# Abstract (300words)

# Introduction

## Aging and ROS

## Aging and LOH {McMurray, 2003 #244;McMurray, 2004 #419}

## ROS and LOH?

## Significance of this project

The link between ROS and LOH and mitotic asymmetry

We hypothesized that the frequency of loss of heterozygosity events will occur in a dosage dependent manner of hydrogen peroxide (H2O2).

# Materials and Methods

## Strains

Strains with heterozygous Met15+/- were described previously {Qin, 2008 #516}.

Strains were grown overnight at 30°C in 5 mls of YPD using autoclaved glass tubes. Following incubation, a spectrometer was used to determine saturation of yeast in the glass tubes at an optical density of 600 (OD600). The yeast culture was restaged to 0.6 at OD600 in fresh YPD in new autoclaved glass tubes.

## Hydrogen peroxide treatment (protocol)

## Viability and LOH quantification

# Results

Natural variations of hydrogen peroxide (hydrogen peroxide, is by some not considered a form oxidative stress. However, it is an intracellular chemical, that under certain conditions can change into a free radical).

## The induction of LOH by H2O2 varies in strain backgrounds

### Selective strains, high, long life span etc

## Contrasting switching pattern of H2O2 and chronological aging on LOH

### CLS trigger LOH after , H2O2 trigger LOH before on mid viability

## Significant correlation between CLS and the relative timing of the H2O2 trigger on LOH

### L0 (ratio of half black / full black) at time zero

### L0 ~ Cb/Cv negative correlation?? (a negative correlation would mean that the more resistant the strain is to hydrogen peroxide the shorter the lifespan)…opposite direction of what we thought

### Mitotic asymmetry (half black/full black; Cb (middle concentration of black colonies/Cv(middle concentration of viability)

# Discussion

Yeast in log phase, log phase 🡪point at which the yeast grows the fastest). In stationary phase, cells resistant to oxidative stress🡪difference between strains will be amplified. Difficult to compare strains if all are resistant to oxidative stress.

-Future direction

Test gene deletion mutants with hydrogen peroxide.

# References

Richter C, (1995) Oxidative Damage to Mitochondrial DNA and its Relationship to Ageing. *Science Direct: The International Journal of Biochemistry and Cell Biology.* 647-653.

Manuscript Format

<http://www.genetics.org/site/misc/ifora.xhtml>

#2012Feb25, Tg.vs.Tc ~ ln(R0) + G

# Partial correlations are all negative, this is agaisnt my realibity model.

# Tg/Tc is a measure of ability to maintian recombiation rate during aging

rm( list = ls() );

#Load previous results

tb = read.table("summary.new.by.strain.csv", header=T, sep="\t");

tb.old = tb;

labels = names( tb.old );

tb = tb.old[c(1:11), c("strain","ARLS","R0","G","CLS","Tc", "Tg","Tmmax","Tbmax", "Td", "Tdmax","TLmax","Lmax",

"b.max", "b.min", "strains", "L0.all", "L0.small" , "Pbt0","Pb0.5t0", "Pbt0.b") ];

tb$CLS.vs.Tc = tb$CLS / tb$Tc;

tb$Tg.vs.Tc = tb$Tg / tb$Tc;

tb$strain = as.character(tb$strain)

#load exg06 data

nat = read.table("062705.rls.cls.tab", sep="\t", header=T, colClasses=c("character", rep(NA,4)) );

#Load H2O2-LOH results

tb2 = read.csv("H2O2\_Log\_Plot\_Summarized\_data,2012Jan24.csv")

tb2$Strain = as.character(tb2$Strain)

names(tb2) = c("Date", "Strain", "Cv", "Cb", "OD600nm","Notes","repeat")

tb2.old = tb2;

tb2 = tb2[, c(1,2,3,4)]

tb2$Cv.vs.Cb = tb2$Cv / tb2$Cb

head(tb2)

#check strains names, do they match?

strains2 = unique(tb2$Strain)

intersect( strains2, tb$strain)

intersect( nat$strain, strains2)

#### analyze H2O2-LOH

#raw values

hist(tb2$Cv.vs.Cb, br=10)

hist(log2(tb2$Cv.vs.Cb), br=10)

summary(tb2)

hist(log2(1/tb2$Cv.vs.Cb), br=10)

