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# Mutations in the Voltage-Dependent Anion Channel of the Mitochondrial Outer Membrane Cause a Dominant Nonlethal Growth Impairment

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Point mutations at K234 and K236 in the yeast voltage-dependent anion channel 1 (VDAC1) of the mitochondrial outer membrane have been shown to markedly impair the membrane insertion of this protein (Smith *et al.*, 1995; Angeles *et al.*, 1998). Mutants of this type were expressed *in vivo* in a strain of yeast with a disruption in the VDAC1 gene. Expression of the various VDAC1 forms was under the control of a Gal1 promoter. Wild-type VDAC1 expression fully complemented the slow growth phenotype caused by the disruption. VDAC1 mutants in which K234 and K236 were replaced by arginine, glutamate, or glutamine caused a more severe negative effect on growth. This effect appeared to be dominant since the mutant VDAC1 forms suppressed growth in a yeast strain that retained its VDAC1 gene. This apparent dominant negative effect on growth did not seem to be specific for any stage of the cell cycle. However, the growth defect was not lethal as the affected cells still could accumulate the vital stain, FUN1. Expression of a mutant in which K234 had been replaced by glutamate had more serious negative growth effects than did a similar mutation at K236. Expression of Δ71-116 VDAC1 complemented the VDAC1 disruption; however, expression of the same deletion mutant in which the lysines corresponding to K234 and K236 were mutated to glutamate severely impaired growth. These results have shown that a deficiency of lysine at positions 234 and 236 in VDAC1 causes a nonlethal growth defect that is more severe than deletion of 45 amino acids from VDAC1 or disruption of the VDAC1 gene. They also indicate that there is a hierarchy in the importance of these lysines with mutations at K234 being the more serious.

**KEY WORDS:** VDAC1; Mitochondria.

## INTRODUCTION

The voltage-dependent anion channel of the mitochondrion (VDAC1 or eukaryotic porin) forms large (3–4 nM) channels in the outer membrane that allow passage of charged biochemicals (Rostovtseva and Colombini, 1997). These biochemicals are important as substrates for oxidative phosphorylation and other reactions. In yeast mitochondria, there are two VDAC isoforms (Blachly-Dyson *et al.*, 1997), VDAC1 (283 residues) and VDAC2 (281 residues); VDAC1 is capable of forming channels while VDAC2 apparently is not. Genetic disruption of VDAC1 in yeast results in seriously impaired growth while disruption of VDAC2 has no obvious growth phenotype. These observations suggest that channel formation is crucial; however, VDACs also serve other functions. In humans, one isoform, VDAC1, provides binding sites for hexokinase I on the cytosolic side of the outer membrane, while another isoform, VDAC2, does not (Blachly-Dyson *et al.*, 1993). It has been proposed that VDAC interacts with creatine phosphokinase and the adenine nucleotide translocator on the mitochondrial side of

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the outer membrane (Beutner *et al.*, 1996). The observation that VDAC exists in various isoforms in the outer membrane implies that its functions aside from channel are important (Mannella, 1996). This argument is supported by the fact that disruption of the genes for both VDAC1 and VDAC2 in yeast causes a more severe growth defect than disruption of either gene singly (Blachly-Dyson *et al.*, 1997). In addition, overexpression of yeast VDAC2 (no detectable pore formation) has been shown to complement the growth defect caused a VDAC1 disruption, while overexpression of a channel forming VDAC from *Drosophila melanogaster* failed to complement (Blachly-Dyson *et al.*, 1997).

VDAC1, like most mitochondrial proteins, is encoded by a nuclear gene and is synthesized on ribosomes in the extramitochondrial cytoplasm. The VDAC1 precursor is identified by the mitochondria and, subsequently, is inserted into the outer membrane. Previous experimentation has shown that two lysyl residues in VDAC1, K234, and K236, participate in the membrane insertion of the VDAC1 precursor. Point mutation of these residues markedly impairs insertion into the outer membrane both *in vitro* and *in vivo* (Smith *et al.*, 1995; Angeles *et al.*, 1998). In the experiments described here, mutant forms of VDAC1 were expressed in *Saccharomyces cerevisiae* to evaluate the physiological effect of these mutations. Previous work suggested that the growth rate might be affected by the mutant VDAC1 forms (Blachly-Dyson *et al.*, 1990). Surprisingly, *in vivo* expression of VDAC1 with point mutations at either or both K234 and K236 resulted in an immediate and severe dominant negative effect on growth. This growth impairment was not lethal, but was more severe than that caused by disruption of the VDAC1 gene. The growth defect occurred immediately after expression of the mutants and did not appear to be specific for a particular phase of the cell cycle. This unusual toxicity cannot be accounted for entirely by inefficient membrane insertion or by impaired channel function. It was concluded that K234 and K236 in VDAC1 are involved in other unknown functions.

