Notes on papers relating to yeast aneuploidy, dosage compensation, gene expression

*Identification of Aneuploidy-Tolerating Mutations – Eduardo Torres et al 2010[1]*

**Key Words:** aneuploidy, mutation, yeast, proteasomal degradation

**General subject:** aneuploidy, yeast

**Specific subject:** how do highly proliferative aneuploid yeast have higher fitness than the ancestral strain?

**Hypothesis:** They have some sort of gene that helps them regulate their expression/protein production or there is dosage compensation occurring

**Methodology:**

* did it in haploids, that were disomic for certain chromosomes
  + 2x more DNA
  + mine: diploids with 1,3,4 copies: 0.5, 1.5, 2x more DNA
  + they also selected for the extra chromosome using auxotrophs and antibiotic resistance
  + markers
* \*got disome for chromosome XIII
* comparative genome hybridization analysis – to look at karyotypes.
* wanted to know if the catalytic or noncatalytic function of UBP6 was increasing the fitness of the lines, so replaced the cysteine 110 with alanine to get rid of the catalytic activity, and expressing it in yeast strains
* measured relative protein levels using SILAC-based mass spectrometry

**Results:**

* found fitness gains in several of the lines
* some were only in -His+G318 media, showing that they were environmentally specific
* chromosome composition wasn’t affected
* some descendants lost the entire duplicated chromosome or large chunks of it
  + the breakpoints of the lost chunks were in or near Ty1 elements (due to homologous recombination)
* \*Evidence to back up my claim of no whole-chromosome dosage compensation: found that gene expression of aneuploid chromosome was not reduced even in the strains with higher fitness
  + expr increased average 1.84-fold (compared to rest of genome)
* found a “common transcriptional profile” among aneuploids
  + But “only seen under conditions that eliminated the differences in growth rate between aneuploid strains” (i.e. no competition?) grew them in a chemostat with limited phosphate
* when they compared this profile between descendants and their ancestral strains, they found that the descendants were more similar to each other than they were to their respective ancestral strains
  + these mutations must affect the same pathways (the strains with higher fitness all had a common transcription signature)
* \*\*Questions I could ask for my individual gene analysis:
  + Do lines with similar fitness have similar transcription signatures?
  + Lines with same aneuploid chromosome, similar expression patterns on other chromosomes?
* found common SNPs in disomic lines with increased fitness
  + for lines with 2 copies of XVI, found mutations in SVF1
  + \*\*I should look at this gene in my aneuploids for chromosome 16
* Also found that higher-fitness strains with disomes V, VIII, IX, XI, XIV had mutations in proteasomal degradation genes UBP6, RPT1, RSP5, UBR1
* UBP6 deletion didn’t help WT cells but increased fitness of cells with V duplication, but the descendant strain that just had a mutation in the gene still had higher fitness
  + so there must be other stuff going on too not just loss of function of this gene
* low levels of ubiquitin are not the reason for higher fitness in lines with UBP6 mutations
* a mutated UBP6 protein without catalytic abilities increases fitness compared to WT UBP6
  + protease activity increases growth
* if you get rid of the proteasome activity, growth rate decreases in just about every disome
* protein level doesn’t always correlate with DNA level
  + attenuated at either the transcriptional level or posttranscriptionally
* in one disome the proteins are attenuated at the RNA level, and in the other disome, the proteins are attenuated at the posttranscriptional level (prob by increased protein degradation)
* in one disome, deletion of UBP6 results in upregulation of proteins in genes with relatively low expression and downregulation of proteins in genes with relatively high expression. in the other disome it only downregulates proteins in genes with high expression.
  + they think it’s because of higher protein degradation.
  + makes sense, easier to degrade proteins that inhibit transcription than increase the protein product at the end

**Summary of key points:**

* different strains evolved different ways to cope with aneuploidy
* UBP6 mutations were common in 4 different evolved strains
* Ubp6 mutations/deletions increase fitness by increasing the activity of the proteasome
  + this helps the cell get rid of the excess proteins that are produced from the extra chromosome
* because of the increased protein load, the cell cannot degrade proteins as efficiently as normal so proteins that could potentially have detrimental fitness effects build up
  + examples of these are alpha and beta tublin and histones
  + Citations: Anders et al 2009, Katz et al 1960, Gunjan and Verreault, 2003, Meeks-Wagner and Hartwell, 1986
* 12 of 29 genes with mutations in the evolved strains have human homologs, and some of those have been shown to be upregulated in tumors.

