CBS 530 Assignment No 2

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Review of the paper: Functional organization of the yeast proteome by systematic analysis of protein complexes

Abstract

In this report the author gives her own view on the recent articles of [1]. The author shall try to cover the questions that the work of this paper addressed, a critique of the appropriateness of the methodology, the primary results that relate to the question and the discussion of whether the data answer the question.

1 Introduction

Most of the processes of cell are carried out by multiprotein complexes. The identification and analysis of multiprotein components provide how the group of performers working together to expressed protein which is organized into functional units for that Gavin et al used tandem-affinity purification(TAP) and mass spectrometry(MS) methods to characterize multiprotein complexes in yeast. In medicine, genetic information is reflected in the numerous disorders based on polygenic characterization and indication is that the number of human diseases exceeds than the number of genes in the genome. Postmortem of the genetic and biochemical circuits of a cell is a fundamental problem in biology. At the biochemical level, protein act alone, rather than they interact in a group to perform particular cellular tasks. The hidden truth gained from the analysis has been fundamental for the biological understanding of their functions and well beyond the limits of genetic analysis. Important examples are the spliceosome, the cyclosome, the proteasome, the nuclear pore complex and the synaptosome.

2 Questions Related To Work

Using TAP/MS methods how Gavin et al have characterized the protein complexes relies on the conditions used for the assemblies and retrieval of the complexes? What are two methodologies address different aspects of protein interaction?

3 Methodology

For purification of multiprotein complexes, Gavin et al developed a strategy, according to that the TAP cassette is inserted at the C terminus of a given yeast ORF by homologous recombination, generating the TAP-tagged fusion protein. TAP complexes purified from different subcellular

compartments separated on denaturing protein gels and stained with Coomassie.
They processed 1, 739 genes, in which 1, 143 human genes representing eukaryotic orthologeus to human biology and purified tagged 589 protein assemblies. These assemblies defined 232 different multiprotein complexes(annotated TAP complexes) and also suggest new cellular functions for 344 protein, in which 231 protein has no previous functional reference and for comparison also targeted a nonorthologous set of 596 genes from chromosomes 1, 2 and 4, and used haploid cells to test the tagged genes in the absence of the wild-type allele and also from 1, 739 genes, a library of 1, 548 yeast strains generated of which 1, 167 expressing the clones. After growing cells to mid-log phase, assemblies were purified from total cellular lysates by TAP.
This technique combine a • first high-affinity purification using a site- specific protease and a
• second affinity purification to obtain protein complexes. The purified protein assemblies were separated by denaturing gel electrophoresis, individual bands were digested by trypsin, analyzed by matrix-assisted laser desorption or ionization time-of-flight mass spectrometry and identified by database search algorithms, and only 70 of the membrane-associated protein were analyzed, of which 40 were purified successfully. Analysis covers proteins of various subcellular compartments.

From 589 tagged purified proteins, 78% presents combine partners and also generated samples for mass spectrometry and those who were unable to purify and identify the reason behind that they may not form sufficient stable or soluble complexes. Furthermore, the method fail to detect transient interactions, low stoichiometric complexes and in about 30% cases failed to purify complexes around a given protein.

For reproducibility Gavin et al purified 13 large complexes at least twice. The probability of detecting the same protein in two different purifications was 70%, when complexes were retrieved from only one entry point.

On the basis of overlapping obtained 589 different entry points in which 98 known for non-repeated multiprotein compartments presented in the yeast protein database and 242 purification were joined into 134 new complexes. The remaining 102 protein showed no detectable association with other protein when purified directly, or as part of other complexes.
Among the complexes that were assigned to the known YPD complexes, specified amount of components was very high. Of all 232 TAP complexes, only 9% had no noval element. The size of the TAP complexes varied from 2 to 83 components, with an average of 12 components per complex, of the 304 protein with no YDP functional references identified for 113 protein that had a functional reference.

The dynamics of complex composition are already defined by the cellular signaling complexes formed around the protein phosphate 2*A*. Tagging different known PP2A components resulted in

the purification of the known trimeric complexes containing Tpd3 either in regulatory A subunit or in regulatory B subunits.

