Supplementary Materials

Roles of *cis*- and *trans*-changes in the regulatory evolution of genes in the gluconeogenic pathway in yeast

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1. The primer and probe sequences for qRT-PCR.

MIG1-BY1
AGTTCTAGTGCTACCAGTATACCAGATTT

MIG1-BY2
TCTACCTTTTCTTGTAGAAGATCGACT

MIG1-RM1
TCTAGTGCTACCAGTATACCAGATTC

MIG1-RM2
CTACCTTTTCTTGTAGAAGATCGAC

MIG1-BY-probe
6FAM-CAACACCTATCGCCT

MIG1-RM-probe
VIC-CAACACTTATCGCCT

CAT8-BY1
CCTGGTCTTCCTGCGGTAAAG

CAT8-BYRM-2
GCTGAGGAATAAACAGAATTTCTTCA

CAT8-RM1
CCTGGTCTTCCTGCGGTAAAA

CAT8- BY-probe

6FAM-AATGACGACTCATCAAC

CAT8- RM-probe
VIC-CAATGACGGCTCATCAA

MLS1-1

TGTTGATTGAAACTTTGCCTGCT

MLS1-3

TTGATTGAAACTTTGCCTGCG

MLS1-4

AATGGGGAAGTCATAGTCACTTGATC

MLS1-6

CCCCTCCGATGACAGGTATTG

MLS1-TaqManprobe

NED-AGT GGG TTG AAT TGC GGA CGT TGG-MGBNFQ

TaqMan-probe-BY-MLS1

6FAM-TCA CTT GAT TTC TAT TGG GC-MGBNFQ

2. Pyrosequencing Primers. For each tested gene, “F” represents the forward primer, “R” the reverse, and “SR” or “SF” the extension primer to exam the SNP. If not indicated, the primers are used for comparisons between BY and RM, or YJM and RM strains.

CAT8: (3661)

F – 5’-ACCAAAATGATCAGAACTCCGC-3’ (22 bp.)

R – 5’-TAAATCGTTGAAATCACAGTTGTCC-3’ (25 bp.)

SR – 5’-TGCCAGTTGAAGGTG-3’ (15 bp.)

MIG1:

BY/RM or YJM/RM (649) -

F – 5’-CATCCGCTTCCACTGCTTTGT-3’ (21 bp.)

R – 5’-GCGTTCAGTTTCAGTCTACTGCC-3’ (23 bp.)

SF – 5’-TGCTTTGTCTTCGTTGA-3’ (17 bp.)

BY/YJM (1196) -

F – 5’-CTAGTGGTACGAATTTGCACACTT-3’ (24 bp.)

R – 5’-TTTTGGAAATCAGGAGATGATGAG-3’ (24 bp.)

SF – 5’-ACCAAAATCACTTGCAT-3’ (17 bp.)

MLS1: (795)

F – 5’-GAGCACCACTTGGAAGCTAAACTA-3’ (24 bp.)

R – 5’-ATATAGTCCCAACGTCCGCAATT-3’ (23 bp.)

SR – 5’-TCTTCCATTTGGAAAGC-3’ (17 bp.)

IDP2: (426)

F – 5’-ATTCGGCGATCAGTACAAAGC-3’ (21 bp.)

R – 5’-ATCTACATCATGAGTTCCGCTCTT-3’ (24 bp.)

SR – 5’-CCTTCTTCAGGGACTATTAC-3’ (20 bp.)

SFC1: (678)

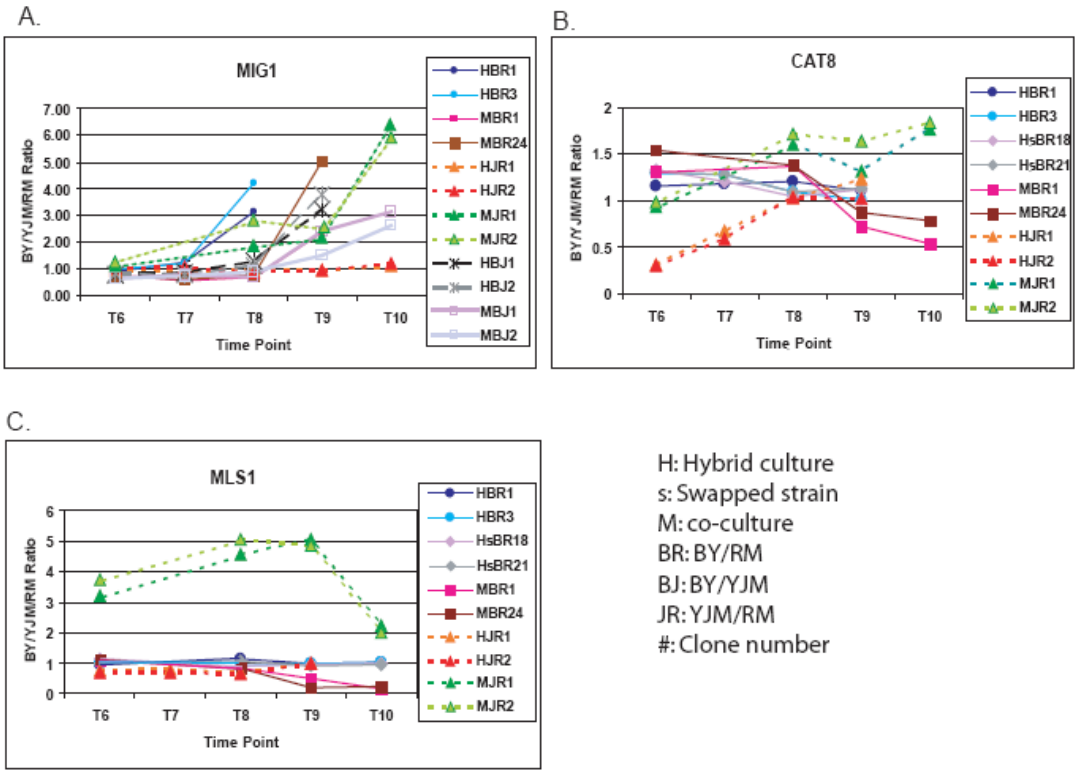
F – 5’-CTTCATGGGAAACTTCTTGCATAG-3’ (24 bp.)

