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Uptake of endocytic markers into mitotic yeast cells

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[³H]α-Factor and Lucifer yellow were used to measure receptor mediated and fluid-phase endocytosis in the yeast *Saccharomyces cerevisiae*, arrested in mitosis by depolymerization of the microtubules or due to a mutation preventing nuclear division (*cdc16*). Both processes continued at roughly the same level as during interphase. This shows that in yeast endocytosis is not interrupted during mitosis like in mammalian cells.

Endocytosis; Mitosis; *Saccharomyces cerevisiae*

1. INTRODUCTION

In animal cells endocytosis and exocytosis are continuous processes during interphase, but stop abruptly at the onset of mitosis with an as yet unknown molecular mechanism [1–3]. Concomitantly, the morphology of intracellular organelles changes dramatically. The nuclear envelope, the Golgi complex and sometimes the endoplasmic reticulum break down to vesicles [4–6]. Membrane traffic is resumed at telophase, coinciding with reassembly of the organelles [1,4]. Though exocytosis occurs in the budding yeast *S. cerevisiae* similarly as in the mammalian cell [7], it appears to be regulated differently. We have shown that in *S. cerevisiae* the secretion of a number of glycoproteins continues during mitosis [8–10]. Golgi functions, like extension of *O*- and *N*-glycans and proteolytic processing, occur similarly during mitosis and interphase, though the Golgi cisternae appear fenestrated and vesicularized [8,9]. Here we show that fluid-phase and receptor mediated endocytosis go on in *S. cerevisiae* cells arrested in mitosis.

2. MATERIALS AND METHODS

The wild-type strain S13 (derivative of S288C), RH448 (*Mata, his4, leu2, ura3, lys2, bar1-1*), RH449 (*Matα, his4, leu2, ura3, lys2, bar1-1*) [11], *cdc16-1* (*Mata, ade1, ade2, ural, tyr1, his7, lys2, gal1*) and its parent strain *CDC*⁺ [12], were grown in YPD medium containing 1% yeast extract (Oxoid Ltd, Basingstoke, UK), 2% bacto peptone (Difco, Detroit, MI, USA) and 2% glucose (BDH Pharmaceuticals Ltd, UK) at 25°C or 30°C in a shaker to densities of $2–4 \times 10^7$ cells/ml. Cells were arrested reversibly in mitosis by methyl[5-(2-thienylcarbonyl)-1H-benzimidazol-2-yl]carbamate (nocodazole) treatment (Sigma Chemical Co., St. Louis, MO, USA) or by using the temperature-sensitive cell cycle deficient mutant *cdc16*, as described [8]. Binding of [³H]α-factor (Cambridge Research Biochemicals Limited, Cambridge, England) was carried out at a density of 5×10^8 *bar1-1* cells/ml in 50 mM KH_2PO_4, pH 3.5 (pH 3.5-solution). Free and unspecifically bound ligand was removed with cold 50 mM KH_2PO_4, pH 6 (pH 6-solution) [13]. For internalization the cells were then suspended in pre-warmed pH 3.5-solution, containing 2% glucose, the necessary amino acids and nucleotides, and 1.5 mg/ml of N_α-*p*-tosyl-L-arginine methyl ester (TAME, Sigma). In continuous internalization experiments, 2.4×10^{-7} M (1 μCi) of [³H]α-factor was used. Duplicate samples of 200 μl were washed with cold 50 mM sodium citrate solution, pH 2 (pH 2-solution), to release all surface-bound ligand, spheroplasted and measured for radioactivity [13]. In fluid phase endocytosis experiments, 2×10^8 cells/ml were incubated in a shaker in YPD-medium with Lucifer yellow CH (LY) (Fluka Chemie AG, CH-9470 Buchs, Switzerland). Duplicate samples of 500 μl were washed and lysed [14], and measured for fluorescence intensity with a Hitachi F-3000 fluorescence spectrophotometer, or inspected with an Olympus BH-2 fluorescence microscope equipped with Nomarski optics. Unlabelled synthetic α-factor, KF, NaN_3 and cycloheximide were from Sigma.

