Report

Current Biology

Transforming yeast into a facultative photoheterotroph via expression of vacuolar rhodopsin

Highlights

- Vacuolar rhodopsins can be transferred from *U. maydis* to *S. cerevisiae*
- Adding rhodopsin makes *S. cerevisiae* a functional facultative photoheterotroph
- Rhodopsins do not require evolutionary optimization for fitness benefits

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In brief

Microbial rhodopsins are light-driven proton pumps, transducing light into metabolic energy. They have an extensive history of horizontal gene transfer across the tree of life. Peterson et al. find that transferring vacuole-localized rhodopsins from *U. maydis* to *S. cerevisiae* transforms the latter into a functional facultative photoheterotroph.

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Transforming yeast into a facultative photoheterotroph via expression of vacuolar rhodopsin

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SUMMARY

Phototrophic metabolism, the capture of light for energy, was a pivotal biological innovation that greatly increased the total energy available to the biosphere. Chlorophyll-based photosynthesis is the most familiar phototrophic metabolism, but retinal-based microbial rhodopsins transduce nearly as much light energy as chlorophyll does,[1] via a simpler mechanism, and are found in far more taxonomic groups. Although this system has apparently spread widely via horizontal gene transfer,[2–4] little is known about how rhodopsin genes (with phylogenetic origins within prokaryotes[5,6]) are horizontally acquired by eukaryotic cells with complex internal membrane architectures or the conditions under which they provide a fitness advantage. To address this knowledge gap, we sought to determine whether *Saccharomyces cerevisiae*, a heterotrophic yeast with no known evolutionary history of phototrophy, can function as a facultative photoheterotroph after acquiring a single rhodopsin gene. We inserted a rhodopsin gene from *Ustilago maydis*,[7] which encodes a proton pump localized to the vacuole, an organelle normally acidified via a V-type rotary ATPase, allowing the rhodopsin to supplement heterotrophic metabolism. Probes of the physiology of modified cells show that they can deacidify the cytoplasm using light energy, demonstrating the ability of rhodopsins to ameliorate the effects of starvation and quiescence. Further, we show that yeast-bearing rhodopsins gain a selective advantage when illuminated, proliferating more rapidly than their non-phototrophic ancestor or rhodopsin-bearing yeast cultured in the dark. These results underscore the ease with which rhodopsins may be horizontally transferred even in eukaryotes, providing novel biological function without first requiring evolutionary optimization.

RESULTS

Model system

In this paper, we examine whether an obligately heterotrophic eukaryote can gain a fitness benefit by obtaining a rhodopsin, becoming a facultative photoheterotroph. While rhodopsins have been previously engineered to localize to the mitochondrial membrane of eukaryotes via fusions to inner membrane proteins, where they can provide an energetic benefit,[8–11] they have never been observed in this organelle in nature. In contrast, rhodopsins have been found in multiple eukaryotes, which localize to the other organelle containing a rotary ATPase—the vacuole.[12–16] However, the benefit of acquiring vacuolar rhodopsins has yet to be examined directly in the laboratory. To address this knowledge gap, we introduced the vacuolar rhodopsin gene *UmOps2* from the fungus *Ustilago maydis*[7] to *Saccharomyces cerevisiae* strain Y55.

We synthesized and codon-optimized *UmOps2* and *UmOps2-GFP* fusion genes for yeast expression and introduced them to the chromosome via a plasmid-bearing homology to the HO locus (Figure 1A). Chromosomal integration with this vector results in a tandem repeat array of multiple copies of the entire plasmid sequence.[17] Microscopy indicated localization to the vacuolar membrane was maintained in *S. cerevisiae* (Figure 1B). This rhodopsin is oriented to pump protons into the vacuole when illuminated, which may either increase membrane potential sufficiently to run the vacuolar ATPase in reverse and generate ATP, or reduce ATP consumption by the vacuolar ATPase, a significant fraction of the cellular ATP budget[18] (Figure 1C). To absorb light, rhodopsin must be covalently bound to retinal, a chromophore that transduces light energy by switching one double bond from *trans* to *cis* and forcing a proton across the membrane.[19,20] Cells bearing rhodopsin were a purple color when all-*trans* retinal was added, indicating that the protein/pigment complex successfully forms and absorbs green light (Figure 1D). While in our experiment the function of UmOps2 was dependent upon externally supplied retinal pigment obtained from the environment, in nature rhodopsins are known

