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Endocytosis in *Saccharomyces cerevisiae*: internalization of α-amylase and fluorescent dextran into cells

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In the preceding paper I reported that *Saccharomyces cerevisiae* spheroplasts were able to internalize particulate markers, enveloped viruses, into intracellular organelles. Here the internalization of soluble macromolecules into cells having an intact cell wall is described. α-Amylase was taken up into cells in a temperature- and concentration-dependent way. The kinetics of accumulation were linear for the first 20 – 40 min at 37°C and then started to level off. Internalization of α-amylase into spheroplasts displayed similar characteristics, but the accumulation rate was about four times higher than into cells. Fluorescent dextran was used to mark morphologically the compartment into which internalization occurred. This marker was accumulated into the vacuole of the cells in a time-, temperature- and concentration-dependent way. A temperature-sensitive mutant deficient in exocytosis was found to be defective in intracellular accumulation of α-amylase and dextran. At the restrictive temperature, very little α-amylase accumulated into the cells and only faint staining of intracellular organelles with fluorescent dextran could be detected. At the permissive temperatures, accumulation of α-amylase and dextran into the mutant cells was comparable with accumulation into wild-type cells. I conclude that α-amylase and fluorescent dextran were internalized into *S. cerevisiae* cells and directed into the vacuoles.

Key words: endocytosis/membrane traffic/*Saccharomyces cerevisiae*/transport mutants

Introduction

In the preceding paper internalization of vesicular stomatitis virus (VSV) and Semliki Forest virus (SFV) into *Saccharomyces cervisiae* spheroplasts was described. Internalization was time-, temperature- and concentration-dependent. After internalization the viruses could be found in three intracellular compartments which were resolved by Percoll density gradient centrifugation. Spheroplasts were used in these studies since the virions would have been excluded by the cell wall covering the plasma membrane. Yeast cells do not occur at any stage of their life cycle without a cell wall, and thus it is important to show that endocytosis is not restricted to spheroplasts but occurs in intact cells as well.

In the animal cell, endocytosis usually starts at clathrin-coated areas of the plasma membrane giving rise to coated pits. The coated pits appear to pinch off into the cytoplasm to form coated vesicles (Anderson *et al.*, 1977; Helenius *et al.*, 1980). Recently, clathrin-coated vesicles have been found in *S. cerevisiae* (Mueller and Branton, 1984). The origin of the intracellular coated vesicles remained uncertain. It will be interesting to learn whether coated vesicles operate in membrane traffic in yeast. In the animal cell, internalized material is rapidly found in smooth-surfaced irregular-shaped endosomes which have an acidic internal milieu (Willingham and Pastan, 1980; Simons *et al.*, 1982; Tycko and Maxfield, 1982; Galloway *et al.*, 1983). The low pH of the endosomal compartments appears to be important for many of the functions of this organelle, e.g., detachment of ligand from receptor (Pricer and Ashwell, 1971; Gonzales-Noriega *et al.*, 1980; Tietze *et al.*, 1982), detachment of iron from transferrin (Dautry-Varsat *et al.*, 1983; Klausner *et al.*, 1983) and returning internalized receptors and membrane components back to the plasma membrane by recycling (King *et al.*, 1980; van Leuven *et al.*, 1980; Basu *et al.*, 1981; Pesonen and Simons, 1983). Endosomes fuse with lysosomes to deliver material for use or degradation. In transcellular transport endosomes can fuse with another plasma membrane domain (Abrahamson and Rodewald, 1982; Herzog, 1983; Pesonen *et al.*, 1984). In secretory cells the Golgi complex can be involved in the endocytotic pathway (see Farquhar, 1981).

In this paper soluble macromolecules, α-amylase for quantitation and fluorescent dextran for morphological observations, were used to show that endocytosis also occurred in normal yeast cells with an intact cell wall. Both markers appeared to be directed into the vacuole.

Results

α-Amylase as an internalization marker

To study endocytosis in cells having an intact cell wall, α-amylase was used as a marker. To test the assay, different amounts of α-amylase were incubated in the presence or absence of *S. cerevisiae* lysates. Similar linear curves were obtained in both cases (Figure 1). Thus, *S. cerevisiae* did not contain endogenous α-amylase activity, nor were the lysates able to degrade the exogenous marker. When constant amounts of α-amylase were assayed for different periods of time, the assay was found to be linear

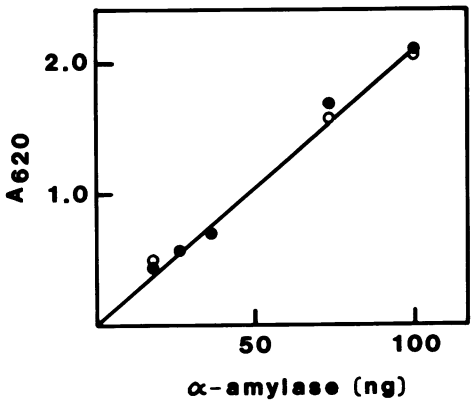

Fig. 1. α-Amylase assay. Different amounts of α-amylase were assayed for 1 h in the absence (●) or presence (○) of spheroplast lysates (100 x 10^6 per sample) and plotted against absorbance at 620 nm.

