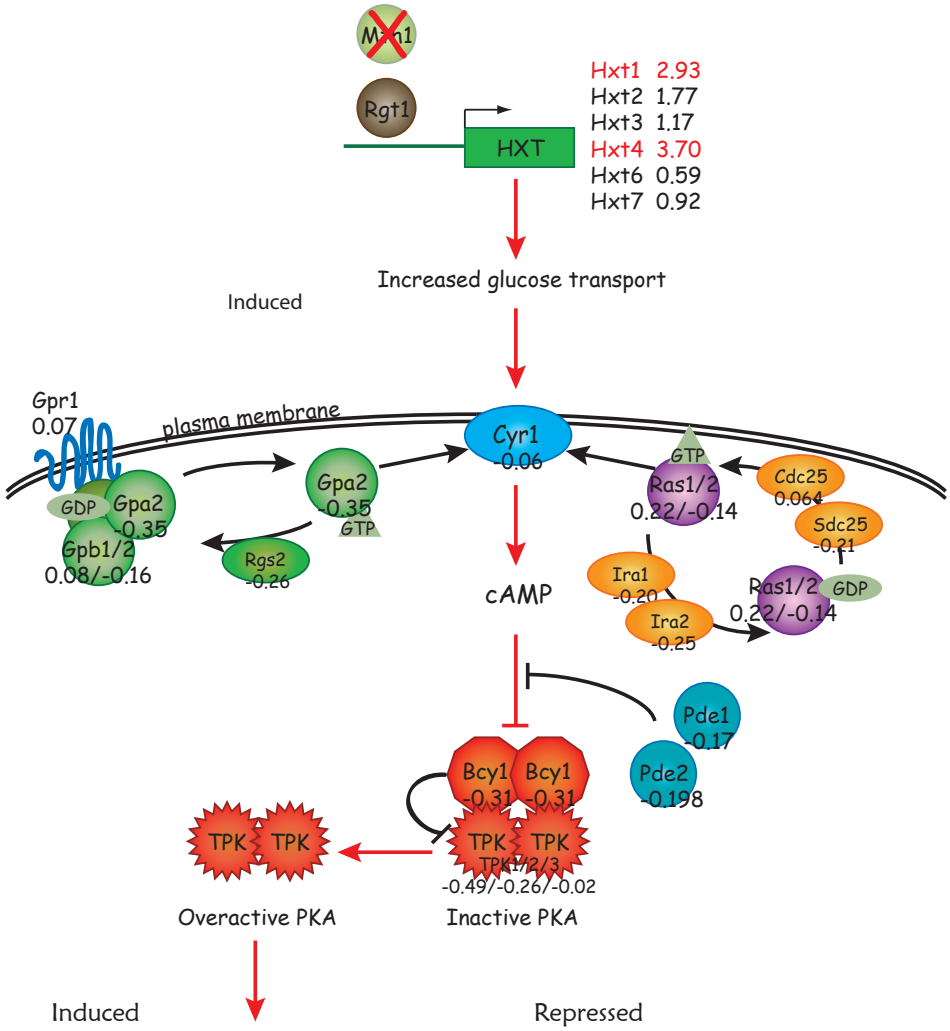
# Molecular Characterization of Clonal Interference during Adaptive Evolution in Asexual Populations of *Saccharomyces cerevisiae*

Katy C. Kao[1] and Gavin Sherlock[2]

[1]Department of Chemical Engineering, Texas A&M University, College Station, TX 77843, USA

