Diploids Heterozygous for a vma13Δ Mutation in Saccharomyces cerevisiae Highlight the Importance of V-ATPase Subunit Balance in Supporting Vacular Acidification and Silencing Cytosolic V1-ATPase Activity*

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The V-ATPase H subunit (encoded by the VMA13 gene) activates ATP-driven proton pumping in intact V-ATPase complexes and inhibits MgATPase activity in cytosolic V1 sectors (Parra, K. J., Keenan, K. L., and Kane, P. M. (2000) J. Biol. Chem. 275, 21761–21767). Yeast diploids heterozygous for a vma13Δ mutation show the pH- and calcium-dependent conditional lethality characteristic of mutants lacking V-ATPase activity, although they still contain one wild-type copy of VMA13. Vacuolar vesicles from this strain have ~50% of the ATPase activity of those from a wild-type diploid but do not support formation of a proton gradient. Compound heterozygotes with a second heterozygous deletion in another V1 subunit gene exhibit improved growth, vacuolar acidification, and ATP-driven proton transport in vacuolar vesicles. In contrast, compound heterozygotes with a second deletion in a V0 subunit grow even more poorly than the vma13Δ heterozygote, have very little vacuolar acidification, and have very low levels of V-ATPase subunits in isolated vacuoles. In addition, cytosolic V1 sectors from this strain and from the strain containing only the heterozygous vma13Δ mutation have elevated MgATPase activity. The results suggest that balancing levels of subunit H with those of other V-ATPase subunits is critical, both for allowing organelle acidification and for preventing unproductive hydrolysis of cytosolic ATP.

V-ATPases2 acidify multiple organelles in all eukaryotic cells and, through their role in organelle acidification, are linked to cellular functions, including protein sorting and degradation, ion homeostasis, and viral entry (1). In addition, certain polarized cells have high levels of apical plasma membrane V-ATPases that pump protons out of the cell; defects in specific plasma membrane V-ATPases have been linked to the human diseases distal renal tubule acidosis and osteopetrosis (1, 2). All eukaryotic V-ATPases consist of 13 or 14 different subunits assembled into a peripheral membrane sector containing the sites of ATP hydrolysis, V1, and a membrane sector containing the proton pore, V0 (1, 3). The individual subunit sequences and the overall subunit composition of the enzyme are very similar in fungi, plants, and animals and also bear considerable resemblance to the V- or A-type ATPases found in certain eubacteria and archaeabacteria (4, 5).

The yeast V-ATPase has emerged as the predominant model for eukaryotic V-ATPases, in part because complete loss of V-ATPase activity is conditionally lethal in fungi but is lethal in higher eukaryotes. Loss of V-ATPase activity in Saccharomyces cerevisiae results in the Vma− phenotype, characterized by sensitivity to elevated pH and calcium concentrations, inability to grow on nonfermentable carbon sources, and sensitivity to many heavy metals, along with an array of other phenotypes (3). Although they exhibit some growth defects under all conditions, yeast vma mutant strains grow optimally at pH 5 and fail to grow at pH 7.5 (6). This pH-dependent growth phenotype has permitted biochemical characterization of multiple loss of function mutations, ranging from full deletions to point mutations in individual subunit genes. Virtually all of these studies in yeast have been carried out in haploid cells in which the sole copy of V-ATPase subunit genes was deleted or mutated.

In addition to loss of function mutations, altering subunit ratios has been shown to result in a Vma− phenotype in certain cases. For example, overexpression of wild-type V1 subunit G in yeast destabilizes V1 subunit E, and results in a Vma− phenotype (7). Low level overexpression of subunits C and H also results in a Vma− phenotype, whereas high level overexpression of these subunits appeared to be lethal (8). These results suggest that balanced stoichiometry of certain V-ATPase subunits is critical to the cell, and perturbing this balance can be more detrimental than a simple loss of V-ATPase function would explain.

Disrupting subunit balance in the F-ATP synthase, the mitochondrial ATP synthase that is evolutionarily related to V-ATPases (9), can also have dramatic consequences. S. cerevisiae can tolerate loss of F-ATPase activity by relying solely on fermentative growth, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so mutants lacking F-ATPase activity can grow on fermentable, but not on nonfermentable, carbon sources, so
sources. However, diploid cells containing only one functional copy of the γ or δ subunit not only fail to grow on nonfermentable carbon sources but also exhibit a high rate of mitochondrial DNA loss that is not seen in heterozygous mutants lacking other subunits of the F-ATPase complex (10, 11). This suggests that a heterozygous deletion mutation in either of these subunits is semi-dominant negative and results in a “gain of function” that is detrimental to the cell. Further characterization of these heterozygous mutants demonstrated that the gain of function was proton leakage through partially assembled ATP synthase complexes in the mitochondrial membrane (11) and that this gain of function could be prevented by the addition of a second mutation that prevented assembly of partial complexes (10, 11).

*S. cerevisiae* can exist in both stable haploid and stable diploid states and thus is ideal for studying the consequences of subunit imbalance that might arise from mutating one of two copies of a subunit gene. Although many heterozygous diploids are indistinguishable from wild-type diploids, some heterozygous deletions, like the deletions in the ATP synthase δ subunits described above, have negative effects on growth. This phenomenon, called haploinsufficiency, was recently examined on a genomic scale in yeast and found to be characteristic of at least 184 different genes, many of which encode members of multimeric subunit complexes (12). Although no genome-wide assessment of haploinsufficiency has been possible in higher eukaryotes, recent work has clearly established that haploinsufficiency in a number of different genes is associated with disease (13–16).

