An In Vivo Map of the Yeast Protein Interactome
Overview

• Genome-wide in vivo screen for PPIs in *S. cerevisiae*

• Identified 2,770 interactions among 1,124 endogenously expressed proteins
  – Confirmed known interactions
  – Most unknown

• PCA detected structural, topological relationships between interactomes (PINs)
  – Map of interacting complexes, extended networks
    • provides insight into cellular processes and pathways
PCAs

*An Alternative Approach*

- Do protein complexes, PINS reconstructed/reconstituted in vitro/removed from normal context of expression reflect their organization in living cells?
- Previously used
  - Y2H
    - Direct binary interactions btw pairs of proteins
  - TAP-MSs
    - Stable protein complexes
Y2H, TAP-MS

• Neither measures PPIs in natural cellular context
• Not easily amenable to protein complexes
  – Transiently associated
  – Dynamic
  – To not survive in vitro purification
  – Cannot be transported to nucleus
  – Form interactions in absence of stabilizing interactions (Y2H)
PCA Strategy

- Alternative approach to detect PPIs in natural context
- 2 proteins of interest fused to complementary fragments of reporter gene

- Proteins interact, reporter fragments brought together
  - Fold into native structure
  - Reconstitute reporter activity of PCA
Genome-wide in vivo screen

- PCA based on mDHFR assay adapted to yeast
- mutant of mDHFR insensitive to DHFR inhibitor methotrexate
- F[1,2], F[3] complementary N-, C-terminal DHFR fragment sequences
- Transformation, ORFs obtained with F[1,2] fragment in MATa strains, F[3] in MATα
- MATa, MATα mated, selected for methotrexate resistance
PPIs were determined based on the growth of the diploid colonies measured by the pixel intensities on the selection plates.
Quality Assessment

• False positives
  – Trapping of complexes due to irreversible folding of mDHFR reporter proteins
  – Potential spontaneous folding of DHFR PCA fragments
    • Adenosine 3’, 5’-monophosphate-dependent dissociation of yeast protein kinase A complex
      – DHFR PCA fully reversible
      – Trapping of complexes unlikely

• Screened strains against F[1,2], F[3]
  – Elimination of highly expressed proteins
    • False positives in affinity purifications
Quality Assessment cont’d

- Threshold of PPI
- MIPS complexes as standards for true positive/negative PPI
  - Filtering, benchmarking, obtained proteins with PPV
- High sensitivity of DHFR PCA assay reflected in abundance of proteins
- Expect many unknown PPIs
General Organization of PIN

- Observed stronger co-regulation of interacting protein pairs than expected for random networks
- PPIs more enriched in PCA determined network compared to TAP-MS
- PCA PPIs detect links among functionally related categories
  - Supported by semantic analysis of full GO hierarchies
Interactions Within GO Categories

(1) Golgi apparatus; (2) cell cortex; (3) cell wall; (4) cellular bud; (5) unknown; (6) chromosome; (7) cytoplasm; (8) cytoplasmic membrane-bound vesicle; (9) cytoskeleton; (10) endomembrane system; (11) endoplasmic reticulum; (12) extracellular region; (13) membrane; (14) membrane fraction; (15) microtubule organizing center; (16) mitochondrial envelope; (17) mitochondrion; (18) nucleolus; (19) nucleus; (20) peroxisome; (21) plasma membrane; (22) ribosome; (23) site of polarized growth; (24) vacuole;
Global Structure and Topology

- Observation of interaction
  - distance between the C termini of 2 proteins
  - length of polypeptide linker separating bait and prey proteins to PCA fragments
- 5.7 times more likely to detect if within 82 Å

![Diagram showing interaction and non-interaction](image)
Global Structure and Topology 
cont’d

- 3.5 times more likely to detect interaction between pair of proteins if C termini closer than 82 Å
- Membrane proteins co-localize to same cellular compartment 12 times more likely to show interaction if parallel
Overview of In Vivo PIN

• General predictions led to specific hypotheses for how protein complexes, networks are organized in living cells

• Unsupervised hierarchical clustering of the 2770 DHFR PCA interactions provides overview of in vivo PIN
  – Proteins with similar interaction patterns, interact grouped together
• PPIs between complexes reflect cross-compartmental, cross-functional interactions visualized as off-diagonal interactions
• Represent links among several network modules
• Identify previously unknown multifunctional PINs, associate, integrate other proteins to processes
Conclusions

• Effects of growth conditions on PINS?
• Genomic tools enable analysis of PPIs, uncover mechanisms of biochemical network regulation
• provide reference constraints for determining architecture of macromolecular assemblies
• integration of results will lead to fuller understanding of how complex cellular processes are orchestrated at molecular, structural level in living cell