## MATERIALS AND METHODS

### The Construction of Expression Vectors Containing Wild-Type VDAC1 and Mutant VDAC1 and Their Transformation into JHRY20-2Ca Yeast Strains

The construction of all of the mutant VDAC1 forms except Δ71-116, K234E, K236E VDAC1 has been previously described (Smith *et al.*, 1995). Δ71-116, K234E, K236E VDAC1 was constructed by ligating a 432 bp *Ncol*/*Xbal* fragment of pSP65 K234E, K236E VDAC1 to the 3486-bp *Ncol*/*Xbal* fragment of pSP65 Δ71-116 VDAC1. Since wild-type VDAC1 and all the mutant VDAC1 forms were contained in the *in vitro* expression vector, pSP65 (Promega, WI), it was necessary to subclone them into the 2μ multicopy *in vivo* expression vector, pYes2.0 (Stratagene, CA). Except in the cases of Δ71-116 VDAC1 and Δ71-116, K234E, K236E, this could be done by ligating the 936-bp fragment obtained by digestion of the appropriate pSP65 construct with *Sacl* and *Xbal* with similarly digested pYes2.0. In the cases of Δ71-116 VDAC1 and Δ71-116, K234E, K236E, VDAC1, a 727-bp fragment containing the open reading frame was obtained by digestion with *BsaBl* and *Xbal*. This fragment was ligated into the 5844-bp fragment obtained by a similar digestion of wild-type VDAC1 in pYes2.0.

The various pYes2.0 constructs were transformed into *wt* (JHRY20-2Ca: wild type for VDAC1) and *dp1* (JHRY20-2Ca: VDAC1 gene disrupted) strains of *S. cerevisial* (see Table I for detailed descriptions of these and other strains) by the lithium carbonate procedure using commercially prepared reagents (Bio 101, CA). The *wt* and *dp1* strains are both auxotrophic for uracil (see Table I). pYes2.0 carries the URA3 gene and transformants were selected on agar plates containing

**Table I.** *Saccharomyces cerevisiae* Strains Used in This Study[a]

| |
|---|
| *wt* JHRY20-2Ca: MATa, *GAL* +, leu2, his3-52, and ura3-52 |
| *wt/y2* wt transformed with pYes 2.0 |
| *wt/wt* wt transformed with pYes 2.0—VDAC1 |
| *wt/2r* wt transformed with pYes 2.0—K234R, K346R VDAC1 |
| *wt/2q* wt transformed with pYes 2.0—K234Q, K346Q VDAC1 |
| *wt/2e* wt transformed with pYes 2.0—K234E, K346E VDAC1 |
| *dp1* JHRY20-2Ca: MATa, *GAL* +, yvdac1::LEU2, his3-52 and ura3-52 |
| *dp1/y2* dp1 transformed with pYes 2.0 |
| *dp1/wt* dp1 transformed with pYes 2.0—VDAC1 |
| *dp1/2r* dp1 transformed with pYes 2.0—K234R, K346R VDAC1 |
| *dp1/2q* dp1 transformed with pYes 2.0—K234Q, K346Q VDAC1 |
| *dp1/2e* dp1 transformed with pYes 2.0—K234E, K346E VDAC1 |
| *dp1/k234e* dp1 transformed with pYes 2.0—K234E VDAC1 |
| *dp1/k236e* dp1 transformed with pYes 2.0—K346E VDAC1 |
| *dp1/d45* dp1 transformed with pYes 2.0—Δ71-116 VDAC1 |
| *dp1/d45-2e* dp1 transformed with pYes 2.0—Δ71-116, K234E, K346E VDAC1 |

[a] The abbreviation for each strain used in this study is given in italics. The name, genotype, and vectors associated with the strain are in normal type.

2% glucose in yeast nitrogen base supplemented with essential nutrients except uracil. Since expression in pYes2.0 is under the control of a Gal1 promoter, the growth phenotype was initially evaluated on similar plates containing 2% raffinose or 2% galactose and 1% raffinose instead of glucose. These plates were incubated at 30°C for 3 to 5 days, depending on the growth of the various strains. The selected clones were grown in a liquid culture (2% glucose in yeast nitrogen base supplemented with essential nutrients except uracil) to an absorbance of about 1.0 at 600 nm. Cultures were stored for up to 2 weeks at 4°C before use.

### Growth Curves

Prior to the beginning of an experiment, the cell density of the stock cultures (glucose) was determined by microscopy. Twenty ml of 2% raffinose in yeast nitrogen base supplemented with essential nutrients except uracil was inoculated at a density of about $10^5$ cells/ml and incubated for 4 h at 30°C with shaking at 300 rpm. During the preincubation with raffinose, expression of very low levels of VDAC1 could be detected by immune blotting in *dp1/wt* cells (but not in *dp1/2e, dp1/2q* or *dp1/2r* cells). At the end of this period, the cells were collected by centrifugation at 1500 $\times$ *g* for 5 min and incubated in 2% galactose and 1% raffinose in yeast nitrogen base supplemented with essential nutrients except uracil. These cultures were incubated for up to 24 h under similar conditions. Samples were removed at various intervals and the cell density was estimated by microscopy. The number of growing cells was determined by plating an aliquot containing approximately 400 cells on YPD plates and counting the number of clones after 3 to 5 days of incubation at 30°C.