In this work, we characterize heterozygous diploids lacking a single copy of several different V-ATPase subunit genes. We find that loss of one copy of the V₁ H subunit gene (VMA13) is particularly detrimental to growth of heterozygous diploid cells and that growth of these cells can be either improved or worsened by a heterozygous deletion in a second subunit gene. We propose that these growth phenotypes arise from a functionally destructive subunit imbalance in both V-ATPase complexes assembled at the vacuole and cytosolic V₁ complexes.

**EXPERIMENTAL PROCEDURES**

**Materials and Media**—Concanamycin A was purchased from Werner Bioagents. 5-Fluoroorotic acid was purchased from Wako Biochemicals, and Zymolyase-100T was obtained from Sigma. ClonNat was purchased from Open Biosystems, and their genotypes are shown in Table 1. The BY4741 vma13Δ::kanMX MATα strain was created by switching the *natr* marker to library *vma* deletion strains (BY4741 vma13Δ::kanMX strains) and selecting for antibiotic resistance to both clonNat and G418 at 200 μg/ml each. The BY4742 vma13Δ::natr strain was created by switching the *kanMX* marker in the BY4742 vma13Δ::kanMX mutant to a *natr* marker as described by Tong et al. (20). The genotypes of these and other strains cited are shown in Table 1.

Genetic instability proved to be a problem for a number of the diploids, particularly the *vma13Δ* × WT and *vma13Δ* × *vma3Δ* diploids, which had the most severe growth defects. Although freshly prepared diploids had consistent growth and biochemical phenotypes, the behavior of these strains changed over time, potentially as a result of selection for mutations that minimized the growth defects. To control this problem of genetic drift, we frequently selected for diploids on a double antibiotic selection and also retained a complementing *VMA13* plasmid in the strains for as long as possible. In order to easily maintain the *vma13Δ* × WT strain under a double antibiotic (diploid) selection, we crossed the BY4742 *vma13Δ::natr* strain to a *kanMX*-marked wild-type partner. The BY4741 ydr029wΔ::kanMX strain served as a marked wild-type strain to allow for double selection of our compound heterozygous mutants. The *YDR029W* open reading frame is designated as a dubious open reading frame, and the haploid *ydr029w* deletion has no known phenotype; freshly prepared *vma13Δ* × WT and *vma13Δ* × *ydr029wΔ* diploids also had indistinguishable phenotypes. As a further protection against genetic drift, all heterozygous *vma13Δ* strains were maintained on SD-uracil with the pRS316-N-myc-*VMA13* plasmid to complement the *vma13Δ* mutation until just before each experiment. Before each experiment, the strains were transferred to medium containing 5-fluoroorotic acid, a counterselection against the *URA3* marker, to select for plasmid loss. Cells that grew on plates containing 5-fluoroorotic acid had lost the plasmid and their ability to grow on SD-uracil and were then used for biochemical experiments. Genetic instability in diploid yeast strains that exhibit haploinsufficiency has been described previously (10).

**Biochemical Methods**—Vacuoles were obtained as described previously by Roberts et al. (21), except that strains containing plasmids were grown in supplemented minimal medium to maintain the plasmid prior to lysis, the cells were grown to an *A*₅₆₀ of ~1.0 before harvesting, and diploids without plasmid were grown in YEPD buffered to pH 5. Yeast whole cell lysates and vacuolar vesicles were prepared for SDS-PAGE, and vacuolar proteins were detected by immunoblotting as described previously using mouse monoclonal antibodies 8B1, 13D11, 7A2, and 10D7, monoclonal antibodies against the A, B, C, and subunits, respectively, of the V-ATPase or monoclonal anti-
body 1D3 against yeast alkaline phosphatase (22, 23). Western blot signals were quantitated used ImageJ (National Institutes of Health). The ratio of signals for V₀ subunit Vph1p and alkaline phosphatase, a vacuolar membrane protein that traffics to the vacuole independently from the V-ATPase, was calculated as a measure of V₀ trafficking to the vacuole. Levels of the V₁ A (catalytic) subunit were quantitated from 2-μg samples of vacuolar protein loaded for each strain.

Protein concentrations were determined by a Lowry assay. ATP hydrolysis rates were assessed using a coupled enzyme assay at 37 °C, as described by Lotscher et al. (24). Proton pumping was measured on a SPEX Fluorolog-3-21 fluorometer by the ACMA quenching assay, as described by Shao and Forgac (25). Concanamycin A-sensitive ATPase activity in vacuolar vesicles was assayed exactly as described previously (22).

Purification of Cytosolic V₁ Complexes—Dilipid strains containing a plasmid-borne copy of FLAG-tagged VMA10 were constructed to allow for isolation of cytosolic V-ATPase complexes in each mutant analyzed. Their genotypes are shown in Table 1. All diploid strains were constructed using antibiotic diploid selection and/or dissection microscopy as described above. Each strain contained one copy of FLAG-tagged VMA10 on a CEN plasmid and one genomic wild-type copy of VMA10. Therefore, FLAG affinity chromatography allowed only a sampling of free V₁ populations present in vivo, not an absolute assessment of the amount of V₁ present.

V₁ complexes were isolated via FLAG affinity chromatography in an abbreviated version of our previous protocol (26). FLAG-tagged cells were grown overnight to midlog phase (1–3 A₆₀₀ units/ml) in SD-Leu medium to select for plasmid. Log phase cells (2000 – 6000 A₆₀₀ units total) were harvested by centrifugation at 4000 x g for 10 min and resuspended in 100 ml of 50 mM Tris-HCl, pH 7.5, 1.2 M sorbitol, 2% glucose, 1% β-mercaptoethanol solution. Cells were converted to spheroplasts by adding zymolyase 100T (50 units/g of cells) to the cell suspension and gently shaking at 30 °C for 20 min. Spheroplasts were then washed twice with 300 ml of YEPD medium containing 1.2 M sorbitol.

