Tarassov et al| *Linda Sung*| *April 2009*

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

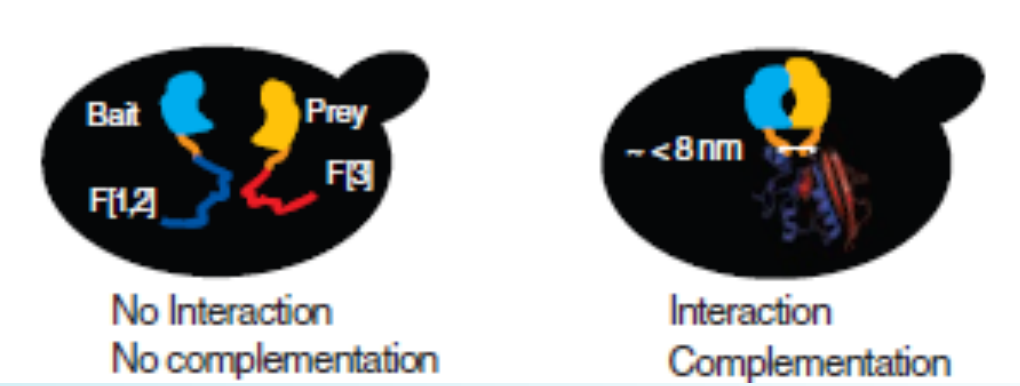
Y2H, TAP-MS

- 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

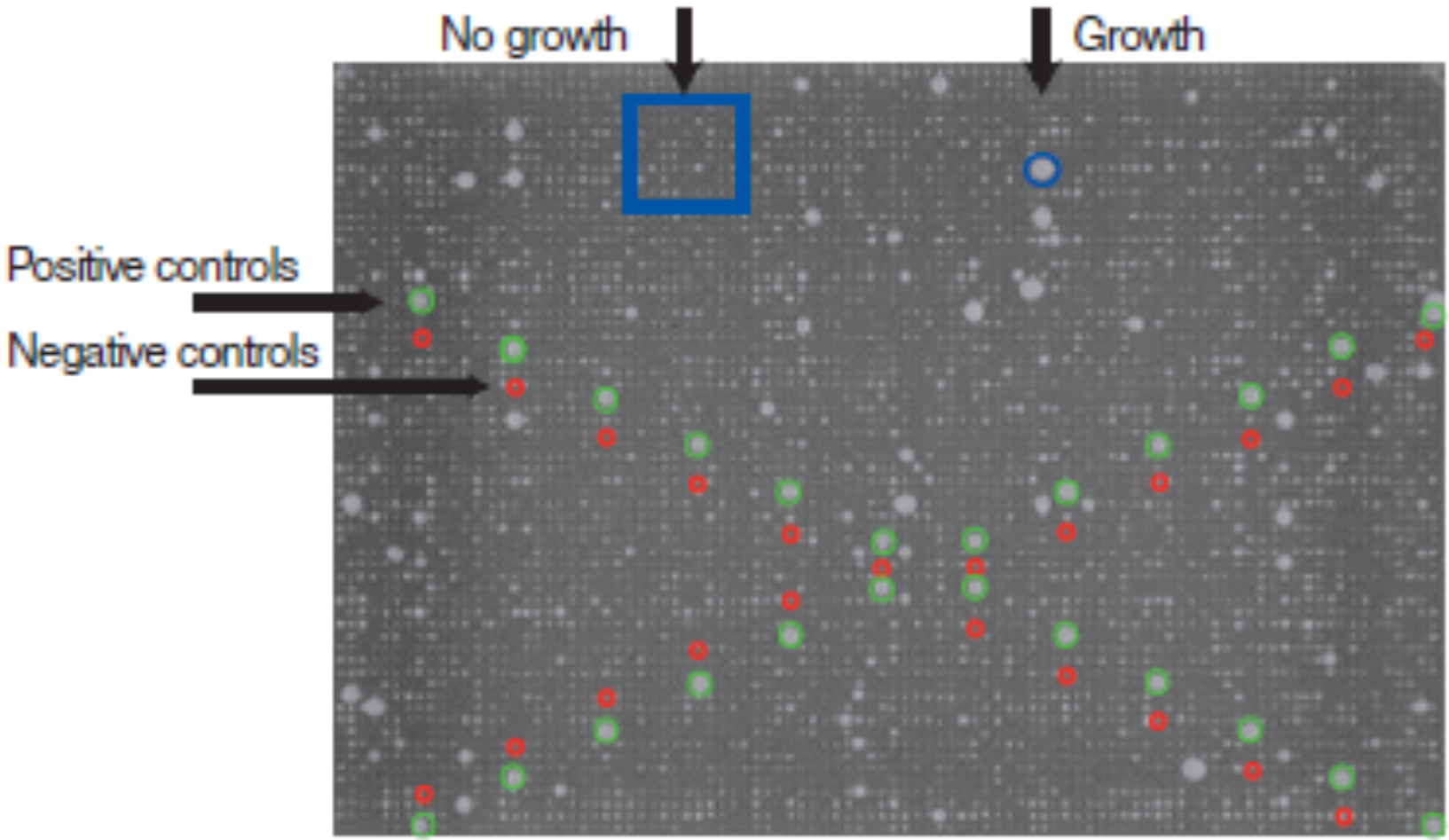
- Alternative approach to detect PPIs in natural context
- 2 proteins of interest fused to complementary fragments of reporter gene