To determine the experiment, purified mock-transformed control strains, in which 17 identified as contaminant proteins. Those were highly expressed because those appeared in more than 20 purification, manly heat-shock and ribosomal proteins. Gavin et al have performed nearly all analysis of protein complexes of baker's yeast. They determined the TAP method to retrieve equivalent multiprotein complexes from yeast and human cells, compared different complexes from different subcellular compartments, Arp2/3 a cytoskeleton-associated complex, Ccr4-Not, a nuclear assembly and Trapp, a Golgi-associated complex. The Arp2/3 complex is a stable multiprotin assembly required for the nucleation of acting filament in all eukaryotic cells and consists of 7 proteins in human and yeast so the TAP combined with MS is an efficient and sensitive method for the retrieval and characterization of human multiproein complexes. The yeast Ccr4-Not complex is involved in the control of gene expression and consists of 8 components. Purification of tagged yeast Ccr4 resulted in the identification of a complex component. This showed human and yeast Ccr4-Not complexes are comparable in subunits. And Purified an orthologous human TRAPP(transport protein particle) complex. This yeast complex contains 10 subunits. These examples show that the complex comparison between yeast and human was largely conserved.

Gavin et al Compared their data with two data base • YDP(yeast protein database)
• Yeast two-hybrid data
and found it is very difficult to compare data with second one because it produces binary interactions, whereas TAP/MS method put data together. But comparison with first one was straight forward. When considered all possible interaction between protein within a complex or a purification with the YDP and yeast two-hybrid data, data cover 10% with YDP and 56% with yeast two-hybrid data and Gavin et al gave two reasons for this difference.

Proteins belonging to defective list generally comprise the weak bonds after staining and identified by peptides(amino acid sequence), and the sensitivity of the TAP/MS methods was high because they found 15 copies per cell of protein.

After assigned individual protein to protein complexes, examined relationship between complexes to understand the combination and coordination of the cellular functions. Represents relationship by linking complexes connections in the network reflect physical interaction of complexes, regulation, localization, architecture and a high-order organization map.

4 Primary Results Related To Question

Several complexes belonging to the same class appear to group, suggesting that sharing of components reflects functional relationship. These relationship are best observed with complexes involved in mRNA metabolism, cell cycle, protein synthesis and turnover, intermediate and energy metabolism, transcription, DNA maintenance, protein and RNA transport.

In comparison they found that orthologous protein prefer to interact with those complexes who has other orthologous and in nonorthologous protein, have a lower tendency for interacting with other complexes and similarly interaction with essential gene product is higher for essential than nonessential in protein.

They found TAP is invaluable for the purification of complexes from different cellular compartments, including those complexes which attached with cellular membrane. TAP allows the purification of very large complexes.

5 Future Work and Whether The Data Answer The Question

Two methodologies used for the
• Assemblies and
• Retrieval
of the complexes of protein interaction.
To retrieve and analysis particular cellular protein complexes under physiological conditions Gavin et. al. studied protein interaction based on
• Biological process(Ex vivo),
• Biochemical process (In vitro) and
• Silico method(Computer based)
such as two-hybrid system and protein chips.
Processed $1,739$ genes, in which $1,143$ human genes representing eukaryotic orthologues to human biology and purified tagged 589 protein assemblies. These assemblies defined 232 different multiprotein complexes(annotated TAP complexes) and also suggest new cellular functions for 344 protein, in which 231 protein has no previous functional references. Comparison of yeast and human complexes showed that preservation of natural environment across species extends from single protein to their larger group of proteins. Analysis provides an rough draft of the eukaryotic proteome working as a network of protein complexes and also provides fundamental biological information form map.

protein complexes can be retrieved from cells by release from constraineds. TAP/MS method does not provide information on the orientation of complex components. They believed that equivalence of human and yeast complexes usefull for multifactorial diseases.

In the future, they will modify the parameters to see environmental impact on complex combination and this will help to understand the functions of complex assembly and disassembly.

References

[1] A.-C. Gavin, M. Bosche, R. krause, P. Grandi, M. Marzioch, A. Bauer, J. schultz, J.M. Rick, A.-M. Michon, et al. *Functional organization of the yeast proteome by systematic analysis*

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