Gresham Lab Strategic Review

January 2014
Our lab studies adaptive evolution, control of cell growth and regulation of mRNA degradation. We primarily use the budding yeast *Saccharomyces cerevisiae* as a model system to study evolution and the molecular basis of cellular processes conserved throughout eukaryotes. Our approach integrates high-throughput genome-scale methods and computational biology with microbiology and molecular biology.
Awarded four year NSF grant
Awarded five year R01
Published 4 peer reviewed papers and 1 commentary
Darach passed quals and joined the lab
High School students Wei and Anise spent the summer mentored by Niki
Hosted Rasmus Bojsen for the summer
Bentley graduated and accepted to NYU PhD program
Lab represented at:
  – SMBE (Jungeui and David)
  – CSHL RNA meeting (Benjy)
  – Gordon Conference (Naomi)
Awards
  – Benjy awarded the Kopac service award
  – Naomi awarded Steve Kazianis award, Fleur Strand award and Deans Dissertation Fellowship
  – Victoria awarded DURF
  – Sarah awarded DURF
Teaching
  – Niki lectured in Applied genomics
  – Darach TA’ed Applied Genomics
  – David taught Applied Genomics
Genetic and Nongenetic Determinants of Cell Growth Variation Assessed by High-Throughput Microscopy

Naomi Ziv, Mark L. Siegal, and David Gresham

Center for Genomics and Systems Biology, Department of Biology, New York University

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Associate editor: Csaba Pal
Design and Analysis of Bar-seq Experiments

David G. Robinson,* Wei Chen,† John D. Storey,*1 and David Gresham‡,1
*Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey 08544, †Berlin Institute for Medical Systems Biology, Max-Delbrück-Center for Molecular Medicine, 13125 Berlin, Germany, and ‡Center for Genomics and Systems Biology, Department of Biology, New York University, New York, New York 10003
Video Article

The Use of Chemostats in Microbial Systems Biology

Naomi Ziv¹, Nathan J. Brandt¹, David Gresham¹
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URL: http://www.jove.com/video/50168
DOI: doi:10.3791/50168

Keywords: Environmental Sciences, Issue 80, Saccharomyces cerevisiae, Molecular Biology, Computational Biology, Systems Biology, Cell Biology, Genetics, Environmental Microbiology, Biochemistry, Chemostat, growth-rate, steady state, nutrient limitation, adaptive evolution

Date Published: 10/14/2013
A sticky solution

Selection favours single-celled mutants that stick together when a sugar needed for growth is in short supply, suggesting that multicellular life may have evolved as a by-product of selection for more efficient usage of resources.

DAVID GRESHAM
The details in the distributions: why and how to study phenotypic variability
KA Geiler-Samerotte, CR Bauer, S Li, N Ziv, D Gresham and ML Siegal
Molecular Specificity, Convergence and Constraint Shape Adaptive Evolution in Nutrient-Poor Environments

Jungeui Hong, David Gresham*

Center for Genomics and Systems Biology, Department of Biology, New York University, New York, New York, United States of America
NSF Specific Aims

The functional basis of genetic interactions underlying quantitative trait variation

Objective 1: Select mutants with increased growth rate in nitrogen-limited environments and identify all acquired genetic variation.

Objective 2: Quantify the individual effect of acquired genetic variants in mutants and test whether the combined effect of genetic variants is explained by an additive or non-additive model.

Objective 3: Generate a testable model of the functional relationships underlying different genetic interaction classes.
NSF Specific Aims

The functional basis of genetic interactions underlying quantitative trait variation

Objective 1: Select mutants with increased growth rate in nitrogen-limited environments and identify all acquired genetic variation. We will perform long-term selection experiments in nitrogen-limited environments and identify spontaneous mutants with increased growth rates. We will quantify the growth rate of mutants in the same condition and identify all acquired genetic variation using high throughput sequencing. Completion of objective 1 will result in a collection of 54 mutants for which we have 1) quantified growth-rate (our quantitative trait of interest) and 2) identified all acquired mutations.