R – 5’-AGTTGAGCACCAATGGTTATGAT-3’ (23 bp.)

SR – 5’-GTTGGAAAACGGCCC-3’ (15 bp.)

Universal biotin-labeled primer:

5’-Biotin-GGGACACCGCTGATCGTTTA-3’ (20 bp.)



H: Hybrid culture
s: Swapped strain
M: co-culture
BR: BY/RM
BJ: BY/YJM
JR: YJM/RM
#: Clone number

Fig. S1.—Pyrosequencing results of *MIG1*, *CAT8* and *MLS1* from co-cultures or hybrids between the BY, RM, or YJM strains with mutiple sampling time points. (A) *MIG1* gene expression ratios between two alleles were tested with all three pairs of co-cultures and hybrid strains. (B) *CAT8* gene was tested between BY/RM and YJM/RM alleles, as well as between BY and RM swapped strains. (C) *MLS1* gene has been done in the same comparisons as *CAT8* gene.

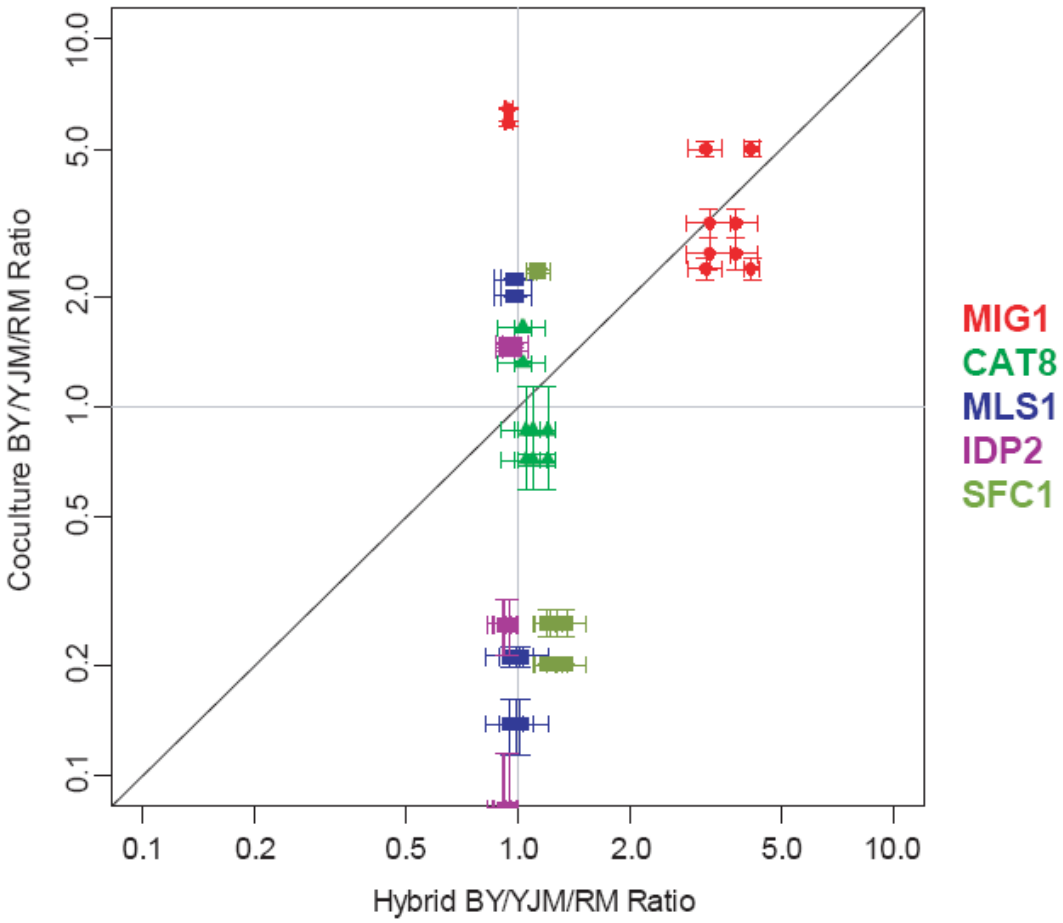


Fig. S2.—Pyrosequencing results of *MIG1*, *CAT8*, *MLS1*, *IDP2* and *SFC1* from the cocultures or hybrids between the BY, RM, or YJM789 strains. Different colors represent indicated genes on the right. Each dot is the allele ratios with the corresponded time pairs of cocultures and hybrids during the diauxic shift in a log scale. The bars show the standard deviations. The diagonal line represents the 1 to 1 ratio, i.e. $BY/RM_{hybrid} = BY/RM_{mixed}$. In other words, when the dots are on or along the diagonal line represents that the gene expression vairances are primarily due to cis-elements. The vertical line on 1 represents that the completely trans-regulatory causes the expression differences.