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A DISTINCT CELL CYCLE IN PSEUDOHYPHAL GROWTH OF *SACCHAROMYCES CEREVISIAE*

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Nitrogen starvation of budding yeast in the presence of a carbon source stimulates a dimorphic transition from round cell (RND) to pseudohyphal (PH) growth. When grown on agar, RND cells form hemispherical mounds fed by nutrient diffusion. In contrast, PH cells form branching chains of spindle-shaped cells which invade the agar, greatly increasing colony boundaries. We performed time-lapse video contrast-enhanced Nomarski-DIC microscopy to compare the growth of RND and PH colonies at high resolution. We found that the growth pattern of PH cells is strikingly different from the classical description of the cell cycle of budding yeast. In RND cells, buds are initiated in G1 after a cell-size checkpoint, START, is passed. Mitosis, septation and separation immediately follow completion of S phase; RND cells spend little or no time in G2. When nutrient starvation limits growth, RND daughters may stall in G0, having been released from their mothers at a size well below the START threshold. By contrast, mother-daughter pairs of PH cells do not septate before doubling size. Upon separation, both cells form new buds in synchrony. In our study, RND cells in low nitrogen/high carbon media required 152+/-6 min (mean+/-SEM, n=18) to progress from nascent buds to first bud initiation; second buds required only 131+/-5 min. For PH cells these times were 142+/-4 and 147+/-5 min (n=11). In contrast to the RND cell cycle, the PH growth pattern favors the daughter, the cell closer to a potential nutrient source. The results of flow cytometry of DNA content and fluorescence imaging of nuclear and spindle morphology suggest that growth of PH daughter cells to the size of the mother follows DNA synthesis but precedes nuclear division. These data provide evidence for a second cell-size checkpoint present in G2.

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ROLE OF PRELAMIN A IN THE REGULATION OF MAMMALIAN CELL CYCLING.

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Most permanent cell lines undergo a G_1 cell cycle arrest upon starvation for mevalonate. This early observation led to the discovery of prenylated proteins, but the particular prenylated protein responsible for this phenomenon has not been identified. We have found that F9 embryonal carcinomas are resistant to the inhibition of DNA biosynthesis by lovastatin. However, incorporation of mevalonate into both sterols and p21**ras** occurs at similar doses of lovastatin as those required to produce these responses in cells in which DNA synthesis is sensitive to lovastatin. Thus, these cells are not resistant to lovastatin inhibition of DNA synthesis due to a lowered sensitivity of isoprenoid synthesis to this compound. It has been observed that F9 cells do not express the A/C lamins. Expression in F9 cells of human prelamin A, a protein which required farnesylation for conversion to mature lamin A, causes these cells to become sensitive to inhibition of DNA biosynthesis by lovastatin. Expression in F9 cells of mature lamin A does not render these cells lovastatin sensitive. We thus propose that accumulation of prelamin A in mammalian cells is responsible for the cell-cycle arrest observed under conditions of mevalonate starvation. We suggest that prelamin A accumulation may also play a physiological role in cell cycle arrest.

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DIFFERENTIAL EFFECTS OF MIMOSINE ON DNA REPLICATION IN SOMATIC AND EMBRYONIC CELLS OF *XENOPUS LAEVIS*

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The plant amino acid mimosine reversibly arrests the cell cycle of cultured mammalian cells near the G_1/S boundary by blocking initiation of DNA replication (Dijkwel and Hamlin, **Mol. Cell. Biol.** 12, 3715-3722, 1992). In our study, mimosine did not inhibit DNA replication in cell-free extracts of *Xenopus laevis* eggs, nor did it block M to S phase transition in these extracts. Incubation of *Xenopus* embryos in 2 mM mimosine had no effect on development for at least 3 days after fertilization. Moreover, microinjection of mimosine (2 mM final concentration) into 4-cell embryos had no detectable effect on embryogenesis through hatching. Neither [^{3}H]-thymidine incorporation nor replication of microinjected DNA during early embryogenesis were inhibited by the microinjected mimosine. Microinjection of mimosine, even after the midblastula transition when *Xenopus* embryonic cells first acquire the G_1 and G_2 phases of the cell cycle, did not inhibit DNA replication or cell cycle progression. In contrast, the growth of *Xenopus* kidney epithelial A6 cells in culture was blocked at the G_1/S boundary by mimosine as were mammalian NRK-49F cells, protozoan (*Tetrahymena*) cells, and plant (Black Mexican sweet maize) cells. We conclude that mimosine affects cell cycle progression and DNA replication differently in somatic cells than in embryonic *X. laevis* cells.

