environmental trauma. Little is known about the kinds of gene function that are required for the establishment of dormancy. Rum1 and Nuc2 proteins which are involved in the inactivation of Cdc2 kinase appeared to be required for the establishment of the G₁ phase in the absence of nitrogen source (Moreno and Nurse, 1994; Kumada et al., 1995).

Fig. 7. Requirement of glucose for the establishment of dormancy and heat resistance. (A-B) Extended viability of nitrogen-starved haploid strain h⁻ 972 requires glucose and vitamins. (A) Log phase culture in EMM2 was transferred directly into EMM2-N (open circles), EMM2-NG (filled circles), EMM2-NGV (open squares) or distilled water (filled squares). (B) Log phase yeast culture in EMM2 was first transferred to EMM2-N. After one day incubation at 26°C, the nitrogen-starved culture was transferred to EMM2-N (open circles), EMM2-NG (filled circles), EMM2-NGV (open squares) or distilled water (filled squares) as well. Arrows in B and C indicate time of transfer to nutrient deficient media. Cultures were continued to be incubated at 26°C. At each time point, the viability of cells incubated in the respective media was determined. Values are averages of three determinations. (C) Haploid fission yeast cells in nitrogen-deficient medium become resistant to heat shock treatment. Log phase culture in EMM2 was transferred to EMM2-N and incubated at 26°C. At various time points, two identical aliquots were taken. One sample was treated at 48°C for 30 minutes (filled circles), whereas a second sample was spared of heat treatment (open circles). Plating efficiency was determined for each sample after appropriate dilution. Values are averages of three determinations.

Fission yeast cannot survive long-term cultivation in media containing a limited amount of nitrogen source: a large fraction of the cell population is dead after two weeks growth to saturation in medium containing 10 mM NH₄Cl (Costello et al., 1986). Saturated growth in 10 mM NH₄Cl yields a significant fraction of cells with a G₂-like DNA content (Costello et al., 1986), which may not be able to establish dormancy and survive extended incubation.

The establishment of a dormant state in the absence of NH₄Cl is accompanied by changes in cell morphology: the loss of cell polarity, the reduction of cell size, the flattening of nuclear chromatin, and the formation of vacuole-like organelles and lipid globules which occupy the cytoplasm. The small cell size associated with dormant cells may be a consequence of macromolecular breakdown. The total protein level of 24 day nitrogen-starved cells is fourfold lower than that of cells starved for one day (unpublished result). Prolonged nitrogen starvation may deplete the cellular protein pool to a minimum.

Heat shock resistance, nuclear chromatin shrinkage and organelle formation are not evident immediately upon nitrogen depletion, but are acquired through days of extended starvation. A carbon source may be required for maintaining nitrogen-starved cells in a metabolically active state to mediate the changes associated with the entry into dormancy. We infer that these properties may be important for preserving the cells’
viability when encountering environmental trauma. The distinct flattened chromatin observed in dormant cells may represent a higher-order structure that is transcriptionally closed and shielded from damage. The chromosome organization of log phase and transcriptionally silent regions has been shown to be different (Piñon, 1978; Orlando and Paro, 1995; Rivier and Pillus, 1994; Roth, 1995; Wolffe, 1994). It remains to be determined how chromatin structure is actually flattened in the dormant nucleus.

The flat chromatin state is clearly not terminal. Once a nitrogen source becomes available, it can be easily converted back to decondensed hemispheres as cells reenter proliferative growth. Remarkably, the flat chromatin changes into a comet-like structure during regeneration prior to S phase entry. The change may mark a transition from a transcriptionally inactive to an active state, and from a nonreplicative state to a replicative state.

Analogous to nitrogen-starved yeast cells, serum-starved mammalian cells also acquire a lag before they commit to proliferative growth (Zetterberg and Larson, 1985; Augenlicht and Baserga, 1974). Serum or growth factor exposure first leads to a burst of gene expression from immediate early genes, and then is followed by the induction of delayed early genes (Hershman, 1991). Some of these genes encode putative transcription factors, metabolic gene products and cytoskeletal-matrix proteins that may be important for triggering cell growth and mediating morphological changes associated with proliferating cells (Bravo, 1991; Bürger et al., 1994; Wick et al., 1994). It is of interest to determine whether similar gene products made by induced transcription mediate the transition from Go-like dormancy to proliferation.

As illustrated in Fig. 8B, yeast cells stimulated to resume mitotic cell cycle undergo cell elongation (CE), DNA synthesis (IDS), nuclear division (ND) and cell division (CD) at a slow rate when cells are recovering from the dormant G0 state. A number of reasons for this delay may be considered. Small cells cannot undergo DNA synthesis and nuclear division until a critical cell size (or cell mass) has been attained (Fantes, 1977; Nurse and Thuriaux, 1977; Russell and Nurse, 1986, 1987). Another possibility is that cells may require a longer time to resynthesize and reorganize proliferative cell structures, since the degree of nuclear and cytoplasmic structural changes is extensive in long-term starved cells. The restoration of cell shape may rely on coordinate activities of several protein kinases as well as phosphatases, which have been shown to affect cell shape by regulating cytoskeletal organiz-
REFERENCES


(Received 22 December 1995 - Accepted 28 February 1996)