In September 2016, the Gresham lab set out to read 3 papers per week. This is a record of their journey.

**Lab meeting: November 3rd, 2016**


**Synopsis:**

Confusions/questions/issues:

**Implications for our research:**

Rajendra Rai, Jennifer J. Tate, Isabelle Georis, Evelyne Dubois and Terrance G. Cooper., Nitrogen Catabolite Repression-sensitive Production of Gat1 Isoforms. [http://www.jbc.org/content/289/5/2918.long](http://www.jbc.org/content/289/5/2918.long)

**Synopsis:**

If you fiddle around with nitrogen-source or rapamycin treatment, you can get differential mobility of Gln3, which folks think is caused by post-tln modifications. You don't see that with Gat1, and you get multiple bands constitutively, so people gave up on that line of experimentation. Cooper's lab, as they are want to do, started of simply by just mutating the M1 to an alanine. Because this is biology and biology is complex with lots of context, that didn't abrogate Gat1p production. Turns out you've got a few methionines to start from, and if you look at full length Gat1 you've got isoformA starting from M40 and you've got isoformB starting from M95 or M102. These are produced a bit differently, but most striking is that if you KO one of these isoforms then the remaining isoform goes nuclear a bit more. Odd. When they truncated the protein with a Myc tag, they got four isoforms. They think these four are each of isoform A and B, but truncated, and each of the two have two isoforms that are a result of post-translational modification. They say tertiary structure (or 4?) masks the post-tln mods. Increased nuclear localization of Gat1 is not associated with increased expression of DAL5.

**Confusions/questions/issues:**

They don't show that these are actually post-tln modifications. Surely there must be a way of completely denaturing Gat1p so you can run it on a
Implications for our research:

Biology is weird. Gat1 and NCR are weirder. Gat1 is probably mostly not nuclear in proline limitation. Gat1 has some isoform choice. Isoform choice is affected by N-source.

Noa Sher, George W. Bell, Sharon Li, Jared Nordman, Thomas Eng, Matthew L. Eaton, David M. MacAlpine, and Terry L. Orr-Weaver, Developmental control of gene copy number by repression of replication initiation and fork progression. http://genome.cshlp.org/content/22/1/64.long

Synopsis:

Confusions/questions/issues:

Implications for our research:

Lab meeting: October 27th, 2016


Synopsis: Metschnikowia reukaufii is a nectar-colonizing yeast species whose genome was sequenced and resolved in this paper. Nectar-colonizing species are an interesting model organism as nectar possesses a very low amount of nitrogen, with concentrations of amino acids similar to many of our own nitrogen-limited experiments. Along with identifying its high level of homology to many other yeast species and conservation of eukaryotic genes, the researchers were able to identify a high rate of tandem gene duplication in many genes, including 227 novel arrays when compared to close phylogenetic species. The arrays related to nitrogen metabolism include four homologues of GAP1
and 3 homologues for PUT4, whose catalytic amino acids were conserved, leading to a proposed conservation of function as well. This was confirmed through expression profiles of GAP1 homologues in synthetic nectar with different limiting amino acids. In relation to priority effects, the heightened level of metabolic activity by these duplications of nitrogen scavenging genes in *M. reukaufii* allows it to affect other colonizing species.

Confusions/questions/issues: Although the scientists managed to find a large number of tandem gene duplications, what would these duplications be as there are very few known TGAs in *S. cerevisiae*?

Despite resolving the genome, the scaffolds created by the scientists do not provide chromosomal information. How could they annotate the genome using chromosomal demarcations?

Implications for our research: It is shown that homologous GAP1 proteins and sequences in other species have a similar function in nitrogen-limited environments as well as experiencing similar nitrogen catabolite repression. Furthermore, *M. reukaufii* possesses GAP1 homologues that appear as a result of tandem duplication events, which lead to an increased fitness of the species in the nitrogen-poor nectar that it inhabits. Therefore, GAP1 copy number possesses similar fitness effects across other species and tracing back these duplication events could be an interesting way to track evolution and divergence of related species.