### Microscopy

Prior to electron microscopy, cells were collected by centrifugation and fixed in 1.5% potassium permanganate for 1 h at 4°C. The fixed cells were washed with water and stained with uranyl acetate. They were serially dehydrated in ethanol and propylene oxide and embedded in Epon-Araldite. Samples were visualized in a JEOL Jem-1010.

The viability of yeast cells was determined using a kit (Molecular Probes, OR) that employs the the vital stain FUN-1. The vacuolar accumulation of FUN-1 was observed through a Zeiss LSM 310 confocal microscope.

### Materials

Mixtures used in the preparation of selective media were obtained from Bio 101, CA. Other reagents not mentioned above were obtained in the highest possible purity from various manufacturers.

## RESULTS

### Exogenously Expressed VDAC1 Complements a VDAC1-Disrupted Yeast Strain

The JHRY20-2Ca auxotrophic strain of *S. cerevisiae* (*wt*) and the same strain with a disruption in the VDAC1 gene (*dp1*) were transformed with pYes2.0, a 2u plasmid. These strains were named *wt/y2* and *dp1/y2*, respectively. These and other strains used here are described in Table I. The strain with the VDAC1 disruption also was transformed with pYes2.0 containing the VDAC1 gene under the control of a Gal1 promoter. This strain was named *dp1/wt*. Each strain was inoculated ($10^5$ cells/ml) into raffinose and, after 4 h, shifted to galactose/raffinose. At 4, 8, and 16 h of incubation in galactose/raffinose, cell density was estimated by microscopy (not shown) and cell growth was estimated by colony formation on YPD plates (Fig. 1). By both measures, the growth of *dp1/wt* (epichromosomal expression of VDAC1)was somewhat better than for *wt/y2* (chromosomal expression of VDAC1). Both of these strains grew much better than *dp1/y2*. Clearly, the expression of the vector borne gene for VDAC1 in *dp1/wt* complemented the chromosomal VDAC1 disruption.

### Expression of Mutant VDAC1 Forms in Wild-Type and VDAC1-Disrupted Yeast Strains Causes Impaired Growth

The JHRY20-2Ca strain in which the VDAC1 gene had been disrupted (*dp1*) was transformed with pYes2.0 containing three mutant VDAC1 genes under the control of a Gal1 promoter. In these mutants, both K234 and K236 had been point mutated to arginine, glutamine, or glutamic acid. These mutations impair the ability of VDAC1 to insert into the mitochondrial

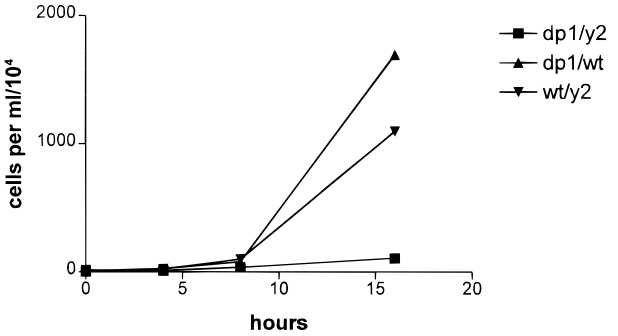



**Fig. 1.** The effect of disruption of the VDAC1 gene and epichromosomal expression of VDAC1 on cell growth. The growth of three yeast strains, *dp1/y2* (VDAC1 gene disrupted), *wt/y2* (VDAC1 gene intact), and *dp1/wt* (epichromosomal expression of VDAC1) were compared. The expression of VDAC1 in *dp1/wt* was under the control of a Gal1 promoter. The cells (initial cell density equal to $10^5$) were precultured in minimal medium (minus uracil) containing 2% raffinose for 4 h and then shifted to the same medium containing 2% galactose−1% raffinose. Portions were removed at the indicated times and applied to YPD plates. The number of colonies on these plates was used to calculate cell density.

outer membrane both *in vitro* and *in vivo* (Smith *et al.*, 1995; Angeles *et al.*, 1998). The growth of the three strains (named *dp1/2r, dp1/2q*, and *dp1/2e*, respectively) was compared to *dp1/y2* and *dp1/wt*.