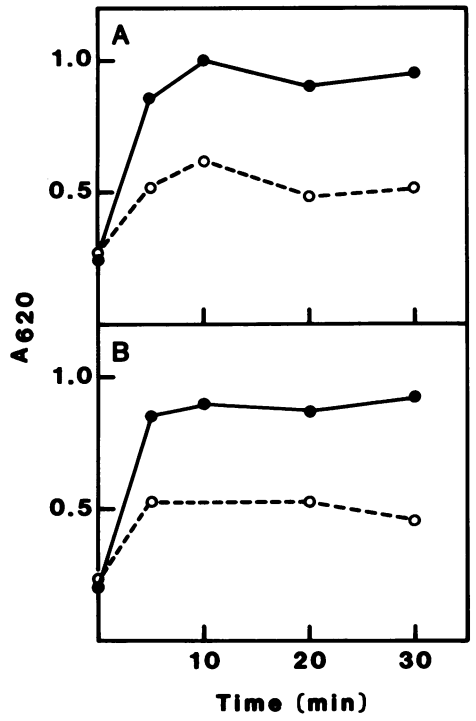

Fig. 2. Binding of α-amylase to spheroplast and cell surfaces. Wild-type *S. cerevisiae* spheroplasts **(A)** or cells **(B)** were incubated at 0°C in growth medium (1500 x 10^6 cells or spheroplasts/ml) containing 270 μg/ml of α-amylase. At different time points triplicate samples of 100 μl were washed twice with cold PBS (1.2 M sorbitol was included in all media up to the lysis step in the case of spheroplasts), lyzed in α-amylase assay buffer and assayed for the enzyme activity for 2 h (●). Parallel samples were treated after the washes with 0.5 mg/ml of proteinase K for 30 min at 0°C prior to lysis and α-amylase assay (○). Cells **(B)** were treated after washes (●) or after proteinase K digestion (○) with zymolyase to achieve complete lysis for the α-amylase assay. Absorbance at 620 nm is plotted against incubation time.

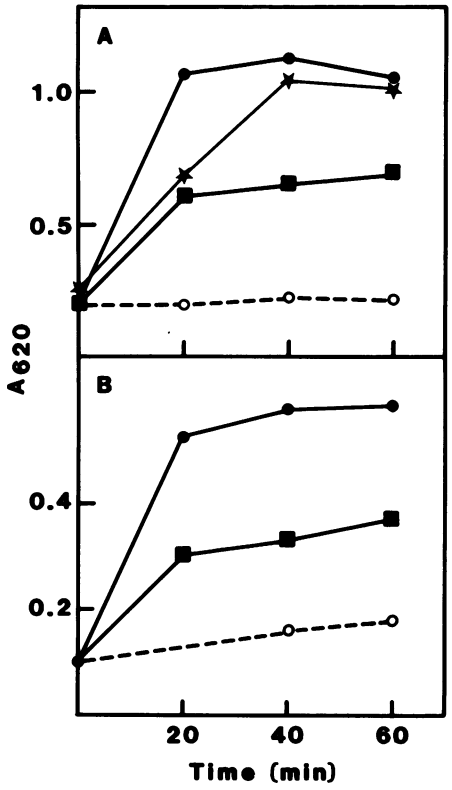

Fig. 3. Uptake of α-amylase into spheroplasts and cells. Wild-type *S. cerevisiae* spheroplasts **(A)** or cells **(B)** were incubated at 37°C (●), 25°C (■) or 6°C (○) in growth medium (1500 × 10^6 spheroplasts or cells/ml) containing 90 μg/ml of α-amylase. (★) Represents parallel experiment for (●), uptake now leveling off after 40 min of incubation (see also Figure 7A). At different time points samples of 100 μl were diluted into 1 ml of cold PBS (prior to lysis all media contained 1.2 M sorbitol), washed twice with 1 ml of cold PBS, digested with proteinase K in the cold, washed twice with cold PBS and lyzed for α-amylase assay (16 h). The number of cells did not increase during the experiment. The cells **(B)** were treated with zymolyase after the proteinase K digestion prior to lysis. Absorbance at 620 nm is plotted against incubation time.

for at least 23 h at 37°C (not shown). Again, the presence of the lysates did not affect the results.