**Context**

* shows that yeast can do something about excess chromosomes/DNA
* indicates that there is not always RNA-level attenuation, and even when there is it is not complete
* identifies some genes that are potentially helping aneuploid cells proliferate faster than their euploid counterparts

**Significance**

* supports my claim that there is no whole-chromosome dosage compensation
* supports my evidence for higher fitness in aneuploid yeast

**Important figures/tables**

* Figure 1c : heat map showing no compensation for extra copies of the chromosomes at the RNA level
* Figure 2b : coculture experiments indicating that most of the time, a mutation in the UBP6 gene results in higher fitness, except in two lines
* Figure 2c : another coculture experiment that indicates that a deletion in the entire UBP6 gene results in higher fitness in all strains tested (same as those tested with the mutation)
* Figure 3C-H : shows that overexpressing ubiquitin in UBP6 mutated or deleted lines does not reduce the growth rate, so low levels of ubiquitin are not the cause of the increased fitness
* Figure 4A-D : shows that if the UBP6 protein loses its catalytic activity, the disomic cells grow faster
* Figure 4E : shows that missing part of the proteasome causes reduced fitness

*Karyotypic Determinants of Chromosome Instability in Aneuploid Budding Yeast[2]*

*Zhu et al 2012 PLOS Genetics*

**Key Words:** aneuploidy, chromosomal instability, yeast

**General subject:** aneuploidy and chromosomal instability

**Specific subject:** aneuploidy in yeast, reasons for chromosomal instability in aneuploid yeast

**Hypothesis:** “is the increased CIN in an aneuploid genome a result of the abnormal chromosome numbers per se or of aneuploidy-driven alteration of the expression of specific genes?”

**Methodology:** produced aneuploid yeast from sporulation of strains with an odd ploidy (3N, 5N, etc)

used FACS to find the overall gene content of the aneuploid cells

used qRTPCR to karyotype the cells

\*I didn’t really like their methods because they specifically chose certain strains that looked more stable and that probably put a good amount of bias in their experiment.

**Results:**

different aneuploid strains displayed different CIN levels

found a wide range of growth rates

“effect of an individual aneuploid chromosome on CIN is dependent on karyotypic context in which the aneuploid chromosome is present”

“heterozygosity of MAD2 ... leads to partial SAC (spindle assembly checkpoint) inactivation and elevated CIN in a diploid background” (Barnhart et al 2011)

**Summary of key points:**

“CIN is not a necessary outcome of aneuploidy”

“no correlation between fitness and CIN among aneuploid strains”

“CIN is significantly linked to distance of karyotype to the haploid state”

“found association of CIN with dosage imbalance between specific chromosome pairs”

“our method does not allow analysis of those highly unstable karyotypes that quickly lead to considerable karyotype diversity within even a small population”

🡪 potential project? Has anyone done this yet?

**Context**

**Significance**

supports my finding that different aneuploid strains have different growth rates, and they are not all negatively affected by aneuploidy in terms of fitness

**Important figures/tables**

Figure 3A: wide range of growth rate

no significant difference between highly unstable and stable karyotypes

*Ploidy Variation in Fungi – polyploidy, aneuploidy, and genome evolution[3]*

*Todd et al 2017 Microbial Spectr*

**Key Words:** aneuploidy, polyploidy, genome size changes

**General subject:**

genome evolution

**Specific subject:**

genome evolution mediated by changes in genome size in fungal species

**Hypothesis:**

**Methodology:**

review

**Results:**

**Summary of key points:**

**Context**

**Significance**

“fungal ploidy is often context dependent and can rapidly change from one environment to the next” – could be backing my finding of no DC 🡪 not enough time spent in aneuploid state to evolve mechanism

aneuploidy in clinical isolates of S. cerevisiae:

“whole genome sequencing of 145 S. cerevisiae clinical isolates found that 34% were triploid or tetraploid and 36% of them were aneuploid”

source: ref 16

Whole genome analysis of 132 clinical Saccharomyces cerevisiae strains reveals extensive ploidy variation Zhu et al 2016 G3

ploidy changes with changes in environment in a different fungal species:

“dramatic ploidy changes have been observed during infection. Polyploid ‘titan’ cells that range from 4N to >64N make up 20% of the infectious population in host tissue”

sources: ref 36, 37

Dynamic chnges in the morphology of Cryptococcus neoformans during murine pulmonary infection Feldmesser et al 2001 Microbiology

The cryptic sexual strategies of human fungal pathogens. Ene and Bennett 2014 Nat Rev Micriobiol.

adaptive aneuploidy to antifungal drugs in C. albicans:

“the amplification of chromosome I has been observed in response to antifungal drug stress”

sources: ref 42,43

Cryptococcus neoformans overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes Sionov et al 2010 PLoS Pathog.