Both *dp1/y2* and *dp1/wt* grew well on agar plates containing either raffinose or galactose/raffinose (Fig. 2A). The more robust growth of dp1/wt was probably due to low levels of expression of VDAC1 during growth on raffinose (see Materials and Methods). However, the strains expressing the doubly mutated VDAC1 forms failed to grow on galactose/raffinose. The growth of the three strains expressing the doubly mutated VDAC1 forms was compared to *dp1/y2* in galactose/raffinose liquid culture. As can be seen in Fig. 2B, the number of cells that were able to grow on YPD plates in all three of the mutant cultures had dramatically decreased after only 4 h in galactose/raffinose. Compared to *dp1/y2* (which was unable to express VDAC1), all three mutants grew poorly over the entire 24-h incubation period (Fig. 2C). The three double mutants also were expressed in wild-type JHRY20-2Ca cells (i.e., containing an intact chromosomal gene for VDAC1). As can be seen in Fig. 3, the strains expressing mutant VDAC1 forms still were severely growth impaired compared to *wt/y2* and *wt/wt* (i.e., both chromosomal and epichromosomal expression of VDAC1).

The doubly mutated VDAC1 forms caused an overt growth defect that was more severe than disruption of the VDAC1 gene and independent of the presence of the chromosomal expression of wild-type VDAC1. Replacement of K234 and K236 in VDAC1 with another positively charged amino acid (arginine), a negatively charged amino acid (glutamate), or uncharged glutamine all caused the severe growth defect. It seemed likely that the toxicity was due to the absence of lysine rather than the nature of the amino acid that substituted for it.

Mutation of either K234 or K236 impairs the ability of VDAC1 to undergo membrane insertion *in vitro* (Smith *et al.*, 1995). VDAC1 mutants in which either K234 or K236 had been point mutated to glutamate were expressed in the *dp1* strain (*dp1/k234e* and *dp1/k236e*, respectively). The growth of these mutant strains in galactose/raffinose liquid cultures was compared to that of *dp1/y2* (Fig. 4). Over the first 16 h, *dp1/k234e* appeared to grow as well as *dp1/y2*; however, by 24 h, the number of growing cells had decreased. The *dp1/k236e* strain was less growth impaired, and at 8 and 16 h, it was growing faster than *dp1/y2*. By 24 h, however, the VDAC1-disrupted strain had outstripped *dp1/k236e*. These experiments show that mutation of either K234 or K236 was less toxic than mutating both and that mutation at K234 produced the more severe growth impairment. It should be pointed out, however, that mutation at either lysine eventually caused a more severe growth defect than disruption of the VDAC1 gene.

All of the mutations in VDAC1 that caused growth impairment *in vivo* have also been shown to interfere with the *in vitro* insertion of VDAC1 into mitochondria (Smith *et al.*, 1995). It seemed possible that any form of VDAC1 that was less insertion competent than wild type might cause the same type of growth defect. To test this argument, two VDAC1 mutants, Δ71-116 VDAC1 and K189E; K191E Δ71-116 VDAC1 were expressed in *dp1* (*dp1/d45* and *dp1/d45-2e*, respectively). The deletion of residues 71-116 in VDAC1 has been shown to impair but not prevent the membrane insertion of VDACl (Smith *et al.*, 1995). In K189E; K191E Δ71-116 VDAC1, the lysines that correspond to K234 and K236 in wild-type VDAC1 were mutated to glutamate. In liquid galactose/raffinose cultures (Fig. 5), *dp1/d45* grew about as well as *dp1/y2* until about 16 h when Δ71-116 VDAC1 began to complement the VDAC1 disruption. This finding indicated that impaired membrane insertion does not necessarily cause the negative growth phenotype. On the other hand, *dp1/d45-2e* was severely growth impaired by 16 and 24 h. It seemed that the absence of lysine at the positions equivalent to K234 and K236 was the cause of the growth defect.

