Proteomics Course

LECTURE-26
Interactomics:
Yeast Two-Hybrid
Immunoprecipitation
Protein microarrays

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Lecture outline

- Interactomics
- Yeast Two-Hybrid
- Immunoprecipitation
- Protein microarrays
Interactomics

Impact of interactions

Tremendous effect of interactions
Protein interactions with biomolecules

- Complex formation
- Protein modifications
- Protein transport
- Many more…

Interactomics to identify

- Function of uncharacterized proteins
  - New roles for characterized proteins
- Mechanisms to regulate protein activity
- Networks of protein interactions
Different methods of interactions

- Transient
- Weak
- Obligate
- Permanent
- Strong
- Non-obligate
- Homo-oligomer
- Hetero-oligomer

Physical reasons for interactions

- Electrostatic
- Steric
- Hydrophobic
- Hydrogen bonds
Several multi-protein complexes in biological pathways

Proteins involved in many binary interaction
Potential effects of protein-protein interactions

- Protein Inactivation
- Altered kinetics
- Substrate binding
- New binding site formation
- Catalysis

Protein interactions essential for any event in cell

- DNA replication
- Signal transduction
- Translation
- Splicing
- Cell Cycle control
- Morphology
- Metabolism
- Transcription
- Growth
- Motility
Significance of protein interactions

• Dynamic, interact with a wide variety of biomolecules
  – lipids, nucleic acids, small drug inhibitors, and many others

• Proteins also interact with one another
  – form macromolecular complexes to regulate signal transduction & gene regulation

Methods to study protein-protein interactions
Methods to study protein-protein Interactions

Traditional approaches

Yeast two hybrids
Affinity chromatography
Immunoprecipitation

High throughput approaches

Protein microarrays
Label-free technologies

Methods to study protein-protein interactions:
Traditional approaches
(I) Immunoprecipitation

Immunoprecipitation (IP)

• Purification of protein complexes by Immunoprecipitation (IP) or tandem affinity purification (TAP)

• Target protein and its interacting partner isolated from complex sample
Immunoprecipitation

Antigen (known)  Unknown ligands

Antibody
Non-denaturing conditions

Denaturing conditions

Immunoprecipitation: Merits

- Proteins in native state
- Interactions are natural
- Large order complexes can be observed
Immunoprecipitation: Demerits

- Sticky proteins appear regularly
- Unclear whether interaction is direct or indirect
- Expensive
- Applications in other organisms challenging

(II) Yeast two hybrid
Yeast two hybrid (YTH or Y2H)

- YTH system demonstrated by Fields and Song (1989)
  - to detect protein interactions
- The bait-BD and prey-AD hybrid proteins are jointly expressed in yeast nucleus
- If protein-protein interaction is established
  - results in activation of reporter gene transcription

Yeast two hybrid

AD

Bait

Prey

BD

Lac Z Reporter
Yeast two hybrid

AD

Bait Prey

BD

Transcription

Lac Z Reporter

Yeast two hybrid: advantages

- Used for protein-RNA, protein-DNA interactions, analysis of particular complexes, construction of protein interaction network
- Simple protocol
- No expensive equipment requirement
- Ability to screen large libraries
Yeast two hybrid: disadvantages

- High false positive/negative rates
- Proteins must localize and interact in nucleus
- Application in a non-yeast environment questionable
- Sensitive to toxic gene
- Limited to pair-wise interaction

Methods to study protein-protein interactions:
Protein microarrays
(III) Protein microarrays: HT approach

Protein microarrays

- Microscopic arrays comprising thousands of discrete proteins
  - High throughput platform
  - biomarker discovery
  - protein-protein interactions
  - functional characterization
**Protein microarrays**

**Antibody Array**
- Microscopic array of antibodies
- Measure levels of proteins or other biomolecules in samples

**Protein Array**
- Microscopic array of proteins
- Assay protein function, Protein interactions, Small molecule interactions, Identify substrates

**Protein microarrays**

- Spotting purified proteins (*E. coli*)
- Limited number of proteins

Protein microarrays

5800 yeast clones (His-tagged)

- Screening for known and novel Calmodulin & lipid binding partners

Zhu et al. 2001 Science, 293, 2101

Protein microarrays: Various platforms
Protein microarrays:
Direct labeling

Protein microarrays:
Sandwich immunoassay
Protein microarrays: Reverse phase protein blot

Protein microarrays: Chemically linked
Protein microarrays: Peptide fusion tags

Protein microarrays: Nucleic acid programmable protein array
Protein microarrays:
Multiple spotting technique

Protein microarrays:
An overview

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<th>Abundance based</th>
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MIST

NAPPA

NPTEL

12/11/12
Protein microarrays

Achievements
High density
Small volume
Multifunctional assays

Challenges
Protein purification
Protein functionality

Summary
- Interactomics
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References

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