Molecular Biology of the Cell
Vol. 4, 21–36, January 1993

Patterns of Mitochondrial Sorting in Yeast Zygotes

Ricardo Azpiroz and Ronald A. Butow

Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Submitted June 12, 1992; Accepted November 2, 1992

Inheritance of mitochondrial DNA (mtDNA) in *Saccharomyces cerevisiae* is usually biparental. Pedigree studies of zygotic first buds indicate limited mixing of wild-type (ρ^+) parental mtDNAs: end buds are frequently homoplasmic for one parental mtDNA, while heteroplasmic and recombinant progeny usually arise from medial buds. In crosses involving certain petites, however, mitochondrial inheritance can be uniparental. In this study we show that mitochondrial sorting can be influenced by the parental mtDNAs and have identified intermediates in the process. In crosses where mtDNA mixing is limited and one parent is prelabeled with the matrix enzyme citrate synthase 1 (CS1), the protein freely equilibrates throughout the zygote before the first bud has matured. Furthermore, if one parent is ρ^0 (lacking mtDNA), mtDNA from the ρ^+ parent can also equilibrate; intracellular movement of mtDNA is unhindered in this case. Surprisingly, in zygotes from a ρ^0 CS1$^+$ $\times$ ρ^+ CS1$^-$ cross, CS1 is quantitatively translocated to the ρ^+ end of the zygote before mtDNA movement; subsequently, both components equilibrate throughout the cell. This initial vectorial transfer does not require respiratory function in the ρ^+ parent, although it does not occur if that parent is ρ^-. Mouse dihydrofolate reductase (DHFR) present in the mitochondrial matrix can also be vectorially translocated, indicating that the process is general. Our data suggest that in zygotes mtDNA movement may be separately controlled from the movement of bulk matrix constituents.

INTRODUCTION

The faithful segregation of organelles is an essential feature of cell division. Although much is known about the process of nuclear division and inheritance, our knowledge of the mechanism and control of segregation of other organelles is minimal. In virtually all eukaryotes mitochondria and mitochondrial DNA (mtDNA) must be partitioned during cell division to maintain the respiratory competence of the progeny. The rules that govern this process are largely unknown.

The yeast *Saccharomyces cerevisiae* has proved to be useful for studies on mitochondrial inheritance because these cells can be propagated without functional mitochondria. In vegetatively growing yeast cells, mitochondria and mtDNA are apportioned to the emerging bud even before nuclear division has occurred (Stevens, 1981). Over the years, a number of nuclear mutations have been described that lead to the loss or destabilization of mtDNA (Weislogel and Butow, 1970; Lisowsky and Michaelis, 1988; Foury, 1989; Lundin *et al.*, 1991), but a comprehensive picture of mitochondrial transmission in vegetatively growing cells has yet to emerge. Also, a number of mutants have been described that are defective in segregation of mitochondria and grouped into a set of genes designated *MDM*, (mitochondrial distribution and morphology) (McConnell *et al.*, 1990). One of these, *MDM2*, is the *OLE1* gene encoding Δ9-fatty acid desaturase (Stewart and Yaffe, 1991), thus implicating a role for unsaturated fatty acids in mitochondrial movement in yeast. Two other genes have recently been identified that appear to affect the maintenance or segregation of mtDNA; the product of one of these genes is related to the dynamin family of microtubule binding proteins (Jones and Fangman, 1992) and the other encodes the HMG1-like protein, ABF2 (Diffley and Stillman, 1991).

Yeast also provides a very tractable system for mitochondrial genetic analysis in crosses, because its mitochondrial genome is highly recombinogenic (Thomas and Wilkie, 1968). In matings, the inheritance of mtDNA is usually biparental, the exceptions being crosses between cells containing wild-type mtDNA (ρ^+) and those that either lack mtDNA (ρ^0) or that are included among a subclass of mitochondrial mutants harboring large deletions of their mtDNA (ρ^- petite) (Dujon, 1981). In the first case, the progeny of the cross is vir-

tually 100% ρ^+; that is, ρ^0 cells are rarely found among the progeny. This could be due to as few as one mtDNA molecule segregating into a zygotic bud, followed by its amplification. In the second case, conversely, the progeny of the cross are virtually 100% ρ^-, and their mtDNA is indistinguishable from the petite parent. This subclass of ρ^- cells, known as hypersuppressive petites (Dujon, 1981), are thought to exert their effect by outcompeting the ρ^+ genome in the zygote for some factor(s) essential for mtDNA transmission. Although the mitochondrial genomes of hypersuppressive petites have in common repeat units containing putative origins of mtDNA replication (Blanc and Dujon, 1980; de Zamaroczy *et al.*, 1981), it has not been convincingly established whether the effect of hypersuppressivity is mediated exclusively as a replicative or segregational advantage of the petite genome over ρ^+. A recessive mutation of a nuclear gene, *MGT1*, has been identified that largely eliminates hypersuppressivity in crosses (Zweifel and Fangman, 1991), but the molecular basis for the phenomenon has yet to be clarified.

A second feature of mtDNA inheritance in yeast crosses is that segregation of mixed genomes occurs very rapidly. Yeast cells contain between 50 and 100 mtDNA molecules; random segregation of individual molecules would predict that more than 1000 generations are necessary for complete segregation from a zygote (Michaelis, 1967). However, mtDNA segregation is usually complete in less than 20 generations and, in individual cells, is often accomplished in a single cell division. This rapid segregation is most readily explained only if the mitochondrial segregating unit is not the individual mtDNA molecule but, rather, a subset of those molecules (Dujon *et al.*, 1974; Birky *et al.*, 1978). The mechanistic relationship between mtDNA molecules and segregating units, however, is not known at present.

Another feature of mitochondrial inheritance in yeast is that mtDNA segregation is not random, and the site of first bud formation can affect the segregation pattern of individual zygotic clones. The genetic composition of a first bud and its mother zygote can be determined by pedigree analysis. By this means Strausberg and Perlman (1978) observed in $\rho^+ \times \rho^+$ crosses that if the first bud emerges from one end of the zygote it is often pure for the mitochondrial genotype contributed by the parent cell that gave rise to that end of the zygote. Furthermore, the remaining zygotic clone is often pure for the other parental genotype, indicating that the bud depleted the mother zygote of one parental genotype; only in those cases where the first bud arises from the neck of the zygote do both bud and zygote almost always inherit both parental alleles, often in recombinant configurations (Birky *et al.*, 1978; Strausberg and Perlman, 1978; Zinn *et al.*, 1987). The conclusion from these observations is that mixing of the parental mtDNAs in $\rho^+ \times \rho^+$ crosses is limited in yeast zygotes and is confined to a region of the zygote neck, where the parent cells have fused. Operationally, then, this region of the cell has been referred to as the zone of mixing (Zinn *et al.*, 1987).

In this study, we have followed the distribution of mitochondria and mtDNA in various crosses by immunofluorescence techniques to determine whether mtDNA mixing is related to the behavior of other components of mitochondria and to detect possible intermediates in the mixing process. We find that mixing of mitochondrial components is dependent, in part, on the mitochondrial genotypes of the parent cells. Some implications of these findings in mitochondrial inheritance are discussed.

MATERIALS AND METHODS

Growth Media

Cells were grown in 1% yeast extract, 1% bacto-peptone with 2% dextrose (YPD) or galactose (YPG). Diploids were selected on 0.67% yeast nitrogen base without amino acids and 2% dextrose (YNBD). Yeast transformants were selected on YNBD plus 0.5% casaminoacids and supplements as required. For genetic analyses, ρ^+ cells were selected on regular glycerol (RG) medium, containing 0.2% yeast extract, 0.2% bacto-peptone, 0.05% NaCl, 0.1% $(NH_4)_2SO_4$, 0.05% $MgCl_2$, 0.05% KH_2PO_4, pH 6.5, and 3.2% glycerol. Mitochondrial drug resistance markers were scored by replica plating on RG containing chloramphenicol at 3 mg/ml or oligomycin at 3 μg/ml. Solid media contained 2% agar. Spent medium was obtained from overnight cultures in YPD of high-efficiency mating MAT**a** and MATα strains grown separately, mixed at equal volumes, and pelleted. The supernatant was filter-sterilized and dextrose was added to 2%.

Strains and Plasmids

BWG 1-7A (MAT**a** *ade1 his4 ura3 leu2*) and BWG1-7A-L (MAT**a** *ade1 his4 ura3 leu2 CIT1::LEU2*) (Kim *et al.*, 1986; Rosenkrantz *et al.*, 1986) were kindly provided by Paul Srere (VA Medical Center, Dallas). BWG 1-7A ρ^0 and BLρ^0 were generated by ethidium bromide mutagenesis (Slonimski *et al.*, 1968). Derivatives of the above strains with different mitochondrial genotypes, all derived from COP 161 mtDNA (ρ^+ *cap*^{r} *oli1*^{r} ω^-), were obtained by cytoduction (Conde and Fink, 1976). They are BL ρ^+ (*cap*^{r} *oli1*^{4} ω^+), BL PZ27 (mit$^-$, *cob* deletion) (Perlman *et al.*, 1980), and BL F11 (ρ^-, 12 kb repeat containing the 21S rRNA gene) (Sor and Fukuhara, 1983). WB 102 (MATα *trp1 ura3 leu2 CIT1::LEU2*) is a spore from a cross between BWG1-7A-L and W303-1b (MATα *ade2 his3 ura3 trp1 leu2*). Its derivatives are WB ρ^+ (*cap*^{s} *oli1*^{s} ω^+), cytoducted from D 273-10B/A2 (MATα *met* [ρ^+ *cap*^{s} *oli1*^{s} ω^+]) and WB ρ^0. pFCS1 contains the entire *CIT1* gene in a 2-μ-based plasmid (Suissa *et al.*, 1984). To create pGCS1, a *Sma* I-*Afl* II fragment from pFCS1 was replaced by a fragment containing the GAL1-10 UAS (West *et al.*, 1984), placing the start codon of the *CIT1* coding sequence ~180 bp downstream of the *GAL1* transcriptional start site. pGAL-OTC-DHFR, supplied by A. Horwich, contains a chimeric gene (OTC-DHFR) encoding mouse dihydrofolate reductase (DHFR)[1] with the mitochondrial targeting peptide from human ornithine transcarbamoylase at its amino terminus, under GAL 1-10 control (Horwich *et al.*, 1985). Plasmids were introduced into yeast by the lithium acetate method (Ito *et al.*, 1983; Baker, 1991).

Antibodies

Rabbit anti-yeast CS1 was a gift from Paul Srere. Rabbit anti-DHFR was obtained from Gottfried Schatz (Brozentrum, Basel). Fluorescein

[1] Abbreviations used: DAPI, 4',6-diamidino-2-phenylindole; DHFR, dihydrofolate reductase; FITC, Fluorescein isothiocyanate.

isothiocyanate (FITC) labeled anti-rabbit IgG was purchased from Jackson Immunoresearch (West Grove, PA). Sheep anti-mouse Ig-rhodamine was obtained from Boehringer (Indianapolis, IN). Anti-chicken brain tubulin antibody was obtained from Sigma (St. Louis, MO). Antibody dilutions were preadsorbed to nitrocellulose filters saturated with yeast protein extract prepared from cells lacking CS1. Anti-DHFR was affinity purified from nitrocellulose-bound bovine enzyme (Sigma), as described by Olmsted (1986).