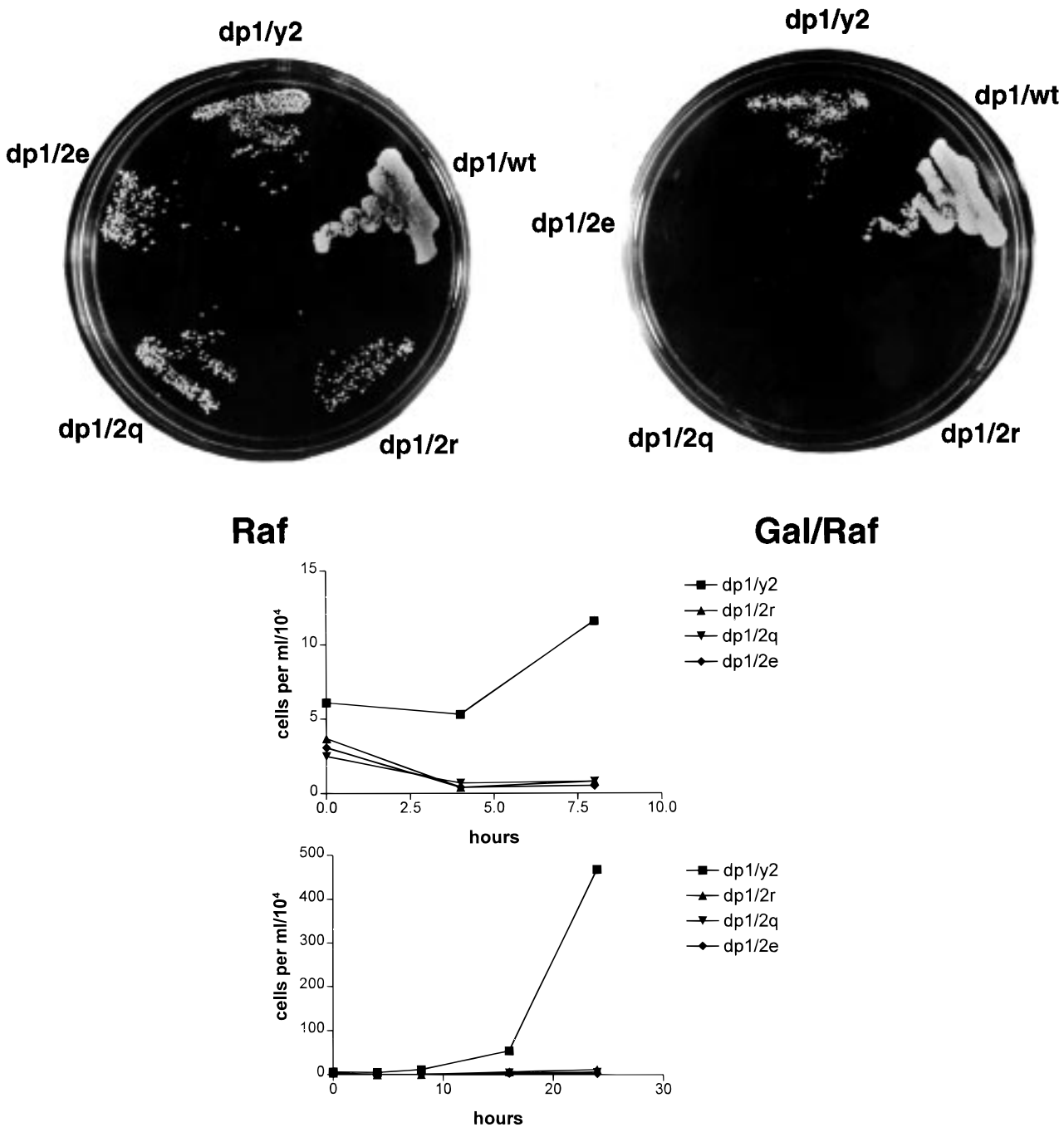



**Fig. 2.** The effect of the expression of VDAC1 mutated at both K234 and K236 on growth of *dp1* cells. (A) Cultures of *dp1/y2*, *dp1/2r*, *dp1/2q*, and *dp1/2e* were streaked on agarose plates containing minimal medium (minus uracil) containing 2% raffinose or 2% galactose–1% raffinose. The plates were incubated for 5 days at 30°C. (B) and (C) The same strains (initial cell density equal to $10^5$) were precultured in minimal medium (minus uracil) containing 2% raffinose for 4 h and then shifted to the same medium containing 2% galactose–1% raffinose. Portions were removed at the indicated times and applied to YPD plates. The number of colonies on these plates was used to calculate cell density.