- 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 *MAT***a** strains, F[3] in *MAT*α
- *MAT***a**, *MAT*α mated, selected for methotrexate resistance



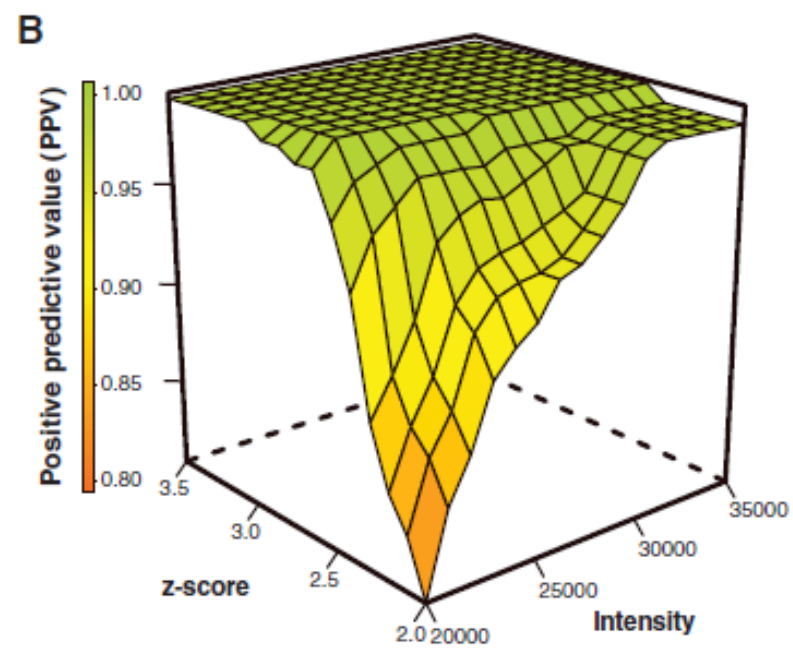
PPIs were determined based on the growth of the diploid colonies measured by the pixel intensities on the selection plates

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'-monophophate-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*