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3. RESULTS AND DISCUSSION

α-Mating type cells secrete a pheromone, α-factor, into the growth medium. It is bound to specific receptors on the plasma membrane of the opposite mating type, *a*-cells, internalized by receptor mediated endocytosis, and transported to the vacuole where it is degraded [11,15,16]. A synthetic [³H]α-factor preparation was used to assay receptor-mediated endocytosis during mitosis. The specificity of its binding to α-factor receptors was tested in competition experiments. 10^{-10} M of unlabelled α-factor completely inhibited binding of 2×10^{-8} M of [³H]α-factor to *a*-cells (Fig. 1). Control cells and nocodazole-treated mitotic cells internalized similar amounts of [³H]α-factor, showing that receptor-mediated endocytosis was not interrupted during mitosis (Fig. 2). α-Factor receptors are subject to down regulation, which temporarily reduces α-factor uptake, before newly synthesized receptors reach the plasma membrane by exocytosis [17]. Cycloheximide, a protein synthesis inhibitor, was

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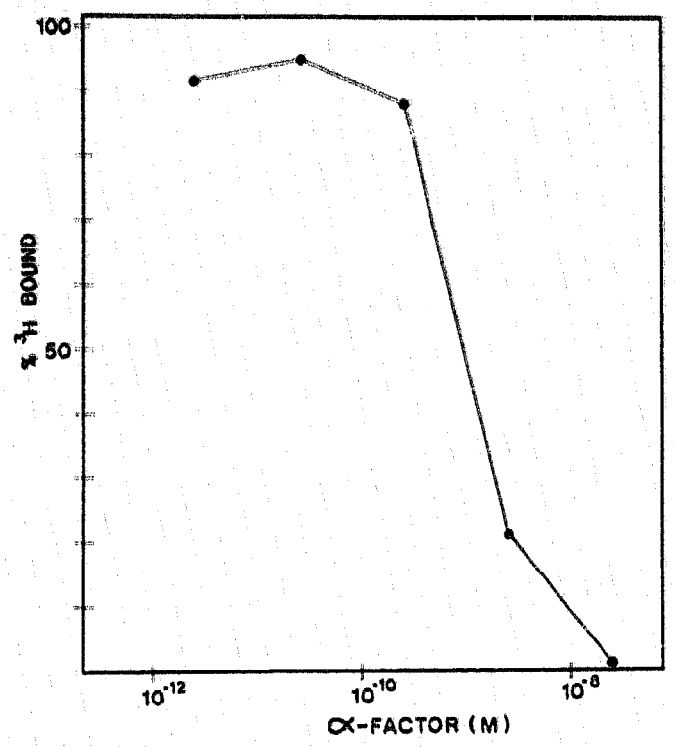


used to uncouple endocytosis from exocytosis. In the presence of cycloheximide, [^{3}H]α-factor uptake levelled off, indicating that the initial set of receptors had been used in 20–30 min (Fig. 2). No uptake occurred into α-cells lacking α-factor receptors, or into a-cells under energy depletion conditions (Fig. 2A, B).

To uncouple binding and internalization, [^{3}H]α-factor was first bound to receptors in the cold. Free and non-specifically bound ligand was removed, and cells were transferred to glucose-containing medium to start endocytosis. [^{3}H]α-Factor was efficiently internalized into mitotic and unsynchronized cells (Fig. 2C). Mitotic doublet cells bound, and subsequently internalized, about 1.2 times more ligand than the unsynchronized cells. By dry weight and protein content, nocodazole arrested cells were about twice as large as average unsynchronized cells (3.0 mg and 1.7 mg of dry weight/10^8 cells; 1.6 ng and 0.8 ng of protein/10^8

Fig. 1. Inhibition of [^{3}H]α-factor binding. a-Cells were incubated in the cold with 2×10^{-8} M of [^{3}H]α-factor with the indicated concentrations of unlabelled α-factor. Binding was calculated as percentage of binding in the absence of the unlabelled ligand.