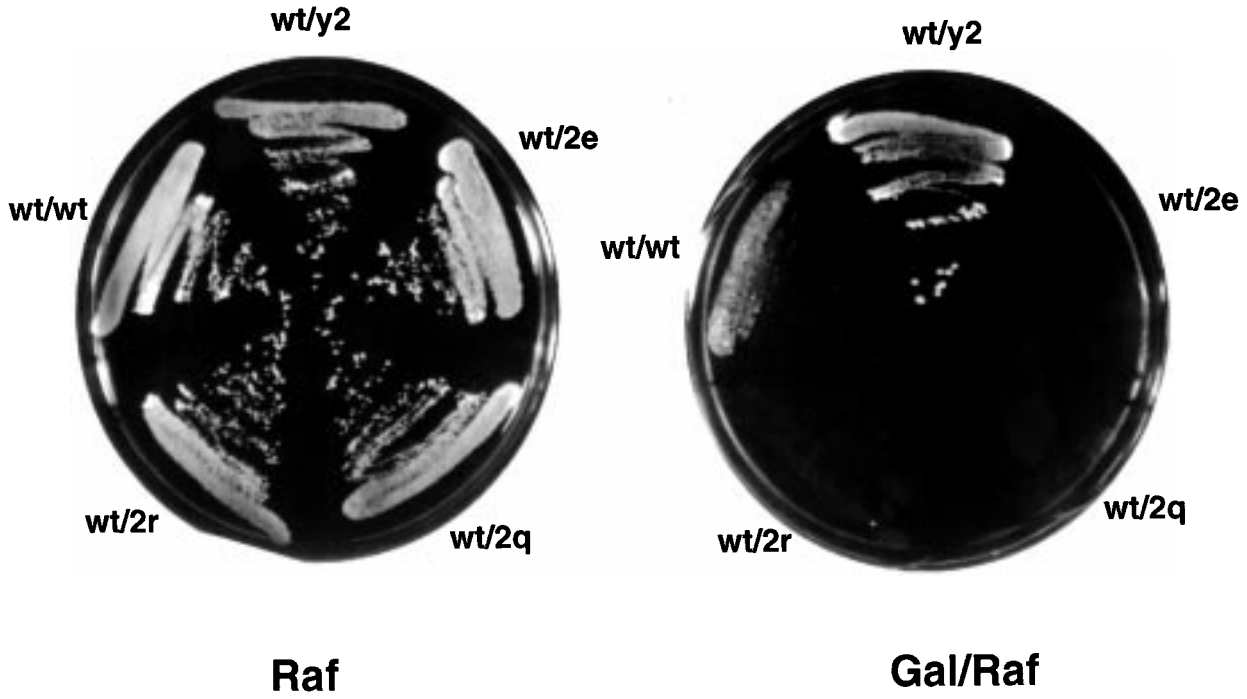



**Fig. 3.** The effect of the expression of VDAC1 mutated at both K234 and K236 on growth of *wt* cells. Cultures of *wt/y2*, *wt/wt*, *wt/2r*, *wt/2q*, and *wt/2e* were streaked on agarose plates containing minimal medium (minus uracil) containing 2% raffinose or 2% galactose–1% raffinose. The plates were incubated for 5 days at 30°C.

## The Growth-Impaired Phenotype Is Not Lethal

Cultures of *dp1/y2* (unable to express VDAC1), *dp1/wt* (expresses *wt* VDAC1), and each of the strains expressing doubly mutated VDAC1 (i.e., *dp1/2r, dp1/2q*, and *dp1/2e*) were grown on galactose/raffinose. After 4 and 16 h, samples were taken for electron microscopy. In Fig. 6 the only mutant shown is *dp1/2e*; however, all of the strains expressing doubly mutated VDAC1 forms were similar in appearance. The most striking difference among these cultures was that after 4 h, there were a number of *dp1/y2* and *dp1/2e* cells

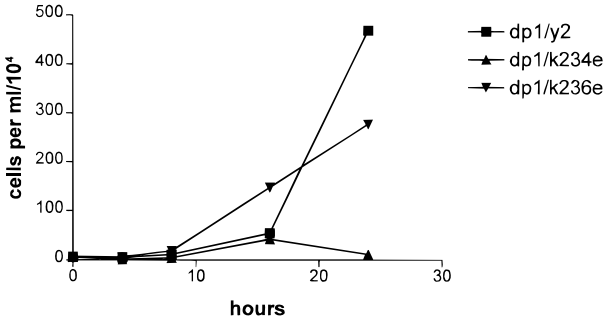



**Fig. 4.** The effect of the expression of VDAC1 mutated at either K234 and K236 on growth of *dp1* cells. Cultures of *dp1/y2, dp1/k234e*, and *dp1/k236e* (initial cell density equal to $10^5$) were precultured in minimal medium (minus uracil) containing 2% raffinose for 4 h and then shifted to the same medium containing 2% galactose–1% raffinose. Portions were removed at the indicated times and applied to YPD plates. The number of colonies on these plates was used to calculate cell density.