Objective 2: Quantify the individual effect of acquired genetic variants in mutants and test whether the combined effect of genetic variants is explained by an additive or non-additive model. We will measure the phenotypic effect of individual acquired mutations and pairwise combinations. We will perform statistical tests to define the class of genetic interaction for each pair of QTL tested. Completion of objective 2 will result in the rigorous definition of interactions between QTL as either additive or one of the three possible classes of epistatic interactions.

Objective 3: Generate a testable model of the functional relationships underlying different genetic interaction classes. We will identify the functional relationships that underlie different genetic interaction classes and experimentally test our model. Completion of objective 3 will result in a model that predicts the type of interaction between QTL on the basis of functional relationships between genes and tests that model.
NIH RO1 specific aims

Regulation of Quiescence in Eukaryotic Cells

- **Aim 1.** Define the conserved quiescence program.

- **Aim 2.** Determine how signaling pathways coordinately regulate initiation of quiescence.

- **Aim 3.** Test the role of regulated variation in mRNA stability as cells enter quiescence.
NIH RO1 specific aims

Regulation of Quiescence in Eukaryotic Cells

• **Aim 1. Define the conserved quiescence program.** Using multiplexed analysis of genome-wide libraries of mutants in budding and fission yeast and high throughput sequencing, we will quantitatively define the role of each gene in quiescence initiated by carbon, nitrogen or phosphorous starvation (nutrients essential for cell growth that are also depleted in some tumor microenvironments). We will study physiological hallmarks of quiescence in homologous mutants defective in quiescence to define their functional roles.

• **Aim 2. Determine how signaling pathways coordinately regulate initiation of quiescence.** We hypothesize that diverse signals are integrated by a network of interactions between signaling pathways. To test this hypothesis, we will identify the targets and interactions of conserved protein kinases that regulate quiescence using quantitative genome-wide genetic interaction mapping in both species in different quiescence-inducing conditions. In addition, we will test the role of subcellular localization of signaling pathway components in regulating quiescence using protein mislocalization experiments.

• **Aim 3. Test the role of regulated variation in mRNA stability as cells enter quiescence.** We will quantify variation in the rate of mRNA synthesis and degradation for each transcript in both species as cells enter quiescence using *in vivo* metabolic labeling of mRNAs and RNA-Seq in chemostat cultures. We will identify factors that contribute to variation in mRNA degradation rates using computational analyses. In addition, we will test the functional role of mRNA stabilization and storage in P bodies in quiescent cells using imaging studies.
NIH RO1 subcontract

- Alexander Statnikov (NYU Med School)

- Sub-aim 3: Assess pathway discovery accuracy and experimental efficiency of both new and existing state-of-the-art methods in real biological data using an experimentally-derived gene regulatory network of S. cerevisiae and a unique database of gene knockout experiments covering a significant part of the yeast genome.

- Perform experimental validation of the predicted local causal pathway of TOR1, a conserved regulator of cell growth that is frequently dysregulated in human tumors.
Declined grants

- mRNA degradation (NIH RO1)
- ts prediction (NIH RO1)
AIM 1. Identify the proteins and pathways that regulate the degradation of NCR transcripts.

AIM 2. Identify cis-regulatory sequences that control the degradation of NCR transcripts.

AIM 3. Determine the substrate specificity of mRNA degradation pathways.
Mechanisms of Environmentally Regulated mRNA Degradation in Yeast Cells

**AIM 1. Identify the proteins and pathways that regulate the degradation of NCR transcripts.** We will comprehensively identify the proteins and cellular processes that regulate the degradation of NCR transcripts by screening a genome-wide library of mutants using a high throughput mRNA degradation assay. To identify proteins that physically associate with NCR transcripts we will perform mRNA affinity purification experiments using transcripts tagged with the MS2 aptamer sequence. To test whether the identified mRNA-binding proteins coordinately regulate NCR transcripts we will use photoactivatable-ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP) coupled with high throughput sequencing to identify co-precipitating mRNAs and define sites of protein binding within transcripts.