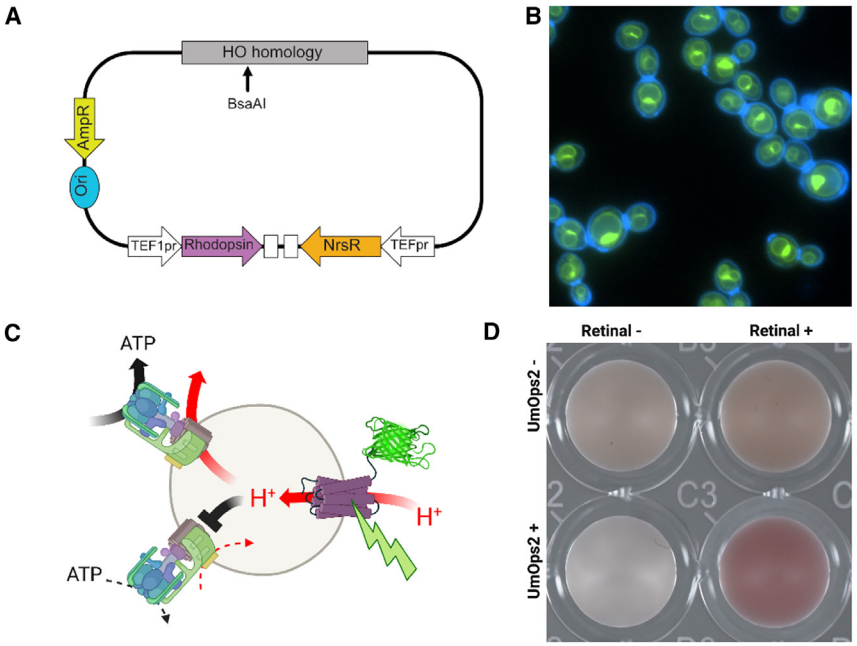

Figure 1. Constructing rhodopsin-bearing yeast

(A) *UmOps2* rhodopsin was put under the control of the *TEF1* promoter on a plasmid with the *NatMX6* selectable marker and a segment of homology to the *HO* gene of yeast. This homology region was cut with restriction enzyme BsaAI for ends-in cloning to produce a multicopy repeat array at the *HO* locus.

(B) The UmOps2-GFP fusion protein localizes to the vacuolar membrane (cell walls stained with calcofluor in blue).

(C) In the presence of green light, rhodopsin pumps protons from the cytoplasm into the vacuolar compartment. This either reduces ATP consumption by the rotary ATPase or pushes the vacuolar membrane to a high enough voltage to allow ATP production rather than consumption.

(D) Confirmation of UmOps2 protein expression and function. Yeast bearing the transgenic rhodopsin gene exhibit a purple color, but only when both the transgene is present and the yeast are supplemented with all-*trans* retinal.

to frequently transfer in multigene cassettes bearing a retinal synthesis pathway.[21] Organisms with rhodopsins that lack retinal production pathways are also known, typically acquiring the pigment by consuming bacteria.[22]

Physiological analysis

To determine whether rhodopsins affect the physiology of *S. cerevisiae*, we measured the effects of illuminating yeast with and without UmOps2, measuring the response via Ura7 fiber dynamics (Figures 2A and 2B). Ura7 is a cytidine triphosphate (CTP) synthase[23] which, under conditions of starvation, polymerizes in the cytoplasm into insoluble, catalytically inactive fibers. This process is mediated by changes in the surface charge of the protein resulting from changes in cytoplasmic pH, and can be used as an indicator of metabolic state.[24,25] Under ordinary circumstances, when a dormant cell containing Ura7 fibers becomes metabolically active, the vacuolar ATPase consumes ATP to move protons from the cytoplasm to the vacuole interior. This raises the pH of the cytoplasm, dissolving the Ura7 fibers. This is a special case of a more general phenomenon in which cytoplasmic pH and macromolecular crowding affect the bulk physical properties of the yeast cytoplasm in a metabolically dependent manner.[26,27]

To test the effects of light on the metabolic state of rhodopsin-bearing yeast, we grew *Ura7-GFP* yeast, both with and without *UmOps2*, for 24 h in the presence and absence of green LED illumination (see STAR Methods). Yeast bearing *UmOps2* displayed a significant decrease in filament area under green light, while those without rhodopsins did not. This effect was observed in as little as 30 min (Figure 2C; one-way ANOVA, $F_{3,16} = 5.1$, $p = 0.012$, pairwise differences assessed with Tukey's HSD with $a = 0.05$) and was maintained for at least 24 h (Figure 2D; one-way ANOVA, $F_{3,16} = 9.6$, $p = 0.0007$, pairwise differences assessed with Tukey's HSD with $\alpha = 0.05$). The reduction in filament area demonstrates a deacidification of the cytoplasm sufficient to alter the function of metabolic enzymes. While this indicates a measurable effect on cellular physiology and pumping of protons across the vacuole membrane to directly alter cytoplasmic pH, this does not necessarily result in ATP synthesis. The V-ATPase pumps fewer protons per ATP than mitochondrial ATPase and requires a high membrane potential (circa 240 mV) to be reversed,[28–30] which is known to significantly decrease the activity of microbial rhodopsins.[31] However, rhodopsin function may result in reduced total ATP consumption and increased ATP availability, as the proton motive force generated by rhodopsin replaces the work of ATP hydrolysis. To determine whether rhodopsins provide a benefit, we examined the relative fitness of rhodopsin-bearing yeast in both light and dark conditions.