Binding of α-amylase to spheroplast and cell surfaces

S. cerevisiae cells or spheroplasts were incubated with α-amylase at 0°C, washed, lyzed and assayed for α-amylase activity. Similar amounts of the marker were bound to cell and spheroplast surfaces (Figure 2). Binding reached a plateau within 10 min in both cases. Proteinase K digestion in the cold was able to remove about two-thirds of the surface-bound marker. However, α-amylase was not inactivated by proteinase K. When α-amylase was incubated with proteinase K for 60 min at 37°C, the activity of the α-amylase was retained quantitatively. Thus, probably a surface-polypeptide was responsible for binding of α-amylase, and this polypeptide was degraded by proteinase K, resulting in removal of α-amylase from the surface. Being a secretion product of *Bacillus*, α-amylase is very resistant to proteases.

Internalization of α-amylase into spheroplasts and cells

Upon incubation at 37°C with *S. cerevisiae* spheroplasts, increasing amounts of α-amylase became spheroplast-associated (Figure 3A). At each time point the spheroplasts were treated with proteinase K in the cold to remove most of the surface-bound marker. Thus, most of the α-amylase remaining spheroplast-associated thereafter was probably internalized. The uptake curves leveled off after 20−40 min at 37°C in different experiments. Uptake was temperature-dependent. At 25°C accumulation was ~50% of that at 37°C. At 6°C no uptake could be observed (Figure 3A). Uptake was also concentration-dependent. A linear curve was obtained when 1−80 μg of α-amylase was incubated with a constant number of spheroplasts and the amount of the marker taken up was plotted against the amount of the marker added to the incubation mixture (not shown).

α-Amylase was taken up also into cells having an intact cell wall. The kinetics and temperature-dependency of internalization into cells were similar to those described for spheroplasts (Figure 3B). However, the rate of accumulation of the marker into cells appeared to be about one-fourth of that internalized by spheroplasts. In six different experiments where spheroplasts were incubated for 30 min at 37°C in growth medium (1.5 x 10^8/ml) containing 90 μg/ml of α-amylase, the average uptake rate was 4.3 ng/h/10^8 spheroplasts (range 2.9−5.7). In three experiments the corresponding average uptake rate for cells was 1.1 ng/h/10^8 cells (range 0.9−1.3).

Internalization of fluorescent dextran into the vacuole of cells

Organelle fractionation studies showed that part of the viruses internalized by spheroplasts were found co-migrating with vacuolar markers in density gradients (accompanying paper). Part of internalized α-amylase was also found to co-sediment with the vacuole markers (not shown). To see whether soluble markers internalized from the extracellular fluid were transported into the vacuole, fluorescent dextran was used as a marker. Wild-type

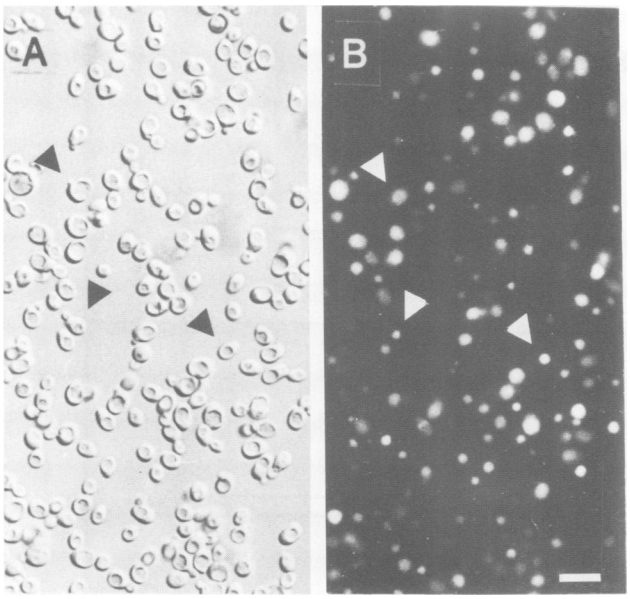

Fig. 4. FITC-dextran staining of vacuoles. Wild-type *S. cerevisiae* cells (150 x 10^6/ml) were incubated in 100 μl of growth medium in the presence of 100 mg/ml of FITC-dextran for 40 min at 37°C. The same live cells were viewed with Nomarski optics **(A)** or through fluorescence filters **(B)**. Arrows point to vacuoles of the same cells in **(A)** and **(B)** panels. Bar: 8 μm, magnification x620.

cells were incubated in the presence of FITC-dextran and prepared for microscopy. When the cells were viewed through Nomarski optics, the vacuoles appeared very clearly as distinct, often very large, organelles (Figure 4A). When the same cells were viewed through the fluorescence filters (Figure 4B), spots were seen, which coincided with the vacuoles visible with Nomarski optics. No surface staining could be observed. In three different experiments, where 200 cells were counted in each, ~90% of the cells were stained. The spots appeared bright in part of the cells and dim in others.