Synchronized Mating

The method is a modification of the procedure of Rogers and Bussey (1978). Cells were grown in YPD or YPG (to induce expression of CS1 or OTC-DHFR) overnight. Equal optical density (OD_{600} units of exponentially growing cultures were mixed, pelleted, and resuspended at 2.4 OD_{600} units/ml in spent media adjusted to pH 3.5 with citric acid. The mixture was incubated on a roller drum at 30°C for 2 h to induce mating factor arrest. No zygotes were formed at this stage. The cells were then diluted 50-fold in water, sonicated briefly to disrupt clumps, and concentrated on 25 mm Millipore HA filters (effective area = 2 cm^2) at 2.4 OD_{600} units per filter. The filters were placed cell side up on YPD plates adjusted to pH 4.5 with citric acid and incubated at 30°C. It is only after transfer to these plates (time = 0) that zygotes begin to form.

Pedigree Analysis

Synchronized mating was performed between BL ρ^+ and WB ρ^+, both transformed with pFCS1. After 1.5 h of incubation of the filters at 30°C zygotes typically constituted 5% or less of the total cell population, with <10% of them containing one bud. No zygotes with more than one bud were observed. At this time, cells were resuspended in 25 mM EDTA to disrupt aggregates and were streaked on solid YPD. Zygotes displaying an end bud were separated from the remaining cells by micromanipulation and incubated at room temperature until the buds matured, typically 1.5 h. Buds were separated from mother zygotes and the plate was incubated at 30°C until visible colonies had formed. These cells were resuspended in water and spread on YNBD plates. The resulting colonies were replica-plated onto RG, RG plus oligomycin, and RG plus chloramphenicol and scored for growth.

Indirect Immunofluorescence Microscopy

The procedure is based on that described by Kilmartin and Adams (1984) and Adams and Pringle (1984). Cells from mating mixtures were resuspended directly from filters into P buffer (40 mM KH_2PO_4, pH 6.5, and 0.5 mM $MgCl_2$) containing 4% formaldehyde and fixed for 1 h at 30°C. After three washes in P buffer and one in PS (P buffer plus 1.2 M sorbitol) the cells were gently spheroplasted with 2 mg/ml zymolyase 20T (ICN Immunobiologicals, Costa Mesa, CA) in PS at 30°C. Spheroplasts were washed three times in PS and placed on poly-L-lysine-coated multiwell slides. All further steps were carried out at room temperature. After adhesion the samples were incubated in 0.2% NP-40 in PS for 15 min to permeabilize mitochondria and then were washed extensively in PS. After a 15 min incubation in phosphate-buffered saline/bovine serum albumin (PBS/BSA) (PBS is 0.8% NaCl, 0.02% KCl, 0.114% Na_2HPO_4, 0.02% KH_2PO_4, pH 7.3; PBS/BSA is the same plus 0.1% BSA) primary antibodies diluted in PBS/BSA were applied and incubated for 1.5 h. Samples were washed extensively with PBS/BSA and incubated a further 1.5 h in the secondary antibody dilution. After washing with PBS/BSA cellular DNA was stained by brief incubation in 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) in PBS followed by a wash in PBS. The samples were then air dried and mounted. Microscopy was performed with the use of a Zeiss Axioplan (Thornwood, NY) microscope equipped for epifluorescence by using filter sets G365/FT395/LP420 for DAPI, BP450-490/FT510/LP520 for FITC, and BP530-585/FT600/LP615 for rhodamine.

Scoring of Zygotes

In any sample from a mating mixture, and especially in samples from early time points, the most abundant cells are unmated haploids. Furthermore, because the procedure for immunofluorescence produces some distortion of cell borders, precautions must be taken to ensure accurate identification of zygotes. For this reason, the criteria used to score a zygote required the presence of the following features: 1) a smooth curve connecting the bodies of the parent cells, 2) the absence of a visible septum dividing the parents, 3) the presence of either a single nucleus or two nuclei in close proximity in the neck connecting the parent cells, and 4) clear staining for both fluorochromes, where the signals could be unambiguously distinguished from background. All such cells in a given field were scored. Each zygote was analyzed and scored for the location and extent of fluorescent signal and the degree of colocalization of CS1 or DHFR (FITC) and mtDNA (DAPI) fluorescence in all focal planes. For some experiments zygotes were also scored for the presence or absence of buds. In most cases, for each individual experiment, the minimum number of zygotes scored per time point was 80; for the 1-h samples the minimum was 40. Cells were photographed on Kodak T-Max p3200 film (Rochester, NY), which was developed at ISO 800. In many instances zygotes were further analyzed by tracing the outline of the cell and nearby landmarks (such as neighboring cells or debris) from phase contrast prints onto transparency sheets and, using the landmarks, transferring the tracing to the DAPI and FITC prints, marking the fluorescence profiles with a felt pen. The results obtained were invariably consistent over a number of repeat experiments; results presented here are the average of up to seven independent repetitions. Repeat scoring of the same sample indicates that our scoring error (cells scored as different types on different occasions) is <3%.

RESULTS

Experimental Strategy

Our experimental approach is based on the premise that new insights into the process of mitochondrial inheritance can be obtained by direct microscopic observation of intracellular sorting of mitochondria and mtDNA within the time-frame in which these components are initially contributed from haploid cells to a newly formed zygote and are subsequently apportioned to the emerging diploid bud. To this end, we developed an assay for the timing and the course of changes in the distribution of parent-specific mitochondrial labels in newly formed zygotes from synchronous matings involving cells of different mitochondrial genotypes. In addition to the mtDNA, two such labels were employed for this work: the mitochondrial matrix enzyme CS1 encoded by the *CIT1* gene (Suissa *et al.*, 1984) and mouse DHFR directed to the mitochondrial matrix by the targeting peptide from human ornithine transcarbamoylase (Cheng *et al.*, 1987).

An obvious requirement for this approach is to ensure that the marker proteins assayed are not synthesized de novo in the zygote itself, so that only protein preexisting in the labeled parent is detected. For this reason the parental haploid strains were disrupted in the *CIT1* gene and this protein was expressed under control of the GAL1-10 UAS (West *et al.*, 1984) in one or the other parent from a plasmid-borne gene (pGCS1); plasmid-borne DHFR (pGAL-OTC-DHFR) was expressed in a similar fashion. In control experiments, we have estab-

lished that the *CIT1* mRNA decreases to <3% of the galactose-induced level after 2 h following a shift to glucose-containing medium, well before zygotes are observed in the synchronous matings. Finally, because the experimental objective was to describe intracellular events as a function only of the mitochondrial genotypes of the parent cells, all strains were derived by cytoduction from a single MAT**a** and a single MATα strain, as described in MATERIALS AND METHODS.

The basic experimental design is as follows: parent strains are grown to early exponential phase in rich medium containing either glucose, for the nontransformed, unlabeled parent, or galactose to induce expression of CS1 or DHFR in the transformed parent strain. The cells are then transferred to glucose-containing medium to repress the synthesis of the marker protein and then subjected to a synchronized mating protocol. Samples are removed and fixed at 1-h intervals and, after processing for immunofluorescence, individual zygotes are scored during the time course of zygote maturation for the location of both the mtDNA and the protein marker as described in MATERIALS AND METHODS.

Limited Mixing of ρ^+ mtDNA in Zygotes

The full extent to which the phenomenon of limited mtDNA mixing in zygotes derived from mating between ρ^+ haploid cells (Birky *et al.*, 1978; Strausberg and Perlman, 1978; Zinn *et al.*, 1987) is strain dependent has not been studied systematically. Therefore, we first performed end-bud pedigree analysis in a cross between BL ρ^+ (*oli1*^{s} *cap*^{s}) and WB ρ^+ (*oli1*^{r} *cap*^{r}) to establish that our strains exhibit this phenomenon. As shown in Figure 1, of eight zygote-bud pairs analyzed, seven first buds (2–8) were pure for at least one marker, and five of them depleted the mother zygote of one allele at one (4) or both (2, 5, 7, and 8) loci. Five of the buds were pure for both markers: all of their progeny were either *oli1*^{r} *cap*^{r} (3, 4, 6, and 8) or *oli1*^{s} *cap*^{s} (7), indicating that the first end bud from these six zygotes received mtDNA from only one parent. Two of the buds (2 and 5) were pure for the *oli1* marker but showed some recombination at the *cap* locus (6.2 and 11.1%, respectively). The zygotes that gave rise to these buds produced progeny with either exclusively or predominantly the other parental allele. These results indicate that first end-buds are largely uniparental and frequently deplete the mother zygote of one parental allele. This is in agreement with the original observation by Strausberg and Perlman (1978), and therefore indicates that, when mated, our strains exhibit limited mixing of parental mtDNAs when both parents contain ρ^+ genomes.

Limited mtDNA Mixing in a $\rho^+ \times \rho^+$ Cross is not Coincident with Mixing of a Mitochondrial Matrix Marker

There are two alternative explanations for the phenomenon of nonrandom segregation of ρ^+ mtDNAs, which cannot be distinguished by pedigree analysis: on the one hand, there may be limited interaction between the parental mitochondria within the zygote; that is, neither mitochondria nor mtDNA mix extensively in the zygote. On the other hand, mixing of certain mitochondrial constituents may occur, as would be the case if the parental mitochondria fuse in the zygote, but the mtDNA is not among them.