Fitness consequences of rhodopsin expression

Rhodopsins in nature are typically used as a supplemental energy source during starvation.[32] We thus examined the potential benefit of rhodopsins in our yeast under energy limiting conditions. Yeast were grown in yeast extract peptone glycerol (YEPG) media supplemented with all-*trans* retinal. This medium forces respiration, a state which limits available energy to yeast due to the presence of non-fermentable glycerol as the sole carbon source. When grown in YEPG, yeast cultures typically become oxygen-limited, even during shaking incubation, respiring at a fraction of their theoretical maximum possible rate.[33] Further, we allowed our yeast to experience an extended stationary phase by transferring to fresh media once every 48 h. This maximizes the potential for phototrophic energy production to supplement metabolism.

We mixed rhodopsin-bearing yeast with controls in a 1:1 ratio, then calculated their relative fitness over a 72-h competition (measured 24 h after one 48-h growth period) under both dark and illuminated conditions (see STAR Methods). Our marked control strain was otherwise isogenic, expressing GFP under

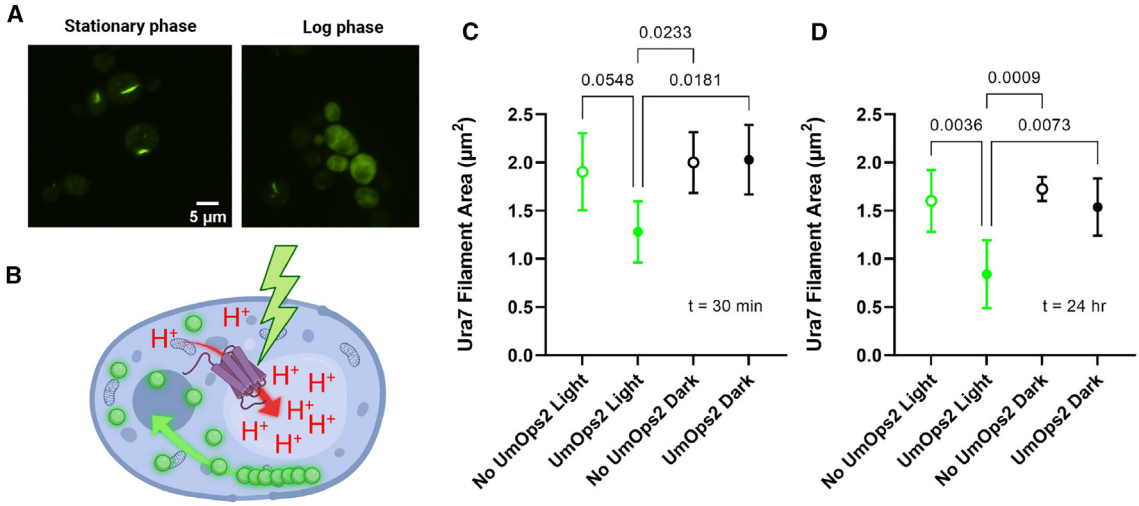

Figure 2. Examining the effect of rhodopsin function on yeast physiology

(A) Yeast-bearing Ura7-GFP fusion proteins exhibit a single fluorescent fiber of polymerized inactive enzyme in stationary phase, which dissociates into free active enzyme in log phase due to cytoplasmic deacidification. This can be used as a marker of physiological state, with filaments indicating starvation.

(B) Model of the effect of vacuolar rhodopsin on yeast physiology. Excitation of rhodopsin with green light pumps protons from the cytoplasm to the vacuole, deacidifying the cytoplasm and dissociating Ura7-GFP fibers.