**AIM 2. Identify cis-regulatory sequences that control the degradation of NCR transcripts.** We will test the role of 5’ and 3’-untranslated regions (UTRs) and gene promoters in NCR mRNA degradation regulation using reporter constructs. To identify the exact sequences that contribute to NCR transcript degradation we will construct and study systematic deletion alleles of 5’ and 3’ UTRs. We will generate nucleotide-resolution maps of mRNA stability determinants in the UTRs of NCR transcripts by engineering libraries of UTR variants and studying their effect on mRNA degradation rates in vivo using pooled assays and high throughput sequencing.

**AIM 3. Determine the substrate specificity of mRNA degradation pathways.** We will test whether different mRNA decay pathways preferentially degrade specific transcripts using in vivo metabolic labeling of mRNAs with 4-thiouracil (4sU) and RNASeq. We will determine genome-wide mRNA degradation rates in cells mutant for core components of known mRNA degradation pathways in two different environmental conditions and identify transcript properties that explain variation in degradation pathway affinities. We will complement these studies with affinity purification of mRNA decay factors and identification of associated mRNAs using high throughput sequencing.
Rationale design of temperature sensitive proteins (with Kris Gunsalus and Rich Bonneau)

• **Aim 1.** Generate a dataset comprising thousands of sequence-verified *ts* and non-*ts* mutations.

• **Aim 2.** Develop a computational algorithm for the rational design of *ts* alleles.

• **Aim 3.** Construct and test predicted *ts* alleles *in vivo* in *S. cerevisiae* and *C. elegans*. 
Rationale design of temperature sensitive proteins (with Kris Gunsalus and Rich Bonneau)

• **Aim 1. Generate a dataset comprising thousands of sequence-verified ts and non-ts mutations.**
  To test the hypothesis that specific types of structural perturbations confer temperature sensitivity we will:
  – Construct and test exhaustive libraries of sequence variants for temperature sensitivity for five conserved essential genes in yeast representing distinct protein classes.
  – Identify ts alleles in a collection of 787 yeast ts strains and 800 worm ts strains, and identify ts, neutral, and null alleles in a sequenced collection of 2,007 homozygous EMS/ENU-mutagenized *C. elegans* lines.
  – Curate ts and non-ts amino acid replacements from published studies and databases.

• **Aim 2. Develop a computational algorithm for the rational design of ts alleles.** We will use machine-learning to discriminate amino acid substitutions that cause temperature-sensitivity from those that do not.
  – Using training data from Aim 1 we will identify compositional features based on conservation, chemical (sequence), secondary, and tertiary structural elements that are predictive of temperature sensitivity.
  – We will predict new ts alleles for the complete essential gene complement in yeast and worm and make these results available to the research community on a web server.
  – We will distribute our code and provide a website for designing ts mutants and predicting the effect of mutations in protein-coding sequences.

• **Aim 3. Construct and test predicted ts alleles in vivo in *S. cerevisiae* and *C. elegans**; We will experimentally test ts mutations predicted with our algorithm by constructing novel predicted ts alleles and assaying for ts phenotypes. We will focus on proteins involved in essential aspects of cell polarity establishment and maintenance.
  – We will construct and test 5 novel ts alleles for each of 10 essential genes in yeast.
  – We will construct and test 5 novel ts alleles for each of 10 essential genes in *C. elegans*. 
2014

• Lab composition
• Grants
• Papers
• Meetings
Lab composition

Lab Manager
  Nathan Brandt (Joined December 2009)

Postdocs
  Niki Athanasiadou (joined May 2011)

PhD Students
  Benjy Neymotin (joined April 2010)
  Naomi Ziv (joined April 2010)
  Jungeui Hong (joined December 2010)
  Darach Miller (joined April 2013)

Masters Students
  None

Undergraduate Students
  Victoria Ettore (joined January 2012)
  Sarah Choksi (joined April 2012)

High School Students
  Wei Wu (joined May 2013)
  Anise Ru (joined May 2013)
Lab composition