To determine whether limited mixing of mtDNA also extends to components of the mitochondrial matrix we performed an experiment to see if mitochondrial CS1 is capable of mixing in zygotes from a $\rho^+ \times \rho^+$ cross in which only one of the parents has been prelabeled with the enzyme. Figures 2 and 3 show the results of a synchronized mating between WB ρ^+ CS1$^+$ and BL ρ^+ CS1$^-$

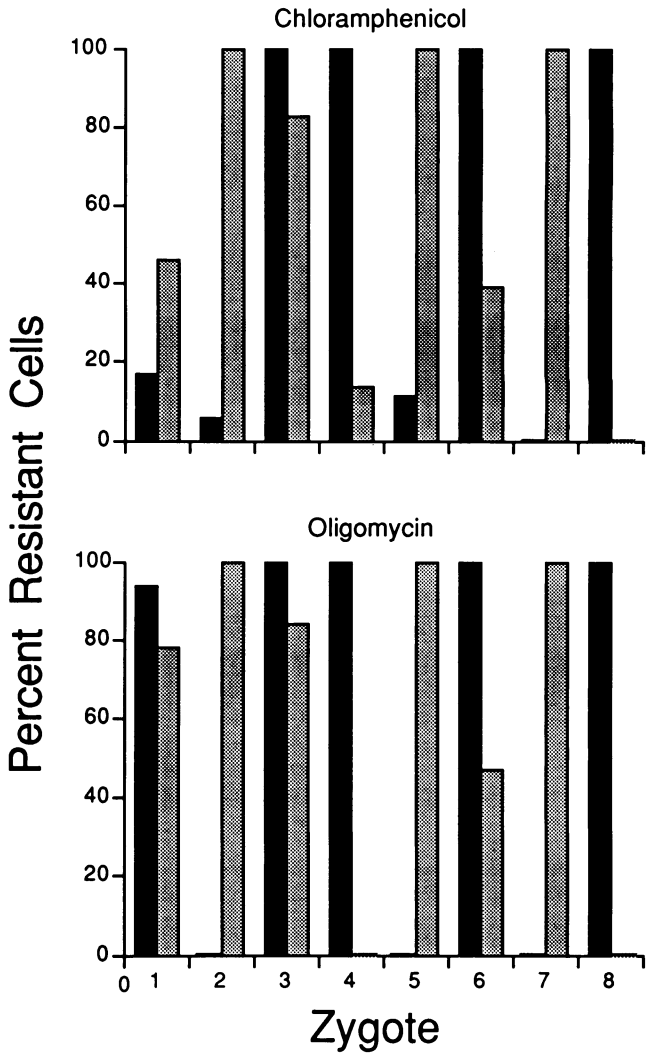

Figure 1. Pedigree analysis of 8 zygotes from a BL (ρ^+ *oli1*^{s} *cap*^{s}) $\times$ WB (ρ^+ *oli1*^{r} *cap*^{r}) cross. Shown is the percent of clones resistant to chloramphenicol (top) and oligomycin (bottom) derived from each first end bud (■) and its corresponding mother zygote (□).

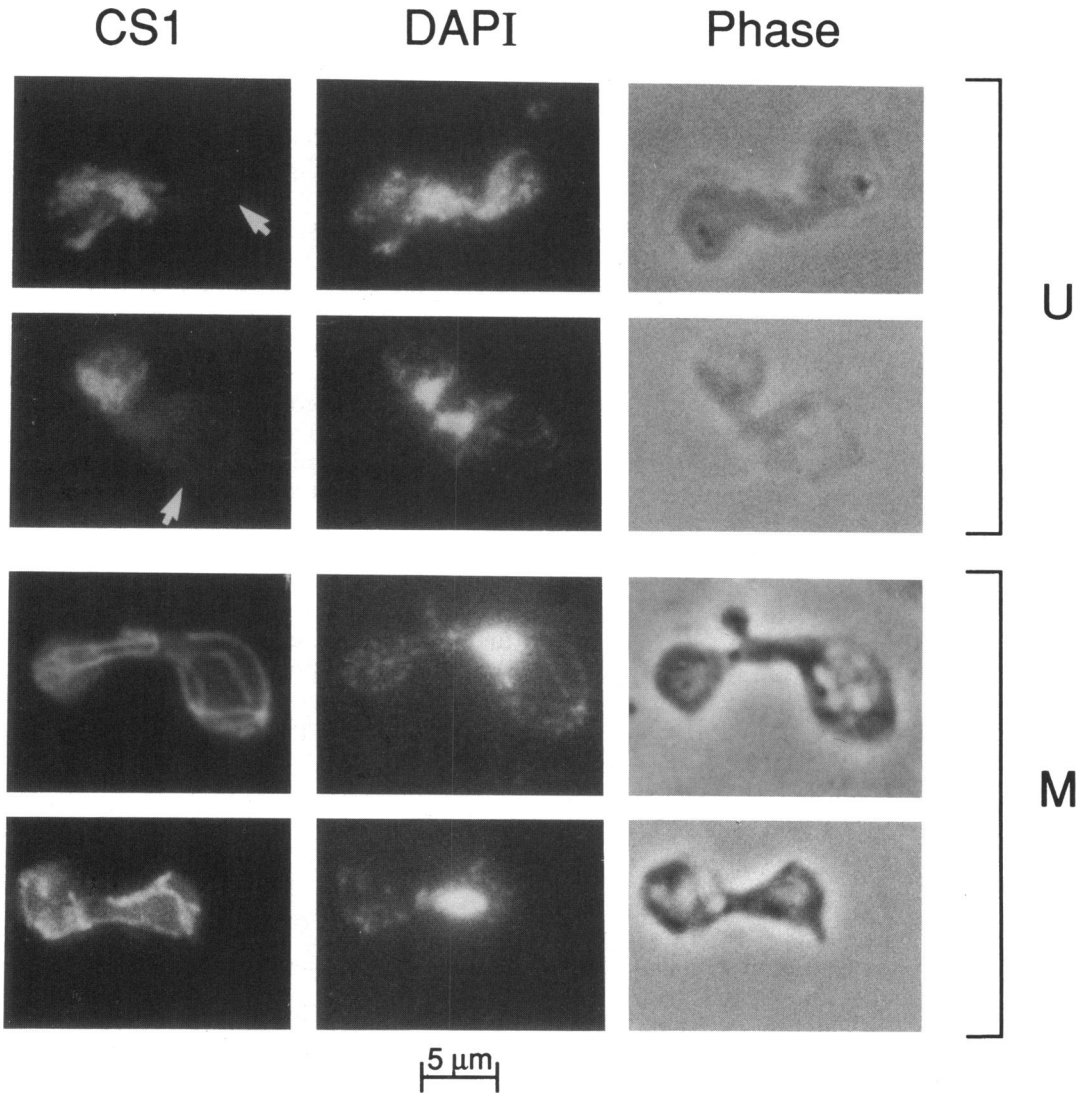

Figure 2. Zygotes from a ρ^+ CS1$^+$ × ρ^+ CS1$^-$ cross, showing fluorescence from citrate synthase 1 (CS1) and DAPI staining, as well as phase contrast images. Note the filamentous or punctuate appearance of CS1 and its colocalization with the punctate staining of mtDNA. Arrows point to the CS1$^-$ end of the zygote. U, unmixed; M, mixed.

cells. A schematic summary of all the types of zygotes observed in this and in subsequent experiments is shown in Figure 4.

As expected, mtDNA is detected throughout the cell in all zygotes of a $\rho^+ \times \rho^+$ cross (Figure 2). Also, zygotes are found with CS1 signal present only in one-half of the cell, that derived from the CS1$^+$ parent. Surprisingly, however, a second type of zygote is also observed in which mitochondria stained with CS1 are found throughout the cell, making it impossible to distinguish which end of the zygote is derived from either of the parent cells. Figure 3 shows that cells of the first type, which we term unmixed (type U), predominate only initially and, over time, are quantitatively replaced by zygotes of the second type. Because there is no de novo synthesis of CS1 during the course of the experiment, CS1 distribution throughout the zygote can only be the result of its equilibration following mating. This zygote form is termed mixed (type M). In all of the type M zygotes, the CS1 and mtDNA signals appear to colocalize to the same structures. The apparently complete equilibration of CS1 within the mitochondria of a $\rho^+ \times \rho^+$ zygote is in sharp contrast to the situation observed for the mtDNA: within 3 h of the start of zygote formation 90% of the zygotes display mixing of CS1, although it is at this time that first buds are removed for pedigree analysis (see MATERIALS AND METHODS), where we find that the parental mtDNAs are still largely unmixed (see above). Indeed, older zygotes with mature-sized buds invariably contain CS1 signal through-

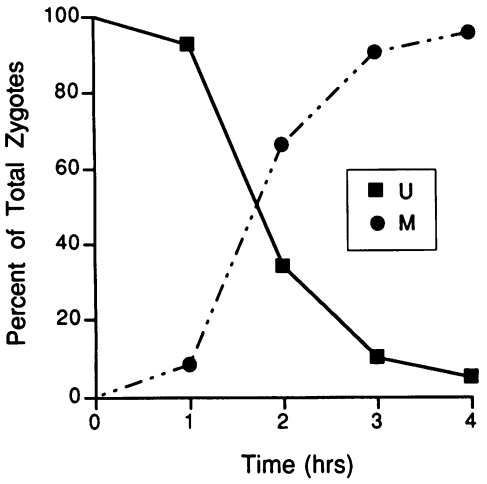

Figure 3. Time course of mixing for a ρ^+ CS1$^+$ × ρ^+ CS1$^-$ cross (same as Figure 2). Numbers depict the abundance, at each time point, of each zygote type as a percentage of all zygotes. U, mixed; M, mixed.

out the cell and also in the bud itself. This experiment suggests that both the rate and extent of mixing are uncoupled for different mitochondrial constituents, because in a $\rho^+ \times \rho^+$ cross, for which pedigree analysis indicates that the mtDNAs do not mix extensively, CS1 is able to equilibrate completely in the zygote and into the emerging diploid bud.

Unusual Zygote Forms in a $\rho^0 \times \rho^+$ Cross

ρ^0 CS1$^+$ × ρ^+ CS1$^-$. In light of the apparent complete equilibration of CS1 in a $\rho^+ \times \rho^+$ cross when one of the parent cells lacks this enzyme, the possibility arises of a similar redistribution of the mtDNA throughout the cell in a $\rho^0 \times \rho^+$ cross, because the progeny of such crosses is virtually 100% ρ^+ regardless of bud position. Further, because equilibration of CS1 appears to be uncoupled from mixing of mtDNA, as shown above, a cross between ρ^0 CS1$^+$ and ρ^+ CS1$^-$ cells would permit the measurement of the relative rates of mtDNA and CS1 movement from an initial situation in which these constituents are located in opposite ends of a zygote. Figures 5 and 6 show the results of such an experiment, where we followed the time-course of CS1 and mtDNA movements in a cross between BL ρ^0 CS1$^+$ × WB ρ^+ CS1$^-$. Several types of zygotes can be observed based on the intracellular location of both CS1 and mtDNA (Fig. 5). For this cross a newly formed, unmixed, zygote is predicted to contain mtDNA only in one half and CS1 only in the other half; as expected, these are the most abundant zygotes found initially, and they disappear from the population over time (Fig. 6). Also, the mixed zygotes increase in abundance after 1 h and become the predominant type shortly after 2 h, with a maximum of ~90% at 6 h. Unexpectedly, however, two additional types of zygotes are observed (Fig. 5): one of them (type P) contains CS1 in both halves of the cell but mtDNA in only one half. Most striking is the appearance of zygotes in which both the mtDNA and CS1 are located entirely in one half of the cell, with no significant signal for either component in the other half (type A). These cells, therefore, in which the mtDNA and CS1 were each initially found only in one-half of the zygote, appear to have undergone quantitative movement of one of the components from one side of the zygote to the other. Once this movement has occurred, giving rise to the type A zygotes, we always observe precise colocalization of CS1 and mtDNA.

