ground (measured in the presence of $6 \times 10^{-7} M$ unlabeled IL-1α) was 921 ± 60 molecules per cell (100% inhibition). On the EL4 6.1 C10 cells maximal binding was $1.33 \pm 0.02 \times 10^4$ molecules per cell and background (see above) was 47 ± 2 molecules per cell.
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8 March 1988; accepted 10 June 1988

Intervacuole Exchange in the Yeast Zygote: A New Pathway in Organelle Communication

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A new pathway of vesicle traffic between organelles has been identified. The vacuoles (lysosomes) of *Saccharomyces cerevisiae* zygotes rapidly exchange their contents at a specific point in the cell cycle. With the use of fluorescence microscopy, "tracks" were observed that connect the original parental vacuoles to the newly forming bud vacuoles. These observations suggest that vacuole-derived vesicles rapidly move along the tracks in both directions, equilibrating vacuole contents. This rapid vesicle movement may be responsible for vacuole formation in newly developing cells.

CELL DIVISION REQUIRES THAT ALL cellular constituents be replicated and segregated faithfully. Whereas mitosis ensures the equal partitioning of chromosomes between two daughter cells, little is known about how other cellular components are segregated. Previous observations of yeast vacuoles strongly suggest that their distribution between mother and daughter cells does not occur by a random process (*1*). *Saccharomyces cerevisiae* grows by an asymmetric process of budding. The bud receives a small vacuole when it is approximately 1% of the mother cell volume (*1*). Although obvious growth is seen only in the bud vacuole and there is no synchronous fragmentation of the maternal vacuole, about 50% of the bud's vacuole contents are derived directly from the mother cell vacuole (*1, 2*). To account for this pattern of inheritance, we proposed that vacuole formation entails continual vesicular traffic between the mother and bud vacuoles. We now report that parental vacuoles within a zygote mix their contents without fusing, presumably by a vesicle-mediated process. This "communication" between parental vacuoles may be related to the process of vacuole formation.

The two parental vacuoles in a zygote can be distinguished by mating an *ade2* strain with an *ADE* strain. Vacuoles of *ade2* yeast accumulate a fluorescent dye when the cells are deprived of adenine. Synthesis of the dye is suppressed by adenine, and the previously accumulated dye is stable (*1, 3*). When yeast zygotes first form, the endogenous *ade2* fluorophore remains with the original parental vacuole (Fig. 1, a and b). The fluorophore appears in the *ADE* parental vacuoles sometime after bud formation (Fig. 1, c to f). Often vesicles can be seen along a "path" from each major vacuole into the bud (Fig. 1c). Although the vacuole contents mix, the original parental vacuoles never fuse (Fig. 1, e and f).

The transfer of vacuole contents occurs at a specific point in zygote development. Zygotes were scored both for bud size and for dye transfer (Fig. 2). At 2.9 hours after mating, most of the zygotes had no bud or a small bud. None of the zygotes scored had dye present in the *ADE* parental vacuoles. In contrast, many of the zygotes scored at time points ranging from 3.4 to 4.8 hours after mating had mixed the contents of the parental vacuoles. In all of the zygotes with no bud and most of the zygotes with a small bud, the *ade2* fluorophore appeared exclusively in the *ade2* parental vacuole. In contrast, both parental vacuoles are labeled in almost all of the large-budded zygotes. In zygotes with medium-sized buds, about 50% have label in both parental vacuoles. From these observations we conclude that transfer of fluorescent dye to the unmarked parental vacuole generally occurs when the bud is medium-sized. To more precisely determine the size of the bud at transfer, we observed and photographed individual zygotes until fluorescence appeared in the *ADE* parental vacuole. During observations of nine such zygotes, we measured the size of the bud at the time that transfer occurred. The volume of the bud at transfer was 9 ± 3% (SD) of the zygote (two fused parental cells plus the bud). Buds eventually grow to a maximum volume of 31 ± 4% (SD) of the zygote. Thus, transfer occurs when the bud is about one-third of its final volume. In addition, transfer occurs in a