(C and D) (C) Experimental effect of green light on Ura7-GFP fiber formation in yeast, with and without UmOps2 protein (5 replicates, mean and standard deviation visualized). Cells bearing the vacuolar rhodopsin exhibit significantly smaller Ura7 fibers when grown in green light, while those without rhodopsin do not (see Tables S1 and S2 for statistical analysis). The effect is apparent within 30 min (C) and is maintained for at least 24 h (D).

the same promoter as the rhodopsin. Rhodopsin-bearing yeast had 2.2% higher fitness in light relative to dark (Figure 3A), calculated using the ratio of Malthusian growth parameters[34–36] (two-tailed t test, $t = 3.35$, $n = 10$, $p = 0.017$). This fitness benefit in light for rhodopsin-bearing yeast required that rhodopsin be localized to the vacuole; when delocalized by *APM3* deletion (involved in vacuolar transport,[37] see Figure S2), the 2.2% fitness benefit of illumination becomes a 2.5% fitness defect (Figure 3B). We hypothesize that this may be due to disruption of membrane energetics and ion homeostasis of other endomembranes, such as the endoplasmic reticulum or plasma membrane. Similarly, poisoning of the V-ATPase with concanamycin A also led to illumination engendering a fitness defect (see Figure S3). This is likely also due either to mislocalization of rhodopsin or a broader vacuole dysfunction preventing rhodopsin function. V-ATPases are implicated in yeast vacuole biogenesis,[38,39] and disruption of the V-ATPase is known to broadly affect endosomal and secretory pathway protein trafficking in other systems,[40] such that poisoning of the V-ATPase can trigger vacuolar protein mislocalization. Taken together, our results demonstrate that rhodopsins can provide a fitness advantage for yeast under illumination, but this depends on proper localization to the vacuole membrane and a normally functioning V-ATPase.

Cell viability

Evolutionary processes are fundamentally a birth-death process. As a result, adaptations (which result in a lineage increasing in frequency within the population) must either increase birth rates and/or decrease death rates.[41] While rhodopsin-bearing yeast grown in light have an advantage during competition (Figure 3), it was not clear whether this benefit stemmed from greater reproduction or reduced mortality. To test this, we measured the viability of cells from each strain during extended culture. We inoculated GFP (control) and UmOps2-bearing yeast into YEPG supplemented with all-*trans* retinal, with and without illumination. We allowed them to grow for 72 h without passaging, sampling every 24 h and measuring viability with the vital stain propidium iodide (Figure 4). Cell death across all populations and time points was relatively low, $\leq 3\%$, and yeast incubated at stationary phase accumulated dead cells at $\sim 0.3\%$ per 24 h or slower. However, yeast bearing rhodopsins experienced 78% higher spontaneous mortality than controls, and culture under continuous illumination (Figure 4A) increased mortality by 282% compared with dark conditions (Figure 4B; ANOVA summary in Table S1, all parameter estimates significant at $p < 0.001$). Taken together, this indicates that the selective advantage experienced by rhodopsin-bearing yeast under illumination is due to increased proliferation sufficient to overcome modestly higher mortality.

Interestingly, the increased mortality of rhodopsin-bearing yeast observed in YEPG media was not observed when cells were incubated in YEPG media (Figure S4), or when yeast were incubated with glycerol without all-*trans* retinal (Figure S5). The increased mortality of rhodopsin-bearing yeast is thus dependent upon both the cells involved undergoing aerobic respiration and the absorption of light by the retinal chromophore. Further work will be required to determine the mechanistic basis of this effect.

DISCUSSION

In this work, we explored the capacity of a heterotrophic eukaryote with no known history of phototrophy, *S. cerevisiae*, to become a facultative photoheterotroph. We transformed

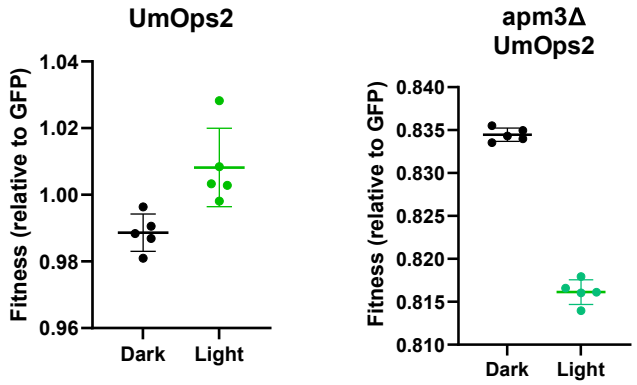

Figure 3. Rhodopsins localized in the yeast vacuole provide a fitness benefit while delocalized rhodopsins do not

Control yeast (GFP, under the same promoter as the rhodopsin strain) and yeast bearing UmOps2 were grown together in dark and light conditions for 2 passages. Rhodopsin-bearing yeast were on average 2.2% more fit, measured as the ratio of Malthusian growth parameters, when grown in green light compared with when they were grown in the dark. Delocalization of rhodopsins by deleting *APM3* causes light to trigger a large fitness defect instead (see also Figures S2 and S3 for effects of rhodopsin delocalization).