Synopsis:

When cells commit to a stable but reversible arrest, the G1/S genes responsible for promoting S phase must be inhibited. This process from yeast to humans, involves the formation of quiescence-specific complex on their promoters. Quiescent yeast cells show pervasive histone deacetylation by the HDAC1 counter part Rdp3. Whi5/srl3 mutant slow down the process of recovery from quiescent cell. Msa1 and Msa2 are quiescence-specific regulator of G1/S phase, which has certain role in DNA synthesis facilitate the cell into quiescent stage not maintain.

Confusions/questions/issues:

Msa1/2’s role are not clearly illustrated in this review. Under high hydroxyurea condition, the cell doesn’t show viability in msa1/msa2 mutant.But this doesn't mean that quiescece is related to this gene.

Implications for our research:

Xbp1 is not expressed in log phase cells, but it’s induced to high levels after the DS and it represses over 800 genes.

After cell grows for 24h, three population can be visualized in flow cytometry. R1: small daughters of asymmetric cell division, R2: non-quiescent mother cell, and R3: quiescent cell.


Synopsis:

Pseudouridine (psU) is a post-trxmodification which is known to stabilize tRNA and rRNA structure. Artificially adding psUto mRNA facilitates non-canonical base pairing in ribosome decoding center. However, natural psUin mRNA was not previously known. This study came up with a protocol called Pseudo-Seq, which involves the use of a chemical called CMC, to identify psUsites. They find hundreds of these within mRNA.
Most of these are assigned via genetic KO’s to 1-7 PUS genes (Pseudouridine synthases). Most of the PUSs are regulated in response to signals from the environment, such as serum starvation in human cells (?) and nutrient deprivation in yeast. This suggests a mechanism for rapid and regulated “rewiring” of genetic code via inducible mRNA modifications.

Confusions/questions/issues:

Implications for our research:

Lab meeting: October 20th, 2016


Synopsis: Untangling complex gene networks, such as the GATA-factor network, requires a prohibitively large number of experiments. Mechanistic modeling using computational approaches enables the study of such highly complex problems. The GATA-factor network is regulated by the 2 activators, GLN3 and GAT1, and the 2 repressors, DAL80 and GZF3. While some of the interactions between the GATA-factors have been established, here, 5 hypothesized interactions are tested for their validity. The 5 hypothesized interactions are 1) Self-repression of DAL80, 2) Self-activation of GAT1, 3) Repression of DAL80 by GZF3, 4) Repression of GZF3 by DAL80, 5) Interaction of GLN3 and GAT1. Bayesian model selection is used to identify the model that best describes this network. This model is then validated experimentally by using GATA-factor promoter-GFP fusion constructs in a single-gene deletion background of each of the GATA-factors during the nitrogen upshift and downshift. Finally, they use their model to predict dynamics such as nuclear concentration, transcriptional contribution, and mRNA levels for different players in the network. According to their model, during the N-upshift, GAT1 and GLN3 exit the nucleus within the first 5 minutes, while DAL80 exits within 15 minutes. During the N-downshift, GAT1 enters the nucleus monotonically, while GLN3 initially overshoots then decreases back down to a lower steady-state level.

Confusions/questions/issues:

In figure 3 where they display the results of the two rounds of model selection. Does excluding all models that excluded hypothesis (4) in the first round affect the results they would otherwise get in the second round after extra information is added to the model?

Implications for our research:
Does this model explain the effects we see in the single-deletion GAP1-GFP fusion library?

Does this model explain the effects on mRNA levels we see upon induction of GLN3?