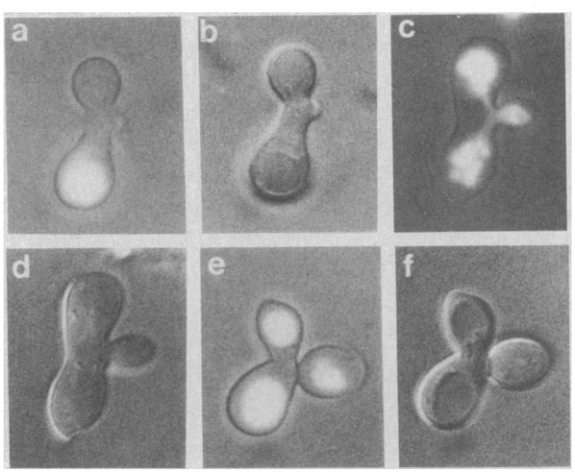

Fig. 1. Zygotes observed after mating *ade2* yeast with *ADE* yeast. The zygotes in this figure are oriented such that the two parental lobes are positioned on the vertical axis and the bud emerges on the horizontal axis. DBY1398 [*MATa ade2 ura3*] were grown in yeast extract–peptone dextrose (YEPD) (*21*) for 3 days until the vacuoles were highly fluorescent. One milliliter of culture was diluted into 25 ml of YEPD with adenine (160 μg/ml) to a final concentration of 2×10^7 cells per milliliter. The culture was aerated at 30°C for 2 hours, then mixed with an equal volume of a culture of X2180-1B and [*MATα*] (Yeast Genetics Stock Center) at the same cell concentration. Cells were incubated with slow shaking at 30°C. Under these conditions, zygotes were observed in 2.5 to 3 hours. Glass slides were treated with concanavalin A (0.5 mg/ml) before application of cells (*5*). Fluorescence of the cells was observed as described (*1*). In addition, the cells were illuminated with visible light so that the cell outlines could be seen. (**a**, **c**, and **e**) Fluorescence micrographs. (**b**, **d**, and **f**) The corresponding Nomarski photographs. (a) In zygotes with no bud, and most zygotes with a small bud, the endogenous *ade2* fluorescence is confined to the original *ade2* parental vacuole. (c) In approximately one-half of the zygotes with a medium-sized bud, fluorescence is seen in both parental vacuoles and in the bud. In addition, vesicles or fluorescent trails appear to connect these areas. (e) When the bud is large, most zygotes contain fluorescence in both parental vacuoles as well as in the bud and the fluorescent trails are no longer visible.

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Table 1. Number of vacuoles labeled with *ade2* and FITC fluorophores; fluorescence distribution in zygotes is shown.

Fluorescence in bud	*ade2*		FITC		Total number
	Bud label	Trail	Bud label	Trail	
None	0	0	0	0	22
FITC only	0	0	14	14	14
ade2 only	14	14	0	0	14
FITC and *ade2*	15	15	15	15	15

narrow range of bud size.

To determine whether there is a vacuole in the bud before transfer of *ade2* fluorophore and whether transfer is influenced by the *ade2* mutation, we devised a double-label technique. Yeast vacuoles can be labeled by exposure of cells to commercial preparations of fluorescein isothiocyanate (FITC)–dextran (*4*, *5*). Because the FITC fluorescence is green and the *ade2* fluorescence is yellow, it is easy to distinguish vacuoles with the FITC fluorophore alone from those with both fluorophores. Zygotes containing one *ade2* parent were labeled by incubation with FITC-dextran. The fluorescence distribution was quantified in zygotes with small to medium buds (2 to 15% of the volume of the zygote) (Table 1). Many buds had no detectable vacuole. Buds were seen with either FITC fluorescence alone or *ade2* fluorescence alone with equal frequency, indicating that the *ade2* fluorophore does not affect the transfer process. In all examples, a fluorescent trail extended from the bud vacuole to the parent vacuole with the same fluorophore, and no such trail was seen to the other parental vacuole. The subsequent transfer of *ade2* fluorescence to those bud vacuoles that had first received FITC was rapidly followed by further transfer to the other parental vacuole, presumably by using the already established pathway. Those buds that had received fluorescence from both parental vacuoles had trails extending to both vacuoles. In large buds, both fluorophores had transferred to the bud, but the trails were no longer visible. The simplest interpretation of these data is that bud vacuoles are initiated by a vesicular traffic, which can initially emanate from either parental vacuole.

Two examples of transfer of the *ade2* dye are shown in Fig. 3. In the first example (Fig. 3, a to d), fluorescence appeared first in the bud (Fig. 3c) and then also in the *ADE* parent (Fig. 3d). Once the fluorescence appeared in the bud vacuole, less than 30 s elapsed before substantial fluorescence had transferred into the *ADE* parental vacuole. In both examples of transfer, the rapid completion of fluorophore transfer to the *ADE* parental vacuole suggests that the bud had already obtained a vacuole and established a nonfluorescent trail to the *ADE* parent. In the second example (Fig. 3, e to h), transfer again occurred in less than 30 s (Fig. 3, f and g). Here, the fluorescence is not observed first in the bud, presumably because that intermediate state occurred at some point within the 30 s that elapsed between photographs. Transfer between vacuoles was never observed during the early stages of zygote development. In addition, the transfer can take place as long as 30 min after the bud's appearance, and it occurs within a few minutes. We never observed direct transfer from the *ade2* parental vacuole to the *ADE* vacuole in the absence of transfer to the bud.