S. cerevisiae with a fungal rhodopsin gene, *UmOps2*, from the corn pathogen *Ustilago maydis*. UmOps2 localized to the yeast vacuole and proved functional, reducing indications of starvation and quiescence under illumination. Under light, UmOps2 increased fitness by 2.2%, due entirely to increased proliferation rather than reduced death, and this benefit was reversed upon disruption of vacuolar membrane protein trafficking.

Our results showing that UmOps2 protein is capable of pumping physiologically relevant quantities of protons, providing a selective advantage during energetically limiting conditions, demonstrate that the vacuole/endosome is a eukaryotic membrane, where a light-driven proton pump may be energetically useful. Here, this activity can alleviate the ATP cost of vacuolar acidification, or potentially raise the membrane potential high enough to run the rotary ATPase in reverse, either way transducing light into biologically available energy. Vacuolar UmOps2 represents one of three described classes of fungal rhodopsins found across the ascomycetes.[12] The vacuole is also known to host proton-pumping rhodopsins in phototrophic marine diatoms,[13] and rhodopsins are also found in both food vacuoles[14] and "birefrigent bodies" (evaginations of food vacuole membranes) of dinoflagellates.[15] Energizing the vacuolar membrane with proton-pumping rhodopsins thus appears to be a general pattern across phototrophic and photoheterotrophic eukaryotes alike.

This work experimentally demonstrates that the horizontal acquisition of novel phototrophic metabolism by a heterotroph is not tightly constrained, even in eukaryotic cells with complex endomembranes and multiple organelles where rhodopsins could theoretically localize. While both the mitochondrion and vacuole represent bioenergetically relevant membranes with rotary ATPases, the vacuolar membrane may be a particularly opportune membrane for rhodopsin-based phototrophy in terms of availability for horizontal transfer. Membrane proteins without any localization signals are typically trafficked into a mix of the endoplasmic reticulum (ER) and Golgi apparatus, and on toward the endosome/vacuole.[42,43] Endomembrane trafficking signal sequences, which specifically send proteins away from the ER along the secretory pathway or to the vacuole, are also typically relatively simple and small[44] compared with the large and specific changes needed to target proteins to the mitochondrion.[8–11] Integration and localization of rhodopsins into elements of the endomembrane system like the vacuole may thus be much more evolutionarily accessible than localization to mitochondria, the other bioenergetically relevant membrane in eukaryotes, upon horizontal transfer.

Although rhodopsin-bearing yeast have a selective advantage when illuminated, we found that they face a slight disadvantage in the dark and exhibit a higher rate of cell death when grown aerobically. This is unlikely to be due to protein biosynthesis costs, as this cost does not manifest in the absence of retinal chromophore. Although the mechanism remains unclear, it is suggestive that this cost specifically manifests in yeast that have both been fed retinal pigment and are growing aerobically. Both respiration[45] and absorption of green light by heme[46] are known to trigger the production of toxic reactive oxygen species (ROS) in yeast. We hypothesize that increased absorption of green light by protein-bound retinal may enhance light-induced ROS production, adding to the production of toxic products of aerobic respiration, though more research is required to confirm this.

The successful transfer of photoheterotrophic metabolism to a model organism via simple synthetic biology techniques may also open novel research avenues. The origin of phototrophy is one of the most impactful innovations in the history of Earth, and it is likely that rhodopsins predate chlorophototrophy as its earliest form.[47] Recent work has reconstructed ancient rhodopsins and probed their likely function.[5,48] Although rhodopsins have been successfully expressed in bacteria,[49,50] a cellular environment likely more akin to the prokaryotic cells of the early Earth, many eukaryotes bear these proteins and they may have been present at the origins of eukaryotes.[51–53] The ability to easily synthesize, express, and characterize the function of ancient rhodopsins in *S. cerevisiae*, a workhorse of modern cell biology, opens a range of new opportunities for understanding the origins and subsequent evolution of retinalophototrophy.

We transformed the yeast *Saccharomyces cerevisiae* into a facultative photoheterotroph by adding a single vacuole-localized rhodopsin. Yeast growing in energetically limiting conditions gained a substantial fitness advantage under illumination, demonstrating that light was being utilized for energy. This required no evolutionary optimization, illustrating the ease of horizontal transfer of this system and providing context for the repeated gain of rhodopsin-mediated phototrophy in eukaryotes. Synthetic construction of photoheterotrophic yeast provides novel insights into the evolution and spread of retinalophototrophy, its optimization and integration into heterotrophic metabolisms, and stands to open new avenues of research and experimentation.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE

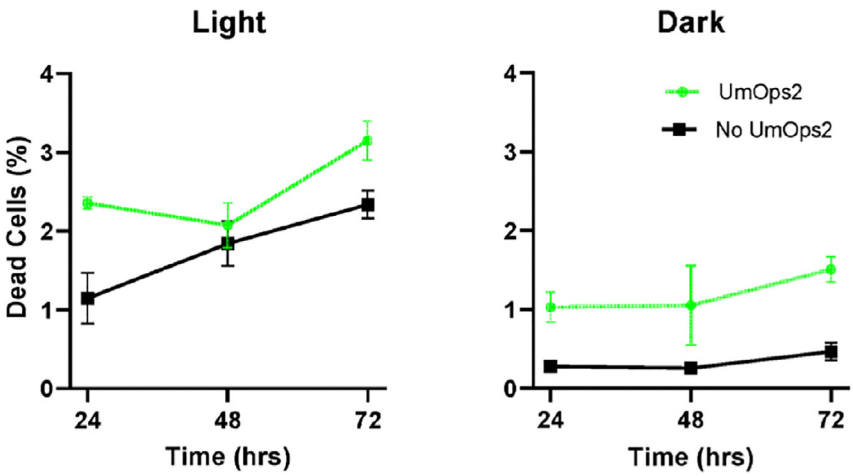

Figure 4. Effect of rhodopsin on cell viability in stationary phase

Control and UmOps2-bearing yeast were grown in YEPG for 3 days in dark and light conditions, stained daily with propidium iodide to measure cell death in each population. Each data point represents the mean of 5 replicate UmOps2+ and UmOps2− populations ± one standard deviation. Rhodopsin increased cellular mortality in both light and dark conditions, suggesting that the higher fitness of rhodopsin-bearing yeast during competition (Figure 3) is due to increased proliferation, not reduced death (see also Figure S4 for cell viability in other growth media).

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.12.044.

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AUTHOR CONTRIBUTIONS

A.B. conceived of the research. A.P., A.B., and W.C.R. planned the experiments. A.P. and A.B. performed the experiments and analyzed the data. All authors contributed to writing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Concanamycin A	VWR	Cat # 102513-630
Propidium Iodide	ThermoFisher	Cat # P130MP
Experimental models: Organisms/strains		
S. cerevisiae: strain yAB1, genetic background Y55HD, genotype *Ura7/Ura7-GFP:HphNT2*	This paper	N/A
S. cerevisiae: strain yAB406, genetic background Y55HD, genotype *ho:UmOps2:NatMX6/ho:UmOps2:NatMX6*	This paper	N/A
S. cerevisiae: strain yAB414, genetic background Y55HD, genotype *ho:UmOps2-GFP:NatMX6/ho:UmOps2-GFP:NatMX6*	This paper	N/A
S. cerevisiae: strain yAB452, genetic background Y55HD, genotype *Ura7/Ura7-GFP:HphNT2 HO/ho:UmOps2:NatMX6*	This paper	N/A
S. cerevisiae: strain yAB703, genetic background Y55HD, genotype *ho:GFP:NatMX6/ho:GFP:NatMX6*	This paper	N/A
S. cerevisiae: strain yAB848, genetic background Y55HD, *genotype apm3Δ:HphNT2/apm3Δ:HphNT2, ho:UmOps2-GFP:NatMX6/ho:UmOps2-GFP:NatMX6*	This paper	N/A
S. cerevisiae: strain yAB851, genetic background Y55HD, genotype *apm3Δ:HphNT2/apm3Δ:HphNT2, ho:UmOps2:NatMX6/ho:UmOps2:NatMX6*	This paper	N/A
Oligonucleotides		
Primer Ura7_S2 for GFP tagging: TAGAAATTTATCTTTGTTAATGCAGTAGACTTTTAATTCT AAAATTTTGATTTAATCGATGAATTCGAGCTCG	This paper	N/A
Primer Ura7_S3 for GFP tagging: GTCATTGAAGGTAAGTACGATCTTGAGGCCGGCGAAA ACAAATTCAACTTTCGTACGCTGCAGGTCGAC	This paper	N/A
Primer Apm3_F for *APM3* deletion: GACTTGGGCAACAACAGAGGCTGTAAACCTTACCAAC CCAACCAAAATAGATCGATGAATTCGAGCTCG	This paper	N/A
Primer Apm3_R for *APM3* deletion: TCTCATTATATTCTATTTAGTTTCGCATGGAATTTCAAG TACACATATAAGACATGGAGGCCCAGAATAC	This paper	N/A
Recombinant DNA		
pYM25: *GFP* for fusion protein production via PCR and a *hphNT2* resistance cassette for gene deletion via PCR	Janke et al.[54]	European Plasmid Repository, Plasmid #268: pYM25
pWR112: *UmOps2* under a *TEF1* promoter, *NATMX6* resistance, *HO* homology for chromosomal insertion	This paper	N/A
pWR113: Expression of *UmOps2-GFP* under a *TEF1* promoter, *NATMX6* resistance, *HO* homology for chromosomal insertion	This paper	N/A
pWR162: Expression of *GFP* under a *TEF1* promoter, *NATMX6* resistance, *HO* homology for chromosomal insertion	This paper	N/A
Software and algorithms		
Excel version 2309	Microsoft	N/A
CellPose 2.0	Stringer et al.[55]	https://www.cellpose.org/
FIJI version 2.14	University of Wisconsin-Madison	https://fiji.sc/
Graphpad Prism 9	Dotmatics	https://www.graphpad.com/

