Advanced Genetics
Lecture 3
Susan Dutcher
Protein trafficking

RNA $\rightarrow$ protein $\rightarrow$ ER folding sugars disulfide bonds

When there is not enough ER $\rightarrow$ accumulate unfolded protein (UPR)

This lead to a transcriptional response

Use a genetic approach to find ER-nucleus signal transduction pathway
Genetic screens for two processes

a. Unfolded protein responses (UPR)

b. TOR pathway

UPR

1. Adapt to ER stress via a transduction pathway to match the "folding capacity" of the ER to the need

   a. Increase ER membranes
   b. Increase chaperones
   c. Increase protein modification enzymes
d. decrease translation

e. decrease entry of peptides/proteins into ER

f. increase targeting of unfolded proteins for degradation (ERAD)

2. This pathway is activated in

a. many cancers

b. Cystic Fibrosis

c. Alzheimer’s

d. Viral infections

e. Sleep deprivation
3. UPR controls transcription of a set of genes (~400 genes)
   - mammals
   - Yeast
   - BiP
   - Kar2

4. Most work was performed in mammalian cells first and then in yeast

5. Upregulated genes share a 22bp motif; mammalian cells have a 28bp
3. Setting up a screen

ER stress results is upregulated

UPRE reporter

22bp motif

β-galactoside

wild-type cells + UPRE reporter

replica plating to x-gal + tunicamycin

screen for white colonies

(fail to turn on β-gal gene)
Secondary screens
1. discard mutations that confer resistance to TUN (ALG9 - transfers Glc-Nac-P in the ER)
   ALG3 asparagine-linked glycosylation
2. Induce UPR with a second method
   2-deoxyglucose
3. Use a different UPR regulated promoter
4. Examine mRNA for KAR2

2 / 40,000 colonies pass all the tests
Genetic tests of mutants

1. Is it recessive? __________

2. Does it show 2:2 segregation? __________

3. Can it be complemented by genomic library plasmid? __________

4. Are these null alleles? __________

Outcomes

- Signal sequence
- ER lumen
- TM
- Kinase domain
- Cytoplasm
- IRE1
It had been identified in another screen as an inositol auxotroph.

This screen identified **ONE** gene.

his3; Δire1; 4x UPRE-HIS

use multi-copy genomic library to find plasmids that increase HIS expression

IRE1 SWI4 HAC bZIP
Secondary tests

1. Do deletions of swi4 or hacl change Kar2 expression w/ UPR?

   Δswi4 - no effect; UPR is wt
   Δhacl - yes; no UPR ≡ Δircl

2. HAC1 binds to UPRE (gel shift assay)

3. HAC1p is only present w/ UPR when protein accumulate in ER
Reminder: only about 52% of genes in yeast have introns.

HAC1 mRNA is spliced in response to UPR.

1. Antibody to last 18 aa detects a protein made "spliced construct". It is constitutive and does not require IRE1.
Splicing

1. HACI splice junctions are not canonical—that is, they are not GU.....AG.

2. Temperature-sensitive allele in a splicing protein does not affect HACI splicing (prp2, prp8).
Summary of pathway so far

UPR (tunicamycin, 2-deoxyglucose) → IRE1 activation

↑ ER ← HAC1 binding ← HAC1 splicing

↓
to UPRE DNA

IRE1 → HAC1 splicing
Synthetic lethal screen
based on tetrad results they knew
rel1 kar2(ΔHDEL motif) was lethal
Reminder 1) HDEL is signal to retain proteins in the ER
2) kar2 - ΔHDEL induces UPR

S.L. kar2 ΔHDEL; ade2; ade3 + plasmid with ADE3 and KAR2

17/20,000 looked for nonsectoring red colonies

looked for nonsectoring red colonies
Secondary Screens

1. Is UPRE-βgal induced?

Only \( \frac{3}{17} \) passed 2\(^{nd}\) screen

\( \frac{2}{3} \) ire1 mutants

\( \frac{1}{3} \) rlg1

Rescue gene by complementation

RLG1 \( \rightarrow \) tRNA ligase

Splice tRNAs with introns
1) Δrlgl is lethal so would not have found it using the deletion collection.