The time course of this cross suggests that both type P and type A zygotes are intermediates in the mixing process (Fig. 6). This is suggested by the fact that the abundance of unmixed zygotes decreases continuously, and the only type to increase in abundance throughout mating is the mixed zygotes, indicating that the mixed state is the endpoint for all zygotes. Type P and type A cells, however, appear earlier than mixed zygotes and peak in abundance at 2 and 3 h, respectively, where they represent a significant proportion of all zygotes; subsequently, type P and A begin to disappear from the population as mixed zygotes continue to appear. Moreover, the appearance and subsequent disappear-

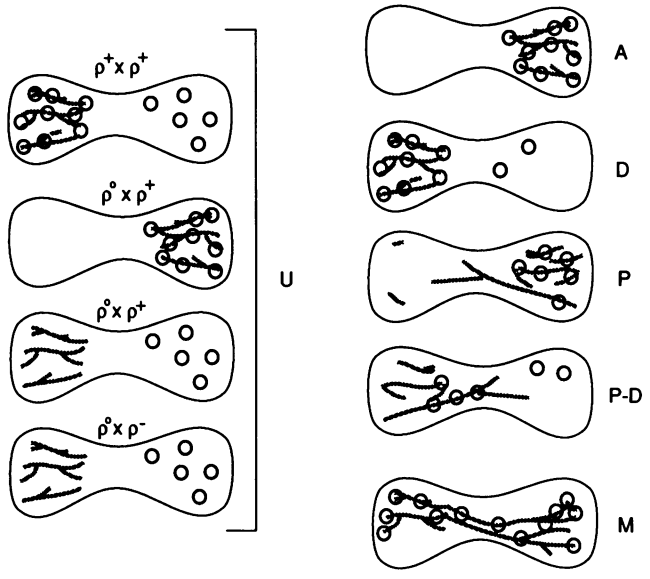

Figure 4. Schematic representation of the various zygote types. The narrowing in the middle represents the zygote neck connecting the 2 parent cells. Circles represent mtDNA and stippled lines represent mitochondrial structures containing citrate synthase. U, unmixed zygotes from the various crosses described in the text; from top: ρ^+ CS1$^+$ × ρ^+ CS1$^-$, ρ^0 CS1$^-$ × ρ^+ CS1$^+$, ρ^0 CS1$^+$ × ρ^+ CS1$^-$, and ρ^0 CS1$^+$ × ρ^- CS1$^-$. A, zygotes in which the distribution of components has become asymmetric (both components in the same side). D, zygotes in which a portion of the mtDNA does not colocalize with the protein label. P, zygotes in which a portion of the protein label does not colocalize with the mtDNA. P-D, zygotes in which both components colocalize partially, only near the neck. M, mixed zygotes.

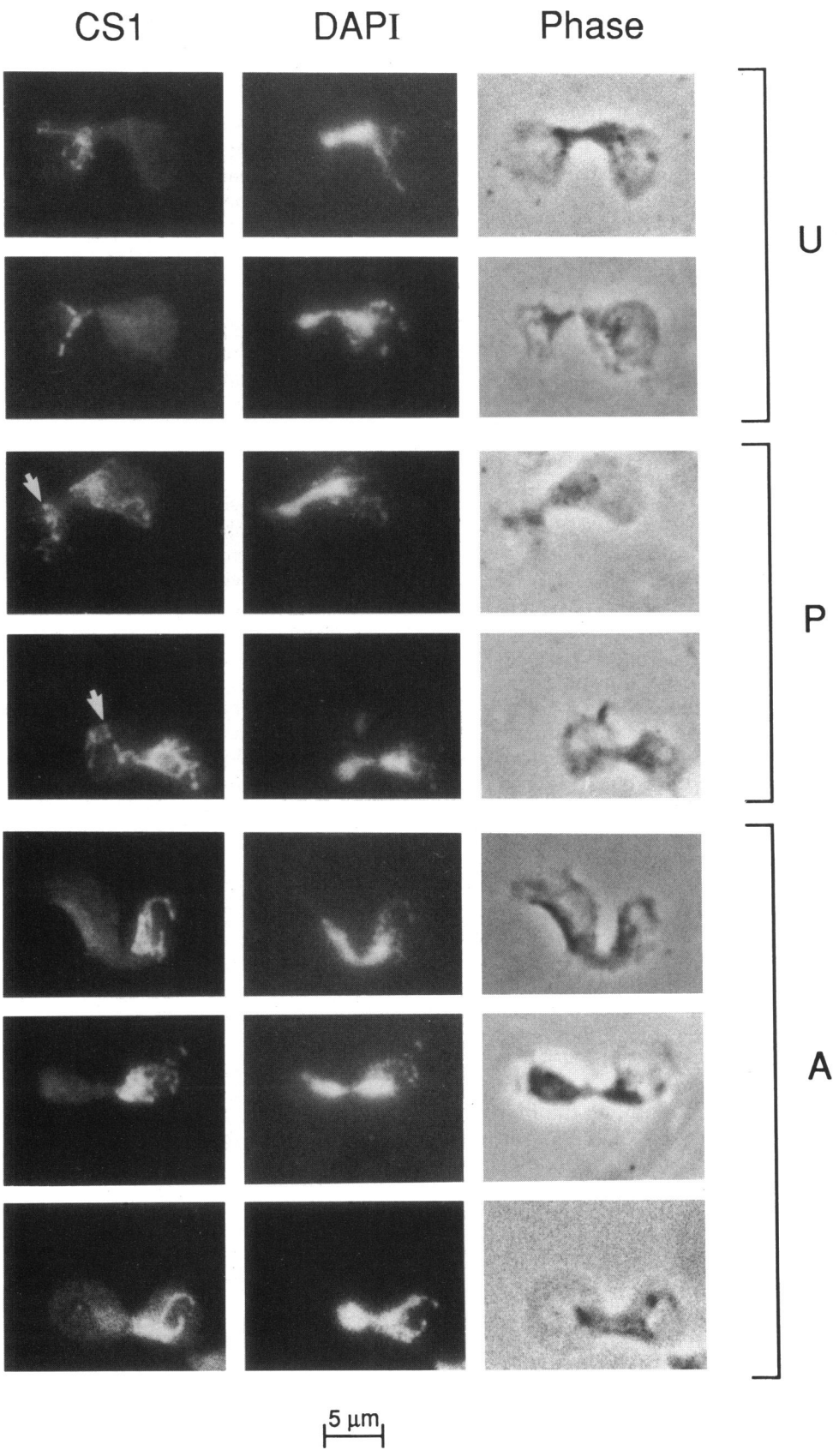

Figure 5. Zygotes from a ρ^0 CS1$^+$ $\times$ ρ^+ CS1$^-$ cross. The various types are described in the text. Mixed zygotes are indistinguishable among the various crosses, so they are not shown in this figure. Arrows indicate CS1-containing mitochondrial structures that do not contain mtDNA in type P zygotes.

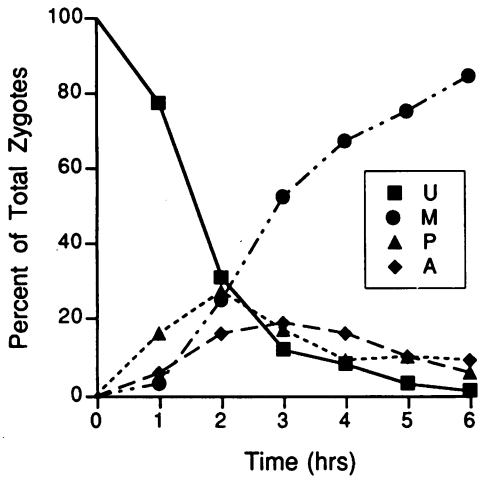

Figure 6. Time course of appearance and disappearance of the various types of zygotes from a ρ^0 CS1$^+$ × ρ^+ CS1$^-$ cross (same as Figure 5). Data depict the abundance of each type as a percentage of all zygotes. Note the appearance of type P and type A, which occurs before the appearance of mixed (M) zygotes.

ance of type A cells is consistently delayed relative to type P cells (Fig. 6). This kinetic relationship between zygote types A, P, and M can also be seen from an analysis of the fraction of each zygote type that has buds (Fig. 7): these data show that the later a particular zygote type appears as determined by the distribution of CS1 and mtDNA, the greater is the fraction of that zygote type with a bud. Taken together, these results suggest a possible precursor-product relationship among zygotes of types P, A, and M.

A possible explanation for the above result is that the initial movement of one component or the delay in movement of the other is due to either the mitochondrial genotype of the ρ^+ parent or the nuclear genotype of one or both parent strains. For example, a particular gene in one of the parent cells may dictate the loss or gain of one mitochondrial constituent or might delay the equilibration of the other. To test this possibility, we analyzed a different ρ^0 CS1$^+$ × ρ^+ CS1$^-$ cross using WB ρ^0 CS1$^+$ and BL ρ^+ CS1$^-$ as the parents. In this case the ρ^+ mtDNA is now in the BL parent, while the WB parent is now ρ^0 and CS1$^+$. We observed the same four types of zygotes in this cross and a time course of their appearance and disappearance similar to that for the previous cross. Therefore, quantitative translocation is due only to the presence of the protein label in one parent and the presence of mtDNA in the other, regardless of the initial nuclear backgrounds.

Finally, it is conceivable, though unlikely, that the presence of type A zygotes reflects some selective impermeability of one end of a zygote versus the other to antibody molecules during the time course of zygote maturation. To rule out such a possibility, we have carried out a double label experiment on zygotes from a ρ^0 CS1$^+$ × ρ^+ CS1$^-$ cross staining for both CS1 (FITC) and tubulin (rhodamine) by indirect immunofluorescence (see MATERIALS AND METHODS). Figure 8 shows that while CS1 staining of types U and A zygotes is localized to only one half of the cells, a typical pattern of tubulin staining is observed across both sides of the same zygote. This pattern of tubulin staining is independent of the disposition of CS1 in zygotes. Thus, asymmetric staining of CS1, particularly in type A zygotes, is likely to reflect the actual intracellular distribution of CS1 molecules.

ρ^0 CS1$^-$ × ρ^+ CS1$^+$. One possibility to account for the type A zygotes in the above crosses is that the mtDNA from the ρ^+ parent quantitatively moves into the ρ^0 end rather than CS1 moving to the ρ^+ before equilibration of both species. Although the presence of the type P zygotes is not fully consistent with this view, it is obvious that simple inspection of the type A zygotes cannot distinguish between these possibilities. To test whether mtDNA exclusively moves during the early phase of zygote maturation, we carried out the same cross as in Fig. 5 except that the ρ^+ parent was prelabeled with CS1. In this case quantitative movement of mtDNA to the ρ^0 end of the zygote would give rise to an initial separation of the two species yielding a zygote type identical to the unmixed form of Fig. 5.