Uptake of FITC-dextran into cells was dependent on the time and temperature of incubation. The intensity of vacuolar staining increased with incubation time (Figure 5A and B). Sometimes incubation for 20 min and 40 min resulted in equally bright staining. Note that uptake of α-amylase also leveled off after 20 or 40 min at 37°C (see above). Incubation at 25°C resulted in weaker staining than incubation at 37°C (Figure 5B and D). However, the fraction of cells stained after an incubation of 40 min at 25°C was 80% (150 cells counted). At 0°C no staining could be observed. Staining of the vacuoles was also concentration-dependent. When cells were incubated with different concentrations of FITC-dextran (1 – 200 mg/ml) for 40 min at 37°C, the intensity of the vacuolar staining correlated with the marker concentration. 20 mg/ml was a threshold concentration; with lower concentrations of FITC-dextran no staining could be observed (data not shown).

Internalization in temperature-sensitive exocytosis-deficient sec 1-1 cells

Growth at permissive and restrictive temperatures. The sec 1-1 mutant of *S. cerevisiae* has been isolated and characterized by Novick *et al.* (1980). The mutant is deficient in exocytosis of secreted glycoproteins at the restrictive temperature (37°C). Under these conditions the proteins are transported from the endoplasmic reticulum *via* the Golgi complex to the secretory vesicles, where they accumulate and fail to be exocytosed. At the permissive temperature (25°C) exocytosis occurs normally. Figure 6

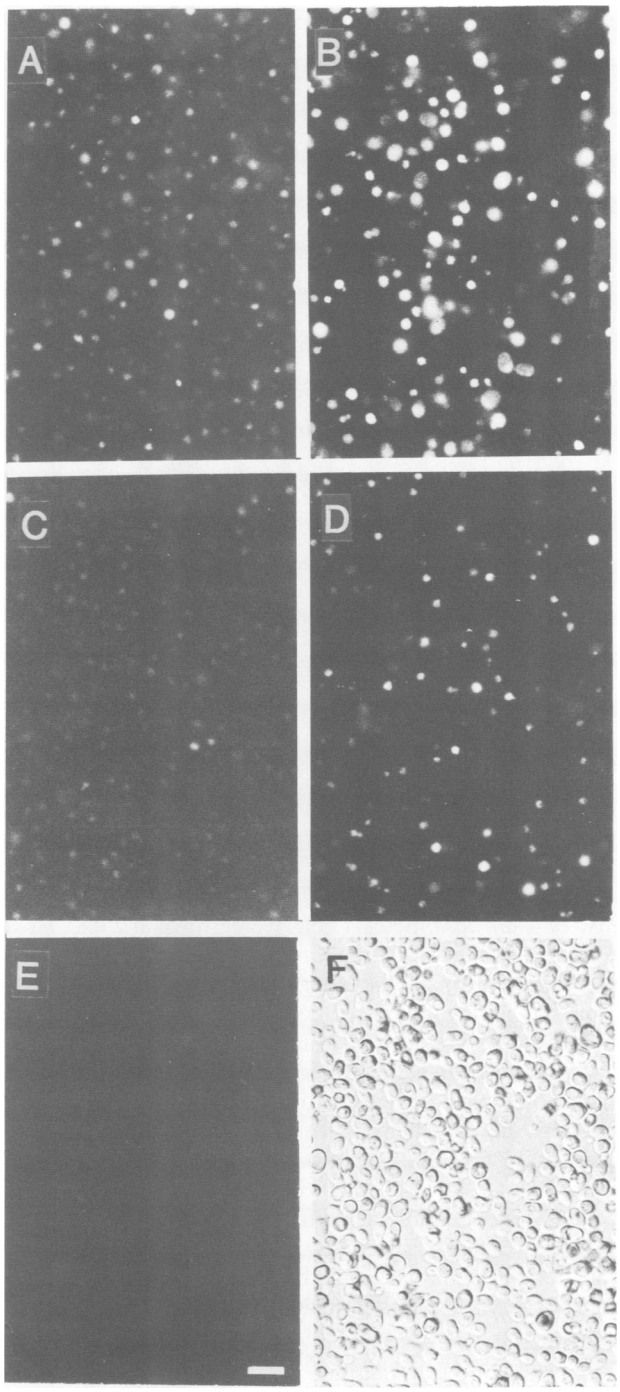

Fig. 5. Uptake of FITC-dextran into cells. Wild-type *S. cerevisiae* cells were incubated in growth medium (1500 x 10^6/ml) containing 200 mg/ml of FITC-dextran for 20 min **(A,C)** or for 40 min **(B,D,E)** at 37°C **(A,B)**, 25°C **(C,D)** or 0°C **(E)**. The live cells were observed through fluorscence filters **(A – E)**. The cells in **E** are viewed in **F** through Nomarski optics. Bar: 8 μm, magnification x560.

shows the growth curve of sec 1-1 at 25°C (1). When part of the culture is shifted from 25°C to 37°C, growth continued for 1 h and then ceased abruptly (2). To see whether the inhibition in growth was reversible, part of the culture shifted to 37°C was shifted back to 25°C after 3 h (3). Growth now continued after a lag of 1 h with a rate comparable with that of the original culture kept at 25°C. Cultures incubated from the start at 37°C did not grow at all (4). A shift of these cultures to 25°C did not start growth (5).