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### Table 1: Genotypes of yeast strains used

| Strain name (name in text if different) | Genotype | Source |
|-----------------------------------------|----------|--------|
| BY4741                                  | MATαhis3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | OpenBiosystems |
| BY4742                                  | MATαhis3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 | OpenBiosystems |
| BY4743 (WT diploid)                     | MATα/MAAα his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/ura3Δ0 | OpenBiosystems |
| BY4744 vma13Δ::kanMX (vma13Δ::kanMX)    | MATα his3Δ1 leu2Δ0 met15Δ0 vma3Δ::kanMX | OpenBiosystems |
| BY4745 vma3Δ::kanMX                     | MATα his3Δ1 leu2Δ0 met15Δ0 vma3Δ::kanMX | OpenBiosystems |
| BY4746 vma4Δ::kanMX                     | MATα his3Δ1 leu2Δ0 met15Δ0 vma4Δ::kanMX | OpenBiosystems |
| BY4747 vma5Δ::kanMX                     | MATα his3Δ1 leu2Δ0 met15Δ0 vma5Δ::kanMX | OpenBiosystems |
| BY4748 vma8Δ::kanMX                     | MATα his3Δ1 leu2Δ0 met15Δ0 vma8Δ::kanMX | OpenBiosystems |
| BY4749 vma10Δ::kanMX                    | MATα his3Δ1 leu2Δ0 met15Δ0 vma10Δ::kanMX | OpenBiosystems |
| BY4750 vma13Δ::kanMX                    | MATα his3Δ1 leu2Δ0 met15Δ0 vma13Δ::kanMX | OpenBiosystems |
| BY4751 vma13Δ::nat/VMA3 vma10Δ::VMA1    | MATα his3Δ1 nat/VMA3 vma10Δ::VMA1 | OpenBiosystems |
| BY4752 vma13Δ::nat/VMA13 (vma13Δ::WT)   | MATα his3Δ1 nat/VMA13 vma10Δ::VMA1 | OpenBiosystems |
| BY4753 vma13Δ::VMA10 (vma13Δ::WT)       | Same as BY4743 except vma10Δ::kanMX/VMA13 | This study |
| BY4754 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::kanMX/VMA13 | This study |
| BY4755 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4756 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4757 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4758 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4759 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4760 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4761 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4762 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4763 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4764 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4765 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4766 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4767 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4768 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4769 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4770 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4771 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4772 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4773 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4774 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
| BY4775 vma13Δ::VMA13 (vma13Δ::VMA13)    | Same as BY4743 except vma13Δ::nat/VMA13 vma10Δ::VMA1 | This study |
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ugal filter device kit by Millipore (final protein concentration was −5 mg/ml). The concentrated high speed supernatant samples were then run twice through an anti-FLAG-M2 affinity column (~0.5-ml bed volume), and FLAG-tagged \( V_1 \) was subsequently eluted with 2.5 ml of 100 \( \mu \)g/ml FLAG peptide in TBSE.

ATPase activity of isolated fractions was analyzed as described previously; however, N-ethylmaleimide (NEM) was used as a specific inhibitor of the \( V_1 \)-ATPase (27). Inhibition by 50 \( \mu \)M NEM of free \( V_1 \) ATPase activities was determined by mixing ~10 \( \mu \)g of protein from column fractions with 50 \( \mu \)M N-ethylmaleimide and incubating for 20 min on ice. This mixture was then added to the ATPase assay mixture, and hydrolysis rates were determined as described above. Almost all MgATPase activity eluted in the first two 0.5-ml fractions collected after the addition of FLAG peptide. Fractions containing ATPase activity were also analyzed for the presence of \( V_1 \) subunits by Western blotting and Coomassie Blue staining (7).

Quinacrine Staining and Vacuolar pH Measurement in Whole Cells—Quinacrine labeling was performed as described (22), and labeled cells were observed within 10 min of labeling. Quinacrine fluorescence was observed using a Zeiss Axioskop II fluorescence microscope equipped with a Hamamatsu ORCA CCD camera. Vacular \( \text{pH} \) was quantitated using the 2',7'-bis-(2-carboxymethyl)-5-(and 6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) labeling method described by Ali et al. (28). Briefly, cells were grown to log phase in YEPD, pH 5, medium and collected by centrifugation, and 100 mg of cell mass was resuspended in 100 \( \mu \)l of the same medium. The \( \text{pH} \)-sensitive ratiometric dye BCECF-AM (Invitrogen) was added to a final concentration of 50 \( \mu \)M, and cells were incubated for 30 min at 30 °C with shaking. The cells were then washed two or three times to remove the dye and resuspended in YEPD, pH 5, at 1 g of cell mass/ml; 10−15 \( \mu \)l of this suspension was combined with 2 ml of 1 mM MES-triethanolamine, pH 5, for fluorescence measurements. Fluorescence intensity at excitation wavelengths of 450 and 490 nm was measured in triplicate for each sample at a constant emission wavelength of 535 nm in a SPEX Fluorolog-3-21 fluorometer. Calibration of fluorescence with pH was carried out for each strain as described by Ali et al. (28).