Synopsis: The authors are interested in the spectrum of possible mutations and their fitness effects during adaptive evolution. To address this question, they used a pooled approach with 5 different libraries: the haploid deletion collection, the heterozygous diploid deletion collection, diploids with low copy number plasmids, diploids with high copy number plasmids, and a control collection (barcodes randomly inserted at neutral genomic locations). They grew each library in phosphate, glucose, and sulfate limiting conditions and measured the proportion of each strain at various time points over 20 generations using BarSeq. Approximately 500 strains (460 genes) had significant increases in fitness. Several genes have been implicated before in evolution studies including SUL1 and SGF73 (sulfate), and MTH1, WHI2 and GPB2 (glucose). They compared these results to a set of mutations identified in evolution experiments and found that ~35% of mutations are beneficial. This indicates that many mutations occurring in LTEE are neutral (hitchhikers). They conclude that screens such as this one have the potential to predict adaptive mutations during experimental evolution.

Confusions/questions/issues:

- Figures 4/5 --> how do they conclude which mutations are drivers? how do they get to their conclusion that most mutations are hitchhikers?

- The plasmid collections only recreate gross dosage changes and doesn't take chromosomal context into account (this is a known consequence of their screen)

Implications for our research:

- 73% of mutations increasing fitness found in the pooled screens were from the plasmid collections --> duplications may be more likely to produce fitness gains than deletions in these conditions

- We could perform LTEE and compare our results to those found in this screen to better understand the fitness effects of individual mutations (and potentially distinguish drivers from hitchhikers)

- Further implicates that diploid backgrounds are more likely to acquire large CNVs and GOF
mutations whereas haploids are more likely to acquire LOF mutations

- There are alternative paths to evolve in sulfate-limitation besides acquiring CNVs with large fitness benefits

Costanzo et al., (2016) A global genetic interaction network maps a wiring diagram of cellular function
http://science.sciencemag.org/content/353/6306/aaf1420.long

Synopsis:

This paper described the construction and analysis of a comprehensive genetic interaction in interaction for a eukaryotic cell. They tested ~6000 genes in the yeast *Saccharomyces cerevisiae* for all possible pairwise genetic interactions (in total ~ 23,000,000 double mutants) that constructed by synthetic genetic array. By quantifying the fitness variation in double mutation and single mutation as in, they identified nearly 1 million interactions, including ~550,000 negative and ~350,000 positive interactions, spanning ~90% of all yeast genes. The genetic interaction profiles they generated enables assembly of a hierarchical model of cell function, including modules corresponding to protein complexes and pathways, biological processes, and cellular compartments. Furthermore, negative interactions connected functionally related genes, mapped core bioprocesses, and identified pleiotropic genes, whereas positive interactions often mapped general regulatory connections among gene pairs, rather than shared functionality. Genes with similar interaction profiles can also be predicted to share similar biological functionalities.

Confusions/questions/issues:

Implications for our research:

Mathematic method for quantifying and normalizing the fitness and variations.

Lab meeting: October 13th, 2016


Synopsis: Previous studies had shown that total mRNA content is decreased in starved cells (refs 5, 8-16, 24 in this paper). They show that the total amount of transcription decreases when cells enter stationary phase by measuring labeled UTP incorporation in sarkosyl treated cells (i.e. a run on assay). Cells that are removed from YPD and placed in GAL show a decrease in transcription within 10 minutes and they show that there is about a 4 hour lag until they start transcribing in Gal. The measure the phosphorylation status of RNA pol II CTD and this is altered. By shifting the rbp1-1 allele from glucose to galactose then doing the temperature shift to 37 they infer that mRNA decay is slowed in galactose (i.e. in slow growth rates). They also claim that mRNA decay is reduced in starved cells.
They claim that pol II initiation is reduced in starved cells and it is not because there is insufficient nucleotide precursors. They also show that there is a decrease in ATP levels in cells when starved and suggest that this may promote reduced transcription.