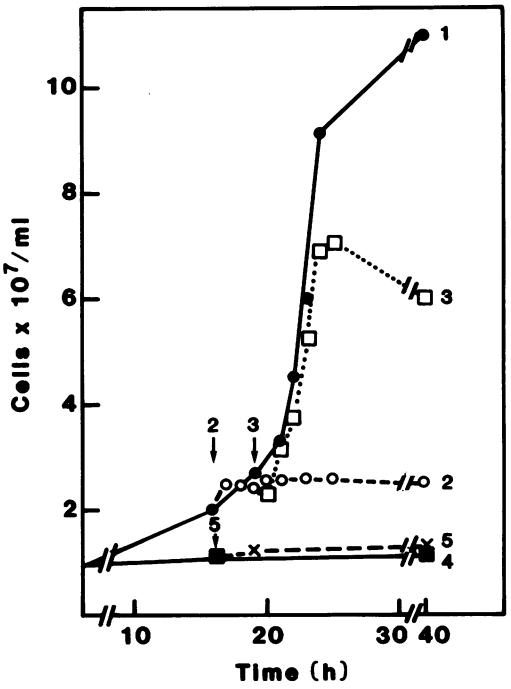

Fig. 6. Growth curves of sec 1-1. The temperature-sensitive exocytosis-deficient sec 1-1 cells were grown under different conditions. **(1)** 40 h at 25°C (permissive temperature), **(2)** 16 h at 25°C, then 24 h at 37°C (restrictive temperature). **(3)** 16 h at 25°C, then 3 h at 37°C, then 21 h at 25°C. **(4)** 40 h at 37°C. **(5)** 16 h at 37°C, then 24 h at 25°C. The number of cells is plotted against incubation time.

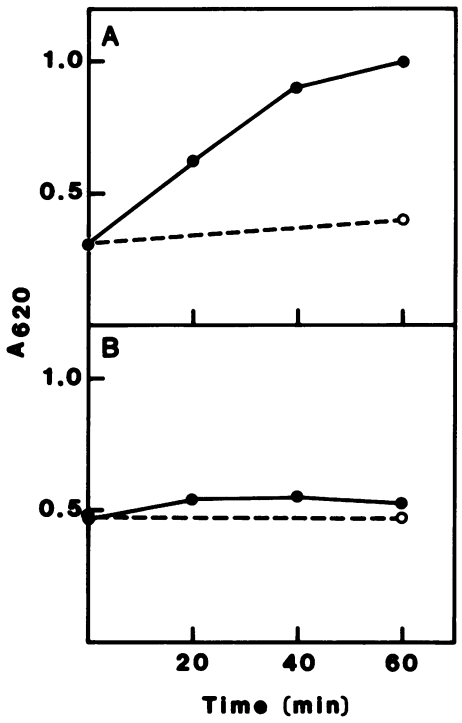

Fig. 7. Uptake of α-amylase into sec 1-1 cells. Sec 1-1 cells were grown for 18 h at 25°C **(A)** or for 16 h at 25°C and then for 2 h at 37°C **(B)**. Both cultures were collected, washed and resuspended into growth medium (1500 x 10^6/ml) containing 90 μg/ml of α-amylase. Parallel samples were incubated at 25°C (●) or 6°C (○) **(A)** and at 37°C (●) or 6°C (○) **(B)**. Samples of 100 μl were diluted at different time points into 1 ml of cold PBS, washed twice with the same buffer, treated with proteinase K in the cold, digested with zymolyase to achieve complete lysis and assayed for α-amylase content (17 h). Absorbance at 620 nm is plotted against incubation time.

Uptake of α-amylase. Sec 1-1 cells were grown at 25°C. At 16 h (see Figure 6) half of the culture was shifted to 37°C for 2 h (restrictive culture) and the other half continued at 25°C for 2 h (permissive culture). Cells of both cultures were collected and incubated in the presence of α-amylase, the permissive cells at 25°C or at 6°C and the restrictive cells at 37°C or at 6°C. Samples were taken at different time points for α-amylase assay. Figure 7A shows that the permissive culture accumulated α-amylase at 25°C with a rate of 0.59 ng/h/10^8 cells. In two other experiments rates of 0.43 and 0.61 ng/h/10^8 cells were obtained. The restrictive culture accumulated α-amylase at a rate of 0.11 ng/h/10^8 cells (Figure 7B); in another experiment no accumulation of the marker could be observed. At 6°C internalization of α-amylase into both cultures was inhibited (Figure 7).