[2]Department of Genetics, Stanford University, Stanford, CA 94305-5120, USA

## Supplementary Information



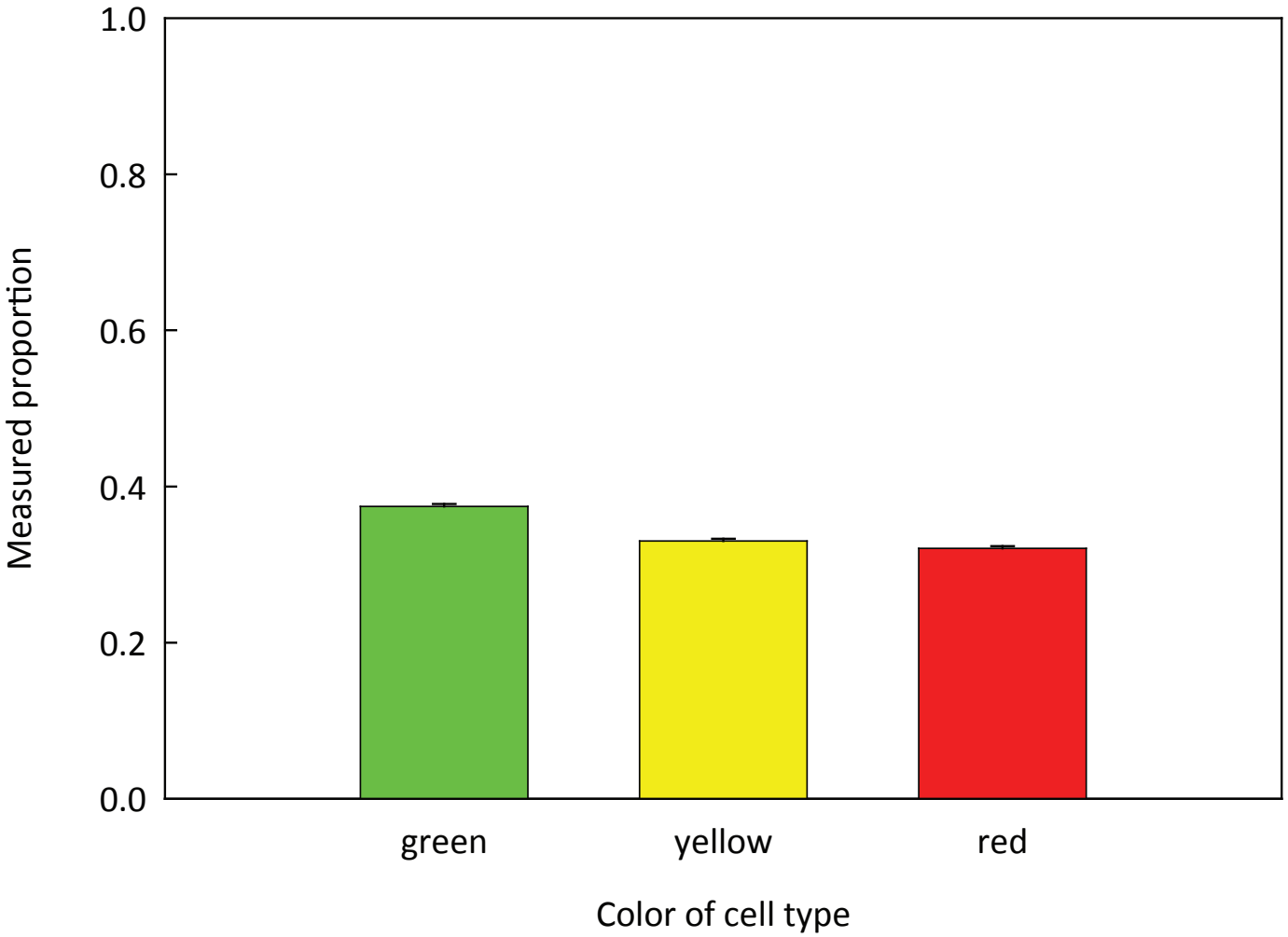

Induced

| geneName | M1 | geneName | M1 |
|---|---|---|---|
| YDL014W | 0.86453 | YPL012W | 0.61844 |
| YEL055C | 0.4553 | YPL217C | 0.63579 |
| YER043C | 0.49913 | YPR010C | 0.6304 |
| YER055C | 0.5562 | YGL008C | 0.67454 |
| YER110C | 0.7621 | YGR279C | 0.49907 |
| YGR151C | 0.64637 | YGL202W | 0.53264 |
| YHR019C | 0.40517 | YHR143W | 0.62188 |
| YHR128W | 0.66575 | YCR031C | 0.44045 |
| YJL050W | 0.44949 | YOR133W | 0.61391 |
| YKL014C | 0.58164 | YER086W | 0.43071 |
| YKL056C | 0.56998 | YJL193W | 0.60967 |
| YKL081W | 0.50801 | YLR180W | 0.90027 |
| YLR048W | 0.44339 | YDL055C | 0.56743 |
| YLR197W | 0.61414 | YJL130C | 0.47205 |
| YLR432W | 0.74487 | YOL136C | 1.20308 |
| YML056C | 1.0567 | YJL158C | 0.5109 |
| YMR217W | 0.48361 | YGR138C | 1.33834 |
| YMR305C | 0.84736 | YJL109C | 0.60099 |
| YNL112W | 0.73777 | YPL126W | 0.40016 |
| YNL141W | 0.98249 | YGL012W | 0.69571 |
| YNL209W | 0.69188 | YOR327C | -0.52334 |
| | | YLR214W | 0.64082 |