2) Three activities:
   a) Poly nucleotide kinase
   b) Cyclic phosphodiesterase
   c) RNA Ligase

3) Does rlgl1 -100 (the allele found) play a role in HAC1 splicing?
Northern blots

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HAC1 splicing is blocked in the rgll11-100 mutant
ER lumen

inactive IRE1 kinase and RNase

HAC1 protein

HAC1 RNA

arrested on polysomes

IRE RNase

Pgl11 ligase

HAC1 spliced RNA
Bernaless et al 2006
Walter MBOC 2010
TOR signaling - TOR target of rapamycin - macrolide - rapamycin
- Streptomycin hygroscopic
- found on Easter Island in 1965
  Rapu-Nui is the indigenous name
  T-cell inhibitor blocked tumor growth blocked angiogenesis
fungicide
Rapamycin

Yeast

G1 arrest

Can you find the target of the drug?
First studies were biochemical

Rapamycin binds to FKBP12

1991 column with FK506 binding protein peptidyl-prolyl cis-trans isomerase

FPRI protein folding for proteins with proline residues

5470 identical to the mammalian protein

Suprisingly Δfpri1 is viable
Model

\[ \text{RAP + FPR1} \rightarrow \text{toxic activity that blocks cell cycle progression} \]

1. Genetic selection for rapamycin resistance
   a. lots of mutants in fpr1
   b. few in two genes now called TOR1, TORZ (first called DRA1, 2)
Yeast is unique in having paralogs, mammals have only 1 TOR gene.

Phosphatidylinositol 3 kinase LIKE
but no lipid kinase activity

Resistance is dominant.
TOR1 + TORZ
or rapamycin

Cell growth
Size/mass

Biochemistry found two complexes

TORC1
TOR1 or TOR2
Rapamycin sensitive

TORC2
(TOR2)
Rapamycin insensitive

TOR2
Actin cytoskeleton
Endocytosis
Sphingolipid biosynthesis
Regulate cell size

Tyers Lab (Science 2002) screened the deletion collection (4812 strains) smallest (40% of wild-type) smallallest (40% of wild-type)

sch9

sfpl

protein kinase (AGC family) regulates transcription of ribosomal protein and biosynthetic genes
Sch9 is a target of TOR1
rapamycin $\rightarrow$ dephosphorylated
depletion of C,N,P, or amino acids
Regulates ribosome biogenesis
Entry into Go
G1 progression
Rapamycin treatment $\rightarrow$ growth arrest

How do cells go from Go $\rightarrow$ cycling

Exit from Go or EGO

Screen yeast deletion collection for the inability to resume growth when rapamycin is removed
Eight mutants phospholipid or amino acid metabolism pib2 sac3 hom2 molecular chaperonin ?? ydj1 yd172c

Three more EGO1, GTR2, EGO3

1. nonessential 4. Interact by Y2H tests
2. enter into Go 5. Co IP
3. retain viability in Go
Glutamine $\leftarrow$ RTG I

Vacuole

rtg1; gtr2 lethal
rtg3; gtr2 lethal

RTG1/RTG3

TF needed for biosynthesis and homeostasis of glutamate and glutamine in mitochondria and peroxisomes

microautophagy

TORC
Autophagy genes -

Yoshinori Ohsumi - post doc at Rockefeller University

Christian de Duve (1974) received the Nobel Prize for his EM and biochemistry showing lysosomes and autophagosomes.

In 1988, Ohsumi set out to find mutants defective in autophagy.
- started with strain from Beth Jones lacking vacuolar degradation enzymes (four mutants)

- started

- reasoned if cells lack proteases, the organelles/complexes delivered to vacuole would accumulate and would be visible

- within 30 minutes, observed structures in vacuole
- screened for absence of these vesicles
- 1992 published 100 mutants that fall into 14 complementation groups

Thumm screened with antibodies for reduction of proteins with starvation

Klionsky

maturation of amino peptidase Ape I
41 genes found in forward genetic screens