Anticipated additions
Christina Nunez (undergraduate)
PhD student(s)
Post-doc

Anticipated departures
Victoria
Sarah
2014 Meetings

1. NYU Abu Dhabi Genome Meeting Feb 19-21
2. RNA Society Meeting (Quebec) June 3-8
3. SMBE (Puerto Rico) June 8 -12
4. ASM Experimental Evolution (Washington DC) June 19-22
5. GSA Yeast Genetics Meeting (Seattle) July 29-August 3)
6. ICSB (Melbourne) Sept 14-18
8. EMBL Evo Meeting (Heidelberg) Oct 12-15
2014 Grants

• mRNA decay RO1 – June 5th
• NSF CAREER award – July 27th
Papers for 2014
Gresham and Hong

• Review of selection in chemostats
• Revision due
Neymotin et al

- RATE-seq
- Near submission
Airoldi et al

- Nitrogen regulated gene expression
- Near submission
Gresham and Dunham

• Opinion piece on why do experimental evolution in chemostats
• First version due Jan 31
The Gresham Laboratory
@ The Center for Genomics and Systems Biology,
New York University

Welcome to the Gresham Lab @ NYU

Our lab studies the regulation of cell growth and the regulation of mRNA decay at The Center for Genomics and Systems Biology in the Department of Biology at New York University.

Research

The Gresham lab studies the regulation of cell growth and the regulation of mRNA decay. We use the budding yeast (Saccharomyces cerevisiae) as a model system and a combination of genetic, genomic and computational methods to address these questions.

Systems biology of cell growth and quiescence

We are interested in the regulation of cell growth and how it is coordinated with progression through the cell cycle, metabolism and the synthesis and degradation of macromolecules. To control the growth rate of cells we use chemostats, which allows us to systematically study the metabolic, physiological and molecular programs associated with different rates of cell growth. We are taking a variety of approaches to study how cell growth is regulated including 1) the study of mutants with increased growth rates selected from long term nutrient-limited chemostats, 2) mapping QTL that underlie variation in growth rate in natural isolates of yeast and 3) using massively parallel phenotyping to identify genetic networks that regulate cell growth and quiescence.

Regulation of mRNA decay
Protocols

Wiki Markup Code for the Menu Bar
An excellent source of yeast protocols can be found here: http://cshprotocols.cshlp.org/cgi/collection/yeast
And more general molecular biology protocols are here: http://cshprotocols.cshlp.org/site/misc/subject.xhtml

Media

Metals
Leucine and Phosphate Limiting Media
Nitrates Agarose Plates
Variable Nitrogen Source Limitation Carboy
Vitamins
Derivatives’ Media

Growth and Growth Assays

Coulter Counter
Colony Counter
Stroma Chromatid
Chemical Protocols
SYTO9 \\ PI FACS Viability Assay
FACs-based analysis for competition experiments

RNA and Expression Analysis

Yeast RNA Extraction
Preparation of an Exemplary RNAlater RNA Preservation Medium
Protease K-mediated extraction of RNA from yeast
Separation of RNA by electrophoresis-Separation of RNA by electrophoresis
Transfer of Denatured RNA to positively charged nylon membrane
Bioluminescence of dsU labeled RNA
Dot Blot Assay
Whole Genome Transcription Analysis
RNA expression spike-ins, linearizing and in-vitro transcription
Untreated Pull-down of Alkaline-labeled H3P* 4SU RNA

High-Throughput Sequencing Methods

Directional RNA-Seq
BarSeq

DNA Microarrays

Hybridization Mix
Affymetrix Tiling Arrays
Slide Staining Protocol Agilent Yeast Arrays

DNA
Lab Books

- Record of all experiments
- One page per date
- Pen
- No removed pages
- Legible by everyone (including yourself)
- Property of the lab
Data management

• Strain database
• Plasmid database
• Oligo database
• Microarray database

• Next-gen seq database?
Lab meetings

• Weekly lab meeting
  • Reschedule?
  • Format?
  • On time

• One on one meetings
  – Weekly
  – Bring lab book, print outs
  – Brief
  – On time
Support for conferences

- Delill Nasser (GSA)
- Conference-specific
- GSAS
- Bio Department
Career development

• Discussion
• Personal career development statement
Other opportunities

• Visit Max Delbruck Center in Germany
Thanks