RESULTS

Haploinsufficiency of VMA Genes—In order to test for the possibility of haploinsufficiency of VMA (vacuolar membrane ATPase) genes, we initially mated three different haploid \( \text{vma} \) mutants, \( \text{vma}3\Delta, \text{vma}10\Delta, \) and \( \text{vma}13\Delta, \) to a congenic haploid wild-type strain of the opposite mating type, identified zygotes under a dissecting microscope, and then grew up the zygotes as heterozygous diploids. Haploid yeast cells have previously been shown to be sensitive to the copy number of \( \text{VMA}10 \), encoding the G subunit, and \( \text{VMA}13 \), encoding the H subunit (7, 8). \( \text{VMA}3 \) was included, because it is believed to be present at the highest stoichiometry in the assembled V-ATPase and thus might be sensitive to a loss in gene copy number (29). As shown by growth of the serial dilutions in Fig. 1A, the \( \text{vma}13\Delta \times \text{WT} \) heterozygous diploid consistently grew poorly on medium buffered to pH 7.5 and failed to grow on medium buffered to pH 7.5 containing 60 mM CaCl\(_2\). Loss of V-ATPase activity results in poor growth at elevated pH and/or elevated calcium concentration (3, 6, 30). These results suggest a pronounced haploinsufficiency in the \( \text{vma}13\Delta \) heterozygote that is not present in the heterozygotes lacking a single copy of the \( \text{VMA}3 \) or \( \text{VMA}10 \) genes (Fig. 1A).

We next asked whether reducing the copy number of a second \( \text{VMA} \) gene would modify the growth phenotypes of a \( \text{vma}13\Delta \times \text{WT} \) heterozygote. A \( \text{vma}13\Delta \) haploid strain was mated to strains of the opposite mating type containing deletions in several different V-ATPase subunits to form compound heterozygous diploids that lack one copy of each of two different V-ATPase subunit genes. The growth of several compound heterozygotes (designated as \( \text{vma}13\Delta \times \text{vma} \)) is shown in Fig. 1B. \( \text{VMA}1, \text{VMA}2, \text{VMA}4, \text{VMA}5, \text{VMA}8, \) and \( \text{VMA}10 \) all encode subunits of the peripheral \( \text{V}_1 \) sector of the V-ATPase (3). Notably, compound heterozygotes combining deletions in these genes with a \( \text{VMA}13 \) deletion grow better than the \( \text{vma}13\Delta \times \text{WT} \) heterozygote (Fig. 1B) at pH 7.5, both with and without added calcium. In contrast, a compound heterozygote combining the \( \text{vma}13\Delta \) mutation and a \( \text{vma}3\Delta \) mutation (\( \text{vma}13\Delta \times \text{vma}3\Delta \)) consistently showed a more severe growth phenotype than the simple \( \text{vma}13\Delta \times \text{WT} \) heterozygote. This suggests that loss of one copy each of the \( \text{VMA}13 \) and the membrane-bound \( \text{V}_1 \) subunit \( \text{VMA}3 \) results in a synthetic haploinsufficiency, in which the
compound heterozygote exhibits growth defects more severe than either of the single heterozygotes.

We asked whether the growth phenotypes for the simple and compound heterozygotes of the vma13Δ mutant were reflections of a loss of vacuolar acidification in vivo by assessing accumulation of the fluorescent lysosomotropic amine quinacrine. Quinacrine is able to cross membranes in its uncharged form but becomes protonated and retained in acidic compartments. As shown in Fig. 2, most vacuoles in wild-type diploid cells are stained with quinacrine. Quinacrine staining is reduced in a vma13Δ × WT heterozygous diploid, but the addition of a plasmid-borne copy of the wild-type VMA13 gene to this strain increases quinacrine accumulation to wild-type levels, confirming that the acidification defect arises from loss of the H subunit. Quinacrine staining in the compound heterozygote vma13Δ × vma2Δ was brighter than in the simple vma13Δ × WT heterozygote, and there was no quinacrine uptake at all in the vma13Δ × vma3Δ mutant, consistent with its severe growth phenotype. The vma3Δ × WT simple heterozygote showed an intermediate staining level. Overall, the quinacrine uptake experiments are consistent with the pattern of growth, but these experiments are difficult to quantitate.

In order to provide more quantitative measurement of the acidification defect in the vma13Δ × WT strain, we measured vacuolar pH in cells grown at pH 5 by the BCECF labeling method described by Ali et al. (28). As shown in Fig. 2B, the wild-type diploid grown at pH 5 had a vacuolar pH of 5.60 ± 0.07, consistent with previous measurements of vacuolar pH in haploid wild-type cells (28, 31). In contrast, the vma13Δ × WT diploid had a vacuolar pH of 6.08 ± 0.11, consistent with the defect in quinacrine uptake suggested by Fig. 2A. Interestingly, the vma2Δ mutation does appear to restore wild-type vacuolar pH in the vma13Δ × vma2Δ heterozygote, and the vma3Δ mutation further increases the vacuolar pH in the vma13Δ × vma3Δ heterozygote.

**Biochemical Basis of Haploinsufficiency**—The V-ATPase H subunit occupies a unique position in the enzyme. In strains lacking this subunit, V1V0 complexes assemble but are inactive and unstable, suggesting that the H subunit serves as an activator of the intact V-ATPase (32). In contrast, soluble V1 sectors lacking subunit H are active in MgATP hydrolysis, unlike V1 sectors containing subunit H; this suggests an inhibitory role for the H subunit in free V1 sectors (27). Both loss of ATP-driven proton pumping in intact V-ATPases and a gain in MgATP hydrolysis in V1 sectors could contribute to the phenotypes of the vma13Δ × WT heterozygote or the vma13Δ × vma3Δ compound heterozygote. In order to further investigate these possibilities, we biochemically characterized both the intact V-ATPase in vacuolar membranes and cytosolic V1 sectors in the different haploid and diploid strains.

Vacuolar vesicles were isolated from a wild-type diploid strain, from the simple vma13Δ × WT and vma3Δ × WT heterozygotes, and from compound heterozygotes vma13Δ × vma2Δ and vma13Δ × vma3Δ. (As described above, the compound heterozygotes have one wild-type and one mutant copy of each gene.) The concanamycin A-sensitive V-ATPase activity measured in vacuolar vesicles from each strain is shown in Table 2. (Concanamycin A is a highly specific inhibitor of V-ATPases (33).) The wild-type diploid strain and the vma3Δ × wild-type heterozygote vesicles had very similar levels of ATPase activity. The vma13Δ × WT and vma13Δ × vma2Δ vesicles also had very similar activities, approximately half the specific activity of the wild-type vesicles. In contrast,
the \( \text{vma13} \times \text{vma3} \) vesicles had almost no V-ATPase activity.