# generate the means

tb2m = data.frame(cbind(strains2 ))

tb2m[,1] = as.character(tb2m[,1])

i=1;

for( i in 1:length(strains2) ){

sub = tb2[tb2$Strain==strains2[i], ]

tb2m$Cv.vs.Cb[i] = mean(sub$Cv.vs.Cb, na.rm=T)

tb2m$Cv[i] = mean(sub$Cv, na.rm=T)

tb2m$Cb[i] = mean(sub$Cb, na.rm=T)

}

tb2m$Cv.vs.CbByMean = tb2m$Cv / tb2m$Cb ;

hist( 1/ tb2m$Cv.vs.Cb, br =10)

hist( 1/ tb2m$Cv.vs.CbByMean, br =10)

plot( tb2m$Cv.vs.Cb ~ tb2m$Cv.vs.CbByMean )

# compare Cb/Cv and Tg/Tc

t.test( 1 / tb2m$Cv.vs.Cb, mu=1, alternative="less") #p=0.051

t.test( 1 / tb2m$Cv.vs.CbByMean, mu=1, alternative="less") #p=0.32

t.test( log2(1 / tb2m$Cv.vs.Cb), mu=0, alternative="less") #p=0.022

t.test( log2(1 / tb2m$Cv.vs.CbByMean), mu=0, alternative="less") #p=0.10

wilcox.test( 1/ tb2m$Cv.vs.Cb, mu=1, alternative="less") #p=0.053

wilcox.test( 1/ tb2m$Cv.vs.CbByMean, mu=1, alternative="less") #p=0.38

# Cb/Cv < 1

hist(tb$Tg.vs.Tc, br=10)

t.test( tb$Tg.vs.Tc, mu=1, alternative="greater") #p=0.00072

wilcox.test( tb$Tg.vs.Tc, mu=1, alternative="greater") #p=0.00098

#Tg/Tc > 1

### side by side bar-plots of Tg/Tc Cb/Cv

mystep=0.2

my.breaks = seq( 0.1, round(max( c(tb2m$Cb.vs.Cv, tb$Tg.vs.Tc ) + 0.1, 1)) ,by= mystep );

h.H2O2 <- hist(tb2m$Cb.vs.Cv, br= my.breaks, xlab = "Cb/Cv", ylab = "relative density", freq=F ) ;

h.aging <- hist(tb$Tg.vs.Tc, br= my.breaks, xlab = "Tg/Tc", ylab = "relative density", freq=F ) ;

#generate the comparison table

bins <- data.frame( rbind(h.H2O2$density,h.aging$density) ) ;

my.mids = my.breaks[-length(my.breaks)] + mystep/2

#my.mids

names( bins ) <- my.mids

row.names(bins) <- c( "H2O2", "Chronological Aging" )

bins

pdf("Figure\_sideBYside.pdf", width=8, height=5)

barplot( as.matrix(bins), beside=T, col=c("black","gray"), ylab="Relative Frequency", xlab="Ratios",

legend= c( "Cb/Cv H2O2", "Tg/Tc Aging" ) );

title(main="H2O2 and chronological aging elevate LOH at different modes" )

dev.off();

### merge tb tb2m

tb$Cb.vs.Cv = tb2m$Cb.vs.Cv[match(tb$strain, tb2m$strains2)]

tb$Cb = tb2m$Cb[match(tb$strain, tb2m$strains2)]

tb$Cv = tb2m$Cv[match(tb$strain, tb2m$strains2)]

### regression analysis

summary( lm( tb$Cb.vs.Cv ~ tb$ARLS + tb$Tg + tb$Tc + tb$Tg.vs.Tc + tb$CLS ) )

summary( lm( tb$Cb.vs.Cv ~ tb$ARLS ) ) #p = 0.134

summary( lm( tb$Cb.vs.Cv ~ tb$R0 + tb$G ) )

summary( lm( tb$Cb.vs.Cv ~ tb$Tg.vs.Tc) )

summary( lm( tb$Cb ~ tb$Tg.vs.Tc + tb$ARLS + tb$CLS ) )

summary( lm( tb$Cb ~ tb$CLS ) ) #p=0.23

summary( lm( tb$Cv ~ tb$CLS ) ) #p=0.85

summary( lm( tb2m$Cb ~ tb2m$Cv ) ) #p0.09

m = lm( tb2m$Cb ~ tb2m$Cv )

plot( tb2m$Cb ~ tb2m$Cv )

abline( m , col='red')