Figures 9 and 10 show the results from a synchronized mating performed between BL ρ^0 CS1$^-$ × WB ρ^+ CS1$^+$ cells. One hour after mating the predominant zygote type is the predicted unmixed form, with both CS1 and the mtDNA localized only in the ρ^+ CS1$^+$ half of the zygote (Fig. 9). The DAPI and FITC signals are invariably superimposable, indicating that both components

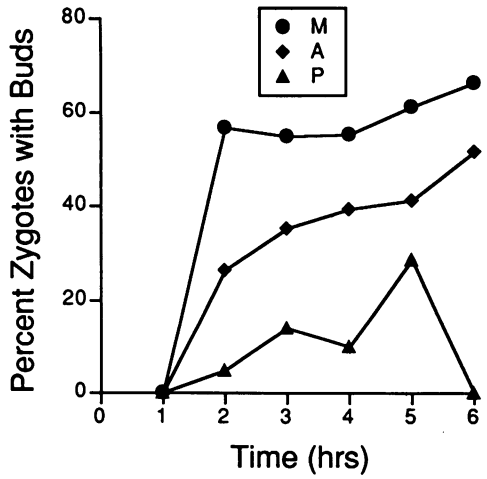

Figure 7. Abundance of zygotes of each type that display one or more buds, as a percentage of all zygotes of that type for each time point, for a ρ^0 CS1$^+$ × ρ^+ CS1$^-$ cross (same as Figures 5 and 6). For each time point the fraction of type M zygotes with buds is always larger than the corresponding fraction of type A zygotes; this, in turn, is larger than for type P. Unmixed zygotes never contained buds.

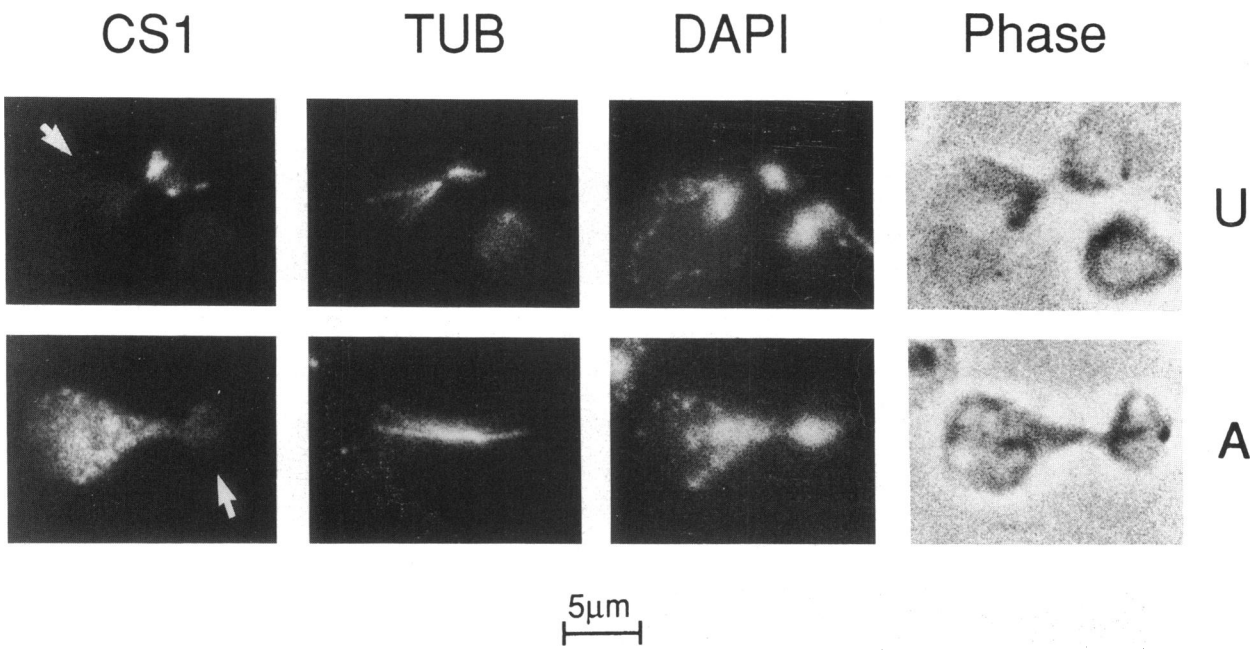

Figure 8. CS1 and tubulin stain differently in type U and A zygotes from a ρ^0 CS1$^+$ × ρ^+ CS1$^-$ cross. Cells were stained for DNA with DAPI and for CS1 and tubulin as described in MATERIALS AND METHODS. The arrows point to the ρ^0 end of the zygotes.

are localized in the same compartment. Mixed zygotes are also found, in which the DAPI and FITC signals are still colocalized but detectable in both halves of the zygote. Two additional types of zygotes are observed in this cross. One hour after mating a small proportion of zygotes show CS1 and mtDNA predominantly colocalized in one half of the cell but with a very small number of structures stained with either DAPI alone (type D) or FITC alone (type P) in the other half. We interpret these few structures to represent portions of mitochondria that have received either mtDNA or CS1, but not both. Their presence suggests that either component is capable of moving into the ρ^0 CS1$^-$ end of the zygote independently of the other. Importantly, however, this movement is not quantitative for either mtDNA or CS1, as zygotes containing each constituent in only one half, to the exclusion of the other, are never observed. As expected, the kinetics of this cross show a decrease in the frequency of unmixed zygotes and their ultimate replacement by mixed zygotes (Fig. 10). Type D and type P zygotes appear in small numbers early in the cross and become even less abundant later on, until none are found after 4 h. Therefore, these zygotes also become mixed over time, so that ultimately all zygotes in the mating population achieve complete equilibration of both mtDNA and CS1. The results from this experiment argue that vectorial translocation is not the result of early and exclusive mtDNA movement, and we conclude, therefore, that CS1 is quantitatively translocated towards the mtDNA.

Vectorial Protein Translocation is not Protein-Specific

A logical question raised by the vectorial translocation of CS1 is whether it is a general phenomenon of proteins in the mitochondrial matrix, and if so, whether it is dependent on the presence of CS1. To address these questions, we carried out a cross between ρ^+ and ρ^0 cells, both CS1$^-$, where the ρ^0 parent is prelabeled with a foreign, nonmitochondrial protein targeted to the mitochondrial matrix before mating to unlabeled ρ^+ cells. For this purpose we chose to use a chimeric protein resulting from a fusion between mouse dihydrofolate reductase and the mitochondrial targeting peptide from human ornithine transcarbamoylase (OTC-DHFR), which is efficiently targeted in vivo to the yeast mitochondrial matrix (Cheng *et al.*, 1987). This labeling is carried out similarly to CS1 labeling as described in MATERIALS AND METHODS.

Figures 11 and 12 show the results of a cross between BL ρ^0 OTC-DHFR$^+$ × WB ρ^+ OTC-DHFR$^-$ cells. Inspection of the photomicrographs (Fig. 11) and a summary of the kinetic behavior of various zygote forms (Fig. 12) show clearly that OTC-DHFR mimics almost exactly the behavior of CS1 in the ρ^0 CS1$^+$ × ρ^+ CS1$^-$ cross (Figs. 5 and 6): in addition to type U and M zygotes, types P and A are observed in which the rise and fall of the type P zygotes precedes that of type A. To directly compare the behavior of OTC-DHFR and CS1, pGAL-OTC-DHFR was introduced into BWG 1-7A, a *CIT1* strain, and a time course was followed for a ρ^0 CS1$^+$

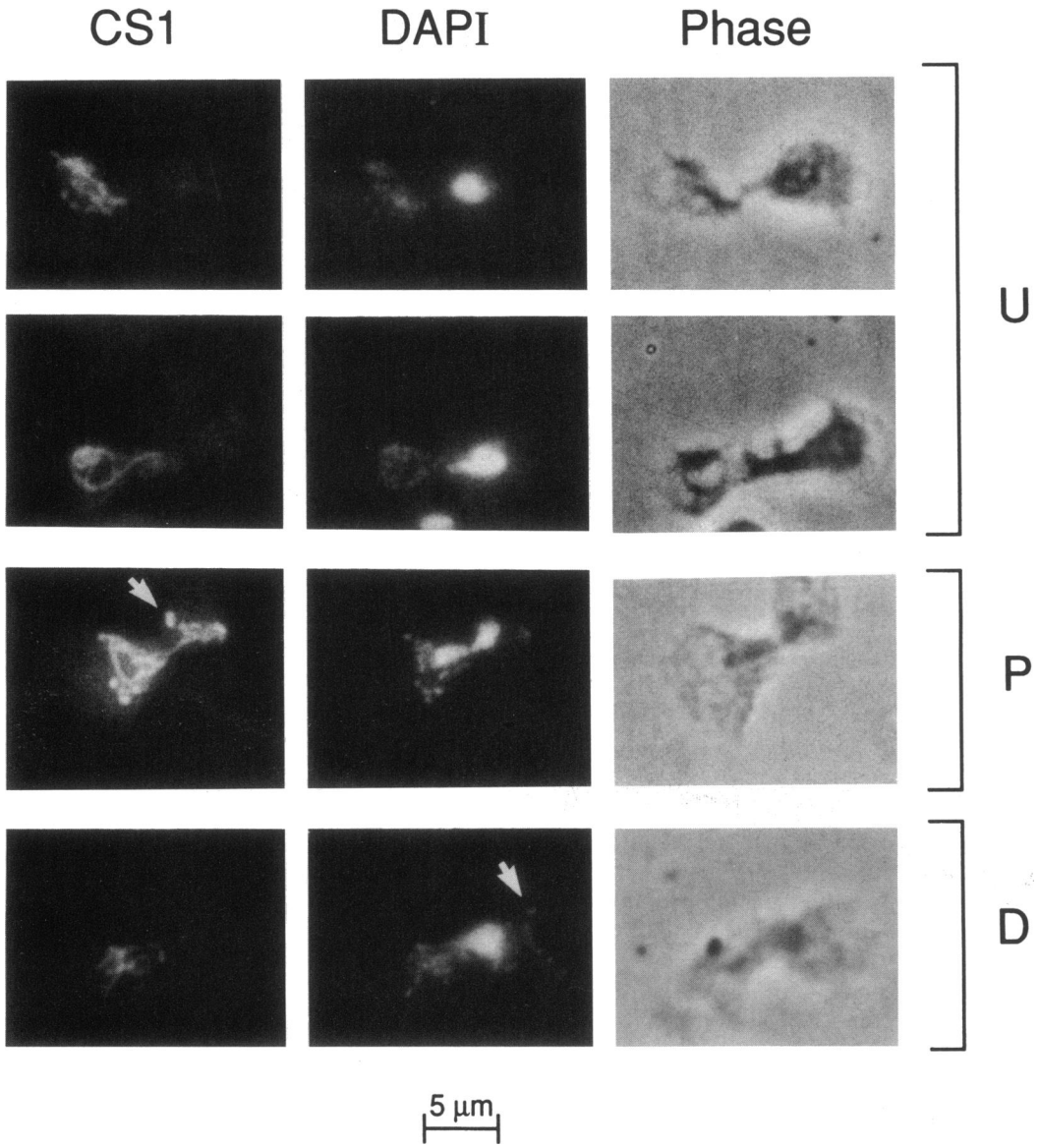

Figure 9. Zygotes from a ρ^0 CS1$^-$ × ρ^+ CS1$^+$ cross. The various types are described in the text. As in Figure 5, mixed zygotes are not shown. Arrows indicate CS1-containing mitochondrial structures that do not contain mtDNA (Type P) and mtDNA-containing structures (nucleoids) that lack CS1 (Type D).

