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The cost of gene expression underlies a fitness trade-off in yeast

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Natural selection optimizes an organism’s genotype within the context of its environment. Adaptations to one environment can decrease fitness in another, revealing evolutionary trade-offs. Here, we show that the cost of gene expression underlies a trade-off between growth rate and mating efficiency in the yeast Saccharomyces cerevisiae. During asexual growth, mutations that eliminate the ability to mate provide an ~2% per-generation growth-rate advantage. Some strains, including most laboratory strains, carry an allele of GPA1 (an upstream component of the mating pathway) that increases mating efficiency by ~30% per round of mating at the cost of an ~1% per-generation growth-rate disadvantage. In addition to demonstrating a trade-off between growth rate and mating efficiency, our results illustrate differences in the selective pressures defining fitness in the laboratory versus the natural environment and show that selection, acting on the cost of gene expression, can optimize expression levels and promote gene loss.

Results
Sterility Increases Growth Rate by Eliminating Unnecessary Gene Expression. We tested the hypothesis that sterile strains generally have a growth-rate advantage by isolating sterile mutants and testing their fitness. Haploid, a-mating type (MATa) cells, arrest in G1 when exposed to the mating pheromone, alpha-factor (αF), and thus cannot form colonies on media containing αF. We initiated, from a single colony of haploid MATa cells, a large number of parallel cultures that were plated onto either rich media or rich media containing αF. On rich media, the vast majority of cells form colonies, but on αF, only the small fraction of cells that have acquired mutations in pheromone-induced signaling can form colonies. From each culture, we randomly chose a single alpha-factor resistant (αFR) or unselected colony and measured its relative growth rate by using a FACS-based competitive growth-rate assay that can detect growth-rate differences as small as 0.5%. The growth-rate coefficient is a measure of the growth-rate advantage over wild type. Fig. 1A shows the growth-rate coefficient (s) for 27 unselected clones and 45 αFR clones. As a control we measured the relative growth rates of 24 similarly selected mutants that were resistant to canavanine, a toxic arginine analog. In each case, several clones have a low growth rate (s < −1%), suggesting that these strains have become mitochondrial deficient or have acquired a deleterious mutation. Excluding clones with s < −1%, the growth-rate coefficients of the unselected clones follow a tight distribution (Fig. 1A, s = 0.08% ± 0.35%) indistinguishable from the distribution of the canavanine-resistant mutants (Fig. 1A, s = 0.36% ± 0.48%, P > 0.05, Wilcoxon rank sum test); however, the growth-rate coefficients of the αFR mutants show greater variation and a positive growth-rate advantage (Fig. 1A, s = 1.48% ± 0.85%, P < 10−7, Wilcoxon).

It appears from these data that at least some sterile mutants have a clear growth-rate advantage over wild type. To determine whether all sterile strains have a similar advantage, and to determine the basis for any growth-rate advantage in the sterile strains, we used a combination of 4 methods: Phenotypic characterization of the spontaneous αFR mutants, growth-rate assays on targeted gene deletions within the mating pathway, mapping of the mutations in the most fit sterile strains, and expression analysis on αFR strains both with and without a growth-rate advantage.

The yeast mating pathway is one of the best studied mitogen-activated protein (MAP) kinase cascades (12). At the beginning of the pathway is a pheromone receptor (Ste2 in MATa or Ste3 in MATa) that binds the cognate mating pheromone. Receptor stimulation activates a heterotrimeric G protein (consisting of Gpa1, Ste18, and Ste4), which in turn, activates a MAP kinase cascade (consisting of the MAP kinase kinase, Ste11, the MAP kinase, Ste7, the MAP kinases Fus3 and Kss1, and...
growth-rate coefficients indicating that a growth-rate advantage exists for the identified spontaneous αFR mutants with the greatest growth-rate advantage. In strains αFR-2, αFR-8, and αFR-20 we identified mutations in known mating genes: Ste11, Ste5, and Ste7, respectively. In strains αFR-4, αFR-7, and αFR-17 we did not identify any mutations from the yeast tiling arrays; however, subsequent expression analysis suggested that αFR-4 and αFR-7 contain mutations in Ste7 and Ste4, respectively. Sequencing of these genes revealed coding changes in each gene resulting in the following protein modifications: Ste11P656H, Ste7E3ochre, Ste4framshell, Ste5C1098S, and Ste7L7ochre in αFR-2, -4, -7, -8, and -20, respectively. Fig. 2A shows a mapping of growth-rate coefficients for the identified spontaneous αFR mutants and gene deletions onto the mating pathway; 5 of the 6 fast-growing spontaneous αFR mutants contain a single mutation that reduces basal-signaling-dependent gene expression. Strain αFR-1 is the exception; it is the only instance where we found multiple mutations and mutations outside of the mating pathway. In this strain we found mutations in Apc1 (an essential component of the anaphase promoting complex) and Eds1 (an uncharacterized, putative zinc-cluster protein). Apc1 and its G1 cofactor Cdh1 play a conserved and critical role in maintaining G1 arrest and mutations in Apcl result in premature entry into S phase (15, 16). This strain was selected for αF resistance, has an expression profile identical to that of canonical ste mutants, and arrests after overexpression of Far1. Because the pheromone signaling pathway is repressed outside G1, mutants that reduce the duration of G1 will reduce the basal expression of mating genes, as APC mutants have been shown to do (17).