summary( lm( tb$Cb.vs.Cv ~ tb$Tc ) ) #p=0.3995

summary( lm( tb$CLS ~ tb$Cb.vs.Cv ) ) #p=0.024 !!!!! negative !!!why

m = lm( tb$CLS ~ tb$Cb.vs.Cv )

plot( tb$CLS ~ tb$Cb.vs.Cv )

abline( m, col='red')

plot( tb$CLS ~ tb$Cb )

plot( tb$CLS ~ tb$Cv )

summary( lm( tb$L0.all ~ tb$Cb.vs.Cv ) ) #p=0.054 !!!!! positive

#this suggest H2O2 effect ~ asymetry

#summary( lm( log(tb$L0.all) ~ tb$Cb.vs.Cv ) ) #p=0.06

plot( tb$L0.all ~ tb$Cb.vs.Cv )

quit("yes")

####

### END

####

###2011Feb25:

summary( lm( tb$Tg ~ tb$ARLS) )

summary( lm( tb$Tg ~ tb$G + log(tb$R0)) )

summary( lm( tb$Tc ~ tb$G + log(tb$R0)) )

summary( lm( tb$ARLS ~ tb$G + log(tb$R0)) )

summary( lm( tb$G ~ log10(tb$R0) + tb$Pbt0 + tb$L0.all + tb$Tg.vs.Tc ) )

# robustness to hyper recombination 1/b.max 1/L0

#summary( lm( tb$b.max ~ log10(tb$R0) + tb$G ) )

summary( lm( 1/tb$b.max ~ log10(tb$R0) + tb$G ) )

summary( lm( -tb$b.max ~ log10(tb$R0) + tb$G ) )

summary(lm( - tb$L0.all ~ tb$R0 + tb$G ))

summary(lm( 1/ tb$L0.all ~ tb$R0 + tb$G ))

summary(lm( 1/ tb$Pbt0 ~ tb$R0 + tb$G ))

summary( lm( tb$Tg.vs.Tc ~ tb$ARLS ) )

#p=0.008, R2=0.56

summary(lm( (tb$ARLS ~ log(tb$R0) + tb$G )))

#both negaive correlation

summary( lm( tb$Tg.vs.Tc ~ log10(tb$R0) ) ) #p=0.11

plot( tb$Tg.vs.Tc ~ log10(tb$R0) )

summary( lm( tb$Tg.vs.Tc ~ tb$G ) ) #p=0.68, negative

plot( tb$Tg.vs.Tc ~ tb$G )

# negative is opposite to GG realibility model

#tb$Tg.vs.Tc[4] = NA; #remove outliers

summary( lm( tb$Tg.vs.Tc ~ tb$G ) ) #p=0.79, remove one outlier, it is positive, However, partial correlation is still negative.

plot( tb$Tg.vs.Tc ~ tb$G )

summary( lm( tb$Tg.vs.Tc ~ tb$R0 + tb$G ) )

#p=0.059, which is similar to ARLS

summary( lm( tb$Tg.vs.Tc ~ log10(tb$R0) + tb$G ) )

#p=0.013, R2=0.66 good p-value !!!!!

#p=0.22, negative correlations

q("no")

######### L0 ~ CLS

#summary( lm( tb$L0.all ~ tb$CLS) ) #p 0.011 R2=0.53 <--

#summary( lm( tb$L0.all ~ tb$ARLS + tb$Tc + tb$CLS + tb$R0 + tb$G ) ) # none

# only CLS shows connection

#summary( lm( tb$L0.small ~ tb$ARLS + tb$Tc + tb$CLS + tb$R0 + tb$G ) ) #none

#summary( lm( tb$L0.all ~ tb$ARLS) ) #p 0.49

#summary( lm( tb$L0.all ~ tb$ARLS + tb$CLS) ) #p 0.05, L0~CLS partial is p=0.02

#summary( lm( tb$L0.all ~ tb$Tc) ) #p 0.33

#summary( lm( tb$L0.all ~ tb$Tg) ) #p 55

####### b.max ~ ARLS

summary( lm( tb$b.max ~ tb$ARLS + tb$Tc + tb$CLS + tb$R0 + tb$G + tb$L0.all ) ) #p 0.50

summary( lm( tb$b.max ~ tb$ARLS ))