OTC-DHFR$^+$ × ρ^+ CS1$^-$ OTC-DHFR$^-$ cross (BWG 1-7A ρ^0 OTC-DHFR$^+$ × WB ρ^+). In this cross both CS1 and OTC-DHFR are initially present in the ρ^0 parent, although now CS1 is expressed from the genomic, wild-type copy of CIT1 and OTC-DHFR is under GAL control. Using anti-DHFR in parallel with anti-CS1, both proteins display very closely matched kinetics of movement within zygotes. Taken together, these results provide strong evidence that the phenomenon of vectorial protein translocation in this type of cross is general and not dependent on the presence of CS1 in either parent.

Attempts to Perturb Vectorial Translocation of CS1

Effects of a mit$^-$ mutation and inhibitors of mitochondrial function. What controls the apparent unidirectional movement of mitochondrial matrix protein from the ρ^0 to the ρ^+ end of a zygote early in zygote maturation? As a first approach to this question, we studied the effects of mitochondrial-specific drugs and a mtDNA mutation on vectorial translocation of CS1. Antimycin A, an inhibitor of mitochondrial electron transport, and oligomycin, an inhibitor of the mitochondrial ATPase, were tested in a BL ρ^0 CS1$^+$ × WB ρ^+ CS1$^-$ cross. For these experiments, CS1 was induced

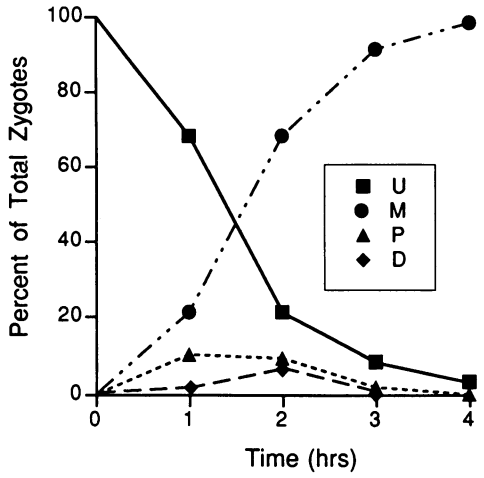

Figure 10. Time course of appearance and disappearance of the various types of zygotes from a ρ^0 CS1$^-$ × ρ^+ CS1$^+$ cross (same as Figure 9). Data depict the abundance of each type as a percentage of all zygotes.

in the BL ρ^0 cells as before; however, when these cells and the ρ^+ parent were induced for synchronous mating factor arrest (see MATERIALS AND METHODS) the medium contained either 1 μg/ml antimycin A or 5 μg/ml oligomycin during the 2-h incubation period; the drugs were also included in the mating plates on which the synchronous matings occur. These concentrations of drugs are sufficient to completely inhibit the growth of ρ^+ cells on glycerol medium, but they have no effect on either the kinetics of mating or the extent of zygote formation. In these experiments, neither drug had a noticeable effect on the movement of mitochondrial components: type P and type A zygotes were observed in proportions similar to those in control matings, and the time course of appearance and disappearance of the various types of zygotes was similar in the presence or absence of the drugs.

We next tested the effect of a mit$^-$ mtDNA mutation on CS1 vectorial translocation. In this experiment the ρ^+ mitochondrial genome of BL cells was replaced by cytoduction with a mutant mitochondrial genome, PZ27, that harbors a deletion of *cob*, the gene encoding apocytochrome b (Perlman *et al.*, 1980). This deletion results in a loss of respiratory function, but has no effect on mitochondrial transcription or translation. The results of this experiment were also negative in that type P and type A zygotes appeared and disappeared with kinetics indistinguishable from the ρ^0 CS1$^+$ × ρ^+ CS1$^-$ cross.

Complex kinetics of zygote forms in a ρ^0 CS1$^+$ × ρ^- CS1$^-$ cross. A second mitochondrial mutant, the petite F11 (ρ^-), which consists of a deletion of all but ~12 kb of the ρ^+ mitochondrial genome (Sor and Fukuhara, 1983) resulting in the loss of mitochondrial protein synthesis, was tested for its effect on vectorial CS1 translocation. As shown in Figures 13 and 14, the cross WB ρ^0 CS1$^+$ × BL ρ^- CS1$^-$ yields no detectable type A zygotes, although type P and type D zygotes are present. Also, an additional type of zygote (type P-D) is found, which thus far is unique to this cross (Fig. 13). In these cells there is colocalization of the DAPI and FITC signals in the medial region of the zygote, including the neck, but a portion of each signal is also found in one but not the other half of the cell. Due to their appearance and kinetics (Fig. 14), this particular zygote form may be yet a different intermediate in mitochondrial mixing in zygotes of this type, in which the mtDNA and CS1 are equilibrating through the zygote in opposite directions, independent of each other. Although the behavior of mitochondrial constituents appears to be more complex in this cross than in others, the absence of any detectable type A zygotes leads to the conclusion that substitution of a ρ^+ genome by a ρ^- petite genome cannot support quantitative vectorial translocation of matrix protein from the ρ^0 end of the zygote to the end containing mtDNA.

DISCUSSION

The analysis of mitochondrial sorting in yeast zygotes derived from synchronous matings provides an experimental opportunity, not feasible with vegetatively growing cells, to evaluate how different parental mitochondria sort, recombine, and partition to buds. Because genetic studies indicate that mitochondrial transmission in crosses is not random (Michaelis, 1967; Dujon *et al.*, 1974; Birky *et al.*, 1978) and depends, at least in part, on zygote bud position (Strausberg and Perlman, 1978; Zinn *et al.*, 1987), we hoped to be able to time-resolve the sorting of mitochondria and mtDNA to identify intermediates in the process. The fact that mitochondrial genomes contributed by haploid parents recombine promiscuously in zygotes (Thomas and Wilkie, 1968) argues strongly that parental mitochondria must fuse to allow their respective DNAs to mix and recombine. Accepting that premise, pedigree analysis of the transmission of parental and recombinant mitochondrial genotypes shows that for ρ^+ × ρ^+ crosses, mtDNA mixing is not extensive and appears to be limited to that portion of the zygote which gives rise to medial buds. As shown by Strausberg and Perlman (1978) and Zinn *et al.* (1987), and in the present work, end-buds are most often parental for the mtDNA contributed by the parent cell that comprises the half of the zygote from which the end bud is derived. From previous studies, it has been clearly shown that medial buds contain most of the recombinant mitochondrial genomes, which partition there by aliquoting only a small fraction of the total mtDNA present in the zygote (Zinn *et al.*, 1987).

Given the above results, we first asked whether this limited mixing of ρ^+ mitochondrial genomes extends to matrix protein. By following the time course of mixing

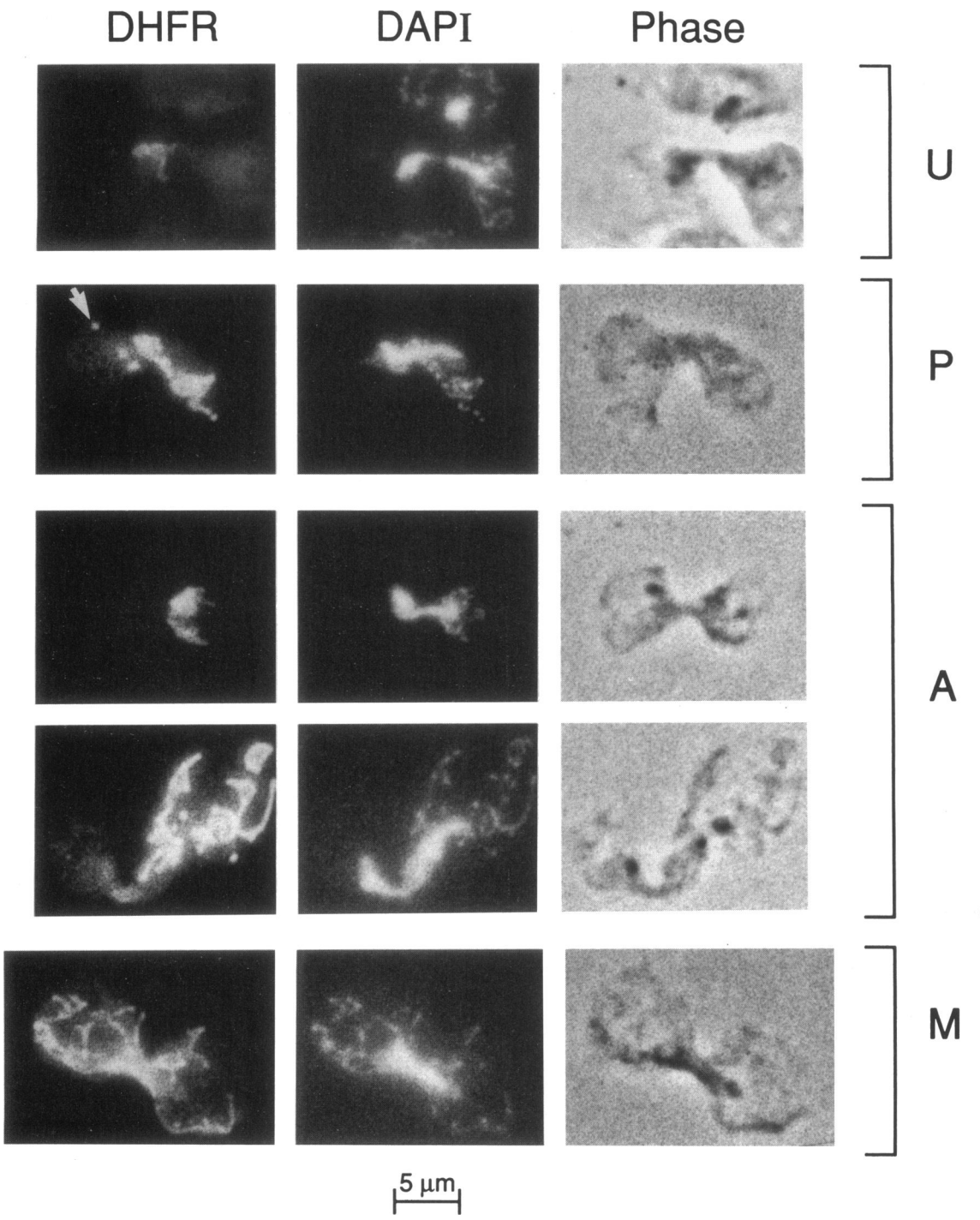

Figure 11. Zygotes from a ρ^0 OTC-DHFR$^+$ × ρ^+ OTC-DHFR$^-$ cross. The various types are described in the text. The arrow points to a mitochondrial structure containing OTC-DHFR but lacking mtDNA in a type P zygote. Compare with Figure 5.

in zygotes of mitochondria prelabeled in one of the parents with the matrix protein CS1 we found, as expected, that at early times mtDNA is distributed throughout the zygote, whereas CS1 is located only in one-half. Over time, however, these unmixed zygotes are quantitatively replaced by a zygote form in which CS1 is distributed throughout the zygote and into all observed buds. This process occurs soon after zygote formation, since up to 90% of the zygotes are mixed at a time when only ~10% display buds. This time frame is in sharp contrast