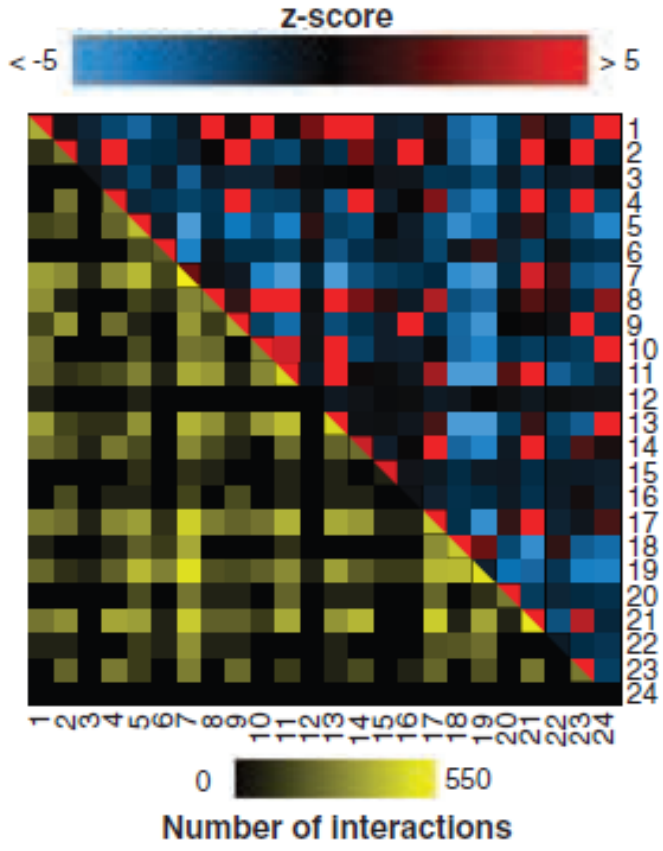
- Threshold of PPI
- MIPS complexes as standards for true positive/negative PPI
  - Filtering, benchmarking, obtained proteins with PPV
- High sensitivity of DHFR PCA assay reflected in abundance of proteins
- Expect many unknown PPIs



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 Catgeories



(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;

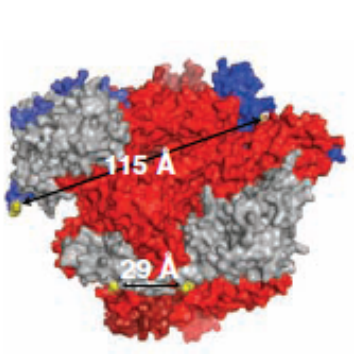
Global Structure and Topology

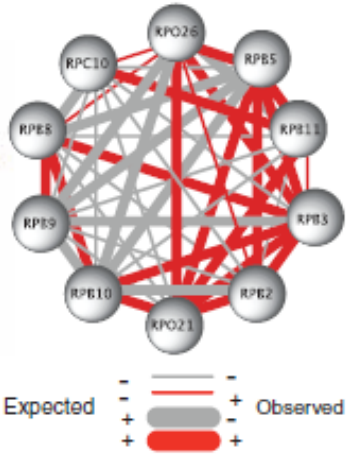
- Observation of interaction
  - distance between the C termini of 2 proteins
  - length of polypeptide linker separating bait and prey proteins to PCA fragments
- 5.7 times more likely to detect if within 82 Å