We assayed for changes in gene expression for the 7 αFR mutants from the upper end of the growth-rate distribution (s₉ > 0), 3 from the lower end of the distribution (s₀ = 0), and the targeted gene disruptions (Fig. 2B). The 7 spontaneous αFR mutants from the upper end of the growth-rate distribution
Eds1P6L mutations in the strain H9251 significantly decrease the expression of 23 genes but did not affect Ste2 or Far1. Identification of the mutations in 5 spontaneous suppression of mating pathway genes in the absence of pheromone is maintained by a transcriptional response through the transcription factor, Ste12 (13). Expression analysis shows that the wild-type GPA1 allele reduces expression of genes downstream of Ste12 relative to the GPA1-G1406T allele, although these genes are not reduced to the levels seen for sterile mutations (ste7Δ, ste4Δ, and ste12Δ), which eliminate signaling through the mating pathway (Fig. 3A). To determine whether reducing the expression of the mating genes by restoring the wild-type GPA1 results in a growth-rate advantage, we measured the relative growth rate by using the FACS-based growth-rate assay and by direct competitions. Strains with the wild-type GPA1 allele have a significant growth-rate advantage over strains with the GPA1-G1406T allele in both assays (Fig. 3B and S4). The wild-type GPA1 allele replacement strains is greater than that observed for strains that do not affect signaling (far1Δ and ste2Δ) and less than that observed for strains that eliminate signaling (ste7Δ, ste4Δ, and ste12Δ) through the mating pathway.

Among the genes up-regulated by the GPA1-G1406T allele are the pheromone genes (MFA1 and MFA2); it has been demonstrated that strains producing more pheromone are more attractive to a mating partner (20). To determine whether the 1% growth-rate disadvantage of strains carrying the GPA1-G1406T allele is offset by an increase in mating efficiency, we measured competitive mating efficiency of strains carrying the 2 alleles of GPA1 by using an assay analogous to the competitive growth-rate assay: MATα cells carrying either the wild-type or G1406T allele of GPA1 were mixed and mated to a limited number of
Mating Pathway

Fig. 3. A trade-off between growth rate and mating efficiency. (A) The wild-type allele of GPA1 down-regulates genes in the mating pathway producing an expression profile intermediate to that of deletions eliminating basal signaling (ste7Δ, ste4Δ, and ste12Δ) and those not affecting signaling (far1Δ and ste2Δ). Shown are 3 independent wild-type GPA1 allele replacement strains. (B) Wild-type GPA1 allele replacement strains have a growth-rate advantage relative to the GPA1-G1406T allele strains (sG = 0.92% ± 0.35% and sM = −0.17% ± 0.34% for the wild-type GPA1 allele and the GPA1-G1406T allele, respectively, P < 2.6 × 10−4, t-test). The points represent 3 independent measurements for each of 3 independent transformants of each GPA1 allele. (C) Wild-type GPA1 allele replacement strains have a mating disadvantage relative to the GPA1-G1406T allele strains (sM = −27.2% ± 6.5%). MATα strains carrying each allele were mixed and allowed to compete for a limiting number of MATα cells. The mating coefficients (sM) were calculated as the change in the natural logarithm of the ratio of the 2 alleles: sM = ln(wild-type GPA1/GPA1-G1406T)postmating − ln(wild-type GPA1/GPA1-G1406T)premating.

MATα cells. The ratio of the 2 GPA1 alleles before and after mating was determined and the mating coefficient (sM) of strains carrying the wild-type GPA1 allele was calculated as the change in the natural logarithm of the ratio of the 2 alleles. The mating coefficients are all negative indicating that strains carrying the wild-type GPA1 allele, which have a growth-rate advantage, have a disadvantage in mating relative to the GPA1-G1406T allele (Fig. 3C, sM = −27.2% ± 6.5%).

Discussion

In bacteria, gratuitous gene expression reduces growth rate (21–26). In the most carefully studied case, deregulation of the lac operon can reduce growth rate by ~10% by diverting ribosomes from the synthesis of other proteins (25). This finding implies that any gene slows cell growth in proportion to how strongly it is expressed. We suspect that the cost of gene expression is not specific to bacterial enzymes or genes in the yeast mating pathway, but rather reflects a universal cost of gene expression and that this cost must be borne in all environments where the gene is expressed. In environments where the protein’s expression increases fitness, this cost is offset by larger benefits, but it is never zero. Previous attempts to demonstrate a general fitness advantage from the elimination of dispensable genes in yeast have been unsuccessful (27, 28). Because 97% proteins are expressed at levels <0.1% of total cell protein (29), the growth-rate advantage for the majority of single gene deletions is below the limit of detection by current assays. However, because mating depends on the expression of 23 genes, mutations that eliminate signaling through the mating pathway eliminate expression of a number of genes and produce a measurable growth-rate advantage.