Polyploid titan cells produce haploid and aneuploid progeny to promote stress adaptation. Gerstein et al 2015 MBio

return to diploidy during periods of starvation – one chromosome lost at a time

“under nutrient starvation, tetraploid cells show an increase in genome instability that leads to the loss of individual chromosomes over time returning progeny cells to either diploidy or (more often) near-diploidy”

sources: 9, 50, 52, 54

Identification of a mating type-like locus in the asexual pathogenic yeast Candida albicans Hull et al 1999 Science

The parasexual cycle in Candida albicans provides an alternative pathway to meiosis for the formation of recombinant strains Forsche et al 2008 PLoS Biol

Parasexual ploidy reduction drives population heterogeneity through random and transient aneuploidy in Candida albicans 2015 Genetics

Completion of a parasexual cycle in Candida albicans by induced chromosome loss in tetraploid strains 2003 EMBO J

do aneuploid cells show a similar response in gene expression to tetraploid cells?

“tetraploid cells had reduced expression of genes encoding G1 cyclins (CLN1 and PCL1) and proteins involved in cytoskeletal organization (e.g. GIC2)”

source: ref 114 [4]

adaptive aneuploidy to fluconazole resistance in C. albicans:

“in virtro evolution of C. albicans in the presence of fluxonazole, acquisitition of multiple aneuploid chromosomes is detected after just ~3.3 generations in the majority of cells in the population. isochromosome 5L, confers fluconazole resistance due to the amplification of two genes, ERG11 and TAC1”

sources: 61, 13, 59

Aneuploidy and isochromosome formation in drug-resistant Candida albicans Selmecki et al 2006 Science

Selmecki et al Acquisition of aneuploidy provides increased fitness during the evolution of antifungal drug resistance PLos Genetics 2009

Selmecki et al an isochromosome confers drug resistance in vivo by amplification of two genes, ERG11 and TAC1 2008 Mol Microbiology

adaptive aneuploidy as buffer time in between stress and finding optimal solution:

“during adaptation to heat stress, S cerevisiae rapidly duplicates chromosome III, however after continued heat stress the chromosome aneuploidy is lost while elevated expression of genes on this chromosome are maintained, suggesting that aneuploidy provides time for cells to search for optimal adaptive solutions”

source: 125

Chromosomal duplication is a transient evolutionary solution to stress. PNAS 2012

could say that this supports my finding of no dosage compensation – again, not enough evolutionary time to evolve a mechanism of compensating for gene dosage, also implies that aneuploidy is NOT the best route to adapting to a stressful environment

fitness effects differ depending on ploidy level:

“fitness effect of a mutation is assumed to be equal across all ploidy levels, however recent experimental evidence suggest that this is not the case for all mutations”

sources: 20, 159-161

Mutational effects depend on ploidy level: all else is not equal. Gerstein Biol Letters 2013

Heterozygote advantage as a natural consequence of adaptation in diploids. PNAS 2011 Sellis et al

Heterozygote advantage is a common outcome of adaptation in Saccharomyces cerevisiae Genetics 2016 Sellis et al

**Important figures/tables**

*Characterization of Chromosome Stability in Diploid, Polyploid, and Hybrid Yeast Cells [5]*

**Key Words:** chromosome stability, aneuploidy, yeast, diploid, hybrids, triploid

**General subject:** chromosomal stability in differing genome states

**Specific subject:** rate of chromosome loss in S cerevisiae diploids, triploids, and S cerevisiae- S bayanus hybrid diploids

**Hypothesis:**

**Methodology:**

**Results:**

* + - negative correlation between chromosome size and rate of loss
    - stability of different chromosomes shows “a consistent pattern”
    - chromosome III is the most unstable (has highest rate of chromosome loss)