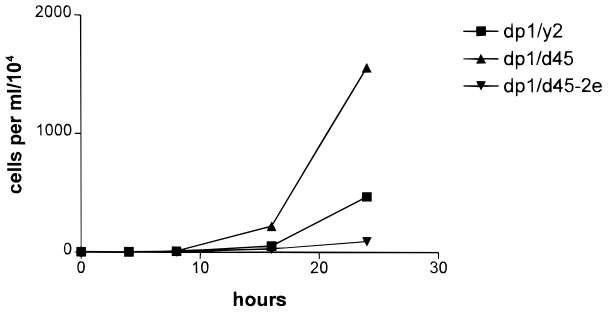
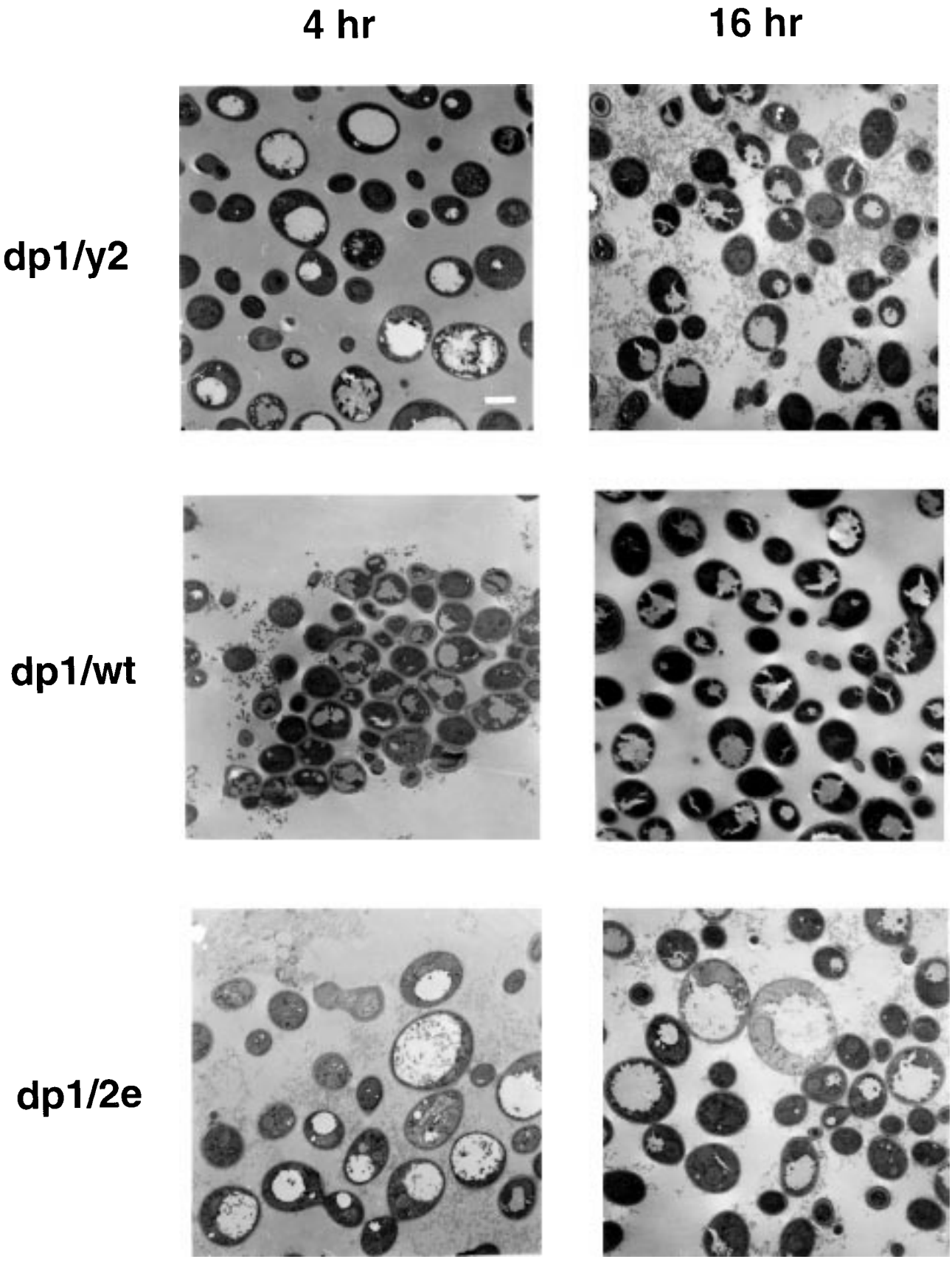



**Fig. 5.** The effect of the expression of Δ45 VDAC1 and K189E; K191E Δ45 VDAC1 on growth of *dp1* cells. Cultures of *dp1/y2, dp1/d45*, and *dp1/d45-2e* (initial cell density equal to $10^5$) were precultured in minimal medium (minus uracil) containing 2% raffinose for 4 h and then shifted to the same medium containing 2% galactose–1% raffinose. Portions were removed at the indicated times and applied to YPD plates. The number of colonies on these plates was used to calculate cell density.


4 hr
16 hr
dp1/y2
dp1/wt
dp1/2e

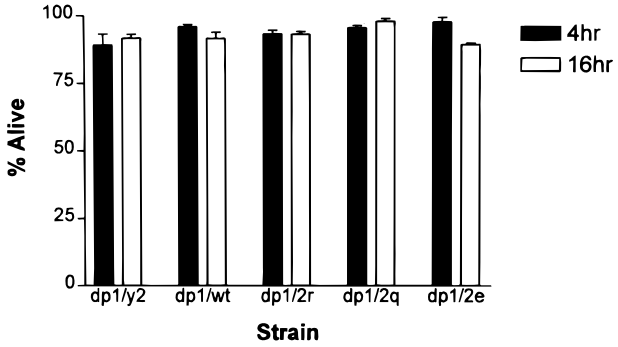



**Fig. 7.** The effect of the expression of VDAC1 mutated at both K234 and K236 on the uptake of the vital stain FUN1 by *dp1* cells. Cultures of *dp1/y2*, *dp1/k234e*, and *dp1/k236e* (initial cell density equal to $10^5$) were precultured in minimal medium (minus uracil) containing 2% raffinose for 4 h and then shifted to the same medium containing 2% galactose–1% raffinose. Portions were removed at the indicated times and incubated with FUN1. The fraction cells capable of accumulating and processing this stain was estimated by fluorescence microscopy. Each point is the average (+/− standard deviation) of five determinations.

that had large clear vacuoles. By 16 h, the *dp1/y2* cells had normally sized vacuoles, but a number of *dp1/2e* cells retained swollen vacuoles. The frequency of mitotic figures was between 5 and 7% in all three strains and there was no obvious morphological change that indicated that the growth defect occurred at a particular stage in the cell cycle.