#Residual standard error: 0.06868 on 9 degrees of freedom

#Multiple R-Squared: 0.4122, Adjusted R-squared: 0.3469

#F-statistic: 6.311 on 1 and 9 DF, p-value: 0.0332

######## Lmax

summary( lm( tb$Lmax ~ tb$ARLS )) #p 0.98

summary( lm( tb$TLmax ~ tb$ARLS + tb$Tc + tb$CLS + tb$R0 + tb$G + tb$L0.all + tb$b.max + tb$b.min + tb$Tdmax ) ) #none

summary( lm( tb$Lmax ~ tb$ARLS + tb$Tc + tb$CLS + tb$R0 + tb$G + tb$L0.all + tb$b.max + tb$b.min + tb$Tdmax ) )

# 0.05 ??? but one degree of freedom?? So, this is questionable.

##### Tg/Tc ~ ARLS

summary( lm( tb$Tg.vs.Tc ~ tb$ARLS) );

# Residual standard error: 0.1861 on 9 degrees of freedom

# Multiple R-Squared: 0.5551, Adjusted R-squared: 0.5057

# F-statistic: 11.23 on 1 and 9 DF, p-value: 0.00851

summary( lm( tb$CLS.vs.Tc ~ tb$Tg.vs.Tc )) #p 0.35

summary( lm( tb$Tg.vs.Tc ~ tb$Lmax + tb$ARLS + tb$Tc + tb$CLS + tb$R0 + tb$G + tb$L0.all + tb$b.max + tb$b.min + tb$Tdmax ) )

## 0.05 ???

postscript("110706.L0-CLS.ps", width=6,height=6, horizontal=F)

m.L0 = lm( tb$L0.all ~ tb$CLS)

plot(tb$L0.all ~ tb$CLS, xlab="CLS",ylab="L0",main="L0 ~ CLS", pch=16, xlim=c(2,18), ylim=c(0.05,0.26) )

abline( m.L0, col="red");

x = numeric( length(tb$CLS) ); names(x) = as.character( tb$strains );

y =x;

y[c("M34", "M5", "M32", "M8")] = c(0.002, -0.01, -0.01, -0.01 )

strains = as.character(tb$strain); names(strains) = tb$strain

#pos = x; pos=NA;

strains["M5"] = " M5";

text( tb$CLS, tb$L0.all+y+0.004, strains);

dev.off();

#########110606Mon regression:

summary( lm( ARLS ~ Tg, data=tb) ); #p = 0.36

summary( lm( ARLS ~ Tc, data=tb) ); # p 0.48

summary( lm( ARLS ~ Tmmax, data=tb) ); #p 0.59

summary( lm( ARLS ~ Tbmax, data=tb) ); #p 0.36

summary( lm( ARLS ~ Td, data=tb) ); #p 0.93

### begin ####ARLS ~ b.max

summary( lm( ARLS ~ b.max, data=tb) ); #p 0.06455

long.arls = tb$b.max[ tb$ARLS > 33 ]

short.arls = tb$b.max[ tb$ARLS < 33 ]

t.test( long.arls, short.arls) # 0.02863

tb.tmp = tb[, c("ARLS", "b.max")]

tb.tmp$by.arls = ifelse( tb$ARLS >33, 1, 0)

tb.tmp$by.b = ifelse( tb$b.max > 0.16, 1, 0)

# wrong:: fisher.test( tb.tmp[,c("by.arls","by.b")] )

tmp = table( tb.tmp[,c("by.arls","by.b")] )

fisher.test( tmp ); #p 0.06

pdf("3c.031908.arls.bmax.pdf", width=6, height=6, )

m1 = lm( b.max ~ ARLS, data=tb);

plot( b.max ~ ARLS, data=tb, pch=16, col="black", xlim=c(22,40), ylim=c(0.02, 0.3) );

abline( m1 , col= "black", lty=2);

y = tb$b.max +0.01 ;

names(y) = as.character( tb$strain );

y[c("M32" )] = y[c("M32" )] - 0.02

y[c("YPS163" )] = y[c("YPS163" )] - 0.02

x = tb$ARLS +0.5 ;

x[c("M1-2")] = x[c("M1-2")] - 2

text( x, y, tb$strain);

text( 25, 0.27, "R2=0.34 p=0.059")

dev.off();