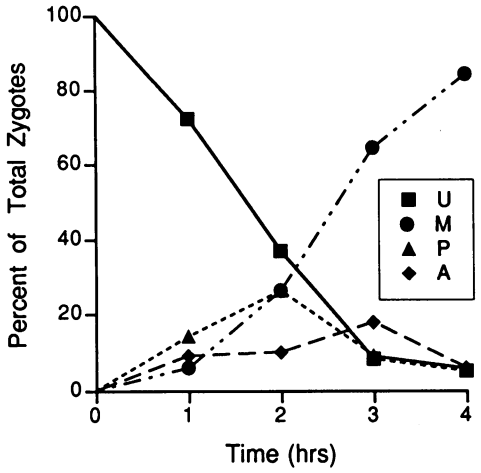

Figure 12. Time course of appearance and disappearance of the various types of zygotes from a ρ^0 OTC-DHFR$^+$ × ρ^+ OTC-DHFR$^-$ cross (same as Figure 11). Data depict the abundance of each type as a percentage of all zygotes. Note the appearance of type P and type A, which occurs before the appearance of mixed (M) zygotes. Compare with Figure 6.

to vacuolar mixing in yeast zygotes, which has been shown to be mediated only through the developing bud vacuole (Weisman and Wickner, 1988). Although our present technology allows us to track mtDNA in $\rho^+ \times \rho^+$ crosses only genetically, the surprising conclusion from this experiment is that the sorting of mtDNA and a mitochondrial matrix protein are not coincident. Because we believe that the CS1 marker is representative of bulk matrix protein (see below), mtDNA must, therefore, not be part of that compartment at least from the standpoint of its sorting properties in zygotes. MtDNA may, for example, be attached at some specific sites to the inner mitochondrial membrane, so that the DNA could be sorted quite differently from bulk matrix protein. Further studies in which the movements of inner membrane components are followed in this experimental system together with mtDNA will be required to settle this point.

In our analysis of a ρ^0 CS1$^+$ × ρ^+ CS1$^-$ cross, two unexpected findings were made. First, unlike the situation in $\rho^+ \times \rho^+$ crosses, mtDNA from the ρ^+ parent equilibrates extensively throughout the zygote and into all buds. This most readily explains the observation that in crosses between ρ^0 and ρ^+ cells, virtually 100% of the progeny are ρ^+. Second, and perhaps more surprising, is that matrix protein prelabeled in the ρ^0 parent moves quantitatively to the ρ^+ end of the zygote as an early event in the sorting process. Importantly, once CS1 moves into the ρ^+ end, the protein invariably colocalizes with the DAPI stain, as it does in a $\rho^+ \times \rho^+$ cross where one side is labeled with the enzyme. This suggests that CS1 movement is not the result of mixing of two different mitochondria within the cytoplasm but, rather, that the parental mitochondria fuse, allowing mixing of at least some of their contents.

Because the kinetic data for unmixed and mixed zygotes are consistent with the expected initial and final states for individual zygotes, the interpretation of type P and type A kinetics leads to the conclusion that they both represent transitional steps in the conversion from unmixed to mixed zygotes and type P, in turn, are precursors of type A. Because the appearance of zygotes in this cross proceeds in the order: unmixed → type P → type A → mixed, with a net outcome of conversion from unmixed to mixed, it seems that the initial event is the transfer of CS1 from the ρ^0 end towards the ρ^+ end of the zygote, without movement of the mtDNA. In this context, the type P zygotes would represent cells in the process of translocation, when CS1 begins to colocalize with mtDNA in the ρ^+ end, but the trailing edge of CS1, before transfer is complete, is still visible in the ρ^0 half. Once this transfer is complete, the ρ^0 end is depleted of its initial content of CS1, giving rise to a type A zygote, which may then equilibrate both components to attain complete mixing.

How is this net transfer giving rise to type A zygotes achieved? Inhibitor studies and the use of a mit$^-$ mitochondrial genome instead of a ρ^+ shows that this vectorial translocation does not require that mitochondria in the "target" end of the zygote be functional. One possibility is that it occurs by simple diffusion, where the ρ^0 matrix marker becomes kinetically trapped in mitochondria of the ρ^+ end before equilibration of all species throughout the zygote. Electron microscopic examination of mitochondrial structures in petite versus ρ^+ cells shows a profound morphological difference between these mitochondria (Stevens, 1981): mitochondria in petite cells have few cristae and lack internal organization, whereas mitochondria in ρ^+ cells have a well organized internal structure with many cristae. It is likely, therefore, that the matrix space in petite mitochondria has a lower protein concentration than ρ^+ mitochondria. Hence it is easy to imagine that movement of molecules in the matrix of petite cells is simply less impeded than in ρ^+. This view would also account for the absence of type A zygotes in a $\rho^0 \times \rho^-$ cross, since the petite mtDNA would also be less impeded in its movement towards the ρ^0 end of the zygote. Indeed, a unique zygote intermediate in such a cross is the type P-D form where both the matrix marker and the ρ^- mtDNA are found in the central portion of the zygote.

At this point, it is worth considering some possible distinctions between bulk mitochondrial movements and the movement of mitochondrial constituents, e.g. matrix protein and mtDNA. Regarding the former, microtubules have been implicated in a number of organisms in mitochondrial motility (Saetersdal *et al.*, 1990; Weissenfels *et al.*, 1990). In yeast, however, it has been noted in the analysis of tubulin mutants that movement of mitochondria into buds is unaffected (Huffaker *et al.*,

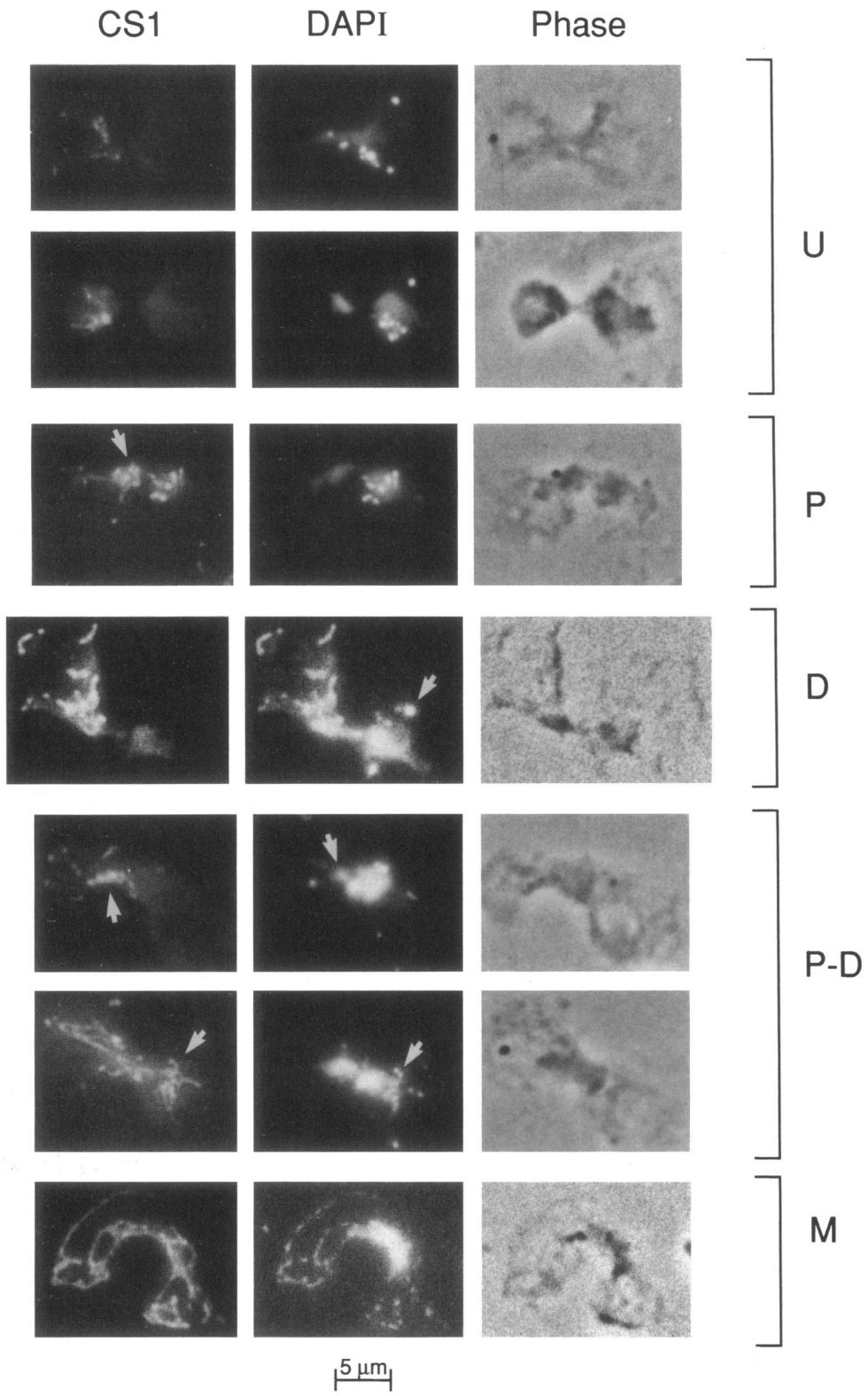

Figure 13. Zygotes from a ρ^0 CS1$^+$ × ρ^- CS1$^-$ cross. Note the distinctive morphology of petite nucleoids, which are larger and brighter than those of ρ^+ cells, but fewer in number. Type P, arrow indicates CS1-containing mitochondrial structures that do not contain mtDNA. Type D, arrow indicates mtDNA structures (nucleoids) lacking CS1. Type P-D, arrows indicate the region (near the neck) where CS and mtDNA colocalize.

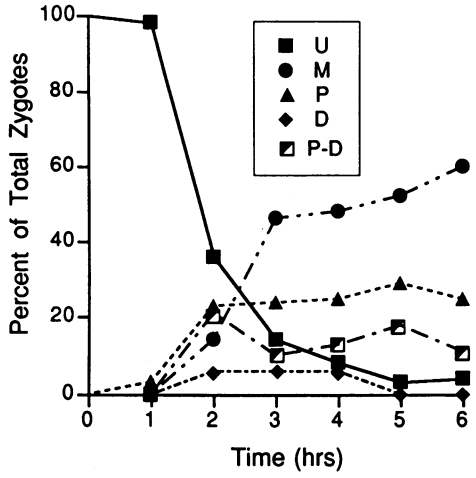

Figure 14. Time course of appearance and disappearance of the various types of zygote from a ρ^0 $CS1^+$ × ρ^- $CS1^-$ cross (same as Figure 13). Note that type P and type P-D zygotes do not entirely disappear from the population, and the yield of mixed (M) zygotes is less than in other crosses.

1988; Hoyt *et al.*, 1990), although the effects of such mutants on sorting of mitochondria in zygotes, to our knowledge, has not been investigated in detail. The results presented here suggest that the movement of mitochondrial constituents is complex and can occur in the absence of bulk mitochondrial movements. The latter conclusion is suggested by the observations that 1) a matrix marker can mix extensively in zygotes without apparent mtDNA mixing, and 2) the movement of a matrix marker from the end of a zygote without mtDNA into the end with DNA results in the colocalization of both species indicating that they are in the same mitochondrial structures. As noted above, a plausible interpretation of this result is that the mitochondria from the two ends of the zygote fuse allowing the net transfer of the matrix marker into the mitochondria of the ρ^+ end.