**Summary of key points:**

**Context**

**Significance**

**Important figures/tables**

*Yeast: a Simple Model Organism to Study the Complex Phenomena of Aneuploidy*

*Mulla et al 2013 FEMS Microbiology Reviews*

**Key Words:** adaptation, chromosomal instability, gene expression, karyotyping, phenotypic variation

**General subject:** aneuploidy

**Specific subject:** yeast as a model system for aneuploidy

**Hypothesis:**

**Methodology:** review

**Results:**

**Summary of key points:**

* hard to separate changes caused by aneuploidy from changes caused by something else
  + MA might be able to help with this. we know all of the other mutations that arose
* yeast are “well tolerant of aneuploidy” 1970 paper
* benefits of looking at this in diploids: can look at loss of a chromosome
  + can’t do that in haploids- kills them
* rate of aneuploidy in lab yeast: 2-8 per 10^6 cell divisions (2-8 x 10^-6/cell division)
* chromosomal instability genes: mutations to them cause CIN
* beneficial aneuploidies under certain conditions – can help “alleviate growth deficiencies caused by gene mutations”
  + environmental stress too ^
* ways to produce aneuploid yeast:
  + environmental stress
  + genetic mutations
  + “meiosis of triploid/pentaploid cells” (mitosis also has issues)
* types of aneuploidy effects on transcriptome:
  + “proportional to DNA dosage – inlier changes” – moderate, most genes on the chromosome
  + “beyond the DNA dosage – outlier changes”

**Context**

**Significance**

Papers from this one:

Context: yeast as model for genetic alterations

Paper: Botstein and Fink 2011

Context: yeast is “well tolerant of aneuploidy”

Paper: Parry and Cox 1970

Context: rate of aneuploidy

Papers: Hartwell and Smith 1985 Klein 2001

Context: beneficial aneuploidies under stressful conditions

Papers: Hughes et al 2000 Rancati et al 2008

Context: “phenotypic effects of aneuploidy directly linked to the changes in expression of many genes”

Papers: Torres et al 2007, Rancati et al 2008, Pavelka et al 2010b, Chen et al 2012

Context: how to plot gene expression data according to chr. location

Papers: Hughes et al 2000, Bouchonville et al 2009

Context: no compensation at protein level

Paper: Springer et al 2010

Context: “aneuploidy causes delay in G1 phase of cell cycle” – reason for slow growth rate

Paper: Niwa et al 2006, Torres et al 2007, Pavelka et al 2010b(gain of chromosome XIII under stress conditions)

Context: “outlier gene expression”

Paper: Rancati et al 2008

Context: return to diploidy

Paper: Waghmare and Bruschi 2005, Chen et al 2012

**Important figures/tables**

The evolution of dosage compensation mechanisms [6]

Marin et al 2000

**Summary of key points:**

DC in C. elegans

males XO hermaphrodites XX

* “downregulation transcription of genes on both hermaphrodite X chromosomes”
* some DPY genes regulate DC in worms
* there is a master regulator of the DC pathway, XO lethal-1, a sex-determining gene
  + negatively regulates the sex determination and dosage compensation genes which regulate dosage compensation
* the Dosage Compensation Complex (DCC) is the main component that enables DC
  + components of this complex condense parts of both hermaphroditic X chromosomes to decrease expression by half
  + condensin IDC is a subunit of the DCC and contains the SMC proteins DPY-27 and MIX1. these work together with 3 chromosome-associated polypeptide proteins
    - condensin is required for chromosome condensation in S. cerevisiae and S. pombe
* “mechanisms related to mitotic chromosome condensation mediate DC”
* “assumed that during evolution, the condensing I subunit SMC-4 duplicated and diverged, giving rise to DPY-27. This resulted in the formation of condensin IDC with the specialized function of dosage compensation. IT is believed that the mechanism of DC is closely linked to condensin’s role in regulating chromosome architecture”

DC in Drosophila

* males XY females XX
* “dosage compensation occurs by doubling the transcription rate of X-linked genes in males”
  + effectively the reverse of what happens in C elegans
* the MS1 genes collectively mediate DC in flies with the help of the ROX genes, which produce non-coding RNAs
* “there is evidence that the products of these genes function together in a complex called the compensasome to alter the chromatin structure of the male X”
  + all components of this complex have to be present for it to work
    - this way, one component can be repressed in females and that way it won’t work in females
* “post-translational modification of histones, and particularly their acetylation, has been implicated in chromatin activation leading to increased transcription”
* “the rox1 and rox2 genes themselves may act as chromatin-entry sites from which epigenetic spreading of the compensasome proceeds along the chromosome”
  + “some X-linked genes are not compensated, despite the fact that their Y-linked homologs have degenerated”
* “the Drosophila X chromosome has some molecular characteristics that make it different from the autosomes”
  + enrichment of some repeats
* not all compensation occurs via compensasome
  + SXL might compensate some genes by down-regulating them in females

**Context**

**Significance**

**Important figures/tables**

*A yeast H2A-H2B promoter can be regulated by changes in histone gene copy number [7]*