In wild-type *S. cerevisiae* cells the ratio of rates of α-amylase internalization at 37°C and at 25°C was 2.0 (1.1/0.55 ng/h/10^8 cells, see above). In the mutant sec 1-1 cells this ratio was at least 10 times lower, 0.2 (0.11/0.55 ng/h/10^8 cells). At the permissive temperature the uptake rates were similar to those of the wild-type cells at 25°C.

Uptake of FITC-dextran. Permissive sec 1-1 cultures were incubated at different temperatures in the presence of FITC-dextran. Figure 8 (A,B,C) shows that the vacuoles of the permissive cultures at 37°C, 25°C and 0°C were stained very much like those of wild-type cells incubated under similar conditions (see Figure 5). During 40 min after shift from 25°C to 37°C no deficiency in FITC-dextran uptake was manifested. During this period the cells were still growing [see Figure 6, curve (2)]. However, in the restrictive culture, which had been incubated for 2 h at 37°C prior to addition of FITC-dextran, accumulation of the marker was decreased. Only faint, vesicular staining could be observed at 37°C and at 25°C (Figure 8, D, E), and no staining at all at 0°C (F).

Discussion

When *S. cerevisiae* cells or spheroplasts were incubated with α-amylase, the marker became cell-associated in a time-, temperature- and concentration-dependent way and could not be released by proteinase K. This suggests that the marker was internalized by *S. cerevisiae*. The uptake leveled off in 20–40 min at 37°C. At 6°C no uptake could be observed.

Accumulation of α-amylase into spheroplasts was four times higher than into cells having an intact cell wall. The binding of the marker to the spheroplast surface in the cold was similar to binding to the cell surface. The reason for the difference in the accumulation is not known. Spheroplasts exocytose in order to build up a new cell wall. If the rate of exocytosis in spheroplasts is similar or higher than in cells, spheroplasts would need accelerated endocytosis to keep the surface area constant. Spheroplasts do not bud, thus there is no loss of plasma membrane into daughter cells as there is in budding cells. A trivial explanation for the difference in accumulation of α-amylase into cells and spheroplasts would be that the cell wall was not readily penetrated by α-amylase. The *Bacillus* α-amylase is a water-soluble globular protein of 54 800 daltons (Takkinen *et al.*, 1983). It is not clear how diffusion through the yeast cell wall correlates with mol. wt. It has been claimed that polyethylene glycol molecules >700 daltons would not cross the cell wall (Scherrer *et al.*,