Given that gene expression is costly, it is surprising that some strains carry a mutation in GPA1, an upstream component of the mating pathway, which increases expression of the mating genes; this polymorphism is one of the strongest trans-acting regulatory polymorphisms between laboratory and wild strains (18). Our results provide a plausible explanation for the existence of the GPA1-G1406T allele: This mutation increases the basal expression of genes in the mating pathway thus increasing mating efficiency by ~30% at a growth-rate cost of ~1% per generation. In the laboratory, cells are mated en masse, a condition that strongly selects for cells that produce more pheromone (20). For these GPA1 variants, where we have determined the effect on growth rate and mating efficiency, we can determine under what conditions each allele will be favored. If these 2 strains are mixed and propagated in a regime where 1 round of mating occurs every 30 generations, these 2 strains would be equally fit (Fig. 4, black trace). If mating is less frequent than every 30 generations, the wild-type allele is favored, whereas if mating is more frequent than every 30 generations, the GPA1-G1406T allele is favored (Fig. 4). During long-term evolution, strains are typically propagated asexually. In this regime, sterile strains, which eliminate basal signaling through the mating pathway, will outcompete mating-proficient strains (Fig. 4, green trace).

The success of different GPA1 alleles in the laboratory versus in wild strains reflects differences in the selective pressures defining fitness in these 2 environments. A similar phenomenon has been observed in E. coli where the laboratory strain B carries an allele of the arginine repressor, argR, that results in deregulation of the arginine biosynthesis pathway, providing a selective advantage under conditions where arginine availability fluctu-
ates rapidly (30). The arg48 allele appears to be a naturally occurring variant that is beneficial under laboratory conditions (31). To follow up on the possibility that the GPA1 mutation arose during laboratory cultivation, we traced the lineage of S288c back to the wild diploid strain, EM93, which was isolated from a rotten fig near Merced, California in 1938 (32). We sequenced the GPA1 genes in EM93, and found, somewhat to our surprise, that EM93 is homozygous for the GPA1-G1406T allele. Thus, we are left with some ambiguity as to evolutionary origin of this allele: If one discounts a strain mix-up, the mutation appears to have arisen in the wild, raising the question as to how much of laboratory domestication is the result of de novo mutation versus the selection of favorable combinations of naturally occurring alleles.

Gene loss is an important process in evolution. Extensive gene loss in protomitochondria and Mycobacterium leprae may have fostered the transition from facultative to obligate intracellular parasites (33), reciprocal gene loss after whole-genome duplication has reinforced species barriers by establishing Dobzhansky-Muller incompatibilities (34), and the loss of key developmental regulators early in vertebrate evolution has been suggested to have played a role in the establishment of modern phyla (35). Here, we provide evidence for a general cost of gene expression and find that elimination of the expression of 23 genes in EM93, and found, somewhat to our surprise, that EM93 is homozygous for the GPA1-G1406T allele. Thus, we are left with some ambiguity as to evolutionary origin of this allele: If one discounts a strain mix-up, the mutation appears to have arisen in the wild, raising the question as to how much of laboratory domestication is the result of de novo mutation versus the selection of favorable combinations of naturally occurring alleles.

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gene. Significant changes reported in Fig. 3 were determined by eye from a volcano plot of log2(red/green) versus P value and correspond to a 1.5-fold change in expression and P < 10^{-3.15} (Fig. 52).

Competitive Mating Assays. Competitive mating assays were performed by competing MATα cells carrying the GPA1-G1406T allele with cells carrying the wild-type allele of GPA1 (marked with NatMX) for a limited number of MATα cells. Each strain was grown to mid-log phase (10^7 cells per mL) then 5 × 10^6 cells of each MATα strain added to 10 mL of YPD + ADE. A sample was diluted, sonicated, and plated to single colonies on -leu media. MATα mating tester (2 × 10^6 cells) was added to the MATα mixture and filtered onto a 25-mm 0.45-µm nylon filter. Cells were mated on a YPD plate at 30°C. After 5 h, the filters were washed and the cells were diluted, sonicated, and plated to single colonies onto minimal media to select for diploids. Colonies on the -leu and minimal plates were replica plated to YPD plates containing ClonNat to determine the ratio of strains carrying the wild-type and G1406T alleles before and after mating. The mating coefficient (s_m) was calculated as the change in the natural logarithm of the ratio of the 2 alleles: s_m = \ln(\text{wild-type GPA1/GPA1-G1406T}_{\text{premating}}) - \ln(\text{wild-type GPA1/GPA1-G1406T}_{\text{postmating}}).

Notebook. The complete laboratory notebook describing these experiments is available at http://www.genomics.princeton.edu/glang/notebooks.htm.

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