**Summary of key points:**

* expression of HTA2 insensitive to the # of histone genes in the cell
* HTA1 gene regulated by histone gene copy #
* functional products of HTA and HTB genes must be made to see effects of gene dosage
* H2A and H2B protein amount is actual feedback signal

*Amplification of histone genes by circular chromosome formation in Saccharomyces cerevisiae [8]*

what we know already:

* when HTA2-HTB2 is deleted, HTA1-HTB1 dosage compensates at the transcriptional level
* a different mechanism can occur when HTA1-HTB1 is deleted
  + HTA2-HTB2 amplifies via creation of a small, circular chromosome
  + duplication includes 39 kb of chrm II and contains HTA2-HTB2, the histone H3-H4 locus HHTI-HHFI, a centromere, and origins of replication
  + formation of new c’some occurs by recombination between 2 Ty1 retrotransposon elements that flank this region
* a decreased level of histones H2A and H2B specifically stimulates this amplification

**Hypothesis:**

unresolved mysteries:

some hta1-htb1delta strains have 2 copies of HTA2-HTB2

hta1delta mutant viable, htb1delta not

hta1delta-htb1delta mutant in S288C background: viable

hta1-htb1delta mutant in W303 background: inviable

**Methodology:**

* for hta1-htb1 delta strains and the hta1 delta strain, HTA2-HTB2 probe hybridized to both chrom II (normal location) and a second prominent band
  + indicates that HTA2-HTB2 exists as a second copy that is not a part of chromosome II
  + amplified region is delimited by the flanking Ty1 elements
  + amplification is required for viability of hta1-htb1delta strains and it occurs in hta1-htb1 delta spores at a high frequency
  + amplification copy # is ~1 per cell
* frequency of amplification: 3 x 10^-5 amplifications/cell
* hta1-htb1 delta strains require 2 copies of HTA2-HTB2 for viability
* all hta1-htb1delta strains produced PCR product expected from Ty1-Ty1 recombinant formed by recombination between 2 Ty1 elements
* upon Ho induction, migration of the amplified DNA shifts markedly, consistent with the linearization of a 39 kb circular chromosome
  + all of this provides evidence that the amplified HTA2-HTB2 locus is a novel circular chromosome
  + the amplification requires both Ty1 elements
    - but they don’t have to be Ty1 elements, they can be any homolgous sequence with adequate length

**Results:**

**Summary of key points:**

**Context**

**Significance**

**Important figures/tables**

*Gene expression analysis of induced pluripotent stem cells from aneuploid chromosomal syndromes [9]*

*Zhang et al 2013 BMC genomics*

**Key Words:** aneuploidy, transcriptome, iPSCs, humans

**Hypothesis:** “how do phenotypic abnormalities develop with aberrant karyotype?”

-RNAseq to answer this question

-How does the missing/extra chromosome affect the transcriptome?

-What is the significance of certain phenomena in aneuploidy during development?

**Methodology:**

generated iPSC lines:

monosomy X

trisomy 8

trisomy 13

partial trisomy 11:22

2 euploid controls

sequenced on SOLiD v3 platform

mapped to human genome using Corona Lite

calculated read densities in RPKM

performed hierarchical clustering and found that aneuploids have similar expression patterns

performed Pearson correlation and found no significant differential expression differences between aneuploids and euploids

DE analysis was performed using DEGseq

MA plot-based method with random sampling was used

M: log ratio of counts between 2 experimental conditions

A: 2-group average of log concentrations of the gene

🡪 using raw count of each gene

**Results:**

* similar #s of up and down-regulated genes in aneuploid lines
* more upregulated genes in trisomies 8 & 13
* more downregulated genes in trisomy 22 and monosomy X
* aneuploidy has dosage effect on gene expression levels: # of DE genes between aneuploid and euploid lines decreases with an increasing log fold-change cutoff
* all other chromosomes besides aneuploid chromosome have some level of gene expression regulation
* found 9 pathways significantly DE in all aneuploid lines

**Summary of key points:**

aneuploidy has effects on the entire transcriptome, not just the chromosomes directly affected

\*\* something I can cite in my paper!

*Paper Title*

**Key Words:**

**General subject:**

**Specific subject:**

**Hypothesis:**

**Methodology:**

**Results:**

**Summary of key points:**

**Context**

**Significance**

**Important figures/tables**

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3. Todd, R.T., A. Forche, and A. Selmecki, *Ploidy Variation in Fungi–Polyploidy, Aneuploidy, and Genome Evolution.* Microbiology spectrum, 2017. **5**(4).

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