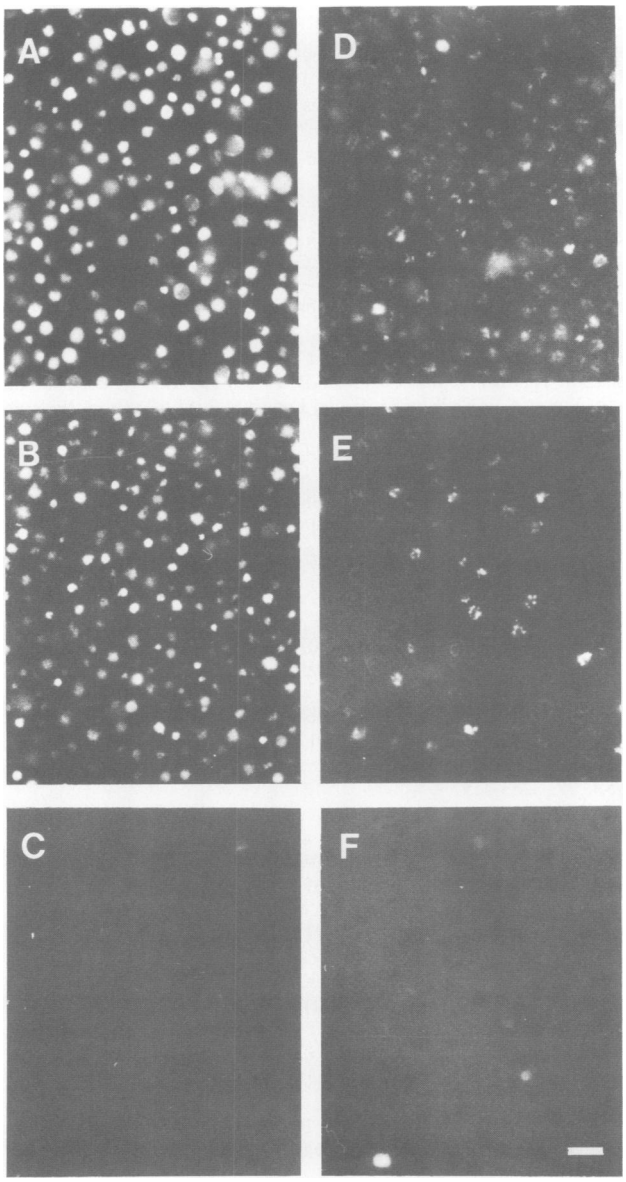

Fig. 8. Uptake of FITC-dextran into sec 1-1 cells. Sec 1-1 cells were grown for 19 h at 25°C **(A,B,C)** or for 17 h at 25°C followed by 2 h at 37°C **(D,E,F)**. The cells were incubated in growth medium (1500 x 10^6/ml) containing 200 mg/ml of FITC-dextran for 40 min at 37°C **(A,D)**, at 25°C **(B,E)** or at 0°C **(C,F)**. The live cells were observed through fluorescence filters. Each panel contains approximately the same cell density. Bar: 8 μm, magnification x560.

1974). On the other hand, wheat α-amylase, a 42 000-dalton protein expressed in *S. cerevisiae*, was efficiently secreted through the cell wall into the growth medium (Rothstein *et al.*, 1984).

Enveloped viruses, VSV and SFV were shown in the preceding paper to be endocytosed by *S. cerevisiae* spheroplasts. The fraction of VSV which was accumulated in the spheroplasts in 30 min at 37°C was ~24 times greater than the fraction of α-amylase accumulated in spheroplasts under similar conditions. The kinetics of uptake differed as well. Uptake of virus leveled off in 90–120 min and uptake of α-amylase in 20–40 min at 37°C. The following phenomena could result in these differences in the characteristics of uptake of the particulate and the soluble markers. (i) α-Amylase, but not VSV, was degraded efficiently after uptake. This appears not to be the case. When cells or spheroplasts were incubated after uptake of α-amylase in the absence of external marker, the activity of α-amylase was recovered nearly quantitatively for at least 2 h at 37°C (unpublished results). (ii) α-Amylase, but not VSV, was recycled back to the medium after uptake. In macrophages and fibroblasts, the fluid phase marker [^{14}C]sucrose has been shown after internalization to recycle constantly back to the incubation medium by exocytosis in a temperature-dependent way (Besterman *et al.*, 1984). (iii) VSV was concentrated from the medium to the spheroplast surface by adsorption prior to endocytosis, as in BHK cells (Marsh and Helenius, 1980), but α-amylase was taken in with the less efficient fluid phase uptake. However, in neither case is it clear whether internalization occurs by fluid phase or by adsorptive endocytosis. Both markers are bound to the plasma membrane in the cold, but it is not known whether these particular areas of the plasma membrane are internalized.

In the accompanying paper I showed by subcellular fractionation that 4–18% of internalized VSV co-sedimented in density gradients with two vacuole markers, non-specific protease and α-mannosidase activity. In similar experiments internalized α-amylase was found to co-sediment with the vacuole markers (data not shown). A fluorescent marker, fluorescein-conjugated dextran, was used to see directly whether there was traffic leading from the exterior of the cells to the vacuole. Incubation of cells with FITC-dextran resulted in intracellular staining, which was time-, temperature- and concentration-dependent. The fluorescence within the cells coincided with the vacuoles which were visible with Nomarski optics.

Schekman and co-workers have isolated and characterized a number of temperature-sensitive transport-deficient mutants of *S. cerevisiae*. These mutants have had a crucial role in the elucidation of the exocytotic pathway in yeast (see Schekman and Novick, 1982). One of these mutants, sec 1-1, was used here to see whether it would also be deficient in endocytosis. The permissive culture internalized α-amylase at 25°C with a rate similar to the wild-type culture at the same temperature. In contrast, the restrictive culture accumulated at least 10 times less α-amylase than the wild-type culture at 37°C. Similar results were obtained with FITC-dextran. In the permissive culture, FITC-dextran clearly stained the vacuoles. In the restrictive culture only faint intracellular staining could be observed. To rule out that the differences in uptake of markers into the permissive and restrictive cells were due to differences in cell wall structure, the two cultures were digested with zymolyase at 25°C and 37°C, respectively, and then assayed for internalization of FITC-dextran. Similar results were obtained to those described above for cells (unpublished data). Thus, endocytosis in sec 1-1 cells appeared to be impaired at the restrictive temperature, although other possibilities like accelerated exocytosis of internalized markers still have to be taken into account.