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
FloMax 2.9	Partec	https://www.sysmex-partec.com
FloMax 3.0	Partec	https://www.sysmex-partec.com
NIS-Elements AR 4.40.00 64-bit	Nikon Instruments	https://www.microscope.healthcare.nikon.com/products/software/nis-elements

RESOURCE AVAILABILITY

Lead contact

Additional information and requests for resources should be directed to and will be fulfilled by the lead contact, Anthony Burnetti (anthony.burnetti@biosci.gatech.edu)

Materials availability

Plasmids and *S. cerevisiae* strains made and used in this study will be made available by the lead contact, Anthony Burnetti, upon request.

Data and code availability

All data utilized in this paper will be shared by the lead contact upon request.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Culture of *S. cerevisiae*

All yeast were grown in liquid Yeast Extract Peptone Glycerol (YEPG) or Yeast Extract Peptone Dextrose (YEPD) media, supplemented with 10 microliters of 10 mM all-trans retinal in 100% ethanol when appropriate for a final concentration of 10 μM of all-trans retinal. When the V-ATPase was inhibited by concanamycin A, it was supplemented to a final concentration of 0.3 μM with 10 microliters of a 0.3 mM stock in pure ethanol.

Genetic modification of *S. cerevisiae*

The *U. maydis* rhodopsin *UmOps2* coding sequence was codon-optimized for expression in *S. cerevisiae* (67% of codons and 26.5% of nucleotides altered) and synthesized via ThermoFisher, as was *GFP*, flanked by restriction sites SpeI and AflII. Both *GFP* and *U. maydis* rhodopsin UmOps2 were ligated into a custom expression vector driven by a *TEF1* promoter and *CYC1* terminator, associated with a *NatMX6* resistance cassette and a fragment of *HO* gene homology bearing the loss-of-function mutations present in the S288C background. By cutting within this *HO* homology using AfeI or BsaAI, *UmOps2* or *GFP* can be introduced as a multicopy tandem repeat of the entire plasmid sequence[17] between regions of *HO* homology at the *HO* locus.

UmOps2 and *GFP* tandem repeats were thus introduced to the *HO* locus of homozygous diploid Y55 derivative Y55HD previously used by the Ratcliff laboratory.[56] These strains were sporulated, and the resultant haploid heterothallic haploids mated with each other to produce homozygous diploid control yeast homozygous for *GFP* at the *HO* locus and experimental yeast homozygous for *UmOps2 at* the *HO* locus.

GFP was fused to the *Ura7* gene at the C-terminus via PCR transformation from plasmid pYM25[54] bearing *GFP* and *hphNT2* in the Y55HD genetic background[56]. Heterozygous yeast were used as a control for metabolic analysis, and after mating with yeast bearing *UmOps2*, double heterozygotes were used as experimental strains for metabolic analysis of the effect of rhodopsin on Ura7 fiber morphology. The *APM3* gene was deleted via standard PCR-based method, using *hphNT2* without *GFP* from plasmid pYM25.

METHOD DETAILS

Yeast competition experiments

For competition experiments, control and rhodopsin bearing yeast were grown separately to saturation shaking at 250 RPM at 30 °C in YEPG + retinal media for 24 hours to acclimatize them. On Day 0, 500 μL of both GFP-bearing controls and rhodopsin-bearing experimental cells were mixed. This mixture was measured via flow cytometry to determine the baseline ratio of control and rhodopsin-bearing yeast. A starting culture of 50 μL of mixed stationary-phase culture was transferred two new tubes of retinal-supplemented YEPG. One was placed in an ordinary shaking incubator at 250 RPM and 30 °C, while another was placed in another shaking incubator with the same settings supplemented with green LEDs and a reflective foil wrapping to provide bright green illumination, measured with an average intensity of 1996 lux and peak wavelength of 515 nm (see Figure S1, measured with a USB4000-UV-VIS spectrometer).