Several lines of evidence argue against the transfer of the *ade2* dye occurring because of dye leakage or new dye synthesis after cytoplasmic mixing. First, we have demonstrated that the *ade2* fluorophore is not a weak base concentrated in the vacuole that would be released if the *p*H of the vacuole fluctuates (*1*). Second, the *ade2* fluorophore is most likely identical or very similar to the characteristic *ade2* red pigments, which are soluble polymers of 1 to 10 kD (*3*) and are presumably too large and polar to diffuse out of the vacuole. Third, before mating, the *ade2* yeast are grown for 1 to 3 hours under conditions in which no new fluorophore is produced. Fourth, the rapid appearance of dye in the originally unmarked vacuole can occur as long as 30 min after appearance of the bud. This argues against new dye formation being responsible for the transfer.

The transfer event does not occur in zygotes exposed to metabolic poisons. Yeast were mated for 3.6 hours, then divided into two portions, a control culture and a culture that was treated with 10 m*M* NaF and 10 m*M* NaN_3. The control culture showed 29% transfer of the *ade2* dye during the first 15 min and 78% transfer of the dye after 1.3 hours. In contrast, the poisoned culture only showed 24% transfer after 2 hours. This requirement of metabolic energy for dye transfer suggests that the process is not simple diffusion.

We are interested in determining which macromolecules are involved in exchange of

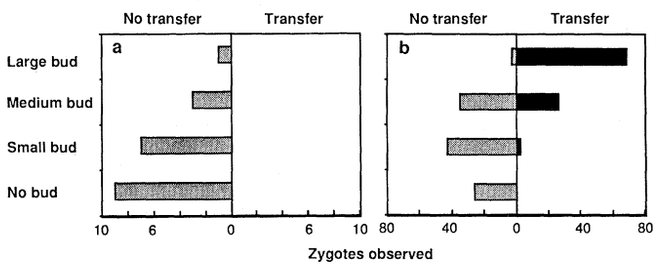

Fig. 2. Vacuole transfer occurs when the zygote contains a medium-sized bud. Yeast cells were mated as described in the legend to Fig. 1. At the times indicated, slides were prepared and all the zygotes in random fields of yeast were scored. For each zygote, the bud size and the location of the fluorophore were scored. Buds comprising less than 5% of the volume of the zygote were scored as small, 5 to 15% were medium, and more than 15% were large. (**a**) Zygotes scored 2.9 hours after mating. (**b**) Zygotes scored 3.4 to 4.8 hours after mating. All the zygotes included here were from a single mating.

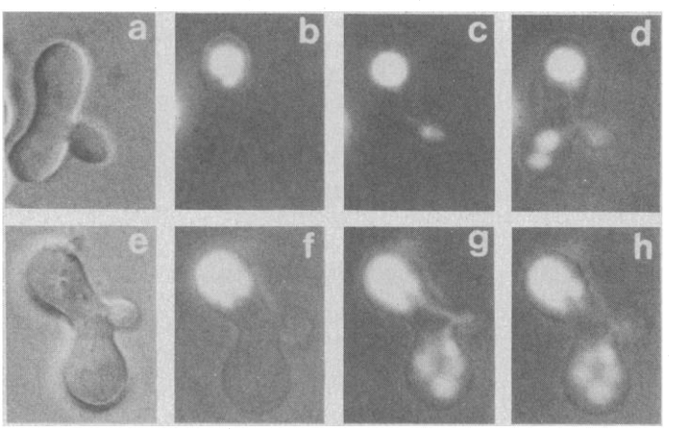

Fig. 3. Two examples of zygotes observed during dye transfer. Cells were mated as described in Fig. 1. Zygotes were photographed every 9 to 200 s. (**a** to **d**) The first example; (**e** to **h**), the second example. The Nomarski photographs are (a) and (e). In the first example, (b) was photographed 90 s before (c), and (c) was photographed 30 s before (d). In the second example, (f) was photographed 30 s before (g), and (g) was photographed 9 s before (h).

vacuole contents after mating, since some of these molecules may be involved in vacuole movement during cell division. We have tested the following mutants for a defect in vacuole mixing in zygotes: *kar1*-1, which is defective in nuclear fusion (*6*), a *pep4* strain, defective in some vacuole proteases (*7*), two *tub2* strains, defective in β-tubulin (*8*), and two *act1* strains, defective in actin (*9*). The matings were performed with the defect present in both mating types. In all cases, dye transfer was normal at both the permissive and, where applicable, restrictive temperatures. Since these mutations may not completely abolish protein function, our studies do not rule out important roles for these proteins in vacuole exchange.