We also examined the levels of the vacuolar membrane marker alkaline phosphatase (ALP), \( V_a \) subunit a, and \( V_1 \) subunits A, B, and C in the isolated vacuolar vesicles. Western blots for each of these vacuolar proteins are shown in Fig. 3. ALP generally runs as a doublet, and the presence of ALP in all of the strains indicates that we were able to obtain vacuolar membranes in each case. There is a higher molecular mass band in the \( \text{vma13} \times \text{vma3} \) lane in Fig. 3A that corresponds to unprocessed ALP (34). Vacuolar protease activity is reduced in cells lacking all V-ATPase activity; thus, a partial loss of ALP processing is consistent with the complete lack of quinacrine accumulation in these cells (23). \( V_a \) subunit a is also present in all of the vacuolar vesicle preparations, but there is a very pronounced reduction of the level in the \( \text{vma13} \times \text{vma3} \) vesicles. In order to compare the level of the \( V_a \) subunit present in the vesicles for the different strains, we normalized the signal for \( V_a \) subunit \( V_{\text{phlp}} \) to that of ALP, a vacuolar membrane protein that traffics to the vacuole independent of the V-ATPase. All of the strains had \( V_{\text{phlp}}/\text{ALP} \) ratios within 17% of wild type except for the \( \text{vma13} \times \text{vma3} \) strain, which had a \( V_{\text{phlp}}/\text{ALP} \) ratio only 33% that of wild-type cells. \( V_a \) subunit A is the catalytic subunit for ATP hydrolysis, which forms the catalytic headgroup of the enzyme with subunit B. 2 and 5 \( \mu \)g of vesicles from each strain were loaded to facilitate comparison of the levels of the A and B subunits in different strains (Fig. 3B). Quantitation of A subunit levels in the 2-\( \mu \)g samples for each vesicle preparation in Fig. 3B indicates that the WT diploid, \( \text{vma3} \times \text{WT} \) diploid, and the \( \text{vma13} \times \text{WT} \) diploid assemble comparable levels of the catalytic subunit on the vacuolar membrane. Somewhat lower levels of the A subunit (72% wild type) were present in the \( \text{vma13} \times \text{vma2} \) vacuoles. Surprisingly, the \( \text{vma13} \times \text{vma3} \) vacuolar vesicles had no measurable A subunit, although we had anticipated that the remaining wild-type copies of these two genes would provide sufficient H and c subunit for assembly of at least some V-ATPase complexes, and there are some \( V_a \) sectors available (Fig. 3A).

Comparison of the levels of ATCase activity in Table 2 with the levels of catalytic subunit present in the membranes in Fig. 3, A and B, suggests that the vacuoles from the \( \text{vma3} \times \text{WT} \) heterozygote are almost indistinguishable from vacuoles obtained from the wild-type diploid. Lower levels of \( V_1 \) assembly may account in part for the lower level of ATCase activity in the \( \text{vma13} \times \text{vma2} \) mutant vacuoles but cannot account for the lower activity in the \( \text{vma13} \times \text{WT} \) mutant vacuoles. Finally, the very low levels of activity in the \( \text{vma13} \times \text{vma3} \) vacuoles are consistent with the absence of the catalytic subunit in vacuoles from this mutant.

In order to address whether the A and B subunits were present in the various diploid strains, particularly the \( \text{vma13} \times \text{vma3} \) strain, we prepared whole cell lysates from the indicated strains, separated proteins by SDS-PAGE, and assessed the levels of the A and B subunits on Western blots. As shown in Fig. 3C, all of the strains, including the \( \text{vma13} \times \text{vma3} \) diploid, contain the A and B subunits. As expected, the \( \text{vma13} \times \text{vma2} \) strain, which has only one copy of the B subunit gene (VMA2) has reduced levels of the B subunit. In addition, quantitation of A subunit levels in the \( \text{vma13} \times \text{vma3} \) lysates demonstrated that these cells have only 41% as much A subunit as the wild-type diploid, but this cannot account for the almost total absence of \( V_1 \) subunits from vacuolar vesicles.

We next used an ACMA fluorescence quenching assay to assess ATP-driven proton pumping activity in vacuolar vesicle preparations from the various mutants. In each assay, vesicles were equilibrated with the dye, and MgATP was added at the indicated point to initiate proton pumping. After ~2 min, the V-ATPase inhibitor concanamycin A (100 nM) was added; this generally restores the fluorescence to near initial levels, because continued proton pumping is required to maintain the proton gradient responsible for the ACMA quenching (22). In addition, a second set of measurements is performed for each sample, in which 100 nM concanamycin A is added at the beginning of the assay, before MgATP; these measurements control for changes in fluorescence derived only from the addition of MgATP. As shown in Fig. 4A, the initial rate and extent of ACMA quenching in the wild-type and \( \text{vma3} \) wild-type diploids was very similar. This is consistent with the very similar ATCase activities between the strains and indicates that the coupling ratio, defined as the ratio between the ATCase activity and the initial rate of proton pumping, is the same in vesicles from these two strains. Fig. 4B compares the proton pumping of wild-type diploid and the \( \text{vma3} \) diploids. Both the initial rate and the extent of proton pumping are reduced relative to wild-type in the \( \text{vma13} \times \text{vma2} \) vesicles. However, because the ATCase activity is also reduced, the coupling ratio of the \( \text{vma13} \times \text{vma2} \) vesicles proved to be virtually identical to the wild-type ratio. ACMA quenching assays for the \( \text{vma13} \times \text{WT} \) and \( \text{vma3} \) vesicles are shown in Fig. 4C. As expected, the \( \text{vma13} \times \text{vma3} \) diploid vesicles show no proton pumping, consistent with a complete lack of ATP hydrolysis activity. Unexpectedly, there was also no pumping in the \( \text{vma13} \times \text{WT} \) vesicle preparations, although there is ATP hydrolysis in these vesicles. We were never able to observe any ACMA quenching in these vesicles. There is evidence of a small amount of vacuolar acidification in vivo for this strain Fig. 2B, although we never observed any ACMA fluorescence quenching in vitro. This may suggest that either the V-ATCase or some other mechanism is