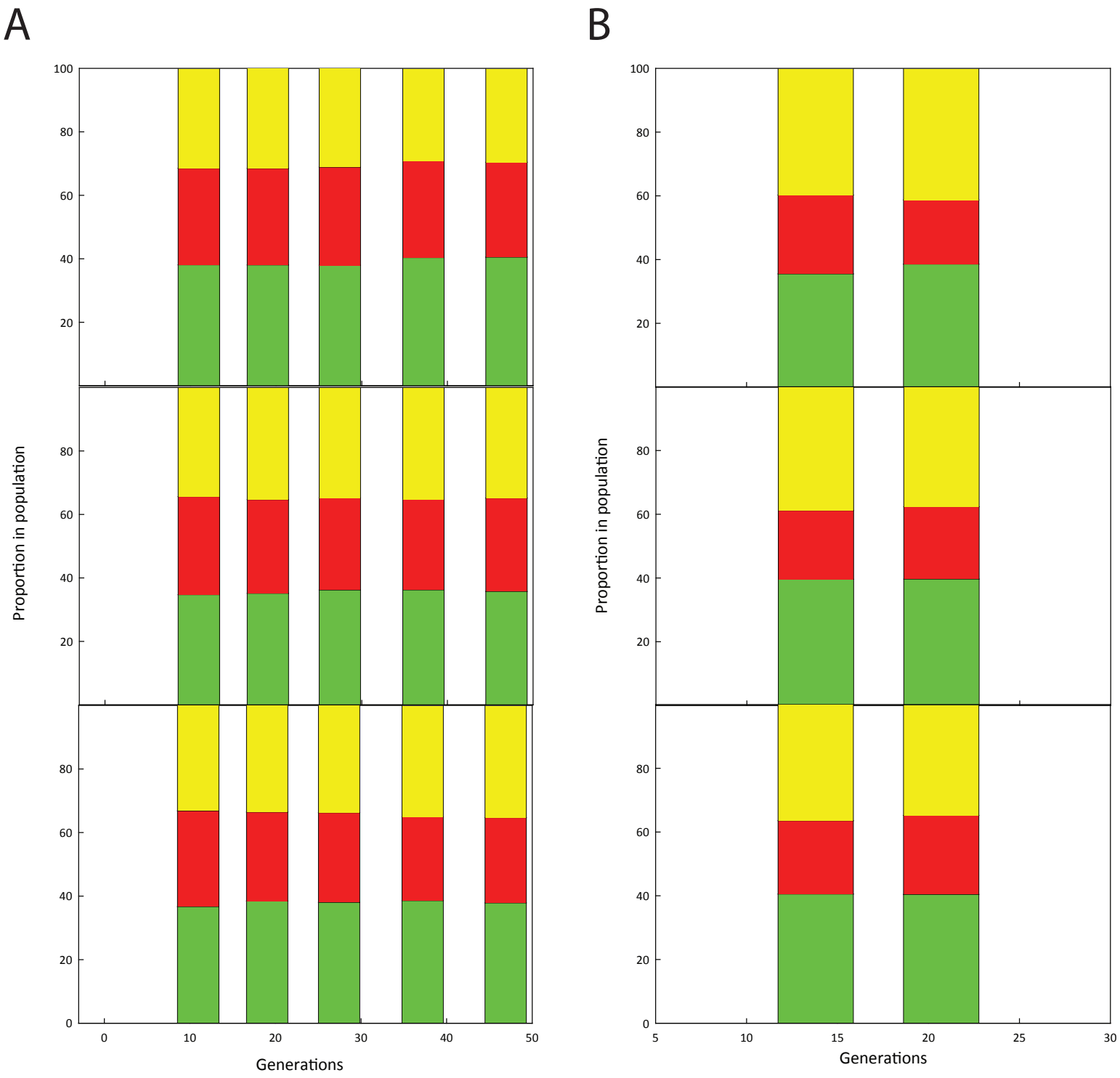
Repressed

| geneName | M1 | geneName | M1 | geneName | M1 | geneName | M1 |
|---|---|---|---|---|---|---|---|
| YDL169C | -0.69273 | YCR061W | -0.6678 | YBR026C | -0.9584 | YMR304W | -0.6431 |
| YDR256C | -3.24056 | YDL214C | -1.1777 | YBR169C | -0.6048 | YMR322C | -1.5675 |
| YKR067W | -0.6086 | YER015W | -1.3453 | YBR241C | -0.5362 | YMR323W | -1.6685 |
| YLR216C | -0.32929 | YGL006W | -0.3579 | YBR285W | -0.8041 | YNL009W | -1.9202 |
| YMR103C | -0.5317 | YGL205W | -2.1596 | YDL085W | -1.837 | YNL115C | -0.6807 |
| YDL215C | -1.18596 | YGR205W | -0.6466 | YDL110C | -0.6915 | YNL200C | -0.6539 |
| YJR148W | -0.54803 | YIL055C | -0.9857 | YDL204W | -1.1627 | YNL270C | -0.8539 |
| YKL148C | 0.39647 | YIL057C | -3.1818 | YDR074W | -0.5417 | YOR161C | -0.7794 |
| YLR438W | -1.20136 | YIL155C | -1.0981 | YDR223W | -1.9054 | YOR173W | -0.7623 |
| YNL117W | -1.67227 | YKR009C | -2.2916 | YDR533C | -1.1467 | YOR185C | -0.7015 |
| YOR100C | -2.16356 | YML089C | -0.9997 | YER121W | -1.9314 | YOR215C | -0.7177 |
| YOR348C | -0.63837 | YMR107W | -2.4425 | YFL006W | -0.4671 | YOR388C | -2.7347 |
| YPL201C | -2.42042 | YPL147W | -2.3221 | YGR043C | -1.9696 | YPL017C | -0.4301 |
| YPR001W | -3.2346 | YER024W | -1.2695 | YGR044C | -0.4277 | YPL036W | -1.5596 |
| YAL054C | -2.71345 | YBR270C | -0.3855 | YGR201C | -0.8227 | YPL113C | -1.0173 |