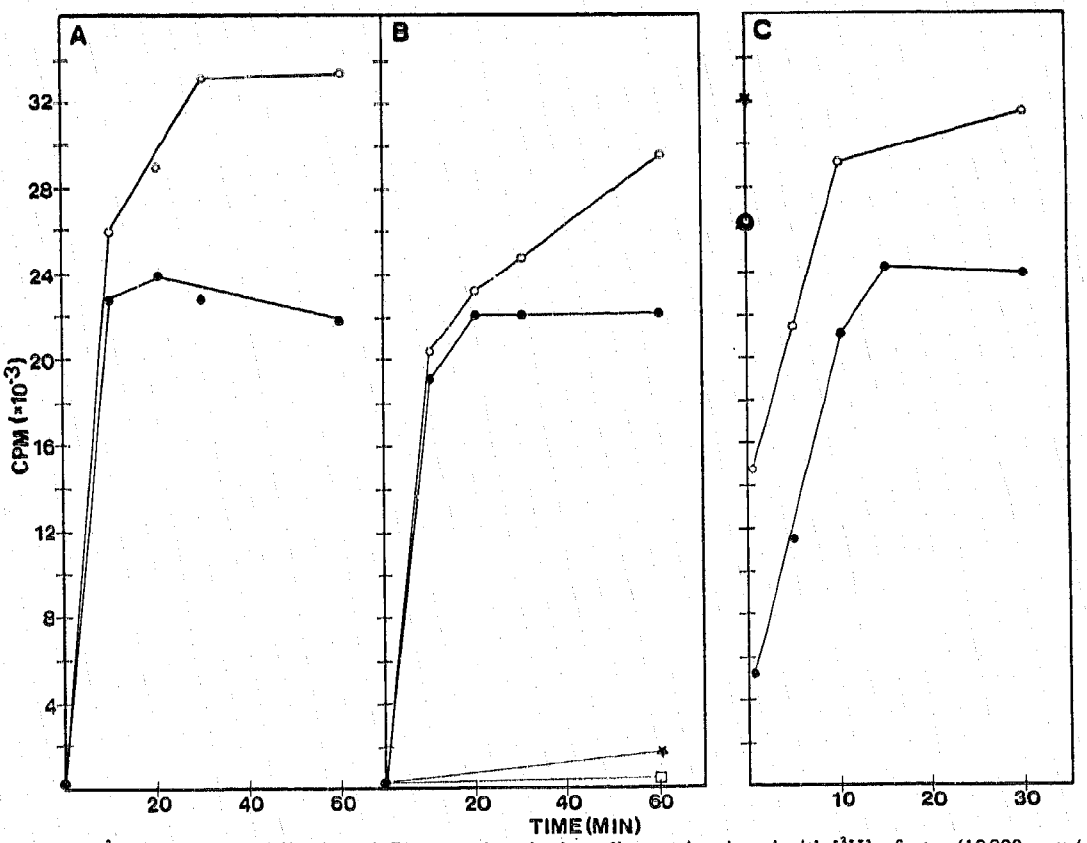
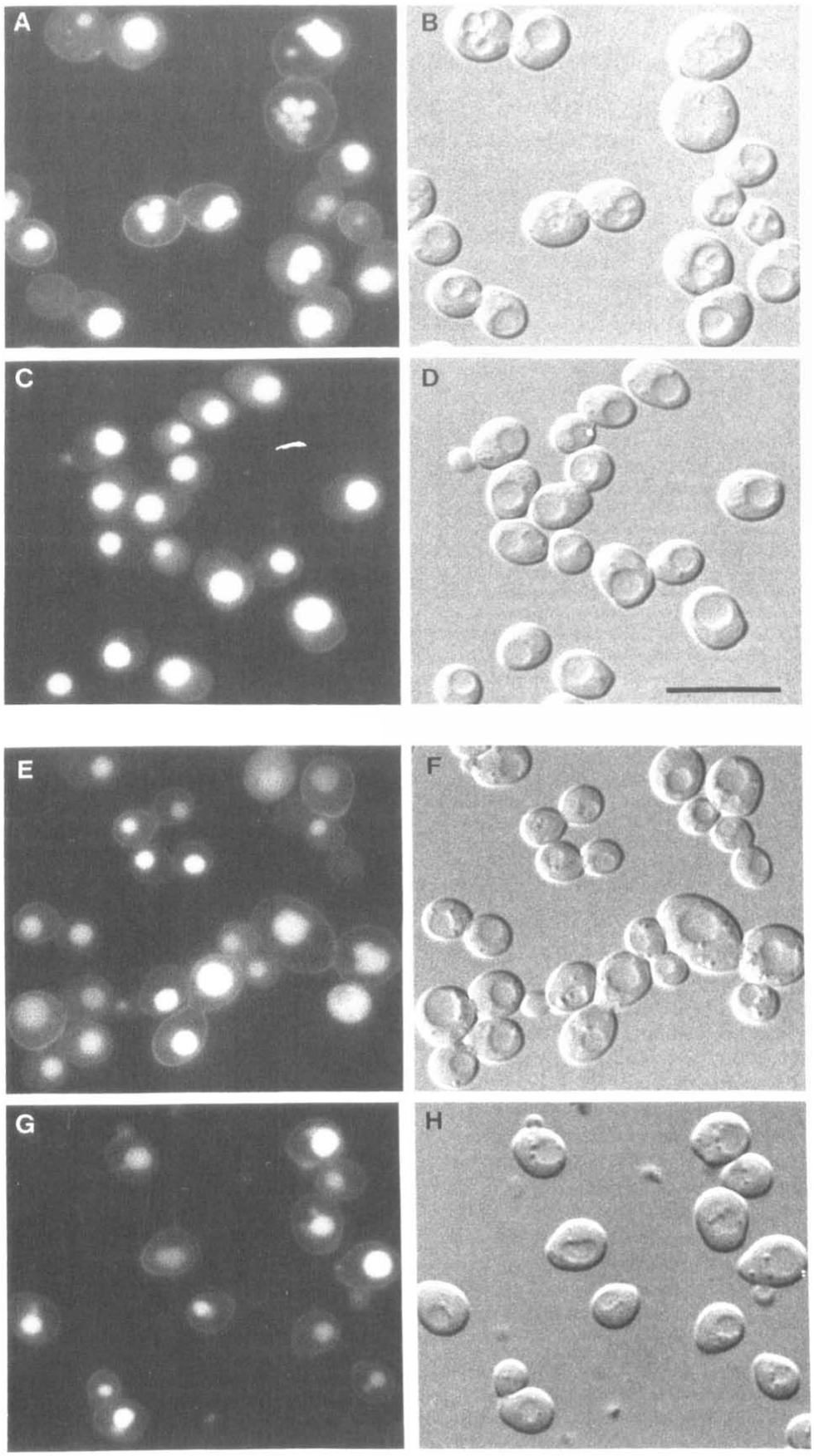