| Strain                     | V-ATCase-specific activity | \( \mu \text{mol/min/mg protein} \) |
|----------------------------|---------------------------|----------------------------------|
| Wild-type diploid          |                           | \( 1.17 \pm 0.17 \) (\( n = 3 \)) |
| \( \text{vma13} \times \text{WT} \) |                           | \( 0.58 \pm 0.09 \) (\( n = 4 \)) |
| \( \text{vma13} \times \text{vma2} \) |                           | \( 0.54 \pm 0.06 \) (\( n = 4 \)) |
| \( \text{vma13} \times \text{vma3} \) |                           | \( 0.03 \pm 0.02 \) (\( n = 4 \)) |
| \( \text{vma3} \times \text{WT} \) |                           | \( 1.09 \pm 0.27 \) (\( n = 4 \)) |
FIGURE 3. Western blots to detect V-ATPase subunit levels in vacuolar vesicles and whole cell lysates from single and compound heterozygotes. A and B, vacuolar vesicles were isolated from the indicated strains as described under “Experimental Procedures.” Vacular vesicle proteins were solubilized, separated by SDS-PAGE, and transferred to nitrocellulose blots. The blots were probed with antibodies to the vacuolar membrane protein ALP as well as V-ATPase subunits a (part of the Vo sector) and C, A, and B (part of the V1 sector). 20 μg of vacular protein from each strain was loaded for detection of the proteins shown in A, B, 5 and 2 μg of vesicle protein was loaded for each strain in order to facilitate comparison of subunit levels in the different strains. The blots shown are representative of results from all of the vacuolar preps summarized in Table 2. Levels of ALP, Vo subunit a (Vph1p), and V1 subunit A in A and B were quantitated using ImageJ. (The 2-μg samples were used for A subunit comparisons, because quantitation of the 5-μg samples suggested that the signals were nearing saturation.) The ratio of Vo subunit a to ALP was determined for each sample and normalized to the level in the wild-type vacuoles for comparison. 

C. whole cell lysates were prepared from the indicated strains. Lysate protein from equivalent cell numbers was separated by SDS-PAGE, transferred to a blot, and probed for the presence of V-ATPase subunits. 

DISCUSSION

Haploinsufficiency is defined as a dominant phenotype in a diploid organism that is heterozygous for a loss of function allele (12). The experiments described above reveal a clear haploinsufficiency for the VMA13 gene, which encodes V-ATPase subunit H. We initially identified a Vma-like growth defect in a haploinsufficient mutant, and subsequent characterization revealed multiple biological and functional defects. The experiments described above reveal a clear haploinsufficiency for the VMA13 gene, which encodes V-ATPase subunit H. We initially identified a Vma-like growth defect in a haploinsufficient mutant, and subsequent characterization revealed multiple biological and functional defects.
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biochemical defects, including reduced vacuolar acidification in vivo, reduced concanamycin-sensitive ATPase activity at the vacuole, apparent uncoupling of ATP hydrolysis and proton pumping in isolated vacuoles, and increased MgATPase activity in free V₁ complexes. Given the importance of the H subunit as a structural and functional regulator of the enzyme and the previously demonstrated sensitivity to subunit H overexpression, some dosage sensitivity was not completely unexpected. However, the extent of the defects in the vma13Δ copy of the subunit gene.

The phenotypes of compound heterozygotes produced from crossing haploid vma13Δ mutants to haploid knockouts in various other VMA genes helped to clarify the underlying biochemical basis for VMA13 haploinsufficiency. Creating an imbalance of both subunit H (Vma13p) and subunit c (Vma3p) in the vma13Δ x vma3Δ diploid exacerbated all of the haploinsufficiency phenotypes seen in the vma13Δ x wild-type diploid. In the vma13Δ x vma3Δ compound heterozygote, growth on all media tested was further slowed, and there was no vacuolar acidification in vivo and no ATPase activity or proton pumping in isolated vacuoles, along with greatly reduced V-ATPase subunit levels in isolated vacuoles and increased cytosolic MgATPase activity. In contrast, the vma13Δ x vma2Δ compound heterozygote showed improvement in every regard over the vma13Δ x wild-type diploid. In addition to a restoration of growth at elevated pH (which was seen in crosses of vma13Δ haploids to all of the V₁ subunit deletions tested in Fig. 1B), the vma13Δ x vma2Δ compound heterozygote showed better in vivo acidification and significantly improved coupling of ATP hydrolysis and proton pumping in isolated vacuoles, although the ATPase activity was similar to that of the vma13Δ x wild-type heterozygote. In addition, there were lower levels of MgATPase in free V₁ sectors in the vma13Δ x vma2Δ mutant. These results suggest that altering the balance of both the H subunit and the B subunit (Vma2p) helps to counteract the effects of subunit H imbalance, perhaps by reducing the population of deleterious complexes, both cytosolic and vacuolar, responsible for the different haploinsufficiency phenotypes.