Confusions/questions/issues:

- they claim that starvation for carbon doesn’t affect 5S rRNA or tRNA transcription, but that doesn’t make sense. Wouldn’t they see UTP incorporation if they were still transcribing these non pol II transcripts?
- The still normalize all their blots with the same quantity of RNA, so getting absolute levels is impossible.
- Actually, Figure 6 is 5e7 cells, and shows decreased mRNA content in starved cells, which is stable

Implications for our research:

- using polyA tail staining David has also found that the amount of mRNA in a YPD stationary phase cell is similar to a rbp1-1 ts allele at the restrictive temperature
- they use a Kin28 ts allele that is a subunit of the general transcription factor TFIIH, which would be a useful strain for us.
- they measure polyA tail length as 75-80 bases in YPD and 26 nucleotides one hour after shifting the rbp1-1 allele to 37C, when they cells are in galactose the decrease in tail length is much slower.
- polyA tail length doesn’t look too different in GAL (Figure 5), which would support the idea that polyA staining in different conditions isn’t affected by total differences in tail length as a function of conditions.

Synopsis: They find that the nonsense mediated decay factor UPF1 is required for responding to oxidative stress in S. pombe. CSX1 is an RNA binding protein that associated with ATF1 mRNA and is required for its stabilization during oxidative stress. Using epistasis they show that CSX1 and UPF1 are in the same pathway

Confusions/questions/issues:

- the number of genes effected by a UPF1 KO using DNA microarrays is only 27 upregulated and 16 downregulated compared with ~500 in cerevisiae. How does this compare with other studies
of the effect of messing with NMD on global gene expression?

Implications for our research:

- UPF1 can stabilize transcripts! (This has also been shown in human cells, ref 22)
- the mechanism by which Upf1 stabilized atf1 mRNA is unclear, could be a variety of mechanisms
- ref 32 showed that Csx1 is an RNA binding protein that controls gene expression in response to oxidative stress from these same authors - good example of affecting mRNA stability to mediate gene expression remodelling

Carmen V. Jack, Cristina Cruz, Ryan M. Hull, Markus A. Keller, Markus Ralser and Jonathan Houseley
"Regulation of ribosomal DNA amplification by the TOR pathway" [http://www.pnas.org/content/112/31/9674]

Synopsis: Amplification of the yeast rDNA locus is effected by TOR. If they use a strain that has 35 copies of rDNA then the amplification following passaging to the ~150 copies in wildtype cells does not occur in the presence of rapamycin. Using genetics they show that this is both RAD52 dependent and also affected by/dependent on SIR2, HST3 and HST4, which are histone deacetylases that affect silencing. When they treat with both rapamycin and nicotimamide, rapamycin doesn’t block rDNA expansion, so they claim that the amplification is through a nicotimamide sensitive pathway. The conclusion is that the three histone deacetylases control rDNA expansion through both homolgous recombination-dependent and -independent mechanisms.

Confusions/questions/issues:

- they claim that amplification of rDNA doesn’t have a fitness effect. Has this really been shown? It would make sense that more rDNA -> more rRNA -> faster growth rates. They claim in figure S1 to show that there is no growth difference.
- the paper is quit confusing and the effects seem to be partial and overlapping making the story quite foggy.

Implications for our research:

- interesting connection between gene expression regulation/epigenetic regulators and CNVs
- they use 25nM rapamycin

Lab meeting: October 6th, 2016

Synopsis: Provides evidence for decoupling between cell growth and cell cycle. Disrupted TOR signalling pathway (rapamycin and mutants) and studied effect on G1 transcripts and cell cycle progression. Found that protein initiation is reduced in response to TOR inhibition. Show that part of the effect on the cell cycle of TOR reduction is reduced translation of the cyclin CLN3. If they drive CLN3 expression then cells progress past START and enter the cell cycle. This paper showed there is a very similar response between starving cells and Tor inhibition (both physiological and transcriptional response).