Fig. 2. Internalization of [^{3}H]α-factor. (A) Mitotic and (B) unsynchronized a-cells were incubated with [^{3}H]α-factor (10900 cpm/sample) at 30°C in the presence (●) or absence (○) of 100 mM cycloheximide. Uptake into α-cells (★). Uptake into a-cells in the presence of 10 mM NaN_3 and KF (□). Cell-associated [^{3}H]α-factor at 0 min has been subtracted from all samples. (C) Internalization of receptor-bound [^{3}H]α-factor. [^{3}H]α-Factor (68100 cpm/sample) was bound to cell surface receptors of mitotic (○) and unsynchronized (●) cells in the cold. Internalization of receptor-bound radioactivity was then followed at 30°C. Radioactivity bound to mitotic cells (★) and to unsynchronized cells (⊖) prior to internalization.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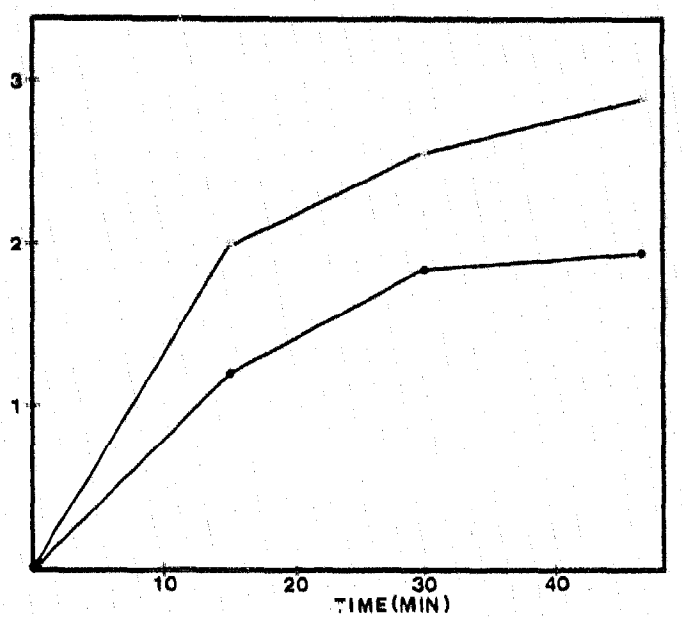