We hypothesize that the biological consequences of loss of one copy of VMA13 in a heterozygous diploid arise from a combination of 1) loss of vacuolar acidification and possibly uncoupling of ATP hydrolysis and proton pumping at the vacuole and 2) a gain in MgATPase activity in cytosolic V₁ sectors. The model shown in Fig. 6 depicts the different complexes that might form in wild-type and the heterozygous diploid deletion strains lacking subunit H and their potential contribution to the overall phenotypes in the different strains. The H subunit appears to act as a “switch” in V-ATPases, capable of activating ATP-coupled proton transport in intact V₁V₀ complexes at the vacuolar membrane and inhibiting MgATPase activity in free V₁ sectors. Because loss of the H subunit prevents ATPase activity in the intact enzyme but does not prevent assembly of other V-ATPase subunits in isolated vacuoles (32), we would predict that vacuolar membranes from the vma13Δ x WT strain contain both fully intact V-ATPase complexes and complexes lacking subunit H. Previous results indicate that the

FIGURE 5. MgATPase activity in cytosolic V₁, fractions from WT diploids and simple and compound heterozygotes. V₁ sectors were isolated from cytosolic fractions derived from the indicated diploid strains. Each strain contained a FLAG-tagged G subunit, which allowed for isolation of V₁ sectors by anti-FLAG affinity chromatography, as described under “Experimental Procedures.” Fractions were collected after the addition of FLAG peptide to elute bound protein, and each fraction was assayed for MgATPase activity in the presence and absence of 50 μM N-ethylmaleimide, a specific inhibitor of V₁-ATPase activity. 20 μg of protein from fraction 1, which contained the highest level of V₁ subunits and MgATPase activity (see “Experimental Procedures”), was separated by SDS-PAGE, transferred to nitrocellulose, and probed for the presence of V₁ subunits A and B to confirm the presence of V₁ in the fraction. The specific NEM-sensitive ATPase activity (V₁-ATPase activity) for each fraction is shown below the blot. 2–5 independent V₁ purifications were done from each strain.

FIGURE 4. ATP-driven proton pumping in vacuolar vesicles isolated from wild-type and heterozygous mutant diploids. For each set of plots, vacuolar vesicles were mixed with 1 μM ACMA in transport buffer (50 mM NaCl, 30 mM KCl, 20 mM HEPES, pH 7) in a fluorometer cuvette. Fluorescence emission intensity was monitored continuously as the mixture was stirred at 25 °C, and at the points marked MgATP, a mixture of MgSO₄ and ATP was added to give final concentrations of 1 and 0.5 mM, respectively. After the fluorescence decrease had stabilized, concanamycin A was added to a final concentration of 100 nM. For each experiment, a parallel control, in which 100 nM concanamycin A was present throughout the assay, is included to evaluate fluorescence changes not due to proton pumping. A, comparison of vacuolar vesicles from the wild-type diploid and vma3Δ x WT heterozygous diploid. 35 μg of vacuolar vesicles were used in each assay. Plots are as follows: wild-type diploid vesicles (dark blue, experiment; pink, control), vma3Δ x WT vesicles (orange, experiment; light blue, control). B, comparison of vacuolar vesicles from the wild-type diploid and vma13Δ x vma2Δ compound heterozygote. 14 μg of vacuolar vesicles were assayed. Plots are as follows: wild-type diploid vesicles (dark blue, experiment; light blue, control), vma13Δ x vma2Δ vesicles (orange, experiment; pink, control). C, comparison of vacuolar vesicles from vma13Δ x WT and vma13Δ x vma3Δ. 14 μg of vacuolar vesicles were assayed. Plots are as follows: vma13Δ x WT (dark blue, experiment; light blue, control), vma13Δ x vma3Δ (orange, experimental; pink, control). The addition of MgATP to the control samples was delayed to allow better separation of the data for this experiment. Similar proton pumping measurements were obtained for multiple vacuolar preparations for each strain.
complexes lacking subunit H do not contribute to the ATPase activity (32), so ATPase activity in the vacuoles also suggests that intact V-ATPase complexes are present. One possible explanation for the decrease in vacuolar acidification in vivo and the loss of coupled proton transport in vacuoles from the vma13Δ × WT strain is that the assembled complexes lacking subunit H are capable of “leaking” protons and thus fully or partially collapsing the pH gradient across the vacuolar membrane. Previous work on haploid vma13Δ mutants, which have no ATPase activity, would not have revealed an increased proton leak, because there was no proton gradient formed in the first place. Further biochemical experiments will be necessary to establish this explanation, but regardless of the biochemical source, uncoupled ATP hydrolysis at the vacuole could certainly contribute to the overall growth defects in the vma13Δ × wild-type strain. In this context, reducing the copy number of the B subunit (Vma2p), which is essential for assembly of V1 complexes, would be predicted to reduce the total number of V1Vo complexes at the vacuole, resulting in a reduction in ATPase activity, but also to better balance the number of V1Vo complexes with the amount of available H subunit, resulting in fewer V1Vo complexes lacking subunit H.

Increased MgATPase activity in cytosolic V1 sectors could also contribute to the growth defects of the vma13Δ × wild-type diploid. Although we and others have provided evidence of release of V1 into the cytosol in response to glucose deprivation, yeast cells (and other eukaryotic cells as well) appear to contain a cytosolic population of V1 sectors even in the presence of glucose (37). In wild-type cells, these V1 sectors are “silenced”; they have little or no ability to hydrolyze MgATP, the physiological substrate, although they can hydrolyze CaATP in vitro (27, 35). The H subunit is key to this silencing, and we have found that the cytosolic population of V1 sectors has greater MgATPase activity in the absence of the H subunit (27, 35). If the supply of H subunit were limited, as in the vma13Δ × wild-type strain, we would also predict that some of the cytosolic V1 sectors might lack subunit H and thus be capable of MgATP hydrolysis. This is supported by Fig. 5, which shows a higher specific MgATPase activity in V1 sectors isolated from the vma13Δ × wild-type diploid. As described above, decreasing the copy number of the B subunit might be predicted to reduce the levels of both vacuolar V1Vo complexes and cytosolic V1 complexes, causing the H subunit levels to be less limiting and resulting in the production of fewer cytosolic V1 complexes lacking subunit H and active in MgATP hydrolysis.