| YBR046C | -0.77676 | YKL199C | -0.3204 | YGR236C | -2.7181 | YPL276W | -2.5318 |
| YCR010C | -4.11999 | YFL055W | -0.8508 | YGR256W | -1.2455 | YFL054C | 0.5886 |
| YDL174C | -0.40931 | YIL136W | -0.9999 | YHR140W | -0.5171 | YDR534C | -0.5597 |
| YDR018C | -0.48702 | YJR061W | -0.477 | YIL124W | -0.5603 | YER054C | -1.5656 |
| YER136W | -0.45876 | YLR174W | -0.9156 | YJR036C | -0.7476 | YHR137W | -2.168 |
| YGL156W | -0.91602 | YML054C | -0.9645 | YJR096W | -0.7211 | YKL107W | -1.6409 |
| YIL087C | -0.40587 | YOL147C | -0.5652 | YKL151C | -0.9238 | YPR002W | -2.176 |
| YIL099W | -0.91588 | YPR006C | -1.743 | YKL187C | -2.7113 | YIL107C | -0.7635 |
| YIL160C | -2.86518 | YPR151C | -1.0318 | YLL026W | -0.6762 | YOR120W | -0.8791 |
| YLR284C | -1.33157 | YER093C | -0.3067 | YLR080W | -0.8144 | YLR290C | -0.4265 |
| YML042W | -2.9355 | YIR016W | -0.7396 | YLR164W | -1.5189 | YDR070C | -2.1763 |
| YMR110C | -0.66489 | YJR008W | -0.9751 | YLR178C | -0.869 | YER096W | -1.1234 |
| YMR160W | -0.34164 | YBR214W | -0.5284 | YLR273C | -0.6689 | YMR196W | -0.7752 |
| YOR180C | -0.97126 | YDR099W | -0.3509 | YML004C | -1.0543 | YAR027W | -0.397 |
| YPL222W | -0.86633 | YER066W | -0.3839 | YMR053C | -0.6913 | YGL121C | -0.966 |
| YNL202W | -1.7003 | YML131W | -1.053 | YMR250W | -0.9725 | YJR099W | -0.4489 |
| YOR328W | -0.95971 | YMR090W | -0.8184 | YMR271C | -0.8459 | YGR294W | -0.6648 |
| | | YHL032C | -1.1805 | | | YDR294C | -0.3903 |