Fig. 4. Kinetics of LY uptake. Mitotic (○), and unsynchronized (●) wild type cells were incubated with 4 mg/ml of LY at 30°C. Internalized fluorescence was quantitated and plotted in arbitrary units against incubation time. The amount of LY in control samples incubated for 45 min at 0°C was less than the background fluorescence of the samples taken immediately after addition of LY (0 min). The background fluorescence has been subtracted from all other samples.

cells, respectively). In both cell populations, more than 90% of pre-bound [^{3}H]α-factor was internalized within 15 min. The number of receptors per cell, calculated from binding data, was 11 550 and 9400 for mitotic and unsynchronized cells, respectively, which is in agreement with earlier estimations [16].

LY was used to study fluid phase endocytosis in mitotic cells [14]. Fluorescence microscopy showed that mitotic nocodazole-arrested and *cdc16* cells internalized LY to the vacuole (Fig. 3). Disruption of the cytoplasmic microtubules caused fragmentation of vacuoles (Fig. 3A, B) [18], which in the *cdc16* mutants appeared intact (Fig. 3E, F). Quantitations showed that in 45 min the mitotic cells internalized about 1.5 times more LY (250 ng/10^8 cells) than unsynchronized cells (170 ng/10^8 cells) (Fig. 4).

Membrane traffic encompasses, in addition to exocytic and endocytic routes, many circuits where organelles of both routes are interconnected by vesicular traffic. At least part of these routes can be uncoupled from each other. In temperature-sensitive Chinese hamster ovary mutant cells, secretion is inhibited at the restrictive temperature, whereas the clathrin coated pit pathway of endocytosis, and recycling, are not [19,20]. The simultaneous inhibition of exocytosis and endocytosis in mitotic animal cells, and the continuation of secretion and endocytosis in mitotic yeast cells, may result from a common regulatory mechanism for all vesicular traffic.

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Fig. 3. Fluid phase endocytosis. Mitotic (A, B) and control (C, D) wild type cells were incubated at 30°C with 40 mg/ml of LY and washed prior to fluorescence microscopy. *cdc16* cells, arrested in mitosis (E, F), and their parent cells (G, H) were incubated at 36°C (the restrictive temperature) with LY. Panels B, D, F and H: Nomarski optics. Bar = 10 μm.