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CELL CYCLE REGULATION OF THE YEAST Cdc7 PROTEIN KINASE BY ASSOCIATION WITH THE Dbf4 PROTEIN.

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The Cdc7 protein kinase of *Saccharomyces cerevisiae* is a key regulator of the mitotic cell cycle, and is essential for the initiation of DNA replication. The function of Cdc7 is stage-specific in the cell cycle, yet total Cdc7 protein levels remain constant. Therefore, the regulation of Cdc7 function appears to be achieved by a post-translational modification. We propose that the post-translational regulation of Cdc7 is the result of stage-specific association with the Dbf4 protein. Through the use of cell cycle mutants and cell cycle inhibitory drugs, we demonstrate that Cdc7 kinase activity is regulated in the cell cycle, with maximal activity occuring at the G1/S boundary. Furthermore, Cdc7 kinase activity is dependent on the presence of an active Dbf4 protein. Therefore, Dbf4 may function as a cyclin-like protein to provide the cell cycle-specific regulation of Cdc7 function for entry into S phase. Preliminary experiments indicate that Cdc7 may function as part of a multi-subunit complex, and we are currently investigating the possibility that Dbf4 is transiently associated with this complex.

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AN UNUSUAL BUDDING PATTERN IN SACCHAROMYCES CEREVISIAE.

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We are reporting here the first definitive example of a haploid strain (GS 1731) of the budding yeast S. cerevisiae that forms both multiple buds and short filaments during the early phases of growth in a rich medium. In all strains examined previously each mother cell produces only one bud at a time. Certain cell cycle mutants and osmoregulation-defective mutants have been shown to arrest as multiple budded cells under non-permissive conditions. Recently diploid, but not haploid, S. cerevisiae cells have been shown to produce short filaments. By the end of lag phase of the GS 1731 growth curve multiple budded cells constituted 50-60% of the budded cells, with slightly more of the cells possessing three or more buds. During log phase the % cells showing multiple buds decreased. We have shown by electron microscopy that the multiple buds are attached to a common mother cell. Comparison of the data from the lag phase of a normal growth curve and those after removal of a hydroxyurea block suggests that during the S-phase after a growth arrest GS 1731 can initiate several buds. We have observed that cells of GS 1731 have multiple bundles of cytoplasmic microtubules. This would explain why the multiple buds on a mother cell all tend to be at the same pole.

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EFFECTS OF EXTRACELLULAR PH ON INTERNAL PH IN CELL CYCLE DEFECTIVE *SCHIZOSACCHAROMYCES POMBE*.

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The regulation of intracellular pH is important in cell division. In many cell types a rise in intracellular pH is permissive for replication and progression of the cell cycle. We have recently shown that the intracellular pH of the fission yeast *Schizosaccharomyces pombe* can be monitored with the fluorescent dye carboxyseminaphthorhodafluor-1 (C.SNARF-1). We examined the effect of extracellular pH on internal pH in both control and mutant strains. The strain pcr1-1 is a temperature sensitive mutant which shows pH-dependent cell cycle arrest. C.SNARF-1-acetoxymethylester was de-esterified and retained by both control (Q250) and mutant (pcr1-1) *S. pombe*. The de-esterified product from C.SNARF-1-AM was sensitive to pH over a physiological range. In Q250, monitoring internal pH while varying external media pH showed that wild type *S. pombe* regulates internal pH tightly at external values between 3.5 to 6.0. Above pH 6.0 internal pH rises slowly and above external pH 7.0 internal pH rises rapidly. In the mutant pcr1-1, however, pH regulation is different. Internal pH is lower at all external pH values. In addition, at low external pH (below 5.5), internal pH in pcr1-1 is markedly reduced compared with the wild type strain. This observation was independent of the temperature at which the cells were grown. Measurement of internal pH using ^{31}P-NMR gave similar results. The results indicate that internal pH regulation by pcr1-1 is defective in comparison to Q250, suggesting an effect of the *pcr1* protein on the plasma membrane H^+-ATPase. Supported by the MRC of Canada.