The exacerbation of phenotypes in the vma13Δ × vma3Δ mutant is partially explained by the model in Fig. 6. Because subunit c, encoded by the VMA3 gene, is essential for assembly and stability of V1o sectors but does not affect the synthesis or assembly of V1 complexes, it would be predicted that the vma13Δ × vma3Δ would have fewer assembled V1Vo complexes at the vacuole and a larger population of cytosolic V1 sectors. Consistent with this, vacuoles from this mutant have very low levels of V1c subunits, although there are only slightly lower levels of the V1 A and B subunits in whole cell lysates (Fig. 3). If these subunits are not at the vacuole, then they may well be contributing to the cytosolic pool of V1 complexes, and in the presence of limiting amounts of H subunit, more of these V1 sectors would have MgATPase activity. This could certainly contribute to the very poor growth of the vma13Δ × vma3Δ diploid and is similar to the explanation given for the lethality resulting from VMA13 overexpression in a mutant that lacks an intact V1 sector (8). However, there appear to be synergistic effects of the vma3Δ and vma13Δ mutations that are not as easily explained. Specifically, the vma3Δ × WT diploid has few if any detectable growth or biochemical phenotypes other than a small elevation in MgATPase activity in isolated V1 sectors. This might suggest that subunit c is generally present in excess, so that halving of the gene copy number does not produce enough change in the pool of total c subunit protein to be detected by the cell and generate haploinsufficiency. The dramatic reduction in both V1 and V1o subunits in vacuoles isolated from the vma13Δ × vma3Δ compound heterozygote is then very surprising. There appears to be some defect in assembly and/or targeting of the V-ATPase that prevents it from reaching the vacuole. Further experiments will be necessary to understand this phenomenon, but the results suggest that perturbation of the balance of certain subunits can affect the overall stability and assembly of the enzyme.

It is also notable that loss of V-ATPase activity in vacuoles from the vma13Δ × vma3Δ compound heterozygote is unlikely to account for the poorer growth phenotypes of this compound heterozygote relative to the simple vma13Δ × wild-type heterozygote. In fact, the uncoupling of ATP hydrolysis from proton transport in vacuoles from the vma13Δ × WT diploid might be predicted to be more damaging to the cell than the total loss of V-ATPase activity in vacuoles from the vma13Δ × vma3Δ diploid, because it represents a source of unproductive ATP hydrolysis not present in the vma13Δ × vma3Δ heterozygote. Based on this reasoning, we would propose that the exacerbation of growth phenotypes in the vma13Δ × vma3Δ het-

![FIGURE 6. Model for the effects of heterozygous deletions of V-ATPase subunits on intact V1Vo ATPase activity. Different complexes that may contribute to the growth phenotypes observed are shown on the left. V1 sectors (except for subunit H) are in white, V1o subunits are in light gray, and the H subunit is dark gray in these models. The distribution of complexes in each strain and the relationship to growth phenotypes are discussed in more detail under “Discussion.”]
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Erozygote is primarily accounted for by an increased population of cytosolic V₁ sectors with a relatively high specific activity for MgATP hydrolysis. However, because there is an apparent synergy between the vma13Δ and vma3Δ mutations, there may be additional effects in the compound heterozygote for which we cannot account at present.

Although a number of studies have examined the effects of deleting or silencing individual V-ATPase subunits on various organisms (38–40), the consequences of losing one of two genomic copies of a subunit gene in a diploid organism have not been investigated thoroughly. Drosophila heterozygous for one of the plasma membrane V-ATPase subunit isoforms do not exhibit the clear Malpighian tubule phenotype characteristic of the homozygote (41), suggesting that a single copy of this gene is sufficient. No adverse phenotypes have been reported in mice heterozygous for the c subunit gene, which were used to generate transgenic animals with the lethal homozygous deletion of this gene (40). Individuals heterozygous for human disease-causing alleles of the a or B subunit do not manifest symptoms of the disease, since the inherited diseases currently attributed to V-ATPase defects are genetically recessive (42). However, these studies examined only one subunit in the case of transgenic mice and assessed 2–4 subunit isoforms in the human genetic studies. Significantly, all of the human diseases are attributed to specific subunit isoforms believed to operate at the plasma membrane in a limited set of tissues.

Significantly, diploids heterozygous for null mutations in all yeast V-ATPase subunits do not exhibit obvious haploinsufficiency phenotypes (Fig. 1), suggesting that haploinsufficiency of VMA13 is quite specific and possibly reflective of unique aspects of H subunit function. Consistent with this, the recent genome-wide yeast haploinsufficiency screen did not identify most vma heterozygotes as exhibiting haploinsufficiency, but the vma13 heterozygote was not tested (12). To our knowledge, no specific tests for haploinsufficiency of the H subunit have been reported for any higher eukaryote.

One of the most intriguing aspects of this work is the very different phenotypes arising from interactions between different vma deletion mutations in compound heterozygotes lacking one copy of each of two different subunit genes. Although this study was done with null alleles, it is certainly possible that other mutant alleles might also show genetic interactions with biochemical consequences for enzyme activity. These results suggest that different subunit alleles in higher organisms might also “mix and match” to form V-ATPase complexes of varied properties and catalytic capabilities. Given the wide range of processes connected to organelle acidification and V-ATPase activity (1), even subtle variations in some V-ATPase populations could have profound consequences for an organism as a whole.

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