The observation that parental vacuoles never fuse within the zygote, yet mix their contents, contrasts with the behavior of other yeast organelles. During mating, the two parental nuclei fuse, mitosis occurs, and the nucleus divides. One nucleus remains with the zygote and the other nucleus is directed into the bud (*10*). Fusion of the parental mitochondria is rare, and uniparental contribution of mitochondria to the bud often occurs (*11–14*).

It seems likely that intervacuolar dye transfer is mediated by vesicular traffic. In mammalian cells, vesicle-mediated traffic occurs both in endocytosis (*15, 16*) and in biosynthetic transport (*17*). Vesicle traffic between homologous organelles has been well documented in mammalian Golgi (*18*). The Golgi do not fuse during inter-Golgi traffic in vitro. Instead, proteins are moved in a vesicle-mediated process (*19*). It has recently been reported that mammalian lysosomes equilibrate their contents after syncytia formation (*20*). The mechanism by which this occurs is as yet unknown. Possibly, the equilibration observed in mammalian lysosomes is analogous to yeast vacuole behavior during mating.

The observations of intervacuole and interlysosome exchange define a new pathway in interorganelle traffic. Further studies of intervacuole exchange are needed to determine why the transfer is rapid and why it does not happen immediately after cytoplasmic mixing. In addition, our observations demonstrate that vacuole contents can be transferred both from parental vacuoles into the bud and from the bud to the parental vacuoles. Perhaps the transfer is triggered by a cytoskeletal rearrangement. Observation of the trails connecting each parental vacuole to the bud suggests that the bud vacuole may form by directed vesicular traffic from the parental vacuoles to a specific vacuole site in the bud. This model is further supported by the observation that the *ade2* dye appears first in the bud and then in the *ADE* parental vacuole. The vesicle movement between the parental and bud vacuoles may continue in both directions until cytokinesis occurs (or until vacuole formation is complete). Thus, the phenomenon observed in yeast zygotes may provide insight into both vacuole and lysosome division and segregation.

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22. We thank B. Guthrie, M. Grunstein, L. Hicke, and G. Payne for helpful discussions, and D. Botstein for providing DBY1398 [*MATa ade2 ura3*]. Supported by a grant from the National Institutes of Health. L.S.W. was supported by an NIH National Research Service award.

3 March 1988; accepted 2 June 1988

Low Plasma Cholesterol Levels Caused by a Short Deletion in the Apolipoprotein B Gene

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Familial hypobetalipoproteinemia is a syndrome in which the plasma levels of apolipoprotein B (apo-B) and cholesterol are abnormally low. A truncated species of apo-B was identified in the plasma lipoproteins of members of a kindred with familial hypobetalipoproteinemia. DNA sequencing studies on genomic clones and enzymatically amplified genomic DNA samples revealed a four–base pair deletion in the apo-B gene. This short deletion, which results in a frameshift and a premature stop codon, accounts for the truncated apo-B species and explains the low apo-B and low cholesterol levels in this family.

FAMILIAL HYPOBETALIPOPROTEINEmia is a condition in which the concentrations of apolipoprotein B (apo-B) and low density lipoprotein (LDL)–cholesterol in the plasma are abnormally low (*1*). In the homozygous form, apo-B and LDL-cholesterol are either absent from the plasma or present in extremely low concentrations. Homozygotes may have multiple medical problems, including fat malabsorption and neurological disorders. Heterozygotes have apo-B and LDL-cholesterol levels approximately half those of normal; these individuals are usually asymptomatic and may actually be protected from premature atherosclerotic disease (*1*).

In 1979, Steinberg and co-workers described a unique kindred with familial hypobetalipoproteinemia (*2*). Recently, our laboratory identified two different apo-B alleles associated with hypobetalipoproteinemia in that kindred (*3*). One apo-B allele yields a truncated apo-B species, apo-B37, whereas the other allele is associated with low plasma levels of the normal apo-B species, apo-B100. Three individuals had both abnormal alleles and were therefore compound heterozygotes for hypobetalipoproteinemia. There were six heterozygotes with apo-B37, and ten with low levels of apo-B100. The mean LDL-cholesterol level in the latter two groups was 31 mg/dl, less than half of the normal value, whereas it was 6 mg/dl in the compound heterozygotes (*3*).

Because apo-B37 is associated with low

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