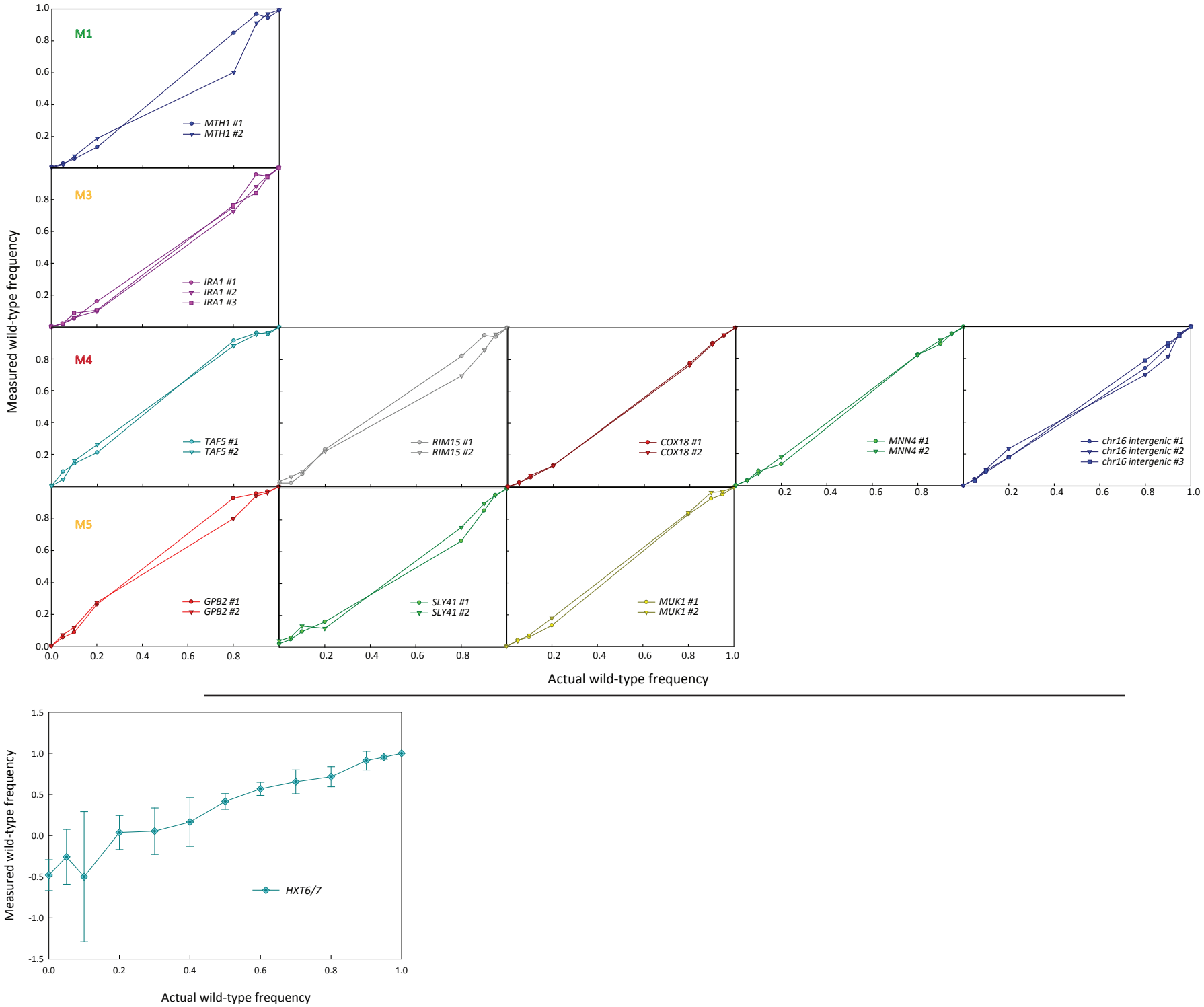
**Supplementary Figure 1.** Simplified diagram of the cAMP signaling pathway showing a potential scenario for the activation of PKA signaling pathway caused by the nonsense mutation in *MTH1* present in the M1 adaptive strain. Mth1 normally represses *HXT* transcription via Rgt1. The log base 2 gene expression data for the components of the signaling pathway are shown below or next to each gene. The tables labeled "induced" or "repressed" are significantly perturbed genes (see Methods) in our dataset that are either induced or repressed in the activated Gpa2 and Ras experiments in Wang et al. The genes whose expression was significantly induced in M1 are highlighted in red, while significantly repressed genes are highlighted in green. Only one gene in the "induced" and two genes in the "repressed" set differ between our data and Wang et al data. The consistency between the datasets suggests that the PKA signaling pathway is increased in M1 relative to the original parent.




**Supplementary Figure 2.** FACS measurement of approximately equal numbers of parental strains expressing GFP, YFP, and DsRED. The average and standard deviation are based on triplicate measurements of 3 separate mixtures.




**Supplementary Figure 3.** FACS measurements of changes in frequency of the different fluorescently expressing cells during A) a serial batch transfer experiment, and B) a short-term chemostat experiment. Each experiment was repeated in triplicate. The three parental strains expressing GFP, YFP, and DsRED were mixed in at equal proportions at the start of the experiment. The green, yellow and red bars represent the proportion of the population




**Supplementary Figure 4.** Allelic frequency test results for a) the different allelic specific primers and b) the HXT6/7 amplification.