###end #######ARLS ~ b.max

### begin ALRS ~ (Tg-Tc)/Tc

tb$frac.gc = (tb$Tg - tb$Tc) / tb$Tc;

tb$frac.bm = (tb$Tbmax - tb$Tmmax) / tb$Tmmax;

summary( lm( ARLS ~ Tg:Tc, data=tb) );

summary( lm( ARLS ~ frac.gc, data=tb)) # p 0.007

summary( lm( ARLS ~ frac.bm, data=tb)) # p 0.01

pdf("3a.031908.Tg-Tc.arls.pdf",width=6,height=6);

plot( tb$Tg.vs.Tc ~ tb$ARLS, pch=16, col="black", xlim=c(22,40), ylim=c(0.8,1.9),xlab="ARLS",ylab="Tg/Tc" );

m2 = lm( tb$Tg.vs.Tc ~ tb$ARLS );

abline( m2 , col= "black", lty=2);

y = tb$Tg.vs.Tc + 0.01

names(y) = as.character( tb$strain );

#y[c("M32" )] = y[c("M32" )] - 0.02

x = tb$ARLS ;

#x[c("M1-2")] = x[c("M1-2")] - 2

text( x, y, tb$strain, pos=4);

text( 27, 1.7, "R2=0.56, p=0.008")

dev.off();

pdf("3b.031908.Trmax-Tmmax.arls.pdf",width=6,height=6);

m3 = lm( frac.bm ~ ARLS, data=tb);

tb$Trmax.vs.Tmmax = tb$Tbmax / tb$Tmmax

m3.2 = lm( Trmax.vs.Tmmax ~ ARLS, data=tb);

plot( Trmax.vs.Tmmax ~ ARLS, data=tb, pch=16, col="black", xlab="ARLS", ylab="Trmax/Tmmax",

# main="(Tmax.g-Tmax.m/Tmax.m) ~ ARLS",

xlim=c(22,40) );

abline( m3.2 , col= "black", lty=2);

y = tb$Trmax.vs.Tmmax + 0.01

names(y) = as.character( tb$strain );

x = tb$ARLS ;

text( x, y, tb$strain, pos=4);

text( 27,1.4, "R2=0.52, p=0.012");

dev.off();

### end

############072307 added changes

#### Tg - Tc

pdf("072307.Tg.Tc.pdf",width=6,height=6);

plot( tb$Tg ~ tb$Tc, pch=16,xlab="Tc",ylab="Tg",xlim=c(3,10),ylim=c(4,14) );

m.gc = lm( tb$Tg ~ tb$Tc );

abline( m.gc , col= "black", lty=2);

y = tb$Tg + 0.01

names(y) = as.character( tb$strain );

#y[c("M32" )] = y[c("M32" )] - 0.02

x = tb$Tc ;

#x[c("M1-2")] = x[c("M1-2")] - 2

text( x, y, tb$strain, pos=4);

text( 5, 13, "R2=0.59, p=0.006")

dev.off();

##### Trmax - Tmmax

pdf("072307.Trmax.Tmmax.pdf",width=6,height=6);

plot( tb$Tbmax ~ tb$Tmmax, pch=16,xlab="Tmmax",ylab="Trmax",xlim=c(4,12),ylim=c(4,14) );

m.bm = lm( tb$Tbmax ~ tb$Tmmax );

abline( m.bm , col= "black", lty=2);

y = tb$Tbmax + 0.01

names(y) = as.character( tb$strain );

#y[c("M32" )] = y[c("M32" )] - 0.02

x = tb$Tmmax ;

#x[c("M1-2")] = x[c("M1-2")] - 2

text( x, y, tb$strain, pos=4);

text( 5, 13, "R2=0.60, p=0.005")

dev.off();

##### Tdmax - Tc

pdf("072307.Tdmax.Tc.pdf",width=6,height=6);

plot( tb$Tdmax ~ tb$Tc, pch=16,xlab="Tc",ylab="Tdmax",xlim=c(3.5,10),ylim=c(4,12) );

m.dc = lm( tb$Tdmax ~ tb$Tc );

abline( m.dc , col= "black", lty=2);

y = tb$Tdmax + 0.1

names(y) = as.character( tb$strain );

y[c("M14" )] = y[c("M14" )] - 0.4

y[c("M2-8" )] = y[c("M2-8" )] + 0.2

x = tb$Tc ;

names(x) = as.character( tb$strain );

x[c("M14")] = x[c("M14")] - 0.5

text( x, y, tb$strain, pos=4);

text( 5, 11, "R2=0.71, p=0.001")

dev.off();

#quit("yes");

1. Qin, H., M. Lu, and D.S. Goldfarb, *Genomic instability is associated with natural life span variation in Saccharomyces cerevisiae.* PLoS One, 2008. 3(7): p. e2670.