The vacuolar changes in the strains with impaired growth seemed remarkable enough to suspect that the cells might be dead. To determine whether the growth impairment was lethal, cultures of *dp1/y2*, *dp1/wt*, and *dp1* cells expressing each of the doubly mutated VDAC1 forms were grown on galactose/raffinose for 4 and 16 h. These cultures were examined by fluorescent microscopy for the vacuolar accumulation and processing of the vital stain, FUN1. As can be seen in Fig. 7, about 90% of the cells in each of the strains were alive at either 4 or 16 h. It is clear that the growth defect did not have lethal consequences.

## DISCUSSION

Lysines at positions 234 and 236 in yeast VDAC1 (283 residues) play an important role in both *in vitro* and *in vivo* membrane insertion of yeast VDAC1 (Smith *et al.*, 1995, Angeles *et al.*, 1998). The effects of these mutations on the growth of *S. cerevisiae* were evaluated. Expression of VDAC1 forms in which both K234 and K236 had been point mutated to arginine, glutamine, or glutamate caused a growth impairment that was considerably more severe than that caused by disruption of the VDAC1 gene. A large reduction in the number of growing cells was evident within 4 h of the time that expression of the mutants was induced. The effect persisted for at least 24 h. The growth arrest was not specific for any stage of the cell cycle and the cells remained viable for at least 16 h after induction of the mutant VDAC1 forms. The fact that all three amino acids that were used to replace lysine caused an equally severe growth defect indicated that the absence of lysine, rather than the nature of the amino acid substitution, was the causative factor. The effect of the mutation of a single lysine was less than mutation of both. The onset of the growth defect also was delayed when only a single lysine was mutated. However, by 24 h after induction, strains expressing either of the single point mutants grew more slowly than the strain with a disruption of the VDAC1 gene. The importance of K234 and K236 in this toxicity seems to be hierarchical since expression of K234E VDAC1 caused an earlier onset and more severe toxicity than did expression of K236E VDAC1.

Although all of the above described VDAC1 mutants have been shown to be poorly inserted into the outer membrane, it is unlikely that failed translocation is sufficient to account for the growth toxicity. Expression of a 45 amino acid deletion mutant that has been shown to poorly insert into the outer membrane (Smith *et al.*, 1995), did not suppress growth. In fact, the deletion mutant, which is able to form electrophysiologically detectable channels (personal communication, M. Colombini), complemented the VDAC1 disruption. On the other hand, if the deletion mutant was further mutated so that the lysines equivalent to K234 and K236 were replaced with glutamate, the toxic phenotype was restored. These observations indicate that the growth toxicity is due to replacement of these particular lysines rather than to a general failure of membrane insertion.

**Fig. 6.** The effect of the expression of K234E; K236E VDAC1 on the morphology of *dp1* cells. Cultures of *dp1/y2*, *dp1/wt*, and *dp1/2e* (initial cell density equal to $10^5$) were precultured in minimal medium (minus uracil) containing 2% raffinose for 4 h and then shifted to the same medium containing 2% galactose–1% raffinose. Portions were removed at the indicated times and analyzed by electron microscopy. Magnification is the same in all six frames and the bar in the upper left hand frame represents 2 uM.

The mechanism of this unusual growth toxicity is not clear. Although the mutant VDAC1 forms were expressed using a 2u plasmid, overexpression is unlikely to be the cause of the growth defect. In fact, both mitochondrial and total cellular content of the toxic doubly mutated VDAC1 forms have been shown to be very low (Angeles *et al.*, 1998). Although the VDAC1 double mutants are localized in the mitochondria, it is unlikely that the toxicity is due to diminished channel function. In fact, the growth toxicity caused by the double mutants is dominant and more severe than that caused by disruption of the VDAC1 gene. Nor is it likely that the failure to grow is due to some other defect in mitochondrial energy metabolism. Cells that exhibit the growth defect are still able to accumulate the vital stain FUN1 in their vacuoles. This accumulation depends on an ATP-driven proton gradient across the vacuolar membrane as well as an intact cellular membrane potential. In addition, 4 h after induction of mutant VDAC1 forms, when only a small fraction of the cells can grow, the cells can still synthesize protein and catalyze the translocation of mitochondria precursor proteins to the matrix (Angeles *et al.*, 1998). Both processes are ATP dependent and the translocation reaction depends on an intact mitochondrial membrane potential.

It is more probable that the growth impairment is due to an overt toxicity of the mutant VDAC1 forms. It seems then that, aside from their importance in membrane insertion, K234 and K236 in yeast VDAC1 have another unknown role and that failure to perform this function has drastic consequences for the cell.

## ACKNOWLEDGMENTS

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