**Supplementary tables**

| Strain | Generation | Subpopulation |
|---|---|---|
| M1 | 56 | Green |
| M2 | 91 | Red |
| M3 | 196 | Yellow |
| M4 | 256 | Red |
| M5 | 385 | Yellow |

**Supplementary Table 1. Description of the adaptive clones analyzed.**

The adaptive clones were isolated at the generations and from the subpopulations specified. See Figure 1A in paper for population structure and details of isolation.

| Strain | Genotype | Source |
|---|---|---|
| FY2 | Matα, *ura3-52, gal+* in S288c background | |
| GSY1135 | FY2 *YBR209W*::Act1p-DsRED.T1.N1-Act1t-*URA3* | this work |
| GSY1136 | FY2 *YBR209W*::Act1p-GFP-Act1t-*URA3* | this work |
| GSY1137 | FY2 *YBR209W*::Act1p-YFP-Act1t-*URA3* | this work |

**Supplementary Table 2.** List of strains used.

| Sequencing Primers used for verification of genotypes | |
|---|---|
| **Primer** | **Sequence** |
| Chr1_39603_for | GATTGTGATTCATTGGCAGG |
| Chr1_39603_rev | TCCTTCCTTAGCTTTGGTAC |
| Chr2_616436_for | GAACGACGCTACTAAATCGC |
| Chr2_616436_rev | GTTTCTCCCCGCTAGGCC |
| Chr4_1014688_for | CCTTGGGAATTTGGAGCTC |
| Chr4_1014688_rev | GAATTCAAACTAACCAGCGC |
| Chr6_73424_for | CTAGTCCAGGATCCTGATC |
| Chr6_73424_rev | CTTGGAAGCACGTGGTATC |
| Chr7_617106_for | TTCTGCCTCTTTCTTGTCTC |
| Chr7_617106_rev | TGAGGTTGTGGAGCTGTTG |
| Chr11_64696_for | AGGGTCCTTATCAAACATTTG |
| Chr11_64696_rev | TTTAGATTTTGGTTCGACTATG |
| Chr15_893328_for | AGGGCTACTGTTTCTAGCG |
| Chr15_893328_rev | TGTTGGATTTGCTTCCATCG |
| Chr16_422264_for | TGAAACTACATGAAATCATGAC |
| Chr16_422264_rev | AGATGCTGAAGATCGTGAAG |
| Chr16_912519_for | TTCTTCCCCATGATAGATAAG |
| Chr16_912519_rev | CATCATACGAAAGTATGTCTG |

| Primers for Allele Frequency Determination | | | |
|---|---|---|---|
| **Gene / location** | **Primer** | **Sequence** | **Comment** |
| *GPB2* | chr1_yellow_wt_for | GGGCATGCGTTGAAGAATCC | common forward primer |
| | chr1_yellow_mut_rev | AATGGAATCCCAACAATTATCTC | mutant allele-specific reverse primer |
| | chr1_yellow_rev | GACCGCACAGTACGATTCAG | wild-type allele-specific reverse primer |
| *SLY41* | chr15_yellow_wt_for | TCTTACTGCCATGTGTTGACC | wild-type allele-specific forward primer |
| | chr15_yellow_mut_for | CTCTTACTGCCATGTGTTGACA | mutant allele-specific forward primer |
| | chr15_yellow_rev | TCGAACATCGTTATTACAATTCC | common reverse primer |
| *MUK1* | chr16_yellow_wt_for | CAGTAGAGTCTCATTGCCATC | wild-type allele-specific forward primer |
| | chr16_yellow_mut_for | CAGTAGAGTCTCATTGCCATA | mutant allele-specific forward primer |
| | chr16_yellow_rev | CTTTGGATAGTAGGAGTCACG | common reverse primer |
| *IRA1* | chr2_yellow_wt_for_new | GCCAAATGATACTGTAGAAGATC | wild-type allele-specific forward primer |
| | chr2_yellow_mut_for | GCCAAATGATACTGTAGAAGATT | mutant allele-specific forward primer |
| | chr2_yellow_rev | TTGAATCTGCAAAAGCATTGGC | common reverse primer |
| *MTH1* | chr4_green_wt_for | GTCTTTTTAAGTTGGACTGTTG | wild-type allele-specific forward primer |
| | chr4_green_mut_for | GGTCTTTTTAAGTTGGACTGTTA | mutant allele-specific forward primer |
| | chr4_green_rev | TTAAATATAGCAGTAGAGGCAC | common reverse primer |
| *TAF5* | chr2_red_wt_for | TAAGGAATAAATGGCATTCTTAC | wild-type allele-specific forward primer |
| | chr2_red_mut_for | ATAAGGAATAAATGGCATTCTTAA | mutant allele-specific forward primer |
| | chr2_red_rev | TAGCGAAGATGGTATTATTAATG | common reverse primer |
| *RIM15* | chr6_red_wt_for | GAAATGGTTCCTGATCTTTACC | wild-type allele-specific forward primer |
| | chr6_red_mut_for | TGAAATGGTTCCTGATCTTTACA | mutant allele-specific forward primer |
| | chr6_red_rev | GGAATTGATCTAACAAAGCATTG | common reverse primer |
| *COX18* | chr7_red_wt_for | CACATATACCATGGATAGTGCT | wild-type allele-specific forward primer |
| | chr7_red_mut_for | CACATATACCATGGATAGTGCA | mutant allele-specific forward primer |
| | chr7_red_rev | AGTTCTTGTTGTTTCAAAATTCTC | common reverse primer |
| *MNN4* | chr11_red_wt_for | GACTACATTAGATCAAGTTACCA | wild-type allele-specific forward primer |
| | chr11_red_mut_for | ACTACATTAGATCAAGTTACCG | mutant allele-specific forward primer |
| | chr11_red_rev | ATGTTGAAAAGTGAAAAATTCCTG | common reverse primer |
| chr16 intergenic | chr16_red_wt_for | ACGATTCGAAATTACTCTTTTCTT | wild-type allele-specific forward primer |
| | chr16_red_mut_for | CGATTCGAAATTACTCTTTTCTG | mutant allele-specific forward primer |
| | chr16_red_rev_new | TTCTTTTCTGTGTTCTTAGTGTC | common reverse primer |
| *HXT6/7* | hxt6_for | AATGGCTTATCATCGTGAGCC | |
| | hxt6_rev | CCTCATGGGTTCCACCATCT | |