More complex models involving specific motors or tethers can also be envisaged. In this connection, Yotsuyanagi (1988) made the intriguing observation that yeast mitochondria contain an intramitochondrial fibrous component (IMF) that can be as long as 8 μm. Interestingly, the morphology of IMF is different in respiratory competent and respiratory deficient cells, and it was speculated that this unusual structure could function to facilitate mitochondrial movement from the mother cell into the emerging bud. Possibly, IMF, which was reported to be sandwiched between cristae, could play some role in the transport of mitochondrial constituents.

Certain matrix proteins, such as those of the TCA cycle, which have been suggested to be organized as a complex or "metabolon" (Srere *et al.*, 1987) could conceivably serve as a tether in ρ^+ mitochondria, effectively contributing to the quantitative movement of matrix protein from a ρ^0 to the ρ^+ end of a zygote. This point is noteworthy in light of recent findings that yeast CS1 can associate with microtubules (Barnes *et al.*, 1992), and that a species of CS in Tetrahymena can form 14-nm filaments (Numata *et al.*, 1991). Our results with OTC-DHFR, however, indicate that the phenomenon of vectorial translocation is neither specific to nor dependent on CS1, since this protein was absent in both parents. If CS1 is functionally part of some motor apparatus in yeast, it is not likely to be directly responsible for the vectorial transport described here. Other mitochondrial tethers can be envisioned, as for example, ρ^+ DNA complexed with certain abundant proteins such as the product of the *ABF2* gene (Diffley and Stillman, 1991). Even so, if specific protein-protein interactions are required, the fact that a foreign protein like DHFR behaves in the same way as CS1 remains an intriguing result. Further studies, tracking the behavior of membrane-specific labels in zygotes, may help clarify the issue.

ACKNOWLEDGMENTS

We thank Art Horwich for providing pGAL-OTC-DHFR, Gottfried Schatz for the Anti-DHFR antibody, and Paul Srere for Anti-CS1 antibody and for yeast strains. We are grateful to Olga Troitskaya for carrying out the immunofluorescence experiments with anti-tubulin antibody. This work was supported by grants GM-33501 from the NIH and I-642 from The Robert A. Welch Foundation.

REFERENCES

Adams, A.E.M., and Pringle, J.R. (1984). Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic mutant *Saccharomyces cerevisiae*. J. Cell Biol. *98*, 934–945.

Baker, R. (1991). Rapid colony transformation of *Saccharomyces cerevisiae*. Nucleic Acids Res. *19*, 1945.

Barnes, G., Louie, K.A., and Botstein, D. (1992). Yeast proteins associated with microtubules in vitro and in vivo. Mol. Biol. Cell 3, 29–47.

Birky, C.W., Strausberg, R.L., Perlman, P.S., and Forster, J.L. (1978). Vegetative segregation in yeast: estimating parameters using a random model. Mol. Gen. Genet. *158*, 251–261.

Blanc, H., and Dujon, B. (1980). Replicator regions of the yeast mitochondrial DNA responsible for suppressiveness. Proc. Natl. Acad. Sci. USA 77, 3942–3946.

Cheng, M.Y., Pollock, R.A., Hendrick, J.P., and Horwich, A.L. (1987). Import and processing of human ornithine transcarbamoylase precursor by mitochondria from *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *84*, 4063–4067.

Conde, J., and Fink, G. (1976). A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. Proc. Natl. Acad. Sci. USA *73*, 3651–3655.

de Zamaroczy, M., Marotta, R., Faugeron-Fonty, G., Gurosot, R., Maugin, M., Baldacci, G., and Bernardiz, G. (1981). The origins of replication of the yeast mitochondrial genome and the phenomenon of suppressivity. Nature *292*, 75–78.

Diffley, J.F., and Stillman, B. (1991). A close relative of the nuclear, chromosomal high-mobility group protein HMG1 in yeast mitochondria. Proc. Natl. Acad. Sci. USA *88*, 7864–7868.

Dujon, B. (1981). Mitochondrial genetics and functions. In: The Molecular Biology of the Yeast *Saccharomyces*, ed. J.N. Strathern, E.W. Jones, and J.R. Broach, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 505–635.

Dujon, B., Slonimski, P.P., and Weill, L. (1974). Mitochondrial genetics IX: a model for recombination and segregation of mitochondrial genomes in *Saccharomyces cerevisiae*. Genetics *78*, 415–437.

Foury, F. (1989). Cloning and sequencing of the nuclear gene *MIP 1* encoding the catalytic subunit of the yeast mitochondrial DNA polymerase. J. Biol. Chem. *264*, 20552–20560.

Horwich, A.L., Kalousek, F., Mellman, I., and Rosenberg, L.E. (1985). A leader peptide is sufficient to direct mitochondrial import of a chimeric protein. EMBO J. *4*, 1129–1135.

Hoyt, M.A., Stearns, T., and Botstein, D. (1990). Chromosome instability mutants of *Saccharomyces cerevisiae* that are defective in microtubule-based processes. Mol. Cell. Biol. *10*, 223–234.

Huffaker, T.C., Thomas, J.H., and Botstein, D. (1988). Diverse effects of β-tubulin mutations on microtubule formation and function. J. Cell Biol. *106*, 1997–2010.

Ito, H., Fukuda, Y., K.M., and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. *153*, 163–168.

Jones, B.A., and Fangman, W.L. (1992). Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin. Genes Dev. *6*, 380–389.

Kilmartin, J.V., and Adams, A.E.M. (1984). Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*, J. Cell Biol. *98*, 922–933.

Kim, K.S., Rosenkrantz, M.S., and Guarente, L. (1986). *Saccharomyces cerevisiae* contains two functional citrate synthase genes. Mol. Cell. Biol. *6*, 1946–1942.

Lisowsky, T., and Michaelis, G. (1988). A nuclear gene essential for mitochondrial replication suppresses a defect of mitochondrial transcription in *Saccharomyces cerevisiae*. Mol. Gen. Genet. *214*, 218–223.

Lundin, M., Baltscheffsky, H., and Ronne, H. (1991). Yeast *PPA 2* gene encodes a mitochondrial inorganic pyrophosphatase that is essential for mitochondrial function. J. Biol. Chem. *266*, 12168–12172.

McConnell, S.J., Stewart, L.C., Talin, A., and Yaffe, M.P. (1990). Temperature-sensitive yeast mutants defective in mitochondrial inheritance. J. Cell Biol. *111*, 967–976.

Michaelis, P. (1967). The investigation of plasmone segregation by the pattern-analysis. Nucleus *10*, 1–14.

Numata, O., Takemasa, T., Takagi, I., Hirono, M., Hirano, H., and Chiba, J. (1991). *Tetrahymena* 14-nm filament-forming protein has citrate synthase activity. Biochem. Biophys. Res. Commun. *174*, 1028–1034.

Olmsted, J.B. (1986). Analysis of cytoskeletal structures using blot-purified monospecific antibodies. Methods Enzymol. *134*, 467–472.

Perlman, P.S., Alexander, N.J., Hanson, D.K., and Mahler, H.R. (1980). Mosaic genes in yeast mitochondria. In: Gene Structure and Expression, ed. D.B. Dean, L.F. Johnson, P.C. Kimball, and P.S. Perlman, Columbus, OH: Ohio University Press, 1–40.

Rogers, D., and Bussey, H. (1978). Fidelity of conjugation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. *162*, 173–182.

Rosenkrantz, M., Alam, T., Kim, K., Clark, B.J., Srere, P.A., and Guarente, L.P. (1986). Mitochondrial and nonmitochondrial citrate synthases in *Saccharomyces cerevisiae* are encoded by distinct homologous genes. Mol. Cell. Biol. *6*, 4509–4515.

Saetersdal, T., Greve, G., and Dalen, H. (1990). Associations between β-tubulin and mitochondria in adult isolated heart myocytes as shown by immunofluorescence and immunoelectron microscopy. Histochemistry *95*, 1–10.

Slonimski, P.P., Perrodin, G., and Croft, J.H. (1968). Ethidium bromide induced mutation of yeast mitochondria: complete transformation of cells into respiratory deficient non-chromosomal "petites". Biochem. Biophys. Res. Commun. *30*, 232–239.

Sor, F., and Fukuhara, H. (1983). Complete DNA sequence coding for the large ribosomal RNA of yeast mitochondria. Nucleic Acids Res. *11*, 339–348.

Srere, P.A., Sumegi, B., and Sherry, A.D. (1987). Organizational aspects of the citric acid cycle. Biochem. Society Symp. *54*, 173–178.

Stevens, B. (1981). Mitochondrial Structure. In: The Molecular Biology of the Yeast *Saccharomyces*, ed. J.N. Strathern, E.W. Jones, and J.R. Broach, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 471–504.

Stewart, L.C., and Yaffe, M.P. (1991). A role for unsaturated fatty acids in mitochondrial movement and inheritance. J. Cell Biol. *115*, 1249–1257.

Strausberg, R.L., and Perlman, P.P. (1978). The effect of zygotic bud position on the transmission of mitochondrial genes in *Saccharomyces cerevisiae*. Mol. Gen. Genet. *163*, 131–144.

Suissa, M., Suda, K., and Schatz, G. (1984). Isolation of the nuclear yeast genes for citrate synthase and fifteen other yeast mitochondrial proteins by a new screening method. EMBO J. *3*, 1773–1781.

Thomas, D.Y., and Wilkie, D. (1968). Recombination of mitochondrial drug resistance factors in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. *30*, 368–372.

Weislogel, P.O., and Butow, R.A. (1970). Low temperature and chloramphenicol induction of respiratory deficiency in a cold-sensitive mutant of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *67*, 52–58.

Weisman, L.S., and Wickner, W. (1988). Intervacuole exchange in the yeast zygote: a new pathway in organelle communication. Science *241*, 589–591.

Weissenfels, N., Wachtmann, D., and Stockem, W. (1990). The role of microtubules for the movement of mitochondria in pinacocytes of fresh-water sponges. Eur. J. Cell Biol. *52*, 310–314.

West, R.W., Yocum, R.R., and Ptashne, M. (1984). *Saccharomyces cerevisiae GAL1-GAL10* divergent promoter region: location and function of the upstream activating sequence UAS_G. Mol. Cell Biol. *4*, 2467–2478.

Yotsuyanagi, Y. (1988). Fibrous component of yeast mitochondria. J. Ultrastruct. Res. *98*, 254–266.

Zinn, A.R., Pohlman, J.K., Perlman, P.P., and Butow, R.A. (1987). Kinetic and segregational analysis of mitochondrial DNA recombination in yeast. Plasmid *17*, 248–256.

Zweifel, S.G., and Fangman, W.L. (1991). A nuclear mutation reversing a biased transmission of yeast mitochondrial DNA. Genetics *128*, 241–249.