It therefore appears that there exists a transport route leading from the plasma membrane of *S. cerevisiae* to the vacuole. Obviously, there must be one or more intermediary compartments which take material from the exterior of the cell and deliver it to the vacuole. Subcellular fractionation studies revealed two compartments, distinct from the vacuole, which contained internalized VSV (accompanying paper). Work is in progress to clarify the role of these compartments in endocytosis in *S. cerevisiae*, and to characterize them, especially with respect to their internal pH. Animal cells take advantage of endocytosis to internalize fluid nutrients like cholesterol and iron from the extracellular to be used inside the cell (Goldstein *et al.*, 1979; Karin and Mintz, 1981). Endocytosis is also used to internalize hormone- and growth factor-receptor complexes and to direct them to lysosomes

for destruction after signal transmittance (Carpenter and Cohen, 1976; Kasuga *et al.*, 1981). It is not clear how the yeast cell makes use of its endocytotic capacity. Are important molecules internalized from the growth medium, or would endocytosis just serve to balance the plasma membrane area? The α-factor is a yeast pheromone, which binds apparently to specific receptors on the surface of opposite mating type cells, leading to the early stages of conjugation (see Thorner, 1982; Jenness *et al.*, 1983). The cells have been reported to recover from cell division arrest by desensitization, which may occur by loss of functional surface receptors for α-factor (Moore, 1984). It would be interesting to know whether such loss of receptors could be brought about by their internalization and subsequent destruction in the vacuole, analogously to the animal cell.

Now that an endocytotic apparatus has been identified in *S. cerevisiae*, the yeast system should prove to be a valuable tool for research of membrane traffic into the cell by providing the arsenal of powerful genetic methodology.

During the preparation of these manuscripts Howard Riezman kindly provided me with a preprint of his recent data (Riezman, 1985). Using an organic fluorescent dye, lucifer yellow, the vacuole of *S. cerevisiae* could be stained. Intracellular staining was inhibited at the restrictive temperature in many of the temperature-sensitive exocytosis-deficient mutants isolated by Novick *et al.* (1980).

Materials and methods

Cells and spheroplasts

Wild-type *S. cerevisiae* cells (derivative of S288C) were grown in YPD-medium (see accompanying paper) at 30°C in a shaker. Sec 1-1 cells (*S. cerevisiae* strain HMSF1; Novick *et al.*, 1980) were grown in the same medium at the indicated temperatures. Cells were used for experiments when the density was 40 – 150 x 10^6 cells/ml unless otherwise stated. Incubation of cells and spheroplasts with internalization markers was performed in YPD-medium supplemented with 20 mM Hepes, pH 7.2. The production and characterization of wild-type spheroplasts is described in the accompanying paper. Sec 1-1 spheroplasts were produced similarly but using 0.1 mg/ml of zymolyase 60000 (Seikagaku, Japan) in the presence of 50 mM dithiothreitol for 80 min at 37°C.

α-Amylase assay

The α-amylase assay was performed in triplicate in 4.2 ml of distilled water containing 0.1% SDS, 10 mM sodium azide and one Phadebas tablet (Phadebas α-Amylase Test, Pharmacia Diagnostics, Sweden; Ceska *et al.*, 1969). After incubation at 37°C for the indicated times the reaction was stopped by adding 1.0 ml of 0.5 M NaOH. After centrifugation for 5 min at 5000 r.p.m. the absorbance of the supernatant was read at 620 nm against a blank (A_{620} maximally 0.050) missing only α-amylase (*Bacillus*; Sigma, USA) with a Shimadzu UV120-02 spectrophotometer. Spheroplasts were first washed twice with cold PBS-sorbitol (phosphate buffered saline, pH 7.2, containing 1.2 M sorbitol), then digested in PBS-sorbitol with 0.5 mg/ml of proteinase K (Sigma, USA) for 30 min at 0°C, washed again twice with cold PBS-sorbitol and then lyzed in the above buffer for α-amylase assay. 1.2 M sorbitol was included in the media for treatment of spheroplasts. Cells were treated after the proteinase K digestion and the last washes with zymolyase, as described above, in 0.5 ml of buffer. Then, 3.7 ml of distilled water, SDS, sodium azide and the Phadebas tablet were added as above for the α-amylase assay. In this way complete lysis of the cells was obtained. Presence of the zymolyase digestion components did not interfere with the α-amylase asay.

Uptake of FITC-dextran

After incubation of cells with fluorescein isothiocyanate-conjugated dextran (FITC-dextran 70S, Sigma, USA), samples were diluted in 1 ml of cold PBS, washed three times with 1 ml of cold PBS, resuspended into a small volume of mounting medium (see accompanying paper) and applied to microscopic slides. The slides were pre-treated with 0.5 mg/ml of concanavalin A (Sigma, USA) to immobilize the living cells. For observation and photography, a Polyvar microscope (Reichert-Jung, Austria) equipped with a 100x oil immersion objective, filters for fluorescein isothiocyanate fluorescence and Nomarski optics was used. Agfapan 400ASA film (Agfa-Gevaert A.G., FRG) was used. Exposure times for fluorescence and Nomarski photographs were 45 s and 2 s, respectively.

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