B

RNApol II small scale

	Distance within 82 Å	Distance above 82 Å
Detected	17	4
Not detected	10	14



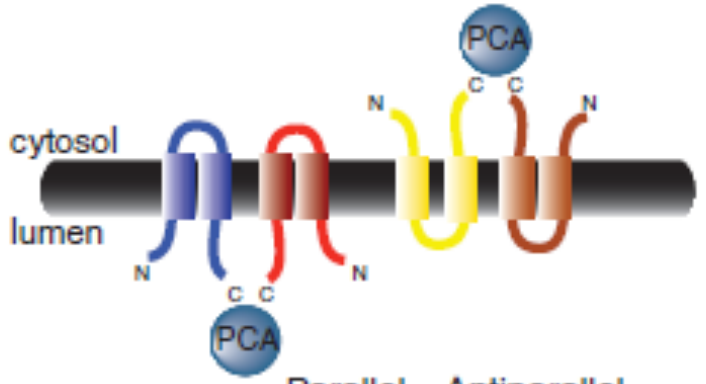


Global Structure and Topology *cont'd*

- 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

C **Protein Data Bank homologs**

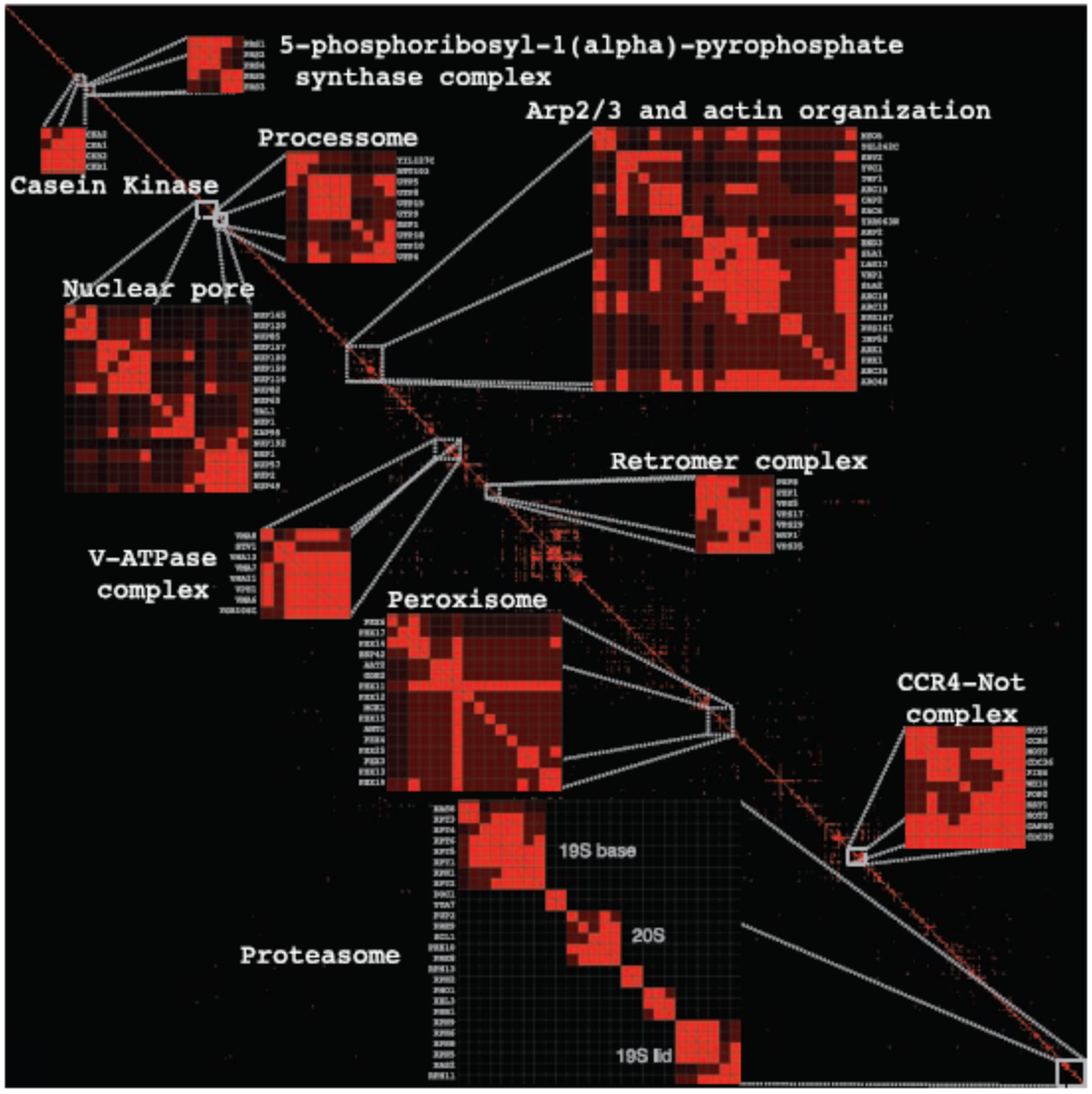
	Distance within 82 Å	Distance above 82 Å
Detected	95	13
Not detected	45	22