These cells were allowed to grow for 48 hours, upon which 50 μL of stationary phase culture was transferred to new tubes and placed into the same incubator. At 24 and 72 hours, samples were taken for flow cytometry to count GFP and rhodopsin bearing yeast. For apm3 yeast and yeast poisoned by concanamycin A, samples were taken at 24 and 48 hours with a 24-hour growth cycle.

Cell viability measurements

To quantify the viability of cells, a propidium iodide vital stain was used to measure the proportion of dead cells as a rhodopsin-bearing population and control population progressed through stationary phase. Five replicate populations were inoculated and grown in YP Glycerol supplemented with all-trans retinal for 72 hours. After 24, 48, and 72 hours, 500 μL of each population was spun down and double washed, resuspending in 1 mL of water for imaging. One μL of propidium iodide stock solution (1 mg/mL) was added and cells were imaged on a TI Nikon Eclipse Ti-Motorized Inverted Microscope. Four fields of view were imaged at 20x objective magnification, capturing a bright-field image, and an image with TRITC filter and 1.3 millisecond exposure to capture propidium iodide stained dead cells. Images were thresholded using MaxEntropy auto thresholding in Fiji Image Analysis[57] to identify and count dead cells, and the bright-field channel segmented via Cellpose[55] to count total cells.

Ura7 filament metabolic analysis

In order to determine whether rhodopsin provides an energy benefit, metabolic state was assessed via polymerization of GFP-tagged Ura7p. A starting culture of 50 μL of control yeast bearing GFP-tagged Ura7p, and experimental yeast bearing this fusion and the UmOps2 rhodopsin, were inoculated and grown for 24 hours in 10 mL Synthetic Complete media supplemented with all-trans retinal. Five replicates each were grown in a dark incubator at 250 RPM and 30 °C, and a green illuminated incubator at 30 °C with light approximately 1996 lux. At 24 hours growth, stationary phase cells were removed and quickly imaged on a TI Nikon Eclipse Ti-Motorized Inverted Microscope. Between 130 and 600 fibers were imaged in the FITC channel with a constant exposure of 1s and analog gain of 64.0x. The average filament area was quantified using Fiji Image Analysis[57] via automatic image segmentation using MaxEntropy auto thresholding of particles $\geq$ 10 pixels in size.

QUANTIFICATION AND STATISTICAL ANALYSIS

Fitness quantification

Frequency of different cell types were determined either via flow cytometry or microscopy using FloMax 2.9, FloMax 3.0 (Partec, Göttingen, Germany), and NIS-Elements AF 4.40.00 (Nikon Instruments, Tokyo, Japan) as described above. Relative fitness can be determined from the change in the frequency genotypes over time. Relative fitness was determined as described in the Lenski long-term evolution experiment.[35,36] The relative fitness W of a rhodopsin-bearing yeast can be defined as the ratio of the number of generations of rhodopsin-bearing yeast (G_R) divided by the number of generations of a control yeast (G_C) over the same time frame using Equation 1.

$$W = \frac{G_R}{G_C} \quad \text{(Equation 1)}$$

The number of generations experienced by a genotype in a population (G) after a number of transfers n during which the population reaches saturation can be calculated from the fraction of the population at passage (F_n), the fraction of the population at passage 0 (F_0), the dilution factor (d), and the number of transfers (n) using Equation 2:

$$\mathrm{G} = log_2\left(\frac{F_n}{F_0}d^n\right) \quad \text{(Equation 2)}$$

By combining Equations 1 and 2, we determine the equation for relative fitness W in terms of number of passages (n), fraction of the population bearing rhodopsin after passage n (Fr_n) and at passage 0 (Fr_0), fraction of the population that are controls after passage n (Fc_n) and at passage 0 (Fc_0), the dilution factor (d), in the form of Equation 3:

$$W = \frac{log_2\left(\frac{fr_n}{fr_0}d^n\right)}{log_2\left(\frac{fc_n}{fc_0}d^n\right)} \quad \text{(Equation 3)}$$

Equation 3 was used to calculate the relative fitness of rhodopsin-bearing yeast in dark and light incubation for all 5 replicate populations each at day 3 of cultivation, such that dilution factor d equals 200 and number of passages n equals 2.

Statistical analysis

Fitness analysis, Statistical analysis, and one-way ANOVA analysis was performed using Excel (Microsoft Corporation, WA, USA) or Graphpad Prism (Dotmatics, MA, USA). Statistical significance ($p<0.05$) is indicated on figures with asterisks. Details of ANOVA analysis parameters and results for comparison of URA7-GFP fiber sizes are detailed in Tables S1 and S2.