Confusions/questions/issues:

- Does this really show that translation initiation is inhibited or could it also be explained by reduced overall ribosome number?
- there is still some low translation when cells are treated with rapamycin. Could this be cap-independent translation (Gilbert/Doudna showed use of IRESs in starved cells)
- claim that S6 kinase is not a target of TOR in yeast, but we now know that SCH9 is the S6 kinase homologue, so this doesn’t hold up?

Implications for our research:

- They look at relative gene expression, but how would these gene expression changes be interpreted in the context of reduced overall transcriptome size, which is what our results suggest.
- TOR inhibition results in G1 arrest and they see a population of large cells, and small cells which they assume are new daughters - we see similar bimodality in size when cells starved particularly with nitrogen starvation.
- The claim that there is not a global repression of transcription b/c ACT1 expression remains the same, but presumably they are just normalizing to total RNA. We should measure total RNA content upon rapamycin treatment using RNA-sytoselect.
- Cycloheximide results in increased polysomes, whereas TOR inhibition results in decreased polysomes.
- interesting that TOR inhibition does not result in sporulation in diploids but PKA/Ras inhibition does.
- the CLN3 result suggests a mechanisms for why unnatural starvations might not arrest as unbudded cells as TOR activity remains high -> CLN3 translation is maintained -> cells keep cycling and get stuck when they ultimately run out of their auxotrophy

Aditya Radhakrishnan, Ying-Hsin Chen, Sophie Martin, Najwa Alhusaini, Rachel Green Jeff Coller.
Synopsis: Codon optimality is correlated with mRNA decay rate, but how is this sensed? They claim that DHH1, a helicase, senses non-optimal codons by sensing slow moving ribosomes and promoting the degradation of those transcripts. In a DHH1 mutant the effect of nonoptimal codons on decay is abrogated. DHH1 is preferentially associated with transcripts containing nonoptimal codons, and is associated with the ribosome.

Confusions/questions/issues:

- Clearly shows that DHH1 is involved in effect, but how does it exert its effect?
- Violin plots binning continuous variables (e.g. codon optimality) incorrectly enhances the apparent effect.
- Northern of decay with different optimal codons are very compelling.
- Both rbp1-1 ts allele and GAL promoter to shut off transcription have issues - would be better to use Tet-off system or 4-tU labeling

Implications for our research:

- Pulling down the mRNA with 3'-UTR biotinylated oligo is a cool method when used with cross-linking to identify associated proteins. We could pull down GAP1 this way and find proteins (but probably not enough material for mass spec analysis. they used northerns)

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Ruli Gao, Alexander Davis, Thomas O McDonald, Emi Sei, Xiuqing Shi, Yong Wang, Pei-Ching Tsai, Anna Casasent, Jill Waters, Hong Zhang, Funda Meric-Bernstam, Franziska Michor & Nicholas E Navin (2016) "Punctuated copy number evolution and clonal stasis in triple-negative breast cancer" [http://www.nature.com/ng/journal/v48/n10/full/ng.3641.html](http://www.nature.com/ng/journal/v48/n10/full/ng.3641.html)

Synopsis: Study CNVs in tumor cells using single cell sequencing. Find that CNVs occur early in tumorigenesis and then undergo clonal expansion. This is in contrast to a continual accumulation of CNVs throughout the tumor evolution.

Confusions/questions/issues:

- Do they look at cell size in their sorting to avoid clumps/multinuclei?
- They looked at a single time point to make evolutionary inferences, what does it look like over time?
• What is the noise in DNA content associated with the whole genome amplification process?
• the do single end sequencing and their resolution of CNVs is poor, i.e. they are only really big CNVs.
• would have been interesting to do whole population sequencing
• they claim that "genome instability is turned on and then off at the earliest stages of tumor evolution" Does this need to be the case.

Implications for our research:

• the dynamics looks very similar to what we see in chemostat selections.
• is there any hope for getting allele frequencies from whole population sequencing. that seems like a really hard problem.
• "unlikely for selective sweeps to occur late in tumor progression". That is probably the same in our selections. Does this imply that "evolution stops"?