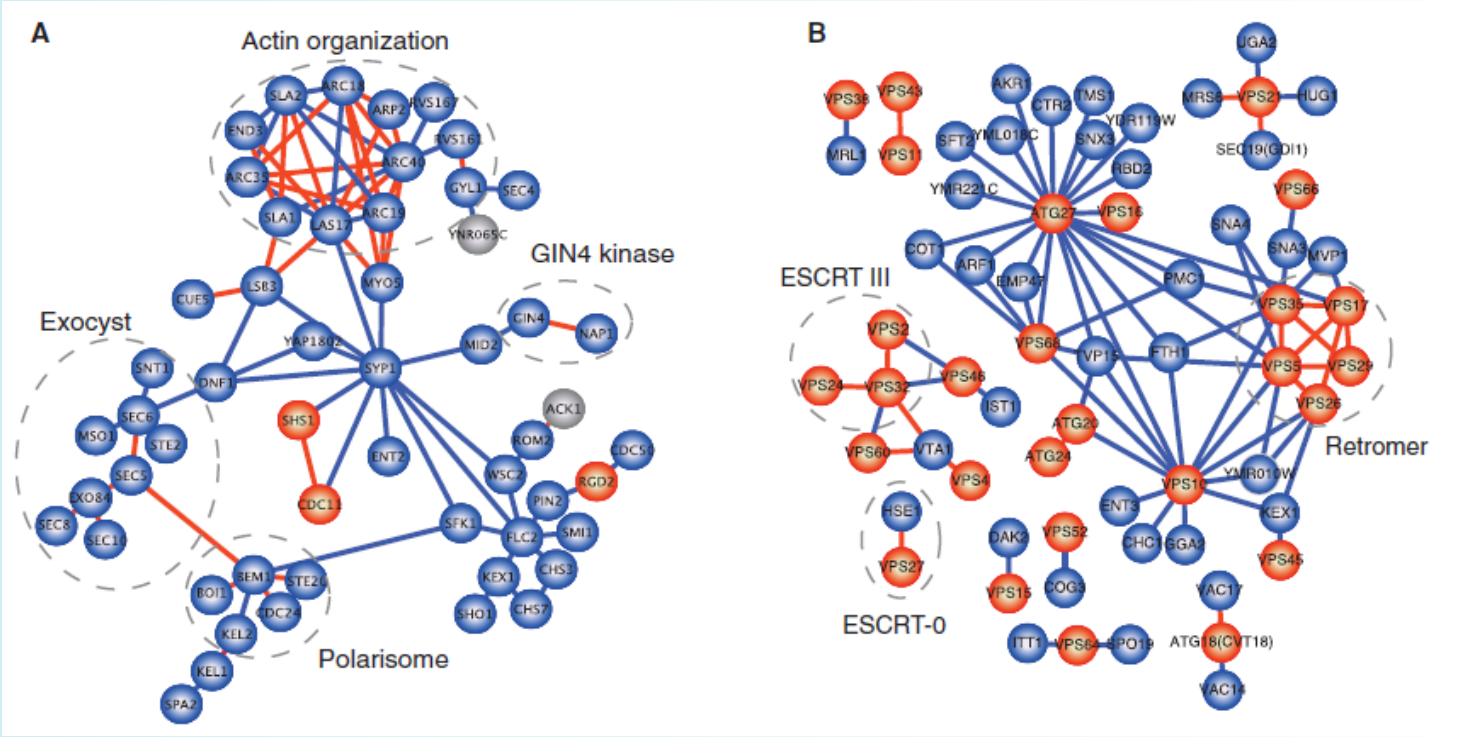


	Parallel	Antiparallel
Detected	148	1
Not detected	1108	83

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


5-phosphoribosyl-1(alpha)-pyrophosphate synthase complex
Arp2/3 and actin organization
Processome
Casein Kinase
Nuclear pore
Retromer complex
V-ATPase complex
Peroxisome
CCR4-Not complex
19S base
20S
Proteasome
19S lid



- 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