**Supplementary Table 3.** Primers used.

## Supplementary Methods

### Construction of Fluorescently Marked Strains

Fluorescently tagged strains were constructed by integrating plasmids pGS62, pGS63, and pGS64 into FY2. These plasmids were constructed from YIplac211 (Gietz and Sugino, 1988) in the following fashion: YIplac211 was digested with *Hind*III, blunt-ended with Klenow polymerase, and then digested with *Sph*I, making it compatible with *Eco*RV and *Sph*I cutting sites. A 157 bp region from the dubious ORF, *YBR209W*, was amplified by PCR, digested with *Eco*RV and *Sph*I, then cloned into this vector. The resulting plasmid was digested with *Eco*RI, blunt-ended with Klenow, then digested with *Kpn*I; into this was then inserted a DNA fragment containing a 161 bp region from the 5' region of *YBR209W* digested with *Eco*RV and *Kpn*I. *Eco*RI and *Sph*I were then used to cut this plasmid in the middle of the *YBR209W* region and into this was then inserted a fragment of DNA containing the *ACT1* promoter amplified from pRB2138 (Doyle and Botstein, 1996), coupled with either Green Fluorescent Protein (GFP) amplified from pFA6a-GFPS65T-HisMX6 (Wach et al., 1997), Yellow Fluorescent Protein (YFP) amplified from pDH5 (http://depts.washington.edu/yeastrc/), or a red fluorescing protein (DsRED1.T1) amplified from pTY24 (http://depts.washington.edu/yeastrc/); each was followed by the *ACT1* terminator amplified from pRB2132 (Doyle and Botstein, 1996). These inserts were generated using fusion PCR followed by digestion with *Eco*RI and *Sph*I. The resulting plasmids were linearized with *Hind*III and transformed into FY2 to integrate at the 5' region of *YBR209W* using a lithium acetate protocol (Gietz et al., 1995). Integration was confirmed by PCR. The resulting strains were GSY1136

(YBR209W::YIplac211-*ACT1*p-GFP-*ACT1*t), GSY31137 (YBR209W::YIplac211-*ACT1*p-YFP-*ACT1*t), and GSY1135 (YBR209W::YIplac211-*ACT1*p-DsRED.T1.N1-*ACT1*t).

**FACS error estimation**

The error of FACS measurements were determined by mixing approximately equal numbers of parental strains expression GFP, YFP, or DsRED, followed by FACS analysis. Three different mixtures were generated, and each mixture was measured 3 times by the FACS. The results are shown in Supplementary Figure 1. The error of FACS measurement is less than 1%.

**Fluor bias**

The potential biases in the different fluorescent proteins were tested by serial batch transfer experiments with equal numbers of parental strains expressing GFP, YFP, or DsRED in the starting population. Approximately 10,000 cells were serially transferred every 24 hours in 200 μl of 0.25% glucose minimum medium aerating on a roller drum at 30 °C. The cells undergo approximately 10 generations per 24 hour period. The results for three replicate experiments are shown in Supplementary Figure 2. No significant bias in fitness was observed between the three fluors from the serial batch transfer experiments.

**Allelic frequency test**

The primers used for the population allelic frequencies were tested for their ability to estimate the proportion of each allele in a mixture. The wild-type genomic DNA was mixed in with the genomic DNA of M1, M3, M4, or M5 (M2 was not used since it contains an identical mutation as in M4) to generate mixtures with the following percentages of wild-type genomic DNA: 100, 95, 90, 80, 20, 10, 5, 0. Each series was tested at least twice. The allele frequency is calculated using the following equations:

$$\text{frequency of wild-type allele} = 1/(2^{(\text{Ct wild-type} - \text{Ct mutant})} + 1)$$

$$\text{frequency of mutant allele} = 1 - \text{frequency of wild-type allele}$$

The results are shown in Supplementary Figure 3A. For the estimation of the *HXT6/7* frequency in the population, M4 genomic DNA was mixed in with a parental genomic DNA to generate mixtures with the following percentages of wild-type genomic DNA: 100, 95, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, and 0. Each series was repeated 3 times. The calculation of allelic frequency in the mixture was estimated by using a copy number of 8 for the *HXT6/7* allele in M4. The following formula was used to estimate the frequency of the *HXT6/7* allele:

$$\text{frequency of wild-type HXT6/7 allele} = -(2^{(\text{Ct wildtype} - \text{Ct mutant})} - 8)/7$$

$$\text{frequency of mutant } \textit{HXT6/7} \text{ allele} = 1 - \text{frequency of wild-type } \textit{HXT6/7} \text{ allele}$$

The results are shown in Supplementary Figure 3B. Note, the measurement of the *HXT6/7* mutant frequency was not as accurate as for the other mutations, and thus the estimation of the *HXT6/7* amplification frequency in the population is only a rough estimate.

**Gene Expression Data**

SAM analysis (Tusher et al., 2001) found a total of 393 genes to be significantly induced or repressed in at least one of the adaptive clones assayed. As expected, the nonsense mutation in *MTH1* in adaptive mutant M1 resulted in an increased expression of *HXT1* and *HXT4*, while the genes whose expression was induced in M3 were also enriched for those involved in hexose transport. Even though these genes are known to be affected by the PKA signaling pathway, it is unclear whether the mutation in *IRA1*, which results in a conservative substitution, was the direct cause of their differential expression. Disruption of *RIM15* has previously been shown to affect genes involved in stress response (*HSP12*, *HSP26*, *SSA1*, *CTT1*Reinders et al., 1998). Indeed, these genes were repressed in M4, indicative of a nonfunctional *RIM15*. In addition, the amplification in *HXT6/7* in M4 also resulted in an increase in gene expression of *HXT6*, but not *HXT7*. Surprisingly, even though an amplification was also observed in the region of *HXT6/7* in M5, the increase in *HXT6* gene expression was not as significant. The mutation in *GPB2* in M5 may result in an increase in PKA activity. Comparison of our gene expression data to a published list of genes whose transcript abundances change in response to activation of RAS or GPA2 (Wang et al., 2004), showed that several genes previously found to be induced or repressed by PKA activation were also similarly induced or repressed in M5.

**Supplementary methods references**


1. Doyle, T. and D. Botstein (1996). Movement of yeast cortical actin cytoskeleton visualized in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **93,** 3886-3891.
2. Gietz, R.D., R.H. Schiestl, A.R. Willems, and R.A. Woods (1995). Studies on the Transformation of Intact Yeast-Cells by the Liac/S-DNA/Peg Procedure. *Yeast* **11,** 355-360.
3. Gietz, R.D. and A. Sugino (1988). New Yeast-*Escherichia coli* Shuttle Vectors Constructed with Invitro Mutagenized Yeast Genes Lacking 6-Base Pair Restriction Sites. *Gene* **74,** 527-534.
4. Reinders, A., N. Burckert, T. Boller, A. Wiemken, and C. de Virgilio (1998). Saccharomyces cerevisiae cAMP-dependent protein kinase controls entry into stationary phase through the Rim15p protein kinase. *Genes & Development* **12,** 2943-2955.
5. Tusher, V.G., R. Tibshirani, and G. Chu (2001). Significance analysis of microarrays applied to the ionizing radiation response (vol 98, pg 5116, 2001). *Proceedings of the National Academy of Sciences of the United States of America* **98,** 10515-10515.
6. Wach, A., A. Brachat, C. AlbertiSegui, C. Rebischung, and P. Philippsen (1997). Heterologous HIS3 marker and GFP reporter modules for PCR-targeting in Saccharomyces cerevisiae. *Yeast* **13,** 1065-1075.
7. Wang, Y., M. Pierce, L. Schneper, C.G. Guldal, X.Y. Zhang, S. Tavazoie, and J.R. Broach (2004). Ras and Gpa2 mediate one branch of a redundant glucose signaling pathway in yeast. *